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COMPARATIVE STUDY OF THE VOLATILE CONSTITUENTS OF SOME HIGH-LAND AROMATIC PLANTS GROWING IN ETHIOPIA

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ABSTRACT

The essential oil of *Plectranthus assurgens* was analysed by GC/MS where several components were identified for the first time. The components of the oil of *P.assurgens* as well as *Callistemon citrinus* prepared from Ethiopian plants were compared with those oils prepared from other species growing abroad.

INTRODUCTION

Plectranthus assurgens (Beaker) Morton, Family Lamiaceae is one of the perennial aromatic herbs commonly found in Addis Ababa region and the surrounding moist Forests. The plant is also cultivated for ornamental purposes in houses specially cooler regions due to its variegated aromatic foliage [1]. Stems of this plant are up to 150 cm long, leaves are triangular to lanceolate to ovate in shape having leathery pubescent laminae covered also with glands that emit an aromatic fragrance especially on crushing the leaves. Flowers are purple blue in whorls of panicles usually observed in September to March where bees forage for nectar and pollen through out the day [1].

Some species of *Plectranthus* have evidenced marked antimicrobial activities in their essential oils [2,3]. The essential oil of the leaves of the Caribbean *P. amboinicus* inhibited the growth of pathogenic bacteria viz., *Vibrio cholera, Escherishia coli, Staphylococcus aureus* and *Mycobacterium smegmatus* (MIC, 0.062 - 0.5 mg/ml) [2]. Inhibition was also evidenced on the fungi viz., *Botrytis cinerea, Asperigillus niger* and *Candida albicans* (MIC 0.125-0.25 mg/ml) by the same oil [2]. *Plectranthus incanus* oil also was active against *Vibrio cholera, Salmonella typhosa, E.coli, Bacillus subtilis, Corynebacterium diphteria, Staphyllococcus aureus and Shigella dysenterica* [3].

The same oil of *P. incanus* when dissolved in propylene glycol has induced marked inhibition of rate and force of cardiac contraction of isolated frogs and rabbit hearts [4]. The antimicrobial activity reported in *P.elegans* was evidenced in the gramme +ve bacteria as well as the spore germination of *Cladosporium curcumerinum* [5]. Meanwhile, their was a negative effect on Gram -ve organisms [5]. These activities were related to the diterpenoid constituents present in the plant [5]. Another antimicrobial properties were also related to the diterpenoids present in *P. hereroensis* [6] and *P. glancolylx* [7]. On the other hand the diterpenoids viz., trichorabdals present in *P. trichocarpus* have showed marked inhibition of Erlich ascites carcinoma cells in mice [8]. In Rwanda the essential oil of *P. barbatus* is used as a remedy for bronchitis and pneumonia [9], while *P. sylvestres* is usually recommended in several skin diseases [9]. P.barbatus growing in Kenya, was found to be the etiology of perianal dermatitis when used as a toilet facility [10].

The essential oil of *P. assurgens* has not so far been investigated for components responsible for its fine fragrance. It may be recommended for perfumary and/or scenting soaps; if the plant is cultivated for commercial use.

Callistemon citrinus (Curt) Skeels (1913) previously known as *C.lanceolatus* DC (1882), Family Myrtaceae is well known plant as bottle brush [11]. The trees are attractive and cultivated for ornamental purposes in parks and town gradens. It is indigenous to New South Wales and Victoria garden in Australia [11].

However, the plant is now very well established as an ornamental throughout the tropics and warm temperate countries in the world. In Ethiopia it is commonly found in gardens of Addis Ababa, Harare and other towns showing many drooping branches with reddish silky leaves and lemon scented when crushed. The flowers are in showy cylindrical spikes up to 10 cm long and appears during the dry season in Ethiopia in the gardens and streets in the high-land up to 2500 m [12].

The flowers provide sufficient quantities of nectar and pollen for honeybees which assist indirectly the production of bee colonies in Ethiopia particularly when other plants are not flowering [13]. *Callistemon citrinus* was reported to be a very polymorphic plant, and hybridises freely with other species and forms the progeny [14]. This will certainly leads to unpredicted changes in the volatile oil composition, which normally varies from locality to another.

The constituents of volatile oil of *C. lanceolatus* and *C. rigidus* growing in Egypt were previously reported with similar oil composition in their terpenoid contents [15]

This study of *C. citrinus* is intended to explore the effect of high-land locality and/or hybridization on the composition of its oil when cultivated in different moist environment like Ethiopian high-lands.

EXPERIMENTAL

a) The plant materials:

The plant materials (viz., *Plectranthus assurgans* and *Callistemon citrinus*) were collected on flowering stage in November from Arat-Kilo area, Addis Ababa. Authentication was performed by **Dr. Dawit Abebe**, Associate Professor of Taxonomy, Institute of Medicinal Plants, Addis Ababa University. Herbarium specimens of the collection are available at the Herbarium Department, College of Science, Addis Ababa University.

b) Preparation of the oils:

Freshly collected leaves of *P.assurgans* and *Callistemon citrinus* were cut into pieces and then subjected to hydrodistillation. The average percentage of three oil preparations were calculated (0.7% for *Callistemon citrinus* and 0.051% for *Plectranthus assurgans*).

c) GC/MS Analysis:

Analysis of the oils were performed using Shimadzu GC/MS-14A, Q P-1000 Ex instrument. Separation of components was performed on a capillary column HP-Innowax cross linked polyethylene glycol and Helium as a carrier gas. Inlet temperature 80°C, programmed rate 10°C/min, and final temperature 250°C, final time 25 min. Mass spectra were recorded by EI mode at 70 ev. and maximum mass units were up to 1000.

d) Identification of components:

The components of the volatile oils of *P.assurgens* and *C. citrinus* were identified by matching of their spectra with reference compounds in the data base and also with mass spectral comparison with those reported in the literature [16] (Table 1 & 2).

e) Chromatographic analysis:

Some authentic oil components viz., linalool, geraniol, camphor, menthol, thymol, α-pinene, cineol were co-chromatographed with the isolated oils on TLC using precoated silica gel PF 254 and developed with $CHCl_3$:MeOH 9:1 and 9.5 : 0.5 . Spots were located with vanilline-H_2SO_4 spray reagent where linalool, camphor and menthol could be verified in the oils.

RESULTS AND DISCUSSION

Analyses of the essential oils of *P. assurgens* and *C. citrinus* revealed several terpenoids being identified by their mass spectra (Table 1, 2). Amongst the terpenoids of *C. citrinus* are linalool and its acetate which constitutes the principle monoterpenoid of the oil (96.9%) of the high-land plant of Ethiopia. The oils prepared from *C. citrinus* and *C. rigidus* growing in Egypt [15] were very low in proportion of these components when compared with the Ethiopian plant. The latter species had instead cineol (68-76%) as the principle terpenoid.

Other important differences revealed among the species of *plectranthus* were evidenced for *P. amboinicus*. While carvacrol and camphor constituted the major components of oils prepared from plants growing in Martinique and Mauritius, a lower percentage was reported for the same plant elsewhere (Table 1.). Carvacrol, however, is not evidenced on other species of *Plectranthus* such as *P.tenuiflorus* or *P. defoliatus* grown in Saudi Arabia or Burundi. Thymol (85.3%) and piperitenone oxide (~88%) instead, constituted the major components of these oils, respectively. Other species like *P.coleoides, P.sylvestres*, however, were lacking all of these compounds in their oils (Table 1.). *Plectranthus assurgens* grown in Ethiopia is also deficient in such a high concentration of these compounds, but instead several sesquiterpenes of the cedran type could be identified in its oil.

These differences may reflect the great influence of soil, locality and/or environmental aspects on the composition of oils of such medicinal plants. Consequently, proper identification of constituents of aromatic plants when grown in different localities is highly recommended, before such plants are used for Pharmaceutical purposes.

Table (1) : Volatile oil constituents of Plectranthus species

Comp. No.	RT. (min)	*P. assurgens*			*P. amboinicus (Coleus aromatics)* [2,17,18]					
		Name of compound	Main fragments	%	Name of compound	%	Name of compound	%	Name of compound	%
1	8.34	Camphor	152,137,108,104, 95,82,09,67,55'	16.3	Carvacrol [a]	72	Carvacrol [b]	13.4	Carvacrol [c]	41.3
2	9.42	Menthol, neo-	138,137,123,109,93,83,71,55	7.6	Z-1,3-Hexadiene	0.1	Camphor	12.3	Camphor	39
3	10.26	Elements , δ	204,189,121,147,121,79,67	2.7	Z-3-α–Hexenol	0.6	Δ^3 carene	16.3		
4	11.01	Cedrene, β-	204,189,175,161,147,121,105,93,79,69,55	18.9	E, Z-α-Farnesene	0.2	γ -Terpinene	11.9		
5	11.42	Cadinol, δ	204,182,161,133,119,105,193,67,69,55	4.4	E, E-α-Farnesene	0.2				
6	11.76	Unknown	134,119,109,92,81,55	3.8	α -Muurolene	0.2				
7	12.51	β isaboline, φ-	204,135,121,93,69,55	4.0						
8	12.92	Hexadecyl acetate	125,111,97,81,83,71,69,55	14.7						
9	14.17	Geranylacetone	177,161,151,136,121,109,104,93,81,55	6.6						
10	14.46	Cedranol, neo-	204,179,175,161,123,138,133,119,109,95,81,67,55	3.1						
11	16.67	Cedrol, β (cedran (8-ol)	205,189,161,151,52,121,95,81,67,7,89,177	5.7						
12	17.17	Cedran-9- one	135,123,121,109,95,82,69,67,55	4.0						
13	17.42	Unknown	137,135,121,109,95,82,69,67,55	2.6						
14	18.01	Cedran, 8,13-Oxide	220,177,169,133,121,105,91,93	5.6						

Table (1) : Volatile oil constituents of *Plectranthus species* (Continued)

Comp. No.	*P. coleoides* [22]	%	*P. sylvestres* [9]	%	*P. barbatus* [9]	%	*P. tenuiflorus* [d] [19]	%	*P. defoliatus* [e] [20]	%
1	Fenchone	14.2	t-β-Ocimene		Fenchylacetate		Thymol	85.3	Piperitenone oxide	53-88
2	Bornyl acetate	9.3	β-Bourbonene		α-capaene					
3	Isbornylacetate	8.2	t-Caryophyllene		Aromdendrene					
4	β-caryophyllene	11.3	Germacrene-D		Borneol					
5			α-Farnesene		γ -Cadinene					
6			δ-Cadinene		t-Caryophyllene					
7					Ledol					
8					T-cadinol					
9					Forregol					

a-plant collected from Martinique [2]
b-Components reported in the essential oil and aqueous extract [18]
c-Pant collected from Mauiritius [17].
d-Plant collected from Saudi Arabia [19]
e- Plant collected from Burundi [20]

Table (2) : A comparison between volatile oil composition of *Callistemon* species growing in Egypt, Ethiopia and Saudi Arabia.

Comp. No.	RT. (min)	*C. citrinus* growing in Ethiopia			*C. citrinus* [15] growing in Egypt		*C. rigidus* [15] growing in Egypt		*C. speciosus* [21] growing in Saudi Arabia	
		Name of compound	Main fragments	%	Name of compound	%	Name of compound	%	Name of compound	%
1	2.86	Linalool	139,109,121,93,71,67,55	51.1	α - Pinene	13.9	α - Pinene	1.3	α-Pinene	15.2
2	8.96	Linalyl acetate	136,121,93,81,80,67,55	45.8	β - Pinene	0.4	β - Pinene	0.8	Terinoline	1.3
3	10.61	Geraniol	139,123,121,111,93,81,69,68,67	0.8	Myrecene	0.09	Myrecene	1.6	d-Fenchyl alcohol	0.8
4	12.46	Bornyl angelate	236,136,121,109,107,95,93,83,67,55		Limonene	5.1	Limonene	6.1	Cineol	37.7
5					Cineol	68.1	Cineol	68.1	Caran-5-01	0.5
6					γ- Terpinene	0.2	γ- terpinene	0.4	Linalool	2.7
7					Linalool	-	Linalool	1.9	Terpenen-5-01	3.7
8					Linalylacetate	2.5	Linalylacetate	10.2	2,2,3-Trimethyl-1-acetyl cyclo pentane	0.5
									α-Terpineol	6.4
									Nerol	1.8
									Isoeugenol	1.6
									Caryophyllene	6.7
									Longifolene	2.0
									α-Ellemene	1.3
									t-β- Farnesene	2.0
									Dihydro eugenol butyrate	3.6
									1-Octadecene	1.2
									Globulol	1.8
									Ledol	3.1
									β-Eudesmol	0.7
									Dihydro-β-eudesmol acetate	1.7
									Spathulenol	0.6
									2,3-dihydro-2-methyl-1-phenyl-4-(1H)-quinazolinone	1.8
									9,13-Dimethyle tetra deca 8,12-dien-2-one	*0.5*
									2,3-dihydro-2,3-dimethyle-1-phenyl-4-(1H) quinazolinone	*0.5*
									2,6-4 α-hydroxy-spathulenol	*0.3*

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دراسة مقارنة للمحتويات الطيارة لنباتى بلكترانثس اشورجنيس وكالستيمون ستوينس التى تنمو على المناطق المرتفعة فى اثيوبيا

عـــلي محـــمـــد الســـيـــد
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فى هذا البحث تم تحضير الزيت الطيار لنبات بلكترانثس أشورجنيس وتحليلة بكروماتوجرافيا الغاز المتصل بمطاف الكتلة . عديد من محتوى هذا الزيت الطيار تم التعرف عليه لأول مرة . وكذلك تم تحضير وتحليل الزيت الطيار لنبات الكالستيمون ستوينس الذى ينمو فى أثيوبيا بنفس الطريقة السابقة وقد قورنت المحتوى التربينى لهذه الزيوت بأخرى المحضرة من أنواع أخرى أو التى تنمو فى مصر والسعودية وبورندى.

وقد ثبت من هذه الدراسة أن الربينات الأولية مثل الينالول وأسيتات الليناليول تكون حوالى ٩ر٩٦٪ من الزيت الطيار لنبات كالستيمون ستوينس الذى ينمو فى أثوبيا بالمقارنه بنفس النبات الذى ينمو فى مصر الذى يحتوى على السنيول بنسبة ٦٨٪ . أما نبات بلكترانثس أشورجنس فهو يفتقر كثير من المركبات التى ذكرت فى كثير من الأنواع بتركيزات عالية مثل الثيمول ، البيريتون ، كارفاكرول ولكن وجد أنه يحتوى على كثير من السسكويتربينات من نوع السدران تم التعرف عليه لأول مرة.