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INFLUENCE OF EXTRACTION METHODS ON THE YIELD AND CHEMICAL COMPOSITION OF ESSENTIAL OIL OF *PLATYCLADUS ORIENTALIS* (L.) FRANCO.

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Abstract

Platycladus orintalis (L.) Franco belongs to Cupressaceae family. This species is an important herb in chinese medicine as a hemostatic, expectorant, and cough remedy. Mono and sesquiterpenoids in essential oils of different parts of the plant were previously reported as chemical constituents of this plant. The aim of this work was to compare the influence of steam distillation (SD) and steam distillation solvent extraction (SDE) methods on the essential oils composition of different parts of the plant. The essential oils from different parts (leaves, branches, fruits) of *P. orientalis* were obtained by SD and SDE methods separately. The essential oils were analyzed for identification of the components by GC and GC-MS instruments. The main components of all essential oils were determined as α-pinene, δ-3-carene, sabinene and cedrol. Significant differences between two methods were recorded in the oxygenated constituents percentages. The SDE method showed a higher recovery of these compounds. The recovery of oxygenated compounds is higher in SDE method than SD method. The results indicated that SDE is more selective than the conventional steam distillation method in the extraction of oxygenated monoterpenes in essential oil.

Keywords:

Platycladus orientalis, Essential oils, Steam distillation, Steam distillation solvent extraction.

Introduction

Platycladus orientalis (L.) Franco. [*Thuja orientalis* L., *Biota orientalis* (L.) Endl., Oriental Arborvitae] is a monoecious and evergreen tree which belongs to Cupressaceae family. *Orientalis* is the only species of genus *Platycladus*. This conifer shrub or tree is 5-12m high, scale leaves minute, closely imbricate, of two types in opposite and decussate paris; composed of an inner, median facial pair and an outer, lateral pair, adnate for much of their length with small, free, obtuse tips, male cones terminal.

Female cones ovoid-pyriform, with 8-0, thick, valvate scales, the apical pair of scales sterile, only the central 4 usually fully fertile, seeds under each scale, ovate, not winged, 3mm thick (1). The plant is indigenous of Korea, Manchuria, north of China and Iran. Its persian names are "Nush" , "Sarv-e-Khomrehi" and sarv-e-tabari(1-3). The leaves and seeds of *P*.

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orientalis are used as a traditional remedies in some eastern asian countries but not in Iran. Dried leaves of *P. orientalis* have been used as a hemostatic, expectorant and hypotensor in Korean folk medicine (4). This species is an important herb in chinese medicine as a hemostatic, expectorant, and cough remedy (5). Fresh leaves of the plant are used as an anti inflammatory drug (6). Seeds are used for bronchitis, insomnia and as antitussive (7). However, to date, some constituents of the plant such as terpenoids and flavonoids were shown to have pharmacological or biological activities (8).
Many chemical components from different parts of *P. orientalis* (L.) Franco have been extensively investigated. Sesquiterpenoids and diterpenoids from the heartwood (9-13), mono and sesquiterpenoids in essential oils of different parts of the plant (14, 15), flavonoids from leaves (14, 15), four bisnor and trinorlabdantype diterpenoids from seeds (16), two monolignol derivatives from pollens (17), some labdane and isopimarane diterpenoids from pericarpes and leaves (4, 5), and some long chain aliphatic compounds (6, 18) were previously reported as chemical constituents of this plant. Among them are a number of compounds which have been reported as new or novel.
There is no routine set for extraction of the essential oils from plant material; the procedures vary with the nature of the source materials (1eaf, petal, bark, root, etc.) and the type of target compound. Apart from "cold-pressing" and direct sampling from secretory structures, most of the techniques employed in the extraction of the essential oils from plants have a significant effect on the final composition of the product obtained (19).
The most commonly used techniques for extraction of essential oils are steam distillation, hydro-distillation and solvent extraction (20). Steam distillation can be carried out directly on the shredded tissue either on the usual steam distillation or by means of a steam distillation-solvent extraction (simultaneous distillation-extraction or SDE) apparatus in which the continuous generated steam distillate is continuously extracted by a suitable solvent (21).
The aim of this work was to compare the influence of steam distillation and steam distillation solvent extraction methods on the essential oils composition of different parts of this plant.

Materials and methods

Plant material

The different parts of *Platycladus orientalis* (L.) Franco consist of fruits, leaves and branchlets (leaves), branches (without leaves) were collected (Oct. 2000) from wild trees in the Soorkesh valley (950m height) near the Aliabad katol, Golestan province, Iran. The plant was identified by Department of Botany, Research institute of Forests and Rangelands, Tehran, Iran (TARI). Voucher specimens were deposited in herbarium of TARI (accession number 72894) and herbarium of Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan, Iran. The plant materials were stored at –20 ºC (22).

Steam distillation

A total 200g of each crushed plant materials were subjected to steam distillation (3 h) in a distillation apparatus (Ashkeh Shishe Co., Tehran, Iran). Therefore, steam is passed through a vessel containing the plant material-water mixture to yield a condensate (20). Then, slightly yellow oils, lighter than water, were separated, dried over anhydrous $Na_2 SO_4$ (Merck, Germany) and stored in sealed container under N_2 and refrigerated (-20ºC).

Steam distillation solvent extraction

A total 200g of each crushed plant materials were subjected to SDE (3h). The SDE apparatus (Ashke Shisheh Co., Tehran, Iran) used in this work was a newly designed modification of the apparatus of Linkens and Nickerson (23) and Godefroot and co-

workers (24, 25). It was constructed for the use of organic solvents with density lower than water (26). Pentane (Merck, Germany) was used as the extraction solvent.
The extraction head chamber was constructed in such away as to provide more complete mixing of solvent and steam vapors, and the condensing surface area was increased to about 500 cm, which allows the use of tap water (10-12ºC) as coolant. After finishing the procedures, the pentane extracts were concentrated with nitrogen. These concentrated solutions were then ready for GC and GC-MS analysis without any further purification (26).

Gas Chromatography (GC) analyses
The GC analyses were performed using a Perkin-Elmer (UK) 8500 Gas chromatograph equipped with Flame Ionization detector (FID). Separations were achieved on a BP-1 fused silica column (25 m×0.33 mm i.d., film thickness 1.0 µm, SGB Co., Australia); temperature programming (mostly), 60-275ºC at 4ºC/min; injector temperature (split: 1/25) 250ºC; detector temperature, 280 ºC; carrier gas, N_2 at 12 psi.

Gas Chromatography-Mass Spectrometry (GC-MS) analyses
The GC-MS analyses were carried out using following apparatus: Hewlett-Packard 6890 Gas chromatograph apparatus (USA) fitted with a HP-5ms fused silica column (30 m×0.25 mm i.d., 0.25 µm film thickness, Agilent HP, USA) interfaced with a Hewlett-Pakared 6890 mass selective detector and a computer equipped with Wiley 275 mass spectra library. Column temperature, 60-275ºC at 4ºC/min; injector temperature, 250ºC; volume injection, 0.1 µl; split ratio, 1:50; carrier gas, Helium at 2 ml/min; mass spectra electronic impact, ionization potential, 70 eV; ion source temperature, 250ºC; mass range, 30-300 mui.

Identification of essential oils components
The relative percentage composition of individual compounds was computed from the GC peak areas obtained without using correction factors. A series of hydrocarbon standards were used to calculate Kovats indices (KI). Kovats indices were calculated by the Kovats equation. The oil components were identified from their GC retention indices obtained with reference to n-alkane series (Sigma, UK) on HP-5ms column, comparison of their mass spectra and fragmentation patterns reported in literature (27,28), comparing with previously published data on essential oils components of this plant (29-33) and by computer matching with Wiley 275, NIST 21&107 libraries, as well as, by peak enrichment on co-injection with authentic standards whenever possible. Co-chromatography using authentic samples was carried out in identification procedure of 11 compounds. The yield percent and composition of the essential oils were expressed in mL/100g fresh plant materials.
The relationships between the different methods of obtaining essential oils were studied by statistical analysis (*t* student).

Results

The essential oils isolated from fruits, leaves and branches of *Platycladus orientalis* with SD and SDE methods were slightly yellow in color and moderately woody in odor.
The yield percentage of the different essential oils obtained with SD and SDE methods are presented in Table 1. The yield results showed that there is no statistically significant difference between two methods ($P \leq 0.05$). The percentage of composition of the essential oils and their grouped components are given in Table 2. The components are listed in order of their retention indices on HP-5ms column.

Table 1:The yield percentage of the essential oils of fruits, leaves and branches of Platycladus orientalis obtained with SD and SDE methods

Method of Extraction	% Yield (v/w), n=3		
	Fruits	Leaves	Branches
SD	1.23 ± 0.07	0.80 ± 0.05	0.51 ± 0.06
SDE	1.21 ± 0.11	0.78 ± 0.07	0.52 ± 0.04

Table 2: The Percentage of composition of the essential oils of fruits, leaves and branches of *Platycladus orientalis* obtained by SD and SDE methods

No	Compounds	KI	Fruits		Leaves		Branches	
			SD	SDE	SD	SDE	SD	SDE
1	α-Thujene	932	1.08	0.90	1.36	1.01	3.28	2.50
2	α-Pinene	943	31.35	31.50	22.69	22.65	12.80	12.70
3	α-Fenchen	954	2.81	2.90	2.05	2.32	1.48	1.38
4	Thuja-2,4(10)-diene	961	0.20	0.17	-	-	-	-
5	Sabinene	980	5.36	6.50	6.32	4.72	12.27	11.37
6	β-Pinene	983	1.71	2.47	1.53	1.39	0.61	0.58
7	Myrcene	995	1.94	1.04	3.70	2.14	1.77	1.33
8	Δ-3-Carene	1012	23.83	24.51	24.40	23.99	18.70	17.85
9	*ortho*- Cymene	1024	0.29	0.31	0.16	0.17	-	-
10	*para*- Cymene	1028	1.11	1.20	1.43	1.56	1.48	2.18
11	D-Limonene	1033	1.34	1.40	-	-	2.06	2.00
12	β-Phellandrene	1036	1.26	1.32	5.90	5.65	0.56	0.20
13	trans- Ocymene	1054	0.11	-	-	-	-	-
14	γ-Terpinene	1065	0.13	0.12	0.08	0.07	0.25	0.22
15	Cis-Sabinene hydrate	1072	0.10	0.90	0.07	0.10	-	0.17
16	Unknown	1083	0.23	-	0.15	0.17	0.18	-
17	Terpinolene	1093	1.55	1.53	1.86	1.75	1.78	1.57
18	trans-Sabinene hydrate	1100	0.35	0.20	-	-	0.18	-
19	Linalool	1103	-	-	0.05	1.08	0.15	0.25
20	an Oxygenated monoterpene	1110	-	-	-	0.17	0.20	0.27
21	α-Campholenal	1129	0.62	0.53	-	-	-	-
22	cis-Limonene oxide	1137	0.46	0.50	-	0.11	-	-
23	cis-Verbenol	1144	0.77	0.81	0.06	0.22	0.21	0.27
24	trans-Verbenol	1149	0.65	0.83	-	0.10	-	-
25	an Oxygenated monoterpene	1163	0.19	0.26	0.06	0.13	-	-
26	Terpinen-4-ol	1181	0.47	0.69	0.23	0.90	1.24	2.13
27	ρ-Cymene-8-ol	1185	0.24	0.37	0.25	0.71	0.44	0.53

28	Myrtenal	1196	0.15	0.16	-	0.11	-	-
29	Verbenone	1207	0.19	0.18	-	-	-	-
30	an Oxygenated monoterpene	1213	0.10	0.11	-	-	-	-
31	an Oxygenated monoterpene	1253	0.17	0.19	0.23	0.28	0.20	-
32	Bornyl acetate	1289	0.62	0.73	0.39	0.43	0.25	0.31
33	Carvacrol	1302	-	-	0.28	0.33	0.22	0.23
34	Terpinen-4-ol acetate	1343	0.75	0.81	0.71	0.79	0.69	0.79
35	α-Terpinyl acetate	1354	0.62	0.75	1.33	1.42	1.39	1.72
36	α-Funebrene	1401	-	-	0.10	0.11	0.11	0.18
37	Longifolene	1406	-	-	0.27	0.22	0.23	0.33
38	α-Cedrene	1409	-	-	-	-	0.12	0.20
39	β-Funebrene	1415	2.07	2.40	1.28	1.32	2.82	2.46
40	β-Caryophyllene	1421	1.54	1.32	4.81	3.92	2.10	1.50
41	β-Cedrene	1423	1.36	0.75	0.91	0.85	1.83	2.21
42	Thujopsene	1432	3.56	2.83	1.83	3.33	10.14	10.40
43	(Z)- β-Farnesene	1445	0.27	-	0.20	0.23	0.31	0.86
44	α-Humullene	1456	1.04	1.90	3.45	2.86	2.34	1.79
45	α-Acoradiene	1465	0.14	0.06	0.32	0.29	0.2	-
46	β-Acoradiene	1469	0.14	-	-	-	0.16	0.63
47	γ-Muurolene	1479	-	-	0.30	0.34	0.21	0.50
48	α-Curcumene	1483	0.44	0.31	1.25	1.19	0.41	0.43
49	Germacrene-D	1485	0.20	0.16	0.53	0.70	0.32	0.62
50	a Hydrocarbone sesquiterpene	1493	-	-	-	-	0.14	-
51	α-Chamigrene	1501	0.14	-	0.18	0.22	0.27	0.36
52	β-Himachalene	1503	0.27	0.19	0.23	0.19	0.60	0.86
53	Cuparene	1506	0.70	0.59	0.73	0.62	1.20	1.42
54	γ-Cadinene	1513	-	-	0.41	0.32	-	-
55	β-Sesquiphellandrene	1526	-	-	-	-	0.15	0.31
56	A Hydrocarbon sesquiterpene	1542	-	-	-	-	-	0.18
57	Caryophyllene oxide	1584	0.45	0.44	0.70	0.88	0.67	0.66
58	an Oxygenated sesquiterpene	1587	-	-	-	-	-	0.40
59	an Oxygenated sesquiterpene	1590	0.51	0.39	0.37	0.47	0.96	1.02
60	Cedrol	1600	5.39	4.38	6.12	6.41	10.85	10.24
61	Cubenol	1631	-	-	-	-	0.20	0.22
62	an Oxygenated sesquiterpene	1638	-	-	-	-	-	0.16
63	an Oxygenated sesquiterpene	1651	-	-	-	0.16	-	0.19
64	an Oxygenated sesquiterpene	1715	0.14	-	-	-	0.61	0.70
	Total (%)		98.88 ±0.43	99.64 ±0.24	99.13 ±0.35	99.13 ±0.31	99.34 ±0.44	99.44 ±0.36

As it can be seen from Table 2, 64 components were detected in all essential oils, of which the 52 most abundant compounds (representing more than 96% essential oils composition) were unambiguously identified. Among these, 37 compounds have been reported in previously published papers (29-33).

The pattern of main grouped components in essential oils was as follows: monoterpene hydrocarbons>> sesquiterpene hydrocarbons> oxygenated sesquiterpenes ≥ oxygenated monoterpenes (with the exception of oxygenated monoterpenes in essential oils of fruits that was obtained with SDE).

Discussion

In the essential oils of fruits obtained with SD and SDE, main components were α-pinene (31.35%, 31.50%), Δ-3-carene (23.83%, 24.51%), sabinene (5.36%, 6.50%) and cedrol (5.39%, 4.38%), respectively. In the essential oils of leaves Δ-3-carene (24.40%, 23.99%), α-pinene (22.69%, 22.65%), sabinene (6.32%, 4.72%), β-phellandrene (5.90%, 5.65%) and cedrol (6.12%, 6.41%) were the major components obtained with SD and SDE, respectively.

The composition of the essential oils showed some similarities with the previous studies. An earlier report from China indicated the presence of 20 mono- and sesquiterpenoides in hydro-distillated essential oils of fruits and leaves with α-pinene (40%), Δ-3-carene and cedrol as major compounds (33). Garg and co-workers reported α-pinene (67.8%) and β-phellandrene (12.3%) as major compounds in hydrodistilled fruit essential oil of Himalayan *P. orientalis* (30).

In Iran, several reports have been published on essential oils obtained with hydro-distillation and steam-distillation of fruits and leaves of the plant, which represented monoterpene hydrocarbons as the most abundant constituents (8,29,31). Hassanzadeh and his colleagues have indicated the presence of 18 components in the essential oils of fruits and leaves with α-pinene, sabinene, Δ-3-carene, limonene and cedrol as major ones (8). These results are in agreement with the results of Nickavar and co-workers with the exception of β-phellandrene, which was reported instead of sabinene (29). Afsharipour and Emami reported that essential oils of fruits and leaves obtained with steam distillation showed Δ-3-carene (19.90%, 15.10%), α-pinene (14.34%, 9.29%) and β-phellandrene (5.68%, 6.35%) as common major compounds, respectively (31).

The main components of branches essential oils obtained with SD and SDE were Δ-3-carene (18.70%, 17.85%), α-pinene (12.80%, 12.70%), sabinene (12.27%, 11.37%), thujopsen (10.14%, 10.40%) and cedrol (10.85%, 10.24%), respectively. It was reported more than 20 mono- and sesquiterpenoides were identified in the essential oils of branches (33). Among them, thujopsene (45.05%) and cedrol (21.32%) were reported as two major compounds.

In order to compare the influence of SD and SDE methods on essential oil composition, 64 volatile components detected, relative standard deviation (RSD, or C.V.%) were calculated for all of constituents and for grouped contents to evaluate the repeatability or the precision of the two methods. The compounds which their mean peak areas (mean percentages) were higher than 0.20%, their RSDs were determined lower than 10% in both methods. The amounts of these compounds were more than 97% of total amount of identified compounds in both methods. Besides, the RSD for all grouped contents were determined from 0.26% to 3.36%. In conclusion, the precision (repeatability) of SD and SDE methods were acceptable in the analysis of essential oils of *P. orientalis*.

According to the results shown in Table 1, the major constituents of the essential oils obtained by two methods from each part of plant were qualitatively and partially quantitatively identical. The effects of different distillation methods on oil content

and composition of aromatic plants have also been previously reported (34).

The differences between the two methods are more clearly revealed in comparison of grouped contents of essential oils obtained by SD and SDE methods (Table 3). Although with the exception of sesquiterpene hydrocarbons of leaves and oxygenated sesquiterpenes of branches, differences ($P \leq 0.05$) were observed among the amounts of other grouped contents, but higher significant differences were recorded in oxygenated monoterpenes grouped contents. As indicated in Table 3 the simultaneous distillation extraction method offered higher percentages amounts of oxygenated monoterpenes than the steam distillation method. The oxygenated monoterpenes yield percentage for fruits (6.45%, 8.02%), leaves (3.67%, 6.87%) and branches (5.17%, 6.67%) were obtained by SD and SDE methods, respectively. In the steam distillation method, volatile components were significantly diluted by water when being collected and among the various grouped contents of essential oils, the oxygenated monoterpenes are more water-soluble rather than the other compounds and probably were partially re-dissolved in the water existing in the separation part of the distillation apparatus returning back to the flask or producing the floral water, therefore reducing the recovery of these compounds in isolated essential oils. This problem was overcome using SDE method by solvent extraction of the distillate. Therefore, more oxygenated compounds were recovered from distillate during the organic solvent extraction with in SDE method. In fact, SDE method is considered as a separation and also sample enrichment technique. The separation and enrichment principles of this method are based on simultaneous steam distillation and solvent extraction, which are controlled by liquid-liquid extraction equilibrium. These findings are almost in agreement with previously published reports (35-37).

In conclusion, the essential oil analyses showed that there is no statistically significant difference between SD and SDE methods in the total yield percentage of essential oils. The simultaneous distillation extraction method was offered higher percentages amount of oxygenated monoterpenes than the steam distillation method.

Table 3: The yield percentage variations of essential oils compounds obtained from different parts of *P. orientalis* by SD and SDE methods

Grouped components	Fruites		Leaves		Branches	
	SDE	SD	SDE	SD	SDE	SD
Monoterpene hydrocarbones	75.87±0.35	74.07±0.95	67.59±0.39	71.48±0.19	53.88±0.29	57.04±0.30
Oxygenated monoterpens	8.02±0.25	6.45±0.11	6.88±0.14	3.66±0.12	6.67±0.15	5.17±0.12
Sesquiterpene hydrocarbones	10.51±0.34	11.87±0.21	16.71±0.42	16.8±0.17	25.24±0.19	23.66±0.34
Oxygenated Sesquiterpenes	5.21±0.14	6.49±0.14	7.92±0.24	7.19±0.15	13.59±0.27	13.29±0.15

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