Antioxidant and antimicrobial activities of *Callistemon comboynensis* essential oil

Mohamed I. Abdelhady [a,b*] and Hamdy A. H. Aly[c]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, Umm Al-Qura University, Makkah, P.O. Box 715, Saudi Arabia;*
[b]*Pharmacognosy Department, Faculty of Pharmacy, Helwan University, Cairo, Egypt;*
[c]*Environmental Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat city – Menofia University – Egypt. P.O. Box 79.*

ABSTRACT

Introduction: The genus *Callistemon* is known in folk medicine for its anticough, antibronchitis, and insecticidal effects and its volatile oils have been used as antimicrobial and antifungal agents. **Methods:** The essential oils obtained by hydrodistillation of the leaves of *Callistemon comboynensis* (*Cc*) was investigated by GC/MS. Antioxidant activity of *Cc* was investigated using 1,1-diphenyl-2-picrylhydrazyl (DPPH). The antimicrobial activity of the essential oil of *Cc* was evaluated against both gram positive (*Bacillus subtilis* and *Staphylococcus aureus*), gram negative (*Proteus vulgaris*, *Pseudomonas aeruginosa*) and a pathogenic fungus *Candida albicans*. **Results:** It was found that *Cc* afford 0.22% volatile oil. The major components of the volatile oil of *Cc* are 1, 8-cineol (53.03%), eugenol (12.1%), methyl eugenol (9.2%) and α-pinene (8.3%). The oil had pronounced antibacterial and antifungal activities on all the tested microbes. Nevertheless, *Cc* leaf oil extract exhibited high antioxidant activity (91.1 ± 0.3 %) at a concentration of 1000 µg.ml^{-1}, comparable to 100 µg.ml^{-1} gallic acid (95.7 ± 2).

Key Words: *C. comboynensis*, essential oil, cineole, antioxidant, and antimicrobial activity.

INTRODUCTION

The genus *Callistemon* (commonly named bottle brush) comprises about 25 species belong to the family Myrtaceae, which are widely cultivated and much used as ornamental shrubs in California, and in warm countries and in green houses[1]. The genus *Callistemon* is known in folk medicine for its anticough, antibronchitis, and insecticidal effects[2,30] and its volatile oils have been used as an antimicrobial[3,4] and antifungal[5] agents. The oil of *C. lanceolatus* was reported as antifungal[6] and *C. citratus*, *C. viridiflorous* and *C. pendulous* as antifungal and antimicrobial[7,8]. Additionally, the oil of *C. lanceolatus* shows insecticidal activity[8]. Essential oils of some *Callistemon* species have been chemically examined i.e. *C. lanceolatus* (syn. *C. citrinus*)[9-12], *C. viminalis*[9,13], *C. speciosus*[14], *C. rigidus*[15-17] and *C. linearis*[29]. In all cases 1,8-cineole has been reported as their major constituent with varying amounts, while *ß*-pinene represents the major constituent incase of *C. polandii*[28], and methyl eugenol represents the major constituent incase of *C. viridiflorous*[25]. The present communication reports on the leaf oil composition of *C. comboynensis* grown in Egypt for the first time. Also it reports the antioxidant and antimicrobial activities of the volatile oil.

***Address for correspondence:**
E-mail: mohibrahem@yahoo.com and miabdelhady@uqu.edu.sa

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MATERIAL AND METHODS

Plant material

Cc leaves were collected from Alexandria-Cairo road, Egypt in April 2011. Identification of the plant was confirmed by Dr. Trease Labeb, senior specialized of plant taxonomy, Orman garden, Giza, Egypt as well as by comparison with reference herbarium specimens.

Material for testing the antimicrobial and antioxidant activities

Gram positive bacteria (*Bacillus subtilis* NCTC6633 and *Staphylococcus aureus* ATCC4175), gram negative bacteria

(*Proteus vulgaris* NCTC4175 and *Pseudomonas aeruginosa* CNCMA21) and yeast (*Candida albicans* ATCC60193) were supplied from Microbiology department, faculty of science, Beni-suif University. DPPH (1,1-diphenyl-2-picrylhydrazyl; Sigma-Aldrich, St. Louis, MO, USA).

Standard antimicrobial agents

Ofloxacin and Amphotricin B antibiotics ready discs (5 ug/disc) used as positive control.

METHODS

Preparation of the essential oil

Freshly comminuted leaves of *Cc* was separately hydrodistilled for 6 hours in a Clevenger type apparatus. The resulting oils were collected, dried over anhydrous sodium sulphate and stored under refrigeration until analysis. Percentage yields were determined according to the Egyptian Pharmacopoeia, 1984[24].

G.C/MS for volatile oil

G.C analyses were performed on a GC/MS system (SHIMADZU GC/MS-QP5050A) with software (Class 5000). Gas chromatograph equipped with a fused silica column (DB5.25 m × 0.53 mm i.d; 1.5 um film thickness). The analyses were carried out under the following conditions: Carrier gas: He with flow rate 2 ml/min; 280 °C; Detector temp. FID: 280 °C; Injector temp.: 250 °C; split ratio; 1:10; Oven temp. Program: initial temp.; 40 °C (0.5 min) increasing to 150 °C (at 7.5 °C/min), 150 °C (1min) then increasing to 250 °C (at 5 °C/min)-250 °C (2min). The capillary column was directly coupled with a quadrupole mass spectrometer (QP5050A). EI-MS were recorded at 70 ev. The analysis has been done in the Analytical biotechnology unit, the regional centre for Mycology and Biotechnology, Alazhar University, Cairo, Egypt. Identification of the components were performed by aid of the computer library search (Class 5000 lab software package) comparison of mass spectra with literature data and by comparison of their retention times and mass fragmentation patterns with those of the library data base (Wiley (Wiley Int. USA))[31-33].

Biological study

The anti-microbial activity of the volatile oil was carried out in the Microbiology department, faculty of science, Beni-suif University, applying the disc agar diffusion method[26]. The oil was diluted with DMSO at concentration 1:5 v/v, then 20 µl (4 ul of pure oil/disc) was aseptically transferred onto sterile discs of Whatmann filter paper (5 mm diameter).

Evaluation of the antioxidant activity

Determination of the free radical scavenging activity of the different extracts was carried out using a modified quantitative assay[53]. Various concentrations of sample oil extract in methanol were prepared (1000, 500, 250, and 100 µg/ml). Gallic acid was used as a positive control at concentrations of 100, 50, 25, and 10 µg/ml. Blank samples were run using 1 ml methanol in place of the test extract. One ml of 0.2 mM DPPH in methanol was added to 1 ml of the test solution or standard plus 1 ml of methanol for dilution and allowed to stand at room temperature in a dark chamber for 30 min. The change in color from deep violet to light yellow was then measured at 517 nm. Inhibition of free radical in percent (I %) was calculated according to the following equation: I% = [(A0 - A1)/ A0] X 100, with A0 being the absorbance of the control reaction (containing all reagents except for the extract) and A1 the absorbance of the extract. Measurements were carried out in triplicates

RESULTS AND DISCUSSION

Essential oil was obtained by hydrodistillation of the leaves of *Cc* which yielded 0.22 was analyzed by GC/MS. Qualitative and quantitative variations of the components in *Cc* are compiled in table (1). GC-MS analysis of the oil under the experimental conditions revealed the presence of 23 components in *Cc* leaves.

This oil is dominated by oxygenated compounds 90.22%. In the same time, the hydrocarbon contents are small (8.48%). Previous studies[9-28] on the composition of the oil from leaves of other *Callistemon* species showed that 1,8-cineole is the main component in those species between (45-80%), while *ß*-pinene represents the major constituent in case of *C. polandii*, methyl eugenol incase of *C. viridiflorous*. In the present study it was observed that 1,8-cineole (53.03%) is the main component in *Cc* followed by eugenol (12.1%), methyl eugenol (8.3%), α-terpineol (4.3%) then carveol (3.4%). It was interesting to know that *Cc* produces high amount of cineole, eugenol and methyl-eugenol as previous studies observed that those compounds are potent naturally occurring antimicrobial agent against broad spectrum of microorganisms and antifungal activity and it has also nematocidal activity and have a good antioxidant activities[25,34-38,39-42]. This directed the study to think about the influence of the volatile oil on the growth of certain microorganisms and as antioxidant activity.

The antimicrobial screening showed that oils under investigation exhibited broad spectrum effect against

gram-positive, gram-negative and yeast (table 2). The highest antimicrobial activity was observed against *Staphylococcus aureus* (107% that of OFX) and *Proteus vulgaris* (102% of that OFX) for volatile oil of *Cc*. The oil of *Cc* possessed a moderate activity against *Bacillus subtilis* (88% of that OFX) and *Pseudomonas aeruginosa* (80% of that OFX) and for *Candida albicans* (77% that of Amp B). This activity may be attributed to the higher content of oxygenated constituents (90.22%).

Antioxidants play an important role in the prevention of human diseases. Antioxidant compounds may function as free radical scavengers, complexing agents for pro-oxidant metals, as well as reducing agents and quenchers of singlet oxygen formation[43,44,51,54].

It is well known that there is a strong relationship between total phenol content and antioxidant activity, as phenols possess strong scavenging ability for free radicals due to their hydroxyl groups. Therefore, the phenolic content of plants may directly contribute to their antioxidant action[45-47,52].

The standardized *Callistemon* extracts were assessed for their capacity to scavenge DDPH free radical along with gallic acid as a positive control. The antioxidant activity data are presented as percent of free radical inhibition in Table (3). The oil extract from leaves of *Cc* exhibited pronounced antioxidant activity (91.1 ± 0.3%) at a concentration of 1000 µg/ml, comparable to 100 µg/ml gallic acid (95.7 ± 2%). It was previously reported that non- phenolic

Table 1: Results of GC/MS analysis of the volatile oils of *C. comboynensis*

Identified compounds	Retention time (min)	M^+	*C. comboynensis* %
2-hydroxy-pentamide	4.947	117	0.2
Heptylhydroperoxide	5.690	132	0.33
ß-pinene	6.433	136	0.12
Myrcene	7.453	136	0.06
Linalyl acetate	8.734	196	0.15
1,8-cineol	9.234	154	53.03
α-terpinolene	10.431	136	0.7
Linalool	10.853	154	1.1
α-Terpineol	11.134	154	4.3
Carveol	11.764	152	3.4
Citronellal	11.973	156	0.4
Maltol	12.327	126	0.1
Geraniol	12.842	154	2.2
Alpha pinene	12.983	136	8.3
Citronellyl acetate	13.254	206	0.14
Neryl acetate	13.546	196	1.4
Cyclopentylmethyl ketone	13.949	112	0.1
Verbanone	14.266	150	0.2
p-Propenylanisole	14.556	148	0.75
Eugenol	15.461	164	12.1
Dihydrocarvyl acetate	16.415	196	0.2
Methyl-Eugenol	18.756	178	9.2
ß-Copaen	21.112	204	0.22
Identified compounds			98.7
Unidentified compounds			1.3
Hydrocarbons			8.48
Oxygenated compounds			90.22

Table 2: results of antimicrobial activity of the essential oil of the leaves *C. Comboynensis*

Tested microorganisms	Diameter of zone of inhibition (mm)		
	Of essential oil of *C. comboynensis*	OFX	AMP B
Staphylococcus aureus ATCC 4175	31 (107%)	29 (100%)	-
Bacillus subtilis NCTC 6633	28 (88%)	32 (100%)	-
Proteus vulgaris NCTC 4175	30.5 (102%)	30 (100%)	-
Pseudomonas aeruginosa CNCMA21	16 (80%)	20 (100%)	-
Candida albicans ATCC 60193	17 (77%)	-	22 (100%)

OFX, Ofloxacin; AMP B, Amphotricin B; -, no inhibition zone.

Table 3: Antioxidant activity of *Cc* volatile oil extract assayed by the DPPH assay

Gallic acid	Conc. of standard µg/ml	Cc	Conc. of extract µg/ml
95.7 ± 2	100	91.1 ± 0.3	1000
86.7 ± 0.7	50	87.4 ± 0.4	500
78.3 ± 0.5	25	70.8 ± 0.4	250
66.4 ± 0.2	10	62.6 ± 0.7	100

Activity is expressed as inhibition of free radical in percent, I% ± SD (n=3). Leaf and cell culture extracts were tested at 1000, 500, 250 and 100 µg/ml and the positive control (gallic acid) at 100, 50, 25 and 10 µg/ml.

antioxidants might also contribute to the antioxidant activity of plant extracts[50,51]. Thus, compounds other than phenolics might be responsible for the pronounced antioxidant activity observed with *Cc* oil, which requires further investigation. The responsible constituents in the *Callistemon* species under study have to be identified and characterized. Phenolic compounds are also believed to have chemo preventive and suppressive activities against cancer cells by inhibition of metabolic enzymes involved in the activation of potential carcinogens or arresting the cell cycle[50]. Nevertheless, a compound with strong antioxidant potential can also contribute to DNA protection and prevent apoptosis[54]. In a previous comparative study of Tamoxifin (a synthetic antiestrogen) and methyl eugenol it was found that they have antitumor effect in case of breast cancer with restricted doses, because at higher doses they cause mutation of DNA leading to increase susceptibility to cause liver tumor[34]. It was reported that chronic oral intake of high dose levels of methyl eugenol was associated with increased of hepatotoxicity and liver and stomach neoplasma in rats and mice[34,36]. Further study of the effect of the volatile oil of *C.comboynensis* as a natural source of cineole, eugenol and methyl eugenol may open the way to treat the breast cancer with no side effects (i.e. herbal medicine). Further studies will therefore be done to detect potential anticancer activity of the extracts reported here.

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