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Full Length Research Paper

The profile and antimicrobial activity of the essential oil from *Callistemon viminalis* (Sol. Ex Gaertner) G.Don Ex Loudon leaves

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Hydrodistillation of the essential oil from the fresh leaves of *Callistemon viminalis* (Sol. Ex Gaertner) G.Don Ex Loudon afforded 0.7% (v/w) essential oil. The chemical composition of the oil was analyzed by GC/MS. Seventeen compounds representing 96.14 % of the total oil were identified. The oil was characterized by the dominance of 1,8-Cineole (66.36 %), *α*-pinene (20.43 %) and *α*-terpineol (6.65 %). The *in vitro* antimicrobial activity of the essential oil was studied against two bacteria and one fungus strain, using disc diffusion method. The oil was highly active against *Escherichia coli* and showed moderate activity against *Staphylococcus aureus* and *Aspergillus niger* when compared with the standard antibacterial gentamicin and antifungal clotrimazole.

Key words: *Callistemon viminalis*, Myrtaceae, essential oil composition, antimicrobial activity.

INTRODUCTION

Myrtaceae is one of the essential oil rich families. The family consists of 130-150 genera from which are the genus callistemon which comprises about 25 species. *Callistemon viminalis* (bottle brush) grows 5 to 7 m tall producing bright red flower spikes which are very rich in nectar. It is native to Australia but now it is widespread throughout the world. In Egypt, it is a common tree on roads and in many gardens, cultivated for its beautiful red-coloured inflorescence. *Callistemon* species are used for forestry, essential oil production, farm tree/windbreak plantings, degraded-land reclamation and ornamental horticulture (Spencer and Lumley, 1991). *Callistemon* species are also used as weed control (Wheeler, 2005) and as bioindicators for environmental management (Burchett et. al., 2002). In China, *Callistemon* species especially *C. viminalis* are used in traditional Chinese medicine as pills for treating hemorrhoids (Oyedeji et. al., 2009).

The volatile oil of different *Callistemon* species has been reported to possess antimicrobial (Sudhakar et. al., 2005 and Abdelhady, 2009), anticandidal (Dutta et al., 2007), bio-repellents for Land Leeches (Nath et. al., 2002), insecticidal (Singh et al., 2001; Sharma et al., 2001; Lee et al., 2004) and antifungal (Pandey et al., 1982; Pandey, 1995; Misra et al., 1997; Sudhakar et al., 2005; Abdelhady, 2009; Dongmo et al., 2010) activities.

Chemical studies of the essential oils of *C. viminalis* from Australia, Egypt, Cameroon, Brazil and India had been previously reported (Brophy et al., 1985; Mahmoud et al., 2002; Srivastava et al., 2003; Ndomo et al., 2010; Silva et al., 2010). 1,8-Cineole (47.9-82.0%) is the predominant constituent of the oils. Other significant components include α-pinene, β-pinene, myrcene, limonene, linalool and menthyl acetate.

Controversy of the oil profile in plants of different geographical sources, lead us to investigate the sample

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growing in Nile Delta aiming to find wether the oil profile is affected by the geographical distribution and to predict a geographic marker or clear-cut prove on the environmental factors which could affect the oil profile.

Materials and Methods

Plant materials

The leaves of *Callistemon viminalis* were collected from Mansoura university gardens, Egypt in March, 2007 in the early morning. The plant identity was confirmed in Department of Botany, Faculty of Science, Mansoura University, Egypt.

Hydrodistillation of the essential oil

Fresh leaves (300 g) of *Callistemon viminalis* were subjected to hydrodistillation for 4 h in a Clevenger-type apparatus. The resulting oils was collected, dried over anhydrous sodium sulfate, preserved in a sealed amber glass ampoule and stored at 4^{o}C until analysis.

GC/MS analysis

GC/MS analysis of the essential oils was carried out at the National Research Center, Dokki, Cairo, Egypt using GC/MS Fenningan Mat SSQ 7000 chromatograph with Digital DEC 3000 work station fitted with a fused silica DB-5 (30 m x 0.25 mm ID, 5% phenyl methyl polysiloxane) capillary column with helium as carrier gas at flow rate of 1.6 ml/min and column head pressure 13 psi. The gas chromatograph is coupled to a mass selective detector (MS) at 70 eV in EI ionization mode. The chromatogram was coupled by holding the oven temperature at 50 °C for 5 min., and then programmed from 50-500 °C at 4 °C/min.

Identification of compounds

Identification of the components was based on the comparison of their retention indices, matching the fragmentation pattern in the resulted mass spectra with those published in literature (Adams, 1995) and using NST mass spectral database of the gas chromatograph computer.

Antimicrobial assay

The essential oil was tested against two reference bacteria and one reference fungus obtained from the Microbiology Department, Faculty of Pharmacy, Mansoura University, Egypt. Gram-positive bacteria: *Staphylococcus aureus*. Gram-negative bacteria: *Escherichia coli*. Fungus: *Aspergillus niger*. The stock cultures were maintained at 4^{o}C in Mueller-Hinton agar (MHA) (Oxoid).

Agar disk diffusion assay

Culture of *S. aureus, E. coli* and *A. niger* (24 hour) were inoculated (0.2 ml each) into flasks containing 50 ml molten nutrient agar at 50-55^{o}C, respectively. The inoculated molten nutrient agar in each flask was poured into petri dishes each of 10 cm diameter (25 ml/dish). Cups, each of 8 mm diameters, are made using a sterile Weatherman tube. Three cups for the essential oil and one for the dimethylformamide (DMF), (mount) as positive control and one for the standard antimicrobial compound (clomitrazole, 0.01 mg/mL and gentamycin, 10 mg/mL, respectively) as negative control. The sample (50 µl of 100 mg/ml) was transferred to the corresponding cups in the different seeded strain agar. The plates are incubated at 37 ^{o}C for 24 hours. Inhibition zone of each tested sample: A, DMF: C; (C= the actual diameter of the inhibition zone produced by DMF + the diameter of the cup) and standard sample were measured using a caliper. The actual diameter of the inhibition zones (X) of each of the tested fractions and compounds were calculated from the equation: X= (A-C)/2

RESULTS AND DISCUSSION

Hydrodistillation of the fresh leaves of *C. viminalis* yielded 0.7% (w/w) of essential oil. The oil components were identified with their percentage composition and relative retention indices, Table 1. Seventeen constituents were identified and quantified in the oil representing 96.14 % of the total oil. The oil is characterized by the dominance of 1,8-cineole (66.36 %), *α*-pinene (20.43 %), *α*-terpineol (6.65 %) in addition to an unknown compound (2.26 %). Minor components are 2,4-dimethyl-1-penten-3-one (0.11 %), *β*-pinene (0.12 %), β-myrcene (0.07 %), *y*-terpinene (0.07 %), linalool (0.32 %), pinocarveol (0.07 %), terpin-4-ol (0.19 %), *trans*-geraniol (0.52 %), isoeugenol (0.43 %), *trans*-caryophyllene (0.06 %), acetyleugenol (eugenol acetate) (0.1 %), geranyl butyrate (0.04 %), spathulenol (0.33 %) and 4-bromo-2-methoxy-phenol (0.23 %).

Although, the essential oil compositions of *C. viminalis* from different countries have been studied including South Africa (Oyedeji et al., 2009), India (Srivastava et al., 2003), Brazil (Silva et al., 2010), Egypt (Mahmoud et al., 2002, Ayoub et al., 2007; Mahmoud and Aly, 2012), Australia (Brophy et al., 1985) and Cameroon (Ndomo et al., 2010), there are differences in the yield and profile of the oil constituents, which could be attributed to many environmental factors viz. latitude, geographical

Table 1. The composition of the essential oil of *Callistemon viminalis* leaves

Retention time (t_r) (min.)	Relative % composition	M^+ peak	Base peak	Fragmentation peaks (*m/z*)	Components
8.4	0.11	112	69	27, 41, 43, 67, 81, 97	2,4-dimethyl-1-penten-3-one
10.54	20.43	136	93	39, 41, 53, 67, 77, 91, 105, 121	*α*-pinene
11.6	0.12	136	93	41, 53, 69, 79, 94, 121	*β*-pinene
12.02	0.07	136	93	41, 69, 77, 80, 91, 121	*β*-myrcene
14.57	66.36	154	154	58, 68, 71, 81, 84, 93, 96, 108, 111, 125	1,8-Cineole
14.74	0.07	136	93	77, 79, 91, 105, 121	*γ*-terpinene
15.71	0.32	136	71	41, 43, 55, 67, 80, 83, 93, 107,121	Linalool
16.88	0.07	134	92	41, 55, 70, 81, 83, 91, 109, 119	Pinocarveol
18.03	0.19	154	71	43, 55, 69, 81, 86, 93, 111	Terpinen-4-ol
19.23	6.65	136	59	43, 55, 68, 81, 93, 107, 121	*α*-terpineol
20.29	0.52	154	69	41, 53, 68, 84, 93, 111, 123, 136	*trans*-geraniol
22.84	0.43	164	164	55, 65, 77, 103, 121, 131, 149	Isoeugenol
24.56	0.06	204	133	55, 69, 79, 93, 105, 119, 147, 161, 189	*trans*-caryophyllene
25.27	0.1	209	164	43, 55, 77, 91, 103, 131, 149	Acetyleugenol (Eugenol acetate)
26.65	0.04	205	69	41, 43, 68, 71, 80, 93, 104, 121, 136	Geranyl butyrate
27.05	2.26	236	166	55, 95, 123, 151, 193, 221	Unknwon
28.51	0.33	220	205	43, 55, 69, 79, 91, 105, 119, 121, 147, 159, 162, 187	Spathulenol
28.7	0.23	222	43	69, 81, 96, 107, 122, 161, 204	4-bromo-2-methoxy-phenol

distribution etc (Simmons and Parsons 1999; Quijano-Célis et al., 2010).

The main qualitative and quantitative differences in the essential oil compositions of *C. viminalis* leaves from different countries are shown in Table 2. 1,8-cineole was higher in South Africa and Egypt samples then in the Equator samples, India, Cameroon and Australia.

The inhibition zones and disc diameters of the essential oil of *C. viminalis* against the tested microorganisms are shown in Table 3. The results obtained from the agar disc diffusion method for the essential oil revealed that the volatile oil was highly active against the gram negative bacteria *E. coli*. It showed moderate activity against the gram positive bacteria *S. aureus* and the fungi *A. niger*, in comparison with the standard antibacterial compound (gentamicin) and antifungal compound (clotrimazole).

Conclusion

The dominance of 1,8-cineole, in different geographical samples, makes it a good marker for the different *Callistemon* species together with the myrtaceae family. The antibacterial activity showed by the essential oil of *C. viminalis* could be attributed to the presence of some major components such as 1,8-cineole, α-pinene and α-terpineol, along with other components in lower amount such as, β-pinene and linalol, which were already known to exhibit antimicrobial and bacteriostatic activities (Carson and Riley, 1995; Tzakou et al., 2000; Mourey and Canillac, 2002; Viljoen et. al., 2003) nevertheless,

Table 2. The profile of the essential oil of *Callistemon viminalis* leaves form different geographical sources.

Components	Egypt [1]			South Africa [2]	India [3]	Brazil [4]	Australia [5]		Cameroon [6]
	I*	II*	III*				Cultivator I	Cultivator II	
1,8-cineole	66.36	47.94	78.31	83.2	61.7	65.0±2.3	64.2	48.7	58.49
α-pinene	20.43	3.29	0.5	6.4	24.2	12.0±1.1	0.7	18	0.93
α-terpineol	6.65	7.56	4.21	4.9	2.3	13.0±1.6	1.2	11.8	7.83
trans-geraniol	0.52	0.84	0.43	0.5	------	---------	0.4	0.1›	-------
Linalool	0.32	13.03	5.16	0.5	0.3	1.1±0.1	16	0.5	1
β-pinene	0.12	1.61	1.3	0.9	0.7	----------	1	1.3	-----
Pinocarveol	0.07	------	------	0.9	0.2	2.3±0.4	0.1	0.3	-----
Terpinen-4-ol	0.19	3.41	------	0.6	-----	1.4±0.2	1.6	0.9	0.79
β-myrcene	0.07	2.06	1.67	------	0.1	---------	1.8	0.3	-----
γ-terpinene	0.07	0.72	0.63	------	0.3	---------	0.6	0.3	-----
4-bromo-2-methoxy-phenol	0.23	------	------	------	------	---------	-----	------	-----
2,4-dimethyl-1-penten-3-one	0.11	------	------	------	------	---------	-----	------	-----
Menthyl acetate	-----	------	------	------	5.3	---------	-----	-------	-----
Iso-amyl-isobutyrate	------	------	-------	-------	0.5	----------	-------	------	-----
p-cymene	------	------	0.4	-------	0.5	3.6±0.1	0.2	0.8	-----
Borneol	------	------	-------	-------	0.7	----------	-------	------	-----
Ledol	------	------	0.05	-------	------	1.5±0.7	-------	------	-----
Limonene	------	10.9	-------	-------	------	----------	2.5	5.4	7.01
Sabinene	------	------	0.7	-------	------	----------	-------	------	-----
3-carene	------	------	-------	-------	------	----------	-------	------	8.61
Ocimene	------	------	-------	-------	------	----------	-------	------	0.81

1- Egypt: * I: Present study, C. viminalis leaves were collected from Mansoura university gardens in March, 2007 in the early morning.
*II: Mahmoud et al., 2002, C. viminalis leaves were collected from Alexandria-Cairo road in May, 1999.
*III: Ayoub et al., 2007, C. viminalis leaves were collected from private gardens by personal communication.
2- South Africa: C. viminalis leaves were collected from Durban and Johannesburg, in the Province of KwaZulu-Natal.
3- India: C. viminalis leaves were collected in the month of February, 2001 from the National Botanical Research Institute (NBRI).
4- Brazil: C. viminalis leaves were collected in February 2008, from plants grown in the arboretum of the Forest Engineering Department, Dendrology Sector, at the Federal University of Viçosa (UFV), Minas Gerais state.
5- Australia: C. viminalis leaves were collected from two cultivars at Randwick, New South Wales.
6- Cameroon: C. viminalis leaves were collected in November 2005 in Dschang city located in the Menoua division of the Western highlands of Cameroon.

Table 3. Antimicrobial screening of the essential oil of *Callistemon viminalis* leaves*

Compounds		The diameter of the inhibition zone (mm)		
		Aspergillus niger	***Escherichia coli***	***Staph. aureus***
Essential oil		3	7	2
Clotrimazole (0.01mg/ml)	Antifungal	10	------	-------
Gentamicin (10mg/ml)	Antibacterial	------	4	10

*mean of three determinations

the presence of minorbcomponent could also play a role in the biological activity.

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