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COMPOSITION OF SOME *EUCALYPTUS* LEAF OILS AND THEIR ANTIMICROBIAL ACTIVITIES

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ABSTRACT
Analysis of the leaf essential oils of *Eucalyptus robusta, E. resinifera* and *E. gomphocephala* by GC and GC-MS resulted in the identification of 20, 14 and 22 components, comprising 88.4 , 89.5 and 89.5 % of the oils, respectively. The composition of the three oils varies both qualitatively and quantitatively. The oils are characterized by high concentration of sesquiterpene alcohols (α-, β- and γ-eudesmol and viridiflorol), oxygenated monoterpenes and phenolic ethers. Cineole ranges from 4.6 to 16.8%. *trans-* and *cis-*Methylisoeugenol were found only in the oil of *E. gomphocephala* , these compounds have not been reported in *Eucalyptus* before. The oil of *E. resinifera* exhibited a significant antibacterial and antifungal effects, while that of *E. gomphocephala* showed a moderate antibacterial and strong antifungal activities. The oil of *E. robusta* showed a weak antibacterial effects and no antifungal activity against the tested microorganisms.

INTRODUCTION

Eucalyptus (family Myrtaceae) is one of the world's most important and most widely planted genera[1]. The genus *Eucalyptus* is native to Australia and closely adjacent islands. It contains about 750 species of evergreen aromatic trees[1], and has been successfully introduced into 90 countries world wide[2].

Eucalyptus species are employed mainly in forestry (timber, fuel and paper pulp) , environmental planting (water and wind erosion control) , amenity planting, as sources of essential oils (medicinal and perfumery oils) and in floriculture.[1,3] However, only 20 species have been exploited for their essential oils.[1] Selected *Eucalyptus* species were used to treat colds, influenza, toothaches, snake bites, fevers, diarrhea and malaria.[1,4] In recent years, some biologically active secondary metabolites have been isolated from *Eucalyptus* species, sparking renewed interest in the phytochemistry of this genus. Several articles reviewing the bioactive acylphloroglucinol derivatives[1], polyphenols[5-9] and essential and non-essential oil constituents[3] have been published. In addition, several medicinally valuable antioxidant, anti-inflammatory, antibiotic, antiulcer and hypolipidemic agents have been isolated from *Eucalyptus* species[3].

As far as the essential oils are concerned, the composition of a large number of *Eucalyptus* essential oils have been studied.[3,10-17] In addition, the antimicrobial action of the essential oils of many *Eucalyptus* species has been investigated[13,15,16], and the correlation between the chemical composition of 21 species of *Eucalyptus* essential oils and their antimicrobial activity has been comprehensively analyzed[1]. In Egypt, about 100 *Eucalyptus* species have been introduced , of which only a small number (11 species) have been studied, regarding their essential oils composition[11,19-21].

In this paper the essential oil composition of three *Eucalyptus* species grown in Egypt, have been studied in a comparative way, and their antibacterial and antifungal effects have been investigated.

EXPERIMENTAL

Collection of Plant Material and Isolation of The Oils :

E. gomphocephala DC. leaves were collected from El-Kassasin Horticulture Research Station, Ismailia, in April 1996 and identification was confirmed by Prof. Dr. Ahmed Abd- El-Dayem, Director of Forestry Department, El-Kassasin Horticulture Research Station, Ministry of Agriculture. *E.robusta* Sm. and *E.resinifera* Sm. were collected from The National Egyptian Zoo, Giza, Egypt, in December 1995 and were identified in the Horticulture Research Institute, Agriculture Research Center, Cairo (by Prof. Dr. A. Okasha, the Director).

Fresh leaves (500g) were collected from different sides of individual trees and subjected to hydrodistillation and the percentage of the different oils were determined following the E.P. method.[22]

Identification of Oil Constituents :

Analytical GC was conducted on a Carlo Erba ICU 600 gas chromatograph equipped with FID detector and Spectraphysics integrator. Column : OV-1, 30m x 0.25mm, 1μm film (J & W, Scientific). Oven temperature : 45° (2 min isothermal) ; 45-100° at 10°/min ; 100 - 250° at 15°/min, then 15 min isothermal. Injector : split injection (1:30), 250°. Detector : 300°. Helium was used as a carrier gas (1.2 bar). For GC-MS analysis : GC conditions as mentioned above, and the capillary column was directly coupled with a mass spectrometer Carlo-Erba HRGC 4160 Finnigan MAT 4500 and spectra were recorded at 70 eV. Individual components of the oils were identified by their retention indices [23-27], by comparison of their mass spectra with those given in the literature[28-29], and sometimes by co-injection with authentic compounds. Kovats retention indices (RI)[30] were calculated using co-chromatographed standard n-alkanes (C_8 - C_{22}). Results are shown in Table 1.

Antibacterial and Antifungal Activities :

The disc agar diffusion method[26] was employed to evaluate the antimicrobial and antifungal activities of the essential oils of the three *Eucalyptus* species. Two Gram positive and one Gram negative bacteria beside three fungi were used in this study. They were isolated , identified and cultured on nutrient agar and the fungi were cultured on Sabouround dextrose agar. Paper discs (6 mm diameter) were impregnated with the individual oils (20 µl/disc). The oil-impregnated discs were applied gently to the surface of the inoculated plates. The plates were then, incubated at 35° (24 hr) for bacteria and at 25° (48 hr) for fungi. The observed zones of inhibition were measured and compared against standard antibiotic discs (Oxoid) as references. The employed bacteria , fungi and antibiotics as well as the results are shown in table 2.

RESULTS AND DISCUSSION

A survey of the literature shows that no previous study of the volatile components of *E. robusta, E. resinifera* and *E. gomphocephala* DC. grown in Egypt, has been reported. However, the chemical composition of only *E. gomphocephala* growing in Morocco has been documented.[13]

Hydrodistillation of the fresh leaves of *E. robusta, E. resinifera* and *E. gomphocephala* yielded 0.7 , 1.1, and 0.4% v/w, respectively, of pale yellow oils with characteristic aromatic odour. Analysis of the volatile oils by GC and GC-MS resulted in identification of 20, 14 and 22 compounds comprising 88.4, 89.5 and 89.5 % of the oils of *E. robusta* , *E. resinifera* and *E. gomphocephala* , respectively. The majority of the unidentified components occurred in small amounts. Table 1 shows the list of constituents identified in the essential oils of the three species. The components are arranged in order of elution from an OV-1 column. The general elution sequence is confirmed by literature Kovats retention indices.[23-27] Positive identifications are based on literature mass spectral data[28-29]and whenever available by co-injection with authentic reference compounds.

The obtained results revealed significant qualitative and quantitative variations among the components of the three oils. However, they are generally characterized by the presence of high percentages of sesquiterpene alcohols and relatively low percentages of cineole.

Among the monoterpenes hydrocarbons , α-pinene is the major component in *E. robusta* (16.9 %), *E. resinifera* (19.1%), while *p*-cymene (6.7 %) is the major one in *E. gomphocephala*. Oxygenated monoterpenes represent 24.9, 42.4 and 34.8 % of the oils of *E. robusta* , *E. resinifera* and *E. gomphocephala* respectively, of which *cis*- and *trans*-menth-2-en-1-ol were found in *E. robusta* only while borneol occurs in both *E. resinifera* and *E. gomphocephala*. Cineole which is a general constituent in *Eucalyptus* oils (previous reports[4,13] showed that it varies from 1.3 to 80% of *Eucalyptus* oils), is also found in the three studied oils in reasonable amounts (Table 1). Oxygenated aromatic compounds are represented by methyleugenol (13.0 %), *trans*-methylisoeugenol (4.7 %)and *cis*-methylisoeugenol (1.3 %) in *E. gomphocephala* ; and by only thymol in *E. robusta*. It should be noted that, while eugenol and methyleugenol were reported in several *Eucalyptus* oils[13] , *trans*- and *cis*-methylisoeugenol have not been previously reported in this genus.

Sesquiterpenes hydrocarbons constitute 7.0 , 3.8 and 7.9 % of the oils of *E. robusta, E. resinifera* and *E. gomphocephala*, respectively. Caryophyllene is the major one in both *E. robusta* (5.1%) and *E. resinifera* (3.8%), but could not be traced in *E. gomphocephala* , which contained germacrene D instead (2.8%). The three oils showed high percentages of sesquiterpene alcohols (27.3, 20.3 and 21.1% for *E. robusta* , *E. resinifera* and *E. gamphocephala*, respectively). Viridiflorol , α-, β- and γ-eudesmol occurred in the three oils. However, both *trans*-nerolidol (2.7%) and globulol (6.2 %) present only in *E. gomphocephala,* the later component was reported in several *Eucalyptus* species in higher proportions.[14]

Previous examination of the leaf oil of *E. gomphocephala* grown in Morocco[13], showed a slight qualitative similarity to the present results of the plant grown in Egypt. This can be represented by the presence of *P*-cymene, *trans*-pinocarveol , borneol, terpinen-4-ol, methyl eugenol and globulol in both plants. However, a significant qualitative as well as quantitative differences were observed in the chemical composition of the oils of the two plants. These include : the relatively higher cineole content (21.6 %), lower terpen-4-ol (2.7%) ; the absence of *cis*- and *trans*-methylisoeugenol, humulene, pinocarveol and germacrene D beside the presence of myrtenol , myrtenal , eugenol and eudesmenyl acetate in the plant grown in Morocco[13]. It should be noted, however, that variation in chemical composition within one *Eucalyptus* species has been documented[5] before. This could be attributed to the presence of different varieties, environmental conditions or undetected hyperidism.[5,31]

Results of the antimicrobial activity (Table 2) revealed that the oil of *E. robusta* has a relatively weak antibacterial effect and no antifungal activity against the tested microorganisms. *E. gomphocephala* oil showed a moderate antibacterial effect and a strong antifungal effect exceeding Nystatin against *candida albicans*. This may be attributed to the presence of a relatively high percentages of terpene alcohols and phenolic ether (19 %). On the other hand, the leaf oil of *E. resinifera* showed a significant inhibitory effect on

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Table 1: Composition of the Essential Oil of the Leaves of *E. robusta*, *E. resinifera* and *E. gomphocephala*.

No	Compounds	RI	M$^+$ and Major MS Ions m/z*	Concentration %		
				E. rob.	*E. res.*	*E. gom.*
1	α-Pinene[@]	929	**136**, 121, 93 , 91 , 77	16.9	19.1	tr.
2	β-Pinene[@]	963	**136**, 93 , 79, 77 , 69	1.4	2.3	tr.
3	Myrecene[@]	983	**136** , 93 , 91 , 79 , 69	1.2	tr.	-
4	*p*-Cymene[@]	1013	**134** , 119 ,117 , 91 , 71	9.4	1.6	6.7
5	Cineole[@]	1018	**154** ,139 , 108 , 93 , 81	10.6	16.8	4.6
6	Unid.	1074	**134** , 92 , 83 , 70 , 55	-	tr.	1.3
7	Unid.	1091	**152** , 137 , 109 , 83 , 67	-	1.2	-
8	Unid.	1104	**152** , 109 , 95 , 82 , 69	-	1.1	-
9	*cis*-p-Menth-2-en-1-ol	1113	**154** , 139 , 111 , 93 , 71	2.2	-	-
10	*trans*-pinocarveol	1124	**n.d.**,134 , 119 , 92 , 83 , 55	1.8	2.3	8.2
11	Pinocarveol (isomer)	1126	**n.d.**,134 , 109 , 92 , 83 , 55	-	-	2.4
12	*trans*-p-Menth-2-en-1-ol	1128	**154** , 139 , 111 , 93 , 71	0.9	-	-
13	Borneol	1149	**154** ,139 , 136 , 110 ,95	-	4.2	4.3
14	Terpinen-4-ol[@]	1161	**154** , 136 , 111 , 93 , 71	6.2	3.0	13.8
15	Unid.	1165	**150** , 135 , 115, 107 ,91	0.7	-	-
16	α-Terpineol[@]	1171	**154**,136 ,121 ,93 ,81,59	2.0	16.1	tr.
17	*trans*-Piperitol	1187	**154**, 139, 84 , 79 , 55	0.5	-	-
18	Unid.	1189	**n.d.**136 , 121,109 , 93 , 69	-	-	0.7
19	Neral	1191	**n.d.**135 , 95 , 81 , 69 , 53	-	-	1.5
20	*cis*-Methylisoeugenol[≠]	1291	**178** ,163 , 147 , 103, 91	-	-	1.3
21	Thymol	1298	**150**, 135 ,115 , 107 , 91	1.0	-	-
22	Methylisoeugenol	1367	**178** ,147 ,163 , 107 , 91	-	-	4.7
23	Methyleugenol	1370	**178**, 147, 107 , 103 , 91	-	-	13.0
24	Caryophyllene[@]	1402	**204** , 133 , 105 , 93, 79	5.1	3.8	-
25	Unid.	1423	**204** , 161 ,133, 119 ,105 , 91	1.5	2.9	-
26	α-Humulene	1439	**204** , 147 , 121 , 93 , 80	0.9	-	2.5
27	Aromadenderene	1454	**204** , 161 , 133, 105, 91	0.4	-	2.6
28	Unid.	1460	**200**, 173 ,160 ,145,105	0.5	-	-
29	Germacrene D	1469	**204**, 161, 119 , 107, 93	0.6	-	2.8
30	Nerolidol (*trans*)	1477	**n.d.**,189 , 161 , 107 , 93, 69	-	-	2.7
31	Globulol	1541	**n.d.** , 204, 189 , 161,69	-	-	6.2
32	Viridiflorol	1545	**n.d.** , 205, 149 ,119 , 91	2.6	3.5	tr
33	γ-Eudesmol	1586	**222**, 204 , 161 ,109 , 81	9.1	12.1	9.5
34	α-Eudesmol	1598	**222** , 204, 161 , 109, 69	8.6	2.5	tr.
35	β-Eudesmol	1613	**222** , 189, 164, 149 , 59	7.0	2.2	2.7
36	Unid.	1693	**222** , 204, 189, 164,149	-	0.9	tr.
37	Unid.	1715	**n.d.**,204 ,189 ,149 , 135,	2.3	-	-
38	Unid.	1898	**252** ,237, 221, 195, 152	-	-	2.3

* Molecular ion peaks are in **bold** face and base peaks are underlined ; **n.d.** , not detected.
@ Also confirmed by co-injection with authentic sample. tr, trace ; Unid., unidentified component ;
≠ tentatively identified on basis of MS only.

Table 2: Results Of The Antimicrobial Activity Of The Leaf Volatile Oils of *E.resinifera* , *E. robusta* and *E. gomphocephala*. (Diameter of Inhibition Zone , mm)

Microorganisms	Eucalyptus Oils*			Standard Antimicrobial agents				
	Rob.	***Res.***	***Gom.***	**G**	**T**	**A**	**M**	**N**
Bacillus subtilis	16	23	17	20	11	27	15	-
Escherichia coli	-	17	-	18	15	18	23	-
Staphylococcus aureus	9	24	11	18	23	14	22	-
Aspergillus niger	-	17	7	-	-	-	-	-
Aspergillus flavus	-	18	-	-	-	-	-	14
Candida albicans	-	18	13	-	-	-	-	13

G, Gentamycin (10 µg / disc); **T**, Tetracycline(30 µg / disc) ; **A**, Amikacin (30 µg / disc) ; **M**, Amoxycillin (25 µg / disc) ; **N**, Nystatin (100 µg / disc).
*All oils were used in a concentration of 20 µl /disc.

all tested bacteria, its antifungal activity was even more pronounced. It exhibited stronger effects than most of the employed standard antimicrobial agents. These results may be explained on basis of the high alcohol content (*E. resinifera* oil contains 42.4 % monoterpene alcohols and 20.3% sesquiterpene alcohols). Previous studies referred the antimicrobial activity of *Eucalyptus* oils mainly to their alcohol contents[13] and not to the generally major component cineol. [13,15] These results sugest that the leaf oils of the studied *Eucalyptus* species (especially *E. resinefera*) may be incorporated in useful antibacterial and antifungal preparations.

Since closely related *Eucalyptus* species exhibit great morphological similarities,[12] investigation of the volatile oils may help in the identification of the three studied species . However, study of the botanical characteristics as well as the non-volatile constituents of the three species will certainly provide a good tool for firm identification of these species. The later studies are in progress and results will be published elsewhere.

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دراسة مكونات الزيوت الطيارة لأوراق بعض نباتات يوكاليبتوس (الكافور) وفاعليتها المضادة للميكروبات

عزة محمد الشافعى

قسم العقاقير - كلية الصيدلة - جامعة الزقازيق - مصر

فى هذا البحث تم دراسة مقارنة للمكونات الكيميائية للزيوت الطيارة لأوراق ثلاث نباتات من جنس الكافور (المنزرعة فى مصر) وذلك باستخدام كروماتوجرافيا الغاز المتصلة بمقياس طيف الكتلة ونتيجة لهذه الدراسة تم التعرف على عدد ٢٠ مركب تكون ٨٨,٤٪ من مكونات زيت نبات يوكاليبتوس روبيستا وعدد ١٤ مركب تكون ٨٩,٥٪ من مكونات زيت نبات يوكاليبتوس رزينيفرا وعدد ٢٢ مركب تكون ٨٩,٥٪ من مكونات زيت نبات يوكاليبتوس جامفوسيفالا. وقد اثبتت الدراسة المقارنة أن هناك تباين كمى وكيفى واضحين فى مكونات الزيوت الثلاثة مما يساعد فى التعرف على الأنواع الثلاثة من نباتات الكافور والتمييز بينها.

كذلك أوضحت الدراسة أن لهذه الزيوت الطيارة فاعليات مضادة لبعض أنواع البكتريا والفطريات.

هذا البحث يعتبر أول دراسة لمكونات وتأثيرات زيوت النباتات الثلاث المنزرعة فى مصر.