Cape Peninsula University of Technology

MORPHOLOGICAL CHARACTERISTICS AND CHEMICAL COMPOSITION OF SKULLCAP (*Scutellaria lateriflora* L.) AND BURDOCK (*Arctium lappa* L.) CULTIVATED UNDER DIFFERENT CONDITIONS

By

MBAPPE TANGA

Thesis submitted in fulfilment of the requirements for the degree

PhD Environmental Health

in the Faculty of Applied Science

At the

Cape Peninsula University of Technology

Supervisor: Prof. F.B. Lewu
Co-supervisor: Prof. A.O. Oyedeji
Prof. O.O Oyedeji

Cape Town
November 2021

DECLARATION

I, **MBAPPE TANGA** declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

November 22nd, 2021

Signed **Date**

ABSTRACT

Burdock (*Arctium lappa* L.) and American skullcap (*Scutellaria lateriflora* L.) are medicinal plants that are highly rich in phytochemicals which contribute towards their therapeutic values. They also possess culinary values as herbal tea. The roots of Burdock are consumed as salad in Europe and Asian continents which are the regions of its origin. Currently, the plant materials of these plants are imported for their medicinal purposes by herbal practitioners and pharmaceutical industries in different countries of the world, including South Africa. However, the cultivation of these plants is lacking in South Africa and Africa as a whole. To achieve consistent supply of quality products and eliminate batch-to-batch variability of plant materials to meet up with the demand. A preliminary study on yield and phytochemical constituents of *Arctium lappa* L. as influenced by potassium and phosphorous fertilizer application and two different factorial experiments were conducted for Burdock on Takinogawa long cultivar with eight treatments (T_1=$N_{423}P_{210}K3_{15}$, T_2=$N_{423}P_{280}k_{315}$, T_3=$N_{635}P_{210}K_{315,}$ T_4=$N_{635}P_{280}K_{315}$, T_5=$N_{846}P_{210}K_{315}$, T_6=$N_{846}P_{280}K_{315}$, T_7=$N_{1058}P_{210}K_{315}$ and T_8=$N_{1058}P_{280}K_{315}$ Kg/ha) and five collection times laid out in a completely randomized design, replicated five times under 40% shade net in pot and field experiments. Fertilizer treatments were split into two equal doses at seedling stage and four weeks after transplanting. Data on morphological characteristics and yield were collected and analysed using SAS software. There was a significant ($P<0.05$) difference across fertilizer treatments on morphological characteristics and time of collection for both pot and field experiments. Significant differences ($P<0.05$) were also recorded on the yield parameters investigated. Treatment ($N_{635}P_{210}$ K_{315} Kg/ha) significantly outperformed the other treatments in the pot experiment. While for the field experiment, treatment ($N_{846}P_{280}K_{315}$Kg/ha) significantly outperformed the other treatments. Furthermore, phytochemical constituents, antioxidant and anti-inflammatory activities of aqueous and methanol leaf and root extracts of burdock as influenced by fertilizer treatments were assessed. Result of phytochemical screening were fairly rich indicating a positive test for phenols, flavonoids, tannins, saponins and glycosides. Significant differences ($p<0.05$) were observed on total phenolic content (TPC), total flavonoids content (TFC) and condense tannins (PAC) on both aqueous and methanol leaf and root extracts in both pot and field studies. The nutritional constituents of the burdock root and leaf samples in both pot and field experiments were significant ($P<0.05$) except for root lipid content for pot experiment and leaf ash content for field experiment which were not significant ($P>0.05$). For antioxidant activities, 2,2-diphyl-1-picrythydraxyl, Nitric oxide and hydrogen peroxide were significant ($p<0.05$) for aqueous and methanol leaf and root extracts for pot and field experiments among treatment combinations. Overall, scavenging activity for treatment combinations with a lower level of phosphorous (P_{210} Kg/ha) recorded a lower IC_{50}

values compared to those with higher level of phosphorous (P_{280} Kg/ha). Furthermore, anti-inflammatory activity for aqueous and methanol dried root and leaf extracts for pot and field experiments demonstrated significant differences ($p<0.05$) among treatment combinations. GC-MS analysis of essential oil for burdock root validated a variation in chemical composition among the different treatment combinations. Interestingly, the greatest variation was demonstrated by T_7 with a total of 20 compounds identified; followed by T_3 with 19 compounds, T_4, T_5, T_6 and T_8 with 14compounds while T_1 and T_2 had 13 compounds. Similarly, a factorial pot experiment was conducted for *S. lateriflora* with the same procedure and application, but with different treatments (T_1=$N_{350}P_{213}K_{213,}$ T_2=$N_{350}P_{320}K_{213,}$ T_3=$N_{525}P_{213}K_{213}$, T_4=$N_{525}P_{320}K_{213,}$ T_5=$N_{700}P_{213}K_{213}$, T_6=$N_{700}P_{320}K_{213}$, T_7=$N_{800}P_{213}K_{213}$ and T_8=$N_{800}P_{230}K_{213}$ Kg/ha). Fertilizer treatments and time of collection had a significant ($p<0.05$) effect on the vegetative growth parameters investigated. Significant ($p<0.05$) effect was also recorded on biomass yield and total flavonoid contents across the different phenological stages. Fertilizer range (N_{525}-N_{700} with P_{213}-P_{320} and K_{213}Kg/ha) had the highest influence on growth and yield attributes. Furthermore, phytochemical constituents, antioxidant, and anti-inflammatory activities of aqueous and methanol leaf extracts of American skullcap as influenced by fertilizer treatments were assessed. The results of phytochemical screening were quite rich at a varying degree, demonstrating the presence of flavonoids, tannins, saponins, phenols, alkaloids, terpenoids and steroids. The highest concentration of total phenolic content was recorded at the post flowering stage (41.65%) followed by the pre-flowering stage (33.83%) and the least at flowering stage (24.52%). Also, total flavonoid content recorded the highest concentration at post flowering stage (38.2%), followed by pre-flowering stage (34.2%) and the least at flowering stage (27.6%). However, for condense tannins, the highest concentration was recorded at the pre-flowering stage (38.5%) followed by the post flowering stage (31.2%) and the least at the flowering stage (30.3%). Also, the nutritional constituents of the leaves were significant ($P<0.05$). The antioxidant and anti-inflammatory activities exhibited in aqueous, and methanol dried leaf extracts had significant ($p<0.05$) scavenging and anti-inflammatory activities demonstrated by the IC_{50} values. T_5 had the lowest IC_{50} values for all the antioxidant assays. However, T_4 recorded the most ideal anti-inflammatory activity with IC_{50} value (352.8 µg/ml) for aqueous extract and T_7 (834.1 µg/ml) for methanol extract. Overall, treatments with lower supplementary phosphorous (P_1=213kg/ha) recorded a higher concentration of total phenolic, flavonoids and condense tannins, ash, and lipid contents than those with higher supplementary phosphorous (P_2=320kg/ha). For carbohydrate content, treatments with high supplementary phosphorus had a higher yield response than those with lower supplementary phosphorous

Keywords: Cultivation, Skullcap, Burdock, medicinal plants, morphology, secondary metabolites,

fertilizer treatments.

ACKNOWLEDGEMENTS

I wish to thank:

- God Almighty for providing me with the opportunity to enrol for this degree, the dedication, courage, hope and faith that sustained me throughout the study.
- My supervisors, Profs. F.B. Lewu, A.O. Oyedeji and O.O Oyedeji for their guidance throughout the study; not only as supervisors, but as parents and believing in me which has been my drive throughout the study.
- My wife, daughter and son for their love, encouragement, and sacrifice, and for their patience in the absence of a husband and a father throughout the study period.
- My entire family back at home for their constant prayers and words of encouragement, making me to understand that I was not alone in the PhD journey.
- My in-laws for their support and words of encouragement to me and my wife that the journey was a family one.
- The Department of Environment and Occupational Studies CPUT for granting me admission to enrol for the degree.
- The Department of Agriculture CPUT for accepting me with all the love and trusting me with their resources throughout the study.
- Dr. Bisi Oladele from Australia for her relentless effort for seed sourcing for the study.
- Dr. Lewu from ARC for her laboratory coaching for my experimental set-up for proximate analysis.
- Mr. Mzwane and his term for the constant support I received during the horticultural and field work of the study.
- Dr. Nell for his relentless effort in his capacity to make sure that all the requisitions concerned are not delayed in this duty.
- The Department of Chemistry Walter Sisulu University (WSU) for granting me assess to their Natural Product laboratory with all the assistance from Mr. Miya Gugulethu making sure I lack nothing during my experimental set-up for phytochemical and biological studies, not forgetting the WSU postgraduate Natural Product Group for all the love and assistance I received throughout the study.
- The Department of Food Technology CPUT Bellville Campus with the assistance of Mr. Ndumiso for his support for the experimental set-up for protein and nitrogen analyses.
- All my friends, most especially Pastor Marinus Alang and the family for their constant encouragement, moral support and unconditional love shown to me throughout my PhD program.

- Prof, F. Nchu, Prof. M. Fanadzo and Dr. M. Saibu for their words of encouragement throughout the PhD Journey
- The postdocs Dr. Ighodaro, Dr. Adetunji and Dr. Chika for their technical assistance and moral support throughout the study
- The Department of Agriculture CPUT Staff, most especially Mr. N. Madolo, Ms A. Bolilitye, Ms N. Mfeka and Ms S. Buyabuye for their love and moral support shown to me throughout the study.
- The financial assistance of the National Research Foundation (NRF/DST Scholarship) awarded to me for the PhD study.

DEDICATION

TO MY FAMILY

PREFACE OF THE THESIS

The research activities presented in the thesis were conducted in the Department of Agriculture, Cape Peninsula University of Technology, Wellington Campus, South Africa. The horticulture and field experimental work were conducted at the Department of Agriculture, Agric-hub research station of the Cape Winelands region of the Western Cape Province of South Africa.

The thesis consists of eight chapters

Chapter 1: The general introduction, consisting of the background, rationale, aim and objectives, hypothesis delineation and significance of the study.

Chapter 2: The literature review, presents detailed perspectives on medicinal plants cultivation as a possible solution for quality assurance and consistent availability of plant materials for commercialisation with a case study on Burdock (*A lappa*) and American skullcap (*S lateriflora*)

Chapter 3: Investigates the growth, yield, and phytochemical constituents of Burdock (*A lappa*) in response to phosphorous and potassium fertilizers application.

Chapter 4: Investigates the yield and morphological characteristics of Burdock (*A lappa* .) as affected by NPK mineral fertilizer application in pot and field experiments

Chapter 5: Examines the yield, morphological characteristics, and the accumulation of flavonoid content in American skullcap (*S lateriflora* L) as influenced by NPK mineral fertilizer application in a pot experiment.

Chapter 6: Investigates the phytochemical constituents, antioxidant, and anti-inflammatory activities of American skullcap (*S lateriflora*) as influence by NPK mineral fertilizer application in pot experiment.

Chapter 7: Evaluates the phytochemical constituents, antioxidant, anti-inflammatory activities, and the variation in essential oil composition of Burdock (*A lappa*) as affected by NPK mineral fertilizer application in pots and field experiments

Chapter 8: Comprises the general conclusions and recommendations which summarises the results and discussions, conclusion, and recommendations of the study.

RESEARCH OUTPUTS

The following are research outputs that acknowledged the candidate contributions to scientific knowledge and development during the PhD program.

Journal Papers

Published

Tanga, M., Lewu, FB., Oyedeji, AO. & Oyedeji, OO. 2018. Cultivation of medicinal plants in South Afriica: A solution to quality assurance and constitent availability of medicinal plant materials for commercialization. Academic Journal of Medicinal Plants, 6(7),168-177. https//doi.org/10.15413/ajmp.2018.0133,(Chapter 2).

Tanga, M., Lewu, FB., Oyedeji, AO. & Oyedeji, OO. 2020. Growth, yield, and phytochemical constituents of *Arctium lappa* L. in response to phosphorous and potassium fertilizers Application. The 18th South Africa International Conference on Agricultural, Chemical, Biological and Environmental Sciences (ACBES-20) Nov.16-17,2020Johannesburg (SA) proceedings. https://doi.org/10.17758/EARES10.EAP1120122,(Chapter 3).

Tanga, M., Lewu, FB., Oyedeji, AO. And Oyedeji, OO. 2020. Yield and morphological characteristics of Burdock (*Arctium lappa* L) in response to mineral fertilizer application. Asian J. Agric. Biol. 8(4): 511-518. https://doi.org/10.35495/ajab.2019.11.524 (Chapter 4). .

Tanga, M., Lewu, FB., Oyedeji, AO. & Oyedeji, OO. 2022. Volatile oil composition of Burdock root (*Arctium lappa* L) in response to mineral fertilizer application.The 33rd South Africa International Conference on Chemical, Biological and Environmental Engineering (JCBEE-22), March 17-18 2022 Johannesburg (South Africa), ISBN-978-989-53228-9-3. (A section of chapter 7).

Submitted /Prepared Manuscripts

Tanga, M., Lewu, FB., Oyedeji, AO. & Oyedeji, OO. 2020. Cultivation of Burdock (*Arctium lappa* L.): a possible solution to quality assurance and consistent availability of plant materials for commercialization Open Access Journal of Medicinal and Aromatic Plants. ID: 104293-09-02 (Chapter 2).

Tanga, M., Lewu, FB., Oyedeji, AO. & Oyedeji, OO. 2021. Cultivation of skullcap (*Scutellaria* l*ateriflora* L.): as a possible solution to quality assurance and consistent availability of plant materials for commercialisation prepared manuscript (Chapter 2).

Tanga, M., Lewu, FB., Oyedeji, AO. & Oyedeji, OO. 2021. Yield, morphological characteristics, and flavonoid content of American skullcap (*Scutellaria lateriflora* L.) in response to mineral fertilizer application. Prepared manuscript (Chapter 5).

Tanga, M., Lewu, FB., Oyedeji, AO. & Oyedeji, OO. 2021. Phytochemical constituents, antioxidant properties and anti-inflammatory activities of American skullcap (*Scutellaria lateriflora* L.) in response to mineral fertilizer application. Prepared manuscript (Chapter 6).

Tanga, M., Lewu, FB., Oyedeji, AO. &Oyedeji, OO. 2021. Phytochemical constituents, antioxidant properties, anti-inflammatory activities, and volatile oil composition of Burdock (*Arctium lappa* L.) in response to mineral fertilizer application. Prepared manuscript (Chapter 7)

B. Conferences Attended

Tanga, M., Lewu, FB., Oyedeji, AO. & Oyedeji, OO. 2019. Yield evaluation, phytochemical constituents, and Essential oil composition of burdock (*Arctium lappa* L.) in response to phosphorous and potassium fertilizer application, Proceedings of 7th international U6 consortium conference, held at University of Calabar, Nigeria, 2nd – 6th September 2019 (Oral presentation)

https://u6conference.unical.edu.ng

Tanga, M., Lewu, FB., Oyedeji, AO. & Oyedeji, OO. 2020. Growth, Yield and Phytochemical Constituents of *Arctium lappa* L. in Response to Phosphorous and Potassium Fertilizers Application. The 18th South Africa International Conference on Agricultural, Chemical, Biological and Environmental Sciences (ACBES-20) Nov.16-17,2020 Johannesburg (SA) proceedings. (Oral presentation). http://earbm.org/proceedingspdf.php?id=404

Tanga, M., Lewu, FB., Oyedeji, AO. & Oyedeji, OO. 2022. Volatile oil composition of Burdock root (*Arctium lappa* L) in response to mineral fertilizer application.The 33rd South Africa International Conference on Chemical, Biological and Environmental Engineering (JCBEE-22), March 17-18 2022 Johannesburg (South Africa), ISBN-978-989-53228-9-3. (Oral presentation). https://www.iicbe.org/2021/11/18/127

TABLE OF CONTENTS

DECLARATION ii
ABSTRACT iii
DEDICATION viii
PREFACE OF THE THESIS ix
Table of Contents xii
LIST OF TABLES i
LIST OF FIGURES i
GLOSSARY ii
1 CHAPER 1 GENERAL INTRODUCTION 1
1.1 Background 1
1.2 Statement of the problem 3
1.3 Rational of the study 3
1.4 Aim of the study 5
1.5 Objectives of the study 5
1.6 Significance of the study 5
1.7 Hypotheses 5
1.8 Delineation of the study 6
1.9 Structure of the thesis 6
References 7
2 CHAPTER 2 LITERATURE REVIEW 10
2.1 Cultivation of medicinal plants in South Africa: a solution to quality assurance and consistent availability of medicinal plant materials for commercialization 10
2.1.1 Introduction 10
2.1.2 Importance of medicinal plants 11
2.1.3 Trade in medicinal plants 11
2.1.4 Overharvesting of medicinal plants 12
2.1.5 Conservation strategy of wild medicinal plants collection 13
2.1.6 Prospects in medicinal plant cultivation 13
2.1.7 Introducing medicinal plants for commercialization in South Africa 14
2.1.7.1 Cultivation of medicinal plants 14
2.1.7.2 .Seed propagation 15
2.1.7.3 Vegetative propagation 16
2.1.8 Cultivation of medicinal plants: A solution for biodiversity conservation in South Africa. 17
2.1.9 Cultivation of medicinal plants: As a possible solution of product quality and commercialization 18
2.1.10 Challenges in medicinal plants cultivation 21

2.1.11 Influence of regulatory policy on the safety and quality of medicinal plants products 21
2.2 CULTIVATION OF BURDOCK (*ARCTIUM LAPPA* L): A POSSIBLE SOLUTION TO QUALITY ASSURANCE AND CONSISTENT AVAILABILITY OF PLANT MATERIALS FOR COMMERCIALIZATION. 22
2.2.1 Introduction 22
2.2.2 Botany and geographical distribution of Burdock 23
2.2.3 Agroecology of Burdock 23
2.2.4 Agronomic practices of Burdock 24
2.2.5 Field cultivation of Burdock 25
2.2.6 Micropropagation and molecular studies on Burdock 26
2.2.6.1 Tissue culture of Burdock 27
2.2.6.2 . Plant culture and biosynthesis of secondary metabolites of Burdock 28
2.2.7 Metabolomic profile of Burdock 29
2.2.8 Medicinal and other impetus for cultivation of Burdock 32
2.2.9 Potential economic benefits of Burdock 37
2.3 Cultivation of skullcap (*Scutellaria lateriflora* l.) As a possible solution to quality assurance and consistent availability of plant materials for commercialisation 38
2.3.1 Introduction 38
2.3.2 Botany and geographical distribution of American Skullcap 39
2.3.3 Agroecology of American Skullcap 40
2.3.4 Agronomic practices of American Skullcap 41
2.3.4.1 Field cultivation of American Skullcap 41
2.3.4.2 Micropropagation and molecular studies on American Skullcap 43
2.3.4.3 Tissue culture and biosynthesis of secondary metabolites of American skullcap 43
2.3.5 Metabolic profile of American Skullcap 44
2.3.6 Medicinal impetus for cultivation of American Skullcap 47
2.3.7 Potential economic benefits of American Skullcap 49
2.3.8 Conclusion 51
References 53
3 CHAPTER 3 GROWTH, YIELD AND PHYTOCHEMICAL CONSTITUENTS OF *ARCTIUM LAPPA* L, IN RESPONSE TO PHOSPHOROUS AND POTASSIUM FERTILIZER APPLICATION 65
3.1 Introduction 65
3.2 Materials and methods 65
3.2.1 Study Area 65
3.2.2 Experimental layout and treatment application 66
3.2.3 Data collection 66
3.2.4 Phytochemical screening and proximate analysis of root and leaf of Burdock 66

3.2.5 Statistical analysis 67
3.3 Results and Discussion 67
3.3.1 Effects of fertilizer treatments on growth parameters of Burdock 67
3.3.2 Effects of fertilizer treatments on yield parameters of Burdock..... 68
3.3.3 Phytochemical screening of root and leaf extract of Burdock 69
3.3.4 Proximate analysis of root and leaf samples of *Arctium lappa* L..... 71
3.3.5 Conclusion..... 72
References..... 73
4 CHAPTER 4 YIELD AND MORPHOLOGICAL CHARACTERISTICS OF BURDOCK (*ARCTIUM LAPPA* L) IN RESPONSE TO MINERAL FERTILIZER APPLICATION 75
4.1 Introduction..... 75
4.2 Materials and methods 76
4.2.1 Study Area..... 76
4.2.2 Soil collection and analysis 76
4.2.2.1 Pot experiment 76
4.2.2.2 Field experiment 77
4.2.3 Experimental layout and treatment application..... 77
4.2.4 Data collections 78
4.2.5 Statistical analysis 78
4.3 Results 78
4.3.1 Influence of fertilizer treatments on morphological characteristics of Burdock. 78
4.3.1.1 Pot experiment 78
4.3.1.2 Field experiment 79
4.3.2 Influence of time of data collection on morphological characteristics of Burdock.. 81
4.3.2.1 Pot experiment 81
4.3.2.2 Field experiment 81
4.3.3 Influence of fertilizer treatments on yield of Burdock 83
4.3.3.1 Pot experiment 83
4.3.3.2 Field experiment 83
4.4 Discussion 84
4.5 Conclusion..... 87
Reference 88
5 CHapter 5 YIELD, MORPHOLOGICAL CHARACTERISTICS AND FLAVONOID CONTENT OF AMERICAN SKULLCAP (*Scutellaria lateriflora* L.) IN RESPONSE TO MINERAL FERTILIZER APPLICATION 90
5.1 Introduction..... 90
5.2 Materials and methods 91
5.2.1 Study Area..... 91
5.2.2 Soil collection and analysis results..... 91

5.2.3 Seeds collection and nursery 92
5.2.4 Experimental layout and treatment application 92
5.2.5 Data collections 92
5.2.5.1 Growth parameters 92
5.2.5.2 Total flavonoid content (TFC) 93
5.2.6 Statistical analysis 93
5.3 Results 93
5.3.1 Influence of fertilizer treatments on morphological characteristics of American Skullcap 93
5.3.2 Influence of time of data collection on morphological characteristics of American Skullcap 94
5.3.3 Influence of fertilizer treatments on biomass yield of American Skullcap 95
5.3.4 Influence of fertilizer treatments on the accumulation of total flavonoid content at the harvesting stage of American Skullcap 96
5.4 Discussions 97
5.5 Conclusion 99
References 101
6 CHAPTER 6 PHYTOCHEMICAL CONSTITUENTS, ANTIOXIDANT PROPERTIES AND ANTI-INFLAMMATORY ACTIVITIES OF AMERICAN SKULLCAP (*Scutellaria lateriflora* L) IN RESPONSE TO MINERAL FERTILIZER APPLICATION 104
6.1 Introduction 104
6.2 Materials and methods 105
6.2.1 Study sites 105
6.2.2 Chemicals 105
6.2.3 Samples preparation and extraction 105
6.2.4 Phytochemical determination 106
6.2.5 Determination of total phenolic content (TPC) 106
6.2.6 Determination of total flavonoid content 106
6.2.7 Determination of proanthocyanidin content (Condense Tannin) 107
6.2.8 Proximate analysis of leaf samples 107
6.2.9 Evaluation of antioxidant activity 107
6.2.9.1 DPPH free radical scavenging assay 107
6.2.9.2 Nitric oxide scavenging assay 108
6.2.9.3 Hydrogen peroxide scavenging assay 108
6.2.9.4 Anti-inflammatory Assay in vitro 109
6.2.10 Data analysis 109
6.3 Results 109
6.3.1 Phytochemical screening of leaf samples of American Skullcap in response to fertilizer treatments 109

6.3.2 Influence of fertilizer treatments on yield of total phenolic content (TPC) at the different phenological stage of American Skullcap. Values are means ((µg/mL GAE/g) at (P-0.05). 110

6.3.3 Influence of fertilizer treatments on yield of total flavonoid content (TFC) at the different phenological stage of American Skullcap 111

6.3.4 Influence of fertilizer treatments on yield of condense tannins (PAC) at the different phenological stages of American Skullcap 112

6.3.5 Nutritional constituents of American Skullcap as affected by fertilizer treatment combinations. Values are means at (P-0.05) 113

6.3.6 Influence of fertilizer treatments on antioxidant activity of aqueous and methanolic dried leaf extracts of cultivated American skullcap 114

6.3.7 Influence of fertilizer treatment on anti-inflammatory activity of aqueous and methanolic dried leaf extracts of cultivated American skullcap 115

6.4 Discussion 116

6.5 Conclusion 119

References 121

7 CHAPTER 7 PHYTOCHEMICAL CONSTITUENTS, ESSENTIAL OIL COMPOSITION, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF BURDOCK (*ARCTIUM LAPPA* L.) IN RESPONSE TO MINERAL FERTILIZER APPLICATION 124

7.1 Introduction 124

7.2 Materials and methods 125

7.2.1 Study sites 125

7.2.2 Chemicals 125

7.2.3 Samples preparation and extraction 125

7.2.4 Phytochemical screening 125

7.2.5 Determination of total phenolic content (TPC) 125

7.2.6 Determination of total flavonoid 126

7.2.7 Determination of proanthocyanidin content (Condense tannin) 126

7.2.8 Proximate analysis of leaf and root samples 126

7.2.9 Evaluation of antioxidant activity 126

7.2.10 DPPH free radical scavenging assay 126

7.2.11 Nitric oxide scavenging assay 127

7.2.12 Hydrogen peroxide scavenging assay 127

7.2.13 Anti-inflammatory Assay (*In vitro*) 127

7.2.14 Determination of volatile oil composition of Burdock root as influence by fertilizer treatments 127

7.2.15 Data analysis 128

7.3 Results 128

7.3.1 Phytochemical screening of aqueous root and leaf extracts of Burdock in response to fertilizer treatments for pot and field experiments 128

7.3.2 Influence of fertilizer treatments on total phenolic content (TPC), total flavonoids content (TFC) and condense tannins (PAC) of root and leaf extracts of Burdock for pot experiment 130
7.3.3 Influence of fertilizer treatment on total phenolic content ((TPC), total flavonoid content (TFC) and Condense tannin (PAC) of root and leaf extracts of Burdock for field experiment. 133
7.3.4 Influence of fertilizer treatments on nutritional constituents of leaf and root samples of Burdock for pot experiment 136
7.3.5 Influence of fertilizer treatments on nutritional constituent of leaf and root samples of Burdock for field experiment 136
7.3.6 Influence of fertilizer treatments on DPPH antioxidant activities for root and leaf extract of Burdock for pot and field experiment 139
7.3.7 Influence of fertilizer treatments on nitric oxide scavenging activity for dried root and leaf extracts of Burdock for pot and field experiments 140
7.3.8 Influence of fertilizer treatments on hydrogen peroxide scavenging activity for dried root and leaf extracts of Burdock for pot and field experiments 142
7.3.9 Influence of fertilizer treatments on anti-inflammatory activities for dried root extracts of Burdock for pot and field experiment 144
7.3.10 Influence of fertilizer treatments on volatile oil composition of Burdock root as determined by GC-MS for field experiment. 146
7.4 Discussion 151
7.5 Conclusion 155
References 156
8 CHAPTER 8 GENERAL CONCLUSIONS AND RECOMMENDATIONS 159
8.1 Summary and Conclusions 159
8.2 Recommendations 162

LIST OF TABLES

Table 2.1 Advances on factors that influence seed germination potentials..... 15
Table 2.2 Cultivation practice and uses of some South African medicinal plant's species..... 17
Table 2.3 Factors that influence the synthesis of secondary metabolites in plants..... 20
Table 2.4 Metabolic Profile of plant extracts and volatile constituents of Burdock (Arctium Lappa L)..... 30
Table 2.5 Therapeutic and pharmacological activities of Burdock (*Arctium lappa* L.)..... 32
Table 2.6 Metabolic Profile of plant extracts of American Skullcap (*S lateriflora*)..... 45
Table 2.7 Therapeutic and pharmacological activities of American Skullcap..... 48
Table 2.8 American Skullcap products in the market..... 50
Table 3.1 Influence of fertilizer treatments on morphological characteristics of Burdock. Values are means at (P-0.05)..... 68
Table 3.2 . Influence of fertilizer treatments on yield related parameters of Burdock..... 69
Table 3.3 Phytochemical screening of aqueous root and leaf extracts of Burdock in response to treatments..... 70
Table 4.1 Chemical properties of potted soil..... 77
Table 4.2 Morphological characteristics of burdock with different fertilizer treatments for pot and field experiment. Values are means at (P-0.05)..... 80
Table 4.3 Correlations on Morphological characteristic at (P-value 0.05)..... 80
Table 4.4 Influence of different fertilizer treatments on yield related parameters of burdock. Values are means at (P-0.05)..... 84
Table 5.1Chemical properties of potted soil..... 91
Table 5.2 Morphological characteristics of American Skullcap with different fertilizer treatments. Values are means at (P-0.05)..... 94
Table 5.3: Influence of fertilizer treatments on biomass yield of American Skullcap. Values are means at (P-0.05)..... 96
Table 6.1 Phytochemical screening of aqueous leaf extract of American Skullcap (*Scutellaria lateriflora* L.) in response to fertilizer treatments..... 110
Table 6.2 Proximate analysis for leaf samples of American Skullcap of different treatments. Values are means at (P=0.05)..... 114
Table 6.3 Influence of fertilizer treatments on antioxidant properties on leaf extract of American Skullcap. Values are means at (P-0.05)..... 115

Table 7.1Phytochemical screening of aqueous root and leaf extract of Arctium lappa L. in response to fertilizer treatments in pot experiment..... 129
Table 7.2Phytochemical Screening of aqueous root and leaf extract of Arctium lappa L. in response to mineral fertilizer treatments in field experiment..... 129
Table 7.3 Influence of fertilizer treatments on Total phenolics, flavonoids and proanthocynadin content of Burdock leaf and root extracts for pot experiment. Values are means at (P-0.05)... 132
Table 7.4 Influence of fertilizer treatments on Total phenolics, flavonoids and proanthocynadin content of Burdock leaf extracts for field experiment. Values are means at (P-0.05)..... 135
Table 7.5 Proximate analysis of root and leaf samples of Burdock as influenced by fertilizer treatments. Values are means at (p=0.05). 138
Table 7.6Influence of fertilizer treatments on DPPH scavenging activity for dried root and leaf extracts of Burdock. Values are means of IC_{50} (µg /ml) at (P-0.05). 139
Table 7.7Influence of fertilizer treatments on nitric oxide scavenging activity for dried root and leaf extracts of Burdock. Values are means of IC50 (µg/ml) at (P-0.05). 141
Table 7.8.Influence of fertilizer treatments on hydrogen peroxide scavenging activity of dried root and leaf extracts of Burdock. Values are means of IC50 (µg/ml) at (P-0.05). 143
Table 7.9 Influence of fertilizer treatments on anti-inflammatory activity of dried root and leaf extracts of Arctium lappa L for pot experiment. Values are means of IC_{50} (µg/ml) at (P-0.05).. 145
Table 7.10 Chemical composition of volatile oil of Burdock root as determined by GC-MS 147

LIST OF FIGURES

Figure 2.1 Different morphological features of Burdock (*A lappa*) 24
Figure 2.2 Structures of the major flavonoids of American skullcap (*Scutellaria lateriflora* L.) ... 39
Figure 2.3 Geographical distribution of Skullcap species(Irvin et al., 2019)..... 40
Figure 2.4The morphological features of *S lateriflora* 41
Figure 3.1 Influence of potassium fertilizer on crude lipid (fig: 3.1a), crude fibre (fig:3.1b) and ash content (fig:3.3c) of Burdock (*Arctium lappa* L). Values are means at (P-values 0.05). 71
Figure 3.2 Influence of phosphorous fertilizer on crude lipid (fig; 3.2a), crude fibre (fig: 3.2b) and ash content (fig: 3.3c) of Burdock (*Arctium lappa* L.). Values are means at (P-values 0.05)..... 71
Figure 4.1 Graph showing the influence of time on data collection on morphological characteristics of burdock for pot experiment. Values are means at (P=0.05). Bars with the same letter on each parameter are not statistically different (P≥0.05). (Least significant difference (LSD) for number of leaves = 0.32, plant height = 0.98, leaf length =1.06, leaf width = 1.24 and leaf chlorophyll=1.67)..... 82
Figure 4.2 Graph showing the influence of time on data collection on morphological characteristics of burdock for field experiment. Values are means at (P=0.05). Bars with the same letter on each parameter are not statistically different (P≥0.05). (Least significant difference(LSD) for number of leaves = 0.32, plant height = 0.98, leaf length =1.06, leaf width = 1.24 and leaf chlorophyll=1.67). 82
Figure 5.1 Influence of time on data collection on morphological parameters of American Skullcap. Values are means at (P-values 0.05). Bars with the same letter on each parameter are not significantly difference (P≥0.05). 95
Figure 5.2 Influence of fertilizer treatments on accumulation of flavonoid content at harvesting stage of American Skullcap..... 97
Figure 6.1 a & b: Accumulation of total phenolic content (TPC) at the different phenological stages of development of American Skullcap. Values are means (µg/mL QE/g) at (P-0.05). .. 111
Figure 6.2 a & b: Acummulation of total flavonoids content (TFC) at different phenological stages of development of American Skullcap. Values are means (µg/mL QE/g) at (P-0.05)..... 112
Figure 6.3 a & b: Accumulation of condense tannins (PAC) at the different phenological stages of development of American Skullcap. Values are means (µg/mL GAE/g) at (P-0.05). 113

GLOSSARY

°C	Degree Celsius
µg	Microgram
TPC	Total phenolic content
TCM	Traditional Chinese medicine
ANOVA	Analysis of variance
ARC	Agricultural Research Council
TFD	Transplanting date
C:N	Carbon to nitrogen ratio
TFC	Total flavonoid content
cm	Centimetre
CPUT	Cape Peninsula University of Technology
WSU	Walter Sisulu University of Science and Technology
TDW	Total dry weight
DF	Degree of freedom
PAC	Proanthocyanidin
TFW	Total fresh weight
DMRT	Duncan`s multiple range test
ha	Hectare
K	Potassium
kg	Kilogram
LSD	Least significant difference
m	Meter
mg	Milligram
min	Minute
mm	Millimetre
N	Nitrogen
NO	Nitric oxide
P	Phosphorus
S	Sulphur
K	Potassium
VIF	Variance inflation factor
T	Fertilizer treatment
DPPH	2,2- diphenyl-1picrythydrazyl hydrate
ASC	Ascorbic acid
H_2O_2	Hydrogen peroxide
GC-MS	Gas chromatography mass spectrometry
HPLC	High performance liquid chromatography
LC	Liquid chromatography
GDP	Gross domestic product

CHAPER 1
GENERAL INTRODUCTION

1.1 Background

There is an increasing interest in the traditional system of medicine in Africa and in different regions of the world due to poor investment in modern infrastructures for western medicine to take care of the increasing population (Schoneveld et al., 2011, Adefolaju, 2014). This has led to a significant increase in the use of herbal-derived medicines in our communities (Alegbeleye, 2020, Luo et al., 2020). Similarly, the demand for herbal products in the production of supplements and cosmetics has increased in the industries that produce them (Novoveská et al., 2019). Currently, the demand for medicinal plant products is gaining attention globally as a source of raw material for pharmaceutical industries and health care systems (Kandari et al., 2012, Chiebuka, 2020). This has resulted in unsustainable harvesting of medicinal plants in the wild without any mitigating measures, which increases pressure on the wild plant`s population (Xego et al., 2016, Van Wyk and Prinsloo, 2018). Similarly, there is an increasing trend in habitat loss locally and globally due to environmental degradation and encroachment of alien invasive plants which are adversely affecting the availability of medicinal plant species in the ecosystem (Phondani et al., 2016).

Furthermore, the increasing impact of global climate change and anthropogenic factors remain the key challenges to our vital flora resources, as well as the beauty, diversity, and natural heritage of the planet (Koohafkan and Altieri, 2011, Egoh et al., 2012). In addition, the collection of medicinal species from wild populations comes with serious challenges of batch-to-batch variability due to differences in weather, climatic conditions, variation in soil fertility, moisture levels, and agricultural practices (Leung, 2006, Salgueiro et al., 2010).

The cultivation of medicinal plants is a mitigating approach toward biodiversity conservation and to problems associated with the quality and quantity of medicinal plant materials for quality assurance (Volenzo and Odiyo, 2020, Labokas and Karpavičienė, 2021). However, this is accompanied by some challenges like an adaptation of plants to a new environment, susceptibility to disease, labour cost and net return to investment (Egharevba, 2019). The use of good propagation techniques in medicinal plant cultivation is imperative to overcome the challenges associated with the production of secondary metabolites which are of interest to our herbal and pharmaceutical industries (Gonçalves and Romano, 2018, Seukep et al., 2020). Also, the implementation of good and standard regulatory policy for the safety and quality of medicinal plants products is critical (Sahoo et al., 2010, Govindaraghavan and Sucher, 2015)

The different habitat types and varying microclimatic conditions found in different regions of the world like in the case of South Africa provide ideal environmental conditions for optimal growth and development of medicinal plants (Hoareau and Dasilva, 1999, Lubbe et al., 2010). South Africa is very rich in plant species and most of them are used for the treatments of various ailments such as fever, flu, cough, arthritis, asthma, cancer, hypertension, sore throat, skin diseases, and also for cosmetics purposes (Philander, 2011, Van Wyk, 2011). However, the use of some non-indigenous medicinal plant species individually or synergistically for the treatments of various chronic diseases by herbal practitioners in the South African community remains. (Gureje et al., 2015, Nyakudya et al., 2020).

American skullcap (*Scutellaria lateriflora* L.) is one of such powerful medicinal herbs of the family Lamiaceae, endemic to the American continent where it grows well in wet places. It is mostly used by herbal practitioners in the treatment of a wide range of nervous conditions including epilepsy, nervous tension, and insomnia (Awad et al., 2003). The dried aerial parts are used as sedatives, nerve tonic, and tranquilizers (Bonnet et al., 2005). In South Africa especially in Eastern Cape Province, they are being used in combination with other herbs for the treatment of arthritis.

Similarly, Burdock (*Arctium lappa* L.) is also one of such medicinal plants of great potential in the family Asteraceae, a native to Northern Asia and Europe. It was introduced to North America by early European settlers and now grows wild across the United States and Canada (Mabey et al., 1988). It is a very healthy and nutritive food in Chinese societies and many other countries (Chan et al., 2011). The roots and young leaves are used as food and for traditional medicine as a diuretic, prebiotics, and helps to reduce body weight, cholesterol, blood sugar level, arthritis sore throat, and many other health-related diseases (Chan et al., 2011). Several studies have reported that Burdock possesses anti-inflammatory antioxidant, antidiabetic and antimicrobial properties (Predes et al., 2011, Salama and Salama, 2016). However, the supply is mostly from Europe and America which is very expensive to the herbal practitioners and pharmaceutical industries that mostly import the plant materials. This unsustainable practice remains unreliable, which keeps the supply uncertain as well as the quality of their plant materials (Matewo, personal communication).

The introduction of these medicinal plants of interest into cultivation using fertilizers is a solution to improve the quality and quantity of medicinal plant materials of standard to our pharmaceutical companies. This will guarantee reliable identification, steady supply of plant raw materials, standardized volume and price agreement between growers and buyers, control of post-harvest handling which will assure quality control, and products standard for consumer's preferences (Macdonald et al., 2004, Onumah et al., 2007). In this regard, a study on the implications of varying farming conditions (propagation techniques) of Burdock and American skullcap of high medicinal

potential will be of great interest to the communities of South Africa at large, since these plants are not found in South Africa. This is a Department of Science and Technology (DST) / Technology Innovation Agency (TIA) initiated project to grow some potential medicinal plants that are not found in South Africa, a solution for livelihood enhancement and poverty alleviation through jobs creation in communities. As part of the global development goal to meet up with the agenda 2030 of the South African economic development and empowerment.

1.2 Statement of the problem

While most attention has been directed towards food security to meet up with the demand of the World's growing population (Godfray et al., 2010, Fan et al., 2012), the cultivation of medicinal plants as a resource to meet up with the increasing demand of medicinal plant materials for pharmaceuticals and traditional healthcare system has not been well documented especially in South Africa (Makunga et al., 2008, Street and Prinsloo, 2013). To ensure the sustainable livelihoods for millions of people in the world, about 80% of the developing countries rely on traditional medicines for their primary health care and more than 85% of herbal medicines used in traditional health care systems are derived from medicinal plants (Phondani et al., 2014). This is due to the growing recognition of natural products having little or no side effects, the availability, and affordability of medicinal plants. In addition, they are a cheap source of health care for many local communities especially in Africa and Asia (Kandari et al., 2012, Mahomoodally, 2013). Over 70% of plant collections involve destructive harvesting because of the use of parts like roots, barks, stems, and leaves or whole plants in the case of herbs. While the demand for medicinal plants is growing, some of them are increasingly being threatened in their natural habitats (Jan et al., 2020, Shafi et al., 2021). In South Africa, there is also the issue of affordability of some medicinal plants which are not found in South Africa but are being used by traditional healers who depend on importation from herbal shops with no assurance of product quality and certainty in supply. Therefore, there is a need for the cultivation of medicinal plants especially those that are not found in South Africa and are only imported by herbal shops with little or no guarantee of their product quality and certainty of supply to traditional medical practitioners. Their cultivation will address the issue of sustainable supply to meet up with future demands for our herbal market (Street and Prinsloo, 2013), and pragmatic information on the propagation of these medicinal plants would enhance their value in agricultural landscapes by helping farmers to improve their livelihoods and ensure environmental sustainability (Gouwakinnou et al., 2011)

1.3 Rational of the study

According to the World Health Organization (WHO), about 80% of the developing countries rely on traditional medicines for their primary health care and more than 85% of herbal medicines used

in traditional health care systems are derived from medicinal plants (Phondani et al., 2016), to ensure the livelihoods of millions of people in the world. According to Mander (1998), about 27 million South Africans depend on traditional medicine for their primary health care needs, and this can be attributed to a number of factors such as the relatively good accessibility to the plants, affordability and extensive local knowledge and expertise amongst the local communities in South Africa. However, many of these medicinal plants are harvested from the wild which tends to threaten medicinal plant`s biodiversity and population stability of medicinal plants in South Africa. Also, there are some medicinal plants that are not grown in or native to South Africa but are being used by some traditional healers who depend on supplies from herbal shops that import these plants from other countries, making the plant materials very costly as well as not being sure of their steady supply and product quality. The product quality is usually influenced during post-harvest processing, compromising the quality of these raw medicinal plant materials as well as secondary metabolites. This may have an influence on the treatment being administered by our South African traditional healers, making their treatment less effective. Therefore, the sourcing of these medicinal plant's seeds, cultivating and acclimatizing these plants to the South African weather conditions using appropriate propagation techniques will be of great importance to mitigate and ensure the sustainable supply as well as the assurance of product quality, efficacy at an affordable price which will help to resolve the challenges faced by South African traditional healers. There is also the issue with regards to the safety and quality of South African medicinal plant species as industrial encroachment has led to contamination of water sources and natural habitats. The deposition of processed and unprocessed mining and industrial waste materials (Naicker et al., 2003, Ochieng et al., 2010) has led to questions of safety for South African medicinal plants that are harvested near these resources. Also, due to intrinsic and extrinsic factors, plants harvested from the wild generally may vary in quality and consistency of active compounds (Bopana and Saxena, 2007). In this context, fertilizers can be used to increase the yield and quality of active compounds of medicinal plants which are going to be cultivated in this study using appropriate propagation techniques (Khalid, 2012). Nowadays, the utilization of chemical fertilizers and nutrients to increase the yield of medicinal plants is becoming very popular (Osman, 2011, Trisilawati et al., 2020). Meanwhile, the effect of different amounts of chemical fertilizers on yield and bioactive compounds (secondary metabolites) in medicinal plants has attracted less attention (Alizadeh et al., 2010). To the best of our knowledge and thorough literature search, there is no baseline data on the cultivation of the medicinal plants used in this study in South Africa.

1.4 Aim of the study

The aim was to investigate the influence of varying growing conditions on the morphological characteristics, chemical composition, and biological activities of American skullcap (*Scutellaria lateriflora* L.) and Burdock (*Arctium lappa* L.) in the Western Cape Province of South Africa.

1.5 Objectives of the study

1. Evaluate the effect of fertilizer application on growth and yield of Skullcap (*Scutellaria lateriflora* L.) and Burdock (*Arctium lappa* L.).
2. Assess the effects of fertilizer application on the metabolite profiles and mineral composition of Skullcap (*Scutellaria lateriflora* L.) and Burdock (*Arctium lappa* L.).
3. Investigate the effect of fertilizer treatments on biological activities of Skullcap (*Scutellaria lateriflora* L.) and Burdock (*Arctium lappa* L.).

1.6 Significance of the study

The Study has the potential of:

Providing baseline protocol for the cultivation of Burdock and American skullcap in the Cape Winelands of the Western Cape province of South Africa.

Providing baseline data on the influence of different fertilizer levels on the growth and yield of Burdock and American skullcap in the Cape Winelands of the Western Cape province of South Africa

Determining the influence of fertilizer treatments on the quality and accumulation of phytochemical constituents by cultivated Burdock and American skullcap through pot and field cultivation practices.

Providing information on the influence of fertilizer treatments on the bioactivity of the extracts of cultivated Burdock and American skullcap through pots and field cultivation practices.

Providing baseline data for essential oil composition of cultivated Burdock roots as influenced by mineral fertilizer application.

Contributing to food security in the community, Burdock and American skullcap are multi-utility plants with medicinal and culinary uses.

Providing awareness on medicinal plant cultivation, especially Burdock and American skullcap as alternative sources of employment to the community.

1.7 Hypotheses

The hypotheses were:

1. Different fertilizer levels have an influence on the growth and development of the cultivated medicinal plants used in the study.

2. Different fertilizer levels have an influence on the metabolite profile and nutrient composition of the cultivated medicinal plants.

3. Different fertilizer treatments have an influence on biological activities of the cultivated medicinal plants.

1.8 Delineation of the study

Field trial on American Skullcap (*Scutellaria lateriflora* L.) and antimicrobial testing of the plant samples are still to be investigated.

1.9 Structure of the thesis

The thesis consists of an introduction, and a literature review, followed by two experimental chapters on growth, yield and morphological characteristics in response to mineral fertilizer application, and two other laboratory experimental chapters on phytochemical constituents, biological properties, and volatile constituents of essential oil composition of root samples of Burdock as influenced by fertilizer treatments.

References

Adefolaju, T. 2014. Traditional and orthodox medical systems in Nigeria: the imperative of a synthesis. *American Journal of Health Research,* 2, 118-24.

Alegbeleye, B. J. 2020. The Use of Herbal Medicines by Cancer Patients in Contemporary African Settings: A Scoping Review. *International Journal of Scientific Advances,* 2.

Alizadeh, A., Khoshkhui, M., Javidnia, K., Firuzi, O., Tafazoli, E. & Khalighi, A. 2010. Effects of fertilizer on yield, essential oil composition, total phenolic content and antioxidant activity in Satureja hortensis L.(Lamiaceae) cultivated in Iran. *Journal of Medicinal Plants Research,* 4, 033-040.

Awad, R., Arnason, J., Trudeau, V., Bergeron, C., Budzinski, J., Foster, B. & Merali, Z. 2003. Phytochemical and biological analysis of skullcap (Scutellaria lateriflora L.): a medicinal plant with anxiolytic properties. *Phytomedicine,* 10, 640-649.

Bonnet, Q., Pimpernel, B., Madweed, M.-D. W. & Flower, H. 2005. Scutellaria spp. *Psychoactive Herbs in Veterinary Behavior Medicine*, 137.

Bopana, N. & Saxena, S. 2007. Asparagus racemosus—Ethnopharmacological evaluation and conservation needs. *Journal of ethnopharmacology,* 110, 1-15.

Chan, Y.-S., Cheng, L.-N., Wu, J.-H., Chan, E., Kwan, Y.-W., Lee, S. M.-Y., Leung, G. P.-H., Yu, P. H.-F. & Chan, S.-W. 2011. A review of the pharmacological effects of Arctium lappa (burdock). *Inflammopharmacology,* 19, 245-254.

Chiebuka, P. 2020. Impacts of Environmental Challenges on Medicinal Plants and the Possibilities for Increased Sustainable Agriculture. *Covenant Journal of Entrepreneurship,* 4.

Egharevba, H. O. 2019. Chapter-4 Progress in Medicinal Plant Research and Traditional Medicine Practice in Africa. *Chief Editor*, 77.

Egoh, B. N., O'farrell, P. J., Charef, A., Gurney, L. J., Koellner, T., Abi, H. N., Egoh, M. & Willemen, L. 2012. An African account of ecosystem service provision: use, threats and policy options for sustainable livelihoods. *Ecosystem services,* 2, 71-81.

Fan, M., Shen, J., Yuan, L., Jiang, R., Chen, X., Davies, W. J. & Zhang, F. 2012. Improving crop productivity and resource use efficiency to ensure food security and environmental quality in China. *Journal of experimental botany,* 63, 13-24.

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S., Thomas, S. M. & Toulmin, C. 2010. Food security: the challenge of feeding 9 billion people. *Science,* 327, 812-818.

Gonçalves, S. & Romano, A. 2018. Production of plant secondary metabolites by using biotechnological tools. *Secondary metabolites, sources and applications, IntechOpen*, 81-99.

Gouwakinnou, G. N., Lykke, A. M., Assogbadjo, A. E. & Sinsin, B. 2011. Local knowledge, pattern and diversity of use of Sclerocarya birrea. *Journal of ethnobiology and ethnomedicine,* 7, 8.

Govindaraghavan, S. & Sucher, N. J. 2015. Quality assessment of medicinal herbs and their extracts: Criteria and prerequisites for consistent safety and efficacy of herbal medicines. *Epilepsy & Behavior,* 52, 363-371.

Gureje, O., Nortje, G., Makanjuola, V., Oladeji, B. D., Seedat, S. & Jenkins, R. 2015. The role of global traditional and complementary systems of medicine in the treatment of mental health disorders. *The Lancet Psychiatry,* 2, 168-177.

Hoareau, L. & Dasilva, E. J. 1999. Medicinal plants: a re-emerging health aid. *Electronic Journal of biotechnology,* 2, 3-4.

Jan, H. A., Jan, S., Bussmann, R. W., Wali, S., Sisto, F. & Ahmad, L. 2020. Complementary and alternative medicine research, prospects and limitations in Pakistan: A literature review. *Acta Ecologica Sinica,* 40, 451-463.

Kandari, L., Phondani, P., Payal, K., Rao, K. & Maikhuri, R. 2012. Ethnobotanical study towards conservation of medicinal and aromatic plants in upper catchments of Dhauli Ganga in the central Himalaya. *Journal of Mountain Science,* 9, 286-296.

Khalid, K. A. 2012. Effect of NP and foliar spray on growth and chemical compositions of some medicinal Apiaceae plants grow in arid regions in Egypt. *Journal of soil science and plant nutrition,* 1, 581-596.
Koohafkan, P. & Altieri, M. A. 2011. *Globally important agricultural heritage systems: a legacy for the future*, Food and Agriculture Organization of the United Nations Rome.
Labokas, J. & Karpavičienė, B. 2021. Development of a Methodology for Maintenance of Medicinal Plant Genetic Reserve Sites: A Case Study for Lithuania. *Plants,* 10, 658.
Leung, P.-C. 2006. Good Agricultural Practice—GAP Does it Ensure a Perfect Supply of Medicinal Herbs for Research and Drug Development? *Current Review Of Chinese Medicine: Quality Control of Herbs and Herbal Material.* World Scientific.
Lubbe, C. S., Siebert, S. J. & Cilliers, S. S. 2010. Political legacy of South Africa affects the plant diversity patterns of urban domestic gardens along a socio-economic gradient. *Scientific Research and Essays,* 5, 2900-2910.
Luo, H., Yang, M., Tang, Q.-L., Hu, X.-Y., Willcox, M. L. & Liu, J.-P. 2020. Characteristics of registered clinical trials on traditional Chinese medicine for coronavirus disease 2019 (COVID-19): a scoping review. *European journal of integrative medicine*, 101251.
Mabey, R., Mcintyre, A. & Mcintyre, M. 1988. *The New Age Herbalist: How to use herbs for healing, nutrition, body care, and relaxation*, Simon and Schuster.
Macdonald, J. M., Perry, J., Ahearn, M. C., Banker, D., Chambers, W., Dimitri, C., Key, N., Nelson, K. E. & Southard, L. W. 2004. Contracts, markets, and prices: Organizing the production and use of agricultural commodities. *USDA-ERS Agricultural Economic Report*.
Mahomoodally, M. F. 2013. Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. *Evidence-Based Complementary and Alternative Medicine,* 2013.
Makunga, N., Philander, L. & Smith, M. 2008. Current perspectives on an emerging formal natural products sector in South Africa. *Journal of ethnopharmacology,* 119, 365-375.
Mander, M. 1998. Marketing of indigenous medicinal plants in South Africa: a case study in KwaZulu-Natal.
Naicker, K., Cukrowska, E. & Mccarthy, T. 2003. Acid mine drainage arising from gold mining activity in Johannesburg, South Africa and environs. *Environmental pollution,* 122, 29-40.
Novoveská, L., Ross, M. E., Stanley, M. S., Pradelles, R., Wasiolek, V. & Sassi, J.-F. 2019. Microalgal carotenoids: A review of production, current markets, regulations, and future direction. *Marine drugs,* 17, 640.
Nyakudya, T. T., Tshabalala, T., Dangarembizi, R., Erlwanger, K. H. & Ndhlala, A. R. 2020. The potential therapeutic value of medicinal plants in the management of metabolic disorders. *Molecules,* 25, 2669.
Ochieng, G. M., Seanego, E. S. & Nkwonta, O. I. 2010. Impacts of mining on water resources in South Africa: A review. *Scientific Research and Essays,* 5, 3351-3357.
Onumah, G., Davis, J., Kleih, U. & Proctor, F. 2007. Empowering smallholder farmers in markets: Changing agricultural marketing systems and innovative responses by producer organizations. *Munich Personal Repec Archive*.
Osman, Y. 2011. Organic vs chemical fertilization of medicinal plants: a concise review of researches. *Advances in Environmental Biology,* 5, 394-400.
Philander, L. A. 2011. An ethnobotany of Western Cape Rasta bush medicine. *Journal of ethnopharmacology,* 138, 578-594.
Phondani, P. C., Bhatt, I. D., Negi, V. S., Kothyari, B. P., Bhatt, A. & Maikhuri, R. K. 2016. Promoting medicinal plants cultivation as a tool for biodiversity conservation and livelihood enhancement in Indian Himalaya. *Journal of Asia-Pacific Biodiversity,* 9, 39-46.
Phondani, P. C., Maikhuri, R. K. & Saxena, K. G. 2014. The efficacy of herbal system of medicine in the context of allopathic system in Indian Central Himalaya. *Journal of Herbal Medicine,* 4, 147-158.
Predes, F. S., Ruiz, A. L., Carvalho, J. E., Foglio, M. A. & Dolder, H. 2011. Antioxidative and in vitro antiproliferative activity of Arctium lappa root extracts. *BMC complementary and alternative medicine,* 11, 1-5.

Sahoo, N., Manchikanti, P. & Dey, S. 2010. Herbal drugs: standards and regulation. *Fitoterapia,* 81, 462-471.
Salama, M. & Salama, S. G. 2016. Arctium Lappa L.(Asteraceae); a New Invasive Highly Specific Medicinal Plant Growing in Egypt. *Pyrex Journal of Plant and Agricultural Research*.
Salgueiro, L., Martins, A. & Correia, H. 2010. Raw materials: the importance of quality and safety. A review. *Flavour and Fragrance Journal,* 25, 253-271.
Schoneveld, G. C., German, L. A. & Nutakor, E. 2011. Land-based investments for rural development? A grounded analysis of the local impacts of biofuel feedstock plantations in Ghana. *Ecology and Society,* 16.
Seukep, A. J., Kuete, V., Nahar, L., Sarker, S. D. & Guo, M. 2020. Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. *Journal of Pharmaceutical Analysis,* 10, 277-290.
Shafi, A., Hassan, F., Zahoor, I., Majeed, U. & Khanday, F. A. 2021. Biodiversity, Management and Sustainable Use of Medicinal and Aromatic Plant Resources. *Medicinal and Aromatic Plants: Healthcare and Industrial Applications*, 85.
Street, R. & Prinsloo, G. 2013. Commercially important medicinal plants of South Africa: a review. *Journal of chemistry,* 2013.
Trisilawati, O., Rizal, M. & Pribadi, E. Organic cultivation of medicinal crops in the efforts to support the sustainable availability of Jamu raw materials. IOP Conference Series: Earth and Environmental Science, 2020. IOP Publishing, 012077.
Van Wyk, A. & Prinsloo, G. 2018. Medicinal plant harvesting, sustainability and cultivation in South Africa. *Biological conservation,* 227, 335-342.
Van Wyk, B.-E. 2011. The potential of South African plants in the development of new medicinal products. *South African Journal of Botany,* 77, 812-829.
Volenzo, T. & Odiyo, J. 2020. Integrating endemic medicinal plants into the global value chains: the ecological degradation challenges and opportunities. *Heliyon,* 6, e04970.
Xego, S., Kambizi, L. & Nchu, F. 2016. Threatened medicinal plants of South Africa: case of the family Hyacinthaceae. *African Journal of Traditional, Complementary and Alternative Medicines,* 13, 169-180.

CHAPTER 2
LITERATURE REVIEW

2.1 Cultivation of medicinal plants in South Africa: a solution to quality assurance and consistent availability of medicinal plant materials for commercialization

(This section of chapter two has been published in Academic Journal of Medicinal Plants, 6(7), 168-177. DOI: https//doi.org/10.15413/ajmp.2018.0133) Please refer to appendix 1).

2.1.1 Introduction

Plants have been of great importance as a source of medicine since the time of human civilization and since then their products are being used for different purposes such as food, shelter, clothing, health care and, as agrochemicals (Kamble et al., 2010). Plant materials used for medicine include the active ingredients of roots, stems, leaves, seeds, and barks of trees in various combinations (Ngarivhume et al., 2015). Medicinal plants are plants that have healing properties and contain mixtures of phytochemicals (Secondary metabolites) that may act individually, additively, or synergistically used to treat some illnesses (Kasso and Balakrishnan, 2013). Presently, medicinal plants are gaining popularity globally as a source of raw material for pharmaceuticals and traditional health care systems (Kandari et al., 2012).

According to the World Health Organization (WHO), about 80% of the world population relies (solely or partially) on traditional medicine for their primary health care and more than 85% of herbal medicines used in traditional health care systems are derived from different parts of medicinal plants (Phondani et al., 2014). In this regard, there has been an increasing interest worldwide in traditional systems of medicine including South Africa, with a substantial increase in the use of herbal-derived medicines, supplements, and cosmetics in communities (Fisk et al., 2014).

In recent decades, there has been indiscriminate harvesting, overexploitation and uncontrolled commercialization of medicinal plant species from the wild (Barata et al., 2011b). Indiscriminate harvesting of medicinal plants has led to an increasing trend in habitat loss locally and globally due to environmental degradation and encroachment of alien invasive species which are adversely affecting the availability of medicinal plants in the wild (Phondani et al., 2016).

In South Africa, millions of people in both rural and urban communities mostly rely on traditional medicine for their primary health care needs due to the easy accessibility and affordability of plant materials with good knowledge and know-how amongst the local communities (Mahomoodally, 2013, Oliver, 2013). This is increasingly putting pressure on the wild population with little or no

mitigating measures like medicinal plant cultivation that may enhance quality, quantity, biomass production and quality control measures such as monitoring of metabolites production and optimization of production techniques.

2.1.2 Importance of medicinal plants

Plants are the primary source of all medicines in the world and provide mankind with new remedies (Beyene et al., 2016). Medicinal plants are the “backbone” of traditional medicine and are considered to have some important ingredients which can be used in drug development and synthesis. They play a critical role in the development of human cultures around the world and also have a promising future because there are millions of plants around the world with yet to be discovered medicinal activities (Singh, 2015). Traditional systems of medicine have become a topic of global importance during the past decade. Presently, in many developing countries, a large proportion of the population relies heavily on traditional practitioners, who use exclusively medicinal plant materials for the treatment of various illnesses on the account that medicinal plants are curative with no side effects (Abdul, 2012). Although there may be the availability of orthodox medicine in these countries, herbal medicines have always upheld strong popularity for historical and cultural reasons (Vishwakarma et al., 2013, Hosseinzadeh et al., 2015). Medicinal plants have many different characteristics. When used in the treatment of diseases, it can be synergic, supportive and preventive (Abdul, 2012). At the moment, there is an increasing interest in the field of research on the metabolomics profile of medicinal plant species due to the remarkable diversity of both chemical and biological activities of naturally occurring secondary metabolites present in different plant species. This has led to the development of novel and sensitive techniques to detect some of these biologically active ingredients with improved isolation techniques to meet human medicinal needs (Hosseinzadeh et al., 2015)

2.1.3 Trade in medicinal plants

Generally, every year more than 500,000 tons of plant materials of about 60,000 plant species are traded based on their medicinal, nutritional, and aromatics properties (Romanelli et al., 2015). According to Barata et al. (2011a), there has been a significant increase in the importation (US$462.8 million) and exportation (US$1034.8 million) of medicinal plants into Europe between the period 1991 to 2002. In the last three decades, there has been substantial growth in herb and herbal product markets across the world. According to the Secretariat of the Convention on Biological Diversity, the global sales of herbal products were estimated to be US$60,000 million in 2002 (Shepherd and Tam, 2007). Every year, about five hundred thousand tons of dried medicinal plants are traded internationally, with substantial quantities in South Africa’s national and local markets. More than 50% of the plants are harvested from the wild, and the demand for

these materials keeps increasing (Barata et al., 2016). The utmost form of traditional medicine has always remained the use of herbs which are highly lucrative in the international market. The annual revenue of Western Europe between 2003 and 2004 was US$ 5 billion (Chaudhary et al., 2010).

The trade in pharmaceutical plants is dominated by only a few countries with three international trade centers; Germany, USA, and Hong Kong. Twelve countries make up 80% of both the exports and imports from the world market. The major markets are in the developed countries, while the bulk of pharmaceutical plants are exported from developing countries, not or only a little processed from developing countries and they are of wild origin (Barata et al., 2011b). The trade also contributes as a source of revenue to millions of families involved in medicinal plant collection, with women often playing the major role in supplying medicinal plant materials to pharmaceutical industries. Although accurate data is lacking, available information indicates that trade is increasingly growing especially in Europe where 90% of commercially used medicinal plant materials are collected from the wild (Barata et al., 2016). In South Africa, medicinal plant trade is a large and growing industry. It operates in both the formal and informal sectors of the economy which involves a few stakeholders in the Eastern Cape but a greater number of commercial harvesters from Kwa-Zulu Natal, Gauteng, and Western Cape provinces (Williams, 2004).

2.1.4 Overharvesting of medicinal plants

Medicinal plants are being harvested in increasing volume from the wild population all over the world creating pressure on plant diversity and population in the wild. In recent decades, the demand for medicinal plants has increased from 8 to 15% per year in Europe, North America, and Asia (Bentley and Trimen, 1981, Ross, 2007). Furthermore, over 25% of prescribed medicines in developed countries are derived from wild plant species and up to 80% of people in developing countries are totally dependent on herbal medicine for their primary healthcare (Hamilton, 2004). In South Africa, about 72% of black South Africans rely exclusively on the traditional health care system for their survival, which involves the use of medicinal plants (Mander et al., 2007). The increasing demand for herbal drugs and natural health products by the growing population, without putting in place a mitigating measure like medicinal plants cultivation may result in some of these plants going into extinction (Chen et al., 2016a). According to the International Union for Conservation of Nature (IUCN), there are between 50,000 to 80,000 flowering plant species used for medicinal purposes worldwide. Among these, about 15,000 plant species are under threat of extinction from overharvesting from the wild and habitat destruction (Bentley and Trimen, 1981) and 20% of these wild resources have already been nearly exhausted with the increasing human population and plant consumption (Ross, 2007). Even though these pressures have been known for years, the accelerated loss of species and habitat destruction worldwide has increased the risk

of extinction of medicinal plants, especially in South Africa because of the high demand by the increasingly large population.

2.1.5 Conservation strategy of wild medicinal plants collection

Extensive studies have been carried out on the conservation and sustainable use of medicinal plants (Larsen and Olsen, 2006, Uprety et al., 2012) and various forms of recommendations have been made regarding their conservation, inventorying, and monitoring systems to coordinate both *in-situ* and *ex-situ* conservation strategies (Hamilton, 2004). *Ex-situ* cultivation is practiced through seed germination and *in vitro* cloning. Recommendations on medicinal plants conservation have been developed on both *in-situ* and *ex-situ* conservation (Huang, 2011) with the knowledge of their geographical distribution and biological characteristics(Chen et al., 2016a) Nurseries and nature reserves are measures put in place to maintain the medicinal efficacy of plants as bioactive compounds can be influenced by intrinsic or extrinsic factors which can then be monitored thereof, while botanical gardens and seed banks are *ex-situ* conservation strategies for future replanting (Kasso and Balakrishnan, 2013, Montenegro De Wit, 2017).

A plant diversity database which is available for an update in all provinces of South Africa has been created in order to produce a national flora that will go live on the world wide web by 2020 (Victor et al., 2014). Through the International Union for the conservation of nature (IUCN) red list category, the South African National Biodiversity Institute (SANBI) has created a comprehensive national plant red list for South African plants (Raimondo et al., 2009). A mandate was made to monitor plant species listed as threatened and protected species of the National Environmental Management Biodiversity Act (NEMBA) Act N0: 10 of 2004 between 2005 to 2020 by provincial and National Authorities. In 2010, the Department of Environmental Affairs launched a R20 million conservation project on medicinal plant species in Limpopo to promote and protect endemic medicinal plant species in South Africa through the Indigenous Knowledge System (IKS) of South Africa Trust. Notwithstanding, the project did encounter financial mismanagement resulting in its collapse. Moreover, despite the tremendous efforts put in place as conservation strategies, the issues of shifting from subsistence to commercial harvesting of medicinal plant materials as an employment opportunity are posing an unprecedented extinction threat to the wild population as a setback to the aforementioned.

2.1.6 Prospects in medicinal plant cultivation

There is a global increase in the demand for medicinal plant products which is estimated to be worth R2.9 billion per annum (Sobiecki, 2014). In South Africa a large business venture has been created in the interest of plant-derived medicines with an estimated value of R270 million per annum (Dold and Cocks, 2002, Wiersum et al., 2006). In KwaZulu-Natal, it is estimated that 1.9

tons of the indigenous African ginger containing 52,000 plants are traded annually (Mander, 1998). The aerial part of Khoi- San`s traditional plant “Buchu” is sold at $56/kg and the seeds are sold for R20000.00 per Kilogram(Moolla and Viljoen, 2008). The ethanolic formulation of *Pelargonium sidoides* extracts (Eps 7630) called “Umckaloaba” is reported to be the most successful phytomedicines in the world (Van Wyk, 2011, Theisen and Muller, 2012) with a very high market potential, especially in Germany recording 80 million Euros per annum (Van Wyk, 2011). The economic benefits of some South African indigenous medicinal plants are an encouragement to promote the cultivation of medicinal plants as a viable option for livelihood enhancement that needs to be addressed especially in rural communities (Lewu et al., 2007a, Lewu et al., 2007b). This will significantly contribute to job creation with the potential to improve South Africa’s economy (Moolla and Viljoen, 2008, Street and Prinsloo, 2013).

In this regard, medicinal plant cultivation is gaining attraction as an emerging sector of self-employment for subsistence and commercial farmers (Rashid et al., 2014). Also, there is a growing interest in medicinal plant research focusing on the cultivation and processing of medicinal plants by some government agencies and academic institutions especially those that are in high demand within and outside the country. The optimization of propagation techniques will definitely be a remedial action to ensure a sustainable supply of good quality plant materials to our local communities and plant-based industries contributing toward biodiversity conservation and environmental health (Davis et al., 2012)

2.1.7 Introducing medicinal plants for commercialization in South Africa

2.1.7.1 Cultivation of medicinal plants

The cultivation of medicinal plants can be a means of sustainable supply of medicinal plant materials with quality assurance to the market. However, there is little available information in this exercise, with much attention on crop cultivation for food security. Currently, the consumption of herbal medicines is widespread due to the presence of useful compounds within plants of medicinal potential. This has raised a lot of interest in medicinal research with increasing support from government and industries, with the interest most specifically to commercialize the products. Different cultivation practices such as planting date, fertilizer application, irrigation systems, and harvesting methods need to be optimized to improve the growth, yield, and quality of medicinal plant materials. Cultivation of medicinal plants with the use of controlled environments will be a viable alternative that offers the opportunity to overcome the problems that are inherent in herbal extracts such as extract variability, contamination, misidentification, instability, and a means to manipulate the yield of bioactive compounds. However, the propagation of some medicinal plants in a new habitat may be challenging due to the local adaptation of some medicinal plants to their

natural environments (Lewu et al., 2007b) and low germination rate (Vines, 2004). Also, specific information about the requirements for seed germination, growth and pollination is usually not available since these plants are introduced into cultivation. The propagation success of new plants in an environment will depend on the understanding of the basic principles of plant propagation which usually takes place through seed and vegetative propagation (Hartmann and Kester, 2002).

2.1.7.2 .Seed propagation

Seed propagation is a means of large-scale multiplication of plants which takes a longer period for plants to reach maturity. It is a less expensive process in which the crop stands a chance to compensate for the time lost in seed germination. Seed germination is a vulnerable stage in the life cycle of plants (Kigel, 1995). It is the process of reactivation of metabolic machinery of seed which starts with imbibition in completion with radicle emergence from the seed coat giving rise to a seedling. It is a complex phenomenon that is influenced by genetic and environmental factors such as light, temperature, moisture and nutrient availability that are annually unstable (Tang et al., 2015). The requirements for seed germination (temperature, soil pH, light, moisture, and nutrients) vary between plants and among species which greatly influence germinability. In general, seed germination remains the key to modern agriculture as a critical event in determining the success of plant species. To meet up with the demand and supply of plant materials for commercialization, a fundamental understanding of the protocol for seed germination is essential to medicinal plant production. Several studies on different plant species have been carried out by some researchers showing the influence of some of these factors on seed propagation (Table 1).

Table 2.1 Advances on factors that influence seed germination potentials

Study	Findings of the study	Sources
Investigated the effects of water and temperature on seed germination and emergence as a seed hydrothermal time model	Temperature diversity influences germination capacity in many species, relating to different strategies adopted as a consequence of the heterogeneity of the habitat and microclimatic seasonality intrinsic to their ecosystems	(Shaban, 2013).
Influence of seed source, pre-chilling, light and temperature on the germination of South African *Pelargonium sidoides.*	Seed age, temperature, light, and pre-chilling conditions had an influence on seed germination of *pelargonium sidoides*. At temperatures higher than 25°C germination was reduced by 60% and at pre-chilling there was a drop by 29% in seed germination compared to other treatments.	(Lewu et al., 2010)
Investigated the effects of seed germination and vegetative propagation of bush tea (*Athrixia phylicoides*),	Germination percentage of bush tea differed with the temperature treatments, with the highest (75%) at 20 - 25°C. There was a high germination percentage at constant	(Araya, 2007)

	temperature than at alternate temperature and in a continuous light than alternate light. Condition.	
Effect of saline water on seed germination and early seedling growth of the *halophyte quinoa*	Saline water had an influence on growth attributes, an efficient antioxidant mechanism was present in quinoa, activated by salts during germination and early seedling growth and total antioxidant capacity was higher under salt stress than in water. Also, osmotic, and ionic stress factors had different degrees of influence on germination and development.	(Panuccio et al., 2014)
Hydrothermal time models for conidial germination and mycelial growth of the seed pathogen *Pyrenophora semeniperda*	This study has demonstrated that the hydrothermal time model framework developed to describe the effects of temperature and water potential on physiological processes in seeds can also be successfully applied to germination and growth processes in ascomycete seed pathogen	(Barth et al., 2015)
Temperature requirements for seed germination and seedling development determine timing of seedling emergence of three monocotyledonous temperate forest spring geophytes	The different stages of development, from embryo growth to leaf development, occur continuously in response to different temperatures. Differences in timing of emergence between the three species studied are caused by a subtle difference in temperature effects on germination and seedling development	(Vandelook and Van Assche, 2008)
Interactive effects of salt, light and temperature on seed germination and recovery of a Halophytic grass-*Phragmites karka*	Toxicity of salts varies with environmental conditions, treated seeds were better in moderate temperatures. The salts did not affect viability of seeds which probably entered dormancy. During extended exposure to high salinity and temperature stress, seeds were prevented from germination.	(Zehra et al., 2013)

2.1.7.3 **Vegetative propagation**

This is the multiplication of plants through vegetative parts; it is a fast, expensive, and more labour-intensive process with a lot of technical applications compared to seed propagation. Vegetative propagation can be through cuttings, grafting, budding and micropropagation. It is a process of plant multiplication in which a portion or fragment of the plant body functions as a propagule and develops into a new individual (Megersa, 2017). Among the different methods of vegetative propagation, cutting is regarded as the most preferred and best method as it retains and conserves the genetic diversity of the plant (Dumroese et al., 2016). Cuttings are any vegetative parts of plants that can fully develop into the parent materials when subjected to favourable conditions for regeneration (Hartmann and Kester, 2002). Several studies on vegetative propagation of medicinal plants have been investigated by some researchers such as Chandra et al. (2015) who reported the highest rooting percentage in the macro propagation of *Holostemma Ade-kodien,* tested in vermiculate and vermicompost growth media using different concentrations of IBA and

IAA growth hormones. Kavita et al. (2015) investigated the sprouting and rooting percentage of *Paris polyphylla* Smith, using various soil compositions with different concentrations and combinations of IBA and GA3 hormones (50, 100 and 150 mg/L) treatments Combination of 100 mg/L GA3 and 100 mg/L IBA demonstrated highest sprouting and rooting percentage in soil: loam: sand (3:2:1).

2.1.8 Cultivation of medicinal plants: A solution for biodiversity conservation in South Africa.

The cultivation of South African medicinal plants has increased over the years due to their high demand in the market by herbal practitioners, pharmaceutical industries, and the increasing awareness of medicinal plant conservation. Opportunities have been created for farmers to cultivate medicinal plants which have helped to reduce the issue of competition between farmers (Reinten and Coetzee, 2002). Research projects have been conducted in South Africa on the cultivation and commercialization of indigenous plant materials to reduce the pressure of wild plant collection. A handful of indigenous South African medicinal plants are under cultivation in the different provinces (Table 2).

Table 2.2 Cultivation practice and uses of some South African medicinal plant's species.

Species	Common Name	Propagation	Place of cultivation	Parts used	Uses
Siphonochilus aethiopicus	African ginger	Seeds and rhizomes	Mpumalanga, Limpopo	Secondary roots and rhizomes	Flu, cold, malaria, charm, appetite suppressant and sedative
Eriocephalus africanus	Wild rosemary	Seeds, cuttings, layering and division of roots	Western cape, Eastern Cape	Leaves	Asthma, throat, and lung infections
Sutherlandia frustescens	Cancer bush	Seeds and cuttings	Northern Cape, Eastern Cape, KwaZulu Natal, Western Cape, Mpumalanga	Leaves and young stem	Fever, ulcer, poor appetite, diabetics, cold, flu, gastritis, cough, asthma, rheumatism, urinary tract infection, stress, anxiety
Harpagophytum procumbens	Devil`s claw	Seeds, secondary tuber	Northern West, Northern Cape, Free state	Root	Treatment of liver, kidney, and bladder diseases, stimulate appetite and for indigestion

Hypoxis hemerocallidea	African potato	Seeds	Eastern cape, KwaZulu Natal, Mpumalanga, Limpopo, Gauteng, Northwest, Free state	Tuber, leaves and bulbs	Treatment of urinary tract infection ,testicular tumour heart weakness
Tulbaghia violacea	Wild garlic	Seeds	Rocky grassland of the Eastern cape, KwaZulu Natal, Limpopo	Rhizome, leaves, and bulbs flower	Fever, rheumatism, asthma, constipation , cough and cold
Moringa oleifera	Drumstick tree	Seeds and cuttings	Limpopo, Free State, Mpumalanga, KwaZulu Natal, Gauteng	Roots, leaves, barks, and immature pods	Headache, ulcers, diarrhoea, wounds
Pelargonium sidoides	Kalwerbossie	Seeds and cuttings	Eastern cape, Free state, Gauteng	whole plant	Cough, chest pain, bronchitis, fever, sore throat, dysentery, diarrhoea
Warburgia salutaris	Pepper Tree	Seeds and cutting	Limpopo, KwaZulu Natal, Northern Gauteng	Whole plant	Abdominal pain, constipation, cancer, rheumatism, stomach ulcer, malaria, influenza
Eucomis antumnalis	Pineapple flower	Seeds and leaf cutting	Limpopo, Mpumalanga, Free State, Eastern Cape	Bulb	Fever, stomach-ache, colic, flatulence, syphilis
Aloe ferox	Bitter aloe	Seeds and stem cuttings	Western cape, Southern KwaZulu Natal, South-eastern part of Free State	leaves	Arthritis, eczema, hypertension, stress,
Artemisia afra	wormwood	Roots and stem cutting	All provinces of South Africa except in the Northern cape province	Leaves, stems, and roots	Cough, fever, headache, loss of appetite, intestinal worms

Source: (Joffe, 2001, Herbert, 2006, Mcmaster, 2007, Mahomoodally, 2013, Street and Prinsloo, 2013)

2.1.9 Cultivation of medicinal plants: As a possible solution of product quality and commercialization

Medicinal plant cultivation is a solution to meet up with the demand in the market and an advantage for pharmaceutical industries and herbal practitioners to have control over their raw materials (Ekor, 2014). Providing a reliable identification prevents the risk of contamination, steady supply of plant materials, control of post-harvest handling, effective monitoring of products standard, easy plant certification procedure, minimized batch-batch product variability, and provides a platform of agreement between wholesalers and growers (Máthé and Máthé, 2006). Cultivation of medicinal

plants provides the opportunity to optimize the production of secondary metabolites of interest with information on the best season and time of harvest and ultimately increase volume. Herbal medicines and supplements derived from medicinal plants are usually assessed for quality assurance to ensure their safety before commercialization (Govindaraghavan and Sucher, 2015). Quality assessment measures need to be put in place to address safety and efficacy as well as, consistent monitoring of the entire value chain to ensure the delivery of quality finished products to local and international herbal markets. However, this depends on the processing and storage conditions, which may have an influence on the quality of the final product. Product quality depends on active ingredients which are influenced by intrinsic and extrinsic factors. These factors bring about major changes in metabolites creating differences in the final content during every production. It is therefore of utmost importance to understand the dynamics in secondary metabolites for consistency in chemical profiles as well as, biological activities of medicinal plants. Recently, some scientific investigations have been carried out on the synthesis of plant secondary metabolites in response to various factors that deviate from the optimal production of plant secondary metabolites (Table 3).

Table 2.3 Factors that influence the synthesis of secondary metabolites in plants

Studies	Finding of the of the study	References
Quality from the field: The impact of environmental factors as quality determination in medicinal plants	Biotic and abiotic stresses are tools to increase health related properties of plant materials and understanding elicitation of induced metabolites and their lifetime is fundamental to quality assessment of secondary metabolite production. Notwithstanding, quality is indeed growing out in the field, harvesting, and processing methods.	(Ncube et al., 2012)
The improvement of bioactive secondary metabolites accumulation in Rumex gmelini Threz through co-culture with endophytic fungi	Bioactive compounds in RGT seedlings could be enhanced significantly through co-culture with fungus *Aspergillus* sp. However, there is the need for further research for larger scale bioactive secondary metabolites production.	(Ding et al., 2018)
Influence of ecological factors on the production of active substances in the anti-cancer plant *Sinopodophyllum hexandrum* (Royle) ST Ying	Secondary production in the root and rhizomes of *Synopodophyllum hexandrum* are greatly influenced by environmental factors contributing to a geographical difference in different production location	(Liu et al., 2015a)
Seasonal variation in the production of secondary metabolites and antimicrobial activity of two species used in Brazil traditional medicine	Secondary metabolites of the plant materials assessed for seasonal variation showed concentration differences as well as differences in antimicrobial activities.	(Chaves et al., 2013)
Influence of abiotic stress signal on secondary metabolites in plants	Environmental factors such as temperature, humidity, light intensity, water, mineral and carbon dioxide influences the growth of plants secondary metabolites production. Drought and freezing temperatures can cause adverse effects on growth of plants and their productivity.	(Akula and Ravishankar, 2011)
Investigated on effects of salts and drought stress on alkaloid production in endophyte-infected drunken horse grass (*Achnatherum inebrians*	Higher alkaloid levels were recorded for plants under salt or drought stress, with levels of ergonovine being higher than those of ergine. Concentrations of both alkaloids increased over the plant growing period	(Zhang et al., 2011)
Influence of growing conditions on metabolite profile of *Ammivisnagaumbels* with special reference to bioactive furanochromones and pyranocoumarins	Growing conditions had an influence on the quali-quuantitative profile of the secondary metabolites, with hydroponics culture having significant increase in the active principal components	(Sellami et al., 2013)
Effects of cultural practices and environmental conditions on yield and quality of herbal plants: prospects leading to research on influence of nitrogen fertilization, planting density and eco-physiological parameters on yield and quality of field- grown bush tea *(Athrixia phylicoides* DC)	Cultural practices such as fertilization and planting density have an influence on growth and productivity, with fertilization improving growth, total polyphenol, tannins and antioxidant content of herbal tea. Nitrogen fertilization increases the production of new shoots and content of nitrogenous compounds, plant density also increases productivity.	(Tshivh et al., 2013).

2.1.10 Challenges in medicinal plants cultivation

Medicinal plants are widely used traditionally throughout human development in the treatment of various illnesses as a source of primary health care in most communities. However, the source of plant material is mostly dependent on the wild, except recently, where there is growing attention towards medicinal plant cultivation for biodiversity conservation, enhancement of secondary metabolite of interest, product quality, and for assurance of a steady supply of plant materials. However, this is associated with some unforeseen challenges that may arise without expectations. In medicinal plant cultivation, the future for some less known species is unpredictable; some of them being perennial will require several years for maturity before harvesting and therefore investment in them could represent a considerable commercial risk(Canter et al., 2005). Medicinal plant cultivation goes with a series of questions like the accessibility of plant materials in the wild, disease susceptibility, labour cost and adaptation to a new environment may be slow. In a situation where there is an excess of a particular medicinal plant in a very large population in the wild, it becomes very easy for medicinal collectors to harvest these materials with little or no cost in the wild to sell at cheaper prices. This makes it difficult and unworthy for such a plant to be cultivated with the application of labour cost of cultivation to sell at a higher price of which buyers will prefer to buy at a cheaper price from those collectors from the wild. Agro-environmental conditions of cultivated medicinal plants are usually not the same as that of the wild habitat. However, secondary metabolite biosynthesis is induced by the extra-optimal influence of biotic and abiotic factors where an optimal environment will tend to increase biomass production, rather than the synthesized secondary metabolite usually needed to cope with stress (Pavarini et al., 2012). Also, post-harvest processing and market availability are very critical because their negligence would lead to an irreversible quality loss of raw materials. Thus, good processing conditions will help to increase the market value of medicinal plant products.

2.1.11 Influence of regulatory policy on the safety and quality of medicinal plants products

The use of many plant products as food or dietary supplement is associated with some problems of classification in some countries (Ekor, 2014). As a result, quality tests and production standards tend to be less controlled and, in some cases, traditional health practitioners may not be licensed. The safety of medicinal plant products is imperative to the general public (Kasilo and Trapsida, 2011) All manufactured herbal products are required to be licensed as “traditional herbal medicinal products” (Raynor et al., 2011, Committee, 2013). Like orthodox medicines, it is compulsory that they are accompanied by comprehensive information like precaution, prescription, side effects, storage, and regulatory information (Ekor, 2014). However, in the developing countries where many unregistered and poorly regulated herbal products are sold freely on the market with little or no restraint, licenses cannot be obtained for some herbal medicines. Furthermore, the myth that

natural products are not poisonous and are devoid of adverse effects often leads to wrong usage and unrestrained intake which may result in poisoning and acute health problems. This is not limited to developing countries but also exists in highly developed countries, where the general public often resorts to “natural” products without any proper information on the side effects; especially in the case of excessive use (Committee, 2013). In South Africa, advances have been made in the quality control of medicinal plant products by creating a Medicinal Control Council to ensure the quality control of herbal medicines, promote the safety of high-quality traditional medicine, and contribute to the capacity building of traditional healers (Felhaber and Gericke, 1996).

2.2 CULTIVATION OF BURDOCK (*ARCTIUM LAPPA* L): A POSSIBLE SOLUTION TO QUALITY ASSURANCE AND CONSISTENT AVAILABILITY OF PLANT MATERIALS FOR COMMERCIALIZATION.

(This section of chapter two has been submitted to Open Access Journal of Medicinal and Aromatic Plants. ID: 104293-09-02).

2.2.1 Introduction

The increase in health consciousness and the preference for natural products by the current human population has initiated the concurrent uses of herbal and orthodox medicine in many communities around the world (Ameade et al., 2018). This has created a potential market for plant-based products of medicinal potential across the world. Nowadays, with this awareness, a lot of money is continuously being spent on phytonutrients and phytomedicines by many people for the treatment of several health problems (Guna, 2019) in different healthcare settings (Who, 2004). This has instigated a great shift of orthodox medicine to Phytomedicines (Hosseinzadeh et al., 2015), most probably because of its affordability, fewer side effects and associated cultural beliefs (Vishwakarma et al., 2013) in the Chinese, Indian and African Communities.

Currently, phytomedicines are used throughout the world as an integral part of our health care systems for disease treatments and sustainable health benefits (Khan, 2015). This is being showcased with the upcoming companies like Green World, Forever Living Products and Herbal Life. Their products are gaining preference, with great marketability in communities. The publicity and use of these products are being harnessed through information dissemination on their products in all the different social community levels with appreciable affordable prices.

Burdock (*A lappa*.) has been traditionally used in various combinations for the treatment of various ailments like burnt, boil, sore throats, rashes, and skin infection because of its medicinal potential. It is known to be very rich in phytochemicals which contribute to its anti-microbial, antidiabetic, antiallergic, anti-inflammatory, sedative and antioxidant properties (Miraj, 2016). It is also consumed as a vegetable in some regions of the world because it is nutritionally reached in

essential minerals (Azizov et al., 2012). The roots are consumed in the form of salad, in a stew, or eaten raw like carrots, while the leaves are consumed as a vegetable in a soup.

The cultivation of this species is very silent, with rare cases of its cultivation practice in the different regions of its origin. In recent times the demand for this plant by herbal practitioners and pharmaceutical industries in the different countries of the world including South Africa keeps increasing (Tanga et al., 2018). The market supply is mostly dependent on wild collection, thereby creating pressure on the wild population. Without any mitigating measures on its cultivation, the huge demand for the plant material could create extinction due to over-harvesting (Lewu et al., 2007a). This review summarizes the currently available scientific literature on botany, geographical distribution, cultivation practices, biotechnology research, photochemistry, therapeutic, pharmacological properties, and the potential of *A lappa* as a commercial medicinal plant. Hence, the measures toward its cultivation and sustainable supply are imperative.

2.2.2 Botany and geographical distribution of Burdock

Burdock (*A lappa*) is a biennial plant of the family Asteraceae. It is the largest family of flowering plants with about 1100 currently accepted genera and 2500 species (Heywood and Skoula, 1997). Burdock is native to the temperate regions of the Old World from Scandinavian to the Mediterranean. Extending from the British Isles through Russia and the Middle East to India, Taiwan Australia, and Japan. It was introduced to North America by early European settlers and now grows wild across the United States and Canada (Mabey et al., 1988). Burdock is usually found in disturbed areas especially in nitrogen-rich soil and stores most of its nutrients during the first year (Chan et al., 2011).

2.2.3 Agroecology of Burdock

Burdock is characterized by large alternating cordiform leaves with long pubescent petiole on the underside. The older leaves are longer and found at the base while the upper leaves are much smaller with an egg shape. The flowers are purple in colour and grouped in globular capitulate united in clusters. The roots are brownish green and can grow up to 1 m long (Salama and Salama, 2016). Burdock is a cool climate temperate plant that grows best at temperatures between 18-28^{o}C in full sun. It is frost sensitive, during winter the leaves will die off and sprout during spring. It prefers a fresh worked, well-drained sandy loamy soil rich in humus or nitrogen with a deep profile for easy root penetration. Propagation can be realized through the nursing of seeds in a greenhouse before transplanting to the field or larger pots. Direct seed sowing can also be done in midsummer in the field. Harvest can take place between 12-16 weeks after seeding until late autumn when the roots become too fibrous (Lim, 2012).

a: *Burdock (A lappa*) Plant

b: Leaf of *A lappa* **.**

c: Inflorescence of *A lappa* .

d: Flower of *A lappa* .

Figure 2.1 Different morphological features of Burdock (*A lappa*)

2.2.4 Agronomic practices of Burdock

The cultivation of burdock is a potential solution to biodiversity conservation and habitat destruction. It will be a suitable practice to reduce pressure from the wild and meet up with the current and future demand for a large volume of this species for plant-based drugs, herbal remedies, and food security. Additionally, its cultivation will be an alternative sector in agriculture to provide supplementary income for the grower of the species and increase local GDP. To the best of our knowledge, there is no record of commercial production of the species in South Africa, hence the need to cultivate and document the agronomic practice for *A lappa.*

2.2.5 Field cultivation of Burdock

Burdock cultivation in the field is mostly through direct seeding in well-drained soil to produce quality roots suitable for eating either raw or after microwave cooking. The cultivation is mostly favorable during spring. According to Soon et al. (2003), a preliminary study was carried out in the field to identify burdock cultivars suitable for Western Australia`s condition and to develop innovative postharvest handling and storage methods for the export market. The best yield was 47 t/ha when 400-600 kg/ha of nitrogen was used with all other cultural practices applied. It was noticed that the marketable yield on all the trials was poor across treatments, therefore further trials were recommended for spring-sown burdock before the optimum rate can be determined. Also, the application of pre-emergent herbicides Kerb (propyzamide) at 4.4 L/ha and trifluralin (20 L/ha) did not affect the total yield. However, Intra-row spacing of 5-8cm was suitable for spring-sown burdock harvested at 20 weeks or 134 days after sowing. Priming of burdock seeds (soaking in water for 12 hours) did not increase the germination rate at planting. However, flatbed formation increased germination rate and marketable yield compared to hilled bed formation.

According toAl-Zyadi (2018), a study on the effect of nitrogen fertilizer and harvesting date on the growth of burdock and total phenolic content was investigated in the experimental research station, College of Agriculture Al-Muthana University of Iraq. Treatments consisted of 3 levels of nitrogen (0, 50, and 100 kg/ha of urea) and harvesting dates at 3 levels (181, 202 and 222 days from seed sowing) in a randomized complete block design replicated 3 times. Fertilization had a significant ($p<0.05$) effect on increasing rates of growth characteristics that were measured. The application of 100kg/ha of Nitrogen (urea) demonstrated the highest effects on all the growth parameters studied, which may be attributed to the superiority of nitrogen as the most important element for plant growth and its role in the formation of amino acids, indole acetic acid (IAA), which stimulate cell division and the growth of apical meristem. Nonetheless, total phenolic content demonstrated the highest significant effect (43.82 mg GAE.g^{-1} dw) compared to the control with the application of 50 kg/ha of nitrogen (urea). Increasing nitrogen application to 100kg/ha negatively affected the phenolic content of the leaves given a lesser value which may be because of primary metabolism which must have used the available plant resources, thereby weakening the production of phenolic compounds due to substrates deficiency (Lattanzio et al., 2009). Furthermore, harvesting dates significantly ($p<0.05$) affected growth parameters and total phenolic content of burdock leaves with the highest effect recorded at 222 days of harvesting time during winter, attributed to the longevity of harvesting time Compared to the other dates which allowed the completion of plant growth(Al-Zyadi, 2018). The increase in total phenolic content may be due to the increase in plant exposure to temperature stress, using different structural and biochemical protective mechanisms (Egigu et al., 2014).

Furthermore, a field experiment was conducted at the research station College of Agriculture Al-Muthanna University during the 2017-2018 winter cropping season on *A lappa*. To investigate the effect of three levels of phosphorous (0, 80, and 120 kg P_2O_5/ha) and three levels of potassium 0, 100 and 150 kg K_2O/ha) on growth, yield, and total phenolic content in roots of *A lappa* in a randomized complete block design replicated three times. The application of P_2O_5 at 120 kg/ha recorded the highest effect on the growth, yield, and total phenolic content (40.57 mg/GAEg/dw) in the roots of *A lappa*. Whereas the application of 150 kg K_2O /ha) recorded the highest effect on growth and yield parameters tested. Nevertheless, the application of 100kg K_2O/ha recorded the highest total phenolic content in roots (44.41 mg/GAEg/dw) (Al-Zyadi, 2019).

Field cultivation of burdock was also reported by Douglas et al. (1992), at five different locations in New Zealand (Redbank, Palmerston, Hastings, Te Hauke and Ohakune). The effects of different sowing dates and harvesting dates on growth and yield were investigated on different cultivars of *A lappa* at different locations. At Redbank, four different cultivars (Shirohada, Yamada-riso, Yamada-wase, and Kieft) of *A lappa* were investigated. Harvesting was done after 218 days and the best cultivar yield (Fresh root yield) of *A lappa* was recorded by Yamada-wase (39 t/ha), followed by Shirohada and Yamada-riso (23t/ha) while the list was recorded by Kieft (17 t\ha). At Palmerston, Yamade-wase, and Yamada-wase and Treflan cultivars harvested at 262 days and the best yield was recorded by Yamada-wase and Treflan. At Hasting location, the four different cultivars were Takinogawa-early, Tokiwa-improved, Watanabe-early, and Watanabe-early were harvested at 124 days, and the best yield was recorded by Watanabe early. At the Te-Hauke location, only one cultivar was cultivated, and harvesting was done at 148days. At Ohakune location, harvesting was done at 83days on three cultivars (Tokiwa-improved, Watanabe-early, and watanabe-early), the highest harvest was obtained on Tokiwa improved. Burdock roots harvested at Redbank were short and very different from those at Palmerston, Hastings, Te-Hauke and Ohakune which were long and thin and may be attributed to differences in soil types across the different regions. Short roots were characterized by the dense loamy silt soil with an eluvia zone at 27-53cm depth which resisted penetration. While the long root harvest was characterized to easy root penetration into the soil beneath 53-63cm. The total yield of *A lappa* for the study was recorded by Yamada-wase (39 t/ha), which was considerably higher than the expected mean production of 13 t/ha in Japan.

2.2.6 Micropropagation and molecular studies on Burdock

The increase in demand for burdock plant materials by our pharmaceutical industries and herbal practitioners is causing a progressive depletion of the native population. This calls for an urgent need for conservation and domestication of the species. Also, since the acquisition of plant

material is mostly from the wild population; it is at times associated with some potential problems of pest infestations, environmental pollution, and seasonal variability. This may result in the changeability and or degradation of the active ingredients of interest. Furthermore, the issue of proper identification is critical at the beginning of an extensive process of quality assurance and is important for the characterization of genetic diversity, phylogeny, phylogeography, and the protection of endangered species. Therefore, the propagation of the species and the optimization of the bioactive compounds through micropropagation leading to more investigations and applications is imperative. Micropropagation is one of the plant tissue culture techniques to produce many genetically superior transplant and pathogen-free transplants in a limited time and space. Up to now, little research has been reported on the tissue culture of burdock which can be an alternative method for consistent availability and faster access to its products (He et al., 2006).

2.2.6.1 Tissue culture of Burdock

This technique is widely used for rapid multiplication, genetic modification, germplasm preservation, cell culture for secondary metabolites, and other studies of scientific investigation (Ravindran et al., 2005). The production of secondary metabolites and the standardization of bioactive compounds can be easily achieved through in vitro plant propagation techniques. The study of burdock has increased in recent times because of its nutritional and curative properties. The reporting on its pharmaceutical properties is increasingly being documented on plant materials obtained from the wild population. The reporting on plant materials from tissue culture has remained silent even with the increasing research interest. However, in recent times some advances have been made in plant tissue culture of burdock roots. A protocol for high frequency-plants regeneration on burdock roots from seedling explants and explant-derived calluses has been investigated.

He et al. (2006) documented a protocol for callus induction and high frequency plant regeneration from hypocotyl and cotyledon explant of burdock. Hypocotyls and cotyledons of burdock plants were induced to form callus by culturing on Murashinge and Skoog (MS) medium supplemented with 2.0 mg^{-1} 2, 4-dichlorophenoxyacetic acid and 0.5-2.0mg^{-1} benzyladenine (BA). Adventitious buds were also induced from explants directly by culturing on Murashinge and Skoog (MS) medium with 1.0mg^{-1} indole-3 butyric acids or 3acetic acid in combination with 1.0mg $^{-1}$ α-Naphthaleneacaetic acid (NAA). The regenerated plants after acclimatization followed the normal morphological and growing stages. Flowering and seed production took place during the second year.

Also, callus culture of burdock was tested on three different media (M_1 =1.5 mg/L BA and 1mg/L NAA, M_2= 1 mg/L BA and 2 mg/L 2, 4-D and M_3= 1.5 mg/L NAA and 1 mg/L 2, 4-D). All the media

consisted of Murashinge, and Skoog (MS) salt (1962) supplemented with Gambong`s B_5 vitamin (1968), 30% sucrose and 02% Gelrite at pH 5.7. Callus induction frequency from burdock cotyledon sections was 100% for all three media. M_1 (1.5 mg/L BA and 1 mg/L NAA) exhibited the fastest callus development followed by M_2 (1 mg/L BA and 2 mg/L 2, 4-D). In terms of long term callus culture M_3 (1.5mg/L NAA and 1mg/L 2, 4-D) was the most suitable for long term callus culture, while M_1 and M_2 had browning problems (Ren et al., 2020).

Recently, in vitro propagation was investigated on different explants of *A lappa*. Hypocotyl and cotyledon of the species were cultured on Murashinge, and Skoog (MS) medium supplemented with different levels of 2, 4-Dichlorophenoxyacetic acid (2, 4-D) and 6-Benzylaminopurine (BAP) for callus induction. Different levels of 6- Benzylaminopurine (BAP) and α-Naphthaleneacaetic acid (NAA) on calli derived from cotyledon and hypocotyl were investigated for indirect regeneration. While different levels of 6-Benylaminopurine (BAP) plus 2 mg/l of α-Naphthaleneacaetic acid (NAA) were compared on cotyledon, hypocotyl, and bud for direct generation. Maximum callus induction was observed on the media containing 2mg/l of 2, 4 – Dichlorophenoxyacetic acid (2, 4-D) plus 1 mg/l 6-Benylaminopurine (BAP) (100% and 76.19% respectively). The highest indirect regeneration percentage (65%) was observed at 1 mg/l 6-Benylaminopurine (BAP) plus 0.5 mg/l α-Naphthaleneacaetic acid (NAA) on cells from hypocotyl. The highest percentage of direct regeneration (90.33) was observed in hypocotyl with a lateral bud explant on MS medium supplemented with 0.5 mg/l 6-Benylaminopurine BAP plus 2 mg/l α-Naphthaleneacaetic acid (NAA) (Zebarjadi et al., 2018).

2.2.6.2 . Plant culture and biosynthesis of secondary metabolites of Burdock

Plant tissue culture is a useful technique for the biotransformation of natural compounds in plant cells because of its potential to synthesize plant`s secondary metabolites of interest (Bonhomme et al., 2000). Callus and cell culture techniques have been used for the commercial propagation of plants because of their high capacity to multiply and differentiate. Use of elicitors like jasmonic acid, salicyclic acid, methyl Jasmonate, sucrose and stress agents have been used to enhance the production of some secondary metabolites of interest in plant tissue culture. Exposure to biotic or abiotic elicitors frequently induces secondary metabolites biosynthesis in plants (Naik and Al-Khayri, 2016, Thakur et al., 2019). Methyl Jasmonate is a signaling compound that elicits plant stress resistance response and has been widely used to enhance secondary metabolites in plant cell cultures (Sudha and Ravishankar, 2002). *In vitro* plant cell culture was used to produce phenolics from burdock. The application of 1 mM methyl jasmonic acid and sucrose (90 g/L) increased the total phenolic content in burdock callus in the first 2 weeks then decreased. The trend would have been due to the oxidation of phenolics causing browning or the volatility of methyl

jasmonic acid or the decrease in sucrose in the media resulting in a decrease in carbon supply for secondary metabolism. The application of 0.5 mM methyl jasmonic acid to other species like sweet basil and lettuce increased the total phenolic content (Kim et al., 2007). Recently, leaf explant and seedlings of *A lappa* were used for hairy roots induction using Agrobacterium rhizogenes strain AR15834 on Murashinge and Skoog (MS) media with different concentrations of ascorbic acid, citric acid, polyvinylpyrrolidone and L-cysteine either alone or in combination with each other for the enhancement of arctiin and arctigenin production as anticancer reagents (Soleimani et al., 2012).

2.2.7 Metabolomic profile of Burdock

Metabolomics is simply the qualitative and quantitative analysis of metabolites (Kim and Verpoorte, 2010, Jung et al., 2015). It is highly useful for studying the biochemical origins of stress, as changes in the metabolic profile are the ultimate results of such external influences (Bailey et al., 2003). The many health benefits of *A lappa* have been reported due to the different classes of bioactive secondary metabolites (e.g. flavonoids, lignans and phenolic compounds)(Ferracane et al., 2010) (Table 1). Various compounds from these different phytochemical groups have been identified in the roots, leaves and seeds of Burdock extracts. Also, the volatile constituents (essential oil) have demonstrated differences in their chemical composition when analysed. The roots of *A Lappa*. contain up to 50% inulin, polyacetylenes, non-hydroxyl acids (lauric, palmitic, stearic, myristic), volatile acids (isovaleric. acetic, butyric, propionic), tannin and polyphenolic acids while the seeds have 15-30% fixed oils, a bitter glycoside (arctiin) and chlorogenic acid (Mabey et al., 1988). The principal components of *A lappa* are polyphenols such as chlorogenic, isochlorogenic, caffeic, and other derivatives of caffeic acid (Jaiswal and Kuhnert, 2011).

Table 2.4 Metabolic Profile of plant extracts and volatile constituents of Burdock (Arctium Lappa L)

Studies	Identified compounds	N0 of compounds	Reference(s)
Metabolite Profiling of the response of burdock root to copper stress	1) H NMR:-Detected polar metabolites were alanine, arginine, asparagine, fructose, glucose, isoleucine, leucine, phenylalanine, proline, succinate, sucrose threonine, tyrosine, valine, phenol.	15	(Jung et al., 2015)
	2) GC-MS: -Detected non-polar metabolites were linoleic acid, linolenic acid, methyl nonadecanoate, campesterol, stigmasterol, gamma-sitosterol.	6	
Metabolic profile of the bioactive compounds of burdock (*A lappa*) seeds, roots, and leaves	1)LC/MS/MS: Phenolic compounds detected in seeds were Chlorogenic acid (caffeoylquinic acid), caffeic acid, cynarin (dicaffeoylquinic acid), lappaol C, Actiin, Arctignan E, Matareisinol, lappaol A, Lappaol F, Arctigenin,	10	(Ferracane et al., 2010)
	2)) LC/MS/MS: Phenolic compounds detected in the roots were Chlorogenic acids (caffeoylquinic acids), Caffeic acid, cynarin (dicaffeoylquinic acid), quercitrin (quercetin rhamnoside), Arctiin, Quercetin, Luteolin	7	
	3) LC/MS/MS:- Phenolic compounds detected in leaves were Chlorogenic acids(caffeoylquinic acid) caffeic acid, Rutin (Quercetin rhamnosil glucoside), cynarin (dicaffeoylquinic acid) quercitrin (quercetin rhamnoside) quercetin, Luteolin	7	
The Essential oil composition of *A lappa* Root and Leaf	1)GC/MS:-Essential oil composition of leaf were; Octane, m-xylene, 2-penthyl furan, Octanal, 1,8-cineole, phenyl ethanol, y-terpinene, linalool, β-thujone, α-thujone, β-fenchyl alcohol, camphor, borneol, nonanol, 4-terpineol, methyl salicylate, decanal, β-cyclocitral, cuminaldehyde, piperitone, dihydro edulan I carvacrol, theaspirane B, theaspirane A, dihydro edulanII, hexyl tiglate, β-damascenone, 2,6,10-trimethyl dodecane, Isocaryophyllene, E-caryophyllene, α-humulene, γ-gurjunene, γ-muurolene, γ-selinene, β-ionone, valencene, γ-cadinene,	51	(Golbaz et al., 2018)

	9,10,-dehydro-isolongifolene, caryophyllene oxide, benzophenone, isospathulenol,β-eudesmol,santalol, 2,6,14-trimethyl pentadecanone, methyl linolenate, diisobutyl phthalate, butyl isobutyl phthalate,2-methoxyy-3,5,5-trimethyl-1,2-cyclohexene-1,4-doine, 7-tricyclo[5.3.2.0(1,6)dodecane-7-ol, 3,4-dimethyl-cyclohexene-carboxaldehyde,and Z-11(13,14-epoxy) tetradecene acetate.		
	2)GC/MS: - Essential oil composition of root was; Phenyl ethanol, β-maaliene, β-panasinsene, β- elemene, cyperene, E-caryophyllene α-bergamotene, α-humulene, β-selinene, α-selinene, Z-α-bisabolene, γ-cadinene, δ-cadinene, 9,10-dehydro-isolongifolene, 7-methyl 3,4-octadiene, 1,3-cyclooctadiene, edusema-4,11, dien-2ol, 15-copaenol, Allo-aromadendrene, α-bisabolol, diisobutyl phthalate, phytol-n-butylphthalate, 4,4-dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4.1.0]heptanes, (3E,5E,8E)-3,7,11-trimethyl-1,3,5,8,10-dodecapentaene.	25	
Chemical composition and antimicrobial activity of volatile constituents from the roots, leaves and seeds of *A lappa*. (Asteraceae) grown in Egypt	1)GC/MS: - Essential oil composition of the root was α-Pinene, carvomenthone, Aromadendrene, trans-β-farmesene, γ-cadinene, caryophyllene oxide, β-copaen-4α-ol, Isoaromadendrene, β-costol, Eicosane, tetracosane, pentacosane, Hexacosane, Heptacosane,	14	(Aboutabl et al., 2013)
	2)GC/MS:-Essential oil composition of leaves was α-Pinene, limonene, Nonanal, carvomenthone, tetradecene, β-elemene, Trans-β-farnesene, Pentadecane, γ-cadinene, caryophyllene oxide, β-copaen4α-ol, β-costol, Nonadecane, Eicosane, Decosane, Tetracosane, pentacosane, Hexacosane, Heptacosane,	19	
	3) GC/MS: - Essential oil composition of seeds was β-myrcene, limonene, linalool, Nonanal, geraniol, thymol, z-citral, E-citral, β-Elemene, Aromadendrene, γ-cadinene, caryophyllene oxide, β-copaen-4α-ol, β-costol, methyl palmitate, Eicosane, methyl oleate, octadecanoic acid, ethyl oleate, pentacosane, Hexacosane, Squalene.	22	

2.2.8 Medicinal and other impetus for cultivation of Burdock

Burdock contains many active components of therapeutic values for the treatment of various diseases (Chan et al., 2011) (Table 2). For centuries, this plant has been used to treat a host of ailments. Traditionally it has been used as a “blood purifier” to clean the bloodstream, increase urine flow, treatment of cancer, hypertension, digestive ulcers, gout, and as a good remedy for skin problems such as eczema, acne, and psoriasis (Salama and Salama, 2016). It is often used with other herbs for the treatment of sore throat and common cold, dizziness and reproductive problems (Johnson et al., 1995). It has been reported that the lyophilized extract of *A. lappa* leaves exhibits antimicrobial activity against oral micro-organisms and is most effective against bacteria related to endodontic pathogens such as*: Bacillus subtilis*, *Candida albicans*, *Lactobacillus acidophilus* and *Pseudomonas aeruginosa* (Pereira et al., 2005). Also, chlorogenic acid isolated from the leaves had restraining effects on *Staphylococcus aureus, Micrococcus lutes and Escherichia coli* (Lin et al., 2004).

Burdock is also recognized internationally for its culinary uses. The carrot-like roots are eaten as salad and cooked as a vegetable in Asia (Da Silva et al., 2013). The young leaves and stems are also consumed as condiments in some foods, especially in modern European cuisine (Haghighi and Mozafariyan, 2011). Also, the stalks are well peeled and consumed either raw or when boiled with salt (Szczawinski and Turner, 1978), due to their moderate amount of dietary fibre, calcium, potassium and amino acids (Chan et al., 2011).

Table 2.5 Therapeutic and pharmacological activities of Burdock (*Arctium lappa* L.)

Therapeutics values	**Pharmacological activities and observations**	**Plant part (s) used**	**Reference(s)**
Anti-allergic	1) Butanol fraction demonstrated anti-allergic effect by decreasing β-hexosaminidase release in mast cells and in the secretion of IL-4 and IL-5 in Con A-induced T cells	Roots	(Sohn et al., 2011)
	2) Fruit extract exhibited the release of β-hexosaminidase, a key biomarker of degranulation during an allergic reaction in RBL-2h3 cells	Fruits	(Yoo et al., 2016)
	3) 100μg/ml Ethanol extract (90%) inhibited the degranulation rate by 52%, determined by the level of β-hexosaminidase. ALE suppressed PCA in rats and attenuated anaphylaxis and histamine release in mice	Roots	(Yang et al., 2016)

	4) Anti-allergic actions of PASA and F-PASA, inhibited the lgE/antigen complex (lgE/Ag)-mediated allergic responses in RBL-2H3 cells and passive cutaneous anaphylaxis in mice	Fruits	(Yoo et al., 2019)
Anti-inflammatory	1) Root extract demonstrated suppression of pro-inflammatory cytokine expression, inhibition of the nuclear factor-kappa B (NF-JB) pathway.	Roots	(Chan et al., 2011)
	2) The lignans Arctiidilactone and butyrolactone isolated from fruits exhibited an anti-inflammatory effect	Fruits	(Yang et al., 2015)
	3) Inflammatory status and oxidative stress in patients with knee osteoarthritis was improved by the intake of root tea	Roots	(Maghsoumi-Norouzabad et al., 2016)
	4) Leaf aqueous extract had a dose dependent anti-edematogenic activity on carrageenan-induced paw oedema, which persisted for up to 48hours	Leaves	(Carlotto et al., 2016)
	5) Crude extract significantly reduces the LPS-induced increase of plasma 1L-1β in the mouse peritonitis model	Roots	(Shi et al., 2018)
	6) LPS-induced inflammation through accumulating myeloid derived suppressor cells was ameliorated by arctigenin	Roots	(Kim et al., 2018)
	7) The circulating levels of the pro-inflammatory cytokines, TNF-α and IL-1β markedly increased in Pb (II) - intoxicated rats.	Roots	(Alhusaini et al., 2019)
Anticancer	1) Flavonoids-types antioxidant and some other active polyphenol antioxidants found in the roots of burdock accounts for the suppressive effects on cancer metastasis	Roots and leaves	(Tamayo et al., 2000)
	2) Arctigenin , an active compound found in seeds, has the ability to eradicate nutrient-deprived cancer cells	Seeds	(Awale et al., 2006)
	3) Dichloromethane extracts showed selective antiproliferative activity against K562, MCF-7, and 786-0 human cancer cell line	Roots	(Predes et al., 2011)
	4) Arctigenin and many other chemicals from *Arctium lappa* L. were found to have cytotoxic activity against human pancreatic cancer PANC-1 cells in nutrient-deprived medium	Roots leaves and seeds	(Tezuka et al., 2013)

	5) *lappaol* F from *Arctium lappa* L suppressed cancer cell growth in a time and dose-dependent manner in human cancer cell line of various tissue types	Roots	(Sun et al., 2014)
	6) PANC-1 and AsPC-1 arctigenin exhibit a highly preferential cytotoxicity to cancer cells that are bathed in glucose-deprived conditions	seeds	(Yoo et al., 2016)
	7) Arctigenin inhibits and regulate the growth of cancer activities in the stomach, lungs, liver and colon	Root, leaves and seeds	(He et al., 2018)
	8) Ethyl acetate root of *Arctium lappa* L. demonstrated a strong anticancer potential and induced intrinsic apoptosis via loss of ψ_m and activation of caspase 3/7	Roots	(Don and Yap, 2019)
Anti-Viral	1) Arctigenin (ATG) inhibits the PCV2 proliferation in PK-15 cells	Root leaves and seeds	(Chen et al., 2016b)
	2) Arctigenin (ATG) interferes with the integration of HIV virus.	Roots leaves and seeds	(Vlietinck et al., 1998)
	3) ATG(dibenxylbutyrolactone lignanolides) inhibits HIV-1	Roots, leaves and seeds	(Eich et al., 1996)
	4) ATG and trachelogenin, lignanolides from Ipomoea cairica inhibit HIV-1 replication at 500nM inhibiting topoisomerase II	Roots, leaves and seeds	(Schröder et al., 1990)
Antibacterial	1) Chlorogenic acid isolated from the leaves showed restraining effects on Escherichia coli, *staphylococcus aureus* and *Micrococcus luteus*	leaves	(Lin et al., 2004)
	2) The root extract had inhibitory activity on Proteus mirabilis through disc diffusion method	Roots	(Keyhanfar et al., 2012)
	2) Root extract influenced *staphylococcus aureus*	Roots	(Habibipour and Rajabi, 2015)
	3) Leaf fraction inhibits the growth and biofilm development of *Escherichia coli* and *Samonella Typimurium*.	leaves	(Lou et al., 2016)
	4) Methanol extract restrained the biofilms (Urinary tract pathogens) on polystyrene and glass surfaces	Roots	(Rajasekharan et al., 2015)

Antioxidant	1) Crude extract fractions exhibited a strong scavenging activity on 1,1diphenyl-2-picryhydrazyl, hydroxyl and superoxide radicals against ascorbic acid.	Roots	(Jiang et al., 2019)
	2) In vivo antioxidant assays of ALP1 administration enhanced antioxidant enzyme activities and total antioxidant capacity as well as decreased the levels of MDA in serum and liver ageing mice	Roots	(Liu et al., 2014)
	3) Cellular antioxidant levels were decreased in the liver of Pb (II) induced rats. Extract of *Arctium lappa* L boosted antioxidant defenses in Pb (II) induced rats, inhibiting liver injury in Pb (II) intoxicated rats by attenuating oxidative injury.	Roots	(Alhusaini et al., 2019)
	4) Aqueous Extract (water root extract and hot water root) of burdock exhibited a 60.4-65% scavenging effect on superoxide and 80.5% scavenging effect on hydrogen peroxide.	Roots	(Duh, 1998)
Anti-aging	1) Natural *Arctium lappa* L. fruit extract improves metabolism of the dermal extracellular matrix which leads to a visible wrinkle reduction in vivo. Can be an effective treatment option for mature skin	fruits	(Knott et al., 2008)
	2) Isolated Lignans arctigenin and Matareisinol from *Arctium lappa* L. seeds have demonstrated an anti-aging activity	leaves	(Corrêa et al., 2018)
	3) Leaves of *Arctium lappa* L. possesses a marked Tyrosinase and elastase inhibitory activities with its antielastase activity stronger compared to its antitryosinase activity	leaves	(Horng et al., 2017)
Anti-hypertensive	1) Arctigenin from *Arctium lappa* L. increases NO production and inhibits the expression of NADPH oxidase in the thoracic aorta of SHRs.	Root	(Liu et al., 2015b)
Anti-diabetic	1) The roots of *Arctium lappa* L are used to slow down the digestion of carbohydrates, reduce the absorption of glucose and control conditions of hyperglycemia	Roots	(Chan et al., 2011)
	2) The lignans from *Arctium lappa* L. are safe antidiabetic agent and prevents diabetic complications	Fruits	(Xu et al., 2008)

	3)Ethanolic extract of *Arctium lappa* L. decrease the blood glucose and increase insulin level in diabetic rats	Roots	(Xu et al., 2008)
Aphrodisiac	1)Aqueous root extract of *Arctium lappa* L. enhances sexual behaviour of male rats, thus a potential treatment for impotence and sterility	Roots	(Jianfeng et al., 2012)
	2) Root extracts of *Arctium lappa* L. increases sperm count, luteinizing hormone, follicle-stimulating hormone, and testosterone in non-diabetic animal.	Roots	(Ahangarpour et al., 2015)
	3)Leaf extracts of *Arctium lappa* L. improves the effect of pentoxifylline in enhancement fertility in rats	leaves	(Alwan and Hasan, 2015)
Anti-constipation	1) A new pectin (ALP-2) extracted from roots of *Arctium lappa* L. exhibited strong anti-constipation activity in vivo with the dosage of 200mg/kg and 400mg/kg.	Roots	(Li et al., 2019)
Anti- acne activity	1) *Arctium lappa* L was effective against acne vulgaries	Root	(Dodov and Kulevanova, 2009)
Antitoxic activity	1) Fibre from *Arctium lappa* L. alleviate toxicity of amaranth in rats	Roots	(Takeda and Kiriyama, 1979)
	2) *Arctium lappa* L (Gobo) prevents bad effect of rancid soya beans oil in diet.	Roots	(Kimura et al., 1984)
	3)*Arctium lappa* L stimulates fecal excretion of toxic furans	Roots	(Morita et al., 1993)
Contraindications	1) *Arctium lappa* L. in some cases causes allergic contact dermatitis	Roots	(Ganter et al., 1997)
	2) Burdock root tea sometimes causes anticholinergic poisoning	Roots	(Rhoads et al., 1984)
	3) *Arctium lappa* L tea needs to be taking into consideration as it has a deleterious effect on the endocrine system of organism	Roots	(Sharma and Goyal, 2014)
	4) Hot aqueous extract of *Arctium lappa* L. inhibits binding platelets activating factor to rabbit platelets	Roots	(Iwakami et al., 1992)

2.2.9 Potential economic benefits of Burdock

The susceptibility to pathogenic infections by the current human population is very alarming in our present time. Even with the presence of sophisticated orthodox medical facilities, people still prefer traditional or herbal medicine because of the little or no side effects. Burdock is one of such detoxifying herbs gaining traction to consumer's attention. Research has proven that the roots, leaves and seeds have many therapeutic values (Chan et al., 2011, Guna, 2019). Given a great opportunity for manufacturers to increase their supply in other to meet up with the demand of the growing population. Burdock`s potential as a multi-utility herb will always safeguard its demand that keeps growing in the market. Thus, increasing the cultivation in a broader spectrum will be a possible solution to meet up with the demand of pharmaceutical industries and herbal practitioners. The supply can be easily achieved from the flexible growing practices since the roots grow very deep in sandy loamy soil rich in nitrogen (Lim, 2012). The cultivation of *A.lappa* as a vegetable has been going on for a long period in the Asian continent (Morita et al., 1993), Australia, America (Guna, 2019), and Egypt where it was characterized as an invasive plant of high medicinal potential (Salama and Salama, 2016). The plant materials are mostly imported to those countries or communities which are not of their origin, with much of the plant material being derived from the wild creating pressure on its natural habitat. In South Africa, for instance, the plant material is imported by the pharmaceutical and herbal industries. The plant materials are being used individually or synergistically with other plant materials as herbal tea. Dried burdock root is being consumed synergistically with dried sarsaparilla (Smilax sp.) and dried Elderberries (Sanbusi nigra), with approximate current prices of R69.00/75 g for dried burdock root, R195.00/75 g of dried sarsaparilla and R115.00/100 g of dried elderberries. The cost price of these plant materials is expensive and inconsistent due to fluctuation in the importation expenses. Moreover, the product quality is not guaranteed even the certainty in supply. Therefore, the cultivation of this species in South Africa and the other parts of the continent will help to address the issue of price, quality, and certainty in the supply of plant materials. Also, the sector of medicinal plant cultivation will be a new enterprise for both our subsistence and commercial farmers to venture into, which has always remained silent. This will help to boost the regional as well as global economy through job creation by entrepreneurs who are willing to invest in this new sector of a more rather monopolistic enterprise

2.3 Cultivation of skullcap (*Scutellaria lateriflora* l.) As a possible solution to quality assurance and consistent availability of plant materials for commercialisation

2.3.1 Introduction

Plants are known to play a considerable role in the field of complementary and traditional medicine across different regions and cultures of the world (Payyappallimana, 2010). With the growth in human needs, population and commercial trade, the demand for plant based products in recent times has increased (Ghimire et al., 2016). This is being achieved at times with the over exploitation of some plant species, which creates pressure on the wild plant population in their natural habitat (Lewis and Elvin-Lewis, 1995). Plant materials harvested from the wild for medicinal purposes at times lack uniformity because they grew under different environmental conditions and could be collected at different stages of maturity (Azaizeh et al., 2005). The variation in seasons and environmental factors across the different regions of the world may have an influence on the therapeutic properties of these species owing to fluctuation in the quality and quantity of bioactive compounds (Yang et al., 2018).

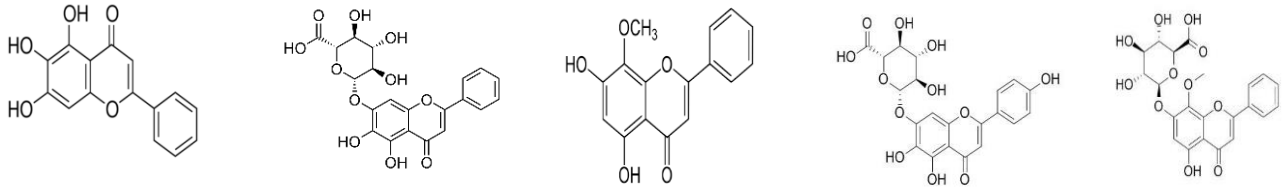
Agencies on biodiversity conservation are recommending that threatened plant species be brought into cultivation as part of mitigating measures toward medicinal species (Lambert et al., 1997, Sharrock et al., 2018). This initiative will help in the sustainable supply of plant materials since they play a significant role in the local economy (Schippmann et al., 2002). The cultivation of such species may result in sustainable supply, greater uniformity, and providing more awareness on the growing conditions and how they may affect the production of target secondary metabolites (Hadacek, 2002). The enhancement of secondary metabolites will also be possible, which may improve their medicinal potential through the manipulation of growing conditions(Bourgaud et al., 2001, Azaizeh et al., 2005).

American Skullcap is one of the most widely used nervines by the native Americana and Europeans (Upton and Dayu, 2012). It is a perennial plant of the mint family and grows naturally in moist loamy soil with partial shade (Awad et al., 2003). It is also known to grow well in full sunlight. In the field it can be successfully grown from seeds or propagated through root division. Traditionally, it is used in the treatment of many diseases and nervous conditions like anxiety, neuralgia, epilepsy and as a sedative (Brock et al., 2010). It is also used for digestive problems, diarrhea and rabies, (Greenfield and Davis, 2004). The herb is sold as a tea in health food stores and as a tonic or in combination with other herbs such as passion flower and valerian as a sleep inducer (Awad et al., 2003).

The phytochemical constituents of American Skullcap include flavonoids, tannins, phenolics, volatile oil, diterpenoids, waxes and iridoids (Wills and Stuart, 2004). This plant has been found to

contain high flavonoid content which is responsible for the diverse useful therapeutic properties (Joshee et al., 2013), such as anti-inflammatory, antioxidant, antiviral, antithrombotic, and sedative activity (Shang et al., 2010). The different types of the major flavonoids (Fig.2.2) identified in *S lateriflora* are flavonoid glycosides baicalin, wogonin baicalein, (Similien et al., 2016), ikonnikoside I, dihydrobaicalin, lateriflorin, scutellarein, oroxylin A-7-O-glucuronide, aglycones, oroxylin, and 5,6,7-trihydroxy-2-methoxyflavone (Bergeron et al., 2005, Lin et al., 2009). The major flavonoids found in this plant are scutellarein and glycosides scutellarein s (Wills and Stuart, 2004). However, the aerial part is known to contain baicalin as the major flavonoid glycoside which is responsible for its anxiolytic effects (Xu et al., 2006), followed by dihydrobaicalin (Bergeron et al., 2005).

In recent time, the demand for this species for its anxiolytic and sedative properties have surpassed all the other categories of its therapeutic values (Block et al., 2004). However, most of the plant materials are mostly obtained from the wild without any guarantee for premium quality and consistent supply, regardless of the silent practices of its cultivation which is still in the nascent stage of development in the region of its origin (Tanga et al., 2018). This review summarizes the currently available scientific literature on its botany, geographical distribution, cultivation practices, biotechnology, phytochemistry, therapeutic properties, and its potential as a possible medicinal herb for commercialization.



Baicalein Baicalin Wogonin Scutellarin Wogonoside

Figure 2.2 Structures of the major flavonoids of American skullcap (*Scutellaria lateriflora* L.)

2.3.2 Botany and geographical distribution of American Skullcap

American skullcap is a hardy perennial herbaceous plant of the family Lamiaceae. It is a member of the mint family and comprises of 360-400 Scutellaria species worldwide (Paton, 1990, Cole et al., 2008). It is native to North America, endemic to the marshy forests, extending from Canada to Florida, Northern and Eastern United States, and westward to the British, Oregon, and New Mexico (Bergeron et al., 2005, Gafner, 2015). The generic name Scutellaria is derived from the Latin word *scutella* meaning little dish, from the lid of the calyx. There are other species of skullcap

with different geographical locations (Fig. 2.3),some of which are used in traditional medicine, include *S baicalensis*, the Chinese skullcap and *S barbata* (Awad et al., 2003).

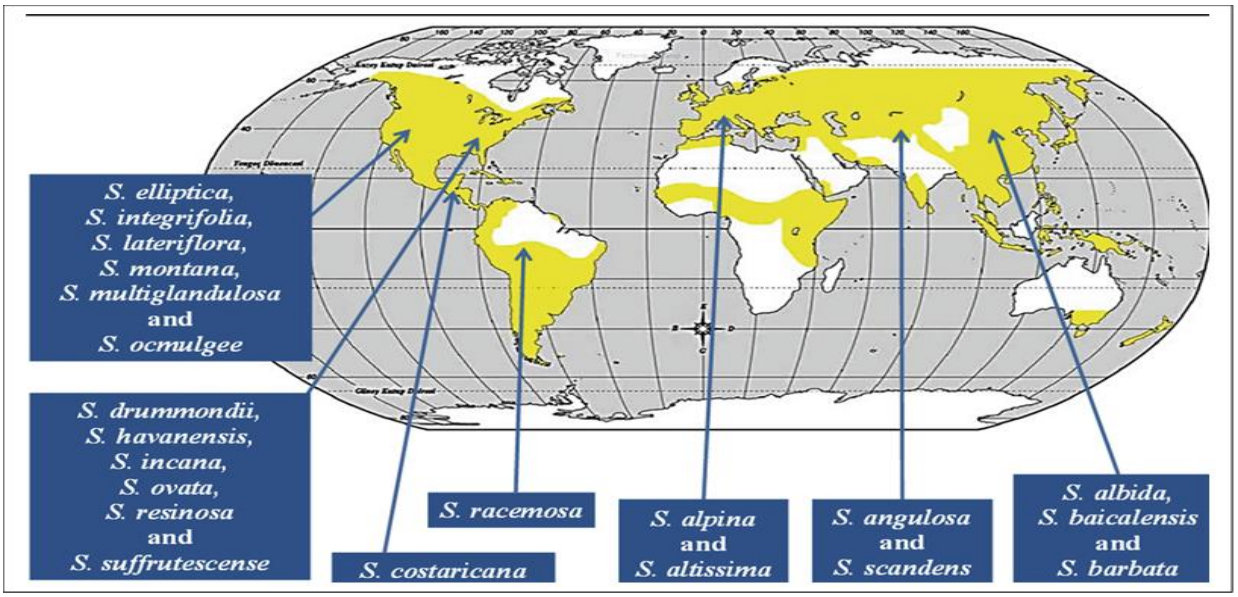


Figure 2.3 Geographical distribution of Skullcap species(Irvin et al., 2019)

2.3.3 Agroecology of American Skullcap

American Skullcap has a fibrous root system with a short creeping hairy stolon. It has an upright habit with a slender branching stem that grows between 30 and 60cm in height, with opposite, ovate and serrate leaves (Fig.3a-b). The leaves are about 3 to11cm in length and 1.5 to 5.5cm in width (Upton and Dayu, 2012). In summer the plant produces small violet, pink to white-blue flowers that resemble a helmet or cap in a shape, giving way to its name *S lateriflora* (Fig. 2.4c). It thrives well near marshes, meadows, shores of rivers, lakes, and other wet habitats with a light shade to full sunlight having a well-drained soil with abundant organic matter of a pH 6.2 to7.0. Propagation can be realized through stratification of seeds (Fig.2.4d) in a refrigerator for one week in a paper towel and nursed in a green house before transplanting in the field in spring. It can also be done through nursing of basal cuttings of the underground stems in trays under light shade or greenhouse until they are well rooted then transplanted to the field in spring. Flowering generally occurs from 8 to 10 weeks and harvesting can take place between 12 to 14 weeks after seedling transplant. The above ground parts are collected during summer around bloom time where they are dried in shade and stored for later use as medicinal herbs (Joshee et al., 2002).

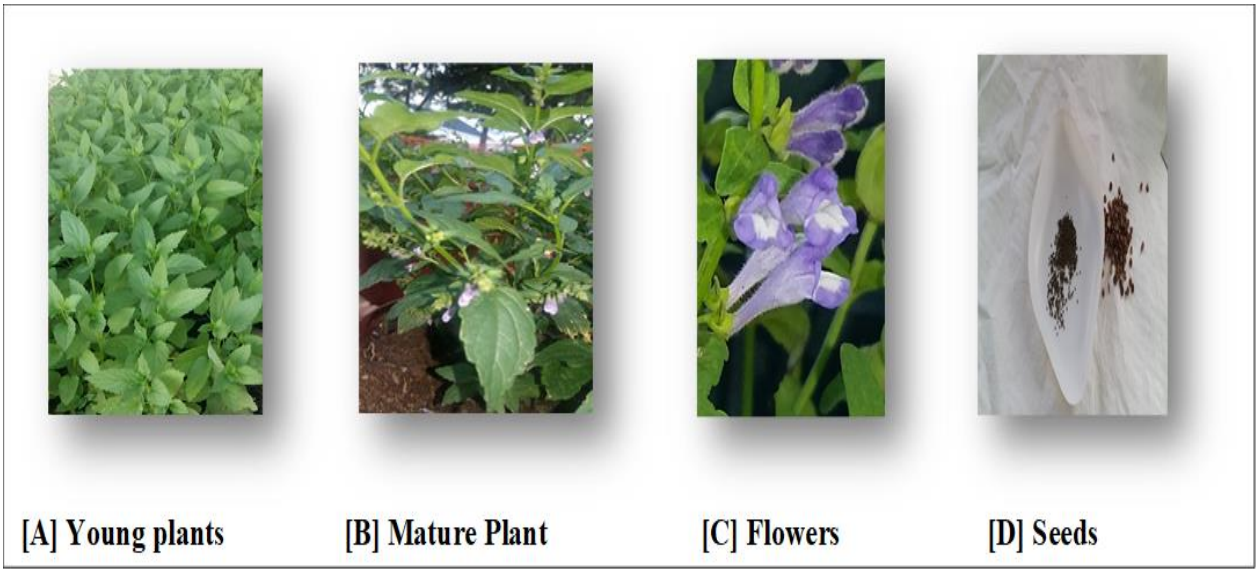


Figure 2.4The morphological features of *S lateriflora*

2.3.4 Agronomic practices of American Skullcap

The cultivation of American Skullcap is a prospective response to biodiversity conservation and habitat degradation.(Joshee et al., 2013) The cultivation of this species will be a plausible solution to reduce pressure on the wild plant population and to meet up with the current and future demand for the plant materials for herbal remedies and food security (Van Wyk and Prinsloo, 2018) Additionally, its cultivation will be an alternative farming practice in agriculture and a source of employment for the enhancement of livelihood to the growers of the species. This will have an appreciable impact on the local GDP, especially in the case of South Africa where to the best of our knowledge, there is no record of the commercial cultivation of this species. Therefore, it is imperative to document the agronomic practices of this species.

2.3.4.1 Field cultivation of American Skullcap

The field cultivation of American skullcap is limited, despite the long-term benefits and widespread which account for its usefulness as a medicinal herb because of its many therapeutic values. The acquisition of this species is mostly from the wild plant population with limited information on filed or horticultural production (Eraso et al., 2007). However, some appreciable amount of information on its production has been provided by Wills and Stuart (2004) and guidelines for field production for small farmers in Kansas (Similien et al., 2012). The cultivation of American Skullcap is most favorable during spring and it requires cold stratification for seven days and light for the germination of seeds (Greenfield and Davis, 2004). This is mostly for the harvest of the aboveground leaves and stems, whereas the other species of *Scutellaria* are cultivated for their roots as well (Bochořáková et al., 2003, Kosakowska, 2017). The nutrient requirement for this

species is not well documented, however, fertilizer is required for its cultivation (Shiwakoti, 2012, Shiwakoti et al., 2016). A plant spacing distance of 20 x 30 cm and a row spacing distance of 45 x 90 cm are recommendable for field cultivation (Greenfield and Davis, 2004). However, a spacing distance of 30 x 30 cm was used which yielded a population density of 53,800 plants/ha assuming a full stand (Similien, 2009).

A study on the cultivation practices has successfully been investigated in the field at Central Alabama to evaluate the effect of partial shade, irrigation, and nutrient application on dry matter yield of American Skullcap and the necessary growing conditions needed to optimize the total dry matter yield (Similien et al., 2012). All the growth variables investigated performed better under shade than in full sun except for dry matter. The highest yield was obtained at irrigation plus manure and irrigation plus fertilizer treatments under shade, which is consistent in the natural habitat of moist soil with cool shady conditions (Awad et al., 2003), and lowest with fertilizer and control treatments in full sun. Dry matter was increased by 45% in shade, 61% by irrigation and, 22% by nutrient application (Similien et al., 2012).

The effect of shade, irrigation, and nutrients on yield and flavonoid concentration on American Skullcap has also been investigated at the Horticulture Unit of the E.V. Smith Research Center. It was observed that flavonoid concentration was 72% higher in full sun but 15% higher with irrigation. Flavonoid yield was 25% higher under shade, 92% higher with irrigation and 43% higher with added nutrients. The increase in biomass yield had a greater effect on total flavonoid harvested than the increase in flavonoid concentration (Similien et al., 2016).

The response of nitrogen, phosphorus and potassium fertilizer on biomass yield and flavonoid content on American skullcap was investigated in a greenhouse of plant Science Research Center at Auburn University, Al, USA. The highest dry matter yield was obtained at 446 kg N/ha, while the flavonoid baicalein and chrysin content, had their highest response at 412 kg N/ha for baicalein and 351 Kg N/ha for chrysin. The yield of baicalein, chrysin, baicalin and scutellarein increased with the addition of phosphorus. The highest dry matter yield was observed at 208 Kg/ha for potassium fertilizer, with a linear response for scutellarein concentration. Phosphorus application had the greatest effect on flavonoid concentration while potassium demonstrated the least effect which was attributed to the presence of fritted clay (Shiwakoti et al., 2016).

Furthermore, in Alabama a field experiment has been conducted to determine the effect of timing and frequency of harvest on shoot yield and flavonoid content on American Skullcap. Plant height, density, percentage of dry matter and shoot yield were higher during the first harvest than in the second harvest. The yield and concentration of baicalin was highest followed by baicalein and

apigenin but there were no differences in flavonoid yield between early and late harvest.(Shiwakoti et al., 2013)

2.3.4.2 Micropropagation and molecular studies on American Skullcap

The harvesting of medicinal plants from the wild is at an alarming rate due to the shift from subsistence use to commercialization resulting in overexploitation causing pressure on the wild plant population in their natural habitat without any mitigative measures(Van Wyk and Prinsloo, 2018). American Skullcap is among the medicinal plants of such category facing this challenge since most of the plant materials are obtained from the wild. As the demand for plant materials of this species keeps on increasing, there is a call for interest in the propagation of healthy plants and biomass on a large scale (Tascan et al., 2007, Tascan et al., 2010). This will help to meet up with the need to produce marketable plant materials as per the demand of pharmaceutical industries and consumers. The application of micropropagation for the growing of this species can help in the optimization of the bioactive compounds of interest. This culture technique has the potential to produce a good number of genetically superior plants which are pathogen free in a short period of time. However, tissue culture has remained silent with limited reports on this species.

2.3.4.3 Tissue culture and biosynthesis of secondary metabolites of American skullcap

Tissue culture is known to be among the fastest and most efficient methods to increase commercial production of plants. The application in plant propagation helps to maintain uniformity and quality in plant growth at a faster speed than traditional methods. It is widely used for rapid multiplication, genotype modification, germ plasma preservation and cell culture for bioactive compounds (Ravindran et al., 2005). The quest for phenolic compounds for medicinal purposes has attracted the establishment of hairy root cultures of *S lateriflora* in vitro (Kim et al., 2014, Marsh et al., 2014). It has been successfully cultivated through micropropagation from hairy root culture for secondary metabolites enhancement, with the use of *Agrobacterium rhizogenes* A4 (Wilczańska-Barska et al., 2012).

The effect of auxin concentrations in American Skullcap has been investigated on biomass and the production of flavonoids (Baicalin, Bacalein and Wogonin) in Hairy root culture (Kim et al., 2017). Biomass varies significantly among the treatments and the auxins treatments responded positively to increase flavonoid production. This was attributed to the fact that secondary metabolites biosynthesis in transformed roots are largely controlled genetically but can also be affected by nutrition and environmental factors. Furthermore, a similar or slightly different trend has been reported in the comparative analysis of flavonoids and polar metabolites from hairy roots

of *S lateriflora* and *S baicalensis* which showed that the growth rates of hairy roots did not vary significantly between auxin treatments (Kim et al., 2014).

The effect of light, methyl jasmonate and cyclodextrin on the production of phenolic compounds in hairy root cultures of *S lateriflora* has also been investigated. The results of this study demonstrated that hairy root cultures of *S lateriflora* have the biosynthetic capacity to produce known Scutellaria flavones and suggested that light may have a selected regulatory effect on the synthesis or regulation of these phenolic compounds (Marsh et al., 2014).

The influence of culture medium composition and light conditions on the bioactive compounds in the shoot cultures of *S lateriflora* growth in vitro have also been investigated. The obtained results indicated that metabolites accumulation was highly stimulated by blue light, under which the extracts were found to contain the highest total amount of flavonoids with glucuronides, baicalin, wogonoside and, verbascoside as the highest amount of flavonoids (Kawka et al., 2017)

2.3.5 Metabolic profile of American Skullcap

The qualitative and quantitative analysis of metabolites in organisms is known as metabolomics (Kim and Verpoorte, 2010). American Skullcap is well known for its medicinal potential due to the presence of many bioactive compounds of flavonoids and alkaloids (Tuan et al., 2018). These metabolites have demonstrated anti-inflammatory, antibacterial, antiviral, anti-obesity, anxiolytic and antioxidant properties (Wang et al., 2019). The anxiolytic properties of *S lateriflora* with no side effects are thought to occur through its flavonoid or amino acid activity (Awad et al., 2003). This species is known to be very rich in various types of flavonoids which have been identified from the extracts of the different parts using different chromatographic methods (Table. 2.6) where baicalein, baicalin, scutellarin and wogonin are the major flavonoids (Brock et al., 2010).

The increase in demand for plants with anxiolytic activity has obliged this species as a prime candidate since the extracts contain numerous phenolic compounds of potential therapeutic effects (Awad et al., 2003). Of the well-known flavones, wogonin, baicalein and, baicalin are the three compounds of greater interest present in this species attributed to their anti-inflammatory, anti-tumor, and antioxidant properties (Marsh et al., 2014).

Table 2.6 Metabolic Profile of plant extracts of American Skullcap (*S lateriflora*).

Studies	Plant parts	Identified Compounds	Methods	Number of compounds	Reference(s)
Comparative analysis of bioactive Phytochemicals from *Scutellaria baicalensis*, *Scutellaria lateriflora* *Scutellaria racemose*, *Scutellaria tomentosa* and *Scutellaria wrightii* by LC-DAD-MS	Leaf	Scutellarin, Baicalin,	LC-DAD	8	(Islam et al., 2011)
	Stem	Acteoside, Scutellarin			
	Root	Baicalin, Baicalein, wogonin, Oroxylin A			
	Leaf	Scutellarin, Scutellarin, Baicalin, wogonin, chrysin	LC-MS/MS	16	
	Stem	Acteoside, Scutellarein, Baicalin, wogonin			
	Root	Scutellarin, scutellarein, Baicalin, Baicalein, Wogonin, chrysin, oroxylin A			
	Leaf	Scutellarin, Scutellarein, Baicalin, wogonin, chrysin	LC-MS/MS	16	
	Stem	Acteoside, Scutellarin, Baicalin, wogonin			

	Root	Scutellarin, Scutellarein, Baicalin, Baicalein, wogonin, chrysin, oroxylin A			
Influence of culture medium compound and light conditions on the accumulation of Bioactive compounds in shoot cultures of *Scutellaria, lateriflora* L.(American Skullcap) Grown In vitro.	Aerial part	Baicalein, wogonin, Baicalin, Wogonoside, verbascoside, 2,4-Dihydroxyphenylacetic acid,	HPLC	6	(Kawka et al., 2017)
Enhanced accumulation of secondary metabolites in hairy root cultures of *Scutellaria lateriflora* L following elicitation	Hairy root cultures	Acteoside, Scutellarin, Baicalin, Wogonoside, Wogonin, Chrysin	HPLC	6	(Wilczańska-Barska et al., 2012)
Molecular characterization of flavonoid biosynthetic genes and accumulation of baicalin, baicalein, and wogonin in plant and hairy root of *Scutellaria lateriflora* L	Aerial part	Baicalin, Baicalein, Wogonin	HPLC	3	(Tuan et al., 2018)
Shade, Irrigation and Nutrients Affect Flavonoid concentration and yield in American Skullcap	Aerial part	Baicalin, Baicalein, Wogonin, Chrysin	HPLC	4	(Similien et al., 2016)

Harvesting Number and Timing effects on Shoot Yield and Flavonoid Content in Organically growth American Skullcap (*Scutellaria lateriflora* L)	Aerial part	Scutellarein, Baicalin, Apigenin, baicalein, Wogonin, Chrysin	HPLC	6	(Shiwakoti et al., 2013)
Nitrogen, Phosphorous and Potassium effects on biomass yield and flavonoid content of American Skullcap (*Scutellaria lateriflora* L)*.*	Aerial part	Scutellarein, Baicalin, Baicalein, Chrysin,	RP-HPLC	4	(Shiwakoti et al., 2016)
Effect of light, methyl Jasmonate and cyclodextrin on production of phenolic compounds in hairy root cultures of *Scutellaria lateriflora* L	Hairy root cultures	Verbascoside, Scutellarin, Baicalin, Scutellarein, Wogonoside, Baicalein, Wogonin	HPLC	7	(Marsh et al., 2014)

2.3.6 Medicinal impetus for cultivation of American Skullcap

American Skullcap contains several biologically active compounds that are of therapeutic value, obtained from the aerial part of the plant (Joshee et al., 2013). The dried aerial part is widely used by the native American herbalists as a sedative, nerve tonic (Awad et al., 2003), and also as antispasmodic agent for the treatment of epilepsy and anxiety (Wilczańska-Barska et al., 2012). The flavonoids of this species are strong antioxidants, free radical scavengers and possess antibacterial, antiviral, antitumor and antiallergic activities (Li-Weber, 2009, Wang et al., 2018), as demonstrated by the different plant materials (Table.2.7). They act on different brain receptors; wogonin demonstrates the potential of its anxiolytic properties (Bergeron et al., 2005) and antiviral activity against the hepatitis type B virus, hepatoprotective and antitumor properties (Gasiorowski et al., 2011). Baicalin is used in Chinese traditional medicine to treat hepatitis (Chan et al., 2000).

Table 2.7 Therapeutic and pharmacological activities of American Skullcap.

Therapeutic value	**Pharmacological activities**	**Plant part(s) used**	**Reference(s)**
Anxiolytic	A double blind, placebo-controlled study *S. lateriflora* on healthy subjects demonstrated a noteworthy anxiolytic effect.	Aerial part	(Hollander et al., 2003)
	Rats treated with aqueous *S. lateriflora* extract entered and spent more time in an open space than the control rats. Treated Rats entered the center part demonstrating anxiogenic effect.	Aerial part	(Awad et al., 2003)
	A pilot survey conducted on herbal medicine practitioners, results indicated that *S lateriflora* is effective for reducing anxiety and stress and is commonly prescribe for these conditions and related co-morbidities.	Aerial part	(Brock et al., 2012)
Anticonvulsant	Four doses of *S. lateriflora* extracts (30, 60, 90 and 150mg/kg) were tested in rodent models of acute seizures. 90mg/kg demonstrated the highest anticonvulsant effect.	Areal part	(Zhang et al., 2009)
Antioxidant	Different solvent extractions of S. *lateriflora* were tested for their antioxidant capacity. Overall, methanolic and aqueous methanolic	leaves	(Wojcikowski et al., 2007)

	extracts demonstrated the highest radical scavenging activity.		
	Antioxidant potential was determined in clonally propagated tissues of *S. lateriflora* having the capacity to detoxified oxygen free radicals as determined by DPPH bioassay.	Areal part	(Cole et al., 2008)
	The antioxidant potential of aqueous/ethanolic extracts of *S. lateriflora* was determined in mouse brain tissues using various biochemical assays, the results unveiled significant antioxidant effects posited against various oxidative stress associated mental disorders.	Areal part	(Lohani et al., 2013)
Antispasmodic	Both hot water and fluid extract of the S. *lateriflora* demonstrated a weak uterine relaxation effect in female guinea pigs	Areal part	(Pilcher et al., 1916)
Antimicrobial	Extracts of dichloromethane and methanol of S. *lateriflora* were screen using TLC bioautography and both extracts were active against *Candida albicans*, *cladosporium cucumerinum, bacillus subtilis and Escherichia coli*	Areal part	(Bergeron et al., 1996)

2.3.7 Potential economic benefits of American Skullcap

The growing world market for American skullcap has generated the great potential for its cultivation to meet up with the demand. The areal parts are used as a sedative in the form of

herbal teas, oral liquid preparations, tablets and in combination with other medicinal plants (Table 2.8). The issue of quality on skullcap products is being driven by consumers who consistently ask for the supply of quality products as preferential access to first-class market with the maximum economic return. Thus, increasing the cultivation of this species using modern technology will help the inconsistent availability of plant materials of good quality. This will help to provide quality plant materials that will give an ultimate concentration of active constituents which is beneficial to the health of consumers. In 2001 the sales of Skullcap in the world market increased 2.5 times from approximately 6.4 kg in 1997 to 15 kg in 2001 (Greenfield and Davis, 2004). The annual consumption of skullcap between the years 2000 and 2001 in the world market was estimated to have increased by 23%. In 2001 the dollar value of Skullcap was between $185,000 and $195,000 which was 3.5 times higher than that of 1997 (Greenfield and Davis, 2004). In Canada originally grown skullcap fetched a premium price of 17.60-33 dollars/kg (Porter and Kramer, 2006). The price of Skullcap had a steady increase in the world market with an annual growth rate of 20-30% (Greenfield and Davis, 2004).

Table 2.8 American Skullcap products in the market

Product (s)	**Description**	**Price**
Herbal Tea Skullcap Herb	Dried *Scutellaria lateriflora* L cut Leaves (75g), used for tea, infusion, and tincture	R220
Nature`s way Skullcap Herbs	Nature`s way *Scutellaria lateriflora* L. herbs in capsule form 425mg-100capsule made in USA	R177
Nature`s answer since 1972	Authentic Botanical finger print *Scutellaria lateriflora* L.	R162.89
Hawah Pharm Skullcap	*Scutellaria lateriflora* L Liquid Extract 2 oz	R848.00
Nature`S Answer,skullcap, alcohol free	*Scutellaria lateriflora* L. alcohol free 2000mg	R162.82

Frontier Natural Products Skullcap Herbs	Frontier organic cut and sifted *Scutellaria lateriflora* L. herbs (453g)	R357.93
Phyto-force Skullcap	Phyto-force *Scutellaria lateriflora* L 50ml (Tincture for used for epilepsy, insomnia, muscular spasms, alcoholism, and drug addiction)	R124.95
Electic, raw , dried Skullcap	Raw, dried *Scutellaria lateriflora* L, (350mg), 90 Non-GMO Veggie-Caps	R247.69

https://www.essentiallynatural.co.za/products/dried-scullcap-scutellaria-lateriflora (accessed on the 3rd February 2021) [*Google. Scholar*].

2.3.8 Conclusion

The high and growing interest in medicinal plant products as a means of a primary health care system in both developed and developing countries gives a prospect to medicinal plants as a suitable substitute for crop cultivation, an option for a source of employment and livelihood enhancement, with little or no competition in product quality, efficacy, and commercialization process. The manipulation and optimization of secondary metabolites of interest will rest assured of a consistent supply of plant materials, price affordability and efficacy in their functions. The propagation process will be accompanied by modern technology to meet up with the high demands of pharmaceutical industries to quality medicinal plant materials. In addition, there will be problem-solving such as misidentification, contamination, pest infestation, harvesting, scarcity in supply and, processing methods of plant materials.

Furthermore, according to a literature search, there is little information on the commercial cultivation of Burdock. The demand for this plant is mostly accomplished through the supply from the wild population, exacerbating pressure in the natural habitats. However, the preference for the plant materials for medicine and culinary uses has initiated some preliminary cases of its cultivation practice which is still in the nascent stage of development. Several agencies and stakeholders are recommending that wild plant species be brought into cultivation systems for sustainable harvesting and supply to meet up with the demand. This is needed for most wild-

harvested plant species and their habitats as one of the conservation strategies, given their current and potential contributions to harvesters over the long term and greater value to the local economies. However, this can be achieved only if the cultivation is understood in relation to cultivation processes, practices, nutrient requirements, and phenological stages of development of the species. Research in biotechnology of the species needs to be harnessed especially in the biosynthesis of the metabolic pathways of the secondary metabolites of interest. The development of a newer technological approach will assist the plea of herbal industries on quality assurance and consistent availability of plant materials for commercialization.

Similarly, American Skullcap is highly valued for its medicinal properties which are associated with its high flavonoid content. According to the literature, the availability of the plant material is mostly from the wild from the regions of its origin. However, efforts on its cultivation /propagation practice have been underway through seeds sowing and stem cuttings. Some cases of micropropagation, pot and field experiments on this species have been done on research levels. However, this has remained silent since it is still in the nascent stage of development. Harnessing the cultivation of this species in the different regions of the world will help to address the issue of a consistent supply of plant materials of good quality for the premium market. This will have an economic drive on the country's GDP, through the value chain at micro and macro levels. Also, the reduced pressure in the wild will enhance conservation status with sustainable harvest and supply of plant materials. This can be successful only if the cultivation practices, nutrient requirements and phenological stage of development are well understood with the application of modern technology at subsistence and commercial levels.

References

Abdul, R. B. 2012. Medicinal plants (Importants and uses). *Pharm. Anal. Acta,* 3, e139.

Aboutabl, E. A., El-Tantawy, M. E. & Shams, M. M. 2013. Chemical composition and antimicrobial activity of volatile constituents from the roots, leaves, and seeds of Arctium lappa L.(Asteraceae) grown in Egypt. *Egyptian Pharmaceutical Journal,* 12, 173.

Ahangarpour, A., Oroojan, A. A., Heidari, H., Ghaedi, E. & Taherkhani, R. 2015. effects of hydro-alcoholic extract from arctium lappa l.(burdock) root on gonadotropins, testosterone, and sperm count and viability in male mice with nicotinamide/streptozotocin-induced type 2 diabetes. *The Malaysian journal of medical sciences: MJMS,* 22, 25.

Akula, R. & Ravishankar, G. A. 2011. Influence of abiotic stress signals on secondary metabolites in plants. *Plant signaling & behavior,* 6, 1720-1731.

Al-Zyadi, Q. A. 2018. Effect of nitrogen fertilization and harvesting date on growth of burdock plant (Arctium lappa L.) and total phenols content in leaves. *Biochemical and Cellular Archives,* 2, 2131-2134.

Al-Zyadi, Q. A. 2019. Effect of phosphorus and potassium fertilization on growth of burdock plant (Arctium lappa L.) and its roots content of total phenols. *Jornal of Al-Muthanna for Agricultural Sciences,* 7, 61-67.

Alhusaini, A., Fadda, L., Hasan, I. H., Ali, H. M., El Orabi, N. F., Badr, A. M., Zakaria, E., Alenazi, A. M. & Mahmoud, A. M. 2019. Arctium lappa Root Extract Prevents Lead-Induced Liver Injury by Attenuating Oxidative Stress and Inflammation, and Activating Akt/GSK-3β Signaling. *Antioxidants,* 8, 582.

Alwan, H. D. & Hasan, H. F. 2015. A comparative study between the effects of Arctium lappa L. leaves extract and pentoxifylline on fertility of male rats treated with gentamicin. *Int J Adv Biol Res,* 5, 293-8.

Ameade, E. P. K., Ibrahim, M., Ibrahim, H.-S., Habib, R. H. & Gbedema, S. Y. 2018. Concurrent use of herbal and orthodox medicines among residents of Tamale, Northern Ghana, who patronize hospitals and herbal clinics. *Evidence-Based Complementary and Alternative Medicine,* 2018.

Araya, H. T. 2007. *Seed germination and vegetative propagation of bush tea (Athrixia phylicoides).* University of Pretoria.

Awad, R., Arnason, J., Trudeau, V., Bergeron, C., Budzinski, J., Foster, B. & Merali, Z. 2003. Phytochemical and biological analysis of skullcap (Scutellaria lateriflora L.): a medicinal plant with anxiolytic properties. *Phytomedicine,* 10, 640-649.

Awale, S., Lu, J., Kalauni, S. K., Kurashima, Y., Tezuka, Y., Kadota, S. & Esumi, H. 2006. Identification of arctigenin as an antitumor agent having the ability to eliminate the tolerance of cancer cells to nutrient starvation. *Cancer research,* 66, 1751-1757.

Azaizeh, H., Ljubuncic, P., Portnaya, I., Said, O., Cogan, U. & Bomzon, A. 2005. Fertilization-induced changes in growth parameters and antioxidant activity of medicinal plants used in traditional Arab medicine. *Evidence-Based Complementary and Alternative Medicine,* 2, 549-556.

Azizov, U., Khadzhieva, U., Rakhimov, D., Mezhlumyan, L. & Salikhov, S. 2012. Chemical composition of dry extract of Arctium lappa roots. *Chemistry of Natural Compounds,* 47, 1038-1039.

Bailey, N. J., Oven, M., Holmes, E., Nicholson, J. K. & Zenk, M. H. 2003. Metabolomic analysis of the consequences of cadmium exposure in Silene cucubalus cell cultures via 1H NMR spectroscopy and chemometrics. *Phytochemistry,* 62, 851-858.

Barata, A. M., Rocha, F., Lopes, V., Bettencourt, E. & Figueiredo, A. C. 2011a. Medicinal and aromatic plants–Portugal. *Medicinal and aromatic plants of The World, Encyclopedia of Life Support Systems (EOLSS). Developed under the Auspices of the UNESCO, Eolss Publishers, Oxford, http://www. eolss. net*.

Barata, A. M., Rocha, F., Lopes, V., Bettencourt, E. & Figueiredo, A. C. 2011b. Medicinal and aromatic plants—Portugal. *Medicinal and Aromatic Plants of the World. Encyclopedia of Life Support. Systems (EOLSS), Developed under the Auspices of the UNESCO*.

Barata, A. M., Rocha, F., Lopes, V. & Carvalho, A. M. 2016. Conservation and sustainable uses of medicinal and aromatic plants genetic resources on the worldwide for human welfare. *Industrial Crops and Products,* 88, 8-11.

Barth, C. W., Meyer, S. E., Beckstead, J. & Allen, P. S. 2015. Hydrothermal time models for conidial germination and mycelial growth of the seed pathogen Pyrenophora semeniperda. *Fungal biology,* 119, 720-730.

Bentley, R. & Trimen, H. 1981. Medicinal plants. *Medicinal Plants.* J & A Churchill.

Bergeron, C., Gafner, S., Clausen, E. & Carrier, D. J. 2005. Comparison of the chemical composition of extracts from Scutellaria lateriflora using accelerated solvent extraction and supercritical fluid extraction versus standard hot water or 70% ethanol extraction. *Journal of agricultural and food chemistry,* 53, 3076-3080.

Bergeron, C., Marston, A., Gauthier, R. & Hostettmann, K. 1996. Screening of plants used by North American Indians for antifungal, bactericidal, larvicidal and molluscicidal activities. *International Journal of Pharmacognosy,* 34, 233-242.

Beyene, Y., Semagn, K., Mugo, S., Prasanna, B. M., Tarekegne, A., Gakunga, J., Sehabiague, P., Meisel, B., Oikeh, S. O. & Olsen, M. 2016. Performance and grain yield stability of maize populations developed using marker-assisted recurrent selection and pedigree selection procedures. *Euphytica,* 208, 285-297.

Bochořáková, H., Paulová, H., Slanina, J., Musil, P. & Táborská, E. 2003. Main flavonoids in the root of Scutellaria baicalensis cultivated in Europe and their comparative antiradical properties. *Phytotherapy Research,* 17, 640-644.

Bonhomme, V., Laurain-Mattar, D., Lacoux, J., Fliniaux, M.-A. & Jacquin-Dubreuil, A. 2000. Tropane alkaloid production by hairy roots of Atropa belladonna obtained after transformation with Agrobacteriumrhizogenes 15834 and Agrobacterium tumefaciens containing rolA, B, C genes only. *Journal of biotechnology,* 81, 151-158.

Bourgaud, F., Gravot, A., Milesi, S. & Gontier, E. 2001. Production of plant secondary metabolites: a historical perspective. *Plant Science,* 161, 839-851.

Brock, C., Whitehouse, J., Tewfik, I. & Towell, T. 2010. American skullcap (Scutellaria lateriflora): an ancient remedy for today's anxiety? *British Journal of Wellbeing,* 1, 25-30.

Brock, C., Whitehouse, J., Tewfik, I. & Towell, T. 2012. The use of Scutellaria lateriflora: a pilot survey amongst herbal medicine practitioners. *Journal of Herbal Medicine,* 2, 34-41.

Canter, P. H., Thomas, H. & Ernst, E. 2005. Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology. *TRENDS in Biotechnology,* 23, 180-185.

Carlotto, J., De Souza, L. M., Baggio, C. H., Werner, M. F. D. P., Maria-Ferreira, D., Sassaki, G. L., Iacomini, M. & Cipriani, T. R. 2016. Polysaccharides from Arctium lappa L.: Chemical structure and biological activity. *International journal of biological macromolecules,* 91, 954-960.

Chan, F. L., Choi, H., Chen, Z., Chan, P. S. & Huang, Y. 2000. Induction of apoptosis in prostate cancer cell lines by a flavonoid, baicalin. *Cancer Letters,* 160, 219-228.

Chan, Y.-S., Cheng, L.-N., Wu, J.-H., Chan, E., Kwan, Y.-W., Lee, S. M.-Y., Leung, G. P.-H., Yu, P. H.-F. & Chan, S.-W. 2011. A review of the pharmacological effects of Arctium lappa (burdock). *Inflammopharmacology,* 19, 245-254.

Chaudhary, S. A., Gadhvi, K. V. & Chaudhary, A. B. 2010. Comprehensive review on world herb trade and most utilized medicinal plant.

Chaves, T. P., Santana, C. P., Véras, G., Brandão, D. O., Felismino, D. C., Medeiros, A. C. D. & Trovão, D. M. D. B. 2013. Seasonal variation in the production of secondary metabolites and antimicrobial activity of two plant species used in Brazilian traditional medicine. *African Journal of Biotechnology,* 12.

Chen, S.-L., Yu, H., Luo, H.-M., Wu, Q., Li, C.-F. & Steinmetz, A. 2016a. Conservation and sustainable use of medicinal plants: problems, progress, and prospects. *Chinese medicine,* 11, 1-10.

Chen, S.-L., Yu, H., Luo, H.-M., Wu, Q., Li, C.-F. & Steinmetz, A. 2016b. Conservation and sustainable use of medicinal plants: problems, progress, and prospects. *Chinese medicine,* 11, 37.

Cole, I. B., Cao, J., Alan, A. R., Saxena, P. K. & Murch, S. J. 2008. Comparisons of Scutellaria baicalensis, Scutellaria lateriflora and Scutellaria racemosa: genome size, antioxidant potential and phytochemistry. *Planta medica,* 74, 474.

Committee, U. I. B. 2013. *The Principle of Respect for Human Vulnerability and Personal Integrity: Report of the International Bioethics Committee of UNESCO (IBC)*, UNESCO.

Corrêa, R. C., Peralta, R. M., Haminiuk, C. W., Maciel, G. M., Bracht, A. & Ferreira, I. C. 2018. New phytochemicals as potential human anti-aging compounds: Reality, promise, and challenges. *Critical reviews in food science and nutrition,* 58, 942-957.

Da Silva, L. M., Allemand, A., Mendes, D. a. G., Dos Santos, A. C., André, E., De Souza, L. M., Cipriani, T. R., Dartora, N., Marques, M. C. A. & Baggio, C. H. 2013. Ethanolic extract of roots from Arctium lappa L. accelerates the healing of acetic acid-induced gastric ulcer in rats: Involvement of the antioxidant system. *Food and chemical toxicology,* 51, 179-187.

Davis, A. S., Hill, J. D., Chase, C. A., Johanns, A. M. & Liebman, M. 2012. Increasing cropping system diversity balances productivity, profitability and environmental health.

Ding, C.-H., Wang, Q.-B., Guo, S. & Wang, Z.-Y. 2018. The improvement of bioactive secondary metabolites accumulation in Rumex gmelini Turcz through co-culture with endophytic fungi. *brazilian journal of microbiology,* 49, 362-369.

Dodov, M. G. & Kulevanova, S. 2009. A review of phytotherapy of Acne vulgaris. *Maced. pharm. Bull,* 55, 3-22.

Dold, A. P. & Cocks, M. L. 2002. The trade in medicinal plants in the Eastern Cape Province, South Africa. *South African Journal of Science,* 98, 589-597.

Don, R. a. S. G. & Yap, M. K. K. 2019. Arctium lappa L. root extract induces cell death via mitochondrial-mediated caspase-dependent apoptosis in Jurkat human leukemic T cells. *Biomedicine & Pharmacotherapy,* 110, 918-929.

Douglas, M., Burgmans, J., Burton, L. & Smallfield, B. The production of Burdock (Arctium lappa L.) root in New Zealand-a preliminary study of a new vegetable. Proceedings Agronomy Society of NZ, 1992. 67.

Duh, P. D. 1998. Antioxidant activity of burdock (Arctium lappa Linne): its scavenging effect on free-radical and active oxygen. *Journal of the American Oil Chemists' Society,* 75, 455-461.

Dumroese, K. R., Landis, T. D., Pinto, J. R., Haase, D. L., Wilkinson, K. W. & Davis, A. S. 2016. Meeting forest restoration challenges: using the target plant concept. *Reforesta,* 1, 37-52.

Egigu, M. C., Ibrahim, M. A., Riikonen, J., Yahya, A., Holopainen, T., Julkunen-Tiitto, R. & Holopainen, J. K. 2014. Effects of rising temperature on secondary compounds of yeheb (Cordeauxia edulis Hemsley). *American Journal of Plant Sciences,* 2014.

Eich, E., Pertz, H., Kaloga, M., Schulz, J., Fesen, M. R., Mazumder, A. & Pommier, Y. 1996. (−)-Arctigenin as a lead structure for inhibitors of human immunodeficiency virus type-1 integrase. *Journal of medicinal chemistry,* 39, 86-95.

Ekor, M. 2014. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in pharmacology,* 4, 177.

Eraso, I., Downing, K. & Stutte, G. Feasibility of controlled environment production of Scutellaria species. International Symposium on Medicinal and Nutraceutical Plants 756, 2007. 213-220.

Felhaber, T. & Gericke, N. 1996. Tramed final narrative report: 1 June 1994–30 April 1996. *Traditional Medicines Programme at the University of Cape Town*.

Ferracane, R., Graziani, G., Gallo, M., Fogliano, V. & Ritieni, A. 2010. Metabolic profile of the bioactive compounds of burdock (Arctium lappa) seeds, roots and leaves. *Journal of pharmaceutical and biomedical analysis,* 51, 399-404.

Fisk, W. A., Agbai, O., Lev-Tov, H. A. & Sivamani, R. K. 2014. The use of botanically derived agents for hyperpigmentation: a systematic review. *Journal of the American Academy of Dermatology,* 70, 352-365.

Gafner, S. 2015. Skullcap adulteration laboratory guidance document. *Austin, TX: ABC-AHP-NCNPR Botanical Adulterants Prevention Program*, 1-12.

Ganter, G., Disch, R., Borelli, S. & Simon, D. 1997. Contact dermatitis and stomatitis due to amine fluoride. *Contact dermatitis,* 37, 248-248.

Gasiorowski, K., Lamer-Zarawska, E., Leszek, J., Parvathaneni, K., Bhushan Yendluri, B., Blach-Olszewska, Z. & Aliev, G. 2011. Flavones from root of Scutellaria baicalensis Georgi: drugs of the future in neurodegeneration? *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders),* 10, 184-191.

Ghimire, S. K., Awasthi, B., Rana, S., Rana, H. K., Bhattarai, R. & Pyakurel, D. 2016. Export of medicinal and aromatic plant materials from Nepal. *Botanica Orientalis: Journal of Plant Science,* 10, 24-32.

Golbaz, F., Zarei, S., Garakani, F. & Mojab, F. 2018. The essential oil composition of Arctium lappa root and leaf. *Iranian Journal of Pharmaceutical Sciences,* 14, 1-6.

Govindaraghavan, S. & Sucher, N. J. 2015. Quality assessment of medicinal herbs and their extracts: Criteria and prerequisites for consistent safety and efficacy of herbal medicines. *Epilepsy & Behavior,* 52, 363-371.

Greenfield, J. & Davis, J. 2004. Medicinal Herb Production Guides. *North Carolina Consortium on Natural Medicines and Public Health. North Carolina State University, Raleigh, NC*.

Guna, G. 2019. Therapeutic value of Arctium lappa Linn-a review

Asian J Pharm Clin Res, 12, 53-59.

Habibipour, R. & Rajabi, M. 2015. Antibacterial effects of Arctium lappa and Artemesia absinthium extracts in laboratory conditions. *Journal of herbmed pharmacology,* 4.

Hadacek, F. 2002. Secondary metabolites as plant traits: current assessment and future perspectives. *Critical Reviews in Plant Sciences,* 21, 273-322.

Haghighi, M. & Mozafariyan, M. 2011. The introduction of extinct endemic vegetables of Iran. *Journal of Medicinal Plants Research,* 5, 7085-7107.

Hamilton, A. C. 2004. Medicinal plants, conservation and livelihoods. *Biodiversity & Conservation,* 13, 1477-1517.

Hartmann, H. T. & Kester, D. E. 2002. *Hartmann and Kester's plant propagation: Principles and practices*.

He, W., Hou, S. & Wang, C. 2006. Callus induction and high-frequency plant regeneration from hypocotyl and cotyledon explants of Arctium lappa L. *In Vitro Cellular & Developmental Biology-Plant,* 42, 411-414.

He, Y., Fan, Q., Cai, T., Huang, W., Xie, X., Wen, Y. & Shi, Z. 2018. Molecular mechanisms of the action of Arctigenin in cancer. *Biomedicine & Pharmacotherapy,* 108, 403-407.

Herbert, D. 2006. Commercializing medicinal plants: a Southern African guide. *Sun Press. South Africa. Herb. Med. Pharmacol,* 1, 1-2.

Heywood, V. & Skoula, M. 1997. International council for medicinal and aromatic plants (ICMAP). *Identification of Wild Food and Non-Food Plants of the Mediterranean Region*, 11-12.

Hollander, J. E., Go, S., Lowery, D. W., Wolfson, A. B., Pollack, C. V., Herbert, M., Mower, W. R. & Hoffman, J. R. 2003. Interrater reliability of criteria used in assessing blunt head injury patients for intracranial injuries. *Academic emergency medicine,* 10, 830-835.

Horng, C. T., Wu, H. C., Chiang, N. N., Lee, C. F., Huang, Y. S., Wang, H. Y., Yang, J. S. & Chen, F. A. 2017. Inhibitory effect of burdock leaves on elastase and tyrosinase activity. *Experimental and Therapeutic Medicine,* 14, 3247-3252.

Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A. & Armand, R. 2015. The application of medicinal plants in traditional and modern medicine: a review of Thymus vulgaris. *International Journal of Clinical Medicine,* 6, 635.

Huang, H. 2011. Plant diversity and conservation in China: planning a strategic bioresource for a sustainable future. *Botanical Journal of the Linnean Society,* 166, 282-300.

Irvin, L., Jackson, C., Hill, A. L., Bajaj, R., Mahmoudi, C., Vaidya, B. N. & Joshee, N. 2019. Skullcaps (Scutellaria spp.): Ethnobotany and Current Research. *Medicinal Plants*, 141-168.

Islam, M. N., Downey, F. & Ng, C. K. 2011. Comparative analysis of bioactive phytochemicals from Scutellaria baicalensis, Scutellaria lateriflora, Scutellaria racemosa, Scutellaria tomentosa and Scutellaria wrightii by LC-DAD-MS. *Metabolomics,* 7, 446-453.

Iwakami, S., Wu, J.-B., Ebizuka, Y. & Sankawa, U. 1992. Platelet activating factor (PAF) antagonists contained in medicinal plants: lignans and sesquiterpenes. *Chemical and pharmaceutical bulletin,* 40, 1196-1198.

Jaiswal, R. & Kuhnert, N. 2011. Identification and characterization of five new classes of chlorogenic acids in burdock (Arctium lappa L.) roots by liquid chromatography/tandem mass spectrometry. *Food & Function,* 2, 63-71.

Jianfeng, C., Pengying, Z., Chengwei, X., Taotao, H., Yungui, B. & Kaoshan, C. 2012. Effect of aqueous extract of Arctium lappa L.(burdock) roots on the sexual behavior of male rats. *BMC complementary and alternative medicine,* 12, 8.

Jiang, Y.-Y., Yu, J., Li, Y.-B., Wang, L., Hu, L., Zhang, L. & Zhou, Y.-H. 2019. Extraction and antioxidant activities of polysaccharides from roots of Arctium lappa L. *International journal of biological macromolecules,* 123, 531-538.

Joffe, P. 2001. Creative gardening with indigenous plants. *Pretoria: Briza*.

Johnson, D., Kershaw, L., Mackinnon, A. & Pojar, J. 1995. Plants of the western boreal forest and aspen parkland. *Front cover*, 44.

Joshee, N., Patrick, T. S., Mentreddy, R. S. & Yadav, A. K. 2002. Skullcap: Potential medicinal crop. *Trends in new crops and new uses. ASHS Press, Alexandria*, 580-586.

Joshee, N., Tascan, A., Medina-Bolivar, F., Parajuli, P., Rimando, A., Shannon, D. & Adelberg, J. 2013. Scutellaria: biotechnology, phytochemistry and its potential as a commercial medicinal crop. *Biotechnology for medicinal plants.* Springer.

Jung, Y., Ha, M., Lee, J., Ahn, Y. G., Kwak, J. H., Ryu, D. H. & Hwang, G.-S. 2015. Metabolite profiling of the response of burdock roots to copper stress. *Journal of agricultural and food chemistry,* 63, 1309-1317.

Kamble, S., Patil, S., Sawant, P. S., Sawant, S., Pawar, S. & Singh, E. 2010. Studies on plants used in traditional medicine by Bhilla tribe of Maharashtra.

Kandari, L., Phondani, P., Payal, K., Rao, K. & Maikhuri, R. 2012. Ethnobotanical study towards conservation of medicinal and aromatic plants in upper catchments of Dhauli Ganga in the central Himalaya. *Journal of Mountain Science,* 9, 286-296.

Kasilo, O. & Trapsida, J. 2011. Decade of African traditional medicine, 2001–2010. *Afr. Health Mon.(Special Issue),* 14, 25-31.

Kasso, M. & Balakrishnan, M. 2013. Ex situ conservation of biodiversity with particular emphasis to Ethiopia. *International Scholarly Research Notices,* 2013.

Kavita, D., Adhikari, R. S., Pande, V., Kumar, M. & Pratibha, R. 2015. Vegetative propagation of an endangered medicinal plant of Himalayan region, Paris polyphylla Smith. *International Journal of Current Microbiology and Applied Sciences,* 4, 660-665.

Kawka, B., Kwiecień, I. & Ekiert, H. 2017. Influence of culture medium composition and light conditions on the accumulation of bioactive compounds in shoot cultures of Scutellaria lateriflora L.(American Skullcap) grown in vitro. *Applied biochemistry and biotechnology,* 183, 1414-1425.

Keyhanfar, M., Nazeri, S. & Bayat, M. 2012. Evaluation of antibacterial activities of some medicinal plants, traditionally used in Iran. *Iranian Journal of Pharmaceutical Sciences,* 8, 353-358.

Khan, H. 2015. Brilliant future of phytomedicines in the light of latest technological developments. *J. Phytopharmacol,* 4, 58-60.
Kigel, J. 1995. *Seed development and germination*, CRC press.
Kim, H.-J., Fonseca, J. M., Choi, J.-H. & Kubota, C. 2007. Effect of methyl jasmonate on phenolic compounds and carotenoids of romaine lettuce (Lactuca sativa L.). *Journal of agricultural and food chemistry,* 55, 10366-10372.
Kim, H. H., Park, J. S., Park, S. U. & Park, N. I. 2017. Production of baicalin, baicalein and wogonin in hairy root culture of American skullcap (Scutellaria lateriflora) by auxin treatment. *Biosciences Biotechnology Research Asia,* 14, 673-677.
Kim, H. K. & Verpoorte, R. 2010. Sample preparation for plant metabolomics. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques,* 21, 4-13.
Kim, J. K., Kim, Y. S., Kim, Y., Uddin, M. R., Kim, Y. B., Kim, H. H., Park, S. Y., Lee, M. Y., Chung, S. O. & Park, S. U. 2014. Comparative analysis of flavonoids and polar metabolites from hairy roots of Scutellaria baicalensis and Scutellaria lateriflora. *World Journal of Microbiology and Biotechnology,* 30, 887-892.
Kim, Y.-K., Koppula, S., Shim, D.-W., In, E.-J., Kwak, S.-B., Kim, M.-K., Yu, S.-H., Lee, K.-H. & Kang, T.-B. 2018. Inhibitory effect and mechanism of arctium lappa extract on nlrp3 inflammasome activation. *Evidence-Based Complementary and Alternative Medicine,* 2018.
Kimura, T., Iida, K. & Takei, Y. 1984. Mechanisms of adverse effect of air-oxidized soy bean oil-feeding in rats. *Journal of nutritional science and vitaminology,* 30, 125-133.
Knott, A., Reuschlein, K., Mielke, H., Wensorra, U., Mummert, C., Koop, U., Kausch, M., Kolbe, L., Peters, N. & Stäb, F. 2008. Natural Arctium lappa fruit extract improves the clinical signs of aging skin. *Journal of cosmetic dermatology,* 7, 281-289.
Kosakowska, O. 2017. Intrapopulation variability of flavonoid content in roots of Baikal skullcap (Scutellaria baicalensis Georgi). *Herba Polonica,* 63.
Lambert, J., Srivastava, J. & Vietmeyer, N. 1997. *Medicinal plants: rescuing a global heritage*, World Bank Publications.
Larsen, H. O. & Olsen, C. S. 2006. Unsustainable collection and unfair trade? Uncovering and assessing assumptions regarding Central Himalayan medicinal plant conservation. *Plant Conservation and Biodiversity*, 105-123.
Lattanzio, V., Cardinali, A., Ruta, C., Fortunato, I. M., Lattanzio, V. M., Linsalata, V. & Cicco, N. 2009. Relationship of secondary metabolism to growth in oregano (Origanum vulgare L.) shoot cultures under nutritional stress. *Environmental and Experimental Botany,* 65, 54-62.
Lewis, W. H. & Elvin-Lewis, M. P. 1995. Medicinal plants as sources of new therapeutics. *Annals of the Missouri Botanical Garden*, 16-24.
Lewu, F., Adebola, P. & Afolayan, A. 2007a. Commercial harvesting of Pelargonium sidoides in the Eastern Cape, South Africa: striking a balance between resource conservation and rural livelihoods. *Journal of arid environments,* 70, 380-388.
Lewu, F., Grierson, D. & Afolayan, A. 2010. Influences of seed source, pre-chilling, light and temperature on the germination of South African Pelargonium sidoides. *Nong Ye Ke Xue Yu Ji Shu,* 4, 18.
Lewu, F. B., Grierson, D. & Afolayan, A. 2007b. Morphological diversity among accessions of Pelargonium sidoides DC. in the Eastern Cape, South Africa. *Genetic Resources and Crop Evolution,* 54, 1-6.
Li-Weber, M. 2009. New therapeutic aspects of flavones: the anticancer properties of Scutellaria and its main active constituents Wogonin, Baicalein and Baicalin. *Cancer treatment reviews,* 35, 57-68.
Li, K., Zhu, L., Li, H., Zhu, Y., Pan, C., Gao, X. & Liu, W. 2019. Structural characterization and rheological properties of a pectin with anti-constipation activity from the roots of Arctium lappa L. *Carbohydrate polymers,* 215, 119-129.

Lim, T. K. 2012. *Edible medicinal and non-medicinal plants*, Springer.
Lin, L. Z., Harnly, J. M. & Upton, R. 2009. Comparison of the phenolic component profiles of skullcap (Scutellaria lateriflora) and germander (Teucrium canadense and T. chamaedrys), a potentially hepatotoxic adulterant. *Phytochemical Analysis,* 20, 298-306.
Lin, X.-Z., Liu, C.-Y. & Chen, K.-S. 2004. Extraction and content comparison of chlorogenic acid in Arctium lappa L. leaves collected from different terrain and its restraining bacteria test. *Natural Product Research and Development,* 16, 328-330.
Liu, W., Liu, J., Yin, D. & Zhao, X. 2015a. Influence of ecological factors on the production of active substances in the anti-cancer plant Sinopodophyllum hexandrum (Royle) TS Ying. *PloS one,* 10, e0122981.
Liu, W., Wang, J., Zhang, Z., Xu, J., Xie, Z., Slavin, M. & Gao, X. 2014. In vitro and in vivo antioxidant activity of a fructan from the roots of Arctium lappa L. *International journal of biological macromolecules,* 65, 446-453.
Liu, Y., Wang, G., Yang, M., Chen, H., Yang, S. & Sun, C. 2015b. Arctigenin reduces blood pressure by modulation of nitric oxide synthase and NADPH oxidase expression in spontaneously hypertensive rats. *Biochemical and biophysical research communications,* 468, 837-842.
Lohani, M., Ahuja, M., Buabeid, M. A., Schwartz, D., Shannon, D., Suppiramaniam, V., Kemppainen, B. & Dhanasekaran, M. 2013. Anti-oxidative and DNA protecting effects of flavonoids-rich Scutellaria lateriflora. *Natural Product Communications,* 8, 1934578X1300801019.
Lou, Z., Li, C., Kou, X., Yu, F., Wang, H., Smith, G. M. & Zhu, S. 2016. Antibacterial, antibiofilm effect of Burdock (Arctium lappa L.) leaf fraction and its efficiency in meat preservation. *Journal of food protection,* 79, 1404-1409.
Mabey, R., Mcintyre, A. & Mcintyre, M. 1988. *The New Age Herbalist: How to use herbs for healing, nutrition, body care, and relaxation*, Simon and Schuster.
Maghsoumi-Norouzabad, L., Alipoor, B., Abed, R., Eftekhar Sadat, B., Mesgari-Abbasi, M. & Asghari Jafarabadi, M. 2016. Effects of Arctium lappa L.(Burdock) root tea on inflammatory status and oxidative stress in patients with knee osteoarthritis. *International journal of rheumatic diseases,* 19, 255-261.
Mahomoodally, M. F. 2013. Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. *Evidence-Based Complementary and Alternative Medicine,* 2013.
Mander, M. 1998. Marketing of indigenous medicinal plants in South Africa: a case study in KwaZulu-Natal.
Mander, M., Ntuli, L., Diederichs, N. & Mavundla, K. 2007. Economics of the traditional medicine trade in South Africa care delivery. *South African health review,* 2007, 189-196.
Marsh, Z., Yang, T., Nopo-Olazabal, L., Wu, S., Ingle, T., Joshee, N. & Medina-Bolivar, F. 2014. Effect of light, methyl jasmonate and cyclodextrin on production of phenolic compounds in hairy root cultures of Scutellaria lateriflora. *Phytochemistry,* 107, 50-60.
Máthé, Á. & Máthé, I. Quality assurance of cultivated and gathered medicinal plants. XXVII International Horticultural Congress-IHC2006: International Symposium on Plants as Food and Medicine: The Utilization 765, 2006. 67-76.
Mcmaster, C. 2007. African potato, a disappearing traditional remedy: wildflower conservation. *Farmer's weekly, national library of South Africa,* 193, 26.
Megersa, H. 2017. Propagation methods of selected horticultural crops by specialized organs: review. *J. Horticulture,* 4, 2376-0354.1000198.
Miraj, S. 2016. Pharmacological effect of Actiumlappa: A review study. *Der Pharmacia Lettre,* 8, 102-106.
Montenegro De Wit, M. 2017. Stealing into the wild: conservation science, plant breeding and the makings of new seed enclosures. *The Journal of Peasant Studies,* 44, 169-212.
Moolla, A. & Viljoen, A. M. 2008. 'Buchu'–Agathosma betulina and Agathosma crenulata (Rutaceae): A review. *Journal of ethnopharmacology,* 119, 413-419.

Morita, T., Ebihara, K. & Kiriyama, S. 1993. Dietary fiber and fat-derivatives prevent mineral oil toxicity in rats by the same mechanism. *The Journal of nutrition,* 123, 1575-1585.
Naik, P. M. & Al-Khayri, J. M. 2016. Abiotic and biotic elicitors–role in secondary metabolites production through in vitro culture of medicinal plants. *Abiotic and Biotic Stress in Plants, Recent Advances Future Perspectives. IntechOpen*, 247-277.
Ncube, B., Finnie, J. & Van Staden, J. 2012. Quality from the field: The impact of environmental factors as quality determinants in medicinal plants. *South African Journal of Botany,* 82, 11-20.
Ngarivhume, T., Van't Klooster, C. I., De Jong, J. T. & Van Der Westhuizen, J. H. 2015. Medicinal plants used by traditional healers for the treatment of malaria in the Chipinge district in Zimbabwe. *Journal of ethnopharmacology,* 159, 224-237.
Oliver, S. J. 2013. The role of traditional medicine practice in primary health care within Aboriginal Australia: a review of the literature. *Journal of ethnobiology and ethnomedicine,* 9, 1-8.
Panuccio, M., Jacobsen, S., Akhtar, S. & Muscolo, A. 2014. Effect of saline water on seed germination and early seedling growth of the halophyte quinoa. *AoB plants,* 6.
Paton, A. 1990. A global taxonomic investigation of Scutellaria (Labiatae). *Kew Bulletin*, 399-450.
Pavarini, D. P., Pavarini, S. P., Niehues, M. & Lopes, N. P. 2012. Exogenous influences on plant secondary metabolite levels. *Animal Feed Science and Technology,* 176, 5-16.
Payyappallimana, U. 2010. Role of traditional medicine in primary health care: an overview of perspectives and challenging.
Pereira, J. V., Bergamo, D. C. B., Pereira, J. O., França, S. D. C., Pietro, R. C. L. R. & Silva-Sousa, Y. T. C. 2005. Antimicrobial activity of Arctium lappa constituents against microorganisms commonly found in endodontic infections. *Brazilian dental journal,* 16, 192-196.
Phondani, P. C., Bhatt, I. D., Negi, V. S., Kothyari, B. P., Bhatt, A. & Maikhuri, R. K. 2016. Promoting medicinal plants cultivation as a tool for biodiversity conservation and livelihood enhancement in Indian Himalaya. *Journal of Asia-Pacific Biodiversity,* 9, 39-46.
Phondani, P. C., Maikhuri, R. K. & Saxena, K. G. 2014. The efficacy of herbal system of medicine in the context of allopathic system in Indian Central Himalaya. *Journal of Herbal Medicine,* 4, 147-158.
Pilcher, J., Delzell, W. & Burman, G. 1916. The action of various female remedies on the excised uterus of the guinea-pig. *Journal of the American Medical Association,* 67, 490-492.
Porter, M. E. & Kramer, M. R. 2006. The link between competitive advantage and corporate social responsibility. *Harvard business review,* 84, 78-92.
Predes, F. S., Ruiz, A. L., Carvalho, J. E., Foglio, M. A. & Dolder, H. 2011. Antioxidative and in vitro antiproliferative activity of Arctium lappa root extracts. *BMC complementary and alternative medicine,* 11, 25.
Raimondo, D., Staden, L. V., Foden, W., Victor, J., Helme, N., Turner, R., Kamundi, D. & Manyama, P. 2009. *Red list of South African plants 2009*, South African National Biodiversity Institute.
Rajasekharan, S. K., Ramesh, S., Bakkiyaraj, D., Elangomathavan, R. & Kamalanathan, C. 2015. Burdock root extracts limit quorum-sensing-controlled phenotypes and biofilm architecture in major urinary tract pathogens. *Urolithiasis,* 43, 29-40.
Rashid, A. M., Tunon, H., Khan, N. A. & Mukul, S. A. 2014. Commercial cultivation by farmers of medicinal plants in northern Bangladesh. *European Journal of Environmental Sciences,* 4.
Ravindran, P., Nirmal Babu, K. & Shiva, K. 2005. Botany and crop improvement of ginger. *Ginger: the genus Zingiber,* 41, 15-85.
Raynor, D. K., Dickinson, R., Knapp, P., Long, A. F. & Nicolson, D. J. 2011. Buyer beware? Does the information provided with herbal products available over the counter enable safe use? *BMC medicine,* 9, 1-9.
Reinten, E. & Coetzee, J. 2002. Commercialization of South African indigenous crops: aspects of research and cultivation of products. *Trends in new crops and new uses. ASHS Press, Alexandria, VA*, 76-80.

Ren, X., Liu, Y. & Jeong, B. R. 2020. Callus induction and browning suppression in tree peony Paeonia ostii 'Fengdan'. *Horticulture, Environment, and Biotechnology*, 1-10.

Rhoads, P. M., Tong, T. G., Banner, W. & Anderson, R. 1984. Anticholinergic poisonings associated with commercial burdock root tea. *Journal of Toxicology: Clinical Toxicology,* 22, 581-584.

Romanelli, C., Cooper, D., Campbell-Lendrum, D., Maiero, M., Karesh, W. B., Hunter, D. & Golden, C. Connecting global priorities: biodiversity and human health: a state of knowledge review. 2015. World Health Organistion/Secretariat of the UN Convention on Biological ….

Ross, I. A. 2007. *Medicinal plants of the world, volume 3: Chemical constituents, traditional and modern medicinal uses*, Springer Science & Business Media.

Salama, M. & Salama, S. G. 2016. Arctium Lappa L.(Asteraceae); a New Invasive Highly Specific Medicinal Plant Growing in Egypt. *Pyrex Journal of Plant and Agricultural Research*.

Schippmann, U., Leaman, D. J. & Cunningham, A. 2002. Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues. *Biodiversity and the ecosystem approach in agriculture, forestry and fisheries*.

Schröder, H. C., Merz, H., Steffen, R., Müller, W. E., Sarin, P. S., Trumm, S., Schulz, J. & Eich, E. 1990. Differential in vitro anti-HIV activity of natural lignans. *Zeitschrift für Naturforschung C,* 45, 1215-1221.

Sellami, H. K., Napolitano, A., Masullo, M., Smiti, S., Piacente, S. & Pizza, C. 2013. Influence of growing conditions on metabolite profile of Ammi visnaga umbels with special reference to bioactive furanochromones and pyranocoumarins. *Phytochemistry,* 95, 197-206.

Shaban, M. 2013. Effect of water and temperature on seed germination and emergence as a seed hydrothermal time model. *International Journal of Advanced Biological and Biomedical Research,* 1, 1686-1691.

Shang, X., He, X., He, X., Li, M., Zhang, R., Fan, P., Zhang, Q. & Jia, Z. 2010. The genus Scutellaria an ethnopharmacological and phytochemical review. *Journal of ethnopharmacology,* 128, 279-313.

Sharma, R. & Goyal, A. 2014. Agro pesticides and andrology. *International Journal of Pharmacy and Pharmaceutical Sciences,* 6, 12-19.

Sharrock, S., Hoft, R. & Dias, B. F. D. S. 2018. An overview of recent progress in the implementation of the Global Strategy for Plant Conservation-a global perspective. *Rodriguésia,* 69, 1489-1511.

Shepherd, A. & Tam, P. Improving the safety of marketed horticultural produce in Asia with particular reference to Vietnam. II International Symposium on Improving the Performance of Supply Chains in the Transitional Economies 794, 2007. 301-308.

Shi, H., Dong, G., Yan, F., Zhang, H., Li, C., Ma, Q., Zhang, J., Ning, Z., Li, Z. & Dai, J. 2018. Arctigenin ameliorates inflammation by regulating accumulation and functional activity of MDSCs in endotoxin shock. *Inflammation,* 41, 2090-2100.

Shiwakoti, S. 2012. *Management Effects on Yield and Flavonoid Content in American Skullcap (Scutellaria lateriflora).*

Shiwakoti, S., Shannon, D. A., Wood, C. W., Joshee, N., Rimando, A., Lawrence, K. S. & Kemppainen, B. 2016. Nitrogen, phosphorus, and potassium effects on biomass yield and flavonoid content of American skullcap (Scutellaria lateriflora). *Journal of Plant Nutrition,* 39, 1240-1249.

Shiwakoti, S., Shannon, D. A., Wood, C. W., Lawrence, K. S., Kemppainen, B., Joshee, N. & Rimando, A. M. 2013. Harvesting number and timing effects on shoot yield and flavonoid content in organically grown American skullcap (Scutellaria lateriflora). *Journal of herbs, spices & medicinal plants,* 19, 248-261.

Similien, A. 2009. *Effect of Shade, Irrigation and Nutrients on Dry Matter Yield and Flavonoid Content of American Skullcap.*

Similien, A., Shannon, D. A., Wesley Wood, C., Rimando, A. M., Kemppainen, B. W., Van Santen, E. & Joshee, N. 2016. Shade, Irrigation, and Nutrients Affect Flavonoid Concentration and Yield in American Skullcap. *Crop Science,* 56, 1213-1224.

Similien, A., Shannon, D. A., Wood, C. W., Van Santen, E., Joshee, N. & Foshee, W. G. 2012. Partial Shade, Irrigation, and Added Nutrients Maximize Dry Matter Yield of American Skullcap (Scutellaria lateriflora L.). *HortScience,* 47, 1705-1709.

Singh, R. 2015. Medicinal plants: A review. *Journal of Plant Sciences,* 3, 50-55.

Sobiecki, J. 2014. The intersection of culture and science in South African traditional medicine. *Indo-Pacific Journal of Phenomenology,* 14.

Sohn, E.-H., Jang, S.-A., Joo, H., Park, S., Kang, S.-C., Lee, C.-H. & Kim, S.-Y. 2011. Anti-allergic and anti-inflammatory effects of butanol extract from Arctium Lappa L. *Clinical and Molecular Allergy,* 9, 1-11.

Soleimani, T., Keyhanfar, M., Piri, K. & Hasanloo, T. 2012. Hairy root induction in Burdock (Arctium lappa L.). *Journal of medicinal plants,* 11.

Soon, Vynka Mcveigh & Hoffmann, H. 2003. Developing new export vegetables with enphasis on burdock, diakon and globe artichoke. *Horticulture Australia.* 50 Carrington Street Sydney NSW 2000: Department of Agriculture Manjimup Hrticulticltural Research Institute South West Highway.

Street, R. & Prinsloo, G. 2013. Commercially important medicinal plants of South Africa: a review. *Journal of chemistry,* 2013.

Sudha, G. & Ravishankar, G. 2002. Involvement and interaction of various signaling compounds on the plant metabolic events during defense response, resistance to stress factors, formation of secondary metabolites and their molecular aspects. *Plant Cell, Tissue and Organ Culture,* 71, 181-212.

Sun, Q., Liu, K., Shen, X., Jin, W., Jiang, L., Sheikh, M. S., Hu, Y. & Huang, Y. 2014. Lappaol F, a novel anticancer agent isolated from plant Arctium Lappa L. *Molecular cancer therapeutics,* 13, 49-59.

Szczawinski, A. F. & Turner, N. J. 1978. *Edible garden weeds of Canada*, Ottawa, Canada: National Museum of Natural Sciences, National Museums of Canada.

Takeda, H. & Kiriyama, S. 1979. Correlation between the physical properties of dietary fibers and their protective activity against amaranth toxicity in rats. *The Journal of nutrition,* 109, 388-396.

Tamayo, C., Richardson, M., Diamond, S. & Skoda, I. 2000. The chemistry and biological activity of herbs used in Flor-Essence™ herbal tonic and Essiac. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives,* 14, 1-14.

Tang, W., Xu, X., Shen, G. & Chen, J. 2015. Effect of environmental factors on germination and emergence of aryloxyphenoxy propanoate herbicide-resistant and-susceptible Asia minor bluegrass (Polypogon fugax). *Weed Science,* 63, 669-675.

Tanga, M., Lewu, F., Oyedeji, A. & Oyedeji, O. 2018. Cultivation of medicinal plants in South Africa: A solution to quality assurance and consistent availability of medicinal plant materials for commercialization. *Academia Journal of Medicinal Plants,* 6, 168-177.

Tascan, A., Adelberg, J., Joshee, N., Yadav, A. & Tascan, M. 2007. Liquid culture system for Scutellaria species. *Acta Horticulturae,* 756, 163-170.

Tascan, A., Adelberg, J., Tascan, M., Rimando, A., Joshee, N. & Yadav, A. K. 2010. Hyperhydricity and flavonoid content of Scutellaria species in vitro on polyester-supported liquid culture systems. *HortScience,* 45, 1723-1728.

Tezuka, Y., Yamamoto, K., Awale, S., Li, F., Yomoda, S. & Kadota, S. 2013. Anti-austeric activity of phenolic constituents of seeds of Arctium lappa. *Natural Product Communications,* 8, 1934578X1300800414.

Thakur, M., Bhattacharya, S., Khosla, P. K. & Puri, S. 2019. Improving production of plant secondary metabolites through biotic and abiotic elicitation. *Journal of Applied Research on Medicinal and Aromatic Plants,* 12, 1-12.

Theisen, L. L. & Muller, C. P. 2012. EPs® 7630 (Umckaloabo®), an extract from Pelargonium sidoides roots, exerts anti-influenza virus activity in vitro and in vivo. *Antiviral research,* 94, 147-156.

Tshivh, I., Mudau, F. N., Soundy, P. & Ngezimana, W. 2013. Effect of cultural practices and environmental conditions on yield and quality of herbal plants: Prospects leading to research on influence of nitrogen fertilization, planting density and eco-physiological parameters on yield and quality of field-grown bush tea (Athrixia phylicoides DC.). *Journal of Medicinal Plants Research,* 7, 2489-2493.

Tuan, T., Thien, N., Nguyen, H., Nguyen, D., Loan, L., Thai, T., Trang, N., Dung, N., Giap, D. & Du, T. 2018. Changes in shoot proliferation and chemical components of in vitro cultured Dendrobium officinale due to organic additives. *Journal of Applied Horticulture,* 20.

Uprety, Y., Asselin, H., Dhakal, A. & Julien, N. 2012. Traditional use of medicinal plants in the boreal forest of Canada: review and perspectives. *Journal of ethnobiology and ethnomedicine,* 8, 1-14.

Upton, R. & Dayu, R. 2012. Skullcap Scutellaria lateriflora L.: an American nervine. *Journal of Herbal Medicine,* 2, 76-96.

Van Wyk, A. & Prinsloo, G. 2018. Medicinal plant harvesting, sustainability and cultivation in South Africa. *Biological conservation,* 227, 335-342.

Van Wyk, B.-E. 2011. The potential of South African plants in the development of new medicinal products. *South African Journal of Botany,* 77, 812-829.

Vandelook, F. & Van Assche, J. A. 2008. Temperature requirements for seed germination and seedling development determine timing of seedling emergence of three monocotyledonous temperate forest spring geophytes. *Annals of Botany,* 102, 865-875.

Victor, J. E., Smith, G. F., Turland, N. J., Le Roux, M., Paton, A., Figueiredo, E., Crouch, N. R., Van Wyk, A. E., Filer, D. & Van Wyk, E. 2014. Creating an online world Flora by 2020: a perspective from South Africa. *Biodiversity and Conservation,* 23, 251-263.

Vines, G. 2004. *Herbal harvests with a future: towards sustainable sources for medicinal plants*, Plantlife International.

Vishwakarma, A., Vishwe, A., Sahu, P. & Chaurasiya, A. 2013. Magical remedies of Terminalia arjuna (ROXB.). *International Journal of Pharmaceutical Archive,* 2, 189-201.

Vlietinck, A., De Bruyne, T., Apers, S. & Pieters, L. 1998. Plant-derived leading compounds for chemotherapy of human immunodeficiency virus (HIV) infection. *Planta medica,* 64, 97-109.

Wang, L., Zhang, D., Wang, N., Li, S., Tan, H.-Y. & Feng, Y. 2019. Polyphenols of Chinese skullcap roots: from chemical profiles to anticancer effects. *RSC advances,* 9, 25518-25532.

Wang, Z.-L., Wang, S., Kuang, Y., Hu, Z.-M., Qiao, X. & Ye, M. 2018. A comprehensive review on phytochemistry, pharmacology, and flavonoid biosynthesis of Scutellaria baicalensis. *Pharmaceutical Biology,* 56, 465-484.

Who 2004. *WHO guidelines on safety monitoring of herbal medicines in pharmacovigilance systems*, World Health Organization.

Wiersum, K. F., Dold, A. P., Husselman, M. & Cocks, M. 2006. Cultivation of medicinal plants as a tool for biodiversity conservation and poverty alleviation in the Amatola region, South Africa. *Frontis*, 43-57.

Wilczańska-Barska, A., Królicka, A., Głód, D., Majdan, M., Kawiak, A. & Krauze-Baranowska, M. 2012. Enhanced accumulation of secondary metabolites in hairy root cultures of Scutellaria lateriflora following elicitation. *Biotechnology letters,* 34, 1757-1763.

Williams, V. 2004. Trade and socio-economic value of forest and woodland resources within the medicinal plant market in Johannesburg. *Indigenous Forests and Woodlands in South Africa: Policy, People and Practice. University of Natal Press, Pietermaritzburg*, 439-472.

Wills, R. B. H. & Stuart, D. L. 2004. *Generation of high quality Australian skullcap products*, Citeseer.

Wojcikowski, K., Stevenson, L., Leach, D., Wohlmuth, H. & Gobe, G. 2007. Antioxidant capacity of 55 medicinal herbs traditionally used to treat the urinary system: a comparison using a sequential three-solvent extraction process. *The Journal of Alternative and Complementary Medicine,* 13, 103-110.

Xu, Z., Wang, F., Tsang, S. Y., Ho, K. H., Zheng, H., Yuen, C. T., Chow, C. Y. & Xue, H. 2006. Anxiolytic-like effect of baicalin and its additivity with other anxiolytics. *Planta medica,* 72, 189-192.

Xu, Z., Wang, X., Zhou, M., Ma, L., Deng, Y., Zhang, H., Zhao, A., Zhang, Y. & Jia, W. 2008. The antidiabetic activity of total lignan from Fructus Arctii against alloxan-induced diabetes in mice and rats. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives,* 22, 97-101.

Yang, L., Wen, K.-S., Ruan, X., Zhao, Y.-X., Wei, F. & Wang, Q. 2018. Response of plant secondary metabolites to environmental factors. *Molecules,* 23, 762.

Yang, W.-S., Lee, S. R., Jeong, Y. J., Park, D. W., Cho, Y. M., Joo, H. M., Kim, I., Seu, Y.-B., Sohn, E.-H. & Kang, S. C. 2016. Antiallergic activity of ethanol extracts of Arctium lappa L. undried roots and its active compound, oleamide, in regulating FcεRI-mediated and MAPK signaling in RBL-2H3 cells. *Journal of agricultural and food chemistry,* 64, 3564-3573.

Yang, Y.-N., Huang, X.-Y., Feng, Z.-M., Jiang, J.-S. & Zhang, P.-C. 2015. New butyrolactone type lignans from Arctii Fructus and their anti-inflammatory activities. *Journal of agricultural and food chemistry,* 63, 7958-7966.

Yoo, J.-M., Park, K. I., Yang, J.-H., Cho, W.-K., Lee, B. & Ma, J. Y. 2019. Anti-allergic actions of F-PASA, a novel herbal cocktail, in IgE/antigen-mediated allergic responses in RBL-2H3 cells and passive cutaneous anaphylaxis in mice. *Phytomedicine,* 55, 229-237.

Yoo, J.-M., Yang, J. H., Yang, H. J., Cho, W.-K. & Ma, J. Y. 2016. Inhibitory effect of fermented Arctium lappa fruit extract on the IgE-mediated allergic response in RBL-2H3 cells. *International journal of molecular medicine,* 37, 501-508.

Zebarjadi, A., Kazem, S. & Kahrizi, D. 2018. Cell dedifferentiation and multiplication of Burdock (Arctium Lappa) as a medicinal plant. *Cellular and Molecular Biology,* 64, 92-96.

Zehra, A., Gul, B., Ansari, R., Alatar, A., Hegazy, A. & Khan, M. A. 2013. Interactive effect of salt, light and temperature on seed germination and recovery of a halophytic grass-Phragmites karka. *Pak. J. Bot,* 45, 725-736.

Zhang, X.-X., Li, C.-J. & Nan, Z.-B. 2011. Effects of salt and drought stress on alkaloid production in endophyte-infected drunken horse grass (Achnatherum inebrians). *Biochemical Systematics and Ecology,* 39, 471-476.

Zhang, Z., Lian, X.-Y., Li, S. & Stringer, J. L. 2009. Characterization of chemical ingredients and anticonvulsant activity of American skullcap (Scutellaria lateriflora). *Phytomedicine,* 16, 485-493.

CHAPTER 3
GROWTH, YIELD AND PHYTOCHEMICAL CONSTITUENTS OF *ARCTIUM LAPPA* L, IN RESPONSE TO PHOSPHOROUS AND POTASSIUM FERTILIZER APPLICATION

(This Chapter has been published in the proceeding of the 18th South Africa Int`l Conference on Agriculture, Chemical, Biological & Environmental Sciences. (ACBES-20) November.16-17, 2020 Johannesburg (SA). DOI: https://doi.org/10.17758/EARES10EAP1120122. Please refer to appendix 2).

3.1 Introduction

Burdock (*Arctium lappa* L.) is an indigenous medicinal herb of the Asian continent and native to Eurasia, which belongs to the family Asteraceae. It has many therapeutic values (Salama and Salama, 2016). Traditionally a mixture of the species with oil and honey is applied on the chest for the treatment of common cold (Badarau et al., 2019). The fruits are used as blood purity and for the treatment of respiratory diseases (Bannai, 2016). The leaves are used for the treatment of rheumatic pain, sunstroke, snake and scorpion bites (Erdemoglu et al., 2009, Neves et al., 2009, Mosaddegh et al., 2012). The roots are used in veterinary medicine for the treatment of mastitis while the infusion extract of the whole plant can be applied against endoparasite in poultry (Lans and Turner, 2011). The species is also consumed as a vegetable in the form of salad and stew because of its high nutritional composition (Chan et al., 2011).

The demand for this plant keeps increasing without assurance of consistent supply since most of its supply is mostly dependent on the wild causing pressure on its natural habitat. The cultivation of this species is given less attention even in the region of its origin not alone in South Africa. South Africa still depends mostly on the importation of the plant material for medicine, with little assurance of consistent supply to satisfy local demand. Also, the non-awareness of this plant as a potential vegetable to help address the issue of food security in our communities not alone its cultivation practices is unpredicted. Therefore, the cultivation of this species in South Africa is imperative. This study was therefore undertaken to investigate the influence of potassium and phosphorous fertilizer application on the growth, yield, and phytochemical constituents of this species.

3.2 Materials and methods

3.2.1 Study Area

The study was conducted at the Research and Teaching Farm, the Agric-Hub, Department of Agriculture Wellington Campus, Cape Peninsula University of Technology (CPUT). The area falls within the Northern part of Wellington at coordinate (S33º37’ E19º 37’), with a Mediterranean climate, and receives about 585 mm of winter rainfall per year. Rainfall is usually from February

to November, with the lowest rainfall in February (10 mm) and the highest in June (105 mm). The average daily temperature for wellington is from 16.5 ^{o}C in July to 28.8 ^{o}C in February.

3.2.2 Experimental layout and treatment application

Burdock seed (Takinogawa long cultivar) was obtained from "The Seed Collection Pty Ltd" company in Australia (Ferntree Gully, Victoria 3156 Australia with permit N0. P0084124). Seedlings were raised in the greenhouse using a plastic tray filled with potting soil. Regular watering was done in the morning and evening to keep the soil moist. At three weeks, 100% germination of the sown seeds was obtained. At six weeks the seedlings were taken outside for acclimatization for two weeks before transplanting to larger pots containing 10kg potting soil obtain from standard farm. The soil had a total nitrogen (0.353%) and phosphorous (418mg/kg) with a moderate amount of potassium (3425mg/kg) and pH of 7.1. The experiment was arranged in a complete randomized design (CRD) with five treatments (140, 210, 280, 350, and 420 kg/ha^{-1}) of phosphorous (Triple superphosphate 20) and potassium (Potassium Chloride 50%) at five levels (210, 315, 420, 520 and 630Kg/ha^{-1}), supplemented with Urea at160 Kg/ha^{-1} as a source of nitrogen. All treatments were replicated four times. Fertilizer treatments were split into two equal doses at seedling transplant and four weeks after transplanting. watering and weeding were conducted as required throughout the cultivation period.

3.2.3 Data collection

Data on plant height, number of leaves per plant, leaf length and leaf width were measured and recorded at two weeks intervals until 16 weeks when the first set of leaves starts to collapse. Plant height, leaf length, and broadest width of plant leaves were measured using a meter rule (in cm). The number of leaves per plant was numerically counted. At maturity, plants were harvested and washed using tap water. Data on yield was collected on plant biomass, root fresh weight, leaf fresh weight using a Lasec, Radwang wagi`s electronic weighing balance Model: WLC 1/A2/C/2, Made in Poland (EU). Root length and root diameter were measured using a thread and subsequently calibrated on a meter rule.

3.2.4 Phytochemical screening and proximate analysis of root and leaf of Burdock

Fresh leaves and roots samples at maturity were harvested from the plants across all the treatments and were used to prepare aqueous root and leaf extracts of Burdock for the different fertilizer treatments. The different extracts were screened for the presence of phenols, tannins, flavonoids, steroids, terpenoids, saponins, alkaloids and glycoside by the following reactions: Ferric chloride test for tannins, Foam test for saponins, NaOH with dilute acid for flavonoids, 5% ferric chloride solution for phenol, Dragendoff`s and Meyer`s reagent for alkaloids, Chloroform, and acetic acid anhydride with Con Sulphuric acid for steroids, glacial acetic acid with a drop of

$FeCl_3$ and Con Sulphuric acid for Glycosides, Chloroform and acetic acid anhydride with Con Sulphuric acid for terpenoids as fully described by (Dyayiya et al., 2016) in the laboratory of the Department of Chemical and Physical Science Walter Sisulu University of Science and Technology. Proximate analysis of the dry powdered root and leaf samples was carried out using Sulphuric acid and Sodium hydroxide for Crude fibre, Diethyl ether for Crude lipid and incineration at 550^{o}C for ash content using the method of (Aoac, 2005) at the Agric-Hub in the Postgraduate Analytical Laboratory of the Department of Agriculture, Wellington Campus, Cape Peninsula University of Technology.

3.2.5 Statistical analysis

Analysis of variance (ANOVA) at 95% confidence limit and comparison of means was carried out on growth, yield, and proximate analysis data of the plant samples using SAS software (SAS Institute 1999). Means separation was done using Fisher`s Least Significant Difference (LSD) and ranking was carried out using Duncan Multiple Range Test (DMRT).

3.3 Results and Discussion

3.3.1 Effects of fertilizer treatments on growth parameters of Burdock

Potassium fertilizer treatments had no significant effect ($p>0.05$) on growth parameters (Table 3.1). The application of potassium (K) above 520 kg/ha significantly ($p<0.05$) affected the number of leaves by 11.8% compared with the control. There is scanty information on the effect of potassium on Burdock. However, potassium is required to promote vegetative growth and development especially in many physiological processes of cell division and elongation (Marschner, 1995). This study indicated that Burdock requires relatively low potassium for optimum growth performance. This result is in contrast to the early study where the elevated application of potassium significantly ($p<0.05$) increased the yield of these growth parameters in different cultivated species (Hossain et al., 2009, Ibrahim and Abd El-Kader, 2015).

Phosphorous (P) fertilizer application on Burdock followed a similar trend as K treatment. The application of different P fertilizer rates did not reveal any significant ($p>0.05$) improvement on growth parameters measured (Table 3.1). Surprisingly, higher application of P at 420 kg/ha significantly ($p<0.05$) depressed plant height, leaf length, and leaf width by 9.5%, 9% and 11.3%, respectively compared with the control. This is in contrast to early the study of Nyoki and Patrick (2013) and that of Nkaa et al. (2014) where significant ($p<0.05$) improvement in growth parameters of cowpea varieties was recorded due to phosphorus application. Phosphorous is a critical element for plant growth and development especially in shoot and root tips where metabolism is high with rapid cell division (Ndakidemi and Dakora, 2007), which improves enzyme activation and

carbohydrate metabolism (Razaq et al., 2017). It is plausible to conclude that Burdock requires limited phosphorus application. Being a wild species, this plant might have adapted to the low utilization of mineral resources for optimum growth

Table 3.1 Influence of fertilizer treatments on morphological characteristics of Burdock. Values are means at (P-0.05).

Treatments (kg/ha)	Number of leaves	Plant height(cm)	Leaf length(cm)	Leaf width(cm)
Potassium				
$T_1(k_{210})$[control]	7.00[a]	26.94[a]	17.75[a]	15.25[a]
$T_2(k_{315})$	6.81[a]	25.75[a]	16.75[a]	15.19[a]
$T_3(k_{420})$	6.44[a]	28.00[a]	16.81[a]	14.63[a]
$T_4(k_{520})$	6.75[a]	26.81[a]	16.56[a]	14.75[a]
$T_5(k_{630})$	6.19[b]	26.94[a]	17.81[a]	15.44[a]
LSD	0.67	NS	NS	NS
Phosphorous				
$T_1(P_{140})$ [control]	6.88[ab]	33.06[a]	20.88[a]	18.25[a]
$T_2(P_{210})$	7.25[a]	34.75[a]	21.19[a]	18.13[a]
$T_3(P_{280})$	6.75[ab]	34.63[a]	21.75[a]	19.69[a]
$T_4(P_{350})$	6.75[ab]	32.81[a]	20.63[ab]	18.19[a]
$T_5(P_{420})$	6.50[b]	29.94[b]	19.00[b]	16.19[b]
LSD	0.63	2.43	1.78	1.58

Means in the same column with the same superscript are not significantly different (P>0.05). LSD= Least significant difference, K= Potassium fertilizer treatments and P=Phosphorous fertilizer treatment. Values are means of four replicates at (P<0.05).

3.3.2 Effects of fertilizer treatments on yield parameters of Burdock

Potassium fertilizer had no significant difference (p>0.05) in total biomass, fresh root weight, fresh leaf weight, and root diameter of Burdock in all the treatments compared with the control (Table 3.2). However, the application of potassium at 420Kg/ha had the highest response with a 15% on total biomass, 13.3% on fresh root weight, and a 32% on fresh leaf weight compared with the control. Thus, an indication of the optimum K requirement for these traits in this experiment. Root length was significant (p<0.05) due to K treatments. Application of K at 315kg/ha increased root length by 34.8%, whereas a 13% reduction was recorded by the application of K at 630kg/ha when compared with the control. The 34.8% increase at K_{315}Kg/ha could be attributed to the optimum K level as a major nutrient for root development (Mcafee, 2008). This conforms with the report of Khan and Sajid (Khan and Sajid, 2007) who reported maximum root length for treatments with increased potassium (Kwizera et al., 2019).

Phosphorous fertilizer had a similar scenario to that of K treatments. However, the application of phosphorous at 420kg/ha demonstrated a slight (0.9%) increase in total biomass compared with the control, demonstrating the highest total biomass yield of 29220 kg/ha in this experiment. Fresh

root weight and fresh leaf weight did not show any progressive increase from the control treatment. Thus, it is reasonable to say these parameters have a better response under low available soil P on Burdock Nevertheless, root length was significant (P<0.05) due to P treatments. Application of P at 280kg/ha demonstrated the highest response with a 59.4% increase compared with the control.

Table 3.2 . Influence of fertilizer treatments on yield related parameters of Burdock

Treatments (Kg/ha)	Total biomass (kg/ha)	Fresh root weight (kg/ha)	Fresh leaf weight (kg/ha)	Root length (cm)	Root diameter (cm)
Potassium					
$T_1(k_{210})$ [Control]	26775[a]	19550[a]	6825[a]	57.50[ab]	2.75[a]
$T_2(k_{315})$	21920[a]	14235[a]	7930[a]	77.50[a]	2.50[a]
$T_3(k_{420})$	30830[a]	22140[a]	9010[a]	64.00[ab]	2.75[a]
$T_4(k_{520})$	24270[a]	17545[a]	6360[a]	69.50[ab]	2.75[a]
$T_5(k_{630})$	27005[a]	19040[a]	7370[a]	50.00[b]	2.50[a]
LSD	NS	NS	NS	22.92	NS
Phosphorous					
$T_1(P_{140})$ [Control]	28950[a]	21110[a]	8325[a]	54.25[b]	2.37[a]
$T_2(P_{210})$	24435[a]	20718[a]	6795[a]	60.75[b]	2.75[a]
$T_3(P_{280})$	28740[a]	17500[a]	7823[a]	86.50[a]	2.75[a]
$T_4(P_{350})$	23590[a]	21005[a]	7178[a]	62.00[b]	2.25[a]
$T_5(P_{420})$	29220[a]	21068[a]	6198[a]	69.75[ab]	3.00[a]
LSD	NS	NS	NS	23.83	NS

Means in the same column with the same superscript are not significantly different (P>0.05). LSD= Least significant difference, K= Potassium fertilizer treatments and P=Phosphorous fertilizer treatment. Values are means at (P-values 0.05).

3.3.3 Phytochemical screening of root and leaf extract of Burdock

Results on phytochemical screening for aqueous root and leaf extracts for P and K treatments were quite rich (Table 3.3). Similar positive (+) and negative (-) test results were observed for aqueous root and leaf extracts, except for saponin that was present in the root, but absent in leaf extracts. Even though there is limited literature on this finding, these results indicates that fertilizer treatments have less influence on the quality of phytochemicals on aqueous root and leaf extracts of Burdock However, these results are in accordance with those of Al-Shammaa et al. (2013), who reported a positive (+) test for saponin, flavonoid, and tannin, but a negative (-) test for Alkaloid on aqueous root and leaf extract of cultivated *Arctium lappa* L. collected from the Department of medicinal plants, College of Agriculture, University of Baghdad, Iraq. The presence of tannin in root and leaf complement the antiplasmodial activity of Burdock (Alshawsh et al., 2007). Also, saponin for its anti-carcinogenic properties and other health-related benefits (Adesokan and

Akanji, 2010). Furthermore, the presence of glycosides for the treatment of heart problems (Fatoba et al., 2003), complementing the therapeutic values of Burdock.

Table 3.3 Phytochemical screening of aqueous root and leaf extracts of Burdock in response to treatments

Root extracts	**Tannins**	**Saponins**	**Flavonoids**	**Terpenoids**	**Glycosides**	**Alkaloids**	**Phenols**	**Steroids**
Phosphorous								
$T_1(P_{140})$(control)	++	+	++	-	+	-	+	-
$T_2(P_{210})$	++	++	++	-	+	-	+	-
$T_3P_{280})$	++	++	++	-	+	-	+	-
$T_4(P_{350})$	++	+	++	-	+	-	+	-
$T_5(P_{420})$	++	+	++	-	+	-	+	-
Potassium								
$T_1(K_{210})$(control)	++	+	+	-	+	-	+	-
$T_2(K_{315})$	++	++	+	-	+	-	+	-
$T_3(K_{420})$	++	++	+	-	+	-	+	-
$T_4(K_{520})$	++	+	+	-	+	-	+	-
$T_5(K_{630})$	++	+	+	-	+	-	+	-
Leaf Extracts	**Tannins**	**Saponins**	**flavonoids**	**Terpenoids**	**Glycosides**	**Alkaloids**	**Phenols**	**Steroids**
Phosphorous								
$T_1(P_{140})$ (control)	+	-	++	-	+	-	+	-
T_2 (P_{210})	+	-	++	-	+	-	+	-
T_3 (P_{280})	+	-	+	-	+	-	+	-
T_4 (P_{350})	+	-	+	-	+	-	+	-
T_5 (P_{420})	+	-	+	-	+	-	+	-
Potassium								
T1 (K_{210})(control)	+	-	+	-	+	-	+	-
T_2 (K_{315})	+	-	+	-	+	-	+	-
$T_3((k_{420})$	+	-	+	-	+	-	+	-
$K_4(k_{520})$	+	-	+	-	+	-	+	-
K_5 (k_{630})	+	-	+	-	+	-	+	-

Legend: (++): Highly present, (+); present, (-): absent.

3.3.4 Proximate analysis of root and leaf samples of *Arctium lappa* L

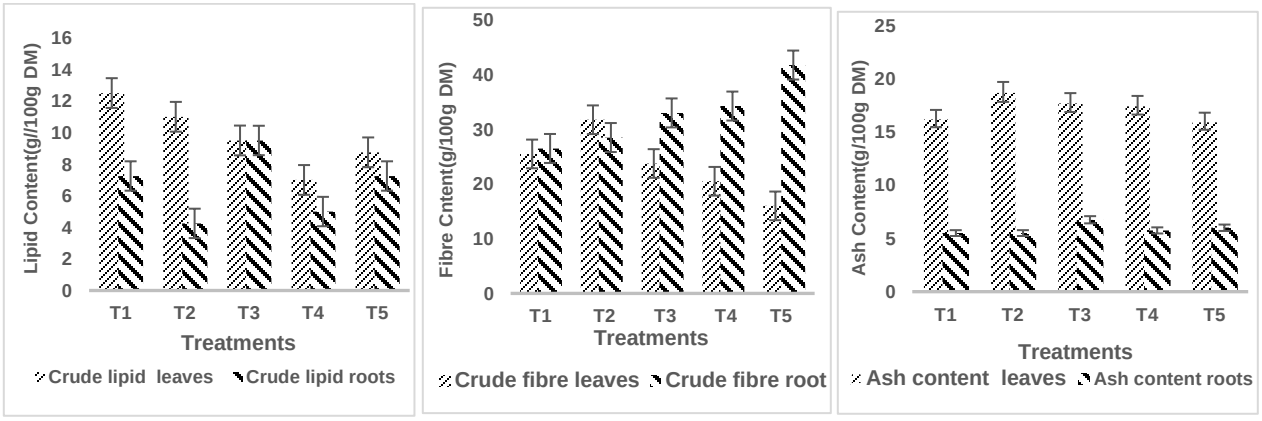


Figure 3.1 Influence of potassium fertilizer on crude lipid (fig: 3.1a), crude fibre (fig:3.1b) and ash content (fig:3.3c) of Burdock (*Arctium lappa* L). Values are means at (P-values 0.05).

Crude lipid and ash content for root and leaf samples were not significant (p>0.05) in response to K fertilizer (Fig.3.1a&c) However, crude fibre content was significant (p<0.05) to K treatments (Fig.3.1b). Higher lipid content was obtained in leaf than root samples. Application of K at 210kg/ha recorded the highest crude lipid content (12.5%) in the leaf whereas, in the root, the highest (9.5%) was recorded by the application of K at 420kg/ha with a 32% increase compared to the control. Ash content demonstrated the same scenario as crude lipid content. Application of K at 315kg/ha recorded the highest ash content (18.8%) in the leaf whereas in root the highest (6.8%) was obtained by K at 420kg/ha. Nevertheless, the crude fibre content in leaf was significant (p<0.05) but not significant (p>0.05) in root in response to fertilizer treatments. Application of K at 315kg/ha recorded the highest crude fibre content (31.8%) with a 25% increase in leaf, whereas, in the root, the highest (41.8%) was obtained by K at 630kg/ha compared with the control.

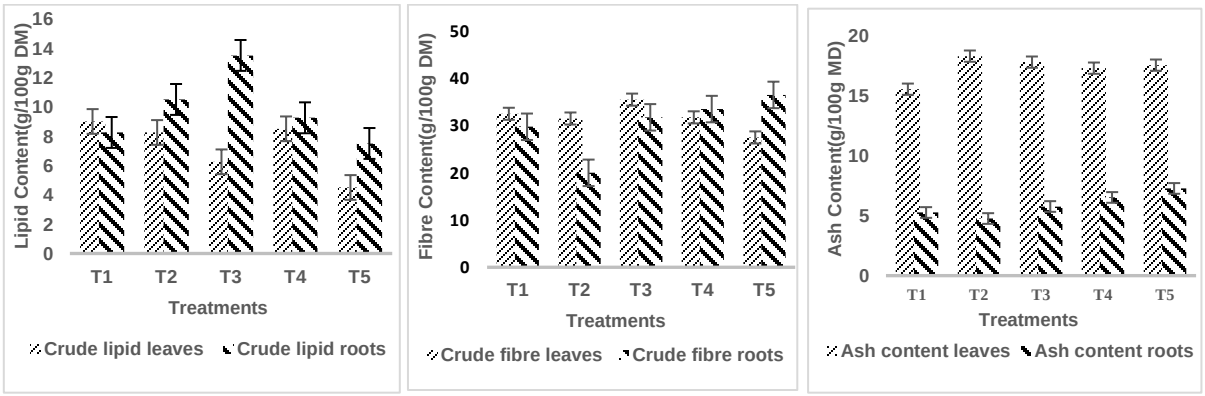


Figure 3.2 Influence of phosphorous fertilizer on crude lipid (fig; 3.2a), crude fibre (fig: 3.2b) and ash content (fig: 3.3c) of Burdock (*Arctium lappa* L.). Values are means at (P-values 0.05).

Phosphorus had no significant effect (P>0.05) on crude lipid and ash content in root and leaf of Burdock (Fig.3.2a &c). However, Crude fibre content was significant (p<0.05) to P treatments (Fig. 3.2b). The application of P at 280kg/ha recorded the highest crude lipid content (13.5%) in root with a 63.6% increase compared to the control which was highest (9%) in the root. On the contrary, ash content was higher in leaf than root. The application of P at 210kg/ha recorded the highest ash content (18.3%) in leaf. Whereas the application of P at 420kg/ha recorded the highest (7.3%) with a 38% increase compared to the control in the root. Also, the application of P at 420kg/ha recorded the highest fibre content (36.5%) in root with a 22.7% increase compared to the control. In leaf, the highest (35.5%) was recorded by the application of P at 280kg/ha with a 9.2% increase compared to the control. The dietary reference intake for lipids in adults is 20-35% of total calories from food. However, the highest crude lipid 13.5% in roots and 12.5% in leaves was recorded. According to Mudi and Muhammad (2009), a moderate amount of lipid has health benefits in the body. The high crude fibre content in the root (41.8%) for K and 35.5% for P recorded in *Arctium lappa* L. suggests it can be a potential source of dietary fibre for anti-tumorigenic and hypocholestrolemic properties (Okoro and Achuba, 2012). This implies it may be recommended for people with cholesterol-related problems (Chihara, 1993). The overall high ash content in leaves of Burdock implies it can be a very nourishing and suitable vegetable for consumption.

3.3.5 Conclusion

This study provided preliminary knowledge on nutrients requirements and cultural practices for the cultivation of Burdock (*Arctium lappa* L.) in the Winelands region of the Western Cape Province of South Africa. It provided an insight on the influence of fertilizer treatments on growth, yield, and phytochemicals of this species with regards to its therapeutic and nutritional values. The study revealed that this species requires a limited amount of potassium and phosphorous application for optimum growth and enhancement of phytochemicals. As a wild species, it might have adapted to low utilization of mineral fertilization for optimum growth and development. However, more studies need to be done on fertilizer treatment combinations especially in the field to scientifically validate a possible protocol for the cultivation of this species in South Africa.

References

Adesokan, A. & Akanji, M. 2010. Antimalarial bioactivity of Enantia chlorantha stem bark. *Medicinal plants: phytochemistry, pharmacology and therapeutics, Volume 1*, 441-447.

Al-Shammaa, D. A., Saour, K. Y. & Abdul-Khalik, Z. M. 2013. Phytochemical investigation for the main active constituents in Arctium lappa L. cultivated in Iraq. *Iraqi journal of pharmaceutical sciences,* 22, 18-24.

Alshawsh, M., Mothana, R., Al-Shamahy, H., Alsllami, S. & Lindequist, U. 2007. Assessment of anti-malarial activity against. *Plasmodium falciparum*, 453-456.

Aoac, C. A. 2005. Official methods of analysis of the Association of Analytical Chemists International. Official Methods Gaithersburg, MD.

Badarau, A. S., Wang, D., Swamy, M. K., Shaw, S., Maggi, F., Da Silva, L. E., López, V., Yeung, A. W. K., Mocan, A. & Atanasov, A. G. 2019. Arctium species secondary metabolites chemodiversity and bioactivities. *Frontiers in Plant Science,* 10, 834.

Bannai, Y. 2016. TCVM Food Therapy for the French Bulldog. *American Journal of Traditional Chinese Veterinary Medicine,* 11.

Chan, Y.-S., Cheng, L.-N., Wu, J.-H., Chan, E., Kwan, Y.-W., Lee, S. M.-Y., Leung, G. P.-H., Yu, P. H.-F. & Chan, S.-W. 2011. A review of the pharmacological effects of Arctium lappa (burdock). *Inflammopharmacology,* 19, 245-254.

Chihara, G. 1993. Medicinal aspects of Lentian Isolated from Lentinus edodes (Berk). Hong Kong. Chinese University Press.

Dyayiya, N. A., Oyemitan, I. A., Matewu, R., Oyedeji, O. O., Oluwafemi, S. O., Nkeh-Chungag, B. N., Songca, S. P. & Oyedeji, A. O. 2016. Chemical analysis and biological potential of Valerian root as used by herbal practitioners in the Eastern Cape Province, South Africa. *African Journal of Traditional, Complementary and Alternative Medicines,* 13, 114-122.

Erdemoglu, N., Turan, N. N., Akkol, E. K., Sener, B. & Abacıoglu, N. 2009. Estimation of anti-inflammatory, antinociceptive and antioxidant activities on Arctium minus (Hill) Bernh. ssp. minus. *Journal of ethnopharmacology,* 121, 318-323.

Fatoba, P., Omojasola, P., Awe, S. & Ahmed, F. 2003. Phytochemical screening of some selected tropical African Mosses. *Nigerian Society for Experimental Biology (NISEB) Journal,* 3, 49-52.

Hossain, A., Islam, M., Bari, M., Amin, M. & Kabir, M. 2009. Effects of mulching and levels of potassium on growth and yield of carrot. *Bangladesh Research Publications Journal,* 3, 963-970.

Ibrahim, E. A. & Abd El-Kader, A. E. 2015. Effect of soil amendments on growth, seed yield and NPK content of bottle gourd (Lagenaria siceraria) grown in clayey soil. *International Journal of Soil Science,* 10, 186-194.

Khan, G. A. & Sajid, M. 2007. Response of dhalia (Dhalia pinnata) to different levels of nitrogen alone and in combination with constant doses of phosphorus and potassium. *American-Eurasian Journal of Sustainable Agriculture*, 25-32.

Kwizera, C., Ong'or, B. T. I., Kaboneka, S., Nkeshimana, F. & Ahiboneye, N. 2019. Effects of Potassium Fertilizer on Bean growth and Yield parameters.

Lans, C. & Turner, N. 2011. Organic parasite control for poultry and rabbits in British Columbia, Canada. *Journal of ethnobiology and ethnomedicine,* 7, 21.

Marschner, H. 1995. Function of mineral nutrients: micronutrients. *Mineral nutrition of higher plants*.

Mcafee, J. 2008. Potassium, a key nutrient for plant growth. *Department of Soil and Crop Sciences: http://jimmcafee. tamu. edu/files/potassium*.

Mosaddegh, M., Naghibi, F., Moazzeni, H., Pirani, A. & Esmaeili, S. 2012. Ethnobotanical survey of herbal remedies traditionally used in Kohghiluyeh va Boyer Ahmad province of Iran. *Journal of ethnopharmacology,* 141, 80-95.

Mudi, S. & Muhammad, A. 2009. Antimalaria activity of ethanolic extracts of leaves of terminalia catappa. l. combretaceae (indian almond). *Bayero Journal of Pure and Applied Sciences,* 2, 14-18.

Ndakidemi, P. A. & Dakora, F. D. 2007. Yield components of nodulated cowpea (Vigna unguiculata) and maize (Zea mays) plants grown with exogenous phosphorus in different cropping systems. *Australian Journal of Experimental Agriculture,* 47, 583-589.

Neves, J. M., Matos, C., Moutinho, C., Queiroz, G. & Gomes, L. R. 2009. Ethnopharmacological notes about ancient uses of medicinal plants in Trás-os-Montes (northern of Portugal). *Journal of ethnopharmacology,* 124, 270-283.

Nkaa, F., Nwokeocha, O. & Ihuoma, O. 2014. Effect of phosphorus fertilizer on growth and yield of cowpea (Vigna unguiculata). *IOSR J. Pharm. Biol. Sci,* 9, 74-82.

Nyoki, D. & Patrick, A. N. 2013. Economic benefits of. *Bradyrhizobium japonicum*.

Okoro, I. & Achuba, F. 2012. Proximate and mineral analysis of some wild edible mushrooms. *African Journal of Biotechnology,* 11, 7720-7724.

Razaq, M., Zhang, P. & Shen, H.-L. 2017. Influence of nitrogen and phosphorous on the growth and root morphology of Acer mono. *PloS one,* 12, e0171321.

Salama, M. & Salama, S. G. 2016. Arctium Lappa L.(Asteraceae); a New Invasive Highly Specific Medicinal Plant Growing in Egypt. *Pyrex Journal of Plant and Agricultural Research*.

CHAPTER 4
YIELD AND MORPHOLOGICAL CHARACTERISTICS OF BURDOCK (*ARCTIUM LAPPA* L) IN RESPONSE TO MINERAL FERTILIZER APPLICATION

(This is pot and field study. The pot study has been published in Asian Journal of Agriculture and Biology 8(4): 511-518. DOI: https://doi.org/10.35495/ajab.2019.11.524. Please refer to the appendix 3)

4.1 Introduction

The increase in demand for plant-based products for food, medicine, shelter, agrochemicals, and clothing by the increasing human population is causing pressure on wild plant populations. This scenario keeps increasing especially for plant species that have been identified to possess valuable phytochemicals of therapeutic values. Presently, the increasing demand for plant-derived natural products has created a large business opportunity for medicinal plant materials especially in developing countries where approximately 80% of both rural and urban populations prefer the use of traditional medicine because of its affordability and cultural acceptability (Maroyi, 2013). This has placed significant pressure on our plant biodiversity with very little or no mitigating measures like the cultivation of medicinal plants of interest. Cultivation and optimum management practice of these species especially defining the best fertilizer application rate for optimum growth and yield of secondary metabolites of interest is imperative. Burdock (*Arctium lappa* L.) is a biennial wild plant of the family Asteraceae and has been documented in the Traditional Chinese medicine (TCM) to contain phytochemicals of phenolic compounds such as chlorogenic, caffeic, isochlorogenic, polyacetylenes, triterpenoids, lignans arctiin and arctigenin (Chan et al., 2011, Tousch et al., 2014). The species has antioxidant, anti-microbial, antidiabetic, antiallergic, anti-inflammatory, sedative, and other health-protecting properties (El-Darier and Sg, 2016). The root is a good source of inulin, a non-starchy polysaccharide that is prebiotic and helps to reduce body weight, sugar and cholesterol level in the blood (Itaya et al., 2017). It is also known for lowering intestinal pH, preventing the establishment and growth of pathogenic bacteria (Wang and Wu, 2013).

Apart from its medicinal potential, Burdock is a nutritious crop that has considerable importance in Europe, Australia and America where it is highly consumed as a vegetable in stew and salad (Imahori et al., 2010, Chan et al., 2011). Nutritionally the root and leaf constitute a significant proportion of iron, manganese, magnesium, selenium, zinc, calcium, phosphorus, folic acid, riboflavin, pyridoxine, niacin and some appreciable amount of potassium which is an important component of cell body fluid regulating heartbeat and blood pressure (Chang et al., 2009). It is

also rich in some valuable amounts of vitamin C and E which are natural antioxidants for the oxidation of easily oxidizable materials (Xu et al., 2017). Morphologically burdock is characterized by heart-shaped leaves that vary in length and width from top to bottom, covering the stem. It is well adapted to full or partial sun growth and grows well in undisturbed areas. The root is fleshy with a slightly thick brown back and grows deep into a well-drained sandy soil (Wu et al., 2017). The availability and supply of these plant materials are mostly limited to the wild, with marginal cases of subsistence cultivation practices restricted mostly to countries and/or regions of its origin. Though of high medicinal and nutritional benefits with high market demand by pharmaceutical companies and herbal practitioners across the world, access to the herb is still restricted to regions of its origin, and countries like South Africa still depend solely on imported plant materials with potential batch-to-batch variability and irregular volume supply (Tanga et al., 2018). The application of modern cultivation techniques to this species will ensure a consistent supply of high-quality materials to both pharmaceutical industries and herbal practitioners. In addition, this approach could present appreciable mitigating measures against population decimation in its natural habitat. To properly adapt and domesticate new species in another environment or climatic condition, evaluation of the morphology and physiological expression of such species is a critical aspect of a preliminary study that must be conducted. This current study evaluates the morphology and yield of Burdock in response to different application rates of N: P: K fertilizer planted in port and field experiments.

4.2 Materials and methods

4.2.1 Study Area

The study consisted of a pot and field experiment. The pot experiment was conducted from August 2018 to March 2019 while the field experiment was conducted from July 2020 to February 2021 cropping seasons at the Research and teaching farm, the Agric-Hub, Department of Agriculture, Wellington Campus, Cape Peninsula University of Technology (CPUT), South Africa. The area falls within the Northern part of Wellington at coordinate (S33^{o} 37` E19$^{o}$ 37`). Wellington has a Mediterranean climate and receives about 585mm of winter rainfall per year. Rainfall is usually from the months of February to November, with the lowest rainfall in February (10mm) and the highest in June (105mm).

4.2.2 Soil collection and analysis

4.2.2.1 Pot experiment

Potting soil for pot experiment was obtained from standard farm and analysed in bemlab, a commercial laboratory in the Western Cape Province, South Africa. The results of soil test (Table

4.1) showed that the soil is sandy, low in total nitrogen (0.353 %) and phosphorous (418 mg/Kg), with a moderate amount of potassium (3425 mg/kg) and a near to neutral pH (7.1).

4.2.2.2 Field experiment

Several soil samples were collected in the field at a depth of 0-30cm at the experimental plot. The soil samples were homogenized to one composite representative soil sample. A baseline analysis of the soil was done in Bemlab. The results of the soil analysis test showed that the soil is loamy, very low in total nitrogen (0.13%), phosphorous (32.0 mg/kg), potassium (93.9 mg/kg) and a pH (4.6). (Table 4.1).

Table 4.1 Chemical properties of potted soil

Experiment	Soil type	pH KCl	Resist Ohm	Stone V%	P mg/Kg Bray II	K mg/Kg	Cu mg/Kg	Zn mg/kg	Mn mg/Kg	B mg/kg	Fe mg/kg	C%	Total N (N %)	Na	K	Ca	Mg
Pot	Sandy	7.1	70	1	418	3425	2.0	33.4	30.3	2 .74	65	3.6	0.35	5.49	8.76	19.73	4 .47
Field	Loam	4.6	870	0.00	32.0	93.9	1.7	7.0	41.1	0.40	404	3.54	0.13	2.56	3.62	54.30	19.61

4.2.3 Experimental layout and treatment application

The pot and field experiments had the same experimental design and treatment application. The design was a factorial experiment of eight treatments $T_1=N_{423}P_{210}K_{315}$, $T_2=N_{423}P_{280}k_{315}$, $T_3=N_{635}P_{210}K_{315}$, $T_4=N_{635}P_{280}K_{315}$, $T_5=N_{846}P_{210}K_{315}$, $T_6=N_{846}P_{280}K_{315}$, $T_7=N_{1058}P_{210}K_{315}$ and $T_8=N_{1058}P_{280}K_{315}$ Kg/ha) and five collection times (T_1, T_2, T_3, T_4 and T_5) of data. The treatments consisted of 4 levels of nitrogen (N_{423}, N_{635}, N_{846} and N_{1058}Kg/ha of Urea 46%), with 2 levels of phosphorous (P_{210} and P_{280}Kg/ha Triple superphosphate 20%) laid out in a completely randomized design replicated five times. A uniform base application of potassium (K_{315}Kg/ha of Potassium Chloride 50%) was applied to all the treatments. The pot experiment was set in pots under a 40% shade net. The field experiment consisted of main plot size (15m x15m) and subplot sizes (2m length x1m width), with a row spacing distance of 1m apart. Each subplot consisted of six plants stand with row spacing distance per plant (70cm x 60cm) apart. Burdock seeds (Takinogawa long cultivar) were obtained from “The Seed Collection Pty Ltd”, a company in Australia (Ferntree Gully, Victoria 3156 Australia with permit NO. P0084124). The seeds were nursed in a greenhouse using a plastic tray filled with potting soil. Regular watering was done in the morning and evening to keep the soil moist. At three weeks, 100% germination of sown seeds was obtained. At six weeks the seedlings were taken outside for acclimatization over a period of two weeks before transplanting to larger pots of 10kg potting soil for pot experiment. This same procedure was replicated for the field experiment and seedlings were transplanted to the field.

The Application of fertilizer treatments was split into two equal doses: one at seedling transplant and four weeks after seedlings transplant for pot and field experiments. At two weeks after the transplanting, fungi treatment was applied using systemic fungicide (Funginex- Reg No, L2469/N-AR0131 Wet Nr.36 van 1947 W130281 of the active ingredient -Triforine) against black spot, powdery mildew, and rust. The treatment application dose was 15ml/L of water. Three treatments were applied on the foliage randomly at weekly intervals using a Trade Air High-Pressure Spraying Gun model TOOS1785.

4.2.4 Data collections

For both pot and field experiments, data were collected on the morphological characteristics and yield parameters. Data on plant height, number of leaves per plant, leaf length, leaf width, and chlorophyll content were measured and recorded at two weeks intervals until maturity at 120 days and above when the first leaves started showing signs of aging and collapsing, indicating senescence phase. Plant height, leaf length and broadest width of plant leaves were measured using a meter rule. The number of leaves per plant was counted and the chlorophyll content of leaves was measured using a chlorophyll meter CCM- 200 plus. Data on morphological parameters were collected five times for both experiments. At maturity, plants were harvested and washed using tap water. Data on yield was collected on plant biomass, root fresh weight and leaf fresh weight using a Lasec, Radwang wagi`s electronic weighing balance, Model: WLC 1/A2/C/2, made in Poland (EU). Root length and root diameter were measured using a thread and subsequently calibrated on a meter rule.

4.2.5 Statistical analysis

The data collected on growth and yield parameters were captured in Excel for pot and field experiments. The data for both experiments were statistically analyzed using two–way ANOVA at a.95% confidence limit using the SAS software. Means separation was done using Fisher`s Least Significant Difference (LSD) and ranking was carried out using Duncan`s Multiple Range Test (DMRT).

4.3 Results

4.3.1 Influence of fertilizer treatments on morphological characteristics of Burdock.

4.3.1.1 Pot experiment

The different fertilizer treatments had a significant ($P<0.05$) influence on the different morphological parameters of burdock (*Arctium lappa* L) investigated (Table 4.2). T_3 recorded the

highest number of leaves per plant with a 17.4% yield increase, followed by T_4 with 13.8% more leaves compared to the control (T_1). Interestingly, there was a 10.8% drop in the number of leaves in T_7 which had a high nitrogen application compared to the control (T_1). Plant height and leaf length demonstrated a similar response to fertilizer treatments as the number of leaves per plant. There was a 52.5% (T_3) and 41.8% (T_4) increase in plant height compared to the control (T_1). Similarly, T_3 and T_4 recorded the highest leaf length of 25.6% and 24.4% respectively. For the same parameter (leaf length), T_7 recorded a 23.3% yield reduction compared to the control (T_1). The highest yield increase was recorded for leaf width at T_4, representing a 31.1% increase, while the least effect was recorded by T_7 with 18.5% reduction compared to the control. Similarly, T_4 recorded the highest leaf chlorophyll content with a 43.2% increase and T_7 recorded a 1.7% reduction compared to the control (T_1). Number of leaves per plant, plant height, and chlorophyll content had a positive correlation to fertilizer treatments, while leaf length and leaf width had negative correlation to fertilizer treatments, with acceptable tolerance values over 0.1 and VIF values less than 10 (Table 4.3).

4.3.1.2 Field experiment

Fertilizer treatments also recorded a significant (P>0.05) influence on all the morphological parameters investigated (Table 4.2). T_4 recorded the highest number of leaves per plant with a 2.78% yield increase followed by T_3 with 1.39% more leaves compared to the control T_1. Excitingly, there was a 2.08% decrease in the number of leaves in T_8. Also, T_4 recorded the highest plant height (3.06%) followed by T_3 (1.82%), while T_7 recorded the lowest with a 14.45% reduction to that of the control (T_1). Leaf length and leaf width recorded a similar pattern with the highest leaf length recorded by T_2 (1.67%) and leaf width T_2(0.13%) while the list was recorded T_7 with a 14.54% decrease for leaf length and 13.72% reduction for leaf width compared to the control (T_1). Furthermore, for leaf chlorophyll content the highest concentrated was recorded by T_8 (17.36%) increase to that of the control (T_1), that recorded the least chlorophyll content. Number leaves per plant, leaf width and leaf chlorophyll content had a positive correlation to fertilizer treatments while plant height and leaf length were negatively correlated to fertilizer treatment, with an acceptable tolerance value of over (0.1) and VIF values less than 10 for number of leaves per plant and chlorophyll content. However, leaf width recorded an acceptable tolerance value of over (0.1) and a VIF value of over (10). (Table 4.3).

Table 4.2 Morphological characteristics of burdock with different fertilizer treatments for pot and field experiment. Values are means at (P-0.05).

Treatments	Number of leaves	Plant height(cm)	Leaf length(cm)	Leaf width (cm)	Leaf Chlorophyll
Pot Experiment					
$T1(N_1P_1\ k_1)$	6.68^{c}	18.36^{e}	18.88^{c}	18.64^{c}	15.46^{d}
$T_2\ (N_1P_2\ k_1)$	6.48^{c}	20.88^{d}	18.56^{c}	18.20^{c}	16.73^{cd}
$T_3(N_2P_1\ k_1)$	7.84^{a}	28.00^{a}	23.72^{a}	24.44^{a}	19.45^{b}
$T_4(N_2P_2\ k_1)$	7.60^{a}	26.04^{b}	23.48^{a}	24.48^{a}	22.14^{a}
$T_5(N_3P_1\ k_1)$	7.16^{b}	24.88^{b}	22.44^{a}	23.12^{ab}	19.99^{b}
$T_6(N_3P_2\ k_1)$	6.84^{bc}	23.04^{c}	20.84^{b}	22.68^{b}	18.64^{cb}
$T_7(N_4P_1\ k_1)$	5.96^{d}	$18,48^{e}$	14.48^{e}	15.20^{d}	15.19^{d}
$T_8(N_4P_2\ k_1)$	6.80^{bc}	19.12^{e}	16.24^{d}	16.12^{d}	18.42^{cb}
LSD	0.41	1.24	1.34	1.56	2.10
Field Experiment					
$T_1(N_1P_1\ k_1)$	5.76^{ab}	20.90^{ab}	21.04^{a}	23.84^{a}	28.17^{c}
$T_2\ (N_1P_2\ k_1)$	5.72^{ab}	21.28^{ab}	21.39^{a}	23.87^{a}	29.89^{bc}
$T_3(N2P_1\ k_1)$	5.84^{ab}	18.92^{bc}	19.82^{ab}	21.97^{abc}	30.82^{ab}
$T_4(N_2P_2\ k_1)$	5.92^{a}	21.54^{a}	20.76^{a}	22.01^{abc}	31.92^{ab}
$T_5(N_3P_1\ k_1)$	5.56^{ab}	19.30^{abc}	20.18^{a}	23.26^{a}	31.98^{ab}
$T_6(N_3P_2\ k_1)$	5.40^{b}	18.78^{bc}	17.71^{c}	20.24^{c}	32.14^{ab}
$T_7(N_4P_1\ k_1)$	5.83^{ab}	17.88^{c}	17.98^{bc}	20.57^{bc}	31.83^{ab}
$T_8(N_4P_2\ k_1)$	5.64^{ab}	20.24^{abc}	20.58^{a}	22.8^{3ab}	33.06^{a}
LSD	0.12	0.05	0.06	0.12	0.09

Means in the same column with the same superscript are not significantly different (P≥0.05). LSD= Least significant difference, T_1=$N_{423}P_{210}$ K_{315}, T_2=$N_{423}P_{280}$ K_{315}, T_3=$N_{635}P_{210}$ K_{315}, T_4=$N_{635}P_{280}$ K_{315}, T_5=$N_{846}P_{210}$ K_{315}, T_6=$N_{846}P_{280}$ K_{315}, T_7=$N_{1058}P_{210}$ K_{315} and T_8=$N_{1058}P_{280}$ K_{315} Kg/ha. Values are means at (P- value 0.05).

Table 4.3 Correlations on Morphological characteristic at (P-value 0.05).

Coefficients[a]

Pot Experiment

Model	Unstandardized Coefficients B	Std. Error	Standardized Coefficients Beta	t	Sig.	95.0% Confidence Interval for B Lower Bound	Upper Bound	Collinearity Statistics Tolerance	VIF
(Constant)	5.567	.720		7.728	.000	4.146	6.988		
Plant height	.092	.046	.274	1.986	.048	.001	.184	.235	4.258
Number of leaves	.053	.163	.039	.326	.745	-.269	.376	.315	3.176
Leaf width	-.007	.059	-.016	-.112	.911	-.122	.109	.225	4.435
Leaf length	-.263	.070	-.618	-3.736	.000	-.402	-.124	.163	6.138
Chlorophyll	.101	.038	.223	2.641	.009	.025	.176	.627	1.594
Field Experiment									
(Constant)	2.629	.731		3.598	.000	1.188	4.071		
Plant height	-.049	.040	-.140	-1.214	.226	-.127	.030	.323	3.098
Number of leaves	.180	.210	.130	.857	.392	-.235	.595	.188	5.313
Leaf width	.004	.074	.012	.054	.957	-.141	.149	.089	11.200
Leaf length	-.189	.089	-.577	-2.126	.035	-.364	-.014	.058	17.109
Chlorophyll	.175	.031	.598	5.640	.000	.114	.237	.384	2.607

a. Dependent Variable: TREATMENT

**. Correlation is significant at the 0.05 level (2-tailed).

4.3.2 Influence of time of data collection on morphological characteristics of Burdock

4.3.2.1 Pot experiment

Most of the morphological parameters of burdock investigated in this study progressively ($P<0.05$) increased with age exponentially (Fig 4.1). Number of leaves and plant height increased significantly ($P<0.05$) with age whereas leaf length and leaf width had no significant ($P>0.05$) increase after the fourth time of data collection (16 weeks). Also, leaf chlorophyll content did not show a significant ($P>0.05$) increase between the third and fourth time of data collection. Overall, between the fourth and fifth time of data collection, there was a significant increase of 22% in the number of leaves per plant, 5.1% in plant height and 6.5% in leaf chlorophyll content. Though not statistically significant, leaf length and leaf width recorded 4.4% and 2.6% respectively between the fourth and the fifth time of data collection. Whereas, between the third and fourth time of data collection on leaf chlorophyll content, a non-significant 5.6% increase was recorded (Fig 4.1).

4.3.2.2 Field experiment

The time of data collection had a significant ($P<0.05$) influence on the morphological parameters of burdock investigated in the field. Similar to pot experiment, the parameters investigated increased exponentially with advancing age (Fig 4.2). Number of leaf and plant high recorded a significant ($P<0.05$) increase with advancing age. However, leaf length, leaf width and leaf chlorophyll content did not follow the same scenario. At fourth and fifth time of data collections (16weeks) a non-significant ($P>0.05$) increase was recorded on leaf length, leaf with and leaf chlorophyll content, even though the first, second and third time of data collections were significant ($P<05$ on these parameters) (Fig 4.2).

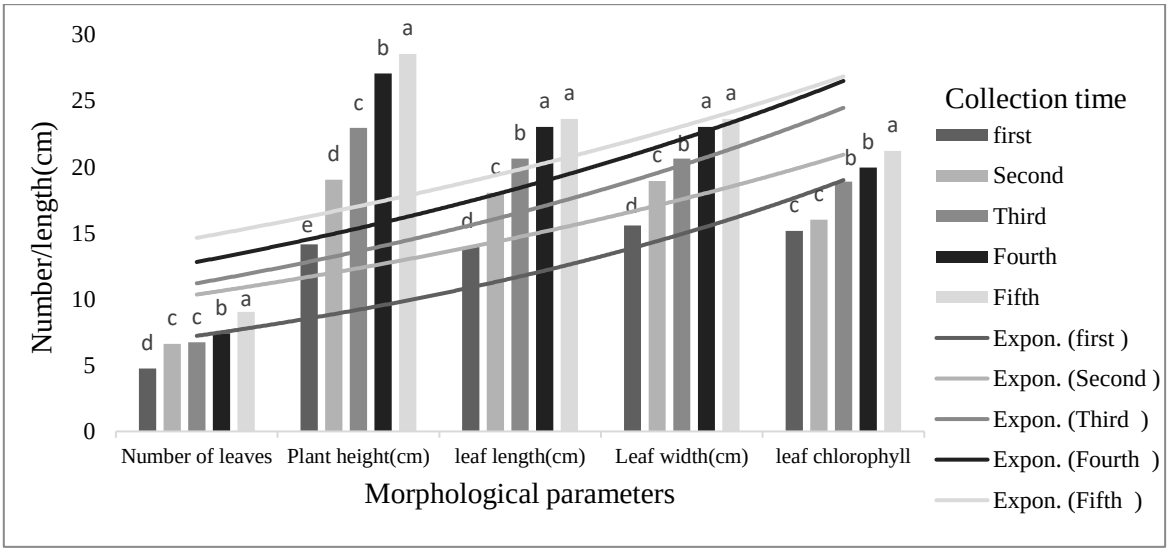


Figure 4.1 Graph showing the influence of time on data collection on morphological characteristics of burdock for pot experiment. Values are means at (P=0.05). Bars with the same letter on each parameter are not statistically different (P≥0.05). (Least significant difference (LSD) for number of leaves = 0.32, plant height = 0.98, leaf length =1.06, leaf width = 1.24 and leaf chlorophyll=1.67).

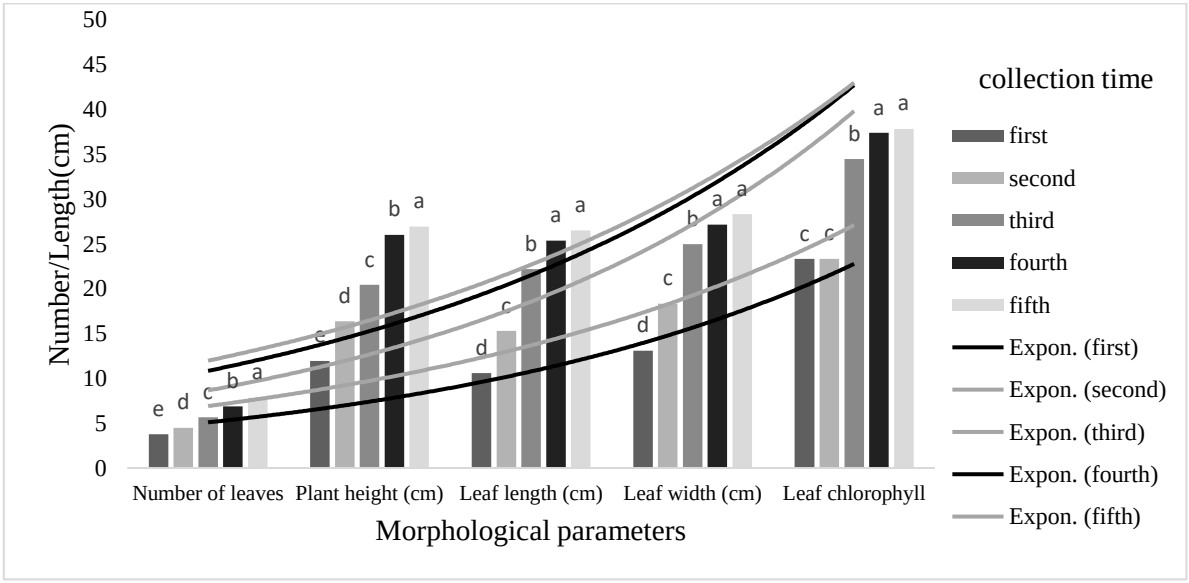


Figure 4.2 Graph showing the influence of time on data collection on morphological characteristics of burdock for field experiment. Values are means at (P=0.05). Bars with the same letter on each parameter are not statistically different (P≥0.05). (Least significant difference(LSD) for number of leaves = 0.32, plant height = 0.98, leaf length =1.06, leaf width = 1.24 and leaf chlorophyll=1.67).

4.3.3 Influence of fertilizer treatments on yield of Burdock

4.3.3.1 Pot experiment

Fertilizer treatments had a significant ($P<0.05$) influence on all the yield-related parameters recorded in Burdock. The overall fresh yield (fresh leaf weight, fresh root weight, and total fresh weight) was significantly influenced by fertilizer treatment levels. Considering the total fresh weight, T_3 recorded the highest (112.46tons/ha) with a 40.4% increase, while T_2 recorded the lowest (53.05 tons/ha) with a 33.7% decrease compared to the control (T_1). Fresh root weight demonstrated a similar response as the case of total fresh weight. However, T_3 had the highest fresh root weight (87.39tons/ha) with a 40.6% increase while, T_2 recorded the lowest fresh root weight (42.47tons/ha) with a 46.4% reduction compared to the control (T_1). In the case of fresh leaf weight, T_5 recorded the highest (28.03tons/ha) with a 62.6% increase while T_2 had the lowest fresh leaf weight (11.36 tons/ha) with a 34.1% decrease compared to the control (T_1). This indicates that T_5 can be the potential treatment requirement for vegetable cultivation of burdock leaf while T_3 is likely to be a potential treatment requirement for burdock root cultivation and that for total yield. Considering root length, T_6 had the highest response on root length (22.1cm) with a 12.2% increase and T_3 (21.6cm) with a 9.6% increase while T_8 recorded the lowest response in length (18.2cm) with a 7.6% reduction compared to the control (T_1). Furthermore, in root collar diameter, T_3 had the highest influence (7.88cm) with a 21.6% increase while T_7 had the lowest effect (6.10cm) with a 5.9% reduction compared to the control (T_1). Overall dry weight (leaf dry weight, root dry weight, and total dry weight) was significantly ($P<0.05$) influenced by fertilizer treatment levels. T_5 produced the highest leaf dry weight (18.84tons/ha) representing a 160.2% yield increase compared with control (T_1). While T_3 produced the highest dry root yield (63.48tons/ha) recording a 99.4% yield increase compared to the control (T_1), profoundly, a total dry yield increase of 102.1% (78.96tons/ha) was recorded at T_3. However, T_7 had the lowest dry root weight (19.28tons/ha) with a 39.4% decrease and total dry weight (27.44tons/ha) with a 29.8% reduction compared to the control (T_1). (Table 4.4).

4.3.3.2 Field experiment

Fertilizer treatments also had a significant ($P<0.05$) influence on all the yield parameters of Burdock investigated in the field. Considering total fresh weight T_6 had the highest yield (161.08tons/ha) with a 150.34% yield increase compared to the control (T_1) followed by T_4 with a 120.9% yield increase to that of the control (T_1), that recorded the lowest yield (64.34 tons/ha). The same scenario was recorded for fresh leaf weight and fresh root weight, T_6 had the highest yield (87.68 tons/ha) with a 140.48% yield increase for fresh root weight while fresh leaf weight

had the highest yield (73.4 tons/ha) with a 163.27% yield increase compared to the control (T_1) with the lowest yield for these parameters. Furthermore, root diameter, dry root weight, dry leaf weight and total dry weight (TDW) also had a significant influence ($P<0.05$) on fertilizer treatment application. The highest yield was obtained by T_6 (12.7tons/ha) for leaf dry weight with a 27% yield increase and dry root yield (18.2 tons/ha) with a 51.67% increase compared to the control (T_1). However, the lowest yield was recorded by T_8 with the highest nitrogen level application. Profoundly for the total dry weight (TDW) 30.9 tons/ha was recorded by T_6 with a 40.45% yield increase compared to the control (T_1) that recorded the lowest yield. Also, for root diameter T_6 recorded the largest diameter (5.7cm) with a 58.33% increase compared to the control (T_1). (T4.4)

Table 4.4 Influence of different fertilizer treatments on yield related parameters of burdock. Values are means at (P-0.05).

Treatments	Total fresh weight (tons/ha)	Fresh root weight (tons/ha)	Fresh leaf weight (tons/ha)	Root length (cm)	Root diameter (cm)	Leaf dry weight (tons/ha)	Root dry weight (tons/ha)	Total dry weight (tons/ha)
				Pot Experiment				
T1(N1P1 k1)	80.12^{bc}	62.17^{b}	17.24^{bc}	19.70^{abc}	6.48^{c}	7.24^{c}	31.84^{b}	39.08^{bc}
T2(N1P2 k1)	53.05^{d}	42.47^{c}	11.36^{c}	18.60^{bc}	6.14^{c}	6.68^{c}	23.56^{c}	30.24^{d}
T3(N2P1 k1)	112.46^{a}	87.39^{a}	24.16^{ab}	21.60^{ab}	7.88^{a}	15.48^{ab}	63.48^{a}	78.96^{a}
T4(N2P2 k1)	95.40^{ab}	67.43^{b}	21.42^{ab}	21.20^{abc}	7.66^{ab}	11.84^{ab}	43.44^{b}	62.88^{ab}
T5(N3P1 k1)	91.12^{ab}	64.01^{b}	28.03^{a}	20.80^{abc}	7.10^{abc}	18.84^{a}	31.80^{b}	42.92^{ab}
T6(N3P2 k1)	83.34^{bc}	57.32^{bc}	25.73^{a}	22.10^{a}	6.58^{bc}	11.16^{b}	30.20^{bc}	41.40^{bc}
T7(N4P1 k1)	61.53^{cd}	39.57^{c}	20.62^{ab}	20.60^{abc}	6.10^{c}	8.16^{bc}	19.28^{c}	27.44^{cd}
T8(N4P2 k1)	64.81^{cd}	43.41^{c}	20.95^{ab}	18.20^{c}	6.7^{bc}	11.12^{b}	22.96^{c}	34.08^{cd}
LSD	29.12	19.96	10.80	3.244	1.102	3.36	20.04	37.56
				Field Experiment				
T1(N1P1 k1)	64.34^{d}	36.46^{d}	27.88^{f}	23.00^{cd}	3.60^{c}	10.00^{bc}	12.00^{b}	22.00^{c}
T2(N1P2 k1)	73.58^{d}	40.70^{d}	32.88^{ef}	18.40^{f}	4.40^{b}	10.60^{b}	12.60^{b}	23.20^{bc}
T3(N2P1 k1)	95.80^{c}	58.80^{c}	37.00^{de}	25.00^{b}	4.50^{b}	10.84^{b}	13.00^{b}	23.84^{bc}
T4(N2P2 k1)	142.80^{b}	80.08^{b}	62.00^{b}	26.00^{b}	4.60^{b}	12.50^{a}	14.40^{b}	26.90^{a}
T5(N3P1 k1)	106.20^{c}	59.08^{c}	47.12^{c}	29.00^{a}	4.60^{b}	10.42^{bc}	11.80^{b}	22.22^{c}
T6(N3P2 k1)	161.08^{a}	87.68^{a}	73.40^{a}	24.00^{bc}	5.70^{a}	12.70^{a}	18.20^{a}	30.90^{a}
T7(N4P1 k1)	138.32^{b}	76.72^{b}	61.60^{b}	25.00^{b}	4.50^{b}	10.60^{b}	13.20^{b}	23.80^{bc}
T8(N4P2 k1)	106.00^{c}	63.20^{c}	42.80^{cd}	22.20^{d}	4.40^{b}	9.20^{c}	11.20^{b}	20.40^{c}
LSD	18.28	7.60	11.40	3.00	0.80	5.90	3.80	5.80

Means in the same column with the same superscript are not significantly different ($P\geq0.05$). LSD= Least significant difference, $T_1=N_{423}P_{210}$ K_{315}, $T_2=N_{423}P_{280}$ K_{315}, $T_3=N_{635}P_{210}$ K_{315}, $T_4=N_{635}P_{280}$ K_{315}, $T_5=N_{846}P_{210}$ K_{315}, $T_6=N_{846}P_{280}$ K_{315}, $T_7=N_{1058}P_{210}$ K_{315} and $T_8=N_{1058}P_{280}$ K_{315} Kg/ha. Values are means at (P- value 0.05).

4.4 Discussion

The results obtained from the pot and field experiments showed that fertilizer treatment combinations had a significant influence on all the morphological parameters of Burdock investigated in the study. This may be attributed to the variation in the different rates of fertilizer application used in the study (Hammed et al., 2019, Ichami et al., 2019). These results are in line

with Al-Zyadi (2018), who reported significant differences in growth and yield parameters of Burdock as influenced by different levels of nitrogen fertilization and harvesting time, but with the highest effect at 100 kg/ha of nitrogen fertilizer application. However, in this study the highest influence was demonstrated by the application of N_{635} kg/ha for the pot experiment while the application of N_{846} Kg/ha had the highest response for field experiment. This may be attributed to the differences in soil type, nutrients availability, environment, type of experiment, cultural practices, soil physical properties, and ecological factors involved, and probably due to cultivar (Aikins et al., 2012, Ncube et al., 2012). Previous studies have also indicated that variation in fertilizer application rate and time of application has an influence on plant growth and yield (Abebe and Feyisa, 2017); nutrient availability, nutrient uptake and photosynthetic activities(Zhao et al., 2008, Razaq et al., 2017). T_3 ($N_{635}P_{210}K_{315}$ Kg/ha) demonstrated the highest influence on plant height, number of leaves per plant, leaf width and leaf length. However, further increase in fertilizer treatment levels had a negative influence on different growth parameters (Benhmimou et al., 2018), for pot experiment. On the other hand, for field experiment T_4 (N_{635} P_{280} K_{315} kg/ha) recorded the highest influence on number of leaves per plant and plant height while T_2 ($N_{423}P_{280}K_{315}$ kg/ha) recorded the highest influence on leaf length and leaf width. This may be attributed to the soil`s physical and chemical properties which might have influence nutrients uptake, plant growth and development in the field to a varying degree among the different treatments (Khalil et al., 2015, Cardone et al., 2020). Results of this study for pot experiment indicate that T_3 (635 kg/ha urea, 210 kg/ha of triple superphosphate and 315 kg/ha of potassium chloride) while for field cultivation T_6(846 kg/ha urea, 280 kg/ha of triple superphosphate and 315 kg/ha of potassium chloride) may be the optimal fertilizer treatment application rates that could potentially produce the best yield of burdock leaves for vegetable production in the Western Cape Province of South Africa under similar environmental conditions. Al-Zyadi (2018), reported the best yield of Burdock at $N_{100}K_{100}$ kg/ha with a uniform application of phosphorous in the field. Also, Ge et al. (2016) reported the best treatment combination of 30% vermicompost combined with nitroxin on growth parameters of burdock. It has been proven that optimum nitrogen supply is critical for growth and development in plants and its availability and concentration in plant tissues affect the partitioning of plant biomass (Bown et al., 2010). This may be due to its role in the formation of amino acid, which is the base for the building of Indole acetic acid (IAA) auxin, which stimulated cell division and elongation, and the growth of apical meristem. Phosphorous, also being a primary nutrient for plant growth, its optimal amount is critical for optimum production and quality (Zapata and Zaharah, 2002). This may have also led to the increase in shoot biomass, influencing the number of leaves, leaf length and leaf width of burdock in these experiments across the different treatments with the highest influence showcased by T_3 for pot experiment and T_6 for field

cultivation of burdock. Moreover, nitrogen and phosphorous are very critical to dry matter partitioning on shoot tip where metabolism is high with rapid cell division and the number of leaves which is a function of plant height (Ndakidemi and Dakora, 2007). The differences in growth parameters in this study may have been a direct result of the differences in the rates of fertilizer application. Generally, this study indicated that T_3 and T_6 are the optimum range of fertilizer treatment combinations for the cultivation of burdock in these different soil types in the Cape Winelands of the Western Cape Province of South Africa. T_4 had the greatest effect on leaf width and chlorophyll content for the pot experiment. However, the results for field experiment indicate T_2 had the greatest influence followed by T_8 which was not significant on leaf width while T_8 had the highest leaf chlorophyll content. This can be attributed to the fact that nitrogen is the most important element in chlorophyll biosynthesis (Razaq et al., 2017) which may influence leaf width. Hence, nitrogen promotes the formation of active photosynthetic pigments by increasing the amount and thylakoid proteins in leaves (Sá Filho et al., 2011). Also, green pigments in leaves depend on phosphorous concentration since the biosynthesis of pigment molecules depends on the uptake of optimal phosphorous levels (Waraich et al., 2015) demonstrated by T_4 for pot experiment and T_2 for field cultivation in this study.

The different times of data collection had a significant influence on all the morphological characteristics investigated in this study. This indicates that growth parameters in burdock are greatly influenced by nutrient availability, time of data collection and plant age. The fifth time of data collection demonstrated the highest effect in all the parameters recorded for both pot and field experiments. However, there was no significant difference in leaf length and leaf width at the fourth and fifth time of data collection regardless of cultivation practice, indicating the potential stage of maturity of this species.

Fertilizer treatments recorded significant effects on yield-related parameters of burdock in this study. T_3 recorded the highest total fresh weight and fresh root weight, indicating the best treatment combination for burdock cultivation in pots while T_6 recorded the best treatment in the field for this study. This may be attributed to the fact that lower phosphorous availability in soil is known to stimulate larger roots in some plant species (Williamson et al., 2001) and root length, since long fine roots are more efficient in nutrient acquisition than short fine roots (Ma et al., 2001), which may have resulted to an increase in total root biomass demonstrated by T_3 for pot experiment. Nevertheless, T_4 and T_5 had no significant difference from T_3. However, for the field experiment, the longest roots were recorded by T_5 with the same lower level of phosphorous application. Furthermore, T_3 recorded the highest dry root weight and total dry weight (TDW) since total dry weight (TDW) is a function of total fresh weight (TFW). T_5 recorded the highest fresh leaf

and dry leaf yield. This indicates that the T_5 combination is a potential treatment for vegetable production of burdock leaves while T_3 can be used for the cultivation of burdock roots as indicated by the pot experiment. However, for the field experiment overall, T_6 had the highest fresh leaf weight, fresh root weight, total fresh weight (TFW) and Total dry weight (TDW). This indicates that T_6 can be used for burdock field cultivation for both burdock root and vegetable production in this study.

4.5 Conclusion

The cultivation of Burdock was highly influenced by fertilizer treatments. The results of the study demonstrated that the crop can successfully grow in the Winelands District of the Western Cape Province of South Africa for medicinal and culinary uses. The cultivation can be done in pots or in the field with all agricultural practices diligently applied. The application of $N_{846}P_{280}K_{315}$ Kg/ha of fertilizer combination could be recommended for potential folia vegetable cultivation. This can be a possible solution to meet up with the demand for daily consumption of burdock leaves as a very nutritive vegetable as a stimulant of appetite and as a good remedy for indigestion problems. Also, the young tender shoots harvested before flowering are used in salads. Furthermore, the application of fertilizer combinations ranging from $N_{635}P_{210}K_{315}$ kg/ha to $N_{846}P_{280}K_{315}$ kg/ha could be recommended for burdock root cultivation. This will be a possible solution to meet up with the demand for burdock roots as a source of food salad and root herbs. The roots are rich in phytochemicals with antioxidant properties, disease prevention and health-promoting properties. This may contribute to the economic growth of South Africa via the provision of medicinal plant materials to pharmaceutical industries and herbal practitioners with the possibility of job creation on the awareness of medicinal plant cultivation by communities as an alternative sector in agriculture. The cultivation of this species can also provide a potential market for vegetable production, a possible solution to food security and job creation for communities. However, more investigation needs to be done in pots and fields as well as optimization studies on secondary metabolites of interest for quality assurance and consistent availability of plant materials. This will provide a good platform for price negotiation for a better buying price of plant materials by our pharmaceutical industries and herbal practitioners.

Reference

Abebe, Z. & Feyisa, H. 2017. Effects of nitrogen rates and time of application on yield of maize: rainfall variability influenced time of N application. *International Journal of Agronomy,* 2017.

Aikins, S., Afuakwa, J. & Owusu-Akuoko, O. 2012. Effect of four different tillage practices on maize performance under rainfed conditions. *Carbon,* 1, 1-51.

Al-Zyadi, Q. A. 2018. Effect of nitrogen fertilization and harvesting date on growth of burdock plant (Arctium lappa L.) and total phenols content in leaves. *Biochemical and Cellular Archives,* 2, 2131-2134.

Benhmimou, A., Ibriz, M., Douaik, A., Lage, M., Al Faiz, C., Chaouqi, S. & Zouahri, A. 2018. Effect of NPK fertilization on the growth, yield, quality and mineral nutrition of new sweet plant in Morocco (Stevia rebaudiana Bertoni). *American Journal of Biology and Life Sciences,* 6, 36-43.

Bown, H. E., Watt, M. S., Clinton, P. W. & Mason, E. G. 2010. Influence of ammonium and nitrate supply on growth, dry matter partitioning, N uptake and photosynthetic capacity of Pinus radiata seedlings. *Trees,* 24, 1097-1107.

Cardone, L., Castronuovo, D., Perniola, M., Scrano, L., Cicco, N. & Candido, V. 2020. The influence of soil physical and chemical properties on saffron (Crocus sativus L.) growth, yield and quality. *Agronomy,* 10, 1154.

Chan, Y.-S., Cheng, L.-N., Wu, J.-H., Chan, E., Kwan, Y.-W., Lee, S. M.-Y., Leung, G. P.-H., Yu, P. H.-F. & Chan, S.-W. 2011. A review of the pharmacological effects of Arctium lappa (burdock). *Inflammopharmacology,* 19, 245-254.

Chang, H.-J., Huang, W.-T., Tsao, D.-A., Huang, K.-M., Lee, S.-C., Lin, S.-R., Yang, S.-C. & Yeh, C.-S. 2009. Identification and authentication of burdock (Arctium lappa Linn) using PCR sequencing. *Fooyin Journal of Health Sciences,* 1, 28-32.

El-Darier, S. & Sg, S. 2016. Arctium lappa L.(Asteraceae); a new invasive highly specific medicinal plant growing in Egypt. *Pyrex Journal of Plant and Agricultural Research,* 2, 44-53.

Ge, C., Radnezhad, H., Abari, M., Sadeghi, M. & Kashi, G. 2016. Effect of biofertilizers and plant growth promoting bacteria on the growth characteristics of the herb Asparagus officinalis. *Applied. Ecology. Environment Research,* 14, 547-558.

Hammed, T. B., Oloruntoba, E. O. & Ana, G. 2019. Enhancing growth and yield of crops with nutrient-enriched organic fertilizer at wet and dry seasons in ensuring climate-smart agriculture. *International Journal of Recycling of Organic Waste in Agriculture,* 8, 81-92.

Ichami, S. M., Shepherd, K. D., Sila, A. M., Stoorvogel, J. J. & Hoffland, E. 2019. Fertilizer response and nitrogen use efficiency in African smallholder maize farms. *Nutrient cycling in agroecosystems,* 113, 1-19.

Imahori, Y., Kitamura, N., Kobayashi, S., Takihara, T., Ose, K. & Ueda, Y. 2010. Changes in fructooligosaccaride composition and related enzyme activities of burdock root during low-temperature storage. *Postharvest biology and technology,* 55, 15-20.

Itaya, N. M., Oliveira, M. G. X. D., Oliveira, M. C. V. D., Porreta, C., Menão, M. C., Borges, R. M., Silva, J. R. M. C. D., Borges, J. C. S. & Knöbl, T. 2017. Prebiotic effects of inulin extracted from burdock (Arctium lappa) in broilers. *Arquivos do Instituto Biológico,* 84.

Khalil, H. A., Hossain, M. S., Rosamah, E., Azli, N., Saddon, N., Davoudpoura, Y., Islam, M. N. & Dungani, R. 2015. The role of soil properties and it's interaction towards quality plant fiber: A review. *Renewable and Sustainable Energy Reviews,* 43, 1006-1015.

Ma, Z., Bielenberg, D., Brown, K. M. & Lynch, J. P. 2001. Regulation of root hair density by phosphorus availability in Arabidopsis thaliana. *Plant, Cell & Environment,* 24, 459-467.

Maroyi, A. 2013. Traditional use of medicinal plants in south-central Zimbabwe: review and perspectives. *Journal of ethnobiology and ethnomedicine,* 9, 31.

Ncube, B., Finnie, J. & Van Staden, J. 2012. Quality from the field: the impact of environmental factors as quality determinants in medicinal plants. *South African Journal of Botany,* 82, 11-20.

Ndakidemi, P. A. & Dakora, F. D. 2007. Yield components of nodulated cowpea (Vigna unguiculata) and maize (Zea mays) plants grown with exogenous phosphorus in different cropping systems. *Australian Journal of Experimental Agriculture,* 47, 583-589.

Razaq, M., Zhang, P. & Shen, H.-L. 2017. Influence of nitrogen and phosphorous on the growth and root morphology of Acer mono. *PloS one,* 12, e0171321.

Sá Filho, M., Baldrighi, J., Sales, J., Crepaldi, G., Carvalho, J., Bó, G. & Baruselli, P. 2011. Induction of ovarian follicular wave emergence and ovulation in progestin-based timed artificial insemination protocols for Bos indicus cattle. *Animal Reproduction Science,* 129, 132-139.

Tanga, M., Lewu, F., Oyedeji, A. & Oyedeji, O. 2018. Cultivation of medicinal plants in South Africa: A solution to quality assurance and consistent availability of medicinal plant materials for commercialization. *Academia Journal of Medicinal Plants,* 6, 168-177.

Tousch, D., Bidel, L. P., Cazals, G., Ferrare, K., Leroy, J., Faucanie, M., Chevassus, H., Tournier, M., Lajoix, A.-D. & Azay-Milhau, J. 2014. Chemical analysis and antihyperglycemic activity of an original extract from burdock root (Arctium lappa). *Journal of agricultural and food chemistry,* 62, 7738-7745.

Wang, H. & Wu, W. 2013. Studies on the physiological functions of burdock inulin. *Medicinal Plant,* 4, 58.

Waraich, E. A., Ahmad, Z., Ahmad, R., Saifullah & Ashraf, M. 2015. Foliar applied phosphorous enhanced growth, chlorophyll contents, gas exchange attributes and PUE in wheat (Triticum aestivum L.). *Journal of Plant Nutrition,* 38, 1929-1943.

Williamson, L. C., Ribrioux, S. P., Fitter, A. H. & Leyser, H. O. 2001. Phosphate availability regulates root system architecture in Arabidopsis. *Plant physiology,* 126, 875-882.

Wu, J., Radnezhad, H., Loni, A., Hassanvand, A., Abari, M. & Zaremanesh, H. 2017. Effect of biofertilizers on the morphological characteristics of Burdock . *Applied Ecology and Environmental Research.,* 15, 1715-1731.

Xu, D.-P., Li, Y., Meng, X., Zhou, T., Zhou, Y., Zheng, J., Zhang, J.-J. & Li, H.-B. 2017. Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. *International journal of molecular sciences,* 18, 96.

Zapata, F. & Zaharah, A. 2002. Phosphorus availability from phosphate rock and sewage sludge as influenced by the addition of water soluble phosphate fertilizer. *Nutrient cycling in agroecosystems,* 63, 43-48.

Zhao, D., Kane, M., Borders, B. & Harrison, M. 2008. Pine growth response to different site-preparation methods with or without post-plant herbaceous weed control on North Florida's Lower Coastal Plain. *Forest Ecology and Management,* 255, 2512-2523.

CHAPTER 5
YIELD, MORPHOLOGICAL CHARACTERISTICS AND FLAVONOID CONTENT OF AMERICAN SKULLCAP (*SCUTELLARIA LATERIFLORA* L.) IN RESPONSE TO MINERAL FERTILIZER APPLICATION

5.1 Introduction

The use of plant-based products in recent years to satisfy the needs of humans has gained popularity (Maia and Moore, 2011, Beverland, 2014, Jeske et al., 2018). These demands from time immemorial keep increasing especially for those plant species with high nutritional and medicinal potentials.(Chongtham et al., 2011, Beyene et al., 2016). This is creating increasing business opportunities for some of these plant species, especially in developing countries where it is estimated that approximately 80% of the rural and urban population depend on and prefer the use of plant-based products for their livelihood (Setshogo and Mbereki, 2011, Chicho et al., 2020). However, their supplies are mostly from the wild plant population, placing significant pressure on their natural habitat, which is causing an alarm about the extinction of such species (Lewis and Elvin-Lewis, 1995, Williams et al., 2013).

The drive towards the cultivation of such species with the implementation of good management practices is necessary for consistent availability and supply of plant materials (Tanga et al., 2018) This initiative will assist in maintaining a sustainable supply of these species since they play a significant role in the local economy (Schippmann et al., 2002). The cultivation practices will enhance the production of good quality plant materials with greater uniformity as a conservation strategy recommended by conservation agencies (Hamilton, 2004, Chen et al., 2016) This will also provide awareness of their growing conditions and how they may influence the production of target secondary metabolites(Hadacek, 2002)

S lateriflora is one of such species with great potential. It is a perennial plant of the mint family, endemic to the American continent where it grows well in wet places of loamy soil with partial shade and full sun (Foster and Duke, 2000) from Canada to Florida and Westward to the British Columbia, Oregon, and New Mexico (Bergeron et al., 2005) Traditionally it is used by the native Americans in the treatment of many illnesses (Wills and Stuart, 2004). It is also used for digestion, diarrhea and rabies (Greenfield and Davis, 2004), and also for the treatment of epilepsy, cholera, and nervous tension kidney malfunction (Newall et al., 1996, Moerman, 1998). The herb is also sold as a tea in health food stores and as a tonic or in combination with other herbs such as passionflower and valerian as a sleep inducer.

The phytochemical constituents available in American Skullcap include flavonoids, tannins, phenolics, volatile oil, diterpenoids, waxes and iridoids (Wills and Stuart, 2004) The high flavonoids contents present in American Skullcap are known to be responsible for its therapeutic properties (Wills and Stuart, 2004, Joshee et al., 2013). Demonstrating its anti-inflammatory, antiviral, antithrombotic, sedative and antioxidant properties (Shang et al., 2010). The demand for this species especially in recent times for its sedative and anxiolytic properties has super passed any other categories of its therapeutic values (Brock et al., 2012). However, most of its plant's materials are mostly obtained from the wild, with silent practice on its cultivation practices which is still in the nascent stage of development in the region of its origin.

To the best of our knowledge, there is no report on the cultivation practice of this species in the African continent not alone in South Africa where the demand for its products keeps increasing (Tanga et al., 2018). Therefore, the aim of this study was to introduce the domestication of American Skullcap, investigating the influence of different fertilizer treatments on its growth and yield in the Western Cape province in the Winelands region of South Africa.

5.2 Materials and methods

5.2.1 Study Area

The study area is reported in section 4.2. 1 (Chapter 4)

5.2.2 Soil collection and analysis results

Potting soil used for this study was purchased from standard farm, a commercial company in Western Cape Province that supplies potting soil for plant nursery and propagation. Analysis of the soil was done in Bemlab, a commercial laboratory in the Western Cape Province. The results of the soil test (Table 5.1) showed that the soil is sandy, low in total nitrogen (0.353 %) and phosphorous (418 mg/Kg), with a moderate amount of potassium (3425 mg/kg) and a near to neutral pH (7.1).

Table 5.1Chemical properties of potted soil

Soil type	pH KCl	Resist Ohm	Stone V%	P mg/Kg Bray II	K mg/Kg	Cu mg/Kg	Zn mg/kg	Mn mg/Kg	B mg/kg	Fe mg/kg	C %	Total N (N %)	Na	K	Ca	Mg
Sandy	7.1	70	1	418	3425	2.0	33.4	30.3	2 .74	65	3.6	0.35	5.49	8.76	19.73	4 .47

5.2.3 Seeds collection and nursery

The seeds of American Skullcap were obtained from the company “Strictly Medicinal Seeds” (medicinal herb Grower`s in USA.S PO box 299, Williams, (541) 846-6704, www. Strictlymedicinalseeds.com. with permit N0. P0084124). The seeds were stratified in a refrigerator for one week after which they were raised in a nursery in the greenhouse using a plastic tray filled with fine sieve potting soil of particle size 0.1 to .0.25mm in diameter. The soil was kept moist by regular watering in the morning and in the evening. Full germination of sown seed occurred at four weeks. At 6 weeks the seedlings were taken outside for acclimatization for two weeks before transplanting into larger pots of 5kg soil.

5.2.4 Experimental layout and treatment application

The study was a factorial experiment of eight treatment combinations ($T_1=N_{350}P_{213}K_{213,}$ $T_2=N_{350}P_{320}K_{213,}$ $T_3=N_{525}P_{213}K_{213}$, $T_4=N_{525}P_{320}K_{213,}$ $T_5=N_{700}P_{213}K_{213}$, $T_6=N_{700}P_{320}K_{213}$, $T_7=N_{800}P_{213}K_{213,}$ and $T_8=N_{800}P_{230}K_{213}$ Kg/ha) laid out in complete randomized design replicated five times. The treatments consisted of 4 levels of nitrogen (N_{350}, N_{525}, N_{700}, and N_{800}kg/ha) of Urea 46% with 2 levels of Phosphorous (P_{213}, and P_{320}Kg/ha) of Triple superphosphate 20% and a uniform base application of potassium (K_{213}kg/ha) of potassium chloride 50%. The application of treatment was split into two equal doses. The first dose was applied during seedling transplants to 5kg potting soil, and the second dose was applied at four weeks after the application of the first dose The experiment was carried out under a 40% shade net with all protocol diligently observed.

5.2.5 Data collections

5.2.5.1 Growth parameters

The collection of data started two weeks after the first treatment application dose. Data were collected on morphological characteristics and yield parameters of the American Skullcap. At two weeks intervals, data was collected on plant height, number of leaves, number of shoots, leaf length, leaf width and chlorophyll content until maturity when the plants start flowering at 10 weeks from the transplanting date (TPD). Five collections were done before the start of flowering. Plant height leaf length and broadest leaf width were measured using a meter rule. Number of leave and shoots per plant were counted and leaf chlorophyll content was measured using a chlorophyll meter CCM-200 plus. Harvesting was done at maturity at almost 100% maturity with signs of leaf falling and total browning of plants at 16 weeks from transplanting date (TPD). During harvest, the plants were washed and data on leaf fresh weight, stem fresh weight and total fresh weight were collected and allowed to dry at room temperature for three weeks after which leaf dry weight, stem dry weight, and total dry weight were measured using a Lasec, Radwang wagi`s electronic weighing balance, Model: WLC1/A2/C/@, made in Poland (EU).

5.2.5.2 Total flavonoid content (TFC)

This was obtained through the colorimetric assay according to Elufioye et al. (2019) with slight modifications. To 1ml each of the extracts (1 mg/ml) was made up to 3 ml with methanol in a test tube. A 5 ml of distilled water, 0.2 ml of 10% aluminum chloride and 0.2 ml of 1M potassium acetate was added into the test tube, thoroughly mixed, and incubated for 30 minutes at room temperature. Thereafter, 250 µL each of the extract solutions and graded concentration of the quercetin (0.031-1.0mg/ml) was pipetted into a 96-welled microplate and the absorbance was obtained at 415 nm using a microplate reader model 680, BIO RAD made in USA. A standard curve was prepared using a graded concentration of quercetin to estimate the total flavonoid content and the results were expressed as mg quercetin equivalent per gram dry weight. The experiment was replicated three times. Total flavonoid was estimated from the standard calibration curve y= 0.0004+0.0585, R^2= 0.9983. Quercetin equivalent (QE/g) of total flavonoids in dry extract.

5.2.6 Statistical analysis

The data collected on growth and yield parameters were captured in Excel Spreadsheet. The data was analysed using a two–way ANOVA at a 95% confidence limit using SPSS software version 22. Means separation was done using Fisher`s Least Significant Difference (LSD) and ranking was carried out using Duncan`s Multiple Range Test (DMRT).

5.3 Results

5.3.1 Influence of fertilizer treatments on morphological characteristics of American Skullcap

Fertilizer treatments had a significant effect ($p<0.05$) on the growth parameters of American Skullcap (Table 5.2). T_4 demonstrated the highest influence on plant height, leaf length and number of leaves per plant. The application of T_4 significantly ($p<0.05$) increased plant height by 30.8%, leaf length (10.2%) and recorded 46.2% more leaves per plant compared to the control (T_1). Interestingly, there was a significant drop in fertilizer influence on leaf length (9.2%) and number of leaves per plant (14.5%) and a similar trend was observed for plant height with a 9.2% decrease from T_4 to T_8.

Leaf width and number of shoots per plant recorded a similar trend on fertilizer treatments. T_6 demonstrated the highest influence on leaf width (15.6%) and with 34.1% more shoots per plant increase compared to the control (T_1). A significant drop in fertilizer influence was also recorded in leaf width (10.8%) and number of shoots per plant (17.9%) from T_6 to T_7. Fertilizer treatments had no significant effect ($p.>0.05$) on leaf chlorophyll content however, the highest effect was demonstrated by T_8 with a 5.4% increase compared to the control (T_1). There was an overall

increasing trend in growth parameters from T_1 to T_4 on plant height, leaf length and number of leaves per plants and from T_1 to T_6 on leaf width and the number of shoots per plant, followed by a decreasing trend to T_8 (Table 5.2).

Table 5.2 Morphological characteristics of American Skullcap with different fertilizer treatments. Values are means at (P-0.05).

Treatments	Plant height(cm)	Number of leaves/plants	Leaf length (cm)	Leaf width (cm)	Number of shoots	Leaf chlorophyll
$T_1(N_1P_1K_1)$	14.9^{b}	119^{c}	5.9^{b}	3.2^{e}	17.0^{d}	24.2^{a}
$T_2(N_1P_2K_1)$	17.5^{ab}	133^{bc}	5.9^{b}	3.3^{de}	21.2^{abc}	24.8^{a}
$T_3(N_2P_1K_1)$	17.5^{ab}	156^{ab}	6.1^{ab}	3.4^{bcde}	21.2^{ab}	24.7^{a}
$T_4(N_2P_2K_1)$	19.5^{a}	174^{a}	6.5^{a}	3.4^{abcd}	22.5^{ab}	23.8^{a}
$T_5(N_3P_1K_1)$	18.9^{a}	162^{a}	6.5^{a}	3.5^{abc}	21.2^{abc}	23.2^{a}
$T_6(N_3P_2K_1)$	17.9^{a}	163^{a}	6.4^{ab}	3.7^{a}	22.8^{a}	25.4^{a}
$T_7(N_4P_1K_1)$	18.6^{a}	164^{a}	6.1^{ab}	3.6^{ab}	19.8^{bc}	23.9^{a}
$T_8(N_4P_2K_1)$	17.7^{a}	149^{ab}	5.9^{b}	3.3^{cde}	18.7^{cd}	25.5^{a}
LSD	2.6	13.0	0.2	0.1	0.3	NS

Means in the same column with the same superscript are not significantly difference (P≥0.05). LSD = Least significant difference, NS= Not significant, $T_1=N_{350}P_{213}K_{213}$, $T_2=N_{350}P_{320}K_{213}$, $T_3=N_{525}P_{213}K_{213}$, $T_4=N_{525}P_{320}K_{213}$, $T_5=N_{700}P_{213}K_{213}$, $T_6=N_{700}P_{320}K_{213}$, $T_7=N_{800}P_{213}K_{213}$, $T_8=N_{800}P_{230}K_{213}$ Kg/ha.

5.3.2 Influence of time of data collection on morphological characteristics of American Skullcap

The growth parameters of the American Skullcap had a progressive increase with advances in age (Fig 5.1). Plant height and the number of leaves per plant increased significantly (P<0.05) with age from the first to the fifth time of data collection. Also, leaf length and leaf width had a similar trend with progressing age. However, leaf length demonstrated a slight resilience increase from the third to the fifth time of data collection (4.5%) while leaf width recorded its maximum (60%) which was the same at the fourth and the fifth time of data collection (16 weeks). Leaf chlorophyll content had an overall progressive increase (25.2%) from the first to the fifth time of data collection. No significant (p>0.05) increase was recorded from the second to the fourth time of data collection

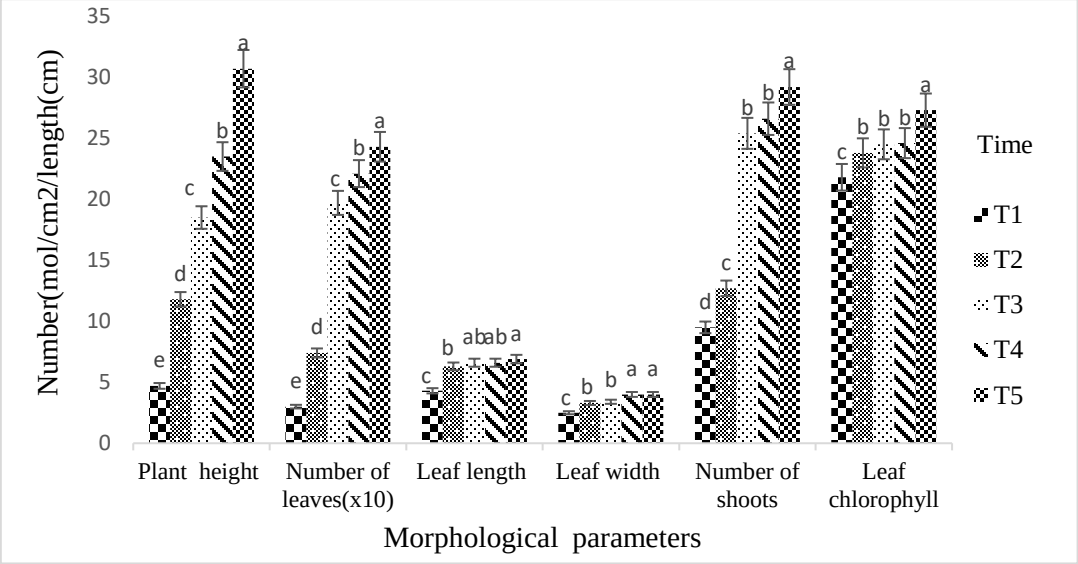


Figure 5.1 Influence of time on data collection on morphological parameters of American Skullcap. Values are means at (P-values 0.05). Bars with the same letter on each parameter are not significantly difference (P≥0.05).

5.3.3 Influence of fertilizer treatments on biomass yield of American Skullcap

The different fertilizer treatments had a significant ($p<0.05$) positive impact on all the yield-related parameters investigated on the American Skullcap (Table 4.3). Fresh leaf weight had a significant ($p<0.05$) positive increase, from T_1 to T_5. The highest weight (201.98 tons/ha) was recorded by T_5 with a 367.76 % increase, followed by T_6 (179.33 tons/ha) with a 315.31% increase compared to the control (T_1) which had the least weight (43.18 tons/ha).

Furthermore, for leaf dry weight, T_6 had the highest influence (102.76 tons /ha) with a 463.9% increase, followed by T_8 (88.76a tons/ha) with a 404.3% increase and T5 (81.84 tons/ha) with a 365% increase compared to the control (T_1) which had the least response (17.60tons/ha) to fertilizer treatments. Interestingly, there was a general similar response on fresh stem weight, leaf dry weight, stem dry weight, total fresh weight (TFW), and total dry weight (TDW) to fertilizer treatments. T_6 recorded the highest impact on weight (146.33tons /ha) with a 684.6% increase followed by T_7 (135.85 tons/ha) with a 628.4% increase which was not significant ($p>0.05$) between the treatments compared to the control for fresh stem weight.

Considering stem dry weight, total fresh weight(TFW) and total dry weight (TDW), T_6 profoundly recorded the highest impact for stem dry weight, (83.89 tons/ha) with a 370.5% increase, total fresh weight (325.68 tons/ha) with a 426.7% increase and total dry weight (186.21 tons/ha) with a 425.6% increase, followed by T_5 with stem dry weight (80.94tons/ha) with a 353.9% increase, total fresh weight (284.69 tons/ha) with a 360.4% increase and total dry weight (162.79 tons/ha) with a 359.5% increase which was not significant ($p>0.05$) among the treatments when compared with

the control (T_1). It can be suggested from this experiment that, T_5 and T_6 had the optimum NPK fertilizer treatments combination for maximum biomass yield for American Skullcap under good production practices.

Table 5.3: Influence of fertilizer treatments on biomass yield of American Skullcap. Values are means at (P-0.05).

Treatments	Leaf fresh weight (tons/ha)	Stem fresh weight (tons/ha)	Total fresh weight (tons/ha)	Leaf dry weight (tons/ha	Stem dry weight (tons/ha)	Total dry weight (tons/ha)
$T_1(N_1P_1K_1)$	43.18^{d}	18.65^{b}	61.83^{c}	17.60^{c}	17.83^{c}	35.43^{c}
$T_2(N_1P_2K_1)$	66.80^{cd}	26.43^{b}	93.21^{bc}	25.16^{bc}	23.57^{bc}	53.35^{bc}
$T_3(N_2P_1K_1)$	67.46^{cd}	27.08^{b}	94.57^{bc}	30.54^{bc}	28.19^{bc}	54.11^{bc}
$T_4(N_2P_2K_1)$	118.46^{abcd}	101.57^{ab}	220.19^{ab}	75.54^{ab}	50.41^{abc}	125.88^{ab}
$T_5(N_3P_1K_1)$	201.98^{a}	82.72^{ab}	284.69^{a}	81.84^{a}	80.94^{a}	162.79^{a}
$T_6(N_3P_2K_1)$	179.33^{ab}	146.33^{a}	325.68^{a}	102.76^{a}	83.89^{a}	186.21^{a}
$T_7(N_4P_1K_1)$	104.06^{bcd}	135.85^{a}	239.87^{a}	73.86^{ab}	54.94^{ab}	137.17^{a}
$T_8(N_4P_2K_1)$	154.23^{abc}	91.01^{ab}	227.59^{a}	88.76^{a}	66.47^{a}	143.78^{a}
LSD	23.62	34.29	31.38	7.56	5.74	11.29

Means in the same column with the same superscript are not significantly difference (P≥0.05). LSD = Least significant difference, NS= Not significant, $T_1=N_{350}P_{213}K_{213}$, $T_2=N_{350}P_{320}K_{213}$, $T_3=N_{525}P_{213}K_{213}$, $T_4=N_{525}P_{320}K_{213}$, $T_5=N_{700}P_{213}K_{213}$, $T_6=N_{700}P_{320}K_{213}$, $T_7=N_{800}P_{213}K_{213}$, $T_8=N_{800}P_{230}K_{213}$ Kg/ha.

5.3.4 Influence of fertilizer treatments on the accumulation of total flavonoid content at the harvesting stage of American Skullcap

Fertilizer treatments recorded a significant ($p<0.05$) influence on the accumulation of total flavonoid content on plant material at harvest (Fig.5.2). Treatments with lower phosphorus levels (P_1=213kg/ha) recorded a higher concentration of total flavonoid than those with a higher level of phosphorus (P_2=320kg/ha) among the different treatment combinations. However, T_3 recorded the highest concentration of total flavonoid with a 3.9% increase compared to the control (T_1) for methanol extract while T_7 recorded the highest concentration with a 7.3% increase compared to the control (T_1) for aqueous extract. Overall, methanol extract recorded the highest concentration of total flavonoid content (67.89%) followed by aqueous extract (32.1%) on leaf extracts of American Skullcap as influenced by fertilizer treatment application (Fig 5.2).

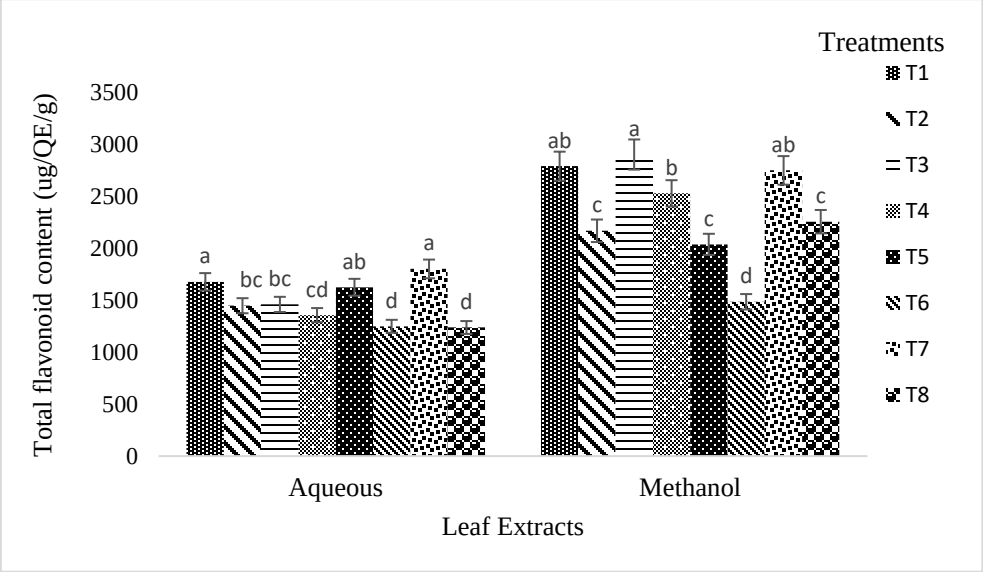


Figure 5.2 Influence of fertilizer treatments on accumulation of flavonoid content at harvesting stage of American Skullcap

5.4 Discussions

Fertilizers are a source of nutrients for plant growth and development, and for increased production and productivity in cultivated plants under good management practices. The results of this study revealed that the different treatments of NPK combinations had a significant impact on the vegetative growth parameters of *S lateriflora*. This may be attributed to the fact that plant growth and productivity of the *S. lateriflora* responded differently to different NPK treatment combinations used in this study (Ichami et al., 2019, Kwon et al., 2019). T_4 demonstrated the highest influence on plant height (30.8%), leaf length (10.2%) and, the number of leaves per plant (46.2%,) with a positive increase from the control (T_1). This may be attributed to the response to macro elements particularly nitrogen which promotes plant growth with an increased number of internodes and leaves. This response may have resulted in the progressive increase in plant height, number of leaves per plant as well as leaf length (Dikr and Belete, 2017, Kwon et al., 2019). However, a further increase in fertilizer levels negatively impacted plant height (9.2%), the number of leaves per plant (14.5%) and leaf length (9.2%). from T_4 to T_8 (Benhmimou et al., 2018). This indicates that T_4 could be a possible treatment combination for optimum growth and productivity of American Skullcap in the Winelands region of the Western Cape province in South Africa. These results are in line with those of Similien et al. (2012) who reported that the application of nutrient combinations increased the growth and yield attributes of *S. lateriflora* It is worth noting to say that a combined application of 525 kg/ha of N (urea 46%), 320 kg/ha of P (Triple supper phosphate 20%) and 213 kg/ha of K (potassium chloride 50%) may be perceived as a crucial fertilizer dose

that can ultimately boost growth and development of this species (Sakakibara et al., 2006), as primary nutrients needed for plant growth (Nedunchezhiyan, 2015, Sahoo et al., 2015). and for photosynthetic activities (Razaq et al., 2017)

Leaf width and number of shoots per plant had a similar trend. T_6 had the highest leaf width (15%) and the number of shoots (34.1%) compared to the control (T_1) (123%). This may be ascribed as the nutrient combination required to promote leaf width and the number of shoots (Mishra and Dash, 2014) of *S. lateriflora* The response of American Skullcap to various fertilizer application levels revealed the optimum nutritional requirements for optimum vegetative growth and development of the crop (Xu et al., 2020). The overall increase in leaf chlorophyll content from T_1 to T_8 may be attributed to the increase in nitrogen levels across treatment combinations known to be crucial in chlorophyll biosynthesis (Razaq et al., 2017). Also, nitrogen being a very important element in a protein molecule, plays a central role in the synthesis of plant constituents via various enzymatic activities (Khalid and Shedeed, 2015). In general, it was observed that the application of N fertilizer between (525 kg to 700 kg/ha), P (320 kg/ha), and K (315 kg/ha) can be suggested as the required fertilizer requirement for optimum growth and development and other vegetative parameters of cultivated *S. lateriflora* in the Winelands region of the Western Cape province of South Africa.

Data collected at the different time intervals had a progressive significant impact on all the vegetative growth parameters investigated with a strong positive correlation. This indicates that growth parameters are greatly influenced by plant age, nutrient availability and harvesting time across the different phenological stages of *S. lateriflora.* Generally, the 5^{th} time of data collection recorded the highest significant impact on all the vegetative growth parameters investigated across the different phenological stages. However, the 4^{th} and 5^{th} times of data collection on leaf length and leaf width were not significant for higher photosynthetic activity (Poorter and Evans, 1998, Niinemets, 2007) and maturity of *S. lateriflora*.

The yield parameters investigated in this study were significantly affected by NPK treatment combinations. T_6 recorded a higher influence, which was not significant from T_5 and T_4 except for leaf fresh weight (LFW) where the highest influence was recorded by T_5 which was also not significant with $T_{6,}$ indicating the possible NPK fertilizer treatment rang for optimum growth and yield of *Scutellaria lateriflora* L in this study. Mineral fertilizer application increases the production of medicinal plants (Liu et al., 2014, Arab et al., 2015). The application of NPK fertilizers in different combinations may possibly provide a proper nutritional balance of nitrogen, phosphorous and potassium requirements for a sustainable cultivation practice (Mishra and Dash, 2014) of American Skullcap in the Winelands region of the Western Cape Province of South Africa. In this

regard, the application of 525 to 700kg/ha of N for metabolism (Sakakibara et al., 2006), in combination with optimum amount of 320kg/ha of P for growth and development (Sadia et al., 2013) and application 213kg/ha of K for physiology and enzymatic activity (Khalid and Shedeed, 2015) can be considered for sustainable cultivation and large scale production for commercialization of American Skullcap.

NPK treatment combination recorded a significant influence on the accumulation of total flavonoid content on leaf extract of the American Skullcap. This may be attributed to the fact that fertilization induces changes in secondary metabolites accumulation in medicinal plants (Azaizeh et al., 2005). According to Gayler et al. (2008) the accumulation of secondary metabolites in plant tissues is affected by factors such as fertilizers treatment application. Also the differences in nutrient released as per treatment combinations with less supplementary phosphorus level having higher accumulation of bioactive compounds than those with more supplementary phosphorus may have led to the differences in carbon/nitrogen ratio which consequently might have resulted to the differences in metabolites accumulation (Bryant et al., 1983, Wu et al., 2013) like the case total flavonoid content at the harvesting stage of *S. lateriflora* . However, Jones et al. (2007) reported reduction of total flavonoids content in broccoli head (*Brassica oleracea* var *italica*) with high N application. Furthermore, P application also demonstrated a negative impact on the accumulation of total flavonoids content in this study, which are in line with Wu et al. (2013). Nevertheless, the overall higher concentration of total flavonoids content in methanol extraction than aqueous extraction in this study indicates that methanol extraction is more effective and reliable as it enabled easy filtration and contained more of total flavonoids than aqueous extract due to the inability of the plant materials been able to completely dissolve in it (Ajuru et al., 2017). Moreover, aqueous extract usually goes bad because it encourages bacterial growth and decay of the plant extract which may have led to the loss of some of the flavonoids which may be present in the methanol extract.

5.5 Conclusion

This is the first report on the establishment and cultivation practice of American Skullcap (*Scutellaria lateriflora* L) in the Winelands region of the Western Cape Province of South Africa. Results of the study revealed that NPK treatment combinations had a significant impact on the vegetative and yield parameters investigated. The overall picture indicates that T_4 to T_6 fertilizer range (N_{525}-N_{700} with P_{213}-P_{320} and K_{213}Kg/ha) demonstrated higher influence across the different parameters of the *S. lateriflora* investigated. It can therefore be suggested from this reciprocated higher yield response that T_4 to T_6 fertilizer applications are the optimum NPK fertilizer treatment combinations required for higher biomass yield *S. lateriflora* under good production practices in

the Winelands region of the Western Cape Province of South Africa. The yield of bioactive compounds responded to fertilizer treatments which significantly affected the accumulation of total flavonoid content (TFC). Overall, it was observed that a shift in N increase was in response to P across treatment combinations. Treatments with P_{213} recorded a higher concentration of TFC compared to those with P_{320} among the different treatment combinations. Furthermore, the highest concentration of TFC was observed in methanol extract than in aqueous extract, indicating that the solvent had the best relative polarity for the extraction of bioactive compounds of this species. In conclusion, further investigation on the cultivation practices of this species needs to be tested in the field as well as optimization measures of bioactive compounds for the supply of quality plant materials for commercialization.

References

Ajuru, M. G., Williams, L. F. & Ajuru, G. 2017. Qualitative and quantitative phytochemical screening of some plants used in ethnomedicine in the Niger Delta Region of Nigeria. *Journal of Food and Nutrition Sciences,* 5, 198-205.

Arab, A., Zamani, G. R., Sayyari, M. H. & Asili, J. 2015. Effects of chemical and biological fertilizers on morpho-physiological traits of marigold (Calendula officinalis L.). *European Journal of Medicinal Plants*, 60-68.

Azaizeh, H., Ljubuncic, P., Portnaya, I., Said, O., Cogan, U. & Bomzon, A. 2005. Fertilization-induced changes in growth parameters and antioxidant activity of medicinal plants used in traditional Arab medicine. *Evidence-Based Complementary and Alternative Medicine,* 2, 549-556.

Benhmimou, A., Ibriz, M., Douaik, A., Lage, M., Al Faïz, C., Chaouqi, S. & Zouahri, A. 2018. Morocco (Stevia rebaudiana Bertoni). *American Journal of Biology and Life Sciences,* 6, 36-43.

Bergeron, C., Gafner, S., Clausen, E. & Carrier, D. J. 2005. Comparison of the chemical composition of extracts from Scutellaria lateriflora using accelerated solvent extraction and supercritical fluid extraction versus standard hot water or 70% ethanol extraction. *Journal of agricultural and food chemistry,* 53, 3076-3080.

Beverland, M. B. 2014. Sustainable eating: mainstreaming plant-based diets in developed economies. *Journal of Macromarketing,* 34, 369-382.

Beyene, B., Beyene, B. & Deribe, H. 2016. Review on application and management of medicinal plants for the livelihood of the local community. *Journal of Resources Development and Management,* 22, 33-39.

Brock, C., Whitehouse, J., Tewfik, I. & Towell, T. 2012. The use of Scutellaria lateriflora: a pilot survey amongst herbal medicine practitioners. *Journal of Herbal Medicine,* 2, 34-41.

Bryant, J. P., Chapin Iii, F. S. & Klein, D. R. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*, 357-368.

Chen, S.-L., Yu, H., Luo, H.-M., Wu, Q., Li, C.-F. & Steinmetz, A. 2016. Conservation and sustainable use of medicinal plants: problems, progress, and prospects. *Chinese medicine,* 11, 37.

Chicho, N., Chanda, R. & Musisi-Nkambwe, S. 2020. Medicinal plants harvesting practices and implications for conservation: insights from the Palapye urban-rural continuum, Botswana. *Indilinga African Journal of Indigenous Knowledge Systems,* 19, 216-231.

Chongtham, N., Bisht, M. S. & Haorongbam, S. 2011. Nutritional properties of bamboo shoots: potential and prospects for utilization as a health food. *Comprehensive Reviews in Food Science and Food Safety,* 10, 153-168.

Dikr, W. & Belete, K. 2017. Review on the effect of organic fertilizers, biofertilizers and inorganic fertilizers (NPK) on growth and flower yield of marigold (Targets' erecta L.). *J. Agri. Sci. Res,* 5, 192-204.

Elufioye, T. O., Olusola, D. M. & Oyedeji, A. O. 2019. Correlation of total phenolic, flavonoid and tannin content of Bryophyllum pinnatum (Lam.)(crassulaceae) extract with the antioxidant and anticholinesterase activities. *Pharmacognosy Journal,* 11.

Foster, S. & Duke, J. 2000. Medicinal plants and herbs of Eastern and Central North America. Houston Mifflin Co. NY.

Gayler, S., Grams, T., Heller, W., Treutter, D. & Priesack, E. 2008. A dynamical model of environmental effects on allocation to carbon-based secondary compounds in juvenile trees. *Annals of Botany,* 101, 1089-1098.

Greenfield, J. & Davis, J. 2004. Medicinal Herb Production Guides. *North Carolina Consortium on Natural Medicines and Public Health. North Carolina State University, Raleigh, NC*.

Hadacek, F. 2002. Secondary metabolites as plant traits: current assessment and future perspectives. *Critical Reviews in Plant Sciences,* 21, 273-322.
Hamilton, A. C. 2004. Medicinal plants, conservation and livelihoods. *Biodiversity & Conservation,* 13, 1477-1517.
Ichami, S. M., Shepherd, K. D., Sila, A. M., Stoorvogel, J. J. & Hoffland, E. 2019. Fertilizer response and nitrogen use efficiency in African smallholder maize farms. *Nutrient cycling in agroecosystems,* 113, 1-19.
Jeske, S., Zannini, E. & Arendt, E. K. 2018. Past, present and future: The strength of plant-based dairy substitutes based on gluten-free raw materials. *Food Research International,* 110, 42-51.
Jones, R., Imsic, M., Franz, P., Hale, G. & Tomkins, R. 2007. High nitrogen during growth reduced glucoraphanin and flavonol content in broccoli (Brassica oleracea var. italica) heads. *Australian Journal of Experimental Agriculture,* 47, 1498-1505.
Joshee, N., Tascan, A., Medina-Bolivar, F., Parajuli, P., Rimando, A., Shannon, D. & Adelberg, J. 2013. Scutellaria: biotechnology, phytochemistry and its potential as a commercial medicinal crop. *Biotechnology for medicinal plants.* Springer.
Khalid, K. A. & Shedeed, M. R. 2015. Effect of NPK and foliar nutrition on growth, yield and chemical constituents in Nigella sativa L. *Journal. Material. Environmental. Science,* 6, 1709-1714.
Kwon, S.-J., Kim, H.-R., Roy, S. K., Kim, H.-J., Boo, H.-O., Woo, S.-H. & Kim, H.-H. 2019. Effects of nitrogen, phosphorus and potassium fertilizers on growth characteristics of two species of bellflower (Platycodon grandiflorum). *Journal of Crop Science and Biotechnology,* 22, 481-487.
Lewis, W. H. & Elvin-Lewis, M. P. 1995. Medicinal plants as sources of new therapeutics. *Annals of the Missouri Botanical Garden*, 16-24.
Liu, C.-W., Sung, Y., Chen, B.-C. & Lai, H.-Y. 2014. Effects of nitrogen fertilizers on the growth and nitrate content of lettuce (Lactuca sativa L.). *International journal of environmental research and public health,* 11, 4427-4440.
Maia, M. F. & Moore, S. J. 2011. Plant-based insect repellents: a review of their efficacy, development and testing. *Malaria journal,* 10, 1-15.
Mishra, P. & Dash, D. 2014. Rejuvenation of biofertilizer for sustainable agriculture and economic development. *Consilience*, 41-61.
Moerman, D. E. 1998. *Native american ethnobotany*, Timber press Portland, OR.
Nedunchezhiyan, M. 2015. Growth and yield of elephant foot yam under integrated nutrient management (INM) in alfisols. *Journal of Root Crops,* 41, 59-64.
Newall, C. A., Anderson, L. A. & Phillipson, J. D. 1996. *Herbal medicines. A guide for health-care professionals*, The pharmaceutical press.
Niinemets, U. 2007. Photosynthesis and resource distribution through plant canopies. *Plant, Cell & Environment,* 30, 1052-1071.
Poorter, H. & Evans, J. R. 1998. Photosynthetic nitrogen-use efficiency of species that differ inherently in specific leaf area. *Oecologia,* 116, 26-37.
Razaq, M., Zhang, P. & Shen, H.-L. 2017. Influence of nitrogen and phosphorous on the growth and root morphology of Acer mono. *PloS one,* 12, e0171321.
Sadia, A., Mahasen, M., Shahrin, S., Roni, M. & Uddin, A. 2013. Phsophorus levels on growth and yield of turnip (Brassica campestris var. rapifera). *Bangladesh Research Publications Journal,* 8, 29-33.
Sahoo, B., Nedunchezhiyan, M. & Acharyya, P. 2015. Productivity potential of elephant foot yam (Amorphophallus paeoniifolius (Dennst.) Nicolson) in alfisols as influenced by fertility levels. *The Bioscan,* 10, 1255-7.
Sakakibara, H., Takei, K. & Hirose, N. 2006. Interactions between nitrogen and cytokinin in the regulation of metabolism and development. *Trends in plant science,* 11, 440-448.

Schippmann, U., Leaman, D. J. & Cunningham, A. 2002. Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues. *Biodiversity and the ecosystem approach in agriculture, forestry and fisheries*.

Setshogo, M. P. & Mbereki, C. M. 2011. Floristic diversity and uses of medicinal plants sold by street vendors in Gaborone, Botswana.

Shang, X., He, X., He, X., Li, M., Zhang, R., Fan, P., Zhang, Q. & Jia, Z. 2010. The genus Scutellaria an ethnopharmacological and phytochemical review. *Journal of ethnopharmacology,* 128, 279-313.

Similien, A., Shannon, D. A., Wood, C. W., Van Santen, E., Joshee, N. & Foshee, W. G. 2012. Partial Shade, Irrigation, and Added Nutrients Maximize Dry Matter Yield of American Skullcap (Scutellaria lateriflora L.). *HortScience,* 47, 1705-1709.

Tanga, M., Lewu, F., Oyedeji, A. & Oyedeji, O. 2018. Cultivation of medicinal plants in South Africa: A solution to quality assurance and consistent availability of medicinal plant materials for commercialization. *Academia Journal of Medicinal Plants,* 6, 168-177.

Williams, V., Victor, J. & Crouch, N. 2013. Red listed medicinal plants of South Africa: status, trends, and assessment challenges. *South African Journal of Botany,* 86, 23-35.

Wills, R. B. H. & Stuart, D. L. 2004. *Generation of high quality Australian skullcap products*, Citeseer.

Wu, C.-S., Gao, Q.-H., Kjelgren, R. K., Guo, X.-D. & Wang, M. 2013. Yields, phenolic profiles and antioxidant activities of Ziziphus jujube Mill. in response to different fertilization treatments. *Molecules,* 18, 12029-12040.

Xu, F., Chu, C. & Xu, Z. 2020. Effects of different fertilizer formulas on the growth of loquat rootstocks and stem lignification. *Scientific reports,* 10, 1-11.

CHAPTER 6
PHYTOCHEMICAL CONSTITUENTS, ANTIOXIDANT PROPERTIES AND ANTI-INFLAMMATORY ACTIVITIES OF AMERICAN SKULLCAP (*SCUTELLARIA LATERIFLORA* L) IN RESPONSE TO MINERAL FERTILIZER APPLICATION

6.1 Introduction

The considerable role played by plants as a source of medicine, food, and agrochemicals in the livelihood of humans are due to the presence of the many phytochemicals present in plant tissues through their different metabolic pathways (Sen and Samanta, 2014). This has created a growing awareness since most people nowadays are becoming more health conscious. They tend to spend more of their income on natural products for food security and health-related issues due to their high antioxidant activities reciprocated by the presence of many phytochemicals with high biological activities (Yuliana et al., 2011).

American skullcap (*S lateriflora*) is one of those medicinal plant species that are been used for centuries in the American continent as a therapy for anxiety, nervous tension, convulsion, and as a mild relaxant (Foster and Duke, 2000). The aerial part is been used by the American herbalist to prepare medicine for the treatment of cholera, epilepsy, diarrhea and to resolve problems of indigestion in humans (Greenfield and Davis, 2004). Information on its medicinal potential has been disseminated in the different regions of the world where it is sold in health food shops as herbal tea. However, the main source of its supply depend mostly on the wild plant population, with fewer cases of domestication practices in the field and horticultural production (Eraso et al., 2007). The domestication of American Skullcap has been investigated in pot experiments looking at the influence of different fertilizer treatments on biomass yield and flavonoid content as one of those several factors which influences yield-related parameters and production of phytochemical constituent (Shiwakoti et al., 2016).

Fertilizer application is known to produce changes in growth parameters, chemical composition, and the antioxidant activities of medicinal plants (Azaizeh et al., 2005). Some metabolites may accumulate in response to different stress levels of fertilizer application and treatment regimens (Dixon and Paiva, 1995, Sharma et al., 2019). The fluctuation in the yield of phytochemicals constituents in medicinal plants may cause a great change in the metabolomics profile thereby compromising the quality and quantity of the bioactive compounds.(Szakiel et al., 2011, Prinsloo and Nogemane, 2018). Hence, influences the bioactivity of the plant in response to the fertilizer treatment levels (Hussain et al., 2018). The variations in chemical composition and bioactivity due to fertilizer treatments may also be attributed to changes in soil fertility, management practices,

and as well as the different phenological stages of development in medicinal species (Yang et al., 2018, Yin-Jheng et al., 2021).

The variation in cultural practices may lead to the loss of increase in the accumulation of some bioactive compounds, which may result in complications in the validation of medicinal efficacy (Pereira and Bartolo, 2016). The growing of this species will provide standardized plant materials with not only a constant concentration of the bioactive compounds but with less variation in its biological activity. Therefore, providing a consistent supply of plant materials of good yield and standardized quality. In these regards, the study was perceived to investigate the influence of different fertilizer applications on the yield of phytochemical constituents and the impact on biological activities of cultivated American Skullcap in the Winelands region of the Western Cape Province of South Africa.

6.2 Materials and methods

6.2.1 Study sites

The details on study side, experimental design, treatment combinations, management practices, source and harvesting of plant materials please refer to section 4.2 1(Chapter Four)

6.2.2 Chemicals

Folin-ciocalteu reagent (Fluka Biochemical), Sodium carbonate (Sigma Aldrich), Gallic acid, Aluminum chloride, Potassium acetate, Vanillin (Fluka Biochemical), Hydrochloric acid, Quercetin, 2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH) (Sigma Aldrich), Ascorbic acid, DDM, Sodium nitroprusside (Thomas baker chemical limited), Naphthalene diamine dichloride (Sigma-Aldrich) Methanol analytical grade, sulfanilic acid reagent (sigma reagent) and Hydrogen peroxide of analytical grade were used for the study.

6.2.3 Samples preparation and extraction

Fresh leaves of American Skullcap were harvested from the experimental plots described in Chapter Five. The well-dried leaves were ground to powder using an electric grinding machine POLYMIX (PX-MFC (90D) made in Switzerland by KINEMATICA. AG at 1.0 mm mesh net. 100g of the grounded leaves from each of the different treatments were weighed and macerated in methanol (analytical grade) and in distilled water for 72hours in a beaker. The extracts were filtered with Whitman's filter paper using suction pressure. Concentrated Methanolic extract was prepared using a rotary evaporator and allowed to dry under cold dry conditions, while the aqueous extract was allowed to dry at room temperature. The weight of extracts was taken to determine the percentage yield of extract per 100g of sample.

6.2.4 Phytochemical determination

Leaves samples obtained from plant materials as described in Chapter four were used to prepare aqueous extract for phytochemical determination. Extracts were screened for the presence of phenol, flavonoids, tannins, terpenoids, saponins, alkaloids, steroids, and glucosides. Foam test was used for saponins, ferric chloride test for tannins, NaOH with dilute hydrochloric acid for flavonoids, Ferric chloride solution for phenol, Dragendoff`s and Meyer`s reagent for alkaloids, chloroform and acetic acid anhydride with the addition of concentrated sulphuric acid for steroids, glacial acetic acid with drops of FeCl3 and concentrated sulphuric acid for glycosides, chloroform with acetic acid anhydride and concentrated sulphuric acid for terpenoids as fully described by (Hossain et al., 2013, Ajuru et al., 2017).

6.2.5 Determination of total phenolic content (TPC)

The total phenolic content (TPC) of leaf and root extracts for the different treatments was determined using the Folin-Ciocalteu assay method (Jimoh et al., 2019) with slight modifications. To 0.5ml each of the extract and standard (1 mg/ml) in separate test tubes, 2.5 ml of 10% Folin–Ciocateus`s reagent was added. Thereafter 2ml of sodium carbonate (7.5% w/v) was mixed and incubated at 40^0C for 30 minutes. Thereafter the 250 ul each of the extract solutions and graded concentration of gallic acid was pipetted into a 96-welled microplate and absorbance was measured at 750 nm with the aid of microplate reader model 680, BIORAD made in USA. A blank was prepared with distilled water. A standard curve was prepared to estimate the total phenolic content using gallic acid at concentration range (0.031-1.0 mg/ml). The total phenolic content was expressed as mg gallic acid equivalent (GAE)/g dry matter from the standard curve y=0.0019X +0.4962, R^2=0.9951. All experiments were carried out in triplicates.

6.2.6 Determination of total flavonoid content

The total flavonoid content was determined by colorimetric assay (Elufioye et al., 2019) with slide modifications. A 1 ml of leaf and root extracts (1 mg/ml) was made up to 3ml with methanol in test tube. 5ml of distilled water, 0.2 ml of 10% aluminum chloride and 0.2 ml of 1 M potassium acetate was added into the test tube and the whole mixture was mixed and incubated for 30 minutes at room temperature. Thereafter, 250 ul each of the extract solutions and graded concentration of the quercetin (0.031-1.0mg/ml) was pipetted into a 96-welled microplate and the absorbance was measured at 415 nm against the blank using a micro plate reader model 680, BIO RAD made in USA. A standard curve was prepared using graded concentration of quercetin to estimate the total flavonoid content and the results expressed as mg quercetin equivalent per gram dry weight from the calibration curve y= 0.0004+0.0585, R^2= 0.9983. All experiments were carried out in triplicate.

6.2.7 Determination of proanthocyanidin content (Condense Tannin)

The proanthocyanidin content was determined according to the previously reported procedure (Elufioye et al., 2019). 0.5 ml of leaf and root extracts (1mg/ml) was mixed with 3 ml of vanillin (4%) in methanol solution. Thereafter, 1.5ml of hydrochloric acid was added, mixed thoroughly, and incubated for 15 minutes at room temperature. Thereafter, the 250 ul each of the extract solutions and graded concentration of the gallic acid were pipetted into a 96-welled microplate and absorbance was measured at 515 nm with the aid of the microplate reader model 680, BIO RAD made in USA. A blank was prepared with distilled water. A standard curve of gallic acid at a concentration range (0.031-1.0 mg/ml) was used to determine the proanthocyanidin content expressed as mg gallic acid equivalent per day weight from the calibration curve $y=0.003X+0.0373$, $R^2=0.9964$ All the experiments were carried out in triplicates.

6.2.8 Proximate analysis of leaf samples

Proximate analysis was done on dry ground leaf samples from the different treatments. Sulphuric acid and sodium hydroxide was used for crude fibre, Diethyl ether for crude lipid and incineration at 550^{o}C for ash content using the method of (Aoac, 2005). Protein was analysed using the laboratory Equipment Corporation (LECO nitrogen and protein analyzer model 630-100-200). Made in USA (St. Joseph M.I 49085-2396 LECO cooperation 300 Lakeview Awe). The analysis was replicated five times and the carbohydrate content was determined using estimation by difference [by subtracting the total ash content, crude lipid, crude protein and crude fibre from 100] (Aoac, 2005).

6.2.9 Evaluation of antioxidant activity

The antioxidant activities of the leaf and root extracts were estimated using DPPH free radical scavenging capacity, nitric oxide (NO) inhibitory activity and hydrogen peroxide scavenging activity methods.

6.2.9.1 DPPH free radical scavenging assay

The 2,2- diphenyl-1picrythydrazyl hydrate (DPPH) free radical scavenging capacity of aqueous and methanolic leaf and root extracts of the different treatments were estimated (Elufioye et al., 2019). Thereafter, 1 ml (mg/ml) of each of the extracts of graded concentrations (0.031 mg/ml-1.0 mg/ml), and 1ml of 0.1 mM DPPH were thoroughly mixed and incubated for 30 minutes in the dark at room temperature. Thereafter, 250 ul of the mixture was pipetted in triplicates into a 96-welled microplate and, absorbance was measured at 515nm against the control containing 1ml of methanol with the aid of a microplate reader model 680-BIORAD, made in USA. Ascorbic acid and DDM were used as standard drugs over concentration range (0.031 mg/ml 1.0mg/ml). The DPPH radical percentage scavenging activity was calculated as follows:

$$\%\text{Inhibition of DPPH radical} = [(A_{control} - A_{test}) / A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of the control reactions (containing all reagents except the test Compound). while A_{test} is the absorbance of the test compound.

6.2.9.2 Nitric oxide scavenging assay

The Nitric oxide scavenging activity of leaf and root extracts of the different treatments was determined according to Jimoh et al. (2019). In this protocol, 2 ml of sodium nitroprusside (10 mM) was prepared in a phosphate buffer saline (0.5 mM) of pH 7.4. was mixed with 0.5 ml of the plant extract. The mixture was incubated at 25^{o}C for 2 hours 30 minutes. After which 0.5 ml of the incubated solution was pipetted and mixed with 0.5 ml Griess reagent containing 0.33% sulphanilamide dissolved in 20% glacial acetic acid and mixed with 1ml of naphthylethylenediamine chloride (0.1%w/v) and incubated at room temperature for 30 minutes. Thereafter, 250 ul of the mixture was pipetted into a 96-welled microplate, and absorbance was measured at 540 nm against the control. Sodium nitroprusside mixed with methanol was used as the negative control. All experiments were done in triplicates of graded concentration (0.031 mg/ml 1.0 mg/ml). The percentage scavenging activity of nitric oxide was calculated as follows:

$$\%\ \text{Scavenged [NO]} = [(A_{control} - A_{test}) / A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of the control reactions (containing all reagents except the test Compound). while A_{test} is the absorbance of the test compound.

6.2.9.3 Hydrogen peroxide scavenging assay

The scavenging activity of hydrogen peroxide of the leaf and root extracts of the different concentrations was determined using the method of Okeleye et al. (2015). A 4 ml of the different extracts prepared in distilled water (mg/ml) of graded concentration (0.031 mg/ml 1.0mg/ml) was mixed with 0.6 ml of hydrogen peroxide solution (4 Mm) prepared in a phosphate buffer (0.1 M: pH 7.4) and incubated at room temperature for 10 minutes. Thereafter, 250 ul of the mixture was pipetted into a 96-welled microplate and absorbance was measured at 405 against the control (Hydrogen peroxide and methanol as negative control) using a microplate reader model 680-BIORAD, made in USA. The percentage of hydrogen peroxide scavenged by the extract was calculated as follows:

$$\%\ \text{Scavenged } [H_2O_2] = [(A_{control} - A_{test}) / A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of the control reactions (containing all reagents except the test compound) while A_{test} is the absorbance of the test compound.

6.2.9.4 Anti-inflammatory Assay in vitro

The anti-inflammatory study was carried out using the method of Chandra et al. (2012) in which 0.2 ml of the egg albumin from the fresh egg was mixed with 2.8 ml of phosphate buffer saline (PBS, pH 6.4) and 2 ml of the test samples or standard drug. The mixture was incubated at 37^{o}C for 15 minutes after which it was boiled in a water bath at 70^{o}c for 5 minutes. The mixture was allowed to cool for 15 minutes. Thereafter, 250 ul of the mixture was pipetted in a 96-welled microplate and absorbance measured at 655 nm using a microplate reader model 680-BIORAD, made in USA, against the reference drug (Diclofenac) at a graded concentration (0.031mg/ml - 1.0mg/ml) and distilled water as the control. The experiment was carried out in triplicates. The percentage inhibition of protein denaturation was calculated using the formula as follows:

$$\% \text{ Inhibition} = [V_{test} / C_{control} - 1] \times 100$$

Where, V_{test} = the absorbance of the test sample, $V_{control}$ = absorbance of the control. The extract concentration for 50% inhibition (IC50) was determined by the dose-response curve.

6.2.10 Data analysis

The data on phytochemical constituents and antioxidant activities were captured in excel and IC_{50} values were calculated. The data was then statistically analysed using the procedures reported in section 5.2. (Chapter Five).

6.3 Results

6.3.1 Phytochemical screening of leaf samples of American Skullcap in response to fertilizer treatments

The screening result of phytochemical constituents of aqueous leaf extract for cultivated American Skullcap in response to fertilizer treatments is presented in (Table 6.1). A more positive (++) result was obtained for flavonoids among the treatments while a positive (+) result was obtained for tannins, saponins, phenols, alkaloids, terpenoids and steroids. However, negative (–) results were recorded on glycosides across treatment combinations.

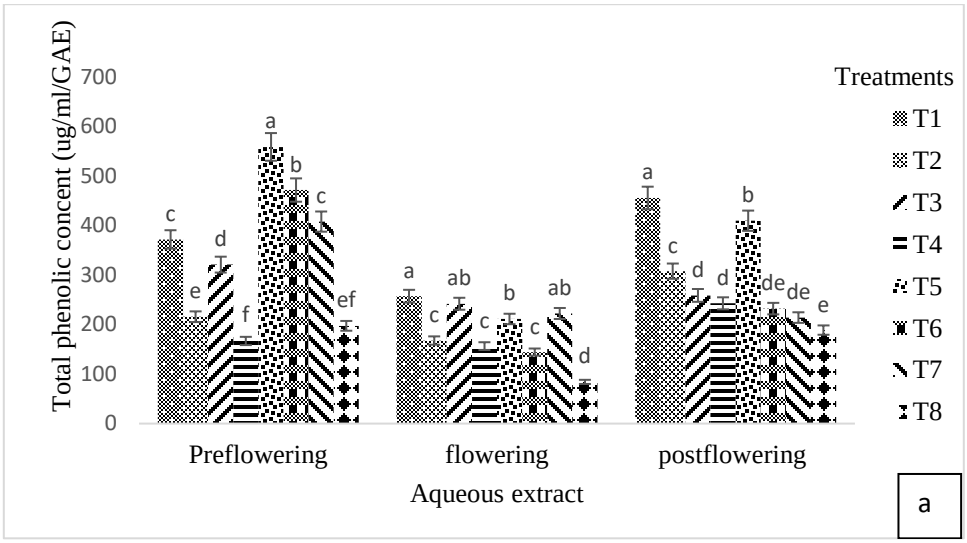
Table 6.1 Phytochemical screening of aqueous leaf extract of American Skullcap (*Scutellaria lateriflora* L.) in response to fertilizer treatments

Trts	Tannins	Saponins	Flavonoids	Phenols	Alkaloids	Glycosides	Terpenoids	**S**teroids
T_1	+	+	++	+	+	-	+	+
T_2	+	+	++	+	+	-	+	+
T_3	+	+	++	+	+	-	+	+
T_4	+	+	++	+	+	-	+	+
T_5	+	+	++	+	+	-	+	+
T_6	+	+	++	+	+	-	+	+
T_7	+	+	++	+	+	-	+	+
T_8	+	+	++	+	+	-	+	+

Legend (++) Highly present, (+) present (–) absent, Trts= treatments, T_1=$N_{350}P_{213}K_{213}$, T_2=$N_{350}P_{320}K_{213}$, T_3=$N_{525}P_{213}K_{213}$, T_4=$N_{525}P_{320}K_{213}$, T_5=$N_{700}P_{213}K_{213}$, T_6=$N_{700}P_{320}K_{213}$, T_7=$N_{800}P_{213}K_{213}$, T_8=$N_{800}P_{230}K_{213}$ Kg/ha.

6.3.2 Influence of fertilizer treatments on yield of total phenolic content (TPC) at the different phenological stage of American Skullcap. Values are means ((µg/mL GAE/g) at (P-0.05).

Fertilizer treatments recorded a significant ($p<0.05$) influence on the concentration of total phenolic content (TPC) on leaves harvested at the different phenological stages of *S. lateriflora*. The highest concentration of total phenolic content was recorded in the post -lowering stage (41.65%) followed by the pre-flowering stage (33.83%) and the least at the flowering stage (24.52%) across the different phenological stages of development. Treatment combinations with the least supplementary phosphorous (P_1=213kg/ha) recorded a higher accumulation of total phenolic content than those with higher supplementary phosphorous ($P_{2=}$320kg/ha). Furthermore, the highest concentration of total phenolic content was recorded by T_1 (18.44%) and the least by T_2 (9.52%) among the different treatment combinations across the different phenological stages of development. Overall, methanol extract recorded a higher concentration of total phenolic content (55.21%) followed by aqueous extract (44.79%) among the different treatment combinations and crop phenology (Fig: 6.1.a & b).



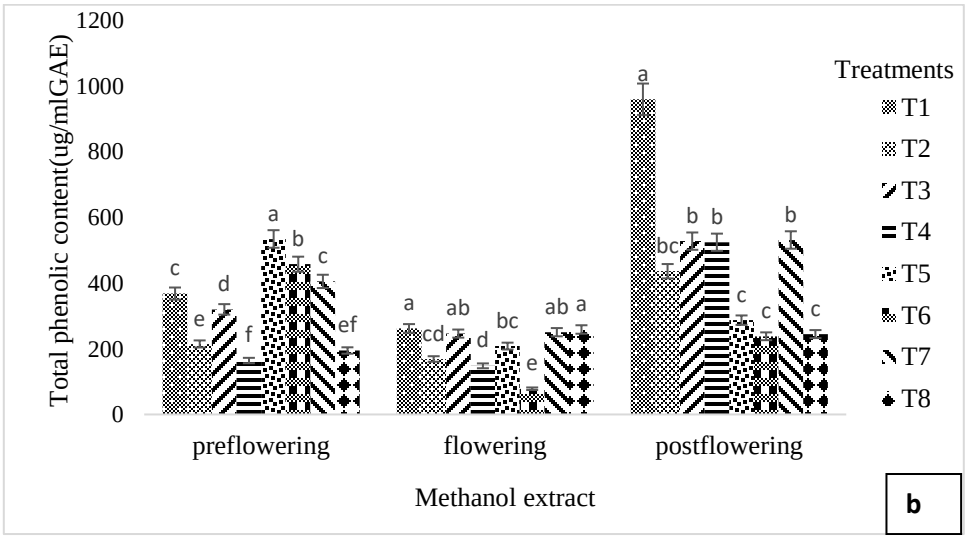


Figure 6.1 a & b: Accumulation of total phenolic content (TPC) at the different phenological stages of development of American Skullcap. Values are means (µg/mL QE/g) at (P-0.05).

6.3.3 Influence of fertilizer treatments on yield of total flavonoid content (TFC) at the different phenological stage of American Skullcap

Fertilizer treatments recorded a significant ($p<0.05$) influence on the accumulation of total flavonoid on plant material harvested at the different phenological stages of development. The highest accumulation of total flavonoid content was recorded in the post-flowering stage (38.2%) followed by the pre-flowering stage (34.2%) and the least at the flowering stage (27.6%). Similarly, the treatments with P_1 (213kg/ha) recorded a higher accumulation of flavonoid content than those with P_2 (320kg/ha) among the different treatment combinations. T_7 recorded the highest accumulation of total flavonoid content with 18.2% increase followed by T_3 (123%) increase compared to the control (T_1) across the different phenological stages. Nevertheless, T_1 recorded the highest accumulation of total flavonoid content at the flowering stage followed by T_7 which was

not significant (P.0.05) among treatment combinations. Overall, methanol extract recorded the highest total flavonoid content (67.9%) followed by aqueous extract (32.1%) among treatment combinations and phenological stages of this species (Fig 6.2. a & b).

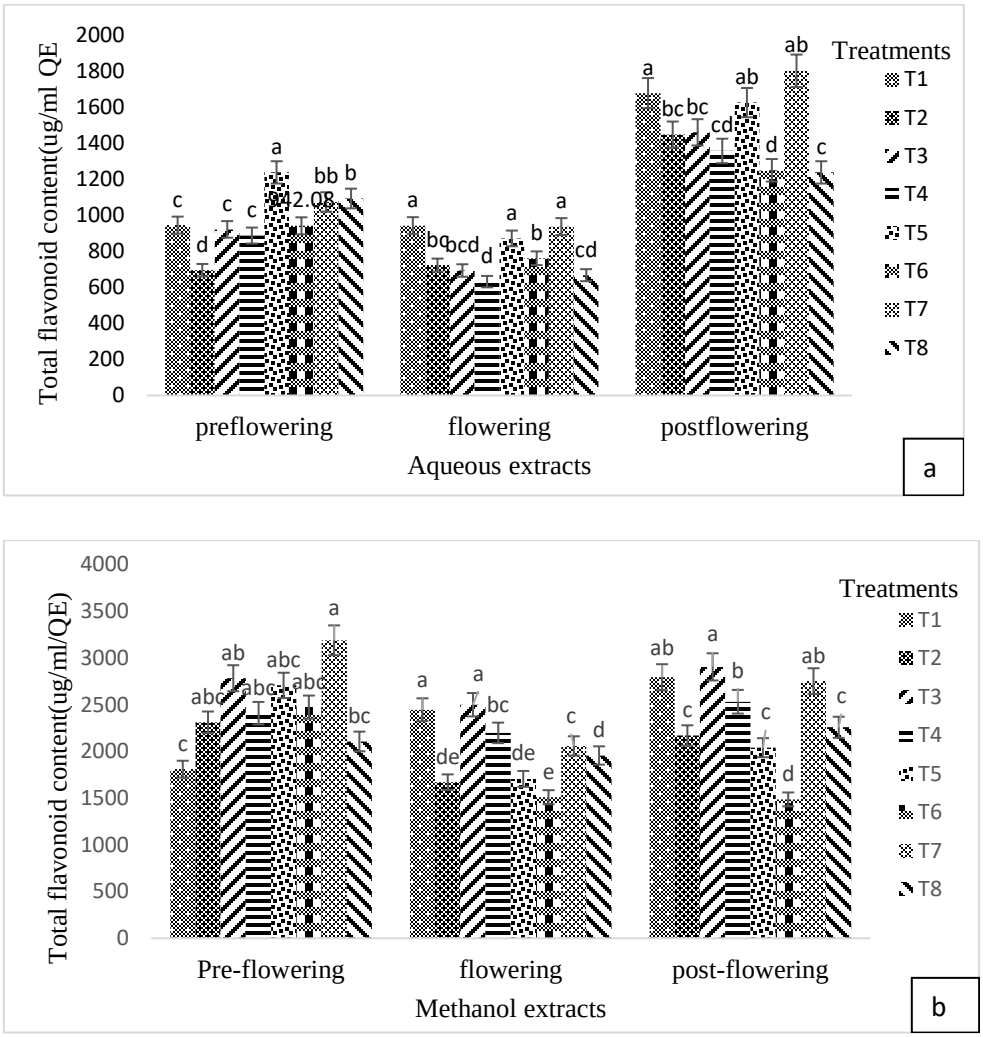


Figure 6.2 a & b: Acummulation of total flavonoids content (TFC) at different phenological stages of development of American Skullcap. Values are means (µg/mL QE/g) at (P-0.05).

6.3.4 Influence of fertilizer treatments on yield of condense tannins (PAC) at the different phenological stages of American Skullcap

Fertilizer treatments also recorded a significant ($p<0.05$) difference in the accumulation of condensed tannins (PAC) at different phenological stages of development except for the methanol extract at the post-flowering stage. Accumulation of condensed tannins was highest at the pre-flowering stage (38.5%) followed by the post-flowering stage (31.2%) and the least at the flowering stage (30.3%) in both aqueous and methanol extraction among the different treatment combinations. Similarly, treatments with the least supplementary phosphorus recorded a higher

concentration of condensed tannins than those with higher supplementary phosphorous in the case of total phenolic and flavonoid content. T_7 recorded the highest concentration of condensed tannins with a 2.7% increase compared to the control (T_1) indicating a more promising treatment combination for accumulation of proanthocyanidin for the management effect of fertilizer treatments on the yield of proanthocyanidin on this species. Overall, methanol extract recorded a higher concentration (56.7%) than aqueous extract (43.3%) among the treatment combination across the different phenological stages of development of this species in the Winelands region of the Western Cape province of South Africa (6.3 a & b).

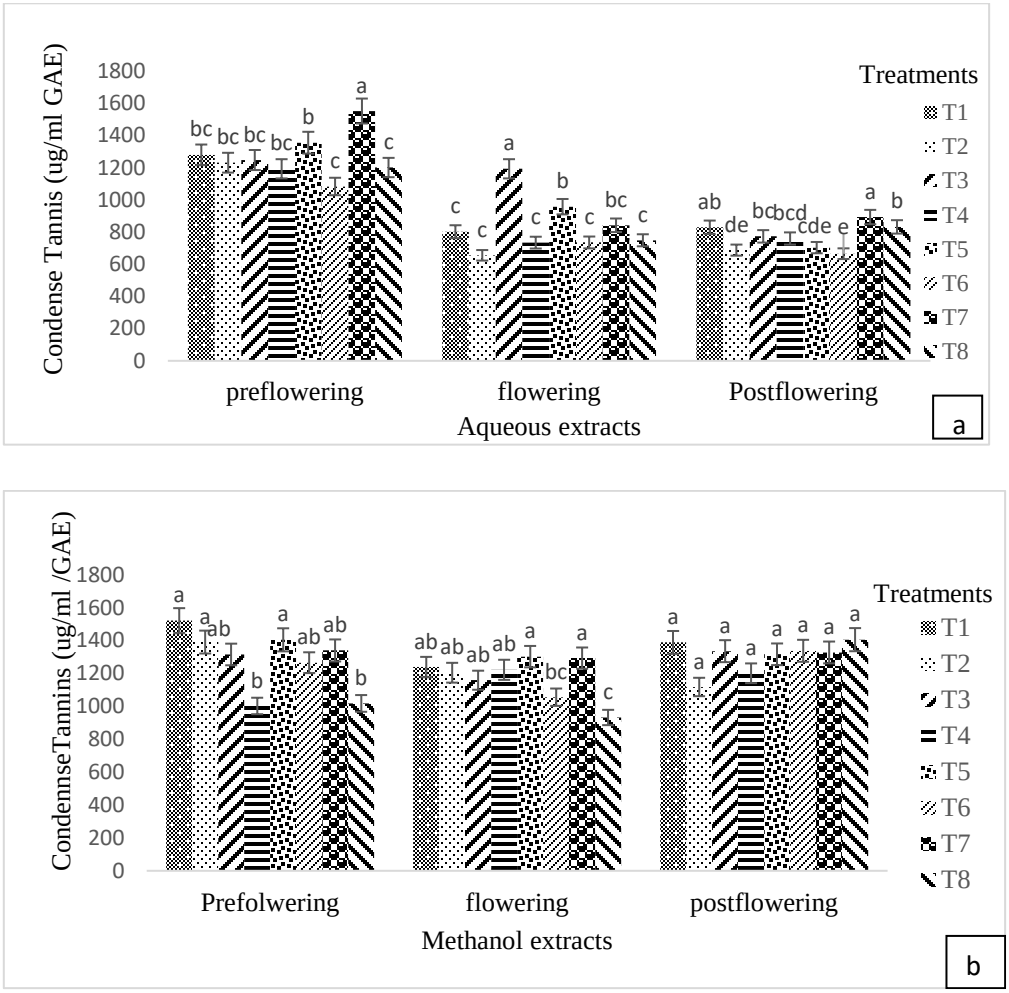


Figure 6.3 a & b: Accumulation of condense tannins (PAC) at the different phenological stages of development of American Skullcap. Values are means (µg/mL GAE/g) at (P-0.05).

6.3.5 Nutritional constituents of American Skullcap as affected by fertilizer treatment combinations. Values are means at (P-0.05)

Overall, fertilizer treatment combinations had a significant influence (P<0.05) on the nutritional constituents of *S. lateriflora* leaves (Table 6.2). Considering the Ash content T_3 recorded the

highest yield (10.6%) with a 3.9 % increase compared to the control (T_1) with the second highest yield. Additionally, for crude lipid, the control (T_1) had the highest yield (10.2 %) followed by T_3 with a 13.7 % reduction from that of the control. It was observed that treatments with the least supplementary phosphorous had a higher yield response than those with more supplementary phosphorous for ash and crude lipid content. However, this was not the case with fibre and protein content. For crude fibre content, T_6 had the highest yield (30.32 %) with a 21.09 % increase compared to the control (T_1) which had the least yield (25.04 %). Additionally, for protein content T_1 had the highest yield (14.44%) with higher protein content recorded by treatments with lower phosphorous levels than those with higher phosphorous levels among treatment combinations. Furthermore, for carbohydrate content T_8 recorded the highest yield with a 27.17 % increase compared to the control (T_1), followed by T_4 (49.02%) with a 22.18 % increase compared to the control (T1) (Table 6.2).

Table 6.2 Proximate analysis for leaf samples of American Skullcap of different treatments. Values are means at (P=0.05)

Treatments	% Ash	% Lipid	% Fibre	% Protein	%Carbohydrate
T_1	10.20 ± 2.30^{ab}	10.20 ± 6.73^{a}	25.04 ± 4.85^{b}	14.44 ± 0.22^{a}	40.12 ± 5.83^{c}
T_2	9.0 ± 1.97^{ab}	6.70 ± 1.60^{ab}	26.70 ± 3.66^{ab}	13.58 ± 0.24^{b}	$44.18\pm2.51b^{c}$
T_3	10.6 ± 1.56^{a}	8.80 ± 2.36^{ab}	28.24 ± 3.40^{ab}	12.82 ± 0.36^{bc}	40.42 ± 4.15^{c}
T_4	6.40 ± 0.82^{c}	8.40 ± 1.95^{ab}	26.88 ± 1.96^{ab}	10.80 ± 0.46^{d}	49.02 ± 5.38^{ab}
T_5	$9.8\pm0.0.97^{ab}$	8.50 ± 3.14^{ab}	26.72 ± 3.63^{ab}	13.20 ± 0.92^{bc}	41.10 ± 6.71^{c}
T_6	8.5 ± 1.90^{ab}	5.30 ± 0.97^{b}	30.32 ± 3.24^{a}	12.50 ± 0.71^{c}	43.38 ± 2.97^{bc}
T_7	$8.1\pm0.0.74^{bc}$	6.50 ± 1.27^{ab}	29.60 ± 2.52^{ab}	11.50 ± 1.08^{d}	44.30 ± 2.59^{bc}
T_8	8.9 ± 0.42^{ab}	7.20 ± 2.31^{ab}	27.55 ± 1.71^{ab}	6.72 ± 0.11^{e}	51.02 ± 2.17^{a}
LSD	0.056	0.106	0.062	0.080	0.069

Means ± SDV, Difference letters showed significant difference using Duncan multiple range test (DMRT) at $p<0.05$ level. $T_1=N_{350}P_{213}K_{213}$, $T_2=N_{350}P_{320}K_{213}$, $T_3=N_{525}P_{213}K_{213}$, $T_4=N_{525}P_{320}K_{213}$ $T_5=N_{700}P_{213}K_{213}$, $T_6=N_{700}P_{320}K_{213}$, $T_7=N_{800}P_{213}K_{213}$,

6.3.6 Influence of fertilizer treatments on antioxidant activity of aqueous and methanolic dried leaf extracts of cultivated American skullcap

The antioxidant effect of aqueous and methanolic dried leaf extracts of American Skullcap as affected by fertilizer treatment combinations was evaluated using the DPPH radical scavenging, nitric oxide scavenging and hydrogen peroxide scavenging assays. The results of IC_{50} values of the different antioxidant activities are presented in Table 6.3. Generally, aqueous and methanolic dried leaf extracts demonstrated a significant ($p<0.05$) DPPH scavenging effect in percentage

inhibition demonstrated by the IC_{50} values among treatment combinations, T_5 had the lowest IC_{50} values for both aqueous and methanol extracts indicating the best scavenging effect among treatments combinations and solvent extraction. Additionally, Nitric oxide also had a significant ($p<0.05$) effect on percentage inhibitions demonstrated by IC_{50} values among treatment combinations (Table 6.3). Interestingly, the lowest IC_{50} value of this assay was still recorded by T_5 among treatment combinations and solvent extracts indicating the best scavenging activity for this trial. Furthermore, hydrogen peroxide also demonstrated a significant ($p<0.05$) scavenging effect in percentage inhibitions in IC_{50} values among treatment combinations and solvent extracts. T_5 still recorded the lowest IC_{50} value among treatment combinations indicating the best scavenging activity (Table 6.3).

Table 6.3 Influence of fertilizer treatments on antioxidant properties on leaf extract of American Skullcap. Values are means at (P-0.05)

Treatments	DPPH IC_{50}		Nitric Oxide IC_{50}		Hydrogen peroxide IC_{50}	
	ME (µg/ml)	AE (µg/ml)	ME (µg/ml)	AE (µg/ml)	ME (µg/ml)	AE (µg/ml)
T_1	631.67±21.65^{c}	680.56±18.86^{ab}	476.04±21.63^{b}	340.63±3.49^{b}	255.66±33.71^{a}	61.31±08.28^{bc}
T_2	710.86±19.04^{b}	724.57±51.28^{a}	433.25±15.19^{c}	459.13±34.11^{a}	262.57±28.62^{a}	68.38±06.34^{b}
T_3	781.23±34.93^{a}	698.29±19.47^{ab}	515.63±18.77^{a}	76.53±14.72^{c}	283.18±32.21^{a}	66.73±01.49^{b}
T_4	583.45±25.25^{d}	653.03±81.52^{ab}	409.88±23.89^{c}	46.89±11.08^{d}	59.19±02.82^{b}	68.93±02.32^{b}
T_5	461.10±17.08^{e}	564.85±20.63^{c}	310.82±17.23^{d}	46.25±01.29^{d}	41.09±02.55^{b}	57.53±04.51^{c}
T_6	461.10±17.08^{e}	646.36±8.21^{b}	407.06±29.22^{c}	79.55±00.66^{c}	58.53±02.72^{b}	69.03±02.99^{b}
T_7	606.98±20.10^{cd}	632.33±20.97^{bc}	529.95±16.01^{a}	62.40±01.48^{cd}	63.53±01.56^{b}	79.79±03.97^{a}
T_8	756.78±33.55^{a}	629.05±29.17^{bc}	531.12±07.94^{a}	330.55±18.69^{b}	72.06±02.71^{b}	67.60±02.89^{b}
ASC	55.76±6.91	55.76±6.91	58.66±3.51	58.66±3.51	75.76±4.34	75.76±4.34
DDM	75.06±5.46	75.06±5.46	85.06±4.46	85.06±4.46	95.06±2.46	95.06±2.46

Means ± SDV, Difference letters showed significant difference using Duncan multiple range test (DMRT) at $p<0.05$ level. Values are means of IC_{50} (µg/ml) at ($p<0.05$). $T_1=N_{350}P_{213}K_{213}$, $T_2=N_{350}P_{320}K_{213}$, $T_3=N_{525}P_{213}K_{213}$, $T_4=N_{525}P_{320}K_{213}$ $T_5=N_{700}P_{213}K_{213}$, $T_6=N_{700}P_{320}K_{213}$, $T_7=N_{800}P_{213}K_{213}$, $T_8=N_{800}P_{230}K_{213}$ Kg/ha, ME= methanol.

6.3.7 Influence of fertilizer treatment on anti-inflammatory activity of aqueous and methanolic dried leaf extracts of cultivated American skullcap

The anti-inflammatory effect of the aqueous and methanol dried leaf extract of American Skullcap as affected by fertilizer treatment combination was evaluated using fresh egg albumin in phosphate buffer saline (pH 6.4) on the standard drug Diclofenac 500 g. Significant ($p<0.05$) anti-inflammatory activity was recorded on leaf extracts among treatment combinations on aqueous and methanolic extracts. T_4 recorded the best anti-inflammatory with an IC_{50} value of 352.8 µg/ml

for aqueous leaf extract. However, for methanolic extract T_7 demonstrated the most ideal with the lowest IC_{50} value of 834.1 µg/ml among treatment combinations indicating the best anti-inflammatory activity. Overall, aqueous extract recorded a more favourable anti-inflammatory response compared to methanolic extract that demonstrated lower IC_{50} values among the corresponding aqueous treatments (Table 6.4).

Table 6.4: Influence of fertilizer treatments on anti-inflammatory activities for dried leaf extract of American Skullcap. Values are means at (P-0.05)

Treatments	ME root(µg/ml)	A E (µg/ml)
T1	1345.47±88.27^{a}	775.68±24.52^{a}
T2	937.67±41.94^{c}	455.15±04.25^{cd}
T3	1225.07±16.46^{b}	473.37±20.29^{bc}
T4	1340.09±08.24^{a}	352.76±12.17^{e}
T5	963.77±25.28^{c}	489.33±15.76^{b}
T6	956.50±55.08^{c}	494.52±03.47^{b}
T7	834.09±18.83^{d}	430.06±19.16^{d}
T8	920.97±28.53^{c}	464.29±18.02^{bc}
Diclofenac	498.55±50.55	498.55±50.55

Means ± SDV, Difference letters showed significant difference using Duncan multiple range test (DMRT) at p<0.05 level Values are means of IC50 (µg/ml) at (P-0.05). $T_1=N_{350}P_{213}K_{213}$, $T_2=N_{350}P_{320}K_{213}$, $T_3=N_{525}P_{213}K_{213}$, $T_4=N_{525}P_{320}K_{213}$ $T_5=N_{700}P_{213}K_{213}$, $T_6=N_{700}P_{320}K_{213}$, $T_7=N_{800}P_{213}K_{213}$, $T_8=N_{800}P_{230}K_{213}$ Kg/ha, ME= methanol extract, AE=aqueous extract.

6.4 Discussion

The results of phytochemical determination of aqueous leaf extracts of American Skullcap were positive except for glycoside which was negative. This indicates that fertilizer treatments had less influence on the quality of secondary metabolites of this species regardless of the cultural practice. Despite the limited literature on phytochemical determination of this species, these results concur with those of Delange et al. (2012) who reported the presence and absence of these phytochemicals in three different extracts of this species. The collective presence of these different phytochemicals investigated may be as a result of the therapeutic values of this species (Shang et al., 2010), most especially in the treatment of nervous disorders (Islam et al., 2011) and the anxiolytic property of this species (Awad et al., 2003).

Significant differences were observed in the concentration of total phenolic (TPC), total flavonoid content (TFC), and condensed tannins (PAC) among the different treatment combinations across crop phenology on aqueous and methanolic extracts. This may be attributed to the fact that,

fertilizer induces changes in secondary metabolites accumulation in medicinal plants (Azaizeh et al., 2005), which can also be influenced by environmental conditions and phenological stages of development (Cao et al., 2019) as observed in this study. The highest concentration of total phenolic and flavonoid contents was recorded at the post-flowering stage followed by the pre-flowering and flowering stage. This may be attributed to the fact that; at the post-flowering stage the soil`s available nutrients may have been used up during crop development and plant growth becomes limited due to the limitation of mineral nutrients which is a tool for induction of secondary metabolites accumulation at the post-flowering stage. Furthermore, according to the carbon/nutrient balance hypothesis when plants are stressed by nutrients limitation, extra carbon is allocated to the production of carbon base secondary metabolites (CBSM) such as phenolics and terpenes (Hamilton et al., 2001), complementing the highest concentration of total phenolic and flavonoids contents at the post flowering stage when plants have attended maturity. However, these results are contrary to those of Feudjio et al. (2020) who reported a significant increase in total phenolic content from the vegetative stage to flowering and to the fruiting stage, which also concurs with those of Berezina et al. (2017). Who reported the rate of production of phenolic compounds was lower during budding compared to flowering and fruiting stages in *Vaccinium macrocarpon*. The increase in total flavonoid content may be attributed to the long period of accumulation of flavonoids during pollens and petal of flower formation through the flowering to the post-flowering stage. However, the highest concentration of tannins at the pre-flowering stage may be attributed to the fact that the ratio of different secondary metabolites varies significantly based on the environment, plant material between and within treatments and taxon, and the duration of stress factors (Jakovljević et al., 2019).

A similar scenario of treatment combinations with least supplementary phosphorous having a higher concentration of total phenolic, flavonoids, and condensed tannins on aqueous and methanolic extracts among treatment combinations across the different phenological stages was recorded in this study. This may be attributed to the fact that a higher concentration of bioactive compounds in plants may be induced under nutrient stress plants compared to non-nutrient plants (Salami et al., 2016, Zali et al., 2018), which may have led to this scenario. Furthermore, the highest concentration of total phenolic content recorded by T_1 and also, T_7 having the highest concentration of total flavonoids, and condensed tannins indicates that these treatments could be the possible preferential low supplementary phosphorous treatments, with limitation of nutrients leading to a higher synthesis of specialized metabolites (Nguyen and Niemeyer, 2008, Kiferle et al., 2013) recorded in this study. Nevertheless, the overall higher concentration of total phenolic, flavonoids and condense tannins in the methanolic extract may be attributed to solvent polarity. Also, the ability of plant materials to be able to completely dissolve in methanol than aqueous

solvent (Ajuru et al., 2017). Moreover, aqueous extracts are more susceptible to spoilage which ultimately may have an influence on the quality and quantity of these metabolites.

The significant difference in nutritional components of cultivated American Skullcap in pots may be attributed to the different fertilizer levels used which might have influenced the yield and the nutritional quality of the leaf samples (Hornick, 1992). This is in agreement with Wang et al. (2008) who reported that fertilizer application in plants generally influences crop yield as well as the nutritional quality of plants. The high fibre content recorded among the different plant samples as affected by fertilizer treatments suggested that leaves can be a potential source of dietary fibre complementing the anti-inflammatory and antitumor properties of this species (Shang et al., 2010, Vaidya et al., 2014). Also, the high carbohydrate content of the different samples with more supplementary phosphorous treatment has high carbohydrate content than those with less supplementary phosphorous with increased nitrogen levels. This may be attributed to the fact that nitrogen is a core nutrient element that influences the process of photosynthesis, protein, and carbohydrate metabolism in plant cells (Krapp, 2015). Furthermore, adult's dietary lipid intake is between 20-35% of the total calories from food. However, the lower crude lipid values of 10.2% and below recorded in this study indicate this species may be beneficial for people with cholesterol related problems (Króliczewska et al., 2011).

The antioxidant effect of aqueous and methanol dried leaf extracts of American Skullcap investigated demonstrated a significant DPPH scavenging, nitric oxide scavenging, and hydrogen peroxide scavenging activities among treatment combinations. This may be attributed to the differences in secondary metabolites concentration among the different nutrient stress levels of the treatment combinations (Borges et al., 2017) applied in this study. The DPPH scavenging activity is one of the most antioxidant assays with relative sensitivity, stability and convenience (Floegel et al., 2011). Aqueous and methanol leaf extracts demonstrated a good DPPH radical scavenging activity attributed to the lowest IC_{50} values of 461.1 µg/ml for methanolic extract and 564.9 µg/ml for aqueous extract. The difference in antioxidant activity of the extracts may be attributed to the differences in the polarity of the solvents (Benzie and Strain, 1996, Meyer et al., 1998, Chang et al., 2002)

Nitric oxide is required for the regulation of several physiological functions (Luiking et al., 2010). The excessive accumulation may result in tissue damage and several disease conditions like inflammation (Sharma et al., 2007). The application of nitric oxide as antioxidants in the management of the disease has been investigated. In this study a significant difference was observed in the scavenging activity of nitric oxide among treatment combinations. This may be attributed to the difference in nutrient stress levels among treatment combinations(Borges et al.,

2017). In this study, T_6 of aqueous leaf extract demonstrated the most ideal NO scavenging effect with an IC_{50} value of 407.1 µg/ml for methanolic extract and 46.3 µg/ml for aqueous extract.

Hydrogen peroxide (H_2O_2) scavenging activity is one of the useful methods used to determine the ability of antioxidants to decrease the level of prooxidants (Sotler et al., 2019). In this study, a significant difference was also observed in hydrogen peroxide scavenging activity among the treatment combinations. This may be attributed to the difference in metabolites concentrations in response to the difference in nutrient stress levels among the treatment combinations (Borges et al., 2017). T_6 recorded the most ideal percentage inhibition with the lowest IC_{50} value of 41.1 µg/ml for methanol extract. However, for aqueous extract the most ideal percentage inhibition was demonstrated by T_4 with the lowest IC_{50} value of 57.5 µg/ml. The scavenging activity of these extracts may be attributed to their phenolics that neutralize hydrogen peroxide to water through electron donation (Halliwell and Gutteridge, 2015). Furthermore, the difference in scavenging capacity is attributed to the structural features of the bioactive components which determine their electron donating abilities(Wettasinghe and Shahidi, 2000).

Inflammation is a complex biological process that is associated with an increase in protein denaturation that causes pain in vascular tissues and membrane alteration (Ferrero-Miliani et al., 2007). Several inflammatory effects usually result in organ injury (Sherwood and Toliver-Kinsky, 2004). The use of plant products with anti-inflammatory activity such as American Skullcap to target inflammatory response can be beneficial for the treatment of inflammation as well as many other chronic diseases. In this study significant differences were observed among the extracts of different treatment combinations. The anti-inflammatory activities of the extracts were found to be effective in their percentage inhibitions with the lowest IC_{50} value (352.8 µg/ml) recorded by T_4 for aqueous extract and 834.1 µg/ml recorded by T_7 for methanolic extract comparable to that of the standards drug diclofenac with IC_{50} value of 498.6 µg/ml in this study. The differences in activity may be attributed to the different treatment effects which may have resulted in the differences in the concentrations of the bioactive compounds that may be responsible for the anti-inflammatory activity of this species (Lin and Shieh, 1996).

6.5 Conclusion

Regardless of the solvent of extraction, phytochemical constituents of the different extracts of American Skullcap (*Scutellaria lateriflora* L.) were greatly impacted to a varying degree by fertilizer treatment combinations. A positive test result was observed for all phytochemicals investigated except for glucoside. This complements the diverse pharmacological activities of this species like antioxidant and anti-inflammatory activities investigated. Nutritionally, the high fibre and carbohydrate contents of this species indicate this species can be recommended as a potential

source of dietary fibre and calorie intake for body functioning. Likewise, the ideal amount of protein content and low lipid content also indicate that this species can be beneficial for people with related cholesterol problems, complementing the therapeutic values. Methanol solvent demonstrated a more ideal solvent than aqueous solvent for extract preparation of this species due to its polarity. Similarly, it was realized that significant differences were recorded on the accumulation of the total phenolic content, total flavonoid, content and condense tannins among the different treatments across the different phenological stages of development of this species in this study. However, some treatments outperformed more on the accumulation of these phytochemicals. Treatment combinations with less supplemented phosphorous recorded a higher concentration compared to those with more supplementary phosphorous among treatment combinations. Antioxidant activity was recorded on aqueous and methanol dried leaf extract of this species which demonstrated a significant influence on DPPH, nitric oxide and hydrogen peroxide scavenging activity, and anti-inflammatory activity as affected by the different fertilizer treatment combinations in this study. This preliminary investigation indicates that *S. lateriflora* cultivated in the Winelands region of the Western Cape Province of South Africa can retain its secondary metabolites of bioactivity and therapeutic values. Nevertheless, more investigation needs to be done using other propagation techniques and, in the field, to potentially validate these findings.

References

Ajuru, M. G., Williams, L. F. & Ajuru, G. 2017. Qualitative and quantitative phytochemical screening of some plants used in ethnomedicine in the Niger Delta Region of Nigeria. *Journal of Food and Nutrition Sciences,* 5, 198-205.

Aoac, C. A. 2005. Official methods of analysis of the Association of Analytical Chemists International. Official Methods Gaithersburg, MD.

Awad, R., Arnason, J., Trudeau, V., Bergeron, C., Budzinski, J., Foster, B. & Merali, Z. 2003. Phytochemical and biological analysis of skullcap (Scutellaria lateriflora L.): a medicinal plant with anxiolytic properties. *Phytomedicine,* 10, 640-649.

Azaizeh, H., Ljubuncic, P., Portnaya, I., Said, O., Cogan, U. & Bomzon, A. 2005. Fertilization-induced changes in growth parameters and antioxidant activity of medicinal plants used in traditional Arab medicine. *Evidence-Based Complementary and Alternative Medicine,* 2, 549-556.

Benzie, I. F. & Strain, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry,* 239, 70-76.

Berezina, E., Brilkina, A. & Veselov, A. 2017. Content of phenolic compounds, ascorbic acid, and photosynthetic pigments in Vaccinium macrocarpon Ait. dependent on seasonal plant development stages and age (the example of introduction in Russia). *Scientia Horticulturae,* 218, 139-146.

Borges, C. V., Minatel, I. O., Gomez-Gomez, H. A. & Lima, G. P. P. 2017. Medicinal plants: Influence of environmental factors on the content of secondary metabolites. *Medicinal Plants and Environmental Challenges.* Springer.

Cao, Y., Fang, S., Fu, X., Shang, X. & Yang, W. 2019. Seasonal variation in phenolic compounds and antioxidant activity in leaves of Cyclocarya paliurus (Batal.) Iljinskaja. *Forests,* 10, 624.

Chandra, S., Chatterjee, P., Dey, P. & Bhattacharya, S. 2012. Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. *Asian Pacific journal of tropical biomedicine,* 2, S178-S180.

Chang, C.-C., Yang, M.-H., Wen, H.-M. & Chern, J.-C. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of food and drug analysis,* 10.

Delange, D. M., Rico, C. L. M., Canavaciolo, V. L. G., Oliver, E. S., Pérez, R. D. L. C. S., Leyes, E. a. R. & Murillo, R. V. 2012. Phytochemical screening of Scutellaria havanensis Jacq. *Revista Cubana de Plantas Medicinales,* 17, 402-407.

Dixon, R. A. & Paiva, N. L. 1995. Stress-induced phenylpropanoid metabolism. *The plant cell,* 7, 1085.

Elufioye, T. O., Olusola, D. M. & Oyedeji, A. O. 2019. Correlation of total phenolic, flavonoid and tannin content of Bryophyllum pinnatum (Lam.)(crassulaceae) extract with the antioxidant and anticholinesterase activities. *Pharmacognosy Journal,* 11.

Eraso, I., Downing, K. & Stutte, G. Feasibility of controlled environment production of Scutellaria species. International Symposium on Medicinal and Nutraceutical Plants 756, 2007. 213-220.

Ferrero-Miliani, L., Nielsen, O., Andersen, P. & Girardin, S. 2007. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation. *Clinical & Experimental Immunology,* 147, 227-235.

Feudjio, C., Yameen, M. A., Singor Njateng, G. S., Khan, M. A., Lacmata Tamekou, S., Simo Mpetga, J. D. & Kuiate, J.-R. 2020. The Influence of Solvent, Host, and Phenological Stage on the Yield, Chemical Composition, and Antidiabetic and Antioxidant Properties of Phragmanthera capitata (Sprengel) S. Balle. *Evidence-Based Complementary and Alternative Medicine,* 2020.

Floegel, A., Kim, D.-O., Chung, S.-J., Koo, S. I. & Chun, O. K. 2011. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of Food Composition and Analysis,* 24, 1043-1048.

Foster, S. & Duke, J. 2000. Medicinal plants and herbs of Eastern and Central North America. Houston Mifflin Co. NY.

Greenfield, J. & Davis, J. 2004. Medicinal Herb Production Guides. *North Carolina Consortium on Natural Medicines and Public Health. North Carolina State University, Raleigh, NC*.

Halliwell, B. & Gutteridge, J. M. 2015. *Free radicals in biology and medicine*, Oxford university press, USA.

Hamilton, J., Zangerl, A., Delucia, E. & Berenbaum, M. 2001. The carbon–nutrient balance hypothesis: its rise and fall. *Ecology letters,* 4, 86-95.

Hornick, S. B. 1992. Factors affecting the nutritional quality of crops. *American Journal of Alternative Agriculture,* 7, 63-68.

Hossain, M. A., Al-Raqmi, K. a. S., Al-Mijizy, Z. H., Weli, A. M. & Al-Riyami, Q. 2013. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. *Asian Pacific journal of tropical biomedicine,* 3, 705-710.

Hussain, M., Gogoi, B., Joshi, B., Borah, B., Lalthafamkimi, L. & Bhau, B. S. 2018. Plant–Environment Interaction: Influence of Abiotic Stress on Plant Essential Oil Yield and Quality. *Metabolic Adaptations in Plants During Abiotic Stress*, 391.

Islam, M. N., Downey, F. & Ng, C. K. 2011. Comparative analysis of bioactive phytochemicals from Scutellaria baicalensis, Scutellaria lateriflora, Scutellaria racemosa, Scutellaria tomentosa and Scutellaria wrightii by LC-DAD-MS. *Metabolomics,* 7, 446-453.

Jakovljević, D., Topuzović, M. & Stanković, M. 2019. Nutrient limitation as a tool for the induction of secondary metabolites with antioxidant activity in basil cultivars. *Industrial Crops and Products,* 138, 111462.

Jimoh, M. O., Afolayan, A. J. & Lewu, F. B. 2019. Antioxidant and phytochemical activities of Amaranthus caudatus L. harvested from different soils at various growth stages. *Scientific reports,* 9, 1-14.

Kiferle, C., Maggini, R. & Pardossi, A. 2013. Influence of nitrogen nutrition on growth and accumulation of rosmarinic acid in sweet basil (Ocimum basilicum L.) grown in hydroponic culture. *Australian Journal of Crop Science,* 7, 321-327.

Krapp, A. 2015. Plant nitrogen assimilation and its regulation: a complex puzzle with missing pieces. *Current opinion in plant biology,* 25, 115-122.

Króliczewska, B., Miśta, D., Zawadzki, W., Wypchło, A. & Króliczewski, J. 2011. Effects of a skullcap root supplement on haematology, serum parameters and antioxidant enzymes in rabbits on a high-cholesterol diet. *Journal of animal physiology and animal nutrition,* 95, 114-124.

Lin, C.-C. & Shieh, D.-E. 1996. The anti-inflammatory activity of Scutellaria rivularis extracts and its active components, baicalin, baicalein and wogonin. *The American journal of Chinese medicine,* 24, 31-36.

Luiking, Y. C., Engelen, M. P. & Deutz, N. E. 2010. Regulation of nitric oxide production in health and disease. *Current opinion in clinical nutrition and metabolic care,* 13, 97.

Meyer, A. S., Heinonen, M. & Frankel, E. N. 1998. Antioxidant interactions of catechin, cyanidin, caffeic acid, quercetin, and ellagic acid on human LDL oxidation. *Food chemistry,* 61, 71-75.

Nguyen, P. M. & Niemeyer, E. D. 2008. Effects of nitrogen fertilization on the phenolic composition and antioxidant properties of basil (Ocimum basilicum L.). *Journal of agricultural and food chemistry,* 56, 8685-8691.

Okeleye, B., Nongogo, V., Mkwetshana, N. T. & Ndip, R. N. 2015. Polyphenolic content and in vitro antioxidant evaluation of the stem bark extract of Peltophorum africanum sond (fabaceae). *African Journal of Traditional, Complementary and Alternative Medicines,* 12, 1-8.

Pereira, R. F. & Bartolo, P. J. 2016. Traditional therapies for skin wound healing. *Advances in wound care,* 5, 208-229.

Prinsloo, G. & Nogemane, N. 2018. The effects of season and water availability on chemical composition, secondary metabolites and biological activity in plants. *Phytochemistry Reviews,* 17, 889-902.

Salami, M., Rahimmalek, M., Ehtemam, M. H., Szumny, A., Fabian, S. & Matkowski, A. 2016. Essential oil composition, antimicrobial activity and anatomical characteristics of Foeniculum vulgare Mill. fruits from different regions of Iran. *Journal of Essential Oil Bearing Plants,* 19, 1614-1626.

Sen, T. & Samanta, S. K. 2014. Medicinal plants, human health and biodiversity: a broad review. *Biotechnological applications of biodiversity*, 59-110.

Shang, X., He, X., He, X., Li, M., Zhang, R., Fan, P., Zhang, Q. & Jia, Z. 2010. The genus Scutellaria an ethnopharmacological and phytochemical review. *Journal of ethnopharmacology,* 128, 279-313.

Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M. & Zheng, B. 2019. Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules,* 24, 2452.

Sharma, J., Al-Omran, A. & Parvathy, S. 2007. Role of nitric oxide in inflammatory diseases. *Inflammopharmacology,* 15, 252-259.

Sherwood, E. R. & Toliver-Kinsky, T. 2004. Mechanisms of the inflammatory response. *Best Practice & Research Clinical Anaesthesiology,* 18, 385-405.

Shiwakoti, S., Shannon, D. A., Wood, C. W., Joshee, N., Rimando, A., Lawrence, K. S. & Kemppainen, B. 2016. Nitrogen, phosphorus, and potassium effects on biomass yield and flavonoid content of American skullcap (Scutellaria lateriflora). *Journal of Plant Nutrition,* 39, 1240-1249.

Sotler, R., Poljšak, B., Dahmane, R., Jukić, T., Jukić, D. P., Rotim, C., Trebše, P. & Starc, A. 2019. Prooxidant activities of antioxidants and their impact on health. *Acta Clinica Croatica,* 58, 726.

Szakiel, A., Pączkowski, C. & Henry, M. 2011. Influence of environmental abiotic factors on the content of saponins in plants. *Phytochemistry Reviews,* 10, 471-491.

Vaidya, B. N., Brearley, T. A. & Joshee, N. 2014. Antioxidant capacity of fresh and dry leaf extracts of sixteen Scutellaria species. *Journal of Medicinally Active Plants,* 2, 42-49.

Wang, Z. H., Li, S. X. & Malhi, S. 2008. Effects of fertilization and other agronomic measures on nutritional quality of crops. *Journal of the Science of Food and Agriculture,* 88, 7-23.

Wettasinghe, M. & Shahidi, F. 2000. Scavenging of reactive-oxygen species and DPPH free radicals by extracts of borage and evening primrose meals. *Food chemistry,* 70, 17-26.

Yang, L., Wen, K.-S., Ruan, X., Zhao, Y.-X., Wei, F. & Wang, Q. 2018. Response of plant secondary metabolites to environmental factors. *Molecules,* 23, 762.

Yin-Jheng, J., Kuan-Hung, L., Chun-Wei, W. & Chang, Y.-S. 2021. Influence of fertilizer and salicylic acid treatments on growth, physiological, and antioxidant characteristics in green and red Perilla frutescens varieties. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca,* 49, 12064-12064.

Yuliana, N. D., Khatib, A., Choi, Y. H. & Verpoorte, R. 2011. Metabolomics for bioactivity assessment of natural products. *Phytotherapy Research,* 25, 157-169.

Zali, A. G., Ehsanzadeh, P., Szumny, A. & Matkowski, A. 2018. Genotype-specific response of Foeniculum vulgare grain yield and essential oil composition to proline treatment under different irrigation conditions. *Industrial Crops and Products,* 124, 177-185.

CHAPTER 7
PHYTOCHEMICAL CONSTITUENTS, ESSENTIAL OIL COMPOSITION, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF BURDOCK (*ARCTIUM LAPPA* L.) IN RESPONSE TO MINERAL FERTILIZER APPLICATION

7.1 Introduction

The importance of medicinal plants is attributed to their healing, culinary and nutritional values in different regions and cultures of the world (Sher and Alyemeni, 2010, Matic et al., 2018, Van Wyk and Wink, 2018). The potentials are in relation to the diverse phytochemical constituents of biological activity, being used for drug development and production of supplements for human health and nutrition. Phytochemicals from medicinal plants play a considerable role in the treatment of various diseases caused by oxidative stress, they have antioxidant properties with the ability to avert and treat diseases to maintain good health (Sofowora et al., 2013).

During the growth and development of medicinal plants, the accumulation of phytochemicals may vary across the different stages of their phenology by several factors. One of such factors that influences the production of phytochemical constituents in plants during growth and development are fertilizer treatment levels and time of application. This may cause notable fluctuations in the quality and quantity of the bioactive compounds which may have an influence on their therapeutic values. This may lead to an increase, decrease, and or loss of activity in medicinal plant materials which may result in medicinal plants being regarded as having no medicinal potential

Different stressed levels of fertilizer application are known to induce changes in the accumulation of secondary metabolites in plants (Dixon and Paiva, 1995). Nitrogen and phosphorous fertilizer stress in plants are known to induce the accumulation of phenolic compounds in plant tissues (Chalker-Scott and Fuchigami, 2018). Also, different fertilizer treatments can induce changes in growth parameters, chemical composition and the antioxidant activities of the bioactive compounds of medicinal plants (Azaizeh et al., 2005). *A. lappa* commonly known as Burdock is one of those medicinal plants used in the treatment of various diseases and is being promoted as a nutritive food. It is considered a very important medicinal plant in American and Chinese traditional medicine. Traditionally, it is used by the Native Americans for the treatment of arthritis, sore throat, urinary tract infections, ringworm, eczema, and common cold (Hutchens, 1992, Cichoke, 2001). In Chinese traditional medicine, it is used in the treatment of cancer, upper respiratory infection, pneumonia and the root tea is known to be a promising beverage because of its extensive therapeutic values (Predes et al., 2011).

The acquisition of the plant material is mostly from the wild whose cultivation practices in the region of its origin and subsistent levels have not been pursued through research despite its potential as medicine and food. Implementing the cultivation of this species with the use of different mineral fertilizer applications is imperative to document on the influence of this abiotic factor on the secondary metabolite's accumulation of bioactivity. This is necessary for the enhancement of product quality and quantity to meet up with the demand of the growing population at a commercial scale through which standardized plant materials of good yield and quality will be provided. In this regard, the current research aimed to investigate the influence of different fertilizer treatments application on the yield of phytochemical constituents and their effect on the biological activities of cultivated Burdock in the Winelands regions of the Western Cape province of South Africa.

7.2 Materials and methods

7.2.1 Study sites

For the details on the study side, experimental design, treatment combinations, management practices, source and harvesting of plant materials please refer to section 4.2 (Chapter Four)

7.2.2 Chemicals

The details on chemicals used pleased refer to section 6.2.2 (Chapter Six).

7.2.3 Samples preparation and extraction

The leaves and root samples of the different treatments were processed for extract preparation, please refer to section 6.2.3 (Chapter Six).

7.2.4 Phytochemical screening

Leaves and root samples were obtained from plant materials as described in Chapter Three were used to prepare aqueous extract for phytochemical screening of phenol, flavonoids, tannins, terpenoids, saponins, alkaloids, steroids, and glucosides as fully described by (Hossain et al., 2013, Ajuru et al., 2017) in section 6.2.4 (Chapter Six).

7.2.5 Determination of total phenolic content (TPC)

The total phenolic content (TPC) of leaf and root extracts for the different treatments was determined using the Folin-Ciocalteu assay method as described by Jimoh et al. (2019) in chapter five (5). A standard curve was prepared to estimate the total phenolic content using gallic acid at the concentration range (0.031-1.0 mg/ml). The total phenolic content was expressed as mg gallic acid equivalent (GAE)/g dry matter from the standard curve $y=0.0019X +0.4962$, $R^2=0. 9951$. All experiments were carried out in triplicates

7.2.6 Determination of total flavonoid

The total flavonoid was determined by the colorimetric assay as described by Elufioye et al. (2019) with slight modifications in Chapter Five. A standard curve was prepared using a graded concentration range (0.031-1.0 mg/ml). Total flavonoid content was expressed as quercetin equivalent per gram dry weight from the calibration curve y= 0.0004+0.0585, R^2= 0.9983. All experiments were carried out in triplicate.

7.2.7 Determination of proanthocyanidin content (Condense tannin)

The proanthocyanidin content was determined according to the procedure as described by Elufioye et al. (2019) in Chapter Six (6). A standard curve of gallic acid at a concentration range (0.031-1.0 mg/ml) was used to determine the proanthocyanidin content expressed as mg gallic acid equivalent per dry weight from the calibration curve y=0.003X+0.0373, R^2=0.9964 All the experiments were carried out in triplicates.

7.2.8 Proximate analysis of leaf and root samples

Proximate analysis of leaf and root samples of cultivated Burdock from pot and field experiment as affected by fertilizer treatments was done according to Aoac (2005) as described in section 6.2.8 (Chapter Six). The experiment was replicated five times. The percentage of Ash, crude fibre, crude lipid, crude protein, and carbohydrate was determined for leaf and root samples for pot and field experiments.

7.2.9 Evaluation of antioxidant activity

The antioxidant activities of leaf and root extracts were estimated using DPPH, Nitric (NO), and hydrogen peroxide scavenging assays.

7.2.10 DPPH free radical scavenging assay

The DPPH free radical scavenging capacity of aqueous and methanolic leaf and root extracts were estimated according to the previously reported procedure by Elufioye et al. (2019) using 2,2-diphenyl-1picrythydrazyl hydrate (DPPH) the stable radical as described in Chapter Five. Ascorbic acid and DDM were used as standard drugs over the concentration range (0.031-1.0 mg/ml). The DPPH radical percentage scavenging activity was calculated using the equation below.

$$\%\text{Inhibition of DPPH radical} = [(A_{control} - A_{test}) / A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of the control reactions (containing all reagents except the test Compound). while A_{test} is the absorbance of the test compound.

7.2.11 Nitric oxide scavenging assay

The Nitric oxide scavenging activity of leaf and root extracts was determined using the previously reported procedure by Jimoh et al. (2019) as described in Chapter Six. All experiments were done in triplicates. The percentage scavenging activity of nitric oxide was calculated by using the equation below.

$$\% \text{ Scavenged [NO]} = [(A_{control} - A_{test}) / A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of the control reactions (containing all reagents except the test Compound), while A_{test} is the absorbance of the test compound

7.2.12 Hydrogen peroxide scavenging assay

The scavenging activity of hydrogen peroxide of leaf and root extracts was determined using the method of Okeleye et al. (2015) as described in chapter six (6). The percentage of hydrogen peroxide scavenged from the extract was calculated using the equation below.

$$\% \text{ Scavenged } [H_2O_2] = [(A_{control} - A_{test}) / A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of the control reactions (containing all reagents except the test Compound). while A_{test} is the absorbance of the test compound

7.2.13 Anti-inflammatory Assay (*In vitro*)

The anti-inflammatory activity was carried out using the method adopted by Chandra et al. (2012) as described in chapter six (6). The experiment was carried out in triplicates. The percentage inhibition of protein denaturation was calculated using the formula below (Banerjee et al., 2014)

$$\% \text{ Inhibition} = ([V_{test} / C_{ontrol}] -1) \times 100$$

Where, V_{test} = the absorbance of the test sample, $V_{control}$ = absorbance of the control. The extract concentration for 50% inhibition (IC50) was determined by the dose-response curve by plotting percentage inhibition against treatment concentration.

7.2.14 Determination of volatile oil composition of Burdock root as influence by fertilizer treatments

The volatile oil was extracted from Burdock roots harvested from the field. This was done through hydrodistillation using the Clevenger apparatus according to Oyedeji et al. (2020). GC/MS analysis of different oils from the different fertilizer treatments was performed on a GCMS-QP2010 Gas Chromatography-mass spectrometer system operating in EL mode at 70eV, equipped with an HP-5 MS fused silica capillary system with a 5 % phenylmethylsiloxane stationary phase. Capillary column parameter was 30 m by 0.25 mm, film thickness 0.25 µm. The initial temperature of the

column was 70^0C and was heated at 250^0C at a rate of 5 ℃/min. The final temperature was kept at 450 ^{0}C and the running time was 68min. Helium was used as the carrier gas at a flow rate of 1ml/min and the split ratio was 100:1 with a scan time of 68min and a scanning range of 35t-450amu.One microliter (1µl) of the diluted oil (in hexane) was injected for analysis. N-alkane of C_8 to C_{30} was run under the same condition for Kovat indices determination. Oil components were identified by the interactive combination of chromatographic linear retention indices and fragmentation pattern of an existing individual constituent, with those reported in the literature (Boelens, 2000, Adams, 2007) and MS data in the computer matching with the WILEY275, NIST 08, ADAMS and FFNSC 2 libraries.

7.2.15 Data analysis

The data on phytochemical constituents and antioxidant activities were captured in excel and IC_{50} values were calculated. The data was then statistically analysed using the procedures are reported in section 5.2. (Chapter Five).

7.3 Results

7.3.1 Phytochemical screening of aqueous root and leaf extracts of Burdock in response to fertilizer treatments for pot and field experiments

The results of phytochemical screening for aqueous root and leaf extracts for cultivated Burdock in pot experiment are presented in Table 7.1 and that for the field experiment is presented in Table 7.2. Aqueous root and leaf extracts for the different treatment combinations investigated were quite rich in phytochemical constituents to a varying degree irrespective of cultivation practice and treatment combinations used. Positive results were observed for phenols, flavonoids, tannins, saponins, and glycosides while negative results were observed for alkaloids, terpenoids, and steroids in root extracts. Also, a positive test was observed on phenols, saponins, flavonoids and glycosides while saponins, terpenoids, steroids, and alkaloids were negative in leaf extracts for the pot experiment irrespective of treatments (Table 7.1). A similar scenario was also observed for phytochemical screening of aqueous root and leaf extracts of field cultivated Burdock, with a varying degree of phytochemical constituents observed across the different treatment combinations investigated (Table 7.2).

Table 7.1Phytochemical screening of aqueous root and leaf extract of Arctium lappa L. in response to fertilizer treatments in pot experiment

Treatments	Plant material/ experiment	Tannins	Saponins	Flavonoids	Phenol	Alkaloids	Glycosides	Terpenoids	Steroids
T_1	Burdock root extract pot experiment	++	++	+	++	-	+	-	-
T_2		++	++	+	+	-	+	-	-
T_3		++	++	+	++	-	+	-	-
T_4		++	++	+	++	-	+	-	-
T_5		++	++	+	++	-	+	-	-
T_6		++	++	+	++	-	+	-	-
T_7		+	+	+	++	-	+	-	-
T_8		+	+	+	+	-	+	-	-
T_1	Burdock Leaf extracts pot experiment	+	-	+	+	-	+	-	-
T_2		+	-	+	+	-	+	-	-
T_3		+	-	+	+	-	+	-	-
T_4		+	-	+	+	-	+	-	-
T_5		+	-	+	+	-	+	-	-
T_6		+	-	+	+	-	+	-	-
T_7		+	-	+	+	-	+	-	-
T_8		+	-	+	+	-	+	-	-

Legend (++) Highly present, (+) present –(absent), $T_1=N_{350}P_{213}K_{213}$, $T_2=N_{350}P_{320}K_{213}$, $T_3=N_{525}P_{213}K_{213}$, $T_4=N_{525}P_{320}K_{213}$, $T_5=N_{700}P_{213}K_{213}$, $T_6=N_{700}P_{320}K_{213}$, $T_7=N_{800}P_{213}K_{213}$, $T_8=N_{800}P_{230}K_{213}$ Kg/ha

Table 7.2Phytochemical Screening of aqueous root and leaf extract of Arctium lappa L. in response to mineral fertilizer treatments in field experiment

Treatments	Plant material/ Experiment	Tannins	Saponins	Flavonoids	Phenol	Alkaloids	Terpenoids	Glycosides	Steroids
T_1	Burdock root extract field experiment	++	++	+	++	-	-	+	-
T_2		++	++	+	++	-	-	+	-
T_3		++	++	+	+	-	-	+	-
T_4		++	++	+	++	-	-	+	-
T_5		++	++	+	+	-	-	+	-
T_6		++	+	+	++	-	-	+	-
T_7		+	+	+	+	-	-	+	-
T_8		+	+	+	+	-	-	+	-
T_1	Burdock Leaf Extracts field experiment	+	+	+	+	-	-	+	-
T_2		+	+	+	++	-	-	+	-
T_3		+	+	+	++	-	-	+	-
T_4		+	+	+	++	-	-	+	-
T_5		+	+	+	++	-	-	+	-
T_6		+	+	+	+	-	-	+	-
T_7		+	+	+	+	-	-	+	-
T_8		+	+	+	+	-	-	+	-

Legend (++) Highly present, (+) present –(absent), $T_1=N_{350}P_{213}K_{213}$, $T_2=N_{350}P_{320}K_{213}$, $T_3=N_{525}P_{213}K_{213}$, $T_4=N_{525}P_{320}K_{213}$, $T_5=N_{700}P_{213}K_{213}$, $T_6=N_{700}P_{320}K_{213}$, $T_7=N_{800}P_{213}K_{213}$, $T_8=N_{800}P_{230}K_{213}$ Kg/ha

7.3.2 Influence of fertilizer treatments on total phenolic content (TPC), total flavonoids content (TFC) and condense tannins (PAC) of root and leaf extracts of Burdock for pot experiment

Results for total phenolic content (TPC), total flavonoid content (TFC), and condensed tannins (PAC) on aqueous leaf and methanol leaf extracts for pot the experiment are presented in Table 7.3. Significant differences ($p<0.05$) were observed in total phenolic content (TPC), total flavonoid content (TFC), and condensed tannins(PAC) on aqueous leaf and methanol leaf extracts among treatment combinations. Overall, methanol extract recorded a higher total phenolic content (79.8%), total flavonoid content (56.2%) and condense tannins (55.3%) compared to aqueous extract with a total phenolic content (20.2%), total flavonoid content (43.8%) and condense tannins (44.7%). It was observed that treatment combinations with the lower phosphorous level recorded a higher accumulation of total phenolic, total flavonoid and condensed tannins across the different leaf extracts. T_7 ($N_{800}P_{213}K_{213}$, kg/ha) recorded the highest accumulation with a 20.4% increase while T_6 ($N_{700}P_{320}K_{213,}$ kg/ha) recorded the lowest accumulation of total phenolic content with a 26.9% decrease compared to the control (T_1) for aqueous leaf extract. However, for methanol leaf extract the highest accumulation of total phenolic content was recorded by T_5 ($N_{700}P_{213}K_{213,}$ kg/ha) with a 64.8% increase, while the lowest was still recorded by T_6 ($N_{700}P_{320}K_{213,}$ kg/ha) but with a 38.3% decrease compared to the control (T_1). A similar scenario was observed for total flavonoids content on aqueous leaf extract. T_5 ($N_{700}P_{213}K_{213,}$ kg/ha) recorded the highest accumulation of 84.4% increase but the lowest accumulation was recorded by T_4 ($N_{525}P_{320}K_{213,}$ kg/ha) with 82.3% decrease compared to the control. The lowest accumulation was recorded by T_4 ($N_{525}P_{320}K_{213,}$ kg/ha) with a 72.5% decrease compared to the control that recorded the highest accumulation of total flavonoids content for methanol extract. For condense tannins T_3 ($N_{525}P_{213}K_{213}$, kg/ha) recorded the highest accumulation of 8.3% increase while the lowest accumulation was recorded by T_4 ($N_{525}P_{320}K_{213,}$ kg/ha) with a 15% decrease compared to the control for aqueous extract. However, for methanol extract, the highest accumulation of condense tannins was recorded by T_5 ($N_{700}P_{213}K_{213,}$ kg/ha) with 18.9% increase while the lowest was recorded by T_4 ($N_{525}P_{320}K_{213,}$ kg/ha) with a 33.3% reduction compared to the control.

The results for total phenolic content, total flavonoid content and condense tannins for aqueous and methanol root extracts for the pot experiment are presented in Table 7.3. An overall significant ($p<0.05$) difference was observed among the treatment combinations for aqueous and methanol root extracts. However, methanol root extracts recorded a higher accumulation of total phenolic content (59.1%), total flavonoids contents (55.8%) and condensed tannins (66.1%) compared to aqueous extracts with total phenolic content (40.9%), total flavonoid content (44.2%) and

condensed tannins (33.9%). The highest accumulation of total phenolic content was recorded by T_5 ($N_{700}P_{213}K_{213,}$ kg/ha) with a 7% increase while least accumulation was recorded by T_8 ($N_{800}P_{230}K_{213}$kg/ha) with a 58.3% decrease compared to the control for aqueous extract. On the other hand, for methanol extract the least accumulation was recorded by T_8 ($N_{800}P_{230}K_{213}$ kg/ha) with a 73% decrease compared to the control (T_1) that recorded the highest accumulation of total phenolic content. Similarly, the control (T_1) recorded the highest accumulation of 414.58 µg/ml of quercetin equivalent (QE/g) for total flavonoids content and the lowest by T_8 with a 42.8% decrease for methanol extract. Likewise, for aqueous extract, the highest accumulation of 337.08 µg/ml of quercetin equivalent (QE/g) was recorded by the control (T_1) even though the lowest accumulation was recorded by T_4 ($N_{525}P_{320}K_{213,}$ kg/ha) with a 55.9% reduction for aqueous extract. Furthermore, for condensed tannins the highest accumulation of 192.33µg/ml of gallic acid equivalent (GEA) per gram of crude extract was recorded by the control (T_1) while the lowest was recorded by T_8 with a 36.4% decrease for aqueous root extract. Whereas, for methanol extract, T_5 recorded the highest accumulation of condense tannins with a 33.6% increase while the least was recorded by T_8 with a 71.4% reduction compared to the control (T_1) of methanol root extracts for the pot experiment (Table 7.3)

Table 7.3 Influence of fertilizer treatments on Total phenolics, flavonoids and proanthocynadin content of Burdock leaf and root extracts for pot experiment. Values are means at (P-0.05).

Treatments	Plant material/ Experiment	AE (TPC) (µg /ml GAE)	ME (TPC) (µg /ml GAE)	A E (TFC) (µg /ml QE)	M E (TFC) (µg /ml QE)	AE (PAC) (µg /ml GAE)	M E (PAC) (µg /ml GAE)
T1(N1P1K1)		236.04±12.07^{b}	766.91±20.00^{cd}	393.75±15.21^{a}	661.25±72.33^{a}	713.44±16.78^{ab}	933.44±47.65^{b}
T2(N1P2K1)		201.12±6.37^{cd}	711.47±21.55^{cd}	98.75±15.61^{a}	203.75±38.97^{ef}	610.11±32.89^{ab}	842.33±32.15^{bc}
T3(N2P1K1)	Burdock	221.82±4.09^{bc}	1152.18±89.14^{ab}	101.25±22.22^{a}	280.42±16.27^{d}	772.33±38.39^{a}	701.22±62.39^{cd}
T4(N2P2K1)	leaf pot	194.46±7.39^{cd}	928.49±86.19^{bc}	69.58±12.83^{a}	182.08±8.77f	623.44±28.45^{ab}	622.33±98.15^{d}
T5(N3P1K1)	experiment	272.35±15.65^{a}	1263.57±19.85^{a}	726.25±10.09^{a}	249.58±20.21^{de}	725.67±11.20^{ab}	1110.11±53.16^{a}
T6(N3P2K1)		172.70±21.83^{d}	473.58±49.66^{d}	113.75±4.33^{a}	197.92±14.43^{ef}	604.56±30.97^{b}	934.56±14.76^{b}
T7(N4P1K1)		284.28±36.80^{a}	1041.65±14.78^{abc}	328.75±39.29^{a}	467.92±32.53^{b}	705.67±12.02^{ab}	875.67±15.52^{b}
T8(N4P2K1)		223.05±7.31b^{c}	800.42±40.78^{cd}	200.42±66.82^{a}	364.58±10.41^{c}	670.11±16.43^{ab}	686.78±84.61^{d}
Total		225.73±39.09	892.29±85.54	254.06±73.78	325.94±161.48	678.17±91.06	838.31±68.45
T1(N1P1K1)		294.63±10.27^{a}	774.63±5.34^{a}	337.08±46.26^{a}	414.58±16.07^{a}	192.33±13.33^{a}	376.78±18.57^{b}
T2(N1P2K1)		239.02±16.78^{b}	386.74±33.88^{b}	283.75±38.48^{ab}	293.75±10.89^{a}	154.56±12.62^{ab}	272.33±11.55^{b}
T3(N2P1K1)	Burdock	313.93±11.70^{a}	393.93±12.64^{b}	331.25±52.38^{a}	354.58±28.98^{a}	127.89±25.46^{b}	371.22±50.04^{b}
T4(N2P2K1)	root pot	219.02±13.95^{b}	298.49±48.54^{c}	148.75±76.28^{d}	343.75±18.97^{a}	134.56±51.89^{b}	193.44±03.85^{b}
T5(N3P1K1)	experiment	315.33±15.47^{a}	298.67±14.02^{c}	280.42±08.78^{ab}	352.08±43.76^{a}	167.89±42.99^{ab}	503.44±18.36^{b}
T6(N3P2K1)		250.25±13.98^{b}	261.12±40.25^{cd}	177.08±44.04^{cd}	272.08±72.86^{a}	155.67±28.48^{ab}	194.56±10.18^{b}
T7(N4P1K1)		218.14±58.93^{b}	228.14±22.09^{de}	256.25±16.39^{abc}	299.58±37.14^{a}	132.33±26.67^{b}	297.89±16.28^{a}
T8(N4P2K1)		123.75±5.01^{c}	209.72±19.24^{e}	222.08±32.24^{bcd}	237.08±77.23^{a}	122.33±10.00^{b}	107.89±13.47^{b}
Total		246.76±63.99	356.43±174.76	254.58±74.72	320.94±50.48	148.44±33.72	289.69±71.89

Means in the same column with the same superscript are not significantly difference (P≥0.05). Values are means at (P-0.05) $T_1=N_{350}P_{213}K_{213}$, $T_2=N_{350}P_{320}K_{213}$, $T_3=N_{525}P_{213}K_{213}$, $T_4=N_{525}P_{320}K_{213}$, $T_5=N_{700}P_{213}K_{213}$, $T_6=N_{700}P_{320}K_{213}$, $T_7=N_{800}P_{213}K_{213}$, $T_8=N_{800}P_{230}K_{213}$ Kg/ha. AE=aqueous extract, ME= methanol extract, GAE= Gallic acid equivalent, QE= quercetin equivalent, TPC= Total phenolic content, TFC=Total flavonoid content, PAC= Proanthocyanidin

7.3.3 Influence of fertilizer treatment on total phenolic content ((TPC), total flavonoid content (TFC) and Condense tannin (PAC) of root and leaf extracts of Burdock for field experiment.

Total phenolic content, total flavonoid content and condensed tannins of leaf and root extracts for the field experiment is presented in Table 7.4. Comparable, significant differences ($p<0.05$) were observed on aqueous and methanol leaf and root extracts for field experiment. Overall, methanol extract recorded a higher accumulation of total phenolic content (50.1%), total flavonoids content (69.5%), condense tannins (64.1%) than that of the aqueous extract with total phenolic content (49.9%), total flavonoids content (35.5%) and condense tannins (35.9%). The highest accumulation of total phenolic content was recorded by T_5 with a 6.2% increase while the least accumulation was recorded by T_4 with a 17.9% reduction compared to the control for leaf extracts. On the other hand, for methanol extract T_4 recorded the highest accumulation with a 225% time increase while the least was recorded by T_8 with a 12.6% reduction compared to the control (T_1). For total flavonoid, the control (T_1), recorded the highest accumulation of 1741.25µg/ml of quercetin equivalent (EQ/g) while T_8 had the least with a 26.2% decrease compared to the control. for aqueous extract. For methanol extract, the highest accumulation was recorded by T_5 with a 26.2% increase while the lowest was recorded by T_4 with a 22.5% reduction compared to the control. The accumulation of condensed tannins recorded the same scenario for both extracts. T_8 recorded the highest accumulation with a 4.8% increase for aqueous extract and a 3.2% increase for methanol extract. Equally, the least accumulation was recorded by T_4 for both extracts with a 37.6% decrease for aqueous extract and a 26.6% decrease compared to the control. Interestingly treatment combination with higher levels of phosphorous demonstrated a positive response with a higher accumulation of total phenolic, flavonoid, and condensed tannins accumulation for leaf extract from field experiment (Table 7.4).

The results for root extracts on total phenolic content, total flavonoid content, and condense tannins for aqueous and methanol extracts for field experiment are presented in Table 7.4. Significant differences ($p<0.05$) were observed among the different treatment combinations in the accumulation of total phenolic content (TPC), total flavonoid content (TFC) and condensed tannins (PAC). Methanol extracts recorded a higher accumulation of total phenolic content (53.9%), total flavonoid content (68.1%), condensed tannins (51%) while aqueous extracts recorded a lower total phenolic content (46.2%), total flavonoids content (31.9%) and condensed tannins (49%). The highest accumulation of total phenolic content was recorded by T_3 with a 6.4% increase while T_2 recorded a 24.9% decrease compared to the control (T_1). For methanol extract T_5 recorded the

highest accumulation with a 6.4% increase while T_8 recorded a 65.5% reduction compared to the control (T_1). However, for total flavonoids content T_1 recorded the highest accumulation of 305.42µg/ml quercetin equivalent (EQ/g), while T_2 recorded a 62.5% decrease compared to the control for aqueous root extract. Similarly, for methanol extract T_1 recorded the highest accumulation of total flavonoid content of 532.92 µg/ml. quercetin equivalent (EQ/g), while T_2 recorded the least of a 62.5% decrease compared to the control. Furthermore, for condensed tannins, T_7 recorded the highest accumulation with a 20.5% increase while T_4 recorded the lowest with a 56.1% reduction compared to the control for aqueous extracts. Similarly, for methanol extract. with a 6.9% increase for T_7 and a 68.3% reduction for T_4 compared to the control Exciting it was also observed that root extracts with treatment combinations of lower levels of phosphorous recorded the highest accumulation of total phenolic content (TPC), total flavonoids content (FTC) and condensed tannins in field cultivated Burdock (*Arctium lappa* L.) (Table 7.4).

Table 7.4 Influence of fertilizer treatments on Total phenolics, flavonoids and proanthocynadin content of Burdock leaf extracts for field experiment. Values are means at (P-0.05).

Treatments	Plant material/ Experiment	AE (TPC) (µg /ml GAE)	ME (TPC) (µg /ml GAE)	AE (TFC) (µg /ml QE)	ME (TFC) (µg /ml QE)	AE (PAC) (µg /ml GAE)	M E (PAC) (µg /ml GAE)
T1(N1P1K1)		311.82±24.01^{a}	182.70±08.95^{e}	1741.25±26.34^{a}	2664.58±52.89^{cd}	1144.56±16.44^{a}	1959.00±94.05^{ab}
T2(N1P2K1)		317.09±17.11^{a}	306.04±15.40^{c}	1570.41±76.41^{ab}	2663.75±60.83^{cd}	832.33±76.38^{bc}	1480.11±30.25^{e}
T3(N2P1K1)	Burdock	322.88±14.93^{a}	277.96±31.29^{cd}	1595.42±57.25^{ab}	3034.58±37.61^{b}	961.22±37.33^{b}	1624.56±25.24^{d}
T4(N2P2K1)	leaf field	255.86±28.87^{b}	595.16±38.43^{a}	1566.25±22.22^{ab}	2465.42±31.25^{d}	714.56±55.41^{c}	1437.89±39.77^{e}
T5(N3P1K1)	experiment	331.29±06.23^{a}	251.12±53.94^{cd}	1632.92±32.15^{ab}	3264.58±81.18^{a}	969.0±40.55^{bc}	1877.89±49.48^{bc}
T6(N3P2K1)		325.33±19.14^{a}	385.16 ±5.34^{b}	1651.25±51.05^{a}	2890.42±91.68^{bc}	959.00±76.76^{c}	1805.67±78.11^{c}
T7(N4P1K1)		259.54±25.91^{b}	243.93±55.42^{d}	1468.75±41.31^{b}	2981.25±98.49^{b}	1173.44±93.39^{a}	2020.11±31.68^{a}
T8(N4P2K1)		269.02±16.81^{b}	159.72±03.17^{e}	1284.58±24.06^{c}	2733.75±79.88^{c}	1200.11±90.51^{a}	2022.11±31.68^{a}
Total		299.11±34.84	300.22±34.99	1563.85±51.45	2837.29±65.44	994.28±80.21	1778.17±49.48
T1($N_1P_1K_1$)		264.11±23.01^{ab}	361.65±48.97^{a}	305.42±16.12^{a}	532.92±74.43^{a}	491.22±30.97^{ab}	512.33±27.28^{ab}
T2($N_1P_2K_1$)		198.32±19.35^{d}	202.18±16.39^{d}	114.58±6.29^{bc}	250.42±18.43^{c}	301.22±36.57^{cd}	375.67±28.87^{c}
T3($N_2P_1K_1$)	Burdock	280.95±21.19^{a}	337.79±14.54^{ab}	201.25±44.23^{ab}	266.25±20.46^{c}	329.00±55.08^{cd}	376.78±91.55^{c}
T4($N_2P_2K_1$)	root field	220.77±21.32^{cd}	287.26±14.03^{c}	132.08±15.88^{c}	139.58±70.90^{d}	215.67±32.62d	162.33±50.44^{d}
T5($N_3P_1K_1$)	experiment	238.14±20.11^{bc}	384.63±35.76^{a}	146.25±25.98^{bc}	348.75±49.18^{b}	385.67±19.77b^{c}	373.44±68.50^{c}
T6($N_3P_2K_1$)		266.56±20.06^{ab}	302.70±11.16^{bc}	137.92±18.43^{c}	360.42±51.98^{b}	229.00±14.53^{d}	417.89±87.83^{bc}
T7($N_4P_1K_1$)		246.39±22.22^{abc}	267.44±36.35^{c}	249.58±37.61^{ab}	503.75±29.47^{a}	592.33±31.79^{a}	547.89±65.52^{a}
T8($N_4P_2K_1$)		233.40±11.96^{bcd}	124.81±17.17^{e}	212.08±11.53^{ab}	369.58±3.82^{b}	536.78±59.75^{a}	442.33±15.28^{abc}
Total		243.58±30.69	283.56±85.29	162.39±11.65	346.46±13.34	385.11±45.59	401.08±12.23

Means ± SDV, Difference letters showed significant difference using Duncan multiple range test (DMRT) at p<0.05 level. Values are means at (P-0.05). T_1=$N_{350}P_{213}K_{213}$, T_2=$N_{350}P_{320}K_{213}$, T_3=$N_{525}P_{213}K_{213}$, T_4=$N_{525}P_{320}K_{213}$, T_5=$N_{700}P_{213}K_{213}$, T_6=$N_{700}P_{320}K_{213}$, T_7=$N_{800}P_{213}K_{213}$, T_8=$N_{800}P_{230}K_{213}$ Kg/ha AE=aqueous extract, ME= methanol extract, GAE= Gallic acid equivalent, QE= quercetin equivalent**,** TPC**=** Total phenolic content, TFC=Total flavonoid content, PAC=Proanthocynadin

7.3.4 Influence of fertilizer treatments on nutritional constituents of leaf and root samples of Burdock for pot experiment

The different nutritional components for leaf and root samples of burdock for the pot experiment were significantly ($P<0.05$) affected by fertilizer treatments, except for root lipid content which was not significant ($P>0.05$) (Table 7.5). Generally, the ash content for leaf samples was higher than those for root samples. T_7 had the highest influence on leaf ash content (18.1%) with a 7.74% increase compared to the control (T_1) and the least effect was recorded by T_4 (14.5%) with a 13.69% decrease compared to the control. However, for root ash content T_2 (8.6%) recorded the highest influence with 11.63% increase compared to the control, while the least was recorded by T_6 (5.5%) with a 27.63% reduction compared to the control. For crude lipid content T_2 (6.1%) had the highest influence with a 90.63% increase while the least was recorded by T_3 (3.0%) with a 6.5% decrease compared to the control for leaf lipid content. Fertilizer treatment had no significant effect on root lipid content. However, T_7 (5.5%) recorded the highest effect with a 7.8% increase compared to the control and the least effect was recorded by T_4 (3.8%) with a 25.49% decrease compared to the control. Interestingly, leaf and root fibre content recorded the same scenario, T_2 (8.48%) with a 70.97% increase compared to the control and the least by T_6(3.44%) with a 30.65% decrease compared to the control for leaf fibre content. On the other hand, for root fibre content T_2 (2.72%) recorded the highest effect with a 17.24% increase compared to the control and the least by T_4 (1.12%) with a 51.72% reduction compared to the control. Overall, the leaf fibre contents were higher than those of roots among fertilizer treatments. Furthermore, leaf protein contents were also higher than those of root among fertilizer treatments. T_7 (20.9%) had the highest effect with a 9.42% increase compared to the control while the least was recorded by T_2 (13.83%) with a 27.64% reduction compared to the control for leaf protein. Equally, for root protein, the highest effect was demonstrated by T_7 (15.12%) with a 29.89% increase compared to the control while the least was recorded by T_8 (10.72%) with a 7.9% reduction compared to the control. Regarding carbohydrate content, T_4 (59.26%) had the highest yield with a 5.93% increase compared to the control for leaf and 76.64% for root with a 4.49% increase compared to the control. Overall, the root carbohydrate contents were higher than those of the leaf among the different fertilizer treatments (Table 7.5)

7.3.5 Influence of fertilizer treatments on nutritional constituent of leaf and root samples of Burdock for field experiment

The nutritional components of burdock root and leaf samples for the field experiment also had a significant ($P<0.05$) influence as affected by fertilizer treatments except for leaf ash content which was not significant ($P>0.05$). (Table 7.5). Overall, leaf ash contents were higher than those of roots among the different fertilizer treatments. T_2 (12.5 %) had the highest leaf ash content with a 2.46%

increase compared to the control. (T_1). Similarly, for root ash content T_5 (6.4%) had the highest yield with a 30.61% increase compared to the control while the least was recorded by T_8 (3.5 %) with a 28.57% decrease compared to the control. Furthermore, for leaf lipid content T_6 (12.4 %) recorded the highest yield with a 51.22 % increase compared to the control. While the least was recorded by T_8 (7.2 %) with a 12.19% reduction compared to the control. In the same way, for root lipid content, T_4 (38.3 %) recorded the highest yield with a 403.95% increase compared to the control, while the least was recorded by T_7 (5.8 %) with a 23.68 % decrease compared to the control. Regarding the fibre and protein content, the leaf samples had higher yields than root samples among the fertilizer treatments. T_2 (6.64 %) had the highest leaf fibre content with a 15.28% increase compared to the control while T_6 (3.2%) had the highest root fibre content with a 185.71% increase compared to the control. All the same for protein content T_7 (28.9 %) recorded the highest with a 2.12% increase compared to the control. While root protein T_5 (13.5 %) recorded the highest with a 27.84 % increase compared to the control. Considering the carbohydrate content, root samples recorded higher yields than leaf samples. Nevertheless, T_8 (51.8 %) recorded the highest yield with 11.45 % increase compared to the control. While the least was recorded by T_6 (41.86 %) with a 9.98% decrease compared to the control for leaf samples. Furthermore, T_2 (77.74 %) recorded the highest yield with a 2.56 % increase compared to the control, while the least was recorded by T_4 (46.66 %) compared to the control for root samples (Table 7.5)

Table 7.5. Proximate analysis of root and leaf samples of Burdock as influenced by fertilizer treatments. Values are means at (p=0.05).

Treatments	% Ash leaf	% Ash root	% Lipid leaf	% Lipid root	% Fibre leaf	% Fibre root	% Protein leaf	% Protein root	%Carbohydrate leaf	% Carbohydrate root
					Pot Experiment					
$T_1(N_1P_1K_1)$	16.80±0.57ab	7.6±1.92ab	3.2±0.84bc	5.1±0.96a	4.96±1.46bc	2.32±0.52ab	19.10±0.32b	11.64±0.68bc	55.94±1.51a	73.34±2.80a
$T_2(N_1P_2K_1)$	16.62±0.85ab	8.6±3.72a	6.1±2.90a	4.6±2.82a	8.48±3.48a	2.72±1.73a	13.82±0.39f	11.48±0.69bc	54.98±2.49a	72.60±4.62ab
$T_3(N_2P_1K_1)$	15.10±1.88bc	6.1±0.74ab	3.0±1.06c	4.4±2.36a	7.12±2.22ab	1.44±0.67ab	15.92±1.03e	11.82±0.18bc	58.86±4.05a	76.24±1.53a
$T_4(N_2P_2K_1)$	14.50±1.97c	5.9±0.65b	5.1±1.08ab	3.8±1.35a	5.16±2.51bc	1.12±0.59b	15.98±0.49e	12.54±0.84b	59.26±4.54a	76.64±2.18a
$T_5(N_3P_1K_1)$	15.80±1.15bc	5.7±1.04b	5.6±2.22ab	4.3±2.23a	5.6±2.21bc	1.60±0.89ab	17.32±0.42d	14.62±1.39a	55.68±4.47a	73.78±3.89a
$T_6(N_3P_2K_1)$	15.00±1.32bc	5.5±1.27b	5.0±0.35ab	4.1±2.27a	3.44±0.83c	2.00±1.23ab	18.10±0.00c	12.20±0.00b	58.46±1.57a	76.20±2.24a
$T_7(N_4P_1K_1)$	18.10±1.19a	8.1±1.71ab	4.1±2.10abc	5.5±2.72a	7.12±1.11ab	2.40±0.49ab	20.9±0.00a	15.12±1.26a	49.78±2.72b	68.88±4.63b
$T_8(N_4P_2K_1)$	16.30±1.68abc	7.5±1.12ab	3.9±1.47abc	5.4±2.13a	5.12±1.11bc	2.08±0.52ab	18.08±0040c	10.72±0.45c	56.60±2.85a	74.30±2.46a
LSD	0.078	0.51	0.056	NS	0.145	0.065	0.846	0.061	0.077	0.079
					Field Experiment					
$T_1(N_1P_1K_1)$	12.2±0.57^{a}	4.9±0.82^{ab}	8.2±0.91^{bc}	7.6±3.42^{d}	5.76±0.96^{a}	1.12±0.34c	28.30±1.11^{a}	10.56±0.39^{c}	46.48±2.67^{ab}	75.80±3.50^{a}
$T_2(N_1P_2K_1)$	12.5±1.36^{a}	5.8±0.91^{ab}	9.2±1.15^{bc}	6.4±1.19^{d}	6.64±0.83^{a}	2.96±2.01^{ab}	26.22±0.64^{b}	7.10±0.35^{e}	45.26±1.54^{ab}	77.74±2.77^{a}
$T_3(N_2P_1K_1)$	11.5±0.50 ^{a}	5.3±1.45^{ab}	10.2±0.91^{ab}	6.7±1.35^{d}	4.32±2.07^{a}	1.52±0.18^{bc}	28.90±2.15^{a}	10.48±0.25^{c}	45.20±4.59^{ab}	75.80±0.99^{a}
$T_4(N_2P_2K_1)$	12.0±1.17 ^{a}	5.3±1.35^{ab}	10.0±1.26^{abc}	38.3±3.09^{a}	5.6±2.62^{a}	1.12±0.52^{c}	26.10±0.35^{b}	8.62±0.29^{d}	45.98±3.29^{ab}	46.66±4.50^{c}
$T_5(N_3P_1K_1)$	11.4±0.89^{a}	6.4±4.12^{a}	8.2±0.84^{bc}	28.5±3.88^{b}	6.56±4.30^{a}	0.96±0.46^{c}	27.78±0.36^{a}	13.5±0.28^{a}	45.16±4.96^{ab}	50.64±2.05^{c}
$T_6(N_3P_2K_1)$	11.6±0.65^{a}	6.0±1.11^{ab}	12.2±4.87^{a}	29.5±6.44^{b}	6.08±2.46^{a}	3.20±1.49^{a}	28.60±0.42^{a}	11.9±0.54^{b}	41.84±3.01^{b}	49.40±7.05^{c}
$T_7(N_4P_1K_1)$	11.7±0.84a	5.3±0.76^{ab}	7.9b±1.25^{c}	5.8±2.77^{d}	6.48±1.91^{a}	1.36±0.61^{c}	27.78±0.36^{a}	10.76±0.05^{c}	45.80±2.50^{ab}	76.78±2.64^{a}
$T_8(N_4P_2K_1)$	11.9±1.08a	3.5±0.61^{b}	7.2±0.76^{c}	20.4±6.05^{c}	6.64±0.36^{a}	1.52±0.52^{bc}	28.20±0.55^{a}	10.7±0.27^{c}	51.80±13.07^{a}	63.88±6.15^{b}
LSD	NS	0.57	0.104	0.519	0.173	0.053	0.108	0.234	0.113	0.165

Means in the same column with the same superscript are not significantly difference (P≥0.05). Values are means at (P -0.05). T1=N350P213K213, T2=N350P320K213, T3=N525P213K213, T4=N525P320K213, T5=N700P213K213, T6=N700P320K213, T7=N800P213K213, T8=N800P230K213 Kg/ha, LSD= Least Significant Different

7.3.6 Influence of fertilizer treatments on DPPH antioxidant activities for root and leaf extract of Burdock for pot and field experiment

The results of antioxidant activity of DPPH scavenging activity for dried root and leaf extracts are presented in Table 7.6. Significant differences ($p<0.05$) were observed among the different treatment combinations for aqueous and methanol leaf and root extracts for cultivated Burdock in the pot experiment. Overall, treatment combinations with a lower level of phosphorous recorded lower IC_{50} values compared to those with a higher level of phosphorous. Interestingly for methanol root extract T_5 recorded the lowest IC_{50} value (15.46 µg /ml) and the highest by T_3 (426.94 µg /ml). However, for aqueous root extract, it was reversed with the lowest IC_{50} value (52.73) recorded by T_3 and the highest (611.76 µg /ml) value recorded by T_5. For methanol leaf extract T_2 recorded the lowest IC_{50} value (26.50 µg /ml) and T_4 the highest (344.4 µg /ml 1) while for aqueous leaf extract the lowest IC_{50} value (25.68 µg /ml) was recorded by T_4 and the highest (267.24 µg /ml) by T_6 (Table 7.6).

Table 7.6.Influence of fertilizer treatments on DPPH scavenging activity for dried root and leaf extracts of Burdock. Values are means of IC_{50} (µg /ml) at (P-0.05).

Treatments	Experiment	ME root(µg /ml)	AE root(µg /ml)	ME leaf(µg /ml)	AE leaf(µg /ml)
T1($N_1P_1K_1$)		250.06±8.10^{c}	240.45±24.86^{ab}	26.91±3.38^{f}	127.98±12.67^{b}
T2($N_1P_2K_1$)		111.12±2.78^{d}	278.54±38.19^{a}	26.50±4.39^{f}	44.01±6.28^{c}
T3($N_2P_1K_1$)	Pot	426.94±60.34^{a}	52.73±8.54^{e}	252.74±8.68^{a}	28.77±3.51^{d}
T4($N_2P_2K_1$)	experiment	392.29±9.10^{a}	166.62±24.45^{d}	344.41±3.13^{a}	25.69±1.98^{d}
T5($N_3P_1K_1$)		15.46±4.39^{e}	61.76±28.61^{e}	153.54±7.72^{c}	258.79±12.21^{a}
T6($N_3P_2K_1$)		31.63±8.83^{e}	69.55±16.51^{e}	110.08±6.96^{d}	267.24±11.05^{a}
T7($N_4P_1K_1$)		305.14±11.43^{b}	70.79±15.19^{e}	53.14±5.33^{e}	39.08±2.83^{cd}
T8($N_4P_2K_1$)		285.59±6.59^{bc}	225.29±21.51^{c}	46.44±13.26^{e}	50.47±4.49^{c}
ASC		55.76±6.91	55.76±6.91	55.76±6.91	55.76±6.91
DDM		75.06±5.46	75.06±5.46	75.06±5.46	75.06±5.46
T1($N_1P_1K_1$)		274.49±3.33^{b}	45.69±5.71^{c}	328.19±7.12^{a}	187.44±4.48^{d}
T2($N_1P_2K_1$)		394.27±15.18^{a}	107.59±10.81^{b}	232.03±7.63^{a}	236.83±13.82^{b}
T3($N_2P_1K_1$)		142.42±21.85^{c}	210.08±9.89^{a}	466.94±14.64^{a}	107.84±4.45^{f}
T4($N_2P_2K_1$)	Field	134.96±23.34^{c}	79.30±6.64^{c}	210.23±4.58^{a}	82.68±4.88^{g}
T5($N_3P_1K_1$)	experiment	24.93±1.87^{d}	114.96±4.94^{b}	318.87±20.24^{a}	35.66±3.13^{h}
T6($N_3P_2K_1$)		39.96±3.83^{d}	38.91±5.32^{cd}	427.22±18.95^{a}	153.71±6.16^{e}
T7($N_4P_1K_1$)		24.96±1.94^{d}	33.73±3.66^{cd}	193.74±2.64^{a}	205.83±8.32^{c}
T8($N_4P_2K_1$)		42.14±3.45^{d}	29.42±3.82^{d}	222.45±18.05^{a}	330±12.29^{a}
ASC		55.76±6.91	55.76±6.91	55.76±6.91	55.76±6.91
DDM		75.06±5.46	75.06±5.46	75.06±5.46	75.06±5.46

Means ± SDV, Difference letters showed significant difference using Duncan multiple range test (DMRT) at ($p<0.05$). Values are means of IC_{50} at (P-0.05). $T_1=N_{350}P_{213}K_{213}$, $T_2=N_{350}P_{320}K_{213}$, $T_3=N_{525}P_{213}K_{213}$, $T_4=N_{525}P_{320}K_{213}$ $T_5=N_{700}P_{213}K_{213}$, $T_6=N_{700}P_{320}K_{213}$, $T_7=N_{800}P_{213}K_{213}$, $T_8=N_{800}P_{230}K_{213}$ Kg/ha, ME= methanol Extract, AE=Aqueous Extract, ASC= Ascorbic acid, DDM=2,6-di-tert-butyl-4-methylphenol.

Results of the antioxidant activity for DPPH scavenging activity for aqueous root and leaf extract for field experiment are presented in Table 7.6. Overall, methanol extract demonstrated higher IC_{50} values compared to those of aqueous extracts among treatment combinations, similar to what

was observed for methanol and aqueous root and leaf extracts. Treatment combinations of lower phosphorous levels recorded lower IC_{50} values than those with higher phosphorous levels. However, for methanol leaf extracts, treatment combinations with lower phosphorous levels recorded higher IC_{50} values compared to those with higher phosphorous levels for extracts of field cultivated burdock (Table 7.6).

7.3.7 Influence of fertilizer treatments on nitric oxide scavenging activity for dried root and leaf extracts of Burdock for pot and field experiments

Nitric oxide scavenging activity for aqueous root and leaf extracts had significant differences $p<0.05$) among treatment combinations for aqueous and methanol root and leaf extracts for pot cultivated Burdock (Table 7.7). The highest IC_{50} value (302.62 µg /ml) for methanol root extract was recorded by T_4 and the lowest (118.84 µg /ml) was recorded by $T_{6.}$ While for aqueous root extract, the highest IC_{50} value (214.13 µg /ml) was recorded by T_3 and the lowest (44.55 µg /ml) by $T_{1.}$ However, for methanol leaf, the lowest IC_{50} value (122.6 µg /ml) was recorded by T_7 and the highest (321.46 µg /ml) by T_8. Likewise, for aqueous leaf extract the lowest IC_{50} value (249.61 µg /ml) was recorded by T_8 and the highest (415.05 µg /ml) by $T_{2.,}$ Generally, Methanol root extracts recorded higher IC_{50} values among treatment combinations than aqueous root extracts. However, leaf, aqueous extracts recorded higher IC_{50} values than methanol extracts (Table 7.7).

Scavenging activity for nitric oxide for aqueous and methanol root extracts for field experiment had significant differences ($p<0.05$) among treatment means (Table 7.7). The lowest IC_{50} value (30.06) µg /ml was recorded by T_2 and the highest (282.76 µg /ml) by T_3 for methanol root extract. While for aqueous root extract the lowest IC_{50} value (145.25 µg /ml) was recorded by T_7 and the highest (323.96 µg /ml) by T_6. For methanol leaf extract T_4 recorded the lowest IC_{50} value (36.98 µg /ml) and highest (320.44 µg /ml) by T_1 while for aqueous leaf extract the lowest IC50 value (158.56 µg /ml) was recorded by T_7 and the highest (598.69 µg /ml) by T5. Overall, aqueous extract recorded higher IC_{50} values than methanol extract for field cultivated Burdock.

Table 7.7Influence of fertilizer treatments on nitric oxide scavenging activity for dried root and leaf extracts of Burdock. Values are means of IC50 (µg/ml) at (P-0.05).

Treatments	Experiment	ME root(µg/ml)	AE root(µg/ml)	ME leaf(µg/ml)	AE leaf(µg/ml)
T1($N_1P_1K_1$)		292.91±10.42^{a}	44.55±12.57^{e}	207.10±34.89^{b}	339.84±23.88^{bcd}
T2($N_1P_2K_1$)		156.14±25.39^{b}	48.58±11.87^{e}	282.52±41.34^{a}	415.05±10.55^{a}
T3($N_2P_1K_1$)	Pot	157.74±29.88^{b}	214.13±29.09^{a}	146.76±25.01^{cd}	293.33±20.81^{def}
T4($N_2P_2K_1$)	experiment	302.62±29.71^{a}	67.44±14.43^{de}	282.71±22.11^{a}	351.48±42.09^{bc}
T5($N_3P_1K_1$)		121.59±24.13^{b}	190.28±27.78^{bc}	198.88±11.56^{bc}	308.15±25.09^{cde}
T6($N_3P_2K_1$)		118.84±14.41^{b}	154.28±18.46^{c}	194.16±33.34^{bc}	285.92±15.53^{ef}
T7($N_4P_1K_1$)		139.44±28.25^{b}	66.98±30.66^{de}	122.60±45.82^{d}	381.29±33.56^{ab}
T8($N_4P_2K_1$)		281.34±13.73^{a}	104.27±28.48^{d}	321.46±20.29^{a}	249.61±20.82^{f}
ASC		58.66±3.51	58.66±3.51	58.66±3.51	58.66±3.51
DDM		85.06±4.46	85.06±4.46	85.06±4.46	85.06±4.46
T1($N_1P_1K_1$)		141.82±38.11^{c}	162.39±23.26^{c}	320.44±28.98^{a}	296.36±40.86^{b}
T2($N_1P_2K_1$)		30.06±17.52^{d}	248.42±14.87^{b}	293.44±28.73^{a}	282.06±38.13^{b}
T3($N_2P_1K_1$)	Field	282.76±25.17^{a}	223.47±25.41^{b}	154.33±25.05^{c}	267.64±16.54^{b}
T4($N_2P_2K_1$)	experiment	183.49±12.41^{b}	249.09±14.80^{b}	36.98±29.46^{d}	251.25±35.63^{b}
T5($N_3P_1K_1$)		34.28±22.84^{d}	239.05±14.77^{b}	183.13±32.43^{bc}	598.69±54.77^{a}
T6($N_3P_2K_1$)		110.83±23.37^{c}	323.96±24.76^{a}	156.15±24.75^{c}	239.17±32.58^{b}
T7($N_4P_1K_1$)		31.37±6.37^{d}	145.35±24.06^{c}	223.06±9.32^{b}	158.56±19.82^{c}
T8($N_4P_2K_1$)		63.57±30.99^{d}	242.30±4.82^{b}	195.11±45.47^{bc}	236.46±35.05^{b}
ASC		58.66±3.51	58.66±3.51	58.66±3.51	58.66±3.51
DDM		85.06±4.46	85.06±4.46	85.06±4.46	85.06±4.46

Means ± SDV, Difference letters showed significant difference using Duncan multiple range test (DMRT) at ($p<0.05$). Values are means of IC_{50} at (P-0.05) $T_1=N_{350}P_{213}K_{213}$, $T_2=N_{350}P_{320}K_{213}$, $T_3=N_{525}P_{213}K_{213}$, $T_4=N_{525}P_{320}K_{213}T_5=N_{700}P_{213}K_{213}$, $T_6=N_{700}P_{320}K_{213}$, $T_7=N_{800}P_{213}K_{213}$, $T_8=N_{800}P_{230}K_{213}$ Kg/ha, ME=methanol extract., AE=aqueous extract.

7.3.8 Influence of fertilizer treatments on hydrogen peroxide scavenging activity for dried root and leaf extracts of Burdock for pot and field experiments

Hydrogen peroxide scavenging activity was significant ($p<0.05$) among treatment combinations for aqueous and methanol leaf and root extracts for Burdock pot experiment (Table 7.8). T_8 recorded the lowest IC_{50} value (24.37 µg /ml) for methanol root extract and the highest (318.23 µg /ml) by T_3. Likewise, for aqueous root extract, the lowest IC_{50} value was recorded by T_8 and the highest (303.89 µg /ml) by T_4. However, for methanol leaf extract the lowest IC_{50} value (98.63 µg /ml) was recorded by T_6 and the highest (317.0 µg /ml 2) by T_1, while for aqueous leaf extract the lowest IC_{50} values (106.65 µg /ml) was recorded by T_2 and the highest (452.95 µg /ml) by T_8. Generally, aqueous extracts recorded higher IC_{50} values compared to those of methanol extracts for Burdock pot experiment.

Table 7.8.Influence of fertilizer treatments on hydrogen peroxide scavenging activity of dried root and leaf extracts of Burdock. Values are means of IC50 (µg/ml) at (P-0.05).

Treatments	Experiment	ME root(µg/ml)	AE root(µg/ml)	ME leaf(µg/ml)	AE leaf(µg/ml)
T1($N_1P_1K_1$)		56.33± 7.98^{e}	56.05±10.04^{d}	317.02±23.74^{a}	235.54±41.56^{d}
T2($N_1P_2K_1$)		46.92± 2.08^{ef}	243.16±27.02^{b}	316.32±23.43^{a}	106.65±9.88^{a}
T3($N_2P_1K_1$)	Pot	318.23±9.68^{a}	258.28±2.73^{b}	289.44±4.49^{ab}	233.28±10.48^{d}
T4($N_2P_2K_1$)	Experiment	201.68±4.18^{c}	303.89±25.06^{a}	264.97±4.53^{b}	284.21±13.68^{c}
T5($N_3P_1K_1$)		100.05±3.41^{d}	164.36±32.24^{c}	222.17±16.16^{c}	249.33±13.67^{d}
T6($N_3P_2K_1$)		264.66±9.20^{b}	272.72±12.87^{ab}	98.63±1.77^{e}	299.28±10.33^{c}
T7($N_4P_1K_1$)		73.13± 9.72^{de}	78.35±32.70^{d}	163.59±17.28^{d}	372.50±5.43^{b}
T8($N_4P_2K_1$)		24.37± 3.06^{f}	36.03±6.58^{a}	301.24±9.79^{a}	452.95±8.97^{a}
ASC		58.66±3.51	58.66±3.51	58.66±3.51	58.66±3.51
DDM		85.06±4.46	85.06±4.46	85.06±4.46	85.06±4.46
T1($N_1P_1K_1$)		29.87± 4.41^{c}	31.53±6.81^{de}	151.00±10.02^{d}	22.82± 1.12^{c}
T2($N_1P_2K_1$)		30.52± 10.25^{c}	22.32±0.64^{e}	187.05±21.07^{bc}	174.14±21.41^{b}
T3($N_2P_1K_1$)	Field	74.29± 16.77^{a}	23.92±6.58^{de}	183.81±23.39^{c}	246.43±7.7$_{a}$
T4($N_2P_2K_1$)	Experiment	23.37± 2.83^{c}	67.25±4.66^{c}	82.46± 13.31^{e}	188.43±10.6^{b}
T5($N_3P_1K_1$)		27.23± 5.77^{c}	39.57±13.40^{d}	192.48±6.18b^{c}	26.58± 2.16^{c}
T6($N_3P_2K_1$)		30.51± 4.48^{c}	37.96±6.51^{de}	33.57± 3.13^{f}	28.96± 5.91^{c}
T7($N_4P_1K_1$)		29.69± 11.22^{c}	99.79±6.31^{b}	257.12±13.48^{a}	171.36±8.37^{b}
T8($N_4P_2K_1$)		54.25± 8.95^{b}	166.37±14.86^{a}	210.79±7.51^{b}	30.19± 5.83^{c}
ASC		58.66±3.51	58.66±3.51	58.66±3.51	58.66±3.51
DDM		85.06±4.46	85.06±4.46	85.06±4.46	85.06±4.46

Means ± SDV, Difference letters showed significant difference using Duncan multiple range test (DMRT) at ($p<0.05$). Values means of IC_{50} at (P-0.05) $T_1=N_{350}P_{213}K_{213}$, $T_2=N_{350}P_{320}K_{213}$, $T_3=N_{525}P_{213}K_{213}$, $T_4=N_{525}P_{320}K_{213}$ $T_5=N_{700}P_{213}K_{213}$, $T_6=N_{700}P_{320}K_{213}$, $T_7=N_{800}P_{213}K_{213}$, $T_8=N_{800}P_{230}K_{213}$ Kg/ha, ME= methanol extract, AE= aqueous extract

The results for hydrogen peroxide scavenging activity for aqueous and methanol root extracts for field cultivated Burdock had a significant difference ($p<0.05$) among treatment combinations (Table 7.8). Overall, IC50 values for aqueous extracts are higher compared to those of methanol extracts. The lowest IC_{50} value (23.37 µg /ml) for root methanol extract was recorded by T_4 and the highest (74.29 µg /ml) by T_3. Likewise, for aqueous root extracts the lowest IC_{50} value (22.32 µg /ml) was recorded by T_2 and the highest (166.37 µg /ml) by T_8. Furthermore, for methanol leaf extract T_6 recorded the lowest IC_{50} value (33.57 µg /ml) and the highest (257.12 µg /ml) was recorded byT_7.However, for aqueous leaf extract the lowest IC_{50} value (22.82 µg /ml) was recorded by T_1 and the highest (246.43 µg /ml) by T_3.

7.3.9 Influence of fertilizer treatments on anti-inflammatory activities for dried root extracts of Burdock for pot and field experiment

Anti-inflammatory activity for aqueous and methanol dried root and leaf extracts for cultivated Burdock in pot experiment demonstrated significant differences ($p<0.05$) among treatment combinations (Table 7.9). T5 recorded the lowest IC_{50} value (832.45 µg /ml) for anti-inflammatory activity and the highest (973.65 µg /ml) was recorded by T_1 for methanol root extract. Nevertheless, for aqueous root extract the lowest IC_{50} value (544.42 µg /ml) was recorded by T_8 and the highest (736.23 µg /ml) by T_6. Likewise, for methanol leaf extract, the lowest IC_{50} value (748.11 µg /ml) was recorded by T_8 while the highest (972.75 µg /ml) was recorded by T_1. Furthermore, in the aqueous leaf extracts the lowest IC_{50} value (167.57 µg /ml) was recorded by T_6 while the highest (739.81 µg /ml) was recorded by T_3. Overall, methanol extracts recorded higher IC_{50} values compared to those of aqueous extracts among treatment combinations for cultivated Burdock from the pot experiment.

Table 7.9 Influence of fertilizer treatments on anti-inflammatory activity of dried root and leaf extracts of Arctium lappa L for pot experiment. Values are means of IC_{50} (µg/ml) at (P-0.05)

Treatments	Experiment	ME root(µg/ml)	AE root(µg/ml)	ME leaf(µg/ml)	AE leaf(µg/ml)
T1($N_1P_1K_1$)		973.65±8.741^{a}	666.20±12.59^{b}	972.75±41.68^{a}	632.96±2.48^{b}
T2($N_1P_2K_1$)		952.13±21.15^{a}	680.94±35.01^{b}	836.99±20.77^{b}	442.03±37.90^{d}
T3($N_2P_1K_1$)	Pot	905.14±19.79^{b}	668.86±8.78^{b}	866.92±30.74^{b}	739.81±21.75^{a}
T4($N_2P_2K_1$)	experiment	908.69±5.15^{b}	736.23±11.21^{a}	891.41±54.7^{b}	590.37±8.55^{b}
T5($N_3P_1K_1$)		832.45±18.22^{c}	613.52±31.43^{c}	791.76±15.71^{cd}	540.69±19.78^{c}
T6($N_3P_2K_1$)		889.49±22.12^{b}	747.84±4.38^{a}	800.49±22.78^{cd}	167.57±20.55^{f}
T7($N_4P_1K_1$)		857.28±5.72^{c}	643.22±3.98^{bc}	797.58±23.07^{cd}	429.29±13.0^{d}
T8($N_4P_2K_1$)		945.99±25.13^{a}	544.42±28.27^{d}	748.11±9.51^{d}	374.70±68.99^{e}
Diclofenac		498.55±50.55	498.55±50.55	498.55±50.55	498.55±50.55
T1($N_1P_1K_1$)		901.76±45.01^{ab}	554.84±23.16^{c}	776.12±9.65^{bc}	676.46±9.82^{b}
T2($N_1P_2K_1$)		813.68±14.15^{e}	576.73±13.64^{bc}	891.51±74.40^{a}	629.43±15.44^{c}
T3($N_2P_1K_1$)	Field	744.67±3.08^{f}	602.87±13.87^{ab}	831.35±12.76^{b}	730.24±26.71^{a}
T4($N_2P_2K_1$)	experiment	839.24±12.26^{de}	327.27±21.93^{f}	806.66±2.23^{bc}	738.44±8.46^{a}
T5($N_3P_1K_1$)		895.99±2.00^{abc}	389.80±10.70^{d}	767.34±7.87^{c}	729.99±37.35^{a}
T6($N_3P_2K_1$)		935.11±36.02^{a}	358.86±21.41^{e}	773.06±20.65^{bc}	635.49±21.89^{c}
T7($N_4P_1K_1$)		867.96±10.78^{bcd}	611.03±10.18^{a}	931.49±20.27^{a}	744.77±9.789^{a}
T8($N_4P_2K_1$)		857.89±3.34^{cd}	592.01±7.12^{ab}	783.02±21.68^{bc}	724.86±5.402^{a}
Diclofenac		498.55±50.55	498.55±50.55	498.55±50.55	498.55±50.55

Means ± SDV, Difference letters showed significant difference using Duncan multiple range test (DMRT) at ($p<0.05$). Values are means at (P-0.05). $T_1=N_{350}P_{213}K_{213}$, $T_2=N_{350}P_{320}K_{213}$, $T_3=N_{525}P_{213}K_{213}$, $T_4=N_{525}P_{320}K_{213}$ $T_5=N_{700}P_{213}K_{213}$, $T_6=N_{700}P_{320}K_{213}$, $T_7=N_{800}P_{213}K_{213}$, $T_8=N_{800}P_{230}K_{213}$ Kg/ha, ME= methanol extract, AE=aqueous extract.

Root and leaf extracts from filed cultivated Burdock demonstrated significant differences ($p<0.05$) among treatment combinations for anti-inflammatory activity (Table 7.9). Nevertheless, for methanol root extract the lowest IC_{50} value (744.67 µg /ml) was recorded by T_3 and the highest (935.11 µg /ml) by T_6. Equally, for aqueous root extract the lowest IC_{50} value (327.27 µg /ml) was recorded by T_4 and the highest (611.03 µg /ml) by T_7. However, for methanol leaf extract the lowest IC_{50} value (773.06 µg /ml) was recorded by T_5 and the highest (931.49 µg /ml) by T_7. Nevertheless, for aqueous leaf extract the lowest IC_{50} value (629.43 µg /ml) was recorded byT_2 while the highest (744.77 µg /ml) was recorded by T_7. Overall, methanol extract recorded higher IC_{50} values compared to aqueous extracts among treatment combinations for field cultivated Burdock (Table 7.9).

7.3.10 Influence of fertilizer treatments on volatile oil composition of Burdock root as determined by GC-MS for field experiment.

The GC-MS analysis of essential oil for burdock root as affected by fertilizer treatments validated a variation in chemical constituents among the different treatment combinations (Table 7.10). Interestingly, the greatest variation was demonstrated by T_7 with a total of 20 compounds identified. Excitingly, seven notable compounds by their peak percentage above 5%. were Cyclohexane-ethyl (18.83%), 2-Propyl-pentanol-trifluoroacetate (16.835), Heptane,2,6-dimethyl (12.72%), 1,1,4-Trmethylcyclohexane (7.26%), Octane-3-methyl (6.21%), Octane,2-methyl (5.36%), and Heptane,4-azido (5.22%) were identified. Furthermore, T_3 recorded 19 compounds with six notable compounds of peak percentages over 5% which were Cyclohexane-ethyl (16.67%), and 3-Trifluoroacetoxyloethyldecane (15.49%), 1,1,4-Trimethyl cyclohexane (7.21%), Octane,3-methyl (6.8%), Hexane,3-ethyl (5.8%) and 4-Undecane,7-methyl (5.84%). Similarly, T_4, T_5, T_6 and T_8 all had 14 compounds. However, for T_4, four notable compounds were identified with peak percentages over 5% which were Cyclohexane-ethyl (26.11%), 3-Trifluoroacetoxy-6-ethyldecane (23.41%), Octane, 2-methyl (16.45%) and Cyclooctane-butyl (9.12%). On the other hand, T_5 had seven compounds with peak percentages of over 5% which were Cyclohexane-ethyl (22.69%), 2-Methyl-1-tetradecane (19.53%), Heptane,2,6-dimethyl (12.94), Cyclohexane,1,13-trimethyl (8.24%), Octane,3-methyl (6.5%), Octane,2-methyl (5.71%), Decane,2,5,6-trimethyl (5.42%) and Heptane, 2,3-dimethyl (5.25). Similarly, T_6 had eight compounds with peak percentages of over 5% which were, cyclohexane-ethyl (20.35%), Decyltrifluoroacetate (17.92%), Heptane, 2,6-dimethyl (12.125%), Cyclohexane,1,1,3-trimethyl (10.85%), Heptane,2,2,3,5-tetramethyl (6.96%), Octane,2-methyl (6.39%), Hexane,2,3,4-trimethyl (6.39%), and Heptane,2,3-dimethyl (5.31%). T8 had seven compounds with notable peak percentages of over 5% which were Cyclopentane, 1-ethyl-3-methyl-trans (22.14%), Aceticacidcyna,2-ethylhexylester (19.93%),

Octane-2-methyl (13.25%), Cyclohexane,1,1,3-trmethyl (8.33%), Acid,2-ethylhexylester (6.56%), Octane,2-methyl (6.1%), and Hexane,3-ethyl (5.43%). Nevertheless, 13 compounds were identified in T_1 and T_2. However, the peak percentages of over 5% for T1 were recorded by Cyclohexane-ethyl (21.83%), 3-hexadecene(z) (18.63%), Cyclohexane1,1,3-trimethyl (7.88%), Heptane, 2,2,3,5-tetramethyl (5.81%) and Ether, hexyl, pentyl (5.22%). Similarly, for T_2 four notable peak percentages identified of over 5% were, Cyclohexane, ethyl (17.34%), Decanal (13.34%), Ether, hexyl pentyl (11.9%), and Hydroxylamine (5.12%). Overall, the two treatments T_3 and T_7 with the highest compound identified were treatments with less supplementary phosphorous.

Table 7.10 Chemical composition of volatile oil of Burdock root as determined by GC-MS

Compounds	**(Treatment 1)**	**PK %**	**RT**	**Cas N0**	**KI**
2-ethyl-1-hexanol		1.06	3.93	25181	1484
Heptafluorobutyrate octane,2-methyl		1,17	3.97	3221-61-2	483
3-hexadecene(z)		18.6	4.09	34303-81-6	1624
Cyclohexane-ethyl		21.8	4.16	1678-91-7	885
Cyclohexane 1,1,3-trimethyl		7.88	4.23	3073663	834
4 methyl-4-nonadecane		2.46	4.31	20915	-
Cyclopropane, 1 hexyl-2-mythyl		1.49	4.42	62238099	963
Heptane, 2,3-dimethyl		3.88	4.51	3074-71-3	846.5
Cyclopentane, 1-buty-2-ethyl		2.26	4.55	72993-32-9	1083
Ether,hexyl pentyl		5.22	4.66	32357-8	886
Undecane, 4,7,-dimethyl		4.47	4.70	17301-32-5	1205
Heptane,2,2,3,5 tetra methyl		5.81	4.85	61868-42-6	-
Octane,3.4 dimethyl		3.05	5.58	15869-92-8	935

T=Fertilizer treatments=T_1 =$N_{350}P_{213}K_{213}$, T_2=$N_{350}P_{320}K_{213}$, T_3=$N_{525}P_{213}K_{213}$, T_4=$N_{525}P_{320}K_{213}$ T_5=$N_{700}P_{213}K_{213}$, T_6=$N_{700}P_{320}K_{213}$, T_7=$N_{800}P_{213}K_{213}$, T_8=$N_{800}P_{230}K_{213}$ Kg/ha., PK= Pick percentage, RT= Retention time, KI=Kovat indices

Compounds	**Treatment 2**	**P k %**	**RT**	**Cas N0**	**KI**
Ether,hexyl pentyl		11.9	3.96	32357-83-8	886
Decanal		13.8	4.08	112-312-2	1502
Cyclopropane, nonyl		3.63	4.09	74663-85-7	1285
Cyclohexane, ethyl		17.3	4.14	1678-91-7	885
Cyclopropane,peptyl		3.69	4.29	2511-91-3	913.7

1,4-1,2,3-Triazole	1.00	4.35	288-36-8	1116
Hexane, 2,3,5-trimethyl	4.86	4.49	1069-53-0	820.6
Heptane,2,3-dimethyl	2.72	4.63	3074-71-3	856
Hydroxylamine	5.20	4.68	5618-62-2	1100
Heptane, 3 ethyl	4.62	4.83	15869-80-4	862.1
2-propenoic acidoxiranylmethylester	1.42	4.91	106-90-1	1784
Cyclopentane bromo	1.00	5.32	2404-35-5	905
Pentane,3-ethyl-3methyl	1.91	5.55	1067-08-9	744.3

T=Fertilizer treatments=T_1 =$N_{350}P_{213}K_{213}$, T_2=$N_{350}P_{320}K_{213}$, T_3=$N_{525}P_{213}K_{213}$, T_4=$N_{525}P_{320}K_{213}$ T_5=$N_{700}P_{213}K_{213}$, T_6=$N_{700}P_{320}K_{213}$, T_7=$N_{800}P_{213}K_{213}$, T_8=$N_{800}P_{230}K_{213}$ Kg/ha., PK= Pick percentage, RT= Retention time, KI=Kovat indices

Compounds (Treatment 3)	**P k %**	**RT**	**Cas N0**	**KI**
Dichloroacetic acid,decylester	1.00	3.92	83005-00-9	1684
Octane,2-methyl	1.00	3.96	3221-61-2	864
3-Trifluoroacetoxyloethyldecane	15.5	4.08	116436590	-
Cylohexane,ethyl	16.7	4.15	1678-91-7	885
1,1,4-Trimethyl cyclohexane	7.21	4.22	7094-27-1	843
1-Undecane,7-methyl	2.33	4.29	74630-42-5	1146
1-Heptanol,4-methyl	1.00	4.38	817-914	1973
Cyclopropane,1-methyl-2-pentyl	1.00	4.39	41977-37-1	864
Heptane,2,3-dimethyl	4.81	4.49	3074-71-3	856
Cyclopentane,1-butyl-2ethyl	2.69	4.53	72993-32-9	1083
Hexane,1(hexyloxy) 3--methyl	1.32	4.56	74421-18-4	-
4-Undecane,7-methyl	5.84	4.64	76441-79-7	-
Hexane,3-ethyl	5.80	4.68	619-99-8	800
Octane,3-methyl	6.80	4.83	2216-33-3	874
3,5-octadien, 2-ol	1.00	5.05	69668-82-2	1038
Cyclopentane, 2-ethyl-1,1-dimethyl	1.00	5.21	54549-80-3	861
Cyclooctane, methyl	1.00	5.23	1502-38-1	1009
1-ethy-3-methylcyclohexane	1.26	5.32	3728-55-0	931
Nonane	4.81	5.55	111-84-2	900

T=Fertilizer treatments=T_1 =$N_{350}P_{213}K_{213}$, T_2=$N_{350}P_{320}K_{213}$, T_3=$N_{525}P_{213}K_{213}$, T_4=$N_{525}P_{320}K_{213}$ T_5=$N_{700}P_{213}K_{213}$, T_6=$N_{700}P_{320}K_{213}$, T_7=$N_{800}P_{213}K_{213}$, T_8=$N_{800}P_{230}K_{213}$ Kg/ha., PK= Pick percentage, RT= Retention time, KI=Kovat indices

Compounds (Treatment 4)	P k %	RT	Cas N0	KI
Octane,2-methyl	16.5	3.89	3221-61-2	864
3-trifluoroacetoxy-6-ethyldecane	23.4	4.02	116436590	-
cyclohexane, ethyl	26.1	4.08	1678-91-7	885
Cyclooctane, butyl	9.12	4.15	16538-93-5	876
Decane,3-chloro	1.00	4.23	1002-11-5	1374
Cyclohexane,1,2,3 trimethyl	1.08	4.32	7667-55-2	898
1,alpha,2,alpha,3,alpha	1.00	4.33	1839-88-9	1697
Octane-4-methyl	4.08	4.42	2216-34-4	823
Undecane,5-methyl	1.64	4.60	1632-70-8	1157
Octane,3-methyl	4.42	4.75	2216-33-3	872
Cyclopentane,1-methyl-2-propyl	1.00	5.13	3728-57-2	913
Cyclooctane, methyl	1.00	5.15	1502-38-1	1009
4,ethyl-2-hydroxyclopent-2-en-1-one	1.62	5.23	28017-62-1	1629
Cyclohexane,1-methyl-3-propyl	3.65	5.33	4291-80-9	983

T=Fertilizer treatments=T_1 =$N_{350}P_{213}K_{213}$, T_2=$N_{350}P_{320}K_{213}$, T_3=$N_{525}P_{213}K_{213}$, T_4=$N_{525}P_{320}K_{213}$ T_5=$N_{700}P_{213}K_{213}$, T_6=$N_{700}P_{320}K_{213}$, T_7=$N_{800}P_{213}K_{213}$, T_8=$N_{800}P_{230}K_{213}$ Kg/ha., PK= Pick percentage, RT= Retention time, KI=Kovat indices

Compounds (Treatment 5)	P k %	RT	Cas N0	KI
2-0ctenal-(E)	1.33	3.91	2548-87-0	1056
Heptane,2,6-dimethyl	12.9	3.95	1072-05-5	834
2-methyl-1-teradecane	19.5	4.07	52254-83-3	1489
Cylohexane,ethyl	22.7	4.13	1678-91-7	885
Cyclohexane,1,1,3-trimethyl	8.24	4.20	3073-66-3	834
1-undecane,8-methyl	2.91	4.28	74630-40-3	1124
Cyclohexane,1,2,3-trimethyl-1alpha,3beta	1.23	4.38	7667-55-2	879
Heptane,2,3-dimethyl	5.28	4.48	3074-71-3	847
Cyclohexane,1,2,4-trimethyl	2.43	4.52	1678804	1297
4-undecane,7-methyl	1.60	4.55	76441-79-7	1146
Decane,2,5,6-trimethyl	5.42	4.63	62108-23-0	1121
Octane,2-methyl	5.71	4.66	3221-61-2	864
Heptane,3-ethyl	1.27	4.77	1586-80-4	862
Octane,3-methyl	6.50	4.81	2216-33-3	872

T=Fertilizer treatments=T_1 =$N_{350}P_{213}K_{213}$, T_2=$N_{350}P_{320}K_{213}$, T_3=$N_{525}P_{213}K_{213}$, T_4=$N_{525}P_{320}K_{213}$ T_5=$N_{700}P_{213}K_{213}$, T_6=$N_{700}P_{320}K_{213}$, T_7=$N_{800}P_{213}K_{213}$, T_8=$N_{800}P_{230}K_{213}$ Kg/ha., PK= Pick percentage, RT= Retention time, KI=Kovat indices

Compounds (Treatment 6)	P k %	RT	Cas N0	KI
Heptane,2,6-dimethyl	12.1	3.98	1072-05-5	834
Decyltrifluoroacetate	17.9	4.10	333-88-0	1367
cyclohexane, ethyl	20.3	4.17	1678-91-7	885
Cyclohexane,1,1,3-trimethyl	10.9	4.23	3073—66-3	834
2-Decene,7-methyl	1.8	4.31	74630-23-2	-
Cyclohexane,1-ethyl-2-methyl-trans	1.0	4.41	4923-78-8	989
Cyclohexane,1,2,3-trimethyl (1, alpha,2,alpha,3,alpha)	1.0	4.43	1839-88-9	920
Heptane,2,3-dimethyl	5.3	4.51	3074-71-3	847
Cyclohexane,1,2,4-trimehyl	2.3	4.55	2234-75-5	881
Decane,2,4,6-trimethyl	2.0	4.58	62108-27-4	1121
Hexane,2,3,4-trimethyl	6.4	4.66	921-47-1	850
Octane,2methyl	6.4	4.71	3221-61-2	864
Heptane,2,2,3,5-tetramethyl	6.9	4.85	61868-42-6	873
Heptane,2,4,6-trimethyl	2.9	5.57	2613-61-8	888

T=FERTILIZER TREATMENTS=T_1 =$N_{350}P_{213}K_{213}$, T_2=$N_{350}P_{320}K_{213}$, T_3=$N_{525}P_{213}K_{213}$, T_4=$N_{525}P_{320}K_{213}$ T_5=$N_{700}P_{213}K_{213}$, T_6=$N_{700}P_{320}K_{213}$, T_7=$N_{800}P_{213}K_{213}$, T_8=$N_{800}P_{230}K_{213}$ KG/HA., PK= PICK PERCENTAGE, RT= RETENTION TIME, KI=KOVAT INDICES

Compounds (Treatment 7)	P k %	RT	Cas N0	KI
Heptane,2,6-dimethyl	12.7	3.97	1072-05-5	834
2-propyl-pentanol,trifluoroacetate	16.7	4.09	#23614	-
cyclohexane, ethyl	18.8	4.16	1678-91-7	885
1,1,4-trmethylcyclohexane	7.26	4.23	7094-27-1	842.9
Cyclopentanone,2-decyl	2.71	4.30	#53495	-
Cis-4-4-dimethylcyclohexane-1-3-dione	1.61	4.41	69841152	-
Hexane,2,3,5-trimethyl	3.43	4.50	1069-53-0	820.6
Heptane,2,3-dimethyl	1.65	4.51	3074-71-3	856
7-dodecen-6-one	2.48	4.54	32064769	2004
1-undecene,7-methyl	1.01	4.56	74630425	1146
Heptane,4-azido	5.22	4.66	27126223	-
Octane,2-methyl	5.36	4.69	3221-61-2	864
Hexane,3-ethyl-4-methyl	1.00	4.79	3074-77-9	860
Octane-3-methyl	6.21	4.84	2216-33-3	872
Cyclohexane,1,2,3-trimethyl (1 alpha, 2, beta, 3 alpha)	1.00	5.06	1678-81-5	920

Cyclohexane, propyl	1.00	5.22	1678-92-8	982
Cyclohexane, 1, 2,3-trimethyl	1.04	5.33	1839-88-9	935
3,5-dimethyl-3-heptene	1.00	5.34	59643684	833
None,2,5-dimethyl	1.88	5.55	17302271	930
Decane,2,5,6-trimethyl	2.63	5.56	62108230	1121

T=Fertilizer treatments=T_1 =$N_{350}P_{213}K_{213}$, T_2=$N_{350}P_{320}K_{213}$, T_3=$N_{525}P_{213}K_{213}$, T_4=$N_{525}P_{320}K_{213}$ T_5=$N_{700}P_{213}K_{213}$, T_6=$N_{700}P_{320}K_{213}$, T_7=$N_{800}P_{213}K_{213}$, T_8=$N_{800}P_{230}K_{213}$ Kg/ha., PK= Pick percentage, RT= Retention time, KI=Kovat indices

Compounds	**(Treatment 8)**	**P k %**	**RT**	**Cas N0**	**KI**
Cylohexane,ethyl		13.3	3.91	1678-91-7	885
Acetic acid,cyna,2-ethylhexylester		19.9	4.02	13361347	-
Cyclopentane,1-ethyl-3-methyl-trans		22.1	4.09	2613-65-2	-
Cyclohexane,1,1,3-trmethyl		8.33	4.16	3073-66-3	834
2-Decene,7-methyl		2.82	4.23	74630232	-
4-undecene,4-methyl		2.02	4.34	61142403	-
Hexane,3-ethyl		5.43	4.43	619-99-8	800
Cyclohexane,1,2,4-trimethyl		2.66	4.48	2234-75-5	881
Heptane,2,4-dimethyl		3.67	4.57	2213-23-2	824
1-undecene,2-methyl		1.33	4.58	18516375	1185
Hydroxylamine,0-decyl		1.00	4.59	29812791	1100
Octane,2-methyl		6.10	4.62	3221-61-2	864
Acid,2-ethylhexylester		6.56	4.76	#25184	1420
Octane-3-methyl		1.04	4.82	2216-33-3	872

T=Fertilizer treatments=T_1 =$N_{350}P_{213}K_{213}$, T_2=$N_{350}P_{320}K_{213}$, T_3=$N_{525}P_{213}K_{213}$, T_4=$N_{525}P_{320}K_{213}$ T_5=$N_{700}P_{213}K_{213}$, T_6=$N_{700}P_{320}K_{213}$, T_7=$N_{800}P_{213}K_{213}$, T_8=$N_{800}P_{230}K_{213}$ Kg/ha., PK= Pick percentage, RT= Retention time, KI=Kovat indices

7.4 Discussion

Results of phytochemical constituents of the different extracts for pot and field experiments investigated on Burdock were quite rich at a varying degree regardless of the cultivation practices or treatment applications. This indicates that fertilizer treatments have less influence on the quality of phytochemical constituents for aqueous root and leaf extracts of cultivated Burdock (*Arctium lappa* L). These phytochemicals are anti-nutrients characterized by protective or disease preventive properties that demonstrate the varying therapeutic values of this species. In literature reports, these results are in accordance with those of Al-Shammaa et al. (2013) who reported the presence of tannins, flavonoids, and saponins, but the absence of Alkaloids for aqueous root and leaf extracts of Burdock collected from the Department of Medicinal Plants, College of Agriculture

University of Baghdad, Iraq. The presence of flavonoids, phenols and glycosides complements the antioxidant properties (Duh, 1998, Liu et al., 2014, Jiang et al., 2019) and anti-inflammatory properties (Carlotto et al., 2016, Alhusaini et al., 2019) of root and leaf extracts of Burdock. Furthermore, the presence of tannins in aqueous leaf and root extracts of the plant also complement its antibacterial properties (Habibipour and Rajabi, 2015, Lou et al., 2016).

Fertilizer application enhances growth and development in plants under good production practices. This ultimately influences the degree of metabolism of bioactive compounds in plant tissues irrespective of the cultivation practices. In this study, significant differences were observed in the accumulation of total phenolic content (TPC), total flavonoid content (TFC) and condensed tannins (PAC) among treatment combinations for pot and field experiments. This may be attributed to the fact that fertilizer treatments influence growth and development in plants which may ultimately induce the accumulation of secondary metabolites in the plant tissues (Azaizeh et al., 2005) of this species. According to Lattanzio et al. (2009) total phenolic content of Oregano (*Origanum vulgare* L.) was increased by nutrient deficiency. The observation of differences in the nutrient release as per the treatment combinations with lesser phosphorous levels having higher accumulation of total phenolic content (TPC), total flavonoids content (TFC) and condensed tannins (PAC) irrespective of plant material and type of solvent for pot and field experiments. This is in accordance with Zamani et al. (2014) who reported that a decrease in phosphorous and an increase in nitrogen fertilizer level in medicinal plants increased the accumulation of phenolic compounds in rose madder (*Rubia tinctorum* L.). However, the accumulation of secondary metabolites in plant tissues is affected by factors such as fertilizer`s treatment application.(Gayler et al., 2008). Furthermore, the treatment combinations with lesser supplementary P had a higher accumulation of total phenolic content (TPC), total flavonoid content (TFC), and condensed tannins (PAC) than those with more supplementary P for pot and field experiments. This may be due to the differences in carbon/nitrogen ratio which consequently might have resulted in the differences in metabolites accumulation (Bryant et al., 1983, Wu et al., 2013), with the highest accumulation of bioactive compounds at the treatment combination range between T_5 to T_7 for pot experiment while for field experiment it was demonstrated between T_5 to T_8.

The overall higher concentration of total phenolic contentment (TPC), total flavonoids content (TFC) and condensed tannins (PAC) in methanol extraction than in aqueous extraction in this study indicates that methanol extraction is more effective and reliable as it enabled the easy filtration and contained more of bioactive constituents than aqueous extraction irrespective of cultivation practices. This may be due to the fact that methanol is a polar solvent and plant materials were able to completely dissolve in it (Ajuru et al., 2017). Moreover, aqueous extract usually goes bad which may have led to the loss of some of the bioactive compounds which may

be present in the methanol extract. Extracts from different plant materials of Burdock (*Arctium lappa* L) are known to have considerable health benefits, by enhancing the body`s immune system and improving metabolic functions (Lin et al., 2002). Furthermore, phenolics, flavonoids and tannins are the main dietary phenolic compounds with unsubstituted OH groups (Ovaskainen et al., 2008) which may have caused an increase in their polarity hence their higher solubility and accumulation in methanol.

Nutritionally, the leaf and root samples of Burdock had an ideal proportion of the different nutrients investigated. The significant differences observed among the different treatment combinations on the leaf and root samples and cultivation practices may be attributed to the fact that fertilizer treatments influence growth and metabolites accumulation which will influence the nutritional constituents. Overall, the higher carbohydrate content identified complements Burdock to be a very good source of energy. This is in agreement with Weckler (1887) who reported that Burdock roots may be an alternative of prebiotic oligosaccharides to bakery products for health benefits. Similarly, the high fibre content of this species also complements its medicinal potential of anti-inflammatory and antitumor properties. Additionally, the essential oil composition of the roots of burdock represented a valuable number of components attributed to the complement as a medicinal and nutritional species.

Significant differences were recorded in antioxidant activities regardless of cultivation practice and solvent extraction among the treatment combinations. This may be attributed to the fact that the application of fertilizers during the cultivation of medicinal plants induces secondary metabolites accumulation which may have ultimately influenced the antioxidant activities of root and leaf extracts to a varying degree. The evaluated antioxidant properties of aqueous and methanol leaf and root extracts using DPPH radical scavenging activity, nitric oxide and hydrogen peroxide assays were significant in their percentage inhibition and IC_{50} values among treatment combinations regardless of cultural practices. This may be attributed to the varying degree of fertilizer influence on the accumulation of bioactive compounds of Burdock. Phenolic compounds are bioactive compounds found in the root and leaf are responsible for the antioxidant activities of extracts of this species. DPPH is one of the most antioxidant assays due to its relative sensitivity, stability and convenience (Floegel et al., 2011). The mechanism involves electron transfer and reduction of coloured oxidants (Nimse and Pal, 2015). Methanol and aqueous root and leaf extracts demonstrated a good DPPH radical scavenging activity. However, T_5 of methanol root extract demonstrated the best DPPH scavenging activity which is attributed to the lowest IC_{50} values of 15.46µg/ml for the pot experiment and 24.93µg/ml for the field experiment. Similarly, the difference in polarity of the solvent may be attributed to the differences in antioxidant activity of the extracts (Benzie and Strain, 1996, Meyer et al., 1998, Chang et al., 2002)

Under normal conditions, nitric oxide is required for the regulation of several physiological functions (Luiking et al., 2010). Nevertheless, excess nitric oxide may result in tissue damage which is usually associated with several disease conditions like inflammation (Sharma et al., 2007), neurodegeneration(Moncada and Bolaños, 2006), and hypertension (Luiking et al., 2010). Hence several applications of natural nitric oxide inhibitors as an antioxidant in the management of diseases has been investigated (Okeleye et al., 2015) In this study T_1 of aqueous leaf extract demonstrated the most ideal NO scavenging effect with an IC_{50} value of 44.55 µg/ml among treatment combinations for the pot experiment. While T_7 of methanol root extract was the most ideal with an IC_{50} value of 31.37 µg/ml among treatment combinations for the field experiment.

Hydrogen peroxide (H_2O_2) scavenging activity is one of the useful methods to determine the ability of antioxidants to decrease the level of prooxidants (Sotler et al., 2019). H_2O_2 scavenging activity of natural antioxidants present in plant extracts has been investigated widely (Keser et al., 2012, Shahriar et al., 2013, Vinodhini and Lokeswari, 2014). In the study, the significant difference in the scavenging activity may be attributed to the difference in fertilizer. treatment combinations. T_8 demonstrated the most ideal scavenging activity for methanol (24.37 µg/ml) and aqueous (36.03 µg/ml) root extracts, while T_6 (98.63 µg/ml) demonstrated the most ideal scavenging activity for methanol leaf and T_2 (106.65 µg/ml) for aqueous leaf extract among treatment combinations for pot experiment. However, for the pot experiment, T_4 (23.37 µg/ml) demonstrated the most ideal for methanol extract and T_2(22.32 µg/ml) for aqueous root extract. Even though for methanol leaf extract T_6 (33.57 µg/ml) demonstrated the most ideal and T_1 (22.82 µg/ml) for aqueous leaf extract among treatment combinations.

Anti-inflammations are protective responses of the body against exogenous pathogens and the repair of damaged tissues resulting from infections (Firuzi et al., 2011). Several inflammatory effects may lead to organ injury or death presenting major management problems (Sherwood and Toliver-Kinsky, 2004). The use of plant products with anti-inflammatory properties such as Burdock to target inflammatory response can be beneficial for the treatment of inflammation as well as many other chronic diseases. In this study, T_5 with IC_{50} value (832.45 µg/ml) of methanol root extract recorded the most ideal inflammatory activity, while T_8 with IC_{50} value (544.42 µg/ml) recorded the most for aqueous root extract. Similarly, for leaf extract, T_5 with IC_{50} vale (791.76 µg/ml) of methanol recorded the most ideal anti-inflammatory activity, even though T_6 with IC_{50} value (167.57 µg/ml) was the most ideal for aqueous leaf extract among treatment combinations for pot experiment. Furthermore, for field the experiment T_3 with IC_{50} value (744.67 µg/ml) of methanol root extract demonstrated the most ideal anti-inflammatory activity, while for aqueous root extract T_4 with IC_{50} value (327.27 µg/ml) demonstrated the most ideal effect. Nevertheless, leaf extract T_5 with IC_{50} value (767.34 µg/ml) recorded the most ideal for methanol while T_2 with

IC_{50} value (629.43 µg/ml) for aqueous leaf extract demonstrated the most ideal anti-inflammatory activity among treatment combinations. These significant differences may be attributed to the differences in fertilizer treatment which ultimately influences the bioactive compounds(Azaizeh et al., 2005). Similarly, the difference in polarity of the solvent may be attributed to the differences in anti-oxidant as well as anti-inflammatory activities of the extracts (Ali et al., 2007, Mhadhebi et al., 2011, Umamahesh et al., 2019). According to Lin et al. (1996), crude extract of Burdock demonstrated anti-inflammatory and radical scavenging effects on carrageenan-induced rat paw oedema. Furthermore, several studies on traditional Chinese medicine have reported that Burdock exhibits anti-inflammatory activities, which is attributed to the presence of the bioactive active compounds (Cho et al., 1999, Kim et al., 2004, Sohn et al., 2011).

7.5 Conclusion

The accumulation of phytochemical constituents was highly impacted by fertilizer treatment combinations qualitative and quantitatively to a varying degree irrespective of the cultivation practices. It was revealed that methanol is a more ideal solvent for the preparation of extract compared to aqueous solvent which was attributed to the solvent polarity. Furthermore, nutrient deficiency in plants influences the metabolism of secondary metabolites, which ultimately increased with phosphorous deficient and nitrogen increased treatment combinations in this study as previously reported that a shift in nitrogen and a decrease in phosphorous fertilizer level in medicinal plants ultimately increased the accumulation of bioactive compounds of this species in this study. Additionally, significant differences were observed in the accumulation of bioactive compounds due to differences in carbon-nitrogen ratio among treatment combinations. In this study T_5 to T_7 demonstrated the best optimum fertilizer combination range for the enhancement of bioactive compounds of medicinal potentials of this species in the Winelands region of the Western Cape province of South Africa. The successful cultivation of the species in pot and in the field with fertilizer treatment combination and for optimum yield of bioactive compounds identified, indicates an alternative source to meet up with the demand of this species by pharmaceutical industries and herbal practitioners in South Africa. This will help to resolve the issue of quality assurance and availability of plant materials of this species for a relatively cheaper price. Also, the nutritional constituents identified demonstrating the nutritional potentials of this species indicates an alternative source of food diversity in South Africa which will accommodate the tourism industry while providing another sector for employment to the community through the awareness of the cultivation practices as part of the country`s development goal for agenda 2030.

References

Ajuru, M. G., Williams, L. F. & Ajuru, G. 2017. Qualitative and quantitative phytochemical screening of some plants used in ethnomedicine in the Niger Delta Region of Nigeria. *Journal of Food and Nutrition Sciences,* 5, 198-205.

Al-Shammaa, D. A., Saour, K. Y. & Abdul-Khalik, Z. M. 2013. Phytochemical investigation for the main active constituents in Arctium lappa L. cultivated in Iraq. *Iraqi journal of pharmaceutical sciences,* 22, 18-24.

Alhusaini, A., Fadda, L., Hasan, I. H., Ali, H. M., El Orabi, N. F., Badr, A. M., Zakaria, E., Alenazi, A. M. & Mahmoud, A. M. 2019. Arctium lappa Root Extract Prevents Lead-Induced Liver Injury by Attenuating Oxidative Stress and Inflammation, and Activating Akt/GSK-3β Signaling. *Antioxidants,* 8, 582.

Ali, A. M., Muse, R. & Mohd, N. B. 2007. Anti-oxidant and anti-inflammatory activities of leaves of Barringtonia racemosa. *Journal of Medicinal Plants Research,* 1, 095-102.

Azaizeh, H., Ljubuncic, P., Portnaya, I., Said, O., Cogan, U. & Bomzon, A. 2005. Fertilization-induced changes in growth parameters and antioxidant activity of medicinal plants used in traditional Arab medicine. *Evidence-Based Complementary and Alternative Medicine,* 2, 549-556.

Benzie, I. F. & Strain, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry,* 239, 70-76.

Bryant, J. P., Chapin Iii, F. S. & Klein, D. R. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*, 357-368.

Carlotto, J., De Souza, L. M., Baggio, C. H., Werner, M. F. D. P., Maria-Ferreira, D., Sassaki, G. L., Iacomini, M. & Cipriani, T. R. 2016. Polysaccharides from Arctium lappa L.: Chemical structure and biological activity. *International journal of biological macromolecules,* 91, 954-960.

Chang, C.-C., Yang, M.-H., Wen, H.-M. & Chern, J.-C. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of food and drug analysis,* 10.

Cho, J. Y., Kim, A. R., Yoo, E. S., Baik, K. U. & Park, M. H. 1999. Immunomodulatory Effect of Arctigenin, a Lignan Compound, on Tumour Necrosis Factor-α and Nitric Oxide Production, and Lymphocyte Proliferation. *Journal of pharmacy and pharmacology,* 51, 1267-1273.

Duh, P. D. 1998. Antioxidant activity of burdock (Arctium lappa Linne): its scavenging effect on free-radical and active oxygen. *Journal of the American Oil Chemists' Society,* 75, 455-461.

Firuzi, O., Miri, R., Tavakkoli, M. & Saso, L. 2011. Antioxidant therapy: current status and future prospects. *Current medicinal chemistry,* 18, 3871-3888.

Floegel, A., Kim, D.-O., Chung, S.-J., Koo, S. I. & Chun, O. K. 2011. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of Food Composition and Analysis,* 24, 1043-1048.

Gayler, S., Grams, T., Heller, W., Treutter, D. & Priesack, E. 2008. A dynamical model of environmental effects on allocation to carbon-based secondary compounds in juvenile trees. *Annals of Botany,* 101, 1089-1098.

Habibipour, R. & Rajabi, M. 2015. Antibacterial effects of Arctium lappa and Artemesia absinthium extracts in laboratory conditions. *Journal of herbmed pharmacology,* 4.

Jiang, Y.-Y., Yu, J., Li, Y.-B., Wang, L., Hu, L., Zhang, L. & Zhou, Y.-H. 2019. Extraction and antioxidant activities of polysaccharides from roots of Arctium lappa L. *International journal of biological macromolecules,* 123, 531-538.

Keser, S., Celik, S., Turkoglu, S., Yilmaz, O. & Turkoglu, I. 2012. Hydrogen peroxide radical scavenging and total antioxidant activity of hawthorn. *Chem J,* 2, 9-12.

Kim, E. K., Li, G. Z., Chai, O. H. & Song, C. H. 2004. Inhibitory effect of Arctium lappa Linne on compound 48/80-induced mast cell activation and vascular permeability. *Korean Journal of Physical Anthropology,* 17, 55-66.

Lattanzio, V., Cardinali, A., Ruta, C., Fortunato, I. M., Lattanzio, V. M., Linsalata, V. & Cicco, N. 2009. Relationship of secondary metabolism to growth in oregano (Origanum vulgare L.) shoot cultures under nutritional stress. *Environmental and Experimental Botany,* 65, 54-62.

Lin, C.-C., Lin, J.-M., Yang, J.-J., Chuang, S.-C. & Ujiie, T. 1996. Anti-inflammatory and radical scavenge effects of Arctium lappa. *The American journal of Chinese medicine,* 24, 127-137.

Lin, S.-C., Lin, C.-H., Lin, C.-C., Lin, Y.-H., Chen, C.-F., Chen, I.-C. & Wang, L.-Y. 2002. Hepatoprotective effects of Arctium lappa Linne on liver injuries induced by chronic ethanol consumption and potentiated by carbon tetrachloride. *Journal of biomedical science,* 9, 401-409.

Liu, W., Wang, J., Zhang, Z., Xu, J., Xie, Z., Slavin, M. & Gao, X. 2014. In vitro and in vivo antioxidant activity of a fructan from the roots of Arctium lappa L. *International journal of biological macromolecules,* 65, 446-453.

Lou, Z., Li, C., Kou, X., Yu, F., Wang, H., Smith, G. M. & Zhu, S. 2016. Antibacterial, antibiofilm effect of Burdock (Arctium lappa L.) leaf fraction and its efficiency in meat preservation. *Journal of food protection,* 79, 1404-1409.

Luiking, Y. C., Engelen, M. P. & Deutz, N. E. 2010. Regulation of nitric oxide production in health and disease. *Current opinion in clinical nutrition and metabolic care,* 13, 97.

Meyer, A. S., Heinonen, M. & Frankel, E. N. 1998. Antioxidant interactions of catechin, cyanidin, caffeic acid, quercetin, and ellagic acid on human LDL oxidation. *Food chemistry,* 61, 71-75.

Mhadhebi, L., Laroche-Clary, A., Robert, J. & Bouraoui, A. 2011. Anti-inflammatory, anti-proliferative and anti-oxidant activities of organic extracts from the Mediterranean seaweed, Cystoseira crinita. *African Journal of Biotechnology,* 10, 16682-16690.

Moncada, S. & Bolaños, J. P. 2006. Nitric oxide, cell bioenergetics and neurodegeneration. *Journal of neurochemistry,* 97, 1676-1689.

Nimse, S. B. & Pal, D. 2015. Free radicals, natural antioxidants, and their reaction mechanisms. *RSC advances,* 5, 27986-28006.

Okeleye, B., Nongogo, V., Mkwetshana, N. T. & Ndip, R. N. 2015. Polyphenolic content and in vitro antioxidant evaluation of the stem bark extract of Peltophorum africanum sond (fabaceae). *African Journal of Traditional, Complementary and Alternative Medicines,* 12, 1-8.

Ovaskainen, M.-L., Torronen, R., Koponen, J. M., Sinkko, H., Hellstrom, J., Reinivuo, H. & Mattila, P. 2008. Dietary intake and major food sources of polyphenols in Finnish adults. *The Journal of nutrition,* 138, 562-566.

Shahriar, M., Hossain, I., Sharmin, F. A., Akhter, S., Haque, M. A. & Bhuiyan, M. A. 2013. InVitro antioxidant and free radical scavenging activity of Withania Somnifera root. *Iosr J Pharm,* 3, 38-47.

Sharma, J., Al-Omran, A. & Parvathy, S. 2007. Role of nitric oxide in inflammatory diseases. *Inflammopharmacology,* 15, 252-259.

Sherwood, E. R. & Toliver-Kinsky, T. 2004. Mechanisms of the inflammatory response. *Best Practice & Research Clinical Anaesthesiology,* 18, 385-405.

Sohn, E.-H., Jang, S.-A., Joo, H., Park, S., Kang, S.-C., Lee, C.-H. & Kim, S.-Y. 2011. Anti-allergic and anti-inflammatory effects of butanol extract from Arctium Lappa L. *Clinical and Molecular Allergy,* 9, 1-11.

Sotler, R., Poljšak, B., Dahmane, R., Jukić, T., Jukić, D. P., Rotim, C., Trebše, P. & Starc, A. 2019. Prooxidant activities of antioxidants and their impact on health. *Acta Clinica Croatica,* 58, 726.

Umamahesh, K., Ramesh, B., Kumar, B. V. & Reddy, O. V. S. 2019. In vitro anti-oxidant, anti-microbial and anti-inflammatory activities of five Indian cultivars of mango (Mangifera indica L.) fruit peel extracts. *Journal of herbmed pharmacology,* 8, 238-247.

Vinodhini, V. & Lokeswari, T. 2014. Antioxidant activity of the isolated compounds, methanolic and hexane extracts of Toona ciliata leaves. *International journal of engineering and technology,* 4.

Weckler, G. A. 1887. Analysis of Burdock Root, lappa Officinalis L. *American Journal of Pharmacy (1835-1907)*, 393.

Wu, C.-S., Gao, Q.-H., Kjelgren, R. K., Guo, X.-D. & Wang, M. 2013. Yields, phenolic profiles and antioxidant activities of Ziziphus jujube Mill. in response to different fertilization treatments. *Molecules,* 18, 12029-12040.

Zamani, Z., Zeinali, H., Masood, S. J. & Madani, H. 2014. Effect of nitrogen and phosphorous fertilizers on the yield and secondary metabolites of medicinal plant Rubia tinctorum L. under salinity conditions.

CHAPTER 8
GENERAL CONCLUSIONS AND RECOMMENDATIONS

8.1 Summary and Conclusions

Medicinal plants are those with identified healing properties in the different regions of the world. Based on the morphology and phytochemical constituents, some medicinal plants also possess culinary properties. The potentials of such plants with medicinal and culinary properties offer prospects to such plants as suitable candidates for cultivation especially those harboured in the wild. The cultivation of such plants is a mitigating measure toward biodiversity conservation. This is also a viable option to overcome problems that are inherent in these plant materials for quality assurance and availability for commercialization. However, this is accompanied by some challenges such as plant adaptation to a new environment, disease susceptibility, labour cost and net financial return. The use of good propagation techniques for the cultivation of such plants will help to overcome the challenges associated with the production of secondary metabolites of interest to our herbal and pharmaceutical industries.

Burdock (*Arctium lappa* L) and American skullcap (*Scutellaria lateriflora* L) are among such plants with identified medicinal and culinary uses. The demand for these plants is mostly accomplished through the supply from the wild plant population, exacerbating pressure in their natural habitats. However, the preference for plant materials for medicine and culinary uses has initiated some induction needs for their cultivation which is still in its nascent stage of development. To ensure good agricultural practice for the cultivation of American Skullcap (*Scutellaria lateriflora* L) and Burdock (*Arctium lappa* L.) for continuous supply and to meet up with the demand of the growing population. This study investigated the yield and morphological characteristics of the two species in response to mineral fertilizer application. The growth and yield parameters were investigated in response to mineral fertilizer application levels, and the accumulation of bioactive compounds was investigated in response to fertilizer treatments. The study also investigated the phytochemical constituents, nutritional potentials, and bioactivities of leaf extracts of American Skullcap as influenced by fertilizer treatments. Similarly, the study further investigated the phytochemical constituent's nutritional potentials and bioactivities of the leaf and root extracts as well as the volatile oil constituents of Burdock roots as affected by mineral fertilizer treatments.

The investigation of yield, phytochemical constituents, and morphological characteristics of Burdock in response to mineral fertilizer led to the conclusion that the cultivation of Burdock was highly influenced by fertilizer treatments. The study demonstrated that burdock can successfully grow in the Winelands District of the Western Cape Province of South Africa for medicinal and culinary uses. The cultivation of this species can be done in pots or fields when all agricultural practices are diligently observed. The application of $N_{846}P_{280}K_{315}$ Kg/ha of fertilizer combination could be recommended for potential folia vegetable production as a possible solution to meet up with the demand for daily consumption of Burdock leaves. Furthermore, the application of $N_{635}P_{210}K_{315}$ kg/ha to $N_{846}P_{280}K_{315}$ kg/ha fertilizer combination range could be recommended for burdock root cultivation as a possible solution to meet up with the demand for Burdock roots as a source of food salad and root herbs. The leaf and root samples of cultivated Burdock were rich in phytochemicals. They demonstrated an ideal nutritional potential to contribute to the issue of food security. Additionally, the samples also demonstrated potential antioxidant and anti-inflammatory activities, rich volatile oil composition, diseases prevention and health promoting properties,

The investigation on yield and morphological characteristics of American skullcap and the accumulation of total flavonoid content in response to mineral fertilizer application revealed that NPK treatment combinations had a significant impact on vegetative and yield parameters investigated. The overall picture indicates that, T_4 to T_6 fertilizer range (N_{525}-N_{700} with P_{213}-P_{320} and K_{213} Kg/ha) demonstrated the highest influence across the different parameters investigated. This reciprocated higher yield response between T_4 to T_6 fertilizer application range, indicates the maximum NPK fertilizer treatment combinations require for maximum biomass yield and production when all agricultural practices are diligently applied in the Winelands region of the Western Cape Province of South Africa.

The application of fertilizer has a significant influence on the accumulation of total flavonoid content (TFC) in the plant. Overall, it was observed that a shift in N increase was in response to P across treatment combinations. Treatments with P_{213} recorded a higher concentration of total flavonoid content (TFC) compared to those with P_{320} among the treatment combinations. Additionally, the highest concentration of total flavonoid content (TFC) was observed from methanol extract than aqueous extract, indicating the solvent with the best relative polarity for extraction of bioactive compounds of this species. In conclusion, further investigation on cultivation practices of this species needs to be tested in the field as well as optimization measures of bioactive compounds for the supply of quality plant materials for commercialization.

Furthermore, the investigation of phytochemical constituents, nutritional potentials, antioxidants, and anti-inflammatory activities of American skullcap in response to mineral fertilizer application

demonstrated that; irrespective of the solvent for extraction, phytochemical constituents of the different extracts were greatly impacted to a varying degree by fertilizer treatment combinations. A positive test result was observed for all phytochemicals investigated except for glucoside. This complements the diverse pharmacological activities of this species like the antioxidant and anti-inflammatory activities investigated. Nutritionally, high fibre and carbohydrate contents were observed in this species, indicating that this species can be recommended as a potential source of dietary fibre and calorie intake for body functioning. Equally, the ideal amount of protein content and low lipid content also indicate this species can be beneficial for people with related cholesterol problems. Methanol solvent demonstrated a more ideal solvent than aqueous solvent for extract preparation of this species due to its polarity. Additionally, it was realized that significant differences were recorded in the accumulation of the total phenolic content, total flavonoid content, and condensed tannins among the different treatments and across the different phenological stages of development of this species. However, some treatments outperformed others in the accumulation of these phytochemicals. Treatment combinations with the lower supplemented phosphorous levels recorded a higher concentration compared to those with higher supplemented phosphorous level among treatment combinations. Antioxidant activity was recorded on aqueous and methanol dried leaf extract of this species which demonstrated a significant influence on DPPH, nitric oxide, hydrogen peroxide scavenging activity and anti-inflammatory activity as affected by the different fertilizer treatment combinations. This preliminary investigation indicates that American skullcap (*Scutellaria lateriflora* L) cultivated in the Winelands region of the Western Cape Province of South Africa can still retain its secondary metabolites of bioactivity and therapeutic values. Nevertheless, more investigation needs to be done using other propagation techniques and, in the field, to potentially validate these findings.

Moreover, the investigation of phytochemical constituents, nutritional potentials, volatile oil composition, antioxidant, and anti-inflammatory activities of Burdock in response to mineral fertilizer application reveals that: The accumulation of phytochemical constituents was highly impacted by fertilizer treatments at a varying degree irrespective of the cultivation practices involved. It was revealed that methanol is a more ideal solvent for the preparation of extract compared to aqueous solvent which was attributed to the solvent polarity. The result obtained in this study complement the culinary and medicinal potentials attributed to this species in the literature. Nutritionally the leaf had ideal ash, protein, and crude lipid content while the root had an ideal carbohydrate content as affected by fertilizer treatments irrespective of the cultural practices involved. Additionally, mineral deficiency in plants has an influence on the metabolism of secondary metabolites. Fertilizer treatments with lower supplementary phosphorous levels among the different treatment combinations recorded an increase in secondary metabolite

accumulation than those with higher supplementary phosphorous levels irrespective of the increase in nitrogen levels used in this study. As previously reported, a shift in nitrogen and a decrease in phosphorous fertilizer levels in medicinal plants ultimately increased the accumulation of bioactive compounds. Furthermore, significant differences were observed in the accumulation of bioactive compounds due to differences in carbon-nitrogen ratio among treatment combinations. In this study T_5 to T_7 demonstrated the best optimum fertilizer combination range for the enhancement of bioactive compounds of this species in the Winelands region of the Western Cape province of South Africa.

The successful cultivation of the Burdock in pots and field and American skullcap in pots with fertilizer application for optimum growth and yield of bioactive compounds was identified. It can therefore be concluded that this is an alternative source to meet up with the demand for these species by pharmaceutical industries and herbal practitioners in South Africa. This will help to resolve the issue of quality assurance and availability of plants materials of this species for a relatively cheaper price while providing another sector for employment to the communities

8.2 Recommendations

South Africa is well known for its rich diversity in fauna and flora. This has attracted the attention of many tourists from different countries of the world, playing a very significant role in the tourism industry. The welfare and satisfaction of these tourists are imperative for the sustainability of the South African tourism industry. Therefore, the establishment of cultivation practices for Burdock and American skullcap in South Africa will increase medicine and culinary option for tourists visiting South Africa, since their availability will be an assurance of food and health security. The cultivation of this species can also provide a potential market for vegetable production, a possible solution for food security, nutrition, and job creation for communities. In this regard, more studies need to be replicated in other provinces to test propagation potential as well as optimization studies on secondary metabolites of interest for quality assurance and consistent availability of plant materials. This will provide a good platform for the advancement of South Africa’s pharmaceutical industries and herbal practitioners. This may contribute to the economic growth of South Africa as part of the country`s development goal for agenda 2030.

APPENDICES

Academia Journal of Medicinal Plants 6(7):
168-177, July 2018 DOI:
10.15413/ajmp.2018.0133
ISSN 2315-7720

Research Paper

Cultivation of medicinal plants in South Africa: A solution to quality assurance and consistent availability of medicinal plant materials for commercialization

Tanga M[1], Lewu FB[2]*, Oyedeji OA[3], Oyedeji OO[4]

Accepted 23rd July 2018

[1]Department of Environmental and
Occupational studies, Faculty of Applied Science, Cape Peninsula University of Technology, P O Box 652, Cape Town 8000, South Africa
[2]Department of Agriculture, Faculty of Applied Science, Cape Peninsula University of Technology, Wellington Campus P O Box X8, Wellington 7654, South Africa
[3]Department of Chemical and Physical Sciences, Faculty of Natural Science, Walter Sisulu University of Science and Technology, P/Bag X1 Mthatha 5099, South Africa
[4]Department of Chemistry, Faculty of Science and Agriculture, University of Fort Hare, Alice, South Africa.

*Corresponding author. E-mail: lewuf@cput.ac.za. Tel: 0218645213;/ 27725515617.

Medicinal plants are plants which have been identified to have healing properties and their cultivation recently is gaining popularity due to the increasing preference towards plant-based products as a source of primary health care in both developing and developed countries. Cultivation of medicinal plant is a mitigative measure towards biodiversity conservation and also a viable alternative to overcome problems that are inherent to medicinal plant materials for quality assurance and availability for commercialization. However, this is accompanied with some challenges of plant adaptation to new environment, disease susceptibility, labour cost and net return. The use of good propagation techniques is imperative to overcome the challenges associated with the production of secondary metabolites of interest to our herbal and pharmaceutical industries and the implementation of good regulatory policy for safety and quality of medicinal plants products of standard is critical.

Key words: Medicinal plants, cultivation, quality, secondary metabolites, commercialization

Growth, Yield and Phytochemical Constituents of *Arctium lappa* L. in Response to Phosphorous and Potassium Fertilizers Application

M Tanga, F B Lewu, AO Oyedeji and OO Oyedeji

***Abstract*—** Burdock (*Arctium lappa* L), is a medicinal plant with many therapeutic values but its cultivation practice is given less attention causing pressure on its wild population. A two separate complete randomized design pot experiment with five treatments of phosphorous (Triple superphosphate 20%) at five levels (140, 210,280, 350 and 420 kg/ha) and potassium (Potassium Chloride 50%) at five levels (210, 315, 420, 520 and 630Kg/ha) with a basal application of Nitrogen fertilizer (Urea,160 Kg/ha) replicated four times was conducted. Fertilizer treatments were split into two equal doses at seedling transplant and four weeks after transplant. Data on growth and yield were collected and analysed using SAS software. Test results were significant ($p<0.05$) in growth parameters in response to phosphorous and the number of leaves in potassium treatments. However, there was no significant difference ($p>0.05$) on yield parameters due to fertilizer application except for root length. Phytochemical screening indicates the presence of Tannins, flavonoids, Terpenoids, glycosides, and phenols across treatments. Crude lipid and ash content were not significant ($p>0.05$) but there was a significant difference ($p<0.05$) on crude fibre content in response to fertilizer treatments.

***Keywords*—**Burdock, cultivation, fertilizer treatments, growth, yield, phytochemicals

Department of Environment and Occupational Studies, Faculty of Applied science, Cape Peninsula University of Technology, PO Box, 652, Cape Town 8000 South Africa
Department of Agriculture, Faculty of Applied Science, Cape Peninsula University of Technology, P.O. Box XB Wellington 7654, South Africa.
Department of Chemical and Physical Sciences, Faculty of Natural Sciences, Walter Sisulu University of Science and Technology
Department of Chemistry, Faculty of Science and Agriculture, University of Fort Hare, Alice, South Africa

I. Introduction

Burdock (Arctium lappa L.) is an indigenous medicinal herb of the Asian continent and native to Eurasia, which belongs to the family Asteraceae. It has many therapeutic values [1]. Traditionally a mixture of the species with oil and honey is applied on the chest for treatment of common cold [2]. The fruits are used as blood purify and for the treatment of respiratory diseases [3]. The leaves are used for the treatment of rheumatic pain, sunstroke, snake, and scorpion bites [4-6]. The Roots are used in veterinary medicine for the treatment of mastitis while the infusion extract of the whole plant can be applied against endoparasite in poultry [7]. The species is also consumed as a vegetable in the form of salad and stew because of its high nutritional composition [8]. The demand for this plant keeps increasing without assurance of consistent supply since most of its supply is mostly dependent on the wild causing pressure to its natural habitat. The cultivation of this species is given less attention even in the region of its origin not alone in South Africa. South Africa still depends mostly on the importation of the plant materials for medicine, with little assurance of consistent supply to satisfy the demand of the needy population. Also, the non-awareness of this plant as a potentials vegetable to help address the issue of food security to our communities not alone its cultivation practices. Therefore, the cultivation of this species in South Africa is imperative. This study was therefore undertaken to investigate the influence of potassium and phosphorus fertilizer application on the growth, yield and phytochemical constituents of this species.

https://doi.org/10.17758/EARES10.EAP1120122 352

Asian J Agric & Biol. 2020;8(4):511-518. DOI: 10.35495/ajab.2019.11.524

AJAB

Original Article

Yield and morphological characteristics of Burdock (*Arctium lappa* L.) in response to mineral fertilizer application

Mbappe Tanga[1], Francis Bayo Lewu[2*], Adebola Omowunmi Oyedeji[3], Opeoluwa Oyehan Oyedeji[4]

[1]Department of Environment and Occupational studies, Faculty of Applied Science, Cape Peninsula University of Technology, Cape Town, South Africa

[2]Department of Agriculture, Faculty of Applied Science, Cape Peninsula University of Technology, Wellington, South Africa [3]Department of Chemical and Physical Sciences, Faculty of Natural Sciences, Walter Sisulu University of Science and Technology, South Africa

[4]Department of Chemistry, Faculty of Science and Agriculture, University of Fort Hare, Alice, South Africa

Received:
November 25, 2019

Accepted:
June 18, 2020

Published:
October 31, 2020

**Corresponding author email:*
lewuf@cput.ac.za

Abstract

Burdock (*Arctium lappa* L.) is a medicinal plant, highly rich in phytochemicals which contribute towards its antioxidant properties and treatment of chronic diseases. It is also consumed as a vegetable in some regions of the world because of its high nutritive value. Currently, the plant material is imported for its medicinal purposes by herbal practitioners and pharmaceutical industries in the different countries of the world, including South Africa. However, the cultivation of this plant is lacking in South Africa and Africa as a whole. In order to achieve consistent supply of quality products and eliminate batch-to-batch variability of plant materials, a factorial experiment was conducted on Takinogawa long cultivar with eight treatments (T_1=$N_{423}P_{210}K_{315}$, T_2=$N_{423}P_{280}k_{315}$, T_3=$N_{635}P_{210}K_{315}$, T_4=$N_{635}P_{280}K_{315}$, T_5=$N_{846}P_{210}K_{315}$, T_6=$N_{846}P_{280}K_{315}$, T_7=$N_{1058}P_{210}K_{315}$ and T_8=$N_{1058}P_{280}K_{315}$ Kg/ha) and five collection time of data laid out in a completely randomized design replicated five times under 40% shade net. Fertilizer treatments were split into two equal doses at seedling transplant and four weeks after transplant. Data on morphological characteristics and yield were collected and analysed using SAS software. There was a significant ($P<0.05$) difference across fertilizer treatments on morphological characteristics as well as the interactions between treatments and time of data collection. Significant differences ($P<0.05$) were also recorded on the yield parameters. Two treatments ($N_{635}P_{210}K_{315}$ and $N_{635}P_{280}K_{315}$Kg/ha) significantly outperformed the other treatments. However, more research needs to be done on the enhancement of secondary metabolites of interest for the sustainable supply of quality plant materials for herbal practitioners and pharmaceutical industries.

Keywords: *Arctium lappa* L., Medicinal plant, Phytochemicals, Morphological characteristics, Fertilizer treatments

How to cite this:

Tanga M, Lewu FB, Oyedeji AO and Oyedeji OO, 2020. Yield and morphological characteristics of Burdock (*Arctium lappa* L.) in response to mineral fertilizer application. Asian J. Agric. Biol. 8(4): 511-518. DO https://doi.org/10.35495/ajab.2019.11.524

Volatile Oil Composition of Burdock Root (*Arctium lappa* L.) in Response to Mineral Fertilizer Application

M Tanga[1], F B Lewu[2], AO Oyedeji[3] and OO Oyedeji[4]

***Abstract*—**This study was undertaken to determine the changes that occur in volatile oil composition of cultivated burdock root as influenced by mineral fertilizer application in the Wine Land Region of the Western Cape province of South Africa. Harvested dry roots from different treatments was used for oil extraction by hydro distillation and the composition of the oil from the different treatments was determined by GC/MS. In total, T_7 recorded the highest number of compounds (20) with 7 of the compounds of peak% over 5. While T_3 had 19 compounds with 6 of the compounds of peak% over 5. However, T_4, T_5, T_6 and T_8, recoded 14 compounds. Nevertheless, T_1 and T_2 had the least number of compounds with 5 compounds of peak % over 5 for T_1 and 4 compounds for T_2. Overall, the two treatments T_3 and T_7 with the highest number of compounds were treatments with less supplementary phosphorous.

***Keywords*—**Burdock, volatile oil, mineral fertilizer, roots, cultivation, compounds.

I. INTRODUCTION

Medicinal plants are those with healing properties due to the presence of the different phytochemicals synthesized in the different organs of the plants [1, 2]. Burdock (*Arctium lappa L.*) is one of such species with several therapeutic values [3] which is associated with the phenolic rich compounds present in the root, leaf and seed [4, 5]. With the advancements of the different analytical techniques [6] more active ingredients of this species have been identified over the last decades [7, 8]. The root is characterized for its multi utility values as food and medicine. The root is consumed as a salad and in stew because of its high nutritional value [9] also the volatile oil from the root is known to boost skin and hair quality [5]. Traditionally, a mixture of the root extract with honey and oil is applied on the chest for treatment of common cold [10]. Historically, the oil from the root has been used to treat many skin conditions like acne, boils, abscesses, and eczema [11]. The demand for the root is critical, most especially for the extraction of essential oils, which requires much of the root to be used during the process of hydro distillation to get a substantial amount of the oil. However, the supply of the root is mostly from the wild with silent cases of its commercial production to meet up with the demand of the root for oil extraction. The cultivation of this species with the use of mineral fertilizer as a management practice to meet demand of the plant material is imperative. Fertilizer is known to have an inducive effect on growth and yield [12], which consequently, will have an influence on the phytochemicals and volatile oil composition. This study was therefore undertaken to investigate the influence of mineral fertilizer application on the volatile oil composition of burdock root cultivated in the Wine Lands region of the Western Cape Province of South Africa.

https://www.iicbe.org/2021/11/18/127