Volatile Oil Composition of Burdock Root (*Arctium lappa* L.) in Response to Mineral Fertilizer Application

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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 is 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.

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II. Materials and methods

A. Study Area and experimental design

The roots of *Arctium lappa* L were obtained from a pilot study conducted at the Research and Teaching Farm of the Department of Agriculture Wellington Campus, Cape Peninsula University of Technology. The area falls within the Northern part of Wellington with coordinate (S33°37' E19° 37'). The study was a factorial experiment of eight treatment combinations of NPK fertilizer in the field (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. Fertilizer treatments for the experiments were split into two equal doses at seedling transplant and at four weeks after transplant. Irrigation and weeding were conducted as required throughout the cultivation period with all protocol diligently observed.

B. Plant material and extraction of volatile oil

Volatile oil was extracted from Burdock roots (Takinogawa long cultivar) harvested from the different treatments. This was done through hydro distillation using the Clevenger apparatus for three hours according to Oyedeji, Lawal [13]. Volatile oil obtained from the roots of the different experiments and treatments were stored at 4^oC in sealed brown vials for GC/MS analysis[14].

C. Analyses of volatile composition

GC/MS analysis of different oils from the different experiments and treatments was performed on GC/MS-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 parameters were 30m by 0.25mm, film thickness 0.25 µm. The initial temperature of the column was 70^0C and was heated to 250^0C at a rate of 5^OC/min. The final temperature was kept at 450^0C 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 scanning range of 35t- 450amu. One microliter (1µl) of the diluted oil (in hexane) was injected for analysis. The identified components of the volatile oils were, retention times, mass spectral percentages, Kovat indices and Cas numbers are tabulated in Tables.

D. GC/MS Data analysis

The components were identified by comparing their retention indices and mass fragmentation patterns of existing individual constituents, with those reported in literature [15, 16] and MS data in the computer matching with the WILEY275, NIST 08, ADAMS and FFNSC 2 libraries. The mass fragmentation patterns were categorized in percentage groups

III. RESULTS AND DISCUSSION

The GC-MS analysis of volatile oil for burdock root as affected by fertilizer treatments validated a variation in chemical constituents among the different treatment combinations. Interestingly, the greatest variation was demonstrated by T_7 with a total of 20 compounds identified. Extraordinarily, seven notable compounds with their peak percentage above 5%. were Cyclohexane-ethyl (18.83%), 2-Propyl-pentanol-trifluoroacetate (16.72), 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%), 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-teradecane (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 (5.28). 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%). Equally, T_8 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 T_1 were recorded for 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 (13.77%), Ether, hexyl pentyl (11.9%) and Hydroxylamine (5.2%). The variations in essential oil composition in the study are similar to those variations reported by [15]. Overall, the two treatments T_3 and T_7 with highest compound identified were treatments with less supplementary phosphorous. Fertilizer application enhances growth and development in plants when all agricultural practices and protocol are diligently observed [16]. This ultimately influences the degree of metabolism of oil compounds in root tissues of the cultivated Burdock root in this study. Nutrient deficiency in plants, influences the metabolism of secondary metabolites [17], which ultimately increased with phosphorous deficiency and increased nitrogen treatment combinations in this study. A shift in nitrogen and a decrease in phosphorous fertilizer level in medicinal plants ultimately increased the accumulation of bioactive compounds which may have an influence on the root oil metabolism.

TABLE I: INFLUENCE OF T_1 ON VOLATILE OIL COMPOSITION OF BURDOCK ROOT

Compounds	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

TABLE II: INFLUENCE OF T_2 ON VOLATILE OIL COMPOSITION OF BURDOCK ROOT

Compounds	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

TABLE III: INFLUENCE OF T_3 ON VOLATILE OIL COMPOSITION OF BURDOCK ROOT

Compounds	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

TABLE IV: INFLUENCE OF T_4 ON VOLATILE OIL COMPOSITION OF BURDOCK ROOT

Compounds	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

TABLE V: INFLUENCE OF T_5 ON VOLATILE OIL COMPOSITION OF BURDOCK ROOT

Compounds	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

TABLE VI: INFLUENCE OF T_6 ON VOLATILE OIL COMPOSITION OF BURDOCK ROOT

Compounds	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

TABLE VII: INFLUENCE OF T_7 ON VOLATILE OIL COMPOSITION OF BURDOCK ROOT

Compounds	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

TABLE VIII: INFLUENCE OF T_8 ON VOLATILE OIL COMPOSITION OF BURDOCK ROOT

Compounds	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

IV CONCLUSION

This study is the first of its kind in the Wine Land region of the Western Cape Province of South Africa. It provides a preliminary knowledge on the influence of mineral fertilizer treatment combinations on the volatile oil composition of Burdock root. The study reveals that a management practice of less supplementary phosphorous application induces the metabolism of Burdock root oil. However, more investigations need to be done under the same conditions to monitor the trend of the results. This will help to potentially validate the results of this study.

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