



## Comparative study of the essential oil and hydrolate composition of *Lythrum salicaria* L. obtained by hydro-distillation and microwave distillation methods

A. Manayi<sup>1</sup>, S. Saeidnia<sup>1</sup>, M. Shekarchi<sup>2</sup>, A. Hadjiakhoondi<sup>1,3</sup>, M.R. Shams Ardekani<sup>3,4</sup>, M. Khanavi<sup>3,4\*</sup>

<sup>1</sup>Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

<sup>2</sup>Food and Drug Control Laboratories and Food and Drug Laboratory Research Center, MOH & ME, Tehran, Iran.

<sup>3</sup>Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

<sup>4</sup>School of Traditional Medicine and Persian Medicine and Pharmacy Research Center, Tehran University of Medical Sciences, Tehran, Iran.

### Abstract

Essential oils are considered as very complex natural mixtures containing numerous components at quite various concentrations. *Lythrum salicaria* has not been previously subjected to examination of its volatile oil. The present study was carried out to evaluate the essential oil composition of the flowering aerial parts of the plant by using conventionally hydro-distillation (HD) and microwave assisted hydro-distillation (MAH) methods along with the aromatic water obtained by hydro-distillation. Components of these three samples were recognized by GC/MS. Identification of components resulted in recognition of 14 (98% oil), 32 (98.4%), and 10 (95.5%) compounds for HD, its hydrolate, and MAH samples, respectively. The predominant constituents in the HD oil were bis (2-ethylhexyl) phthalate (29.2%), *n*-hexadecanoic acid (22%), and pentacosane (9.5%). The major constituents of the hydrolate were elucidated as 2(4H)-benzofuranone-5,6,7,7a-tetrahydro-4,4,7a-trimethyl (6.4%), neryl acetone (6%), pentanoic acid (5.7%), nonanoic acid (5.6%), and octanoic acid (5.5%). Accordingly, bis (2-ethylhexyl) phthalate (43.2%), methyl-cyclopentane (36.5%), and pentacosane (4.7%) were assessed as the main components of MAH oil. Comparing the three samples compositions, it was concluded that the extraction procedure led to variations in quality and quantity of volatiles. Besides, bis (2-ethylhexyl) phthalate, the common plasticizer, was identified as the main pollutant in both HD and MAH oils. The presence of this substance in the oils provided the evidence of contaminations that was absorbed from water into the plant suggesting GC/MS as a suitable method for its detection in plant materials.

**Keywords:** hydro-distillation, hydrolate, Lythraceae, *Lythrum salicaria*, microwave, phthalate contamination

### Introduction

Essential oils are characterized as volatile, natural, complex compounds with a strong odor

which are produced by aromatic plants as secondary metabolites [1]. Major constituents of

the essential oils consist of hydrocarbons, esters, terpenes, lactones, phenols, aldehydes, acids, alcohols, ketones, and esters. The world production and consumption of essential oils and perfumes are quickly increasing [2]. They might be extracted by several methods, for instance using microwave, or mainly low or high pressure distillation by boiling water or hot steam [1].

Extraction of essential oils using microwave energy has been recently elaborated. There are some advantages for this method, which include noncontact heat source, fast energy transfer, decrease in extraction time and solvent used [3]. A traditional way for isolation of volatile compounds is hydro-distillation, in which the material is in direct contact with boiling water to release the essential oil through evaporation [4].

*Lythrum* (Lythraceae) is well represented in the Flora Iranica by 30 species, of which seven are widely growing in Iran [5]. *Lythrum salicaria* is found in the North and North-West of Iran in humid areas as a wild plant which is known as “turbinkwash”, “yerpoose”, and “Surmankhal” by local people [6]. Flowering aerial parts of the plant have been traditionally used in the treatment of diarrhea, chronic intestinal catarrh, hemorrhoid, eczema, varicose veins, bleeding of the gums and vaginitis [7,8]. In the previous phytochemical investigations of this plant, some phenolic components such as tannins, anthocyanins and flavonoids have been reported [7]. The extract has been reported to possess antifungal and antibacterial components and has exhibited antioxidant activity with similar potency as the standard substance [9-12]. Additionally, the plant extract has decreased blood glucose levels in both hyperglycemic and normoglycemic rats and rabbits, and has also showed anti-diarrheal effects [13-15]. Moreover in previous studies, total extract and fractions of the plant have been analyzed for cytotoxic activity against cancer cell lines by MTT assay [16].

As far as our literature survey could ascertain, the composition of the oil of *L. salicaria* has not been studied before. The aim of the present study

was to assess the chemical composition of the plant oil by hydro-distillation (HD) and microwave assisted hydro-distillation (MAH) methods. The hydrolate obtained from hydro-distillation method was also separated and analyzed by GC/MS.

## Experimental

### *Plant material*

Flowering aerial parts of *L. salicaria* were collected from Lahijan province (74 m) in May 2011. The plant was identified by Mr. Yousef Ajani and deposited in Central Herbarium of Medicinal Plants, Jahade-Daneshgahi (ACECR), Karaj, Iran (Ajani 313). The aerial parts of the plant were cleaned and dried in the shade at room temperature. They were crushed for investigation of essential oil constituents in both methods.

### *Isolation of essential oil*

200 g of air-dried aerial parts of the plant were ground and submitted to hydro-distillation in a Clevenger-type apparatus for 4 h to give a colorless oil. Subsequently, the hydrolate was separated using diethyl ether (200 mL). Afterward, diethyl ether was evaporated at room temperature [17], resulting the remained hydrolate with yellowish color. Both the essential oil and the hydrolate were dried over anhydrous sodium sulfate to yield 0.2% and 0.6% oils, respectively.

The microwave oven used for essential oil extraction was OSUN (P.R.C) operating at 2450 MHz. The volume of the interior cavity of the oven was 241 cm<sup>3</sup>. Microwave oven was modified by drilling a hole at the top. A flat bottom flask having a capacity of 500 mL was placed in the oven and connected to the 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 polytetrafloraethylene (teflon) to prevent leakage of microwave. 100 g of the plant powder together with 100 mL distilled water in a flat bottom flask were homogenized and placed at 800 W for 15 min. The essential oil from

microwave method was dried by anhydrous sodium sulfate to yield 0.1 % of colorless oil. All the above mentioned volatiles were stored at 4 °C in the amber sealed glasses.

#### GC/MS analysis

The GC/MS analysis of the essential oils was performed using Agilent Technologies GC equipped with a HP-5MS capillary column (30 m × 0.22 mm id, 0.25 µm film thickness) and a mass spectrometer 5973 from the same company, for GC/Mass detection with an electron ionization system (70 eV). Helium was the carrier gas, at a flow rate of 1 mL/min, the injector and detector temperatures were set at 250 and 230 °C, respectively. Column temperature was initially kept at 50 °C for 5 min then gradually increased to 265 °C at the rate of 2.5 °C/min; Retention indices were calculated using retention times of *n*-alkanes, injected after the oil, at the same chromatographic condition. The compounds were successfully identified by comparison of the retention indices (RI, HP-5) with those reported in the literature together with comparing of their mass spectra to those in the Adams, NIST and Wiley libraries [18-22].

#### Results and Discussion

The compositions of the oils were successfully analyzed by using GC/MS and the components were identified by comparing their mass spectra and retention indices (RI) with those reported previously. The present study described, to our knowledge for the first time, the essential oil composition of the genus *Lythrum*. Analysis of the oils revealed the presence of 14 components in HD oil and 32 components in the hydrolate representing 98 and 98.4% of the total oil, respectively. Additionally, MAH oil consisted of 10 components representing 95.5% of the total oil. The most prominent components found in HD oil were bis (2-ethylhexyl) phthalate (29.2%), *n*-hexadecanoic acid (22%), pentacosane (9.5%), and methyl-cyclopentane (7.5%). The major constituents of the hydrolate were identified as 2(4H)-benzofuranone-5,6,7,7a-

tetrahydro-4,4,7a-trimethyl (6.4%), neryl acetone (6%), pentanoic acid (5.7%), nonanoic acid (5.6%), octanoic acid (5.5%), and docosane (5%). Furthermore, the main components of MAH oil were bis (2-ethylhexyl) phthalate (43.2%), methyl-cyclopentane (36.5%), and pentacosane (4.7%). On the basis of the results, the main parts of these oils were consisted of oxygenated hydrocarbons (47.9-67.7%), although it was obvious, the MAH oil was relatively rich in hydrocarbons (46.4%). Monoterpenes in the oils existed in different amounts, besides the hydrolate contained higher amount of oxygenated monoterpenes. It has been found that 75.3% of the components in the hydrolate have oxygen in their structure which made them more soluble in water. The results revealed that the identified components in all volatiles were virtually different (table 1). It has been reported that furans are greatly formed during thermal degradation of fatty acids [23]. In the present study, furans were just recognized in the hydrolate of the plant. Moreover, sesquiterpenes were only recognized in the HD oil (2.4%). It is well documented that slightly fewer compounds were extracted by microwave compared to the HD method [24]. The current results also showed that microwaved oil was in smaller amount than other volatiles and also fewer components were identified. These differences may be attributed to the different procedures of obtaining the oils with various heat sources. It was previously mentioned that microwave yields essential oil of relatively low volatile fractions as compared to hydro-distillation [2]. Comparison of these three oils has revealed that the main parts of the hydrolate and the HD oil were oxygenated compounds of different structures. It seems that, oxygenated volatiles in the hydrolate had mainly dissolved in water during HD process and could be extracted with diethyl ether. Both, oxygenated monoterpenes and sesquiterpenes were only found in the oils that obtained by HD method. However, in MAH oil, the percentage of methyl-cyclopentane and bis (2-ethylhexyl) phthalate were considerable whereas other components

**Table 1.** Composition of the essential oils obtained from *L. salicaria* by hydro-distillation, its hydrolate, and by microwave assisted hydro-distillation

NO.	Identified Compounds	KI	RT	Content (%)		
				Hydrodistillation	Hydrolate	Microwave
1	methyl-cyclopentane	536	4.8	7.5	3.7	36.5
2	2-methylhexane	637	6.3	2.6	0.9	-
3	2-pentanone, 4-hydroxy-4-methyl	927	13.7	-	2	-
4	pentanoic acid	993	15.7	-	5.7	-
5	1-octen-3-ol	993	15.7	-	-	0.9
6	3-hexenoic acid (trans)	997	15.8	-	2	-
7	2-hexenoic acid (trans)	1020	16.1	-	1.2	-
8	benzyl alcohol	1050	16.4	-	2.6	-
9	limonen	1050	16.4	-	-	1.2
10	<i>o</i> -cresol	1060	16.5	-	0.4	-
11	heptanoic acid	1070	16.6	-	0.8	-
12	<i>p</i> -cresol	1080	16.7	-	0.4	-
13	linalool	1100	16.9	-	1.1	-
14	2-ethylhexanoic acid	1111	17	-	1	-
15	benzeneethanol	1133	17.2	-	3.1	-
16	octanoic acid	1166	17.5	-	5.5	-
17	alpha-terpineol	1212	17.9	-	1.7	-
18	2,3-dihydro-benzofuran	1225	18	-	6	-
19	1H-pyrrole-2,5-dione,3-ethyl-4-methyl	1237	18.1	-	2.3	-
20	nonanoic acid	1257	18.2	-	5.6	-
21	2,4-decadienal (E,E)	1337	18.8	-	1.4	1.7
22	2-methoxy-4-vinylphenol	1342	18.9	-	4.2	-
23	decanoic acid	1357	19	0.9	2.6	0.8
24	5-pentyl-2(5H)-furanone	1371	19.1	-	3.2	-
25	eugenol	1385	19.2	-	2.7	-
26	neryl acetone	1457	19.7	1.3	6	-
27	beta-ionone (trans)	1516	20.1	-	3.9	-
28	dodecanoic acid	1550	20.3	2.3	3.4	1.3
29	2(4H)-benzofuranone-5,6,7,7a-tetrahydro-4,4,7a-trimethyl	1600	20.6	-	6.4	-
30	hexadecane	1600	20.6	-	-	2
31	octadecane	1700	22	-	1.6	-
32	tetradecanoic acid	1783	22.1	6.5	-	-
33	2-pentadecanone-6,10,14-trimethyl	1842	22.5	4.8	-	-
34	4,8,12-tetradecatrienal-5,9,13-trimethyl	1850	22.6	2	-	-
35	nonadecane	1875	22.9	2.3	3	-
36	farnesyl acetone	1922	23.4	2.4	-	-
37	<i>n</i> -hexadecanoic acid	1955	23.7	22	-	-
38	eicosane	2000	24.1	-	4.5	-
39	heneicosane	2100	25.5	-	4.5	-
40	docosane	2200	27.4	-	5	-
41	tricosane	2300	29.8	4.7	-	3.2
42	pentacosane	2500	36.9	9.5	-	4.7
43	bis (2-ethylhexyl) phthalate	2509	40	29.2	-	43.2
monoterpenes				1.3	15.4	1.2
sesquiterpenes				2.4	-	-
C <sub>x</sub> H <sub>y</sub>				26.6	23.2	46.4
C <sub>x</sub> H <sub>y</sub> O <sub>z</sub>				67.7	59.8	47.9
<b>Total</b>				98	98.4	95.5

KI: Kovats Index on HP-5MS with reference to n-alkanes injected after the oil at the same chromatographic condition, RT: Retention Time (min)

were identified in lower amounts. This might be due to the poor stability of these components. The oil of *L. salicaria* obtained by MAH contained greater quantities of volatiles with higher boiling points and fewer quantities of compounds of lower stability. The results of the previous study stated that microwave extraction

of the essential oil from *O. vulgare* was a good alternative for volatile oil preparation comparable to the essential oil obtained by HD method [25]. The results of the present examination are not in consistency with previous studies which had introduced using microwaves as an efficient method for extracting essential oil from plant

material. The HD oil and hydrolate of this plant were rich in volatiles compare to the MAH oil, and these findings indicated that though the microwave method could be a substantial way for energy saving, the hydro-distillation method was superior for extracting the volatile components. The major constituents of HD and MAH oils were identified to be a phthalic acid ester, bis (2-ethylhexyl) phthalate which is widely used in the production of plastics with reproductive and developmental toxicity [26,27]. Although, this chemical is hardly soluble in water (300-400 µg/L), it is present in the environment [27,28], and there are several reports of presence of phthalates in essential oils. The main source of this compound in water is the discharge from rubber and chemical factories. Chemical analysis of plant compositions has revealed the presence of phthalates in the essential oils as major components [29-31]. Since *L. salicaria* principally grows near or in the streams, it could be concluded that bis (2-ethylhexyl) phthalate, the main constituents of the HD and MAH oils, has been absorbed from water as a contaminant. It is a toxic material [32], and based on the results of the present study, GC/MS analysis could be introduced as an effective method for identification of this plasticizer in plant materials.

#### References

- [1] Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils, a review. *Food Chem Toxicol.* 2008; 46(2): 446-475.
- [2] Swami Handa S, Khanuja S, Longo G, Rakesh DD. *Extraction technologies for medicinal and aromatic plants.* Trieste. Italy: ICS-UNIDO, 2008.
- [3] Sawamura M. *Citrus essential oils: flavor and fragrance.* New Jersey: Wiley, 2010.
- [4] Hui YH. *Handbook of food products manufacturing.* New Jersey: Wiley, 2007.
- [5] Rechinger KH. *Flora Iranica.* Graz. Akademische Druck-U Verlagsanstalt, 1968.
- [6] Soltani A. *Encyclopedia of traditional medicine (dictionary of medicinal plants).* Tehran: Arjmand Press, 2011.
- [7] Mantle D, Eddeb F, Pickering AT. Comparison of relative antioxidant activities of British medicinal plant species *in vitro.* *J Ethnopharmacol.* 2000; 72(1-2): 47-51.
- [8] Rauha JP, Remes S, Heinonen M, Hopia A, Kahkonen M, Kujala T, Pihlaja K, Vuorela H, Vuorela P. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *Int J Food Microbiol.* 2000; 56(1): 3-12.
- [9] Becker H, Scher JM, Speakman JB, Zapp J. Bioactivity guided isolation of antimicrobial compounds from *Lythrum salicaria.* *Fitoterapia.* 2005; 76(6): 580-584.
- [10] Lopez V, Akerreta S, Casanova E, Garcia-Mina JM, Cavero RY, Calvo MI. Screening of Spanish medicinal plants for antioxidant and antifungal activities. *Pharm Biol.* 2008; 46: 602-609.
- [11] Coban T, Citoglu GS, Sever B, Iscan M. Antioxidant activities of plants used in traditional medicine in Turkey. *Pharm Biol.* 2003; 41(8): 608-613.
- [12] Tunalier Z, Kosar M, Kupeli E, Calis I, Baser KH. Antioxidant, anti-inflammatory, anti-nociceptive activities and composition of *Lythrum salicaria* L. extracts. *J Ethnopharmacol.* 2007; 110(3): 539-547.
- [13] Lamela M, Cadavid I, Calleja JM. Effects of *Lythrum salicaria* extracts on hyperglycemic rats and mice. *J Ethnopharmacol.* 1986; 15(2): 153-160.
- [14] Torres IC, Suarez JC. A preliminary study of hypoglycemic activity of *Lythrum salicaria.* *J Nat Prod.* 1980; 43(5): 559-563.

- [15] Brun Y, Wang XP, Willemot J, Sevenet T, Demenge P. Experimental study of antidiarrheal activity of Salicairine®. *Fundam Clin Pharm.* 1997; 12(1): 30-36.
- [16] Khanavi M, Moshteh M, Manayi A, Shams Ardekani MR, Vazirian M, Ajani Y, Ostad SN. Cytotoxic activity of *Lythrum salicaria* L. *Res J Biol Sci.* 2011; 6(2): 55-57.
- [17] Shahani S, Monsef-Esfahani HR, Hajiaghache R, Gohari AR. Chemical composition of essential oil and hydrolat of *Geum iranicum* Khatamaz. *J Essent Oil Res.* 2011; 23: 29-33.
- [18] Jalali Heravi M, Zekavat B, Sereshti H. Characterization of essential oil components of Iranian geranium oil using gas chromatography-mass spectrometry combined with chemometric resolution techniques. *J Chromatogr A.* 2006; 1114(1): 154-163.
- [19] Leffingwell JC, Alford ED. Volatile constituents of perique tobacco. *J Environ Agric Food Chem.* 2005; 4: 899-915.
- [20] Pino JA, Mesa J, Munoz Y, Marti MP, Marbot R. Volatile components from mango (*Mangifera indica* L.) cultivars. *J Agric Food Chem.* 2005; 53(6): 2213-2223.
- [21] Pripdeevech P, Machan T. Fingerprint of volatile flavour constituents and antioxidant activities of teas from Thailand. *Food Chem Toxicol.* 2011; 125: 797-802.
- [22] Adams RP. *Identification of essential oil components by gas chromatography/mass spectrometry.* Illinois: Allured Publishing Corporation, 1995.
- [23] Elmore JS, Mottram DS, Enser M, Wood JD. Effect of the polyunsaturated fatty acid composition of beef muscle on the profile of aroma volatiles. *J Agric Food Chem.* 1999; 47(4): 1619-1625.
- [24] Lucchesi ME, Chemat F, Smadja J. An original solvent free microwave extraction of essential oils from spices. *Flavour Fragr J.* 2004; 19: 134-138.
- [25] Bayramoglu B, Sahin S, Sumnu G. Solvent-free microwave extraction of essential oil from oregano. *J Food Eng.* 2008; 88: 535-540.
- [26] Saeidnia S, Abdollahi M. Are medicinal plants polluted with phthalates? *Daru.* 2013; 21(1): 43.
- [27] Turner A, Rawling MC. The behaviour of di-(2-ethylhexyl) phthalate in estuaries. *Mar Chem.* 2000; 68(3): 203-217.
- [28] Arcadi FA, Costa C, Imperatore C, Marchese A, Rapisarda A, Salemi M, Trimarchi GR, Costa G. Oral toxicity of bis (2-ethylhexyl) phthalate during pregnancy and suckling in the Long-Evans rat. *Food Chem Toxicol.* 1998; 36(11): 963-970.
- [29] Srinivasan GV, Sharanappa P, Leela NK, Sadashiva GT, Vijayan KK. Chemical composition and antimicrobial activity of the essential oil of *Leea indica* (Burm.f.) Merr flowers. *Nat Prod Radiance.* 2009; 8(5): 488-493.
- [30] Muthuchelian K, Ramalakshmi S. Analysis of bioactive constituents from the leaves of *Mallotus tetraococcus* (Roxb.) Kurz, by gas chromatography-mass spectrometry. *Int J Pharm Sci Res.* 2011; 2(6): 1449-1454.
- [31] Nadaf M, Halimi khalil abad M, Monfareddi L, Neyestani M. Chemical composition of the essential oil of *Stachys lavandulifolia* (after flowering) growing wild in darkesh protected area (North Khorassan province Iran). *Asian J Plant Sci Res.* 2011; 1(1): 1-4.
- [32] Di Bella G, Saitta M, Pellegrino M, Salvo F, Dugo G. Contamination of Italian citrus essential oils: presence of phthalate esters. *J Agric Food Chem.* 1999; 47(3): 1009-1012.