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ANALYSIS OF THE ESSENTIAL OILS OF *SCHINUS TEREBINTHIFOLIUS* RADDI. CULTIVATED IN EGYPT

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ABSTRACT

The essential oils obtained from different parts of *Schinus terebinthifolius* Raddi. were analysed by GLC and GLC-MS. A total of 53 compounds were identified; 40 of which were reported for the first time in this plant. Qualitative and quantitative differences of different oils have been recorded. In the fruits and stem bark oils, α-pinene was the major compound constituting 27.5% and 52.4%, respectively. The major compound of both flower and leaf oils was α-cadinol and represented by 14.8% and 16.8%, respectively. In the wood oil, α-cadinene (27.5%) was the major constituent. All the prepared oils showed remarkable antimicrobial and antifungal effects.

INTRODUCTION

Several plants of the genus *Schinus* (Anacardiaceae) have been reported to have a powerful bactericidal, emmenagogue [1], contraceptive [2], and herbicidal, activities [3] as well as a substitute for black pepper [4]. Constituents that have been isolated from these plants include volatile oils, sterols, triterpenoids, flavonoids [5,6], glycosides [2] and tannins [5,7].*Schinus terebinthifolius* Raddi. which is a native to Central and South America [8] was introduced to Egypt and cultivated as ornamental plant for its beautiful, red ripe fruits which are of highest decorative value. The chemical composition of *S. terebinthifolius* Raddi. fruits essential oil from Florida [9,10] and Pakistan [11] has been previously studied. Previous study [9] on the neutral oily portion from hexane-ether extraction of crushed berries of the plant growing in Florida, resulted in the identification of nine monoterpene hydrocarbons viz. α-pinene, 3-carene, α-phellandrene, *p*-cymene, limonene, β-phellandrene, β-pinene, terpinolene and sabinene, in addition to *cis*-sabinol, carvotanacetone, β-caryophyllene, α-and β-cubebene. From the same location [10], the essential oil prepared by distillation from the fruits revealed that the oil was rich in α-pinene and α-phellandrene. In the plant growing in Pakistan, Shafiq *et al.*[11] found three major compounds, those identified being α-pinene, α-phellandrene and β-pinene in addition to seven minor ones; camphene, sabinene, 3-carene, *p*-cymene, γ-terpinene, terpinolene and β-caryophellene. The fruits oil of *S. terebinthifolius* Raddi. cultivated in Egypt has not been thoroughly studied with modern analytical methods. Since 1982, fourteen compounds only have been identified in the fruits essential oil of *S. terebinthifolius* Raddi. cultivated in Egypt [12] ; five majors viz. α-pinene, *p*-cymene, α-phellandrene, β-pinene and bornyl acetate and several minors viz. limonene, γ-terpinene, terpinolene, citral, comphor, borneol, geraniol, thymol and carvacrol. The disparity of the above results, as well as the lack of a more detailed analysis of *S. terebinthifolius* Raddi. volatile oil, have motivated our study. As regards the leaf, stem (bark and wood) and flower oils of *S. terebinthifolius* Raddi. nothing could be traced in the current literature concering their composition.

In the present study, the volatile oils prepared from different parts of *S. terebinthifolius* Raddi. were analysed. The qualitative and quantitative variations of their oils have been recorded. Moreover, the antimicrobial activities of these oils were determined.

EXPERIMENTAL

Plant material: Different organs of *S. terebinthifolius* Raddi. (Brazilian pepper-tree, Christmas-berry tree) viz. fruits, leaves, flowers, stem (bark and wood) were collected in October 1997 from plants cultivated in the vicinity of Zagazig. The plant was identified by Dr. A. Al-Nowaihi, the Professor of Plant Taxonomy, Faculty of Science, Ain Shams University, to whom the authors are indebted.

Essential oil isolation: The volatile oils were prepared from fresh plant organs by steam distillation using E. P. method [13] and the percentage yield (V/W) was found to be: fruits (3.8 %), leaves (0.35 %), flowers (0.25 %), stem bark (0.15 %), wood (0.08 %). The oils were dried with anhydrous sodium sulphate and kept at 4°C in sealed vials for analysis.

Essential oil analysis:

GLC: GLC was carried out on a Carlo Erba Miga 5160 gas chromatograph fitted with a fused silica capillary column DB-1 (30m x 0.25 mm i.d., 0.25 μm film thickness) under the following conditions: carrier gas He with flow rate 2 ml $min.^{-1}$; detector, FID, temp. 300 °C, inj. temp. 250 °C; split ratio 1:10; oven temp. program: initial temp. 50 °C for 4 min., 50 - 90 °C at 4 °C $min.^{-1}$, 90-300 °C at 10 °C $min.^{-1}$ then hold for 10 min. Analyses of 2μl of 0.2 % ethyl acetate solution of oils were performed. Kovats retention indices (RI) [14] were calculated using co-chromatographed standard *n*-alkane

mixture (C-9 to C-24). Quantitation of the components was performed on the basis of their GLC peak areas.

GLC-MS. Analyses were carried out on a Finningan MAT 4500 mass spectrometer equipped with a fused silica capillary column OV-1 (30m x 0.25 mm i.d, 0.25 µm film thickness). Conditions: carrier gas He (50 Kpa), split ratio 1:20, inj. temp. 250 °C, oven temp. program: initial temp. 45°C 4 min isothermal, 45-75 °C at 3 °C min^{-1}, 75-115 °C at 4 °C $min.^{-1}$, 115-312 °C at 6 °C $min.^{-1}$. EI-MS were recorded at 45 eV. Identification of the constituents was performed by comparing their retention indices and mass fragmentation patterns with the published data [15-22].

ANTIMICROBIAL ACTIVITY

The antimicrobial activities of the essential oils of different plant parts were carried out, adopting the disc agar diffusion method [23]. Each cup was filled accurately with 50 µl of 20 % oil in dimethylformamide (DMF) for each oil. The sensitivity of the oils was tested against two Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and two Gram-negative bacteria: *Escherichia coli*, *Neisseria meningitidis* and one fungus: *candida albicans* all of them are strains isolated and identified by the staff of Microbiology Department, Faculty of Pharmacy, Zagazig University. Chloramphenicol , penicillin, oxytetracyclin and gentamycin were used as standard antibacterial and nystatin as standard antifungal compounds. The plates were incubated over night at 37 °C in case of bacteria and 30 °C in case of fungi. The observed zones of inhibition were measured (in mm) and compared against the standard discs of antibiotics. Results are recorded in (Table 3).

RESULTS AND DISCUSSION

The qualitative and quantitative analytical results of the essential oils from five different plant parts are shown in (Tables 1 and 2). All the obtained oils exhibited a colourless to pale yellow colour except the stem wood has a dark yellow colour. The oils have a characteristic aromatic pepper-like odour and a pungent taste. Most of their components were identified in each sample. The unidentified parts of the oils were mainly constituted by a mixture of oxygenated monoterpenes and sesquiterpenes, whose individual percentages were only in small or trace amounts. Previous works on *S. terebinthifolius* Raddi. [9-12] enabled the identification of 23 constituents in the oil. In our study, we analyzed the minor constituents as well. We have identified 53 different compounds 40 of which were reported for the first time in the oil of *S. terebinthifolius* Raddi.

In the volatile oil of fruits, 46 compounds were found which represented about 92.3% of the total composition of the oil. As reported before [9,10] the monoterpene hydrocarbons were the main constituents (57.2%), especially α-pinene (27.5%), β-phellandrene (13.6%), β-pinene (4.5%), sabinene (3.9%), *p-cymene* (3.1%) and limonene (2.6%). In the sesquiterpene hydrocarbon content (14.3%), germacrene D is the major (11.7%). Spathulenol (10.3%) and khusinol (3.1%) constitutes the main oxygenated sesquiterpenes. The percentage of α-pinene was quite similar with that reported to be found in the fruit oil of plant from Florida [9] (26%), on the other hand the percentage was found to be lower than that reported to be found in the oil of plants from Pakistan [11] and Egypt [12] (43.2 and 34.25%, respectively). The percentage of β-pinene was higher than that reported to be found in the oil of plants from Florida [9] and Pakistan [11] (2.29 and 1%, respectively) and lower than that of previous report from Egypt [12] (7.29%). α-Phellandrene was detected as traces in fruit oil of our sample. However, it was reported to be found in a higher percentagein plants from Florida [9] , Pakistan [11] and Egypt [12] (16, 18.85, and 19.20%, respectively). Bornyl acetate was reported, as a major oxygenated compound (4.71%), citral, camphor, borneol, thymol and carvacrol (as minors) in previous report from Egypt [12]. These compounds were not detected neither in our sample nor in other reports from Florida [9] and Pakistan [11].

Twenty-eight components which represented about 97.9% of the total composition of the stem bark oil were identified. The oil was richer in early eluting compounds rather than later eluting ones. In particular, the oil is rich in α-pinene (52.4%), β-phellandrene (7.1%) and β-pinene (6.5%). Only, traces from oxygenated monterpenes were detected e. g. α-pinene oxide, terpin-4-ol and *trans* piperitol. Germacrene D (8.5%), β-cubebene (6.4%) and caryophyllene (3.3%) were the major compounds in the sesquiterpene hydrocarbons group.

In the flower oil, 45 compounds were identified representing about 92.5% of the total oil composition. The major compounds were found to be from the oxygenated sesquiterpenes; α-cadinol (14.2%), 1-*epi*-cubenol (9.3%) and spathulenol (3.7%). Other major components include *p-cymene*, α- and β-pinene, β-phellandrene and limonene (from monoterpene hydrocabons).

The leaf oil (37 compounds constituting 84.1%) shows a similar qualitative pattern to that of the flower oil in the oxygenated sesquiterpene contents. The oil of stem wood (39 compounds represeted about 94%) was characterized by high amount of sesquiterpene hydrocarbons (57%) and the oxygenated sesquiterpene (25.4%). A trace of palmitic acid was detected in the

Table 1: MS data of the volatile components identified in the oils of *Schinus terebinthifolius* Raddi.

Components♣	M⁺(abundance %)	B.P.	Major Peaks*
α - Thujene	136(11)	93	91, 77, 92, 79, 105, 41, 121.
α - Pinene	136(13)	93	91, 92, 77, 79, 41, 121, 105.
Camphene	136(7)	93	121, 79, 91, 41, 107, 67, 77.
Sabinene	136(17)	93	91, 77, 79, 41, 121, 107.
β - Pinene	136(10)	93	41, 69, 79, 77, 121, 91, 107.
Myrcene	136(3)	41	93, 69, 91, 67, 53, 107, 121.
α - Phellandrene	136(15)	93	91, 77, 92, 41, 65, 51.
p- Cymene	134(27)	119	91, 77, 65, 41, 103.
β - Phellandrene	136(21)	93	91, 77, 79, 41, 121, 107.
Limonene	136(23)	68	67, 93, 79, 41, 53, 121.
γ - Terpinene	136(29)	93	91, 77, 121, 79, 105, 43.
α -Pinene oxide	n.d.	67	43, 41, 109, 83, 55, 137.
Terpinolene	136(80)	121	93, 91, 41, 79, 67, 107.
Cis- Limonene oxide	n.d.	43	109, 41, 67, 119, 137, 79.
Trans- Limonene oxide	n.d.	43	67, 79, 41, 93, 109, 55.
Cis-β-Dihydro terpineol	n.d.	71	43, 55, 67, 81.
Trans-β-Menth-2-en-1-ol	154(6)	43	93, 139, 111, 71, 79, 121, 69.
Trans-Pinocarveol	n.d.	92	41, 55, 70, 83, 91, 119, 109, 134.
Trans-Pinene hydrate	154(3)	43	139, 41, 69, 93, 111, 121.
Cis-Verbenol	n.d.	109	41, 43, 55, 91, 81, 67, 94, 119.
Terpin-4-ol	154(15)	71	43, 111, 93, 41, 55, 136.
meta-Cymen-8-ol	150(6)	43	135, 91, 65, 109.
α- Terpineol	n.d.	59	93, 43, 121, 136, 81, 67.
Myrtenal	150(1)	79	91, 41, 67, 55, 108, 121, 135.
Cis-Piperitol	154(10)	84	139, 41, 43, 55, 139, 83, 71, 79, 111.
Trans-Piperitol	154(8)	84	83,139, 55, 43, 71, 111, 93, 97, 43.
Cumin aldehyde	148(70)	133	105, 77, 91, 119, 51.
Trans Sabinene hydrate acetate	n.d.	43	93, 81, 55, 69, 107.
Pipertione	152(15)	82	110, 95, 137, 41, 55.
Geraniol	n.d.	69	55, 139, 123, 43, 81.
Dihydro linalol acetate	n.d.	109	43, 81, 55, 95.
α-Terpinen-7-al	n.d.	79	107, 91, 43, 121.

Table 1 : (Continued) .

γ-Terpinen-7-al	150(40)	79	107, 121, 91, 77, 135, 43.
para-Cymen-7-ol	150(53)	135	105, 107, 79, 91, 119.
α-Terpinyl acetate	n.d.	43	121, 93, 136, 79, 59, 107.
Citronellyl acetate	n.d.	81	43, 82, 95, 123, 67, 69, 55, 109.
α-Copaene	204(27)	161	105, 119, 91, 81, 55, 69.
β-Cubebene	204(12)	105	119, 161, 93, 91, 81, 55, 133.
β-Caryophyllene	204(7)	41	93, 69, 79, 91, 133, 119,105, 161, 55, 189.
β-Gurjunene	204(13)	161	105, 91, 147, 133, 55, 79, 147, 189.
α- Humulene	204(8)	93	80, 121, 79, 67, 147, 107, 55, 43, 53.
γ- Muurolene	204(22)	161	91, 105, 79, 119, 133, 55, 67, 147, 189, 43.
Germacrene	204(20)	161	105, 91, 81, 41, 79, 55, 119, 133, 67, 147.
α- Alkaskene	204(20)	121	93, 79, 107, 91, 105, 67, 55, 161, 136, 189.
α- Cadinene	204(26)	105	161, 93, 79, 91, 55, 119, 133, 147, 43, 189.
delta- Cadinene	204(53)	161	134, 119, 105, 91, 81, 189, 55, 145.
α- Agarofuran	220(10)	123	131, 109, 124, 105, 91, 43, 146, 81, 93, 55, 163, 67.
Germacrene B	204(26)	121	93, 105, 107, 67, 79, 161, 189, 147, 133, 81, 55, 43.
Spathulenol	220(11)	43	205, 91, 119, 79, 105, 159, 147, 69, 55, 187, 177.
1-epi-Cubenol	n.d.	119	161, 105, 43, 55, 69, 95, 82, 109, 179.
α- Cadinol	222(5)	43	95, 121, 204, 161, 81, 79, 109, 137, 164.
Khusinol	220(52)	159	177, 91, 105, 81, 67, 131, 55, 43, 117, 202.
Palmitic acid	256(28)	43	73, 60, 57, 55, 41, 70, 83, 97, 129, 115, 213, 185, 157, 171.

M^+, molecular ion peak; B. P., base peak; n.d., not detected.
* Mass fragments are arranged in order of decreasing values of relative intensity %.
♣ Identity are based on MS and / or retention index. Compounds are listed in order of elution from OV-1 column under condition listed in the experimental section.

Table 2: Composition of essential oils from fresh fruits, flowers, leaves and stem (bark and wood) of *Schinus terebinthifolius* Raddi.

Components*	(RI)♣	Percentage				
		Fruits	Flowers	Leaves	Bark	Wood
Monoterpene hydrocarbons						
α - Thujene*	(919)	1.1	-	-	0.6	-
α - Pinene	(924)	27.5	9.6	2.6	52.4	4.1
Camphene	(934)	tr	-	-	4.0	-
Sabinene	(960)	3.9	tr	-	tr	-

β - Pinene	(961)	4.5	5.6	0.7	6.5	tr
Myrcene*	(982)	0.7	0.6	0.9	0.8	2.3
α - Phellandrene	(991)	tr	0.4	0.4	1.3	tr
p- Cymene	(1008)	3.1	6.2	8.9	0.3	tr
β - Phellandrene	(1014)	13.8	5.5	1.4	7.1	tr
Limonene	(1017)	2.6	3.7	5.2	1.7	tr
γ - Terpinene	(1048)	-	-	-	tr	-
Terpinolene	(1077)	-	-	-	tr	-
Oxygenated monoterpenes						
α -Pinene oxide*	(1076)	tr	0.3	-	tr	tr
cis- Limonene oxide*	(1085)	tr	0.4	-	-	tr
trans- Limonene oxide*	(1092)	tr	tr	-	-	tr
cis-β-Dihydroterpineol*	(1092)	tr	tr	-	-	tr
trans-β-Menth-2-en-1-ol*	(1102)	0.5	tr	-	-	tr
trans -Pinocarveol*	(1116)	tr	tr	-	-	tr
trans-Pinene hydrate*	(1120)	0.5	tr	-	-	tr
cis-Verbenol*	(1125)	tr	tr	-	-	tr
Terpin-4-ol*	(1156)	2.8	5.5	0.5	tr	2.0
meta-Cymen-8-ol*	(1159)	tr	tr	-	-	tr
α- Terpineol*	(1168)	0.3	4.2	0.6	-	tr
Myrtenal*	(1176)	tr	tr	0.3	-	tr
cis-Piperitol*	(1176)	tr	2.5	0.3	-	3.5
trans-Piperitol*	(1188)	tr	0.3	tr	tr	0.8
Cumin aldehyde*	(1206)	0.7	tr	0.1	-	-
trans Sabinene hydrate acetate*	(1215)	2.6	tr	0.3	-	-
Pipertione*	(1224)	tr	tr	0.2	-	tr
Geraniol	(1232)	tr	tr	0.3	-	-
Dihydro-linalol acetate*	(1245)	tr	tr	0.4	-	-
α-Terpinen-7-al*	(1253)	tr	0.5	tr	-	-
γ-Terpinen-7-al*	(1258)	tr	0.6	0.5	-	-
para-Cymen-7-ol*	(1271)	tr	tr	0.6	-	-
α-Terpinyl acetate*	(1339)	tr	-	-	-	-
Citronellyl acetate*	(1345)	tr	-	-	-	-

Table 2 : (Continued)

Sesquiterpene hydrocrbons						
α-Copaene*	(1347)	-	-	tr	tr	tr
β-Cubebene	(1373)	tr	0.5	4.1	6.4	5.9
β-Caryophyllene	(1410)	tr	4.6	5.6	3.3	6.4
β-Gurjunene*	(1419)	-	1.2	3.8	0.5	4.7
α- Humulene*	(1445)	tr	0.6	1.7	0.5	5.5
γ- Muurolene*	(1453)	tr	tr	tr	0.3	2.5
Germacrene D*	(1472)	11.7	2.8	1.0	8.5	8.5
α- Alkaskene*	(1486)	tr	1.8	2.1	0.5	tr
α- Cadinene*	(1492)	tr	1.6	1.3	0.5	23.5
delta- Cadinene*	(1513)	0.9	1.7	2.4	0.6	tr
Germacrene B*	(1549)	1.7	1.5	2.2	-	tr
Oxygenated sesquiterpene						
α- Agarofuran*	(1540)	tr	1.7	2.3	-	tr
Spathulenol*	(1561)	10.3	3.7	3.6	-	tr
1-epi-Cubenol*	(1616)	-	9.3	10.7	0.6	11.7
α- Cadinol*	(1641)	-	14.2	16.8	1.5	12.4
Khusinol*	(1664)	3.1	1.1	1.0	-	1.3
Others						
Palmitic acid*	(1955)	-	-	tr	tr	tr
Total identified		92.3 %	92.5 %	84.1 %	97.9%	94 %

tr = traces (con. < 0.1%) - = absent.
RI♣ = retention index; RI data were measured relative to *n*-alkanes (C-9 to C-24) from OV-1 column..
* = New for *Schinus terebinthifolius* Raddi (not previously reported in the essential oil in plants from Florida, Pakistan and Egypt).

stem, (bark, wood), and leaf oils. Palmitic acid has been recently identified in the seed essential oil of *Hibiscus abelmoschus* [24].

Finally, it was evident that there is a considrable qualitative and quantitative differences between the five essential oils of different plant parts.

The essential oil composition of *S. terebinthifolius* was found to be different from data published before [9-12]. Although, we identified much more compounds (Table 1), some of the previously identified compounds were not detected in our sample as: Δ^3-carene, *cis*-sabinol, carvotanacetone, α- cubebene [9, 10], Δ^3-carene [11], citral, camphor, borneol, bornyl acetate, thymol and carvacrol [12]. The differences could be due to seasonal variation, ecological or environmental factors.

Concerning the antimicrobial activity (Table 3), all the tested oils exhibited significant levels of activity aganist the tested strains for both bacteria and fungi. The fruit oil is the most effective oil aganist all the tested organisms. This is in agreement with the previously published data [25, 26].

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Table (3): Results of antimicrobial screening of volatile oils from different plant parts of *Schinus terebinthifolius* Raddi (50 µl were applied in each assay).

Material	Diameter of inhibition zone in mm.				
	Gram neg. bacteria		Gram. pos. bacteria		Fungi
	E. coli	*N. meningitidis*	*S. aureus*	*B. subtilis*	*C. albicans*
Volatile oil of fruits*	20	30	25	20	23
Volatile oil of leaves*	15	25	17	18	16
Volatile oil of bark*	15	27	15	17	17
Volatile oil of flowers*	12	21	10	21	20
Volatile oil of wood*	15	22	20	14	18
Chloramphenicol 30 µg disc.	20	13	-	21	-
Penicillin 10 µg disc.	-	-	22	-	-
Oxytetracycline 30 µg disc.	15	20	-	21	-
Gentamycin 10 µg disc.	15	20	18	-	-
Nystatin 100 µg disc	-	-	-	-	17

– = No zone of inhibition ; Concentration : 20% oil in DMF.

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تحليل الزيت الطيار باستخدام كروماتوجرافيا الغاز الشعريه وكروماتوجرافيا الغاز الشعريه المتصله بمطياف الكتله لنبات شينس تربنثيفوليس مرادى المنزرع فى مصر

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قام الباحثون بفصل الزيت الطيار من الأجزاء المختلفه للنبات وتحديد مكوناته لكل جزء باستخدام كروماتوجرافيا الغاز الشعريه و كروماتوجرافيا الغاز الشعريه المتصله بمطياف الكتله وقد أمكن التعرف على ثلاثة وخمسون مركب من مكونات الزيوت ووجد إختلافا كميا وكيفيا بين محتويات كل جزء. وتبين أن ألفا باينين هو المركب الرئيسى في كل من الثمار وقلف الساق. أما المركب الرئيسى فى كل من الأزهار والأوراق فقد وجد أنه ألفا كادينول أما فى الخشب فظهر أن ألفا كادينين هو المركب الرئيسى. ونتيجة لهذه الدراسة أمكن التعرف على ٩٢,٣% من محتويات زيت الثمار، ٩٢،٥% من محتويات زيت الأزهار ، ٨٤,١% من محتويات زيت الأوراق ، ٩٧,٩% من محتويات زيت قلف الساق ، ٩٤% من محتويات زيت خشب الساق.

كما تم أختبار فاعلية الزيوت ضد بعض البكتريا والفطريات فوجد أن لهم تاثيرا قويا على كل منها.