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Optimized ultrasonic assisted extraction–dispersive liquid–liquid microextraction coupled with gas chromatography for determination of essential oil of *Oliveria decumbens* Vent.

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ABSTRACT

Ultrasonic assisted extraction–dispersive liquid–liquid microextraction (UAE–DLLME) coupled with gas chromatography (GC) was applied for extraction and determination of essential oil constituents of the plant *Oliveria decumbens* Vent. Scanning electron microscopy (SEM) was used to see the effect of ultrasonic radiation on the extraction efficiency. By comparison with hydrodistillation, UAE–DLLME is fast, low cost, simple, efficient and consuming small amount of plant materials (~1.0 g). The effects of various parameters such as temperature, ultrasonication time, volume of disperser and extraction solvents were investigated by a full factorial design to identify significant variables and their interactions. The results demonstrated that temperature and ultrasonication time had no considerable effect on the results. In the next step, a central composite design (CCD) was performed to obtain the optimum levels of significant parameters. The obtained optimal conditions were: 0.45 mL for disperser solvent (acetonitrile) and 94.84 μL for extraction solvent (chlorobenzene). The limits of detection (LODs), linear dynamic range and determination coefficients (R^2) were 0.2–29 ng mL^{-1}, 1–2100 ng mL^{-1} and 0.995–0.998, respectively. The main components of the essential oil were: thymol (47.06%), carvacrol (23.31%), gamma-terpinene (18.94%), *p*-cymene (8.71%), limonene (0.76%) and myristicin (0.63%).

1. Introduction

Essential oils (EOs) are aromatic oily liquids extracted from plants. The most common use of EOs is in food (as flavorings), perfumes and pharmaceuticals (for their functional properties) [1]. The plant *Oliveria decumbens* Vent. (Apiaceae), with the common Persian names of "Mooshkorok", "Den", and "Denak" [2], is found in south and south west parts of Iran and is endemic to Iran. This plant in Iranian folk and traditional medicines is used for indigestion, diarrhea, abdominal pain and feverish conditions [3,4].

Hydrodistillation seems to be the most common technique for extraction and isolation of essential oils from plant materials, but this method is time consuming and needs large amounts of material, which in some situations, especially in cases where enough plant materials is not accessible, is problematic. On the other hand, the volatile and thermally sensitive components of essential oils may be lost in hydrodistillation conditions. There are several developed methods such as solid phase microextraction (SPME) [5–7], single drop microextraction (SDME) [8–12], supercritical fluid extraction (SFE) [13–15], solvent free microwave extraction (SFME) [16], microwave hydrodiffusion and gravity (MHG) [17], thin layer chromatography (TLC) [18], pressurized liquid extraction (PLE) [19], solvent-enhanced headspace sorptive extraction (SE-HSSE) [20], ultrasound solvent extraction (USE) [21], static and dynamic superheated water extraction (S-SWE and D-SWE) [22], enzymatic treatment [23] and controlled instantaneous decompression (DIC) [24] that can be used for preconcentration, extraction and separation purposes.

The aim of present study is to develop an effective, simple, safe (for essential oil components), rapid and low cost method with very low consumption of plant materials and toxic organic solvents.

Ultrasound is simply sound pitched above human hearing (16–18 kHz), which is transmitted through any substance possessing elastic properties generating particles expansion and compression cycles. If the ultrasound intensity is high enough, the expansion cycle can create bubbles or cavities in a liquid. At some point, a bubble can no longer absorb the energy efficiently from ultrasound, so it implodes [25]. When cavitation occurs close to a solid surface, cavity collapse produces high-speed jets of liquid that impact perpendicularly and strongly to the solid surface, and lead to pitting and erosion of the surface [26]. Therefore, ultrasound can be considered a useful alternative for solid sample pretreatment, because the energy imparted facilitates and accelerates some steps, such as dissolution, fusion, and leaching [27–29].

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DLLME was introduced by Assadi et al. [30] which is a modified solvent extraction method and its acceptor-to-donor phase ratio greatly reduced comparing with other extraction methods. Simplicity of the operation, rapidity, low sample volume, and low cost are the main advantages of this method. This method has been applied for preconcentration and extraction of analytes from different matrices [30–40].

In this study, UAE and DLLME were hyphenated to make an efficient method for extraction of essential oils for the first time.

2. Experimental

2.1. Reagents and materials

The plant materials of *O. decumbens* Vent. were collected from Kazeroon located in Fars province, in the south west of Iran, in April 2009. Voucher specimen (No. 6724-TEH) is deposited in the Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences (Tehran, Iran). Thymol, carvacrol, *n*-pentane, anhydrous sodium sulphate, chloroform, chlorobenzene, carbon disulphide, trichloroethylene, acetonitrile, methanol and absolute ethanol with the purity higher than 99% were purchased from Merck chemical company (Darmstadt, Germany). The standard stock solutions (1000 $\mu g\,mL^{-1}$) were prepared in absolute ethanol.

2.2. Instrumentation

GC analyses were performed using a gas chromatograph (Shimadzu-17A, Tokyo, Japan) with a split/splitless injection port and a flame ionization detection system (FID). The injection port was held at 250 °C and used in split mode with a split ratio of 1:25. The gas chromatograph was equipped with a CBP-5 capillary fused silica column (25 m, length; 0.25 mm I.D.; 0.22 μm, film thickness; stationary phase, methyl 5% phenyl polysiloxane). Pure helium (99.999%, from Hiva gas company, Tehran, Iran) passed through a molecular sieve trap was used as the carrier gas at constant flow rate of 1 mL min^{-1}. The oven temperature was initiated at 40 °C (held for 1 min), then raised at the rate of 3 °C min^{-1} to 130 °C, and immediately with the rate of 20 °C min^{-1} raised to 250 °C, held for 10 min at this temperature. An Agilent technologies gas chromatograph–mass spectrometer (GC–MS) system including a 6890 GC coupled with a 5973 mass selective detector was used for separation and determination of the essential oil components. Mass spectra were taken at 70 eV, the ion source was electron impact (EI), and mass analyzer was quadrupole. Separation was carried out on a HP-1 MS capillary fused silica column (30 m, length; 0.25 mm I.D.; 0.25 μm film thicknesses; stationary phase, methyl polysiloxane). The injection port was held at 250 °C and used in split mode with split ratio of 1:25. The temperature program initiated at 40 °C (held for 1 min), then raised at 3 °C min^{-1} to 250 °C, held for 20 min. Other operating conditions were as follows: carrier gas, pure helium (99.999%, from Hiva gas company, Tehran, Iran) passed through a molecular sieve trap was used as the carrier gas at constant flow rate of 1 mL min^{-1}. Scanning electron microscope (SEM), model DSM-960 A, Zeiss (Oberkochen, Germany) was used for SEM imaging. An ultrasonic water bath, working at 50–60 kHz with maximum output power of 350 W (Euronda company, Vicenza, Italy) was used for ultrasonication of the samples. 1 mL insulin syringe was used for injection of extraction and disperser solvents mixtures into the sample solutions. A 1.0 μL in-needle Hamilton microsyringe (Nevada, USA) was used for injection of sample solutions into GC-FID and GC–MS.

2.3. Isolation of essential oil by hydro-distillation

The flowers of *O. decumbens Vent.* were dried under shade at room temperature for 48 h. Then, a 50.0 g of them were separated and ground. The powdered plant materials were immersed in 1 L water in a 2 L round bottom flask and hydro-distilled in a full glass Clevenger-type apparatus as recommended by British Pharmacopeia giving transparent light yellow oil. The extraction was carried out for 3.5 h. When the condensed material cooled down, the water and essential oil were separated and decanted to be used as essential oil. To improve the recovery and the analysis, the essential oil were taken up in *n*-pentane, dried over anhydrous sodium sulphate until the last traces of water were removed and then stored in a dark glass bottle at 4 °C prior to GC–MS analysis. The extraction yield for the essential oil was 6%.

2.4. Identification of essential oil constituents

The components of the essential oil were identified by comparing their mass spectra fragmentation patterns with those stored on a Wiley7n.l MS computer library. Kovats' retention indices of all the constituents were obtained by interpolating between bracketing *n*-alkenes [41,42].

2.5. Ultrasonic assisted extraction–dispersive liquid–liquid microextraction procedure

1.0 g of the powdered sample (dried and ground flowers of the plant) was weighed and placed in a 50 mL beaker, and 20.0 mL of distilled water was added to it and subjected to ultrasonic radiation using an ultrasonic water bath working at 50–60 kHz with maximum output power of 350 W for 10 min at room temperature. Then, the sample was transferred into a conic bottom test tube, and was centrifuged at 3500 rpm for 3 min to separate the plant particles from sample solution. Afterwards, 5.0 mL of centrifuged sample was placed into another test tube, and then 0.50 mL of acetonitrile (disperser solvent) containing 100 μL chlorobenzene (extraction solvent) was injected rapidly into the sample solution using a 1.0 mL syringe, and finally centrifuged at 5000 rpm for 3 min. Thereby, the dispersed fine particles of extraction solvent were sedimented at the bottom of test tube and 0.5 μL of it was taken using a 1.0 μL microsyringe and injected into GC.

3. Results and discussion

3.1. Selection of extraction and disperser solvents

Extraction solvent should be selected based on its higher density than water, good chromatographic behavior, low toxicity and immiscibility with water. Chlorobenzene (density: 1.11 g mL^{-1}), chloroform (density: 1.48 g mL^{-1}), trichloroethylene (density: 1.46 g mL^{-1}) and carbon disulphide (density: 1.26 g mL^{-1}) were selected as extraction solvents. On the other hand, for selection of disperser solvent, miscibility of it with organic phase (extraction solvent) and aqueous phase (sample solution) has great importance. Therefore, methanol, ethanol and acetonitrile were chosen for this purpose. According to the procedure mentioned in section 2.5, different mixtures of the selected extraction and disperser solvents were tested. The mixture of chlorobenzene (as extraction solvent) and acetonitrile (as disperser solvent) showed the highest extraction recovery (Fig. 1).

3.2. Identification of important parameters and interactions

The most popular first order designs are two-level factorial designs. They are used primarily for screening significant factors,

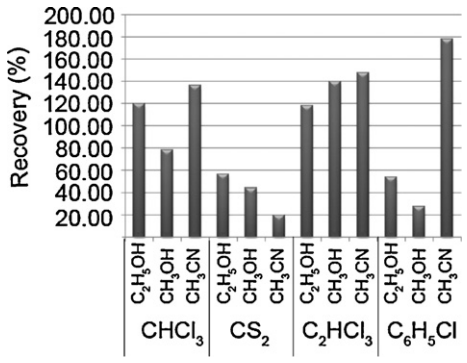

Fig. 1. Selection of disperser and extraction solvents. Total recovery of major essential oil components (thymol and carvacrol) was considered as response. Conditions: volume of disperser solvent, 0.500 mL; volume of extraction solvent, 100.0 μL; ultrasonication time, 10 min; temperature, 25 °C; injection volume, 0.50 μL.

Table 1
Independent variables, their symbols and levels for full factorial design.

Variable	Symbol	Level	
		Min	Max
Temperature (°C)	*T*	20	60
Ultrasonication time (min)	*t*	10	30
Volume of disperser solvent (mL)	*D*	0.200	0.600
Volume of extraction solvent (μL)	*E*	50.0	100.0

but can also be used sequentially to model and refine a process. Due to their simplicity and relatively low cost, full factorial designs are very useful for preliminary studies or in the initial steps of an optimization process [43]. In order to evaluate the method, total extraction recovery of major essential oil components (thymol and carvacrol) was considered as response. Based on our preliminary studies and experiments, four factors that might affect the results are ultrasonication time, temperature, volume of extraction solvent and volume of disperser solvent (Table 1). A full factorial design including 16 runs (2^f, where f is the number of factors) was considered to identify the most important factors and interactions. The experiments were run in a random manner in order to minimize the effect of uncontrolled variables [43]. Since it was not possible to perform all of the experiments in 1 day, the runs were divided into 4 blocks and carried out in four sequential days to remove the expected variations caused by some changes during the course of the experiments. The design matrix and responses are shown in Table 2. Analysis of variance (ANOVA) was obtained using the trial version of software package, Design-Expert 7.1.3 (Table 3). The model *F*-value of 8.33 implies that it is significant. There is only a

Table 2
Design matrix (coded values of variables) and responses for a full factorial (2^4) design.

Run no.	Block	Variable				Response
		T	*t*	*E*	*D*	
1	1	1	−1	1	−1	219.12
2	1	1	−1	−1	1	87.50
3	1	−1	1	1	1	146.46
4	1	−1	1	−1	−1	82.22
5	2	−1	−1	−1	−1	145.23
6	2	1	1	−1	1	48.86
7	2	−1	−1	1	1	95.63
8	2	1	1	1	−1	221.32
9	3	1	−1	−1	−1	121.49
10	3	−1	1	−1	1	36.85
11	3	1	−1	1	1	99.01
12	3	−1	1	1	−1	150.61
13	4	−1	−1	1	−1	213.28
14	4	1	1	−1	−1	152.55
15	4	1	1	1	1	113.24
16	4	−1	−1	−1	1	30.32

1.65% chance that a model *F*-value this large could occur due to noise. The values of prob > *F* less than 0.0500 indicate that model terms are significant and values greater than 0.1000 indicate that model terms are not significant. Therefore, according to Table 3, *D* (volume of disperser solvent) and *E* (volume of extraction solvent) are the most important factors affecting the extraction efficiency, but ultrasonication time and temperature do not have considerable effect on the results.

3.3. Optimization of the method

In the next step, to obtain the model and optimal conditions of the effective parameters, a central composite design (CCD) was applied.

The suggested number of experiments by Design Expert software was 14, but since the time needed to perform each experiment was relatively long, the runs were divided into 2 blocks. The experiments were randomized and carried out in two sequential days for the same reasons as mentioned for full factorial design. The main factors, their symbols and levels are shown in Table 4. The design matrix with the responses is given in Table 5.

The analysis of variance was used to obtain an assessment of the effect of important factors on the response (Table 6). The model *F*-value of 18.68 implies that the model is significant and there is only a 0.26% chance that a model *F*-value this large could occur due to the noise. The lack of fit (LOF) *F*-value of 7.17 implies that it is not significant.

Table 3
Analysis of variance (ANOVA) for full factorial design.

Source	SS[a]	d.f.[b]	MS[c]	*F*-value[d]	Prob > F[e]	
Block	2399.48	3	799.83	0.95	0.4834	Not significant
Model	48,988.52	7	6998.36	8.33	0.0165	Significant
T	1650.19	1	1650.19	1.96	0.2200	Not significant
T	221.04	1	221.04	0.26	0.6299	Not significant
D	26,239.95	1	26,239.95	31.23	0.0025	Significant
E	19,158.02	1	19,158.02	22.80	0.0050	Significant
TD	438.80	1	438.80	0.52	0.5023	Not significant
TE	298.17	1	298.17	0.35	0.5773	Not significant
tD	982.35	1	982.35	1.17	0.3289	Not significant
Residual	4200.84	5	840.17			
Corrected total	55,588.83	15				

[a] Sum of squares.
[b] Degrees of freedom.
[c] Mean square.
[d] Test for comparing model variance with residual (error) variance.
[e] Probability of seeing the observed *F*-value if the null hypothesis is true.

Table 4
Independent variables, their symbols and levels in central composite design.

Variable	Symbol	Level				
		$-a$	−1	0	+1	$+a$
Volume of extraction solvent (μL)	E	30	42	70	98	110
Volume of disperser solvent (mL)	D	0.10	0.23	0.55	0.87	1.00

Table 5
Design matrix (coded value of variables) and responses for central composite design.

Run	Block	E	D	Response
1	1	0	0	123
2	1	−1	−1	50
3	1	0	0	119
4	1	0	0	125
5	1	−1	+1	31
6	1	+1	+1	47
7	1	+1	−1	140
8	2	$+a$	0	180
9	2	0	$-a$	130
10	2	0	$+a$	39
11	2	$-a$	0	33
12	2	0	0	117
13	2	0	0	120
14	2	0	0	124

The second order polynomial with the most reasonable statistics, that is, higher F- and R-values and low standard error was obtained as the satisfactory response surface model to fit the experimental data. This model is shown in Eq. (1) in terms of coded values. It consists of two main effects, one two-factor interaction effect and four curvature effects, where b_0 is the intercept and b terms represent those parameters of the model which are optimized iteratively to fit, or model the data.

$$y = b_0 + b_1E + b_2D + b_3ED + b_4E^2 + b_5D^2 + b_6A^2B + b_7AB^2 \quad (1)$$

where $b_0 = 126.33$; $b_1 = 51.97$; $b_2 = -32.17$; $b_3 = -18.50$; $b_4 = -17.04$; $b_5 = -28.04$; $b_6 = 4.17$; $b_7 = -25.47$.

The F-value of 7.17 for the LOF implies that it is not significant relative to the pure experimental error and confirms the validity of the model. The positive coefficient of E in Eq. (2) shows that when volume of the extraction solvent (chlorobenzene) increases, more analytes from sample solution is extracted into it, thus according to Eq. (2) the extraction recovery increases.

$$\text{Recovery} = \frac{n_s}{n_o} \times 100 = \frac{V_s \times C_s}{V_{aq} \times C_o} \times 100 \quad (2)$$

where n_s is mole numbers of analytes in organic phase, n_0 is mole number of analytes in aqueous sample solution, V_s is volume of organic phase, C_s is concentration of analytes in organic phase, V_{aq} is aqueous sample volume and C_0 is concentration of analytes in aqueous sample solution. The negative coefficient of D in Eq. (1) shows that by increasing volume of the disperser (acetonitrile) the extraction recovery decreases, because solubility of extraction solvent (chlorobenzene) in the sample solution increases. Therefore,

Table 6
Analysis of variance (ANOVA) for quadratic response surface model.

Source	SS	d.f.	MS	F-value	Prob > F	
Block	896.00	1	896.00			
Model	29,711.60	7	4244.51	18.68	0.0026	Significant
E	10,804.50	1	10,804.50	47.56	0.0010	
D	4140.50	1	4140.50	18.23	0.0079	
ED	1369.00	1	1369.00	6.03	0.0576	
E^2	2144.63	1	2144.63	9.44	0.0277	
D^2	5806.78	1	5806.78	25.56	0.0039	
A^2B	34.83	1	34.83	0.15	0.7115	
AB^2	1297.68	1	1297.68	5.71	0.0624	
Residual	1135.83	5	227.17			
Lack of fit	729.17	1	729.17	7.17	0.0553	Not significant
Pure error	406.67	4	101.67			
Corrected total	31,743.43	13				

Table 7
Quantitative and qualitative results of UAE–DLLME–GC-FID and GC–MS for essential oil constituents.

No.	t_R[a]	Compound	%[b]	%[c]	RI[d]	LOD[e]	LOQ[f]	LDR[g]	RR[h] (%)	R^2[i]	RSD (%)
1	11.20	beta-Pinene	0.42	1.83	895	29	285	285–2100	–	0.996	4.0
2	11.93	beta-Myrecene	0.05	0.36	912	6	56	56–2100	–	0.997	11.0
3	13.52	*p*-Cymene	8.71	16.87	945	21	204	204–2100	–	0.995	8.0
4	13.75	Limonene	0.76	1.40	950	9	83	83–2100	–	0.995	6.5
5	15.31	gamma-Terpinene	18.94	15.46	982	16	159	159–2100	–	0.997	7.2
6	26.18	Thymol	47.06	36.99	1194	0.2	1	1–2100	93	0.998	7.8
7	26.54	Carvacrol	23.31	17.35	1203	0.2	1	1–2100	89	0.998	5.6
8	34.99	Myristicine	0.63	7.98	1385	13	134	134–2100	–	0.997	2.1

[a] Retention time (min).
[b] Relative area percent (peak area relative to total peak area) obtained by the proposed method.
[c] Relative area percent (peak area relative to total peak area) obtained by hydrodistillation.
[d] Retention index using a HP-1 MS column (30 m, length; 0.25 mm I.D.; 0.25 μm, film thickness).
[e] Limit of detection (ng mL^{-1}).
[f] Limit of quantitation (ng mL^{-1}).
[g] Linear dynamic range (ng mL^{-1}).
[h] Relative extraction recovery.
[i] Determination coefficient.

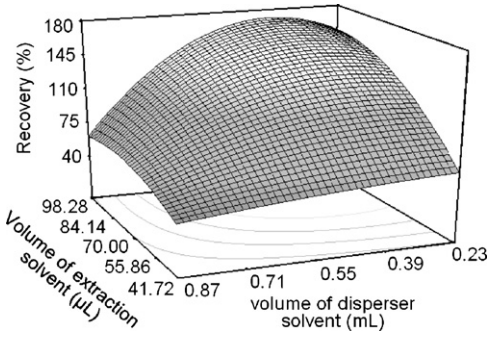

Fig. 2. Three dimensional (3D) plot for extraction–disperser solvents.

volume of the sedimented phase reduces and as a result, extraction efficiency is low.

To visualize the relationship between the response and experimental levels of factors, the model was mapped against two experimental factors while the others were held constant at their central levels. Fig. 2 shows three-dimensional (3D) plot of the model. It represents the change and effect of main variables on the response simultaneously.

Finally, using the optimization option of Design-Expert 7.1.6. software, the optimal set points of the method was obtained as follows: 94.84 μL for volume of extractor solvent and 0.45 mL for volume of disperser solvent.

3.4. Evaluation of the method performance

The analytical figures of merit were obtained under optimal conditions. The correlation coefficient (R^2) was in the range of 0.995–0.998. The relative extraction recovery (RR) was calculated by Eq. (3):

$$RR = \frac{C_{founded} - C_{real}}{C_{added}} \quad (3)$$

where C_{found} is concentration of analyte after addition of known amount of standard solution into real sample, C_{real} is concentration of analyte in real sample and C_{added} is concentration of standard solution that was spiked to real samples, respectively. The limit of detection, based on S/N (signal-to-noise ratio = 3) were in the range of 0.2–29 ng mL^{-1}. Kovat's retention indices and the name of components are given in Table 7. The major components of the essential oil extracted by the proposed method and hydrodistillation and the related chromatograms are presented in this table and Fig. 3, respectively. The results in Table 7 show a good agreement between hydrodistillation and the proposed method. The major components (8 compounds) are same and comprise 99.88% of total components for the proposed method and 98.24% for hydrodistillation. A comparison of this work with other reported methods is shown in Table 8. Results show that the extraction time in the

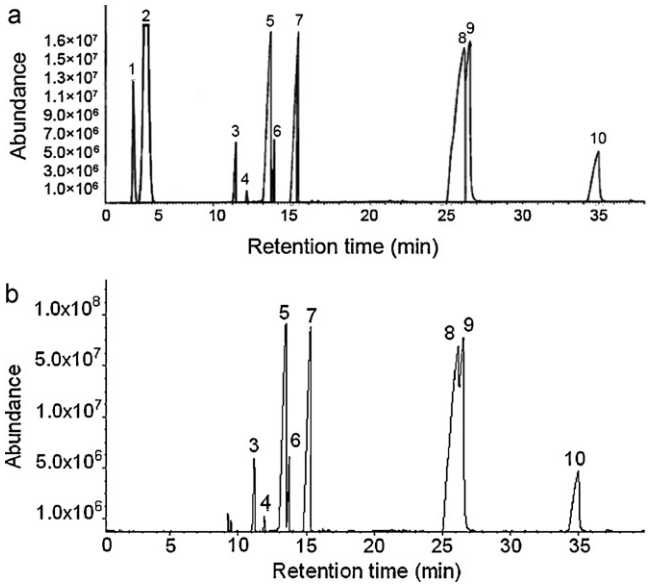

Fig. 3. Total ion chromatogram (TIC) for essential oil main constituents. (a) Obtained by the proposed method; (b) obtained by hydrodistillation. Conditions: 0.260 mL, volume of disperser solvent; 92.5 μL, volume of extraction solvent; HP-1 MS capillary fused silica column (30 m, length; 0.25 mm i.d.; 0.25 μm, film thicknesses; stationary phase, methyl polysiloxane), 0.50 μL, injection volume. (1) Acetonitrile (disperser solvent); (2) chlorobenzene (extraction solvent); (3) beta-pinene; (4) beta-myrecene; (5) *p*-cymene; (6) limonene; (7) gamma-terpinene; (8) thymol; (9) carvacrol; (10) myristicine.

Table 8
Comparison between figures of merits of UAE–DLLME and other methods in essential oil analysis.

EM[a]	QA[b]	IS[c]	RSD (%)	t[d] (min)	SA[e]	T[f] (°C)	P[g] (atm)	LOD (ng mL^{-1})	LDR (ng mL^{-1})	Ref. no.
UAE–DLLME	GC-FID	GC–MS	<11	10	1.0	25	1	0.2–29	1–2100	p.m.[h]
PHWE-SPME	GC–MS	GC–MS	<13	15	0.05	150	50	n.r.[i]	n.r.	[4]
MD-SPME	GC–MS	GC–MS	<9	3	2	25	1	n.r.	n.r.	[5]
MD-HS-SDME	GC–MS	GC–MS	<12	4	2	40	1	n.r.	n.r.	[7]
UNE-HS-SDME	GC–MS	GC–MS	n.r.	20	0.05	25	1	n.r.	n.r.	[8]
HD	GC-FID	GC–MS	<51	240	>25	100	1	n.r.	n.r.	[9]
SFE	GC-FID	GC–MS	<14	30	0.5	70	400	n.r.	n.r.	[9]
SFME	GC-FID	GC–MS	n.r.	95	50	25	1	n.r.	n.r.	[12]
MHG	–	GC–MS	n.r.	15	500	25	1	n.r.	n.r.	[13]
PLE	–	GC–MS	n.r.	10	0.5	100	60	n.r.	n.r.	[15]
SE-HSSE	GC-FID	GC–MS	<14	20	0.05	50	1	n.r.	n.r.	[16]
USE	GC–MS	GC–MS	<11	71.8	2.38	25	1	38–101	n.r.	[17]
S-SWE	–	GC–MS	n.r.	10	0.5	150	50	n.r.	n.r.	[18]
D-SWE	–	GC–MS	n.r.	40	0.5	200	100	n.r.	n.r.	[18]
DIC	–	GC-FID	n.r.	10	100	100	3	n.r.		[20]

[a] Extraction method.
[b] Quantitative analysis.
[c] Identification system.
[d] Extraction time.
[e] Sample amount.
[f] Extraction temperature.
[g] Extraction pressure.
[h] Proposed method.
[i] Not reported.

proposed method is highly shorter than hydro-distillation (HD), USE, SFME, D-SWE and SFE methods. The consumption of plant materials is much less than HD, MHG and DIC. The precision is considerably better than HD method and is slightly better than SFE, PHWE-SPME and SE-HSSE. In comparison with other methods, UAE–DLLME is carried out in normal conditions (room temperature and pressure). The instrumentation relative to SFE is low cost. The proposed procedure is simpler than SDME, SPME. In addition, this method represents good LODs for essential oil major components.

4. Conclusion

In the present study, UAE, DLLME and GC-FID were hyphenated for extraction and determination of essential oils for the first time. Ultrasonic assisted extraction is the most important feature of this work. It helps to extract the essential oil effectively under the moderate conditions (room temperature and pressure) in a short time. Scanning electron microscopy demonstrated the efficiency of the method. Experimental design including two main stages, factor screening and parameter optimization, were applied. Factorial design was used to identify important parameters and possible interactions. Central composite design was applied to find optimal conditions. However, by classical methods it is not possible to see the detailed effect of factors on the efficiency to explain the precise behavior of a system. Compared with other methods, the presented work is fast, simple, low cost and efficient that consumes small amounts of plant materials and is safe for extraction of essential oil components.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.05.037.

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