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Effects of different distillation methods on essential oil content and composition of *Lippiacitriodora* H.B.K.

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Abstract

The world production and consumption of essential oils and perfumes are increasing very fast. Production technology is an essential element to improve the overall yield and quality of essential oil. Hydro-distillation (HD), water and steam distillation (WSD) and steam distillation (SD) are the most traditional and commonly used methods. To study the effect of nine distillation methods (HD by Clevenger type apparatus (HDC), HD by Microwave (HDM), Steam distillation (SD), SD by Microwave (SDM), Water and Steam distillation by Kaiser and Lang type apparatus (Kaiser and Lang WSD), Microwave WSD, Microwave Dry distillation, Industrial SD and Industrial HD.) on volatile oil content and composition of lemon verbena (*Lippiacitriodora* H.B.K.), an experiment was conducted as a Completely Randomized Design (CRD) with three replicates. The essential oils were analyzed by GC and GC/MS. Result showed that the highest level of α-Pinene, Sabinene, 1,8-Cineole, ɣ-Terpinene, cis limonene oxide and citronellal were obtained by HDC, cis limonene oxide and α-terpinyl acetate from HDM, cis limonene oxide and α-terpineol from SD, cis limonene oxide, cissabinol, α-terpineol, neral, geranial and α-terpinyl acetate from SDM, α-terpinyl acetate, geranyl acetate and cubenol from Kaiser and Lang WSD, α-terpinyl acetate, geranyl acetate, cubenol, spathulenol, globulol and epi-α-cadinol from Microwave WSD, α-terpinyl acetate, geranyl acetate, ɣ-elemene and epi-α-cadinol from Microwave Dry distillation, Limonene, trans pinocarveol, cissabinol, α-terpinyl acetate, geranyl acetate, E-caryophyllene and α-humulene from Industrial SD and β-Pinene and cissabinol from Industrial HD. The yield of essential oil was highest in HDC, HDM, SDM and Microwave WSD. Citral is a valuable flavor and scent reagent that is heavily used in the food and perfume industries .According to the results, the SDM method with the highest content of citral and yield of essential oil is suggested as a suitable method for essential oils extraction of lemon verbena. Also, this method had relatively suitable time and temperature for oil extraction and could be proposed as an efficient method considering energy concerns.

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Introduction

Medicinal herbs are some of our oldest medicines. Nowadays increasing use is a clear evidence of public interest in having alternatives to conventional medicine. However, they have not been tested with required methods for conventional pharmaceuticals (Portmann*et al.*, 2012). Aromatic plants represent a renewable source of flavoring substances, which can be used in the food, perfumery and pharmaceutical industries (Gharib, 2006). Currently the Verbenaceae family is composed of 100 kinds and approximately 2000 species of wide geographical distribution, including tropical, subtropical and moderate regions. The family is characterized for including aromatic species mostly used in the traditional and popular medicine (Portmann*et al.*, 2012). The genus *Lippia* (Verbenaceae) includes approximately 200 species of herbs, shrubs and small trees. The genus *Lippia* shows a rich genetic diversity, enabling it to synthesize a variety of essential oil constituents in plants grown in different parts of the world (Agah and Najafian, 2012). Lemon verbena, *Lippiacitriodora* is of Verbenaceae family. This plant is found at South Africa and some parts of Asia and is planted in some area for plant essence production (Mohammadi*et al.*, 2013). Generally, the leaves are being used for flavoring beverages, desserts, fruit salads and jellies and also for seasoning food. A decoction made from the leaves and flowers is given as febrifuge, sedative and anti-flatulent. The plant showed antispasmodic, antimicrobial properties and is traditionally used to treat Candida. The volatile oil obtained from the leaves collected from different places showed differently (Rao*et al.*, 2013).

Essential oils are a diverse group of natural products that are important sources of aromatic and flavoring chemicals in food, industrial and pharmaceutical products. Essential oils are largely composed of terpenes and aromatic polypropanoid compounds derived from the acetate-mevalonic acid and the shikimic acid pathways, respectively. Essential oil composition of plants varies and is due to genetic and environmental factors that influence genetic expression. The essential oil content of plant tissue also varies with developmental stage and can vary by distillation methods. Techniques commonly employed for extracting essential oils include hydro-distillation, steam distillation, solvent extraction, head space analysis and liquid CO_2 extraction (Charles and Simon, 1990). The proportion of different essential oils extracted by steam distillation is 93% and the remaining 7% is extracted by the other methods. Essential oils are multi-component chemicals. The mixture of oil compounds that constitute the essential oil comprises polar and non-polar compounds. Some of the compounds in the composite oil are lost in the wastewaters. During steam distillation of essential oils, the recovery of all organic constituents as the product depends on their partition between water and oil phases of the distillate. In many steam distillation processes, vegetable material is mixed with water and the system is brought to a boil, a process commonly referred to as hydro-distillation (Masango, 2005). The composition of the extracted oil may vary from one extraction method to another (Charles and Simon, 1990). In this paper, we compare the influences of nine extraction methods on measurements of essential oil content and composition in *Lippiacitriodora* and select a rapid and economic extraction method for this aromatic plant among the methods used.

Materials and methods

Lippiacitriodora were field-grown at the medicinal plant Research Farm (Miyaneh, located in East Azerbaijan province, Iran) during summer 2013. The leaves were dried naturally on laboratory benches at room temperature (23-27 °C) until crisp. To evaluation of extraction methods on volatile oil content and composition of lemon verbena, an experiment was conducted as a Completely Randomized Design (CRD) with three replicates in the laboratory of horticulture at Islamic Azad University- Miyaneh branch, Miyaneh, Iran.

Essential oils were obtained by nine distillation methods contained: Hydro-distillation by Clevenger type apparatus (HDC), HD by Microwave (HDM), Steam-distillation (SD), SD by Microwave (SDM) (Fig 1), Water and Steam distillation by Clevenger type apparatus (Kaiser and Lang WSD), Microwave WSD, Microwave Dry distillation (Just soak in water before distillation without mix with water), Industrial SD and Industrial HD. The essential oils were separated from the aqueous layer, dried over anhydrous sodium sulfate and stored in sealed vials at low temperature (4°C) before Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometric (GC-MS) analysis.Essential oil content was defined as followed:

R (%) = (mass essential oil/mass of the dried leaves) x 100 (Agah and Najafian, 2012)

Fig. 1. A View of the Microwave steam distillation (SDM).

GC and GC/MS analysis

GC analyses were performed using a gas chromatograph (GC) (Thermo-UFM). The GC column was ph-5, 10m, 0.1mm (ID), 0.4 (FT), Oven: 60-285 *C/min, Rate: 80 *C/min, Hold time: 3 min, Run Time: 5.8min. Detector: FID, 280 *C, Injector: 280 *C, Carrier Gas: He, 0.5ml/min With Chrom-card soft ware in Research Institute of Forests and Rangelands, Tehran.

GC-MS analyses were carried out on a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d.); Oven temperature was 40°C to 250°C at a rate of 4°C, transfer line temperature 260° C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, Ionization energy 70 eV; scan time 1s and mass range of 40-300 amu. The percentages of compounds were calculated by the area normalization method, without considering response factors. The components of the oil 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 or with data published in the literature (Shibamoto, 1987; Adams, 1995). The retention indices were calculated for all volatile constituents using a homologous series of n-alkanes.

Statistic analysis

The data were analyzed by using SAS ver. 9.1 statistical software. Means were compared using Duncan's Multiple Range Test (DMRT) at 5% probability level.

Result and discussion

In essence performance, methods HD by Clevenger type apparatus, HDM, SDM and Microwave WSD were not significantly different and ranked the highest probability level (Table 3).

More than 23 volatile components have been characterized as constituents of *L.citriodora* oil. The identified constituents with their RIs are summarized in Table 1.

Table 1. Chemical composition of *L.citriodora* identified by GC/MS.

N	Compounds	RI	N	Compounds	RI
1	α-Pinene	940	13	neral	1240
2	Sabinene	976	14	geranial	1269
3	β-Pinene	980	15	α-terpinyl acetate	1352
4	Limonene	1039	16	geranyl acetate	1380
5	1,8-Cineole	1031	17	E-caryophyllene	1420
6	ɣ-Terpinene	1060	18	ɣ-elemene	1438
7	Terpinolene	1090	19	α-humulene	1456
8	cis limonene oxide	1138	20	cubenol	1517
9	trans pinocarveol	1141	21	spathulenol	1580
10	cissabinol	1145	22	globulol	1587
11	Citronellal	1155	23	epi-α-cadinol	1640
12	α-terpineol	1191			

The analysis of variance indicated that all measured compounds except terpinolene were significantly different at 1% of probability (Table 2).

Table 2a. Analysis of variance for volatile oil composition and content of *Lippiacitriodora* H.B.K. affected by extraction methods.

S.O.V.	D.F.	Mean of squares											
		α-Pinene	Sabinene	β-Pinene	Limonene	1,8-Cineole	ɣ-Terpinene	Terpinolene	cis limoneneoxide	transpinocarveol	cissabinol	Citronellal	α-terpineol
distillation method	8	0.45**	0.24**	5.08**	33.17**	35.73**	0.2**	0.02ns	0.11**	0.14**	0.03**	0.12**	0.24**
error	18	0.002	0.0009	0.02	0.044	0.103	0.003	0.019	0.007	0.006	0.006	0.007	0.007
C.V. (%)		7.93	6.78	3.32	1.34	1.83	3.67	10.92	11.69	25.09	27.59	10.33	6.08

ns: non-significant, * and ** : significant at 5% and 1% of probability levels, respectively.

Table 2b. Analysis of variance for volatile oil composition and content of *Lippiacitriodora* H.B.K. affected by extraction methods.

SOV	DF	Mean of squares											
		neral	geranial	α-terpinylacetate	geranylacetate	E-caryophyllene	ɣ-elemene	α-humulene	cubenol	spathulenol	globulol	epi-α-cadinol	Essentialoil%
distillation method	8	12.7**	55.9**	0.04*	0.04**	0.75**	5.77**	0.18**	0.19**	7.76**	11.98**	0.05**	0.03**
error	18	0.01	0.012	0.013	0.007	0.01	0.008	0.008	0.009	0.018	0.008	0.006	0.006
C.V. (%)		0.69	0.56	12.31	20.56	9.98	1.64	13.99	11.43	2.66	1.52	16.53	10.84

ns: non-significant, * and ** : significant at 5% and 1% of probability levels, respectively.

Mean comparison of *Lippia* essence measurement indicated that in HD by Clevenger type apparatus method, α-Pinene, Sabinene, 1,8-Cineole, ɣ-Terpinene, cis limonene oxide and citronellal, in HDM method, cis limonene oxide and α-terpinyl acetate, in SD by Clevenger type apparatus method, cis limonene oxide and α-terpineol, in SDM method, cis limonene oxide, cissabinol, α-terpineol, neral, geranial and α-terpinyl acetate, in Kaiser and Lang WSD method, α-terpinyl acetate, geranyl acetate and cubenol, in Microwave WSD method, α-terpinyl acetate, geranyl acetate, cubenol, spathulenol, globulol and epi-α-cadinol, in Microwave Dry distillation method, α-terpinyl acetate, geranyl acetate, ɣ-elemene and epi-α-cadinol, in Industrial SD method Limonene, trans pinocarveol, cissabinol, α-terpinyl acetate, geranyl acetate, E-caryophyllene and α-humulene and in Industrial HD method β-Pinene and cissabinol were at the highest probability level (Table 3).

Table 3a. The effect of extraction methods on volatile oil composition of *Lippiacitriodora* H.B.K.

Component / Treatment	α-Pinene	Sabinene	β-Pinene	Limonene	1,8-Cineole	ɣ-Terpinene	cis limonene oxide
HDC	1.13a	0.75a	5.53b	18.07b	23.05a	1.91a	0.94a
HDM	0.76c	0.79a	4.52d	15.64d	20.43b	1.57cd	0.8a
SD	0.1g	0.53c	4.62d	17.3c	20.44b	1.69b	0.95a
SDM	0.01h	0.51c	2.68g	9.55g	13.67g	1.66bc	0.91a
Kaiser and Lang WSD	0.35f	0.01f	4.1e	17.68c	16.34e	1.26e	0.65b
Microwave WSD	0.44e	0.61b	3f	12.12f	15.04f	1.53d	0.61b
Dry Microwave	0.52d	0.29e	2.5g	13e	12.66h	1.17ef	0.6b
Industrial SD	1.02b	0.02f	5.28c	19.54a	17.28d	1.15f	0.51bc
Industrial HD	0.77c	0.42d	6.13a	17.65c	18.78c	1.49d	0.45c

Means in each column with the same letters are not significantly different at 5% level of probability DMRT.

Table 3b. The effect of extraction methods on volatile oil composition of *Lippiacitriodora* H.B.K.

Treatment	trans pinocarveol	cissabinol	citronellal	α-terpineol	neral	geranial	α-terpinyl acetate	geranyl acetate
HDC	0.29c	0.23c	1.2a	1.29bc	11.7h	15.1h	0.83bc	0.4bc
HDM	0.35bc	0.25bc	0.71cde	1.4b	12.3f	16.09g	1.04ab	0.35bc
SD	0.33bc	0.25bc	0.69def	1.83a	11.99g	18.4e	0.79c	0.4bc
SDM	0.39bc	0.33abc	0.85bc	1.95a	17.79a	29.39a	0.84abc	0.2d
Kaiser and Lang WSD	0.01d	0.19c	0.55f	1.21cd	11.31i	16.8f	1abc	0.5ab
Microwave WSD	0.01d	0.18c	0.61ef	1.3bc	12.75d	21.03c	1.05a	0.51ab
Dry Microwave	0.25c	0.25bc	0.83bcd	1.09d	13.4c	22.6b	1.01abc	0.56a
Industrial SD	0.7a	0.45a	0.96b	1.35bc	12.51e	19.14d	0.95abc	0.45abc
Industrial HD	0.45b	0.4ab	0.69def	1.33bc	15.15b	19.01d	0.79c	0.33cd

Means in each column with the same letters are not significantly different at 5% level of probability DMRT.

Table 3c. The effect of extraction methods on volatile oil composition of *Lippiacitriodora* H.B.K.

Component / Treatment	E-caryophyllene	ɣ-elemene	α-humulene	cubenol	spathulenol	globulol	epi-α-cadinol	essence %
HDC	0.55ef	4.2f	0.4e	0.69de	3.85f	5.09g	0.29e	0.85a
HDM	0.7de	5.4d	0.5e	0.55e	5.74c	7.2d	0.46bcd	0.83a
SD	0.61ef	4.85e	0.55de	0.69de	4.1e	5.29f	0.35de	0.59c
SDM	0.49f	4.2f	0.4e	0.89c	5.35d	5.49e	0.45cd	0.8ab
Kaiser and Lang WSD	0.99c	6.61b	0.69cd	1.1ab	6.52b	7.9b	0.6ab	0.68bc
Microwave WSD	0.81d	6.59b	0.75bc	1.25a	7.15a	8.95a	0.65a	0.87a
Dry Microwave	1.35b	8.29a	0.9b	1.07b	6.35b	7.5c	0.57abc	0.65bc
Industrial SD	1.95a	5.79c	1.1a	0.55e	2.51h	3.15i	0.3e	0.68bc
Industrial HD	1.49b	4.35f	0.43e	0.8cd	3.3g	3.5h	0.47bcd	0.65bc

Means in each column with the same letters are not significantly different at 5% level of probability DMRT.

Based on Table 4, the SDM method had relatively suitable time and temperature for essence detection (measurement) and could be proposed as an efficient method considering energy concerns. Duration of essential oil extraction affected on the quantity and quality of essential oil. Scientists reported that essential oil percentage and essential oil component of fennel were affected by duration of essential oil extraction (Khorshidi*et al.*, 2009).

Table 4. Time and temperature for different extraction methods.

Distillation method	HDC	HDM	SD	SDM	Kaiser and Lang WSD	Microwave WSD	Dry Microwave	Steam	Hydro-D
Distillation time (min)	90	35	90	45	90	45	35	90	90
Temperature (°C)	94	80	94	80	94	80	80	94	94

The quality and quantity of essential oil isolated from the leaves of *Lippiacitriodora* which were extracted by different methods are shown in Table 3; result clearly shows that the oil traits linked to the extraction method. The analysis of *L. citriodora* essential oil revealed that neral, geranial, limonene and 1,8- cineole were the main components of the oil and each of these compounds is 10 to 25 percent. Citral, the name given to the mixture of the monoterpene aldehydes geranial and neral, imparts a strong "lemony" scent and is known to be emitted or accumulated in such herbs as lemon grass, ginger and some varieties of sweet basil. Citral is a valuable flavor and scent reagent that is heavily used in the food and perfume industries. Several previous investigations have reported that citral is synthesized from geraniol or nerol by an alcohol dehydrogenase or alcohol oxidase, but an enzyme capable of catalyzing such a reaction has not yet been purified and characterized (Iijima*et al.*, 2006). Furthermore, lesser amounts of the other components include α-pinene, sabinene, transpinocarveol, geranyl acetate, α-humulene and epi-α-cadinol were existed in essential oil of this plant. According to research findings, the main components in the essential oils of *L. citriodora* leaves were geranial, neral, limonene, 1,8-cineole, spathulenol, geraniol, trans-β-caryophyllene, nerol and sabinene. The main constituents of the essential oil extracted from fresh leaves of *L. citriodora*, were geranial, neral and limonene. Also, GC-MS analysis of essential oils revealed that 1, 8-Cineole, α-curcumene, geranial,

limonene and caryophyllene oxide were the main components of essential oils of *L. citriodora* leaves, respectively (Khani*et al.*, 2012). The antimicrobial activity of 1,8-cineole has also been reported (Folashade and Omoregie, 2012). The main uses of *Eucalyptus* oils are for the pharmaceutical industry (those that are rich in 1,8-cineole), perfumery (those that are rich in citronellal) and for industrial use (those that have piperitone and α-phellandrene) as their main constituents (Fathi and Sefidkon, 2012). According to the literature, geranial, neral and limonene were the component found to occur in higher quantities in essential oils of the *L. citriodora* (Khani*et al.*, 2012) similarly in our study, neral, geranial, 1,8-cineole and limonene were the major compounds of Lemon verbena and according to result, geranial and neral was significantly increased by SDM.

Although volatile oils are produced by different methods such as solvent extraction, expression and critical fluid extraction, most are produced by steam distillation (Masango, 2005). The effect of different distillation methods on oil content and composition of aromatic plants have also been previously reported. For example, one study showed that the main component in the essential oil of *Eucalyptus dealbata* in three different distillation method (hydro-distillation, water and steam distillation and direct steam distillation) was 1,8-cineole and hydro-distillation gave the highest percentage of 1,8-cineole. Also, hydro-distillation of *Eucalyptus camaldulensis* gave the higher oil yield and 1,8- cineole percentage than steam-distillation (Fathi and Sefidkon, 2012).

Finally it could be concluded that SDM method is suitable for highest essential oil quantity and quality (high level in neral and geranial concentration) and Microwave WSD method is unsuitable for quality (high level in spathulenol, globulol and epi-α-cadinol concentration).

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