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Chemical Composition of the Essential Oils of four *Eucalyptus* Species (Myrtaceae) from Egypt

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ABSTRACT

We have evaluated the medicinal properties of *Eucalyptus* essential oils that are highly flammable and contains compounds which are natural disinfectants and pest deterrents. The essential oils isolated by hydrodistillation from the leaves of four *Eucalyptus* species growing in Egypt were analyzed by GC/MS for their chemical compositions. The major components identified in *E. citridora* Hook oil were 3-hexen-1-ol, cis-geraniol, citronellol acetate, 5-hepten-1-ol, 2,6-dimethyl and citronellal; in *E. camaldulensis* Dehn. were megastigma-3,7(Z),9-triene, dihydrocarveol acetate, cis-nerolidol, kauran-18-al,17-(acetyloxy)-,(4.β.)- and (-)-spathulenol; in *E. gomphocephala* Dc. were dihydrocarveol acetate, p-cymene and citral (isomer 1) cis-, trans-.; and the major components of *E. resinfera* Sm. were eucalyptol, (-)-spathulenol, α-terpineol acetate and trans-nerolidol. The findings demonstrated that there are broad differences in the chemical components for the investigated *Eucalyptus* species.

Key words: *Eucalyptus,* Mhyrtaceae, essential oil composition, leaves, GC-MS

INTRODUCTION

Essential oils are the odorous, volatile products of the secondary metabolism of an aromatic plant which are often concentrated in a particular organ of the plant such as leaves, stems, bark or fruit (Prasad *et al.*, 2011; Monfared and Ghorbanli, 2010; Okiei *et al.*, 2009; Okoh *et al.*, 2007). Essential oils and their components are widely used in medicine as constituents of different medicinal products, in the food industry as flavoring additives and also in cosmetic as fragrances (Cowan, 1999; Dadji *et al.*, 2011; Ogunlesi *et al.*, 2009; Patra, 2011).

Family Myrtaceae comprises 3800 species distributed in 140 genera occurring along tropical and subtropical regions of the world, mainly Australia and central and South Americas (Mabberley, 1997). *Eucalyptus* L'Hér, is a large genus of this family that includes some 900 species and subspecies (Brooker and Kleinig, 2004). This family represents an important source of antimicrobial essential oils (Sartorelli *et al.*, 2007; Musyimi and Ogur, 2008). Although most of the plants are native to Australia, numerous species have been introduced to other parts of the world, including Egypt, as economic and ornamental trees in the forest, where the plants have become source of important fast-growing hardwood trees (Dassanajake, 1981) and Eucalyptus oils (Assareh *et al.*, 2007).

The essential oils of *Eucalyptus* species leaves have been underwent to several studies as antibacterial, antioxidant, antihyperglycemic and antifungal studies (Ghalem and Ali, 2008;

Bendaoud *et al*., 2009; El-Ghorab *et al*., 2003; Oyedeji *et al*., 1999a; Ogunwande *et al*., 2003; Gray and Flat, 2001; Su *et al*., 2006; Akin-Osanaiye *et al*., 2007).

The *Eucalyptus* essential oils could be grouped into three types (medicinal, industrial and perfumery) on the basis of their chemical constituents (Oyedeji *et al*., 1999b). Consequently, *Eucalyptus* oil composition has been extensively investigated due to their numerous uses in the pharmaceutical and cosmetics industries (Ahmad *et al*., 2005). Also, the *Eucalyptus* oil is a complex mixture of a variety of monoterpenes and sesquiterpenes, aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketons; however, the exact composition and proportion of which varies with species (Brooker and Kleinig, 2006).

There are many studies about the composition of essential oil of other *Eucalyptus* species. For example, the essential oils obtained by steam distillation from the leaves of nine *Eucalyptus* species of Moroccan origin (*E. cinerea, E. baueriana, E. smithii, E. bridgesiana, E. microtheca, E. foecunda, E. pulverulenta, E. propinqua* and *E. erythrocorys*) have been analyzed using GC and GC-MS. A total of 83 constituents were identified. All the species investigated were found to possess an oil rich in 1,8-cineole (>68%) (Zrira *et al*., 2004). Moreover, in five species (*E. cinerea* F. Muell., *E. baueriana* F. Muell., *E. smithii* R. T. Baker, *E. bridgesiana* R.T. Baker and *E. microtheca* F. Muell.), the 1,8-cineole content exceeded 80% (Elaissi *et al*., 2011). Furthermore, the essential oil of *E. camaldulensis*, especially from the leaves, has been widely studied. Thus the first two main components were spathulenol and p-cymene detected in trees from Morocco (Zrira and Benjilali, 1996), 1,8-cineole and β-pinene from Mozambique (Pagula *et al*., 2000), p-cymene and spathulenol from Jerusalem (Chalchat *et al*., 2001) and 1,8-cineole and limonene from Burundi (Dethier *et al*., 1994).

Previous studies on the composition of leaf volatile oil of *E. citriodora* have been reported from the plants collected in Morocco (Zrira *et al*., 1992), Madagascar (De Medici *et al*., 1992), Burundi (Dethier *et al*., 1994) and Australia (Boland *et al*., 1991). Therefore, in this study the essential oil of leaves of *E. citridora* Hook., *E. camaldulensis* Dehn., *E. gomphocephala* DC. and *E. resinfera* Sm. collected from Egypt were investigated for their chemical composition and content of the essential oils.

MATERIALS AND METHODS

Plant materials: Fresh leaves of four adapted *Eucalyptus* species were collected in February 2009 from Zoological garden in Giza-Egypt. These species represent the more dominant cultivated plants for ornamentals, medicinal importance and street trees in Egypt. The plant samples were identified in the Flora and Phyto-Taxonomy Researches Department. The herbarial specimens were deposited in the QNA Herbarium (Proposed abbreviation). The plant samples were washed, air-dried for two weeks under laboratory shade prior to the extraction of the oil samples.

Isolation of the volatile oils: The air-dried plant samples were powdered and subjected to hydro-distillation for 3 h using a Clevenger-type apparatus. One hundred gram of the dried samples of each of the plant powdered materials was placed in a round-bottomed flask and 1 L of distilled water was added. After 3 h of steam distillation, the oil layers had separated from the water layers and were collected and anhydrous sodium sulfate was added to remove the water. Yields of the essential oils were determined and the oils were stored in tightly closed dark-colored glass bottle and preserved in refrigerator until use for analysis (Denny, 1991).

Gas chromatography-Mass spectrometry (GC-MS): GC/MS analysis was performed in center lab of Horticulture Research Institute with Shimadzu GC/MS-QP-5000 a system. Column HP5, 30 m, 0.25 mm, 0.5 μm film thickness; Helium was used as carrier gas at flow rate of 1.7 mL min^{-1}; Injector temperature 120°C, detector temperature 210°C; temperature program 50°C (for 1 min) then gradually increasing 190°C at a rate of 3°C m^{-1}. The split ratio was 2:7 and 0.2 μL of sample was injected the mass range was 40-350 m z^{-1}.

RESULTS AND DISCUSSION

As shown in this Table 1, the highest value (2.50 mL) of oil yield was obtained with the dry leaves of *E. camaldulensis*, while the lowest value (1.40 mL) was resulted from *E. resinifera*. Oil odor of *E. citriodora* and *E. gomphocephala* was characteristic perfume, while it was perfume for *E. camaldulensis* and *E. resinifera*. Oil color was found to be pale yellow to yellow colors.

The essential oils were analyzed by gas chromatography/mass spectrometry (GC/MS) and the chemical compositions with their percentage and retention times are present in Table 2-5 of four species under study.

In this respect, Table 2 revealed the major components of the leaf essential oil extracted from *E. citriodora*. The volatile oils of *E. citriodora* leaves were fractionated into 11 peaks as revealed by GC/MS. The major components of volatile oil in *E. citriodora* species were 3-hexen-1-ol (31.26%), cis-geraniol (19.66%), citronellol acetate (13.68%), 5-hepten-1-ol, 2,6-dimethyl (13.14%) and citronellal (9.36 %). Six compounds were found as a minor components (Su *et al.*, 2006; Vahirua-Lechat *et al.*, 2007; Low *et al.*, 1974).

Table 3 revealed the major components of the leaf essential oil extracted from *E. camaldulensis*. The volatile oils of *E. camaldulensis* leaves were fractionated into 20 peaks as revealed by GC/MS. The major components of volatile oil in *E. camaldulensis* species were (4. **β**.)-(5.93%) and (-)-spathulenol (3.12%). Fifteen compounds were found as a minor components. It was found that these results contradicted with some of the previous results, in the research of Pagula *et al.* (2000),

Table 1: The mean essential oils yields (mL 100 g^{-1} of dry leaves powder), odors and colors from *Eucalyptus* investigated species

Species	Oil yield (mL)	Oil odor	Oil color
E. citriodora Hook	1.80	Characteristic perfume	Pale yellow
E. camaldulensis Dehn.	2.50	Perfume	Yellow
E. gomphocephala Dc.	1.80	Characteristic perfume	Yellow
E. resinifera Sm.	1.40	Perfume	Pale yellow

Table 2: Chemical composition of the essential oil isolated from leaves of *E. citriodora*

Components	R.T.	Conc. of the ext. oil (%)	Molecular form
Citronellal	13.717	9.36	$C_{10}H_{18}O$
Cis-geraniol	14.133	19.66	$C_{10}H_{18}O$
2,6-octadien-1-ol,3,7-dimethyl-,(Z)-	14.708	0.49	$C_{10}H_{18}O$
3-hexen-1-ol	17.783	31.26	$C_6H_{12}O$
Citronellol acetate	23.058	13.68	$C_{12}H_{22}O_2$
3-hexenoic acid, butyl ester,(Z)	23.258	1.48	$C_{10}H_{18}O_2$
β-bisabolene	26.425	1.31	$C_{15}H_{24}$
Dihydrocarveol acetate	32.400	4.91	$C_{12}H_{20}O_2$
1,6.10-dodecatrien-3-ol,3,7,11-trimethyl-,S-(Z)-	32.525	4.40	$C_{15}H_{26}O$
Pregn-5-en-20-one,3,17-dihydroxy-,3-acetate	34.708	0.29	$C_{23}H_{34}O_4$
5-hepten-1-ol, 3,6-dimethyl	47.775	13.14	$C_9H_{18}O$

Table 3: Chemical composition of the essential oil isolated from leaves of *E. camaldulensis*

Components	R.T.	Conc. of oil (%)	Molecular form
5-hepten-2-one, 6-methyl-	8.742	4.21	$C_8H_{14}O$
3-heptafluorobutyryl-,delta,-camphor	15.725	0.42	$C_{14}H_{15}F_7O_2$
β-bisabolene	26.417	1.59	$C_{15}H_{24}$
β-farnesol.	27.358	0.84	$C_{15}H_{26}O$
α-farnesene	28.875	2.20	$C_{15}H_{24}$
Farnesyl acetone	31.575	1.12	$C_{18}H_{30}O$
α-limonene diepoxide	31.875	2.39	$C_{10}H_{16}O_2$
Megastigma-3,7(Z),9-triene	32.675	36.50	$C_{13}H_{20}$
Dihydrocarveol acetate	32.875	18.69	$C_{12}H_{20}O_2$
Cis-nerolidol	33.117	12.14	$C_{15}H_{26}O$
Tricycle 5.1.0.02,4 octane-5-carboxylic acid, 3,3,8,8,-tetramethyl-,methyl ester	33.275	1.73	$C_{14}H_{22}O_2$
Kauran-18-al,17-(acetyloxy)-,(4. β.)-	33.442	5.93	$C_{22}H_{34}O_3$
2-butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	33.758	1.13	$C_{13}H_{22}O$
2-naphthalenemethanol,decahydro-, α., α.,4a, 8-tetramethyl-,didehydro derive	34.175	1.88	$C_{15}H_{26}O$
(-)-spathulenol	34.842	3.12	$C_{15}H_{24}O$
Ethanone,1-(octahydro-7a-methyl-1h-inden-1-yl)-,(1. α.,3a., β.,7a.α.)-	34.967	0.23	$C_{12}H_{20}O$
α-bisabolol	35.425	1.54	$C_{15}H_{26}O$
Androst-5-en-7-one,3-(acetyloxy)-4,4-dimethyl-,(3. β.)-	38.117	0.04	$C_{23}H_{34}O_3$
(Z)-. β.-farnesene	38.450	1.74	$C_{15}H_{24}$
Patchulane	39.275	2.56	$C_{15}H_{26}$

Table 4: Chemical composition of the essential oil isolated from leaves of *E. gomphocephala*

Components	R.T.	Conc. of the ext. oil (%)	Molecular form
p-cymene	8.517	10.61	$C_{10}H_{14}$
β-phellandrene	8.625	1.33	$C_{10}H_{16}$
Eucalyptol	8.742	2.50	$C_{10}H_{18}O$
β-citronellal	13.633	2.27	$C_{10}H_{18}O$
2,6-octadien-1-ol,3,7-dimethyl-,(E)-	14.033	1.97	$C_{10}H_{18}O$
Terpinen-4-ol	15.058	2.74	$C_{10}H_{18}O$
α-hexylacrylonitrile	15.625	7.36	$C_9H_{15}N$
Benzaldehyde,4-(1-methylethyl)-	17.967	2.02	$C_{10}H_{12}O$
Citral (isomer 1) cis-, trans-	19.525	8.11	$C_{10}H_{16}O$
3-octyl acetate	23.008	1.04	$C_{10}H_{20}O_2$
β-bisabolene	27.350	2.66	C15H24
Dihydrocarveol acetate	32.608	50.82	$C_{12}H_{20}O_2$
Megastigma-3,7(Z),9-triene	32.708	5.55	C_{13}H20
α-limonene diepoxide	34.783	1.05	$C_{10}H_{16}O_2$

1,8-cineole and β-pinene were the major components detected in trees from Mozambique. Akin *et al*. (2010) mentioned that the major constituents of the essential oil from *E. camaldulensis* were ethanone (25.36%), eucalyptol (13.73%), β-caryophyllene (11.55%) and carvacrol (9.05%). Also Basak and Candan (2010) reported that the major components of leaf oils of *E. camaldulensis* were, p-cymene (68.43 %), 1,8-cineole (13.92%), 1-(S)-α-pinene (3.45%) and R-(+)-limonene (2.84%). Difference in the chemical composition of the extracted oils may be due to one of the following reasons:

- Nutrients of different soils and their accumulation in the leaves may result in different metabolism and production of different bio-products and different volatile oils

Table 5: Chemical composition of the essential oil isolated from leaves of *E. resinifera*

Components	R.T.	Conc. of the ext. oil %	Molecular form
9,12-octadecadienoic acid (*Z,Z*)-, phenyl methyl ester	8.583	0.67	$C_{25}H_{38}O_2$
Eucalyptol	8.783	51.97	$C_{10}H_{18}O$
9,12,15-octadecatrienal	15.583	2.12	$C_{18}H_{30}O$
α-terpineol	15.733	4.03	$C_{10}H_{18}O$
α-terpineol acetate	22.792	8.78	$C_{12}H_{20}O_2$
α-limonene diepoxide	22.867	0.38	$C_{10}H_{16}O_2$
Dihydrocarveol acetate	23.017	1.71	$C_{12}H_{20}O_2$
Benzene,1,2,4,5-tetramethyl-3- (3-phenylpropyl)-	26.050	0.32	C19H24
α-farnesene	26.417	6.03	C15H24
2,6,10,-dodecatrien-1-ol, 3,7,11,-trimethyl-	27.367	1.82	$C_{15}H_{26}O$
19-norpregn-4-ene-3,20-dione, 10-ethyl-	27.417	0.32	$C_{22}H_{32}O_2$
Curan-17-ol,1-ethyl-, (16. α.)-	31.067	1.09	$C_{21}H_{30}N_2O$
(-)-spathulenol	32.333	9.22	$C_{15}H_{24}O$
Trans-nerolidol	32.567	8.75	$C_{15}H_{26}O$
Benzenepropanoic acid, α-oxo-, β., β.-bis (trimethylsilyl)-	45.992	0.93	$C_{15}H_{24}O_3Si_2$
Androst-5-en-7-one, 3- (acetyloxy)-4,4-dimethyl-, (3. β.)-	46.183	0.28	$C_{23}H_{34}O_3$
Undecane,1- (1-naphthyl)-	58.133	1.56	$C_{21}H_{30}$

Conc. of the ext. oil (%) = concentration of the extracted oil (%); R.T. = Retention time (m)

- The change in genes through generations and hybridizations, naturally and induced, may result in production of a variety of volatile oils compared to a nectars or those of different habitat
- Acclimation of species to the environment in which it is growing in the past
- Differences may be due different ecotypes of the species

Table 4 revealed the major components of the leaf essential oil extracted from *E. gomphocephala*. The volatile oils of *E. gomphocephala* leaves were fractionated into fourteen peaks as revealed by GC/MS. The major components of volatile oil in *E. gomphocephala* species were dihydrocarveol acetate (50.82%), p-cymene (10.61%) and citral (isomer 1) cis-, trans-(8.11%). Eleven compounds were found as minor components. There are no results reports in the chemical composition of the essential oils of *E. gomphocephala*. In this study, we will find reports for the first time of the analysis of leaf essential oil of this species which growing in Egypt.

Table 5 revealed the major components of the leaf essential oil extracted from *E. resinfera*. The volatile oils of *E. resinfera* leaves were fractionated into 17 peaks as revealed by GC/MS. The major components of volatile oil in *E. resinfera* were eucalyptol (51.97%), (-)-spathulenol (9.22%), α-terpineol acetate (8.78%) and trans-nerolidol (8.75 %). Thirteen Compounds were found as minor components. It was found that these results were agree with findings of Pino *et al*. (2002). The oil of *E. resinifera* was found to contain 1,8-cineole as the major constituent.

The reason of this variation may be attributed to that the chemical compositions of essential oils depends on climatic, seasonal and geographic conditions, harvest period and isolation technique.

Correlations between the constituents of *Eucalyptus* species and their taxonomic relationship, both within the genus and as a part of the Myrtaceae family, have been attempted. Hegnauer, for example, has addressed the issue in his *Chemotaxonomie der Pflanzen* series (Hegnauer, 1969, 1990). As in natural stands, the oil yields of plantations vary greatly. Factors which affect these yields include: seed provenance and species, soil and nutrient properties, water supply, weather, weeds, pests and diseases (Milthorpe *et al.*, 1994).

The reason of variation between the chemical compositions of oils may be attributed to that the chemical composition of essential oils depends on climatic, seasonal and geographic conditions, harvest period and isolation technique. *E. citriodora* oil, like citronella oil, has a lemon-like odor and contains citronellal as the principal constituent (Ford *et al.*, 1988). The concept of chemical variants (also called chemical forms or chemo-types) has been based on the existence of discontinuities in the chemical composition of certain eucalyptus oils. However, the development of more sensitive analytical methods, such as capillary GC, has indicated that in some of those cases previously considered to be examples of qualitative variation, the variation was only quantitative, as in *E. dives* (Hellyer *et al.*, 1969).

CONCLUSIONS

In this study, we extracted the essential oils from leaves of the four species of *Eucalyptus* trees of *E. citriodora, E. camaldulensis, E. gomphocephala* and *E. resinifera* using a hydrodistillation method. A diverse range of yield and chemical composition has been demonstrated by the kind of volatile oils isolated from *Eucalyptus* species. The yield and chemical compositions of these species have been described above. Such broad differences and the pharmacological profiles provide a stimulus to further research and it is likely that more new compounds will be isolated from an increasing number of *Eucalyptus* species in the future. The ease of cultivation and rapid growth of eucalyptus makes it, potentially, a very valuable natural resource for the commercial production of pharmaceuticals, over and above the present production of eucalyptus oil for medicinal purposes. It is likely that in the years ahead *Eucalyptus* metabolites other than the volatile constituents will form part of the armory of drugs available to the physician for the treatment or prevention of human diseases.

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