Original Article

Comparative gas chromatography-mass spectroscopy, Fourier transform infrared spectroscopy, and high-performance liquid chromatography analysis of essential oils extracted using 4 methods from the leaves of *Eucalyptus globulus* L.

Abstract

Background: *Eucalyptus globulus* L. (family, Myrtaceae) is one of the world's most widely planted genera. *E. globulus* L., commonly referred to as Tasmanian blue gum, is a fast growing, evergreen tree, native to Tasmania and South-East Australia. Apart from its extensive use in pulp industry, it is also produces *Oleum Eucalypti* (eucalyptus oil) that is extracted on commercial scale in many countries such as China, India, South Africa, Portugal, Brazil, and Tasmania, as a raw material in perfumery, cosmetics, food beverage, aromatherapy, and phytotherapy. **Materials and Methods:** Traditional hydrodistillation (HD), solvent extraction (SE), ultrasonication (US), and supercritical fluid extraction (SFE) were conducted for the extraction of essential oil from the leaves of *E. globulus*. Each oil was evaluated in terms of high-performance liquid chromatography (HPTLC) and Fourier transform infrared spectroscopy (FTIR) fingerprinting with qualitative and semi-quantitative composition of the isolated essential oil by gas chromatography-mass spectroscopy (GCMS), the extract yield of essential oil was 2.60%, 2.2%, 2.0%, and 3.6% v/w, respectively, for HD, SE, US, and SFE. **Results:** A total of 53 compounds were identified by GCMS. Comparative analysis indicated that SFE was favorable for extraction of monoterpene hydrocarbon, sesquiterpene hydrocarbon, and oxygenated sesquiterpene hydrocarbon. HD, SE, and US had certain advantages in the extraction of aliphatic saturated hydrocarbons organic acid and esters. Overlay, FTIR spectra of oil samples obtained by four extraction methods were superimposed with each other showing similar components. The maximum separation of compound seen at 254 nm and lesser at 366 nm by HPTLC fingerprinting which again showed superimposed chromatograms. **Conclusion:** It is concluded that different extraction method may lead to different yields of essential oils where the choice of appropriate method is very important to obtained more desired component with higher physiological activities.

Key words:
Essential oil, Eucalyptus globulus L., Fourier transform infrared spectroscopy, gas chromatography-mass spectroscopy, high-performance liquid chromatography, myrtaceae

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Introduction

Eucalyptus globulus (family: Myrtaceae) is one of the world's most widely planted genera.[1] The leaves

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of *E. globulus* contains up to 3.5% w/w essential oil.[2,3] 1,8-cineole (eucalyptol) is the principal constituent found in *E. globulus*. However, other chemotypes such as α-phellandrene, ρ-cymene, γ-terpinene, ethanone, spathulenol, among others have been documented.[1,4,5] Composition pattern of essential oils reflects their nutritional, cosmetic, pharmaceutical, or medicinal values. The essential oils possess many bioactivities (antimicrobial, antiviral, fungicidal, insecticidal, and herbicidal activities), and these bioactivities are highly associated with their unique chemical composition. The novel biological functions of *E. globulus* essential oils suggest research on all *Eucalyptus* species to fully exploit their commercial benefits.[6]

Experimental

Procurement of sample and chemicals

The following plant material (fresh leaves) was collected from Jamia Hamdard (herbal garden), Delhi. Its voucher specimen is deposited in Bioactive Natural Product Laboratory, Jamia Hamdard University as BNPL/JH/YK/MPH: 04 (2012–2013). All the ingredients were taken of pharmacopeial quality and quantity. Standards were obtained from Sami Labs Ltd., Bangalore, (India) as a gift samples and other chemicals and reagents used were of analytical grade (AR) and procured from Merck Ltd., India.

Extraction of essential oil

Hydro-distillation method

The grounded powder (150 g) and 500 mL distilled water were mixed in one L round-bottomed flask and heated for 5.0 h to yield essential oil. The extract was centrifuged at 10,000 rpm for 10 min to separate small water droplets present in the essential oil. The oil was kept at 4°C until further use.

Solvent extraction

The coarse powder (50 gm) was extracted with hexane (100 mL) was kept in a conical flask in 1:2 w/v, ratio for 24 h. The extract was filtered and concentrated under reduced pressure in rotary vacuum evaporator at 40°C. The obtained oil was kept at 4°C until further use.

Ultrasonic assisted extraction

The coarse powder (50 gm) of plant material was taken in a 250 mL conical flask along with 100 mL hexane (1:2, w/v). The flask was covered and then placed in an ultrasound water bath apparatus for 30 min (frequency 33 kHz). The temperature of the water bath was held constant at 25°C. The extract was filtered and concentrated under reduced pressure in rotary vacuum evaporator. The obtained oil was kept at 4°C until further use.

Supercritical fluid extraction

A supercritical fluid extraction (SFE)-1000M1-2-C50 system (Pittsburgh, PA, USA) was used for extractions. The extraction vessel was 200 mL stainless steel. Grounded plant material (50 g) was loaded in the extractor. CO_2 was allowed to pass through the seed matrix at desired pressure (P: 150 bar) and temperature (T: 40°C) and optimized flow rate (5.0 g/min). Total time of extraction was 30 min. After the completion of extraction, the extracted oil was collected from the collecting vessel.[7]

High-performance liquid chromatography

Instrumentation and Procedure

The samples were spotted in the form of band (3.0 mm) with a Camag Microlitre Syringe on thin layer chromatography aluminum plate precoated with silica gel 60F-254 (20 cm × 10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat V sample applicator. The development was carried out mobile phase composed of hexane: Ethyl acetate: Formic acid (7:2:1, v/v/v). The high-performance liquid chromatography (HPTLC) plates were studied at 254 nm and 366 nm as well as in visible range (580 nm) after spraying with anisaldehyde-sulfuric acid reagent. A good separation of constituent was observed at 254 nm and 580 nm.

Gas chromatography-mass spectroscopy analysis

The essential oil obtained by different extraction technique was diluted by adding 1998 µL of hexane to 2.0 µL oil (hydro distilled oil) and 1990 µL hexane to 10 µL oil (other extracted oil). Gas chromatography-mass spectroscopy (GCMS) was equipped with a DB-5 fused silica capillary tubes column (30 mm × 0.25 mm × 0.25 µm). The injection volume was 1.0 µL using autosampler at a carrier gas (helium) flow of 2.0 µL/min helium with a splitless mode. The initial oven temperature was 65°C (3.0 min) then raised to 2.0°C/min–114°C, then to 4°C/min–160°C, 6°C/min–302°C, finally ramped to 310°C at 15°C/min. Other setting of detector type was MS, and its interface temperature was 250°C.

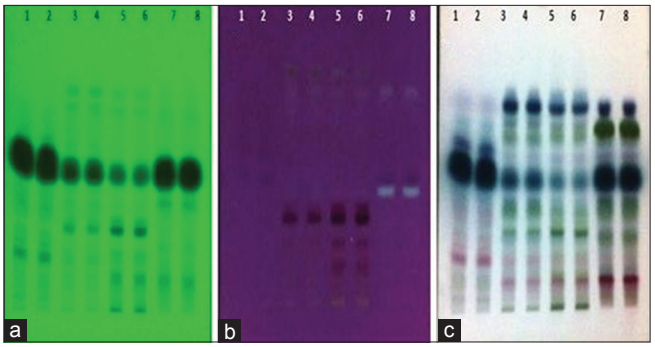

Figure 1: HPTLC fingerprint of different oils of *E. globulus* extracted through different techniques [track 1-2: Hydrodistilled oil, 3-4: Solvent extracted oil, 5-6: Ultra sonication oil, 7-8: SFE oil] visualized at (a) 254 nm, (b) 366 nm, (c) Day light after derivatization using anisaldehyde sulphuric acid reagent

By comparing with the National Institute of Standard and Technology library compounds were detected and identified.[8]

Fourier transform infrared spectroscopy spectral analysis

Fourier transform infrared spectroscopy (FTIR) spectrum was obtained using Shimadzu Bio-Rad FTIR (Kyoto, Japan). The samples were dispersed and triturated with dry potassium bromide (weight 2 μL of sample), grounded well in mortar and pestle and potassium bromide (K Br) disk were at a pressure of 1000 psig. The disk was placed in the FTIR sample holder, where IR spectra in absorbance mode were obtained in the spectral region 4000–400/cm using the resolution 4/cm.

Results and Discussion

Percentage yield (% v/w)

Different extraction techniques were carried out to obtain the maximum yield of oil. The extract yields of essential oil were 2.60%, 2.2%, 2.0%, and 3.6 v/w for hydrodistillation, SE, US, and SFE, respectively.

Development of solvent system for separation of oil sample by TLC

The development of solvent system was carried out by hit and trial method using different solvents considering the results of earlier reports. The solvent system composed

Table 1: Substance with their R_f and area percentage of *E. globulus* essential oil obtained by different extraction techniques

Substance (R_f)	Area percentage(%) 254 nm				Area percentage(%) 366 nm			
	HD*	SE*	US*	SFE*	HD*	SE*	US*	SFE*
A (0.03)	1.37	6.10	4.11	0.90	3.00	2.31	1.75	1.14
B (0.05)	-	-	-	0.70	-	2.41	1.75	-
C (0.07)	-	2.04	2.44	-	-	2.65	3.05	0.76
D (0.08)	-	2.53	2.88	1.31	-	3.59	7.91	2.68
E (0.11)	-	-	2.23	2.04	-	-	-	-
F (0.13)	-	3.68	-	1.58	-	3.92	-	-
G (0.16)	-	14.63	15.26	-	-	8.46	2.89	-
H (0.2)	-	-	-	14.42	-	-	5.85	5.50
I (0.27)	1.17	3.54	16.44	-	-	-	-	8.80
J (0.3)	-	13.52	-	6.19	-	5.10	5.30	-
K (0.34)	-	-	-	4.05	-	4.15	-	7.25
L (0.37)	12.09	-	5.85	-	-	16.86	20.44	8.98
M (0.41)	7.43	5.12	3.11	4.67	-	-	-	-
N (0.46)	-	-	-	5.48	37.27	5.83	5.78	3.97
O (0.5)	72.61	-	11.22	-	31.61	-	-	-
P (0.55)	-	9.79	-	-	-	7.15	8.47	4.98
Q (0.59)	-	5.31	-	18.40	14.48	-	-	-
R (0.68)	-	28.95	25.02	-	13.64	30.92	28.99	55.32
S (0.73)	-	-	6.14	35.81	-	6.66	7.84	-
T (0.78)	5.32	4.79	5.32	4.45	-	-	-	0.61

Substance (R_f)	Area percentage(%) 580 nm			
	HD*	SE*	US*	SFE*
A (0.01)	0.09	0.74	-	-
B (0.04)	-	2.81	1.77	5.52
C (0.11)	-	1.98	1.78	2.37
D (0.11)	-	4.04	1.82	3.49
E (0.15)	-	-	0.57	0.98
F (0.2)	-	2.07	0.81	2.05
G (0.27)	-	8.06	6.17	6.17
H (0.34)	16.13	-	-	-
I (0.41)	35.05	24.70	26.69	40.33
J (0.5)	-	6.28	5.81	8.33
K (0.55)	-	4.02	4.36	-
L (0.6)	12.08	6.22	3.60	-
M (0.67)	-	13.93	19.87	18.14
N (0.71)	-	5.36	5.29	-
O (0.78)	14.59	19.79	21.47	12.62

of hexane: Ethyl acetate: Formic acid (7:2:1, v/v/v) with a maximum separation of constituents and compactness of bands for all the oil samples.

Detection of spots of different samples

Before derivatization at UV 254 nm and 366 nm, and after derivatization by spraying with anisaldehyde-sulfuric acid reagent at 254 nm [Figure 1]. The HPTLC plates were studied at 254 nm and 366 nm as well as in visible range (580 nm) after spraying with anisaldehyde-sulfuric acid reagent. A good separation of a constituent can be observed at 254 nm [Figures 2 and 3]. The comparative results of different oils were showing a dominant constituent showed A-T compounds [Table 1].

Comparative gas chromatography-mass spectroscopy analysis of *Eucalyptus globulus* L. oil obtained by different extraction techniques

Comparative GCMS chromatograms of essential oils of *E. globulus* L. are presented in [Figure 4i and Table 2], which shows the constituents, separated and identified using their retention time and mass from library (Nist and Wiley).

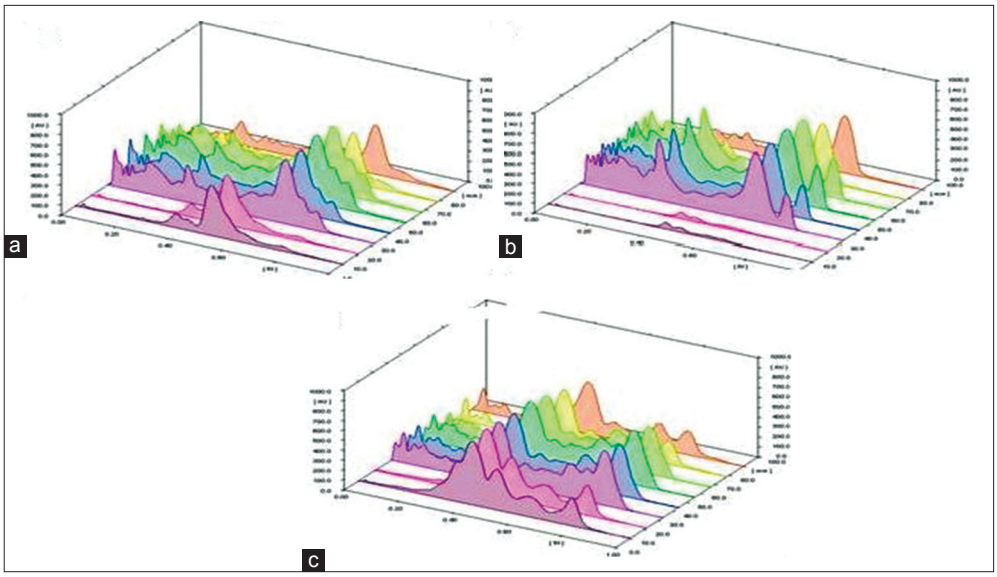

Figure 2: 3D chromatogram of 8 tracks of *E. globulus* oil obtained by different extraction techniques at (a) 254 nm, (b) 366 nm and (c) 580 nm respectively

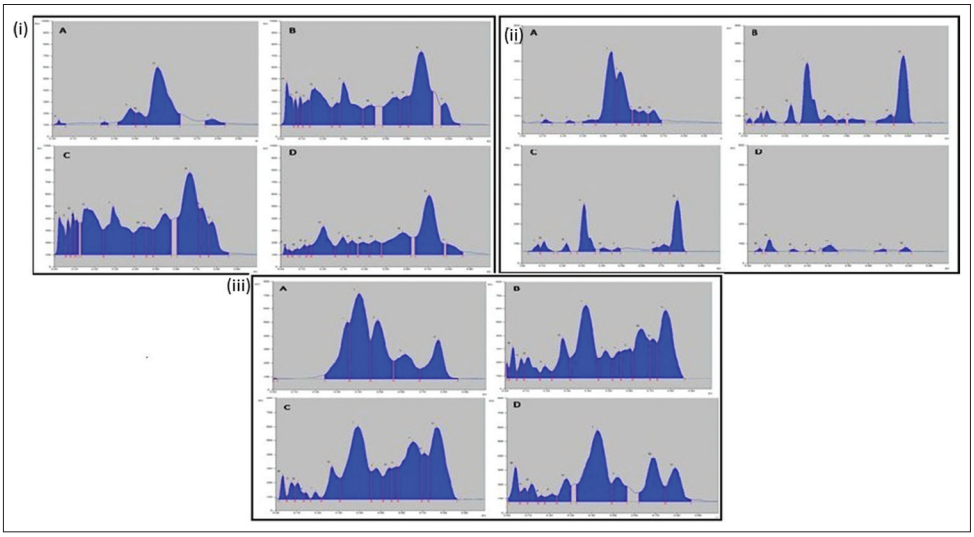

Figure 3: HPTLC chromatograms of different oils of *E. globulus* extracted using different extraction techniques (A) Hydrodistilled oil, (B) Solvent extracted oil, (C) Ultra sonication oil, (D) SFE oil visualized at (i) 254 nm, (ii) 366 nm and (iii) 580 nm

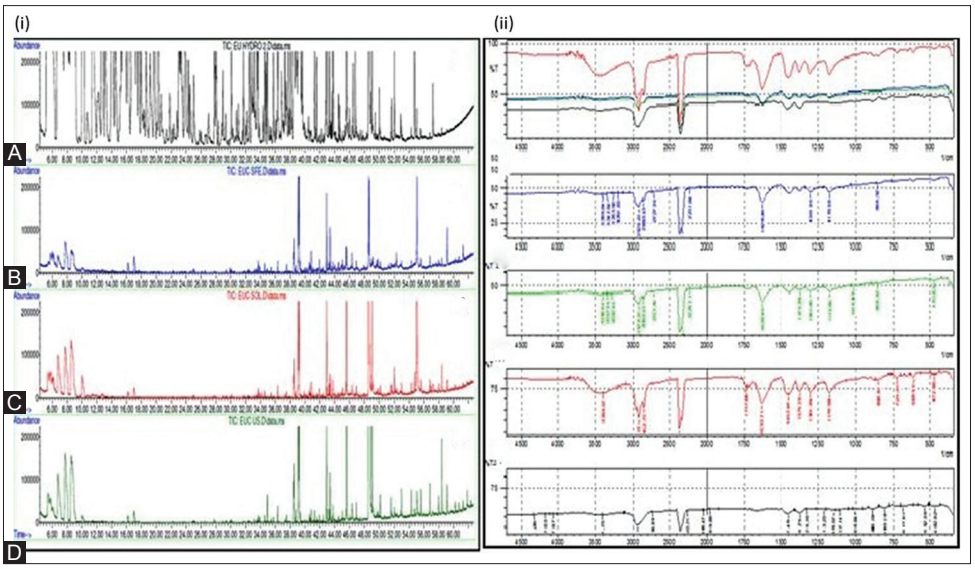

Figure 4: (i) GC–MS Comparative chromatograms of different oils of *E. globulus* L. extracted using different extraction techniques (A) SFE oil, (B) Hydrodistilled oil, (C) Solvent extracted oil, (D) Ultra sonication oil. (ii) Comparative spectra of different oils of *E. globulus* Linn. Extracted using different extraction techniques (A) Hydrodistilled oil, (B) Ultra sonication oil, (C) Solvent extracted oil, (D) SFE oil

Table 2: Results of GC-MS analysis of *E. globulus* oil extracted by different techniques

Component name	Area percent (%)				
	R_T	HD*	SE*	US*	SFE*
Monoterpene hydrocarbon					
α.-pinene	5.45	10.6	0.21	0.65	_
(-)-β-Pinene	6.77	15.21	3.29	2.82	1.86
l-Phellandrene	7.76	10.29	4.01	4.16	11.2
Cymene	8.59	26.39	2.35	2.04	0.82
γ.Terpinene	10.03	1.33	1.34	-	-
α.-terpinolene	11.45	0.5	-	-	-
Oxygenated monoterpene					
D-Fenchyl alcohol	12.68	0.79	-	-	-
Linalool	14.48	0.21	-	-	-
Borneol	15.5	1.32	-	-	-
4-Terpineol	16.19	1.95	-	0.35	-
β-fenchyl alcohol	16.97	-	-	-	3.14
Myrtenol	17.33	0.65	-	-	-
α-phellandrene epoxide	19.71	0.47	-	-	-
Thymol	23.34	0.45	-	-	-
Carvacrol	23.86	0.64	-	-	-
Sesquiterpene hydrocarbons					
Copaene	27.83	0.15	-	-	-
Caryophyllene	30.11	0.14	-		-
α.-Humulene	31.73	0.07	-	-	-
α-amorphene	34.36	0.03	-	-	-
Alloaromadendrene	34.92	0.04	-	0.14	-
β-Selinene	36.19	0.26	2.16	-	-
Valencene	38.47	-	0.32	0.14	1.44
(+)-α.-Selinene	39.22	-	2.36	-	9.83
β-Maaliene	40.96	-	0.61	0.8	2.95
Oxygenated sesquiterpenes derivatives					
3-Cyclohexene-1-methanol	17	4.03	-	0.35	-
2-Cyclohexen-1-one	20.24	0.4	-	-	-
Phellandral	21.82	0.05	-	-	-
Elemol	35.75	0.06	-	-	-
Champacol	37.46	0.05	-	-	-
Eudesm-4 (14)-en-11-ol	40.23	11.41	2.73	0.44	12.64
Non isoprenoid					
1-Tetradecene	28.98	0.08	-	-	-
Phenol, 2,4-bis (1,1-dimethylethyl	34.6	0.16	-	-	-
Dodecanoic acid, methyl ester	34.95	0.11	-	0.3	-

Contd...

Table 2: Contd...

Table 3: Results of FTIR analysis of *E. globulus* L. oil extracted by different techniques

Peak	Functional group	Intensity	HD*	SE*	US*	SFE*
858.32	Aromatic p-disubstituted benzene	Strong	-	+	+	-
1174.65	C-F (fluoroalkane)	Strong, broad	-	+	+	+
1301.95	C-O(ether)	Strong, stretch	-	+	+	+
1375.95	N-O (nitro, aliphatic)	weaker	+	+	-	+
1452.4	C=C (aromatic)	Stretch, medium - strong	+	-	-	+
1629.85	C-C (conjugated diene)	Strong	-	+	+	-
2229.71	-C=-C (alkyne)	Stretch, strong	-	+	+	-
2926.43	methylene	Medium - strong	-	+	+	-
3342.64	O-H (alcohol)	Strong, broad	-	+	+	-

A total of fifty-three compounds were determined. The major terpenoid compounds present were cymene (26.39%), β-pinene (15.21%), eudesmol (12.64%), and in hydrodistilled oil. The oil extracted by SFE contained eudesmol (12.64%), 1, 2 benzenedicarboxylic acid (19.28%). The fatty acid was also present in a significant amount as 9, 12, 15-octadecatrienoic acid, methyl ester (56.70%) in ultrasonicated oil, and 9,12-octadecadienoic acid (34.43%) in solvent extracted oil. α-selinene (9.83%), β-selinene (2.16%), 4-terpineol (1.95%), borneol (1.33%), thymol (0.45%), and carvacrol (0.64%) were other important constituent present.

Comparative Fourier transform infrared spectroscopy analysis of *Eucalyptus globulus* L. oil obtained by different extraction techniques

The interpretation of infrared spectra of *E. globulus* L. essential oil was done by correlating of absorption bands in the spectrum with the known absorption frequency bonds. Comparative spectra of essential oil extracted by different techniques are presented in [Figure 4ii and Table 3] shows the peak common to all the four extraction techniques.

FTIR analysis of eucalyptus oil showed the presence of strong peak of aromatic para-disubstituted benzene ring in solvent extracted and ultrasonicated oil samples. Strong stretching peaks of ether are present in all extracted oil samples except in hydrodistilled oil. Peaks at 1629.85/cm, 2229.43/cm, 2926/cm, and 3342.64/cm are absent in hydrodistilled and SFE oil samples.

Conclusion

In the present investigation, it states that SFE of *E. globulus* L. was proved to have highest percentage yield as compared to other extraction techniques. Furthermore, evidence from HPTLC, GCMS as well FTIR analysis shows that SFE technique is prime technique for extraction of essential oils.

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Conflicts of interest
There are no conflict of interest.

References

1. Akin M, Aktumsek A, Nostro A. Antibacterial activity and composition of the essential oils of *Eucalyptus* camaldulensis Dehn. and Myrtus communis L. growing in Northern Cyprus. Afr J Biotechnol 2010;9:531-5.
2. Bremness L. Herbs. London: Dorling Kindersley; 2004. p. 54.
3. Melka Y, Bekele T, Bauhus J. In *Eucalyptus* species management, history, status and trends in Ethiopia. In: Gil LL, Tadesse WW, Tolosana E, Lopez R, editors. Proceedings from the Congress Held in Addis Ababa. Ethiopia; 2010.
4. Chalchat JC, Kundakovic T, Gorunovic MS. Essential oil from the leaves of Eucalyptus camaldulensis Dehn. Myrtaceae, from Jerusalem. J Essent Oil 2001;13:105-7.
5. Iqbal Z, Bhatti HN, Ahmad IN, Chatha SAS. Content and compositional variations in the essential oils of different Eucalyptus globulus trees from Faisalabad, Pakistan. J Chem Soc Pak 2006;28:397-400.
6. Zhang JB, An M, Wu H, Stanton R, Lemerle D. Chemistry and bioactivity of *Eucalyptus* essential oils. Allelopathy J 2010;25:313-30.
7. Khajeh M, Yamini Y, Bahramifar N, Sefidkon F, Pirmoradei MR. Comparison of essential oils compositions of *Ferula assa-foetida* obtained by supercritical carbon dioxide extraction and hydrodistillation methods. Food Chem 2005;91:639-44.
8. Derwich E, Benziane Z, Taouil R, Senhaji O, Touzani M. Aromatic plants of morocco: GC/MS analysis of the essential oils of leaves of *Mentha piperita*. Adv Environ Biol 2010;4:80-5.