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Microwave Accelerated Distillation of Essential Oils from the Leaves of *Eucalyptus microtheca*: Optimization and Comparison with Conventional Hydrodistillation

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In recent years, a novel technique has been developed for the extraction of natural products of plants based upon exertion of microwave energy at atmospheric pressure namely microwave assisted extraction. Microwave accelerated distillation (MAD) was applied for the extraction of essential oil from the leaves of *Eucalyptus microtheca*. The microwave assisted extraction method has been compared with hydrodistillation (HD) conventional technique, in terms of extraction time, yields and chemical compositions. Also, effect of microwave powers were investigated on extraction of some major constituents of *E. microtheca* essential oil. The obtained results show that, the extraction of essential oils from *Eucalyptus* by microwave assisted extraction is more efficient rather than hydrodistillation especially from yield and time of extraction points of view (10 min *versus* 3 h and 1.72 % *versus* 0.29 %). The extracted essential oils involved 18 and 34 components constituting 94.04 and 89.07 % of the chemical profiles in the hydrodistillation and microwave assisted extraction methods, respectively. The results also show that, each type of *Eucalyptus microtheca* essential oil compounds has an optimum microwave power for efficient extraction and this power should be used to enhance useful components of essential oil and to save energy.

Key Words: Clevenger, Microwave, Extraction, Hydrodistillation, Essential oil, *Eucalyptus microtheca*.

INTRODUCTION

Plants and their derivatives such as essential oils and oleoresins have long been used as food flavoring, beverages and antimicrobial agents[1]. Nowadays, developing countries pay more attention to herbal medicines in treatment due to the noxious side effects of synthetic medicines on patients. In addition, the application of natural antioxidants in food factories has attracted a growing interest. For these reasons, numerous researches are oriented in the extraction field of biologically active compounds from the herbs[2]. The *Eucalyptus* is a diverse genus of flowering in the myrtle family, Myrtaceae. According to the literature, *ca.* 3800 species are found within the Myrtaceae family appearing in 140 genera and occurring along tropical and subtropical regions of the world, mainly in Australia as well as Central and South Americas[3].

In addition, there are more than 600 species of flowering trees of *Eucalyptus*, mostly native to Australia and its Northern islands[4]. In tropical countries, *Eucalyptus* is widely used as ornamentation and a valuable source of wood while in Iranian saunas this plant is used for flavoring the inside space of the baths and in tradition medications it is usually prescribed for treatment of cold, flue, headache, fever and bronchial infections, as well.

In view of the ease of adaptation and rapid growth, this plant is found all around the world particularly in Africa.

The *Eucalyptus* oils rich in cineol, citronellal and piperitone or α-phellandrene are extremely used in medicine, perfumery and industry. Furthermore, the essential oil of *Eucalyptus* and 1,8-cineol as its major component are extensively employed as a flavoring agent in production of softeners, ointments, cough syrup, toothpaste and other medicines. They are also applicable as aromatic substances in soap , detergents and slightly in perfumes. The *Eucalyptus* essential oil has diverse antioxidant and antiswelling effects. On the other hand, the effect of habitat condition and different extraction methods were demonstrated on the quantity and quality of herbal essential according to the previous report[5,6].

Biological activities of some of the most abundant species of *Eucalyptus* genus have been well described in the literature. These reports mainly focus on analgesic and antiinflammatory effects, antibacterial, antimicrobial and antioxidant/lipoxygenase inhibitory activities along with characterization of the chemical profiles of this plant in different parts of the world[7-10].

In a challenging report, the mosquito larvicidal activity of leaf essential oils and their constituents from two *E. camaldulensis* and *E. urophylla* against two mosquito species namely *Aedes aegypti* and *Aedes albopictus* were taken into consideration. Results of this study confirm that *E. camaldulensis* could be considered as a novel candidate against both insects. Moreover, among the six major fractions of *E. camaldulensis*, α-terpinene exhibited the best larvicidal effect toward both *A. aegypti* and *A. albopictus* larvae[11].

As essential oils are used in different industries with a wide variety of regulations and applications, it is necessary to find the best extraction approach, which could provide the appropriate chemical component and better quality of essential oils for each specific request.

In conventional methods such as water distillation or steam distillation a relatively long time is required to approach the optimized temperature for evaporation of volatile compounds. These methods waste power and time and loss many of the effective components. On the other hand, there is a risk of contamination in those methods which use chemical solvents in final steps of extraction.

In recent years, a novel technique has been developed for the extraction of natural products of plants based upon exertion of microwave energy at atmospheric pressure namely "microwave assisted distillation (MAD)". This technique is based on heating in microwave oven with distillation[12]. The MAD method is considered as a green technique, because it could extract essential oil from plants without any solvent just by applying microwave[13].

Regarding the previous studies, the extraction time for this method is fairly less than conventional approaches. In addition, this method is an alternative toward traditional methods in the case of energy consumption and costs. Moreover, the shorter time of extraction causes protection of chemical properties of some components in essential oils such as valuable oxide components, which are hydrolyzed in conventional methods due to longer time and higher temperature during the extraction.

In this paper, effect of microwave power on chemical composition of essential oil of *E. microtheca* was considered for the first time in the literature.

EXPERIMENTAL

The *Eucalyptus* leaves of *E. microtheca*, aged *ca.* 1 year, were collected in the middle of May 2010 from a farm belonging to the agricultural research station of Semnan Province, Iran. A voucher specimen (number 48203) was deposited at the Herbarium of the Research Institute of Forests and Rangelands, Tehran, Iran. To extract the oil, the leaves were separated with a meticulous care and dried in shadow to avoid extra damaging and minimizing cross-contamination of the plant leaves.

Microwave accelerated distillation (MAD): The microwave oven used for MAD provided by Samsung (South Korea) trade mark operating at 2450 MHz. The maximum power of the oven was regulated at 1000W. In order to examine the effect of microwave power, four power levels, 90 % (900 W), 70 % (700 W), 50 % (500 W) and 30 % (300 W) were studied. The dimensions of the interior cavity of the oven were 29 cm × 37 cm × 40 cm. Microwave oven was modified by drilling a hole at the top. Flat bottom flask having a capacity of 1000 mL was placed in the oven and connected to Clevenger apparatus through the hole. After the Clevenger apparatus was placed into the oven, the hole around the neck of the flask was covered with polytetrafluoroethylene (Teflon) to prevent leakage of microwave.

For MAD, 50 g of fresh leaves of *E. microtheca* was soaked in 500 mL distilled water at room temperature (25 ºC) for 1 h in order to hydrate the external layers of the plant material and the excess water was drained off. Soaking time, during which the maximum amount of absorption is achievable, was determined from the preliminary experiment. The moistened plant material was placed in flat-bottom flask combined to a Clevenger apparatus. During the process, the vapor continuously passed through the condenser located at the outside the microwave cavity where it was condensed. The MAD process was performed for different times and was repeated until no more essential oil was obtained. For each condition, experiments were replicated twice. The essential oil was collected in amber coloured vials, dehydrated with anhydrous sodium sulfate, capped under nitrogen and kept at 4 ºC until being analyzed.

Hydrodistillation: Conventional hydrodistillation was carried out with a circulatory commercially available Clevenger apparatus. Clean, mature adult leaves which were dried at shade (one week) were exactly weighed (50 g) and immersed in water in the ratio 1:10, followed by collecting the volatile oils at different process times. For each distillation time, experiments were conducted twice. The maximum distillation period was 3 h since after that time no more essential oil was obtained. Then, the essential oil was collected in amber coloured vials, dehydrated with anhydrous sodium sulfate, capped under nitrogen and kept at -4 ºC until being analyzed.

Gas chromatography and gas chromatography-mass spectrometry: Quantitative and qualitative evaluations of the oils were performed by means of GC and GC-MS instruments. The GC analyses were carried out on a Varian (CP 3800) gas chromatograph equipped with a split/splitless (20:1) injector (250 ºC) and a flame ionization detector (250 ºC). N_2 was used as the carrier gas (1 mL/min). The capillary column used was CP-Sil 5 CB (30 m × 0.25 mm × 0.25 μm film thickness). The oven temperature was held at 60 ºC for 3 min, then heated to 220 ºC with a 7 ºC/min rate and kept constant at 220 ºC for 5 min. Quantitative data were obtained from GC (FID) area percentage.

GC-MS determinations were performed on a HP-6890 GC system coupled with a 5973 network mass selective detector and equipped with a HP5-MS capillary fused silica column (30 m × 0.25 mm I.D × 0.32 μm film thickness). The operating conditions were the same as described above but the carrier gas was He with 1 mL/min flow rate. Mass spectra were taken at 70 eV. Mass spectra were recorded over the range m/z 20-500 amu. All chromatographic measurements were carried out in triplicate and the mean of the retention times and percentage compositions of each component were taken into consideration. Duplicate times were discarded if they differed by more than 1 s and the experiments were repeated again in duplicate.

RESULTS AND DISCUSSION

Identification and quantification of essential oil compounds: The compounds of the extracted essential oils were identified by comparing their mass spectral fragmentation patterns with those of similar compounds from a database (Wiley/NBS library) as well as by comparing their Kova'ts gas chromatographic retention indices[14] with those of the literature. For each compound on the chromatogram, the percentage of peak area relative to the total peak areas from all compounds was determined and reported as relative amount of that compound.

Each essence was separately analyzed by GC to clarify its chemical composition. Results of the GC analysis of the oils are shown in Table-1, where the constituents are sorted in the order of their elution from the HP-5MS column. Accordingly, a total of 34 and 18 components were detected by MAD and HD methods, respectively.

TABLE-1
COMPONENT OF ESSENTIAL OILS FROM *E. microtheca* BY MAHD AND HD TECHNIQUES WITH THEIR PERCENTAGE[a]

No.	Constituents	m.f.	KI[b]	R_t[c]	MAD (%)[d]	HD[e] (%)
1	α-Pinene	$C_{10}H_{16}$	942	10.5	3.67	9.47
2	1,8-cineole	$C_{10}H_{18}O$	1041	13.01	42.10	48.51
3	NI[f]	–	1094	14.33	0.75	0
4	Isoamylisovalerate	$C_{10}H_{20}O_2$	1100	14.46	0.97	0
5	*trans*-Pinocarveol	$C_{10}H_{16}O$	1153	15.68	3.85	2.23
6	Pinocarvone	$C_{10}H_{14}O$	1176	16.20	2.87	1.46
7	NI	–	1195	16.64	0.30	0
8	α-Terpineol	$C_{10}H_{18}O$	1198	16.72	0.75	0
9	NI	–	1239	17.52	0.54	0
10	NI	–	1314	19.04	0.73	0.90
11	NI	–	1321	19.43	0.19	0
12	Bicycloelemene	$C_{15}H_{24}$	1323	19.88	0.74	1.41
13	NI	–	1389	20.67	0.46	0
14	α-Copaene	$C_{15}H_{24}$	1392	20.74	0.74	0
15	β-Elemene	$C_{15}H_{24}$	1403	20.97	0.78	0.64
16	α-Gurjunene	$C_{15}H_{24}$	1430	21.46	0.52	0
17	NI	–	1435	21.55	0.48	0
18	NI	–	1450	21.84	0.79	0
19	Calarene	$C_{15}H_{24}$	1457	21.98	1.27	0.88
20	Aromadendrene	$C_{15}H_{24}$	1464	22.09	19.5	18.31
21	NI	–	1476	22.31	0.88	0.92
22	NI	–	1478	22.36	0.58	0
23	NI	–	1482	22.42	1.72	2.01
24	Allo-aromadendrene	$C_{15}H_{24}$	1486	22.51	5.41	4.67
25	NI	–	1494	22.64	0.78	0.55
26	β-Selinene	$C_{15}H_{24}$	1512	22.97	1.58	1.41
27	Viridiflorol	$C_{15}H_{24}$	1517	23.06	2.94	2.95
28	NI	–	1544	23.36	0.46	0
29	NI	–	1544	23.56	0.32	0
30	NI	–	1578	24.17	0.24	0
31	NI	–	1586	24.31	0.65	0.68
32	Spathulenol	$C_{15}H_{24}O$	1604	24.62	1.38	2.10
33	NI	–	1613	24.77	0.63	0.89
34	NI	–	1648	25.33	0.45	0
Number of total component					34	18
Total (%)					89.07	94.04

[a]The compounds have been sorted according to their Kovats retention indices on HP-5MS capillary column. [b]KI: Kovats retention Index on HP-5MS column. [c]RI: Retention time. [d]Microwave assisted hydrodistillation. [e]Hydrodistillation. [f]Not identified = totally *ca.* 10.93 and 5.96 % against 89.07 and 94.04 % in MAD and HD methods.

In HD method, twelve compounds were identified representing 94.04 % of the leaves oil: including one monoterpenoid hydrocarbons (MH = 9.47 %), three oxygenated monoterpenes (OM = 48.51 %), seven sesquiterpenoid hydrocarbons (SH = 30.27 %) and one oxygenated sesquiterpenoid (OS = 2.1 %). As regards this information, a ranking order of the groups of constituents as OM > SH > MH > OS is observed in the HD method. In this oil, 1,8-cineole (48.51 %), aromadendrene (18.31 %), α-pinene (9.47 %), alloaromadendrene (4.67 %), ledene (2.95 %), *trans* pinocarveol (2.23 %), spathulenol (2.10 %) and pinocarvone (1.46 %) were the major compounds.

In MAD method (power 300 W, time 10 min), sixteen compounds were identified representing 89.07 % of the oil comprising: one monoterpenoid hydrocarbons (3.67 %), four oxygenated monoterpenes (49.57 %), nine sesquiterpenoid hydrocarbons (33.48 %), one oxygenated sesquiterpenoid (1.38 %) and one non-terpenoid hydrocarbon (NH = 0.97 %). Therefore, we can see the classes percentage order as follows: in the MAD method. In this oil, the major constituent was 1,8-cineol (42.10 %) followed by aromadendrene (19.5 %), all-oaromadendrene (5.41 %), *trans* pinocarveol (3.85 %), α-pinene (3.67 %), ledene (2.94 %), pinocarvone (2.87 %) and spathulenol (1.38 %) as the other dominant constituents.

Effect of microwave power on essential oil yield: Essential oil yield was expressed in terms of the volume of the oil collected in mL/g of original plant material.

Essential oil yield decrease with increasing microwave power. The lower yield can be attributed to the loss of some of the volatile compounds due to longer processing time in conventional hydrodistillation process and higher microwave power of MAD. Results tabulated in Table-2, each experiment was repeated three times.

TABLE-2
EFFECT OF MICROWAVE POWER ON ESSENTIAL OIL YIELD

Strategy	Power (watt)	Yield (%)		
		Time (min)		
		10	20	180
HD	–	–	–	0.29
MAD	300	1.72	1.44	–
	500	1.10	1.10	–
	700	0.66	0.20	–
	900	0.20	–	–

Effect of microwave power on extraction yield of the major components of *E. microtheca* oil: Effect of microwave powers on extraction of α-pinene, 1,8-cineole, allo-aromadendrene, spathulenol and viridiflorol were investigated. Times of all experiments are within 10 and 20 min periods. The respective results are given in Figs. 1-5. These figures demonstrate the variation of some major components of *E. microtheca* with time for MAD at different power levels. The increase in sesquiterpenes (allo-aromadendrene) with extraction time is probably due to their higher molecular weights, lower volatilities and lower solubilities in water than the other constituents. More time is required for their concentration to reach their maximum levels in the essential oil. The decrease in monoterpene (α-pinene) concentration during the later stages of extraction may be owing to the enhanced leaching

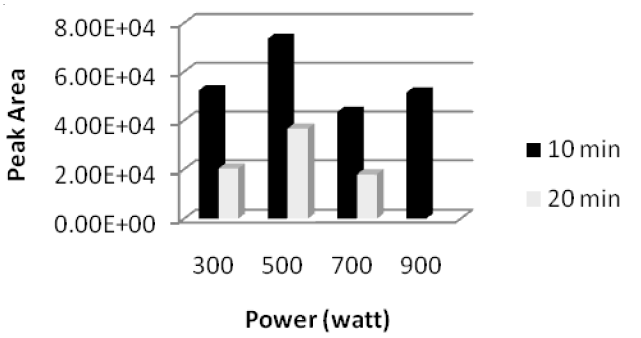

Fig. 1. Effect of microwave power on extraction of a-pinene

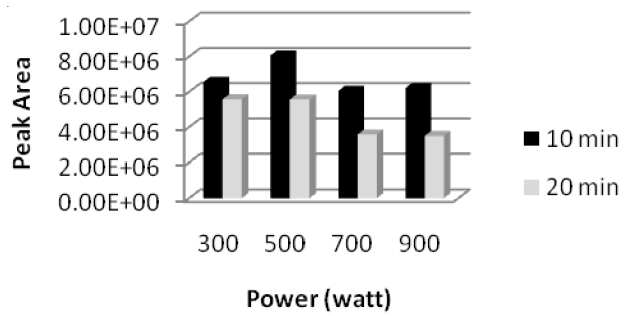

Fig. 2. Effect of microwave power on extraction of 1,8-cineole

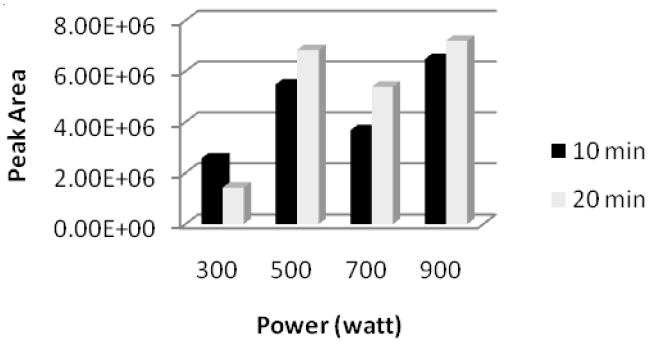

Fig. 3. Effect of microwave power on extraction of allo-aromadendrene

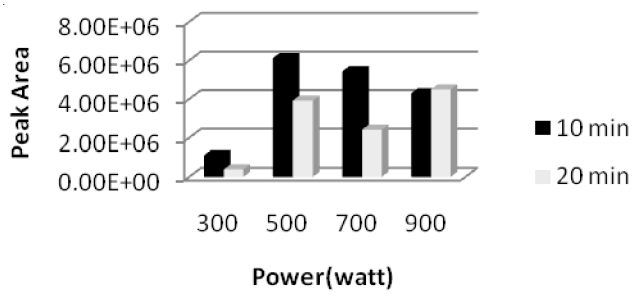

Fig. 4. Effect of microwave power on extraction of spathulenol

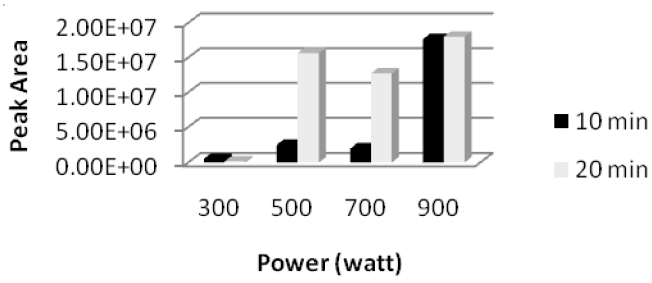

Fig. 5. Effect of microwave power on extraction of viridiflorol

of substances which are more difficult to be extracted. Such substances trend to have a dilution effect on the monoterpenes. Concentration of monoterpenes seems to be reduced with increase in the concentration of oxygenated compounds and sesquiterpenes. In addition, monoterpene hydrocarbons are known to be very unstable against heat and light, so the decrease in their concentration may also due to possible degradation caused by thermal or hydrolytic effects. It is difficult to say that a same trend was obtained in the variation of the amounts of 1,8-cineole with power. The extraction of oxygenated compounds was very rapid in microwave accelerated distillation level because of pressure build-up inside the plant material.

Chemical profiles in other species of *Eucalyptus*: In the comprehensive reports concerning about chemical composition of *Eucalyptus* species essential oils, the most abundant compounds were oxygenated monoterpenes. In all of these oils 1,8-cineole was the main component of the oils[8,10,11,15-17]. The water-distilled essence of *E. globules* was rich in sesquiteropenoids[18] in contrast with the some reports[10,15]. Furthermore, most of the structures were composed of monoterpenoid hydrocarbons in the *E. camaldulensis* leaf essential oil[11] as well as oils of *E. robusta* and *E. saligna*[19].

Conclusion

As can be seen from Table-1, the composition of the essential oils obtained by both methods is approximately the same and comparable. The major compounds present in essential oils of *E. microtheca* by hydrodistillation and microwave accelerated distillation methods showed expected similarities since both samples are of the same genus.

However, the amounts of oxygenated compounds are higher than hydrodistillation in the oil extracted by microwave. The main common components of *E. microtheca* essential oil were determined to be 1,8-cineole, aromadendrene, α-pinene, allo-aromadendrene, ledene, spathulenol and viridiflorol both in hydrodistillation and in microwave accelerated distillation procedures. Microwave accelerated distillation of *E. microtheca* was compared with conventional hydro distillation in terms of process time, yield and composition of the oil. Effects of microwave powers were evaluated on chemical composition of this volatile oil. It was concluded that microwave accelerated distillation could significantly offer higher essential oil yields than conventional hydrodistillation and could reduce the process time. Therefore, it can be concluded that microwave accelerated distillation, which is a simple and time-saving method, is a superior alternative for the extraction of this essential oil. Also, results show that, each type of compounds of microtheca essential oil compounds have an optimum microwave power for efficient extraction and this power should be used to enhance useful components of essential oil and to save energy[19-22].

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