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Effect of Seasonal Variations in the Chemical Composition of Essential Oil of the Leaves of *Croton Macrostachyus.*

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Abstract

The composition of the steam-distilled essential oil from the leaves of C. macrostachyus cultivated in Bule Hora woreda (Ethiopia) was studied by GC-MS. The seasonal variation in the main volatile constituents was also investigated. Analysis of the essential oil resulted in the identification of 21 compounds with sesquiterpene comprising 70% of the total. Germacrene was the most abundant compound, followed by Benzyl benzoate, Caryophyllene, a - Farnesene, β – Farnesene, γ – Muurolene, and Humulene. The monoterpene content was low and was mainly represented by β-copaene, β-pinene, and linalool. The essential oil from the leaves, collected at two different collection periods within 1 year, showed significant differences in composition. Germacrene was the predominant constituent during the collection periods, with a major composition difference. The germacrene content of the oil was high in the dry season (43.57%), and decreased in the rainy season (24.66%). Levels of caryophyllene showed the greatest increase in the rainy season when the temperature was very low.

Keywords*: Germacrene, Caryophyllene, Croton macrostachyus, GC-MS, Germacrene, Seasonal Variation*

Introduction

The presence, yield and composition of secondary metabolites in plants, viz. the volatile components and those occurring in essential oils, can be affected in different ways, from their formation in the plant to their final isolation (Melito S, 2019). Several of the factors of influence have been studied, in particular for commercially important crops, to optimize the cultivation conditions and time of harvest and to obtain higher yields of high-quality essential oils that fit market requirements (Saeb, 2012). In addition to the commercial importance of the variability in yield and composition, the possible changes are also important when the essential oils and volatiles are used as chemotaxonomic tools (P.T. Silva, 2019). Knowledge of the factors that determine the chemical variability and yield of each species is thus very important. These include: (a) physiological variations, (b) environmental conditions, (c) geographic variations, (d) genetic factors and evolution (Saeb, 2012).

Seasonal and maturity factors are interlinked with each other because the specific ontogenic growth stage will differ as the season progresses (Karama Zouari-Bouassida, 2018). There are many reports in the literature regarding the variation in the chemical profiles of essential oils from various plants collected during different seasons (Kofidis, 2004). The essential oil yields varied considerably from month to month and was also influenced by the micro-environment (sun or shade) in which the plant was growing (Juliani, 2002). Pala-Paul et al. (2001) reported month-to-month variations in the essential oil composition and yield of Santolina rosmarinifolia, which could be attributed to precipitation and temperature (Palá-Paúl, 2001). Results obtained by Badi et al., (2004) for Thymus vulgaris (thyme), also indicated that timing of harvest is critical to both yield and oil composition (Saeb, 2012).

C. macrostachys Hochst. ex Del. is specious that belongs to the genus Croton. It is a shrub or deciduous tree that grows up to 30 m high at an altitude of 200-2,000 m and is widely distributed in most parts of Ethiopia. The plant is commonly known as 'Makanissa' or 'Bissana', and is a native species of Eritrea, Ethiopia, Kenya, Nigeria, Tanzania, and Uganda (Edwards, 1995); (Tariku, 2010). In Ethiopia, the bark of *C. macrostachyus* is used as a purgative and vermifuge, the leaves against the itchy scalp, and decoctions of young leafy shoots for jaundice, eruptive diseases resembling small-pox and other skin problems. Crushed leaves and seeds are compounded for tapeworm management and the fruit and root-decoction is drunk for venereal disease. The seeds are known to induce abortion. In some parts of the country, berries of this plant are blended with Brucea anti-dysentrica for local treatment of cutaneous leishmaniasis, a dermatological problem to which currently existing antileishmanial drugs hardly respond (Tariku, 2010).

In the case of Ethiopia, though the essential oils from many plants, including *C. macrostachyus*, which are used as traditional medicine have been studied, there are not many reports regarding the role of the factors that affect the chemical composition of the essential oils obtained.

The present study investigated the chemical constituents of the volatile oil obtained from leaves of *C. macrostachyus* using GC and GC/MS in two different seasons (dry and rainy seasons).

Material and Methods

Plant Material

Fresh leaves of *C. macrostachyus* were collected randomly and purposively from Bule Hora town in two different seasons: the dry season (February to March 26, 2016) and the rainy season (August to September 2016), Ethiopia. The identification of the plant was confirmed and the voucher specimen was housed at the National Herbarium of the Department of Plant Biology and Biodiversity Management at Addis Ababa University, Faculty of Natural and Computational Sciences, Ethiopia.

Isolation and Analysis of the Essential Oils

The plant material was cut into pieces and subjected to hydrodistillation for 3 h in a Clevenger apparatus. The oils were dried over anhydrous sodium sulfate and their yield was calculated based on the volume of oil obtained from the plant material. The essential oils obtained had a yellow color and a characteristic odor. The oils were stored in a refrigerator until the analysis by GC-MS.

Analyses of the volatile oil were achieved using a Varian Star 3400CX gas chromatograph equipped with a fused silica Chrompack WCOT CP-Sil-5 capillary column (30 m × 0.32 mm i.d. with a film thickness of 0.25 μm) and an automatic injector in split less mode, coupled with a Varian Saturn 3 mass spectrometer (ionization voltage of 70 eV). The oven temperature was held at 40°C for 3 min, then programmed from 40 to 220°C at a rate of 3°C/min, and then held at 220°C for 3 min. The carrier gas was helium (5 psi) at 50 mL/min through the injector and 30 cm/sec through the column. The injector and MS detector temperature were both set at 220°C, and a mass range of m/z = 40-400 was recorded. All MS were acquired in EI mode. Essential oil compounds in the volatile oil were identified using a combination of a MS database search (IMS Terpene Library 1989; NIST/EPA/NIH Mass Spectral Library 2005), a relative retention index (ESO Database of essential oils) and a comparison of MS found in the literature. Quantitative analysis (in %) was performed by peak area normalization measurements (TIC = total ion count).

Results and Discussion

C. macrostachyus produces a yellow essential oil with a characteristic odor. The oil yield (ml) in two different seasons and times of extraction is presented in Table 1.

Table 2 shows the composition of the essential oil from leaves of *C. macrostachyus* in the dry season. The chemical class distribution of the volatile constituents of *C. macrostachyus* in the dry season is summarized in Table 3.

Table 4 shows the composition of the essential oil from leaves of *C. macrostachyus* in the rainy season. The chemical class distribution of the volatile constituents of *C. macrostachyus* in the rainy season is summarized in Table 5.

For the essential oil of the leaves in the dry season, a total of 12 compounds were identified (Table 2). Terpenoids accounted for 65.37 % of the compounds analyzed (Table 3). The main compounds found in the oil of leaves collected in the dry season after 3 hours of extraction were Germacrene D (43.57%), benzyl benzoate (31.46%), caryophyllene (3.91%), phytol (3.59%), α-farnesene (3.54%), naphthalene (3.16%), β – farnesene (2.25%) and γ – elemene (2.05%).

For the essential oil from the rainy season, a total of 20 compounds were identified (Table 4). Most of them were terpenoids, representing 69.16% of the total oil (Table 5). Germacrene D (24.66%), caryphyllene (11.90%), 1-methyl-4-(6-methyhept-5-en-2-yl) cyclohexa-1, 3-diene (6.27%), β – Capaene (5.55%), β – Pinene (1.74%), linalool (12.5%) and α – Copaene (1.25%) were the terpenoids identified. Other representative compounds were the ester benzyl benzoate (22.11%), the hydrocarbons Naphthalene (2.23%) and Cyclododecane (1.04%), the heterocyclic compounds Indole (1.08%) and Piperidine (1.06%), the fatty acid Hexadecanoic acid (2.31%), and the amine Phenylephrine (0.74%). The synthesis of some terpenoids occurs specifically in leaf structures called glandular trichomes (Mccaskill, 1999), and studies have shown that in some plants, the full development of these structures is light-dependent (Yamaura, 1989); (Ioannidis, 2002). A greater or lesser light intensity can explain the variation of terpenoids (Tables 2 and 4) when oils from leaves of *C. macrostachyus* are compared at different collection periods. Due to its chemical composition, hydrocarbons, alcohols and esters stand out as the main protective barrier against water loss due to excessive sweating, pathogens action, solar radiation, and chemicals and contaminants inputs due to their chemical composition (Ioannidis, 2002). The concentration of these classes of compounds was higher in the dry season (Table 2), which leads us to believe that these compounds play a protective role in this plant, especially in the leaves which are more subject to loss of water and external injuries when compared to the bark. In the rainy season, when the plant has plenty of water, the number of chemical components produced increases (Table

4). These results further support the fact the chemical composition of the essential oil is in fact affected by seasonal variation.

Conclusion

In different seasonal periods, the plant synthesizes different compounds according to environmental conditions (Gazim, 2010). The data presented here demonstrate this fact (Tables 2 and 4). The results showed the influence of collection time on the chemical composition of the essential oil of the species studied. In the rainy season, the number of chemical constituents of the essential oil and their yields were higher due to the decrease in water stress and flowering period. The major component was Germacrenen D, demonstrating that these phenomena positively affect the production of this metabolite. In the dry seasons, the yields were lower; however, the major component showed a higher yield in the summer, indicating that the plant produces compounds that protect the excess loss of water. The chemical composition of *C. macrostachyus* presented variations when compared with the literature data, in addition to variations throughout the successive seasons. The components identified in the essential oil of *C. macrostachyus* exhibit interesting biological activities, making this essential oil a promising compound for the development of new biodegradable drugs and insecticides. The present study also showed that the extract of *C. macrostachyus* contains many phytochemical components. These compounds have potentially significant applications against human pathogens, including those that cause enteric infections.

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Table 1: Oil yield of *C. macrostachyus* essential oils (CMEO) in two different seasons

Sample	Date of Collection	Season	Time of Extraction	Oil Yield (ml)
CMEO-1	February 23, 2016	dry	3h	1.5
CMEO-2	September 5, 2016	rainy	3h	1.0

Table 2: Chemical composition of the essential oil from the leaves of *C. macrostachyus* in the dry season

No.	Compounds	RT	% GC- MS after 3 h
1	β - Bourbonene	24.967	1.26
2	Caryophyllene	26.414	3.91
3	Humulene	27.822	1.53
4	β - Farnesene	28.004	2.25
5	γ - Muurolene	28.802	1.77
6	Germacrene	28.990	43.57
7	γ - Cadinene	29.526	1.90
8	α – Farnesene	30.085	3.54
9	Naphthalene	30.664	3.16
10	γ – Elemene	31.940	2.05
11	Benzyl benzoate	39.670	31.46
12	Phytol	50.973	3.59

Table 3: Chemical class distribution of the essential oil components from the leaves of *C. macrostachyus* in the dry season.

Functional Group	Oil Percentage (%)
Terpenoids	65.37 (10)
Ester	31.46 (1)
Aromatic Hydrocarbon	3.16 (1)

Table 4: Chemical composition of the essential oil from the leaves of *C. macrostachyus* in the rainy season

No	Compounds	RT	%GC- MS After 3h
1	β – Pinene	6.081	1.74
2	Linalool	8.03	1.25
3	α – Copaene	12.213	1.25
4	β – Bourbonene	12.357	2.17
5	Caryophyllene	12.821	11.90
6	β – Copaene	12.943	5.55
7	γ – muurolene	13.140	2.83
8	Humulene	13.272	3.40
9	Bicyclosesquiphellandrene	13.380	1.49
10	1-methyl-4-(6-methyhept-5-en-2-yl) cyclohexa-1,3-diene	13.499	6.27
11	Germacrenen D	13.616	24.66
12	α – Farnesene	13.790	3.47
13	Naphthalene	13.851	2.23
14	Indole	14.012	1.08
15	Phenylephrine	14.772	0.74
16	Piperidine	15.694	1.06
17	Cyclododecane	15.935	1.04
18	Benzyl benzoate	16.956	22.11
19	Hexadecanoic acid	18.964	2.31
20	O – Veratramide	21.792	1.18

Table 5: Chemical class distribution of the essential oil components from the leaves of *C. macrostachyus* in the rainy season.

Functional Group	Oil Percentage (%)
Terpenoids	63.57 (10)
Ester	22.11 (1)
Hydrocarbons	3.27 (2)
Heterocyclic Compounds	2.14 (2)
Fatty acid	2.31 (1)
Amine	0.74 (1)