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Essential oils Isolated From Leaves of Egyptian *Verbena triphylla* L Herb Using Different Extraction Methods

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Abstract: As a part of an intensive screening program to introduce new source of essential oils (EO) to Egyptian industry, we aimed to evaluate the essential oils content of *Verbena triphylla* L leaves under the conditions of Egypt, Using two different extraction methods; hydro-distillation (HD) and solvent extraction (SE) to conclude whether of them is more efficient for producing high oil percentage with desirable composition. The plant materials were collected from fresh leaves and twigs of *Verbena triphylla* L shrub at full blooming stage. Maximum oil percentage was obtained with HD method compared with SE method. The components of essential oils isolated from leaves and twigs of *Verbena triphylla* L were identified by GC and GC-MS . The essential oil extracted either by water distillation or organic solvent contains the same main components Twenty five compounds were identified in the oil isolated by both methods, they represent approximately 95.69 % and 74.17 % for HD and SE extraction methods respectively. Citral (a+b) (23.25 %) is the major constituents in the oil isolated by DH method followed by D-limonene (16.11%), 1,8 Cineol (8.56%), Caryophyllen oxide (5.1%) and B-Citronellol (5.52%), while in absolute oil extracted from concrete, Citral (a+b) is the main constituent (28.34%) followed by B-Citronellol (9.32%) and Benzene, 1-(1.5 dimethyl1-4-hexenyl)-4-methyl- (5.3%).

Keywords: *Verbena triphylla* L, Hydro-distillation, Solvent extraction Essential oil, Concrete, Constituents, Citral.

Introduction:

There are several natural products used as raw materials in the flavor and fragrance industry. These raw materials might be found in the shape of essential oils. In latest years, the cultivation of some medicinal and aromatic plant species has been perused with increasing interest in Egypt. Their contents of essential oil, which is a very important source of flavoring compounds in foods, industrial and pharmaceutical products[1]. Oil of *Verbena triphylla* is a yellow-greenish liquid with a characteristic fresh, lemon like odor.[2,3]The chemical composition of *V. triphylla* L oil has been the subject of several studies. Several monoterpenes, sesquterpenes, aromatic compounds, alcohols and ketones were identified. Citral, limonene and 1,8 Cineol were found as the main constituents of *Verbena* oil in the leaves of *V. triphylla* L shrub. In addition to these, some other leaf oil contained quantities of, β-citronellene, β-Pinene and neryl acetate, present in the leaves oil[4, 5, 6].

Some investigators found that GC analysis of Geranium oil indicated that, the essential oil of Geranium produced by steam distillation methods produce oil rich in oxygenated terpenes and poor in hydrocarbon[7]. Also Basil oils isolated with various extraction methods varied in their constituents percentage[8,9] .

Our work aimed to evaluate the essential oils content of *Verbena triphylla* L leaves under the conditions of Egypt. Using two different extraction methods; hydro-distillation (HD) and solvent extraction (SE) to conclude whether of them is more efficient for producing high oil percentage with so desirable composition

Materials and Methods

Plant materials

The plant materials were collected from leaves and twigs of *Verbena triphylla* L shrub (two year old plants) growing in (Qalyupia governorate) during flowering stage in September

Extraction Methods

Essential oils from fresh samples were extracted by hydro-distillation technique. While absolute oils of *Verbena triphylla* L were extracted by volatile organic solvents technique.

Hydro-distillation extraction

Essential oils of *Verbena triphylla* L were isolated from fresh leaves and twigs by submitting, the plant materials to hydrodistillation for 3 hours in a Clevenger – type apparatus[10]. The extracted volatile oil (distilled essential oil) was dehydrated over anhydrous sodium sulfate and stored in refrigerator at 4°C until analyzed.

Organic solvents extraction

Extraction with petroleum ether

Plant samples of *Verbena triphylla* L were extracted with petroleum ether at room temperature[11]. The samples macerated 3 folds of its weight with pure petroleum ether (40-60 °C) for 12 hours. The maceration was repeated twice applying the same way each time. The ratio between plant material and petroleum ether was 1 : 3 w/v. The combined petroleum ether extract was dried over anhydrous sodium sulphate, and then filtered over whatman No. 1 after isolation of any existing matters by decantation. The solution was distilled under vacuum at a temperature not exceeding 35 °C by a Rotavapour apparatus. The completely concentrated extract which called concrete is containing the odoriferous principles of the natural perfume, plus a considerable amount of plant waxes, albuminous materials and color pigments therefore; it was a solid waxy and dark brown mass. The obtained concrete from every sample was weight and packed separately.

Extraction of absolute oils:

Verbena absolute oils were extracted from concrete with high – proof ethyl alcohol (ethanol absolute) in three successive washings. The ratio of alcohol by volume to the weight (V/W) of concrete oil was 15:1 in the first washing and 10:1 in the second and third washings. The respective time required for each of the three washings was 20, 15 and 15 hours per each. Then the samples cooled at –20 °C for 20 hours in deep-freezer to facilitate the separation of waxy materials and then were filtered at the same temperature(-20 °C) before the next washing. The filtrate was collected and distilled under vacuum at a temperature not exceeding 35 °C by a Rotavapour apparatus. The obtained absolute oils were then weight and kept in brown dry bottles

Gas chromatography analyses (GC)

GC analyses were performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was held at 40°C for 5 min and then programmed until 250°C at a rate of 4°C/min. Injector and detector (FID) temperature was 260°C; helium was the carrier gas with a linear velocity of 32 cm/s.

Gas chromatography-Mass spectrometry (GC-MS)

GC-MS analyses were carried out on a Varian 3400 system equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d.); Oven temperature was 40 to 240°C at a rate of 4°C/min, transfer line temperature 260°C, injector temperature 250°C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, flow rate 1.1

ml/ min, Ionization energy 70 eV; scan time 1 s ; mass range 40-350 amu. The components of the oils were identified by comparison of their mass-spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds. Kovat's indices were determined by co injection of the sample with a solution containing a homologous series of n-hydrocarbons, in a temperature run identical to that described above.

Qualitative and quantitative analyses

Identifications were made by library searches[12] combining MS and retention data of authentic compounds by comparison of their GC retention indices (RI) with those of the literature [13]or with those of standards available in our laboratories. The retention indices were determined in relation to a homologous series of n-alkanes (C8-C22) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 98 Libraries or with mass spectra from literature. Component relative concentrations were calculated based on GC peak areas without using correction factors.

Physical Properties

The Physical properties of the (EO) of *V. triphylla* were carried out as follows

Determination of Specific gravity.

The specific gravity of the essential oils was determined at 20 C^o using Pycnometer apparatus[14]

Determination of Refractive index.

The refractive index was determined at 20 C^o and correction factor of 0.00044 was used for every degree above or below 20 °C and carried out by Zeiss Refractometer [14]

Determination of Optical rotation.

The optical rotation was determined at room temperature by using a Carl Ziess Polarimeter.[14]

Result and Discussion

In Table (1) The data reveal the supremacy of hydro-distillation method (HD)for obtaining the maximum essential oil yield compared with solvent extraction method (SE). The percentage of *V. triphylla* oil in this respect recorded(0.5%) with HD methods, while extraction with petroleum ether (40 - 60 °c) (nonpolar solvent) followed by extraction of absolute oil from concrete by ethanol at produced less quantity of volatile oil (0.23-0.26 %) equivalent to 55.8-59.0 % from the weight of concrete. However, this is reasonable, since the vast majority of true volatile oils produced by distillation, as steam carries the most volatile aromatic material with it[15] .

As physical and chemical properties of distilled *V. triphylla* leaf essential oil.

It is importance noting that physical properties of any essential oil are of great importance for specification and evaluation this oil

With regards to specific gravity. The different extraction methods are considered negligible. It recorded 0.8970 for HD method against 0.8960 for SE method. These results were equivalent to the recorded by Gunether 1952 [16].

Refractive index of any oil reveals whether it is genuine or mixed. The values of Refractive index recorded.4884 and 1.4850 for HD and SE methods respectively. Also optical rotation gives an indication whether it is natural or artificial. The values of optical rotation of *Verbena triphylla* oil were -10 and- 6 for HD and SE methods respectively

Table (1) Effect of extraction methods on oil percentage and Physical properties of Egyptian *Verbena triphylla* L Herb oil

The properties	Method		
	Water distillation	**Concrete**	**Absolute oil**
Oil %	0.32-0.45	0.38-0.51	0.24-0.28
Absolute oil in concrete (%)			55.8-59.2
Specific gravity	0.8970		0.8960
Refractive index	1.4884		1.4850
Optical Rotation	-10		-6

Effect of extraction methods on the constituents of *Verbena triphylla* oil

GC analysis indicated that the essential oil ***Verbena triphylla*** extracted with various extraction methods varied in their constituent's percentages due to extraction technique. Data in Table (2) indicated that Citral (a+b) is the major constituents in oil isolated by **HD methods** (33.25%) followed by D-limonene (16.11%), 1,8 Cineol (8.56%), β-Citronellol (5.52%) and Caryophyllen oxide (5.3%), while in absolute oil isolated from concrete, Citral (a+b) is the main constituents (28.34%) followed by β–Citronellol (9.32%) and Caryophyllen oxide (3.3%).

Five monoterpenes hydrocarbon compounds were identified in distilled oil against four compounds were detected in absolute oil .The total MCH recorded 21.97 for HD method and reduced to13.07 % only in absolute oil. Limonene was the main constituents of MCH in both oil samples it recorded 16.11 % in HD methods against 9.22% only in absolute oil.

The total oxygenated monoterpene compounds amounted to 50.93 % with distilled oil, while they recorded 46.53 % in absolute oil. Citral (b+a) was found in high concentration (33.25%) in distilled oil while it recorded 28.37% in absolute oil. Also the same trend was observed with 1,8 cineole compound . It recorded 8.52 % in distilled oil against 2.31 % only in absolute oil. In contrary citronellol compound was found in high concentration with absolute oil (9.32 %) , while in distilled oil was 3.52% only.

Dealing with sesquterpenes, distilled oil seemed to have more compounds compared with absolute oil, especially in case of sesquterpenes hydrocarbon. The total of sesquterpenes hydrocarbon compounds recorded 9.68 % in the distilled oil while it recorded 5.27 % in absolute oil. α-Curcumene was the main constituents of sesquterpenes hydrocarbon in both oil. It was found in equally matched ratios in both oil (3.92 and 3.71% for HD and SE, respectively).

Extraction method of volatile solvent produced oil rich in oxygenated terpenes (more slightly than 38.89%) and poor in hydrocarbon terpenes (less slightly than 5.91%)

Various compounds including, Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl- compound found in both oils. It recorded 5.1 and 5.3% for distilled and absolute oil respectively. Several investigator have confirmed that the chemical constituents of essential oils from aromatic species showed qualitative and quantitative variations in relation to the extraction methods employed namely[7, 8, 9,17,18,19].

Table (2) Chemical constituents of the Egyptian *Verbena triphylla* herb isolated by hydro-distillation Method and with volatile solvent

No	Compound	KI*	Hydro-distillation Method (HD)	Volatile solvent(SE)
1	Tricyclene	919	0.21	000
2	α-Pinene	933	1.82	0.73
3	β-Pinene	981	1.42	0.20
4	Decane	999	0.31	0.18
5	Limonene	1031	16.11	9.22
6	1.8-Cineole	1033	8.56	2.31
7	γ-terpinene	1059	2.41	2.92
8	Linalool	1082	1.23	1.19
9	Borneol	1165	0.43	000
10	α-Terpineol	1185	0.31	1.82
11	Nerol	1228	0.41	0.61
12	Citronellol	1230	5.52	9.32
13	citral b	1235	13.14	10.12
14	citral a	1240	20.11	18.22
15	Geraniol	1276	3.22	2.80
16	α-Cubebene	1345	0.41	0.35
17	α-Longipinene	1351	0.21	000
18	α-Cedrene	1409	0.40	0.22
19	β-Caryophyllene	1418	3.72	0.53
20	β-Humulene	1440	0.31	0.21
21	trans-Farnesene	1460	0.71	0.25
22	α-Curcumene	1483	3.92	3.71
23	Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl-	1478	5.1	5.3
24	Caryophyllene oxide	1573	5.3	3.3
25	E-E-Farnesal	1715	0.6	0.55
	Total		95.89	74.06

Table (3) Effect of extraction methods on the :Classification of *Verbena triphylla* herb

No	Group	Hydro-distillation Method (HD)	volatile solvent (SE)
	Monoterpene hydrocarbons		
1	Tricyclene	0.21	000
2	α-Pinene	1.82	0.73
3	β-Pinene	1.42	0.20
4	Limonene	16.11	9.22
5	γ-terpinene	2.41	2.92
	Total	21.97	13.07
	OxygenatedMonoterpene		
1	1.8-Cineole	8.56	2.31
2	Linalool	1.23	1.19
3	Borneol	0.43	000
4	α-Terpineol	0.31	1.82
5	Nerol	0.41	0.61
6	Citronellol	3.52	9.32
7	citral b	13.14	10.17
8	citral a	20.11	18.2
9	Geraniol	3.22	2.80
	Total	50.93	46.53
	Sesquiterpene hydrocarbons		
1	α-Cubebene	0.41	0.35
2	α-Longipinene	0.21	000
3	α-Cedrene	0.40	0.22
4	β-Caryophyllene	3.72	0.53
5	β-Humulene	0.31	0.21
6	trans-Farnesene	0.71	0.25
7	α-Curcumene	3.92	3.71
	Total	9.68	5.27
	Oxygenated Sesquiterpene		
1	Caryophyllene oxide	5.3	3.3
2	E-E-Farnesal	0.6	0.55
	Total	5.7	3.85
	Other		
	Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl-	5.1	5.3

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