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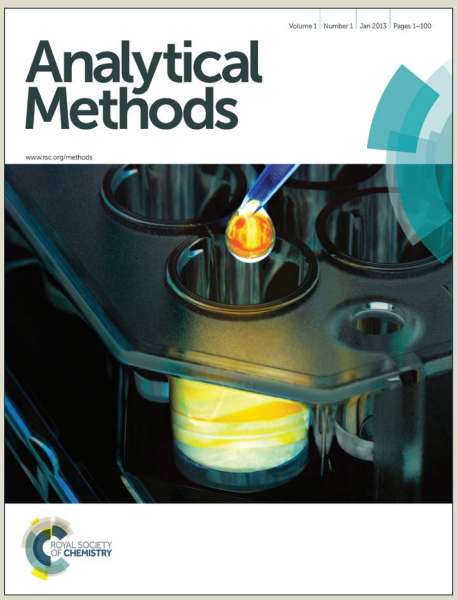

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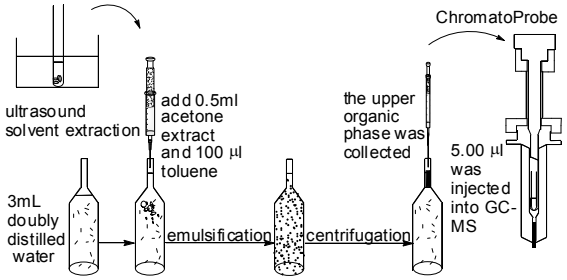
A simple and novel method was successfully developed for determination of essential oil from fresh flowers of *Edgeworthia chrysantha* Lindl. by using ultrasound-assisted extraction/dispersive liquid-liquid microextraction (UAE-DLLME) coupled with gas chromatography-ion trap/mass spectrometry (GC-IT/MS) using a direct sample introduction (DSI) device.



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ARTICLE TYPE

Development of ultrasound-assisted extraction/dispersive liquid-liquid microextraction coupled with DSI-GC-IT/MS for analysis of essential oil from fresh flowers of *Edgeworthia chrysantha* Lindl.

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In this study, a simple and novel method was successfully developed for extraction and preconcentration of essential oil from fresh flowers of *Edgeworthia chrysantha* Lindl. by using ultrasound-assisted extraction/dispersive liquid-liquid microextraction (UAE-DLLME) coupled with gas chromatography-ion trap/mass spectrometry (GC-IT/MS) using a direct sample introduction (DSI) device. The optimum parameters of UAE-DLLME were investigated. The optimum conditions of extraction solvent of toluene, dispersive solvent of acetone, and ultrasound time of 10 min were obtained and applied to the extraction of essential oil compounds from fresh flowers of *Edgeworthia chrysantha* Lindl. The main volatile and semi-volatile compounds from *Edgeworthia chrysantha* Lindl. at different florescences ((A) flower buds; (B) the initial flowering stage; (C) the full flowering stage; (D) the final flowering stage) were studied. The results showed that 36 substances were identified, including alkanes, alkenes, aromatic hydrocarbons, alcohols, aldehydes, ketones, acids, lipids, and compounds containing nitrogen (N), all of which contributing a lot to the fragrance of *Edgeworthia chrysantha* Lindl. In this study, a simple, rapid, and environmental-friendly approach was developed for analysis of essential oil compounds from fresh flowers of *Edgeworthia chrysantha* Lindl. at different florescences.

1. Introduction

Edgeworthia chrysantha Lindl. is distributed in eastern Asia and belongs to *thymelaeaceae* family *edgeworthia* genus. While it is used to make paper in Korea and Japan, the alabastrum is often used as the succedaneum of traditional Chinese medicine which was called "meng hua" in China.[1] According to the literature, rutin and other relevant compounds, separated from *Edgeworthia chrysantha* Lindl. can be used to prevent cerebral hemorrhage by increasing the resistance of blood vessels.[2] In addition, rich essential oil components (such as benzyl alcohol) in *Edgeworthia chrysantha* Lindl. have effect of expectorant, anti-tussive, antipyretic, analgesic, antimicrobial and anti-inflammatory.[3] Therefore, it is necessary to develop a rapid, efficient, and inexpensive method for separation and determination of essential oil from *Edgeworthia chrysantha* Lindl.

Many methods have been developed in the last few years for determination of volatile components of plants. The most widely used methods are gas chromatography-mass spectrometry/flame ionization detection (GC-MS/FID),[4-8] high performance liquid chromatography (HPLC),[8-12] and UV-vis spectrophotometry.[13-15] Gas chromatography (GC) coupled with mass spectrometry (MS) is one of the most important instrumental separation techniques for analysis of volatile components. The main advantages of GC–MS are the abilities of analyzing complex mixtures and identifying the separated components by mass spectra, the high sensitivity, and the low limit of detection (LOD).[16]

For the analysis of volatile components of plants, sample preparations are required to isolate volatile portions of plant tissues, remove interfering compounds and achieve a sufficient sensitivity. Several methods including hydrodistillation,[17] soxhlet,[18-19] and solvent extraction[18] have been used for isolation of volatile components from different plant matrices. However, those traditional methods need large amounts of hazardous organic solvents and plant material which were considered as relatively labour-intensive and time-consuming. In addition, some thermally sensitive compounds may be lost during thermal extraction and/or distillation. So miniaturization and development of environmentally sound methods have become the trend in essential oil extraction and related fields in the past decades. Recently, many kinds of extraction techniques such as ultrasound assisted extraction (UAE),[18,20] microwave-assisted extraction (MAE),[18,21-22] supercritical fluid extraction (SFE),[18,23-24] solid-phase extraction (SPE),[25-29] solid-phase microextraction (SPME),[22,30-31] and liquid-phase microextraction (LPME)[32] have been developed.

UAE is considered as a good alternative for organic compounds extraction from plants which facilitating the release of analytes from plant matrix and intensifies mass transfer. Multiple samples can be performed simultaneously with no specialized experimental equipments required and this extraction technique is relatively inexpensive compared to conventional extraction methods.[33] LPME is a solvent microextraction technique, which offers many advantages such as wide choice and low

consumption of extraction solvents, high extraction efficiency and simplicity in experimental setup. But it also suffers from various shortcomings, including instability of the microdrop (for SDME) and relatively low precision.[34] In 2006, dispersive liquid-liquid microextraction (DLLME) was introduced by Assadi and co-workers, being based on a ternary solvent system such as homogeneous liquid-liquid extraction and cloud-point extraction.[35] DLLME is a high-performance and powerful pre-concentration method which overcomes the problems existed in LLE and SPE. To date, this innovative method has been successfully applied for isolation and preconcentration target analytes in various environmental waters[36-41] and solid matrix (tea,[42-43] Tomatoes,[44-46] cucumber,[46] and corn[47]). Sereshti and co-workers had applied DLLME to extract essential oils from *Elettaria cardamomum* Maton,[48] *Oliveria decumbens* Vent,[49] and tea.[43] However, the main disadvantage of DLLME is insufficient selectivity for complicated matrices. The extract of DLLME is dirty and contains a lot of interferences such as pigment, polysaccharide, and other nonvolatile compounds, which rapidly and severely deteriorate the analytical instruments due to harmful residue accumulation in the GC injector liner and the first portion of the capillary column. Thus, in order to overcome this drawback, it is necessary to employ a clean-up stage and related alternatives after the analyte extraction process and previous to DLLME technique.[50] Direct sample introduction (DSI) was developed by Amirav and co-workers,[51] which is a simple, rapid, and efficient technique for sampling large-volume dirty sample without a further clean-up stage. It is based on the introduction of sample in a disposable microvial which is then placed into a ChromatoProbe vial holder, which is directly inserted into a temperature programmable GC injector. Initially, the extraction solvent is evaporated at a temperature value corresponding to the solvent boiling point minus ~5 °C.[52] Then with elevation of temperature, the target analytes were thermally extracted into the early portion of the GC column, while the impurity and other nonvolatile residues remained in the vial. The technique has been reported in a wide variety of fields.[52-54]

The aim of the present work was to employ UAE-DLLME coupled with DSI-GC-MS for extraction and preconcentration of essential oils of *Edgeworthia chrysantha* Lindl. at different flowering stages. The influence of operational parameters of UAE-DLLME, such as types of extractant and dispersant, different emulsion process, and ultrasound time on the extraction efficiency of volatile components was investigated. To the best of our knowledge, this is the first report describing the combined application of UAE-DLLME as sample preparation and DSI-GC-MS as analysis technique for the extraction and determination of essential oil from *Edgeworthia chrysantha* Lindl.

2. Experimental

2.1. Reagents and materials

Fresh flowers of *Edgeworthia chrysantha* Lindl. were picked from the campus of Zhejiang University of Technology (Hangzhou, China). Methanol, acetone, acetonitrile, cyclohexane, and n-hexane were obtained from Huadong Medicine Company (Hangzhou, China), while toluene was from Tedia Company (Fairfield, OH, USA). Doubly distilled water was obtained from a Purite RO200–Stillplus HP. System, (Purite Oxon, UK).

A homemade glass vial which looks like a soft polyethylene Pasteur pipette was adopted in the pretreatment process. A sketch of the modified glass vial is shown in Fig. 1.

2.2. Instrumentation

GC-MS analysis was carried out using a Varian GC 3800 (Varian, Walnut Creek, CA, USA) equipped with a 1079 temperature-programmable injector connected to a Varian Saturn 2000 ion-trap mass spectrometer. The chromatographic separation was achieved on a 30-m DB-5 fused-silica column (i.d. 0.25 mm, film thickness 0.25 μm) from J&W Scientific. The temperature of the column was held at 40 °C for 7 min, increased at 3 °C min^{-1} to 250 °C and held for 2 min, ramped to 280 °C at 10 °C min^{-1} and held for 2 min. Electronic flow control (EFC) was used to maintain a constant helium carrier gas flow of 0.8 mL min^{-1}. For the essential oils from UAE-DLLME, sample introduction was performed using a large volume direct sample introduction (DSI) device (ChromatoProbe, from Varian) attached to a 1079 programmable injector with injection volume of 5.00 μL. The injector temperature was maintained at 70 °C for 0.5 min, increased at 80 °C min^{-1} to 110 °C and held for 1.5 min with a 50:1 split to evaporate the solvent, then ramped to 280 °C at 100 °C min^{-1} in splitless mode and held for 2.8 min, after which the injector cooled back to 70 °C and the split ratio was 20:1. Full-scan spectra were acquired in electron ionization (EI, 70 eV) or chemical ionization (CI) mode in the mass range of 40–650 m/z with scan time of three uscans, solvent delay of 14 min, ion-trap temperature of 200 °C, manifold temperature of 50 °C and transfer-line temperature of 280 °C. Kovats retention indices (RI) were calculated for all volatile components using a homologous series of C_6–C_{18} n-alkanes (Sigma Chemical, St. Louis, MO, USA).

A TGL-16C centrifuge from Anting Scientific Instrument (Shanghai, China) was used for centrifuging. A KQ-50E ultrasonic bath from Ultrasonic Instrument Company (Kunshan, China) was used to facilitate extraction.

2.3. Ultrasound-assisted extraction/dispersive liquid-liquid microextraction procedure

In the first step, a portion of fresh flowers of *Edgeworthia chrysantha* Lindl. at different flowering period (~ 40 mg) was placed in a small screw cap glass test tube and 1 mL of acetone was added and extracted in an ultrasonic bath for 10 min at room temperature. Then 0.5 mL upper clear liquid was transfered into a small brown glass vial. After this step 100.0 μL toluene (extraction solvent) was injected into it slowly using a 100.0-μL microsyringe (Shanghai, China) and placed in the ultrasonic bath for 1 min to homogenize the solution. In the next step, 3 mL doubly distilled water was placed in a self-made glass vial (which was illustrated in Fig. 1) and 0.6 mL of the mixed extracting solution (prepared in the previous step) was injected immediately into it. A cloudy solution resulted from dispersion of fine droplets of toluene in aqueous solution was formed in the glass vial. In this step, the objective compounds in acetone were extracted into the fine droplets of toluene within a few seconds. The mixture was then centrifuged for 5 min at 5,000 rpm to separate the cloudy solution into two clear phases. The 5.00 μL of the upper organic phase was removed by using a 10.00-μL microsyringe

and injected into GC by direct sample introduction (DSI) device.

2.5. Identification of components

The GC-MS data were operated using the Saturn Software package 5.2.1. Identification of the constituents was based on comparison of the obtained mass spectra with those of reference compounds in the data system of the Wiley library and NIST Mass Spectral Search Program (NIST 2011 version mass spectral database; National Institute of Standards and Technology, Washington, DC, USA) connected to a Saturn 2000 mass spectrometer and homemade library mass spectra built from pure substances and components of known oils and MS literature. The constituents were confirmed by comparing the Kovats retention index with those of authentic standards or in the published literature or GC retention data with those of authentic standards. Moreover, the molecular weights of the identified substances were confirmed by chemical ionization using CH_3CN as a liquid CI reagent. Quantitative analysis in percent was performed by peak area normalization measurements.

3. Results and discussion

3.1. Optimization of UAE-DLLME method

In this study, the experimental parameters of DLLME (types of extraction solvent and dispersive solvent, emulsion process) and UAE (ultrasound time) were optimized by the full flowering stage's flowers using the "single-factor-at-a-time" method. The peak areas of the main representative compounds were employed as the response in the optimization procedure.

3.1.1. DLLME parameters

3.1.1.1. Optimization of extraction solvent

The type of extraction solvent is a critical experimental parameter that governs the extraction efficiency of the DLLME process. For traditional DLLME method, an appropriate extraction solvent should offer the following physicochemical properties: (1) density higher than water, (2) high extraction capability for target analytes, (3) fine formation of cloudy state in an aqueous solution with a disperser, and (4) good chromatographic compatibility. On the basis of these considerations, chlorinated solvent always should be chosen, such as chloroform, carbon tetrachloride, dichlorohexane, and tetrachloroethylene. But in this experiment, lower density solvents were selected as extraction agents. On the one hand, it makes the collection of organic phase more convenient and DLLME devices are not limited to centrifuge tubes with a cone on the bottom; On the other hand, lower density solvents are less toxic and friendly to environment. Thus, cyclohexane (density 0.81 g mL^{-1}), *n*-hexane (density 0.66 g mL^{-1}), and toluene (density 0.87 g mL^{-1}) were investigated as potential extraction solvents for these purposes. A series of experiments were performed by injecting the mixture of 100.0 µL of the selected extraction solvents and 0.5 mL methanol extract into 3 mL ultrapure water quickly. Figure 2 shows the peak areas of six representative compounds with the three different solvents. The results revealed that higher extraction efficiency of most target compounds was obtained when using toluene compared with other solvents. Therefore, toluene was employed as the extraction solvent in further experiments.

3.1.1.2. Optimization of dispersive solvent

Dispersive solvent in this experiment plays two roles: extraction solvent in the step of UAE and dispersive solvent in DLLME step. Therefore, the dispersive solvent should have a good extracting power of the target analytes and be soluble in both water and extraction solvent in order to form fine droplets and increase the contact surface area of target compounds and the selected extraction solvents. Based on these criteria, methanol, acetone, and acetonitrile were evaluated in the following study. The peak areas of the six major compounds were obtained by rapidly injecting 0.5 mL different dispersive solvents and 100.0 µL toluene mixtures into 3 mL ultrapure water. Figure 3 indicated that the peak areas of most target compounds were higher when using acetone as dispersive solvent compared with other solvents for the same extraction conditions. Hence, acetone was selected as the dispersive solvent for the following studies.

3.1.1.3. Optimization of different emulsion process

The formation process of emulsion adopted in previous experiment was directly injecting the mixture of 100.0 µL extraction solvent and 0.5 mL dispersive solvent into 3 mL ultrapure water. In the process of optimization of dispersive solvent, the extraction efficiency of using acetone as dispersive solvent was obviously better than that of using methanol and acetonitrile. In this work, directly emulsion process was compared to ultrasound assisted emulsion (injecting the mixture into ultrapure water with ultrasound at the same time). The peak areas of six major compounds in these two different emulsion processes are shown in Fig. 4. The results showed that there was no obvious difference about the peak areas in these two different emulsion processes. Therefore, directly injection emulsion was chosen while considering the simplicity of the experiment.

3.1.2. UAE parameters

Ultrasonic time is one of the main influencing factors in UAE-DLLME. It affects the mass transfer of target analytes from solid phase to liquid phase process, and then influences the extraction efficiency of the method. In the present study, the effect of ultrasonic time was studied over the time range of 3 to 15 min. Figure 5 shows the peak areas of six major compounds versus ultrasonic time. It can be observed that the peak areas increase with the increase of ultrasonic time from 3 to 10 min. Beyond 10 minutes, there was an obvious decrease of all the analytes. Therefore, 10 min was chosen as the optimum ultrasonic time.

3.2 The method precision of UAE-DLLME

The precision was expressed by relative standard deviation (RSD). Under the optimum conditions, the obtained peak areas of 6 representative compounds which obtained by three replicate analyses of the essential oil in *Edgeworthia chrysantha* Lindl. were used to calculate the relative standard deviation (RSD) values. The RSD values of *β-cis*-Ocimene (OC), Phenethyl acetate (PE), Ketole (KE), Geranyl acetate (GA), Tridecanal (TR), *trans*-Nerolidol (NE) are 8.6%, 9.0%, 5.4%, 7.3%, 8.3%, 4.9%, which are relatively satisfactory. The results showed that the proposed method of UAE-DLLME combined with DSI-GC-MS has a good precision.

3.3 Comparison of essential oil compounds from *Edgeworthia chrysantha* Lindl. at different flowering stages

The optimal UAE-DLLME parameters were applied for isolation of essential oils from *Edgeworthia chrysantha* Lindl. at different flowering stages, followed by DSI-GC-MS analysis. The

chemical composition of the essential oils was identified by the mass fragmentation patterns and/or retention indices, and the relative content was calculated by the ratio of peak areas. Based on computer and manual analysis, the qualitative and quantitative differences in the components of the four different flowering periods from *Edgeworthia chrysantha* Lindl. by UAE-DLLME were represented in Table 1. The number of replicates was three.

As can be see from Table 1, a total of 36 volatile and semi-volatile compounds were identified and the following results: (1) 17, 27, 34, and 20 compounds were identified in the stage of flower buds, the initial flowering stage, the full flowering stage and the final flowering stage, respectively (Table 1), which representing 68.94, 71.51, 75.60, and 72.11%. The same 15 compounds were found at the four flowering stages, including benzaldehyde, *α*-cumyl alcohol, benzyl acetate, *cis*-3-decen-1-ol, *α*-longifolene and *trans*-farnesol; (2)Esters: methyl benzoate and methyl salicylate were found only in the full flowering stage; geranyl acetate and dihydroactinidiolide were found in the initial and full flowering stages; 9-oxononanoic acid methyl ester and 1,4-Dimethylindanyl acetate were found in the full and final flowering stages; phenethyl acetate were found except the final flowering stage. Those methyl and ethyl ester compounds are the main source of the fragrance of *Edgeworthia chrysantha* Lindl. (3)Alcohols: Benzyl alcohol, lemonol and *trans*-nerolidol were found in the initial and full flowering stages. Benzyl alcohol exists in most plants with fruit fragrance. It can be used to make sesame oil and drugs with antibacterial, anti-inflammatory and analgesic function. Lemonol is widely used in flavours and fragrances with soft, sweet scent of roses. It belongs to monoterpenes with functions of antibacterial and expelling parasite. *trans*-Nerolidol also owns a strong aroma which belongs to monoterpene alcohols. (4)Alkanes, alkenes, aromatic hydrocarbons, aldehydes, ketones were also identified. Most of the alkenes are terpenoids. They can also be used to make flavours and fragrances for their volatility and strong fragrance. It's worth mentioning that indole was found in the full flowering stage which has a strong fecal odor in high concentration and a fragrance in low concentration. Indole exists in many essential oils of fruits and flowers and may be one of the reasons of why the flower of *Edgeworthia chrysantha* Lindl. has a distinctive aroma in the full flowering stage.

Compared with previous works on *Edgeworthia chrysantha* Lindl. using SPME[55] or HD[56], the proposed method in this work could extract more semi-volatile compounds in *Edgeworthia chrysantha* Lindl., such as *trans*-Nerolidol, *trans*-Farnesol and 10,13-Eicosadienoic acid, methyl ester. 30 and 61 volatile compounds were identified from SPME and HD, respectively. The main volatiles extracted by SPME were γ-Terpinene (56.4%), benzyl acetate (10.77%), β-phenyl ethyl acetate (9.58%), 3,7-dimethy-2,6-octadien-1-ol acetate (5.61%), methyl salicylate (3.91%), and methyl benzoate (2.85%). Thus, this new method of UAE-DLLME coupled with DSI-GC-MS could be a complementary approach for analysis of the volatile and semi-volatile components of *Edgeworthia, Chrysanta* Lindl.

4. Conclusion

In this work, UAE-DLLME combined with DSI-GC-MS was successfully developed and for the first time applied for extraction and determination of the volatile and semi-volatile compounds in *Edgeworthia chrysantha* Lindl. at different flowering stages. The identified compounds by using the method introduced in this work were more comprehensive than using conventional HD and SPME methods. In addition, the whole sample preparation consumed small amounts of plant material (~ 40 mg) and organic solvents (100 μL toluene and 0.5 mL acetone) and required a much shorter time (~ 10 min). Ultrasound-assisted extraction supplied sufficient energy to destroy plant cells and effectively accelerated the release of target compounds from plants to organic solvent in a short time. In DLLME process, toluene was used as extraction solvent to replace highly toxic organic solvents such as chlorinated solvents. A low-density solvent could also extend the application of DLLME with a wider range of solvents and reduce the risk to human health and the environment. A homemade glass vial was employed as a vessel for extraction, preconcentration and collection of target analytes, which was convenient to withdraw low-density extraction solvent and avoided background interferences (plasticizer such as diethyl phthalate and diethylhexyl phthalate) when using soft polyethylene Pasteur pipett. These results demonstrated that UAE-DLLME coupled with DSI-GC-MS is a simple, rapid, and efficient method suitable for the analysis of essential oil compounds in fresh flowers of *Edgeworthia chrysantha* Lindl. It can also be used as a rapid sample preparation method for analysis of essential oils in other plants and complex matrix.

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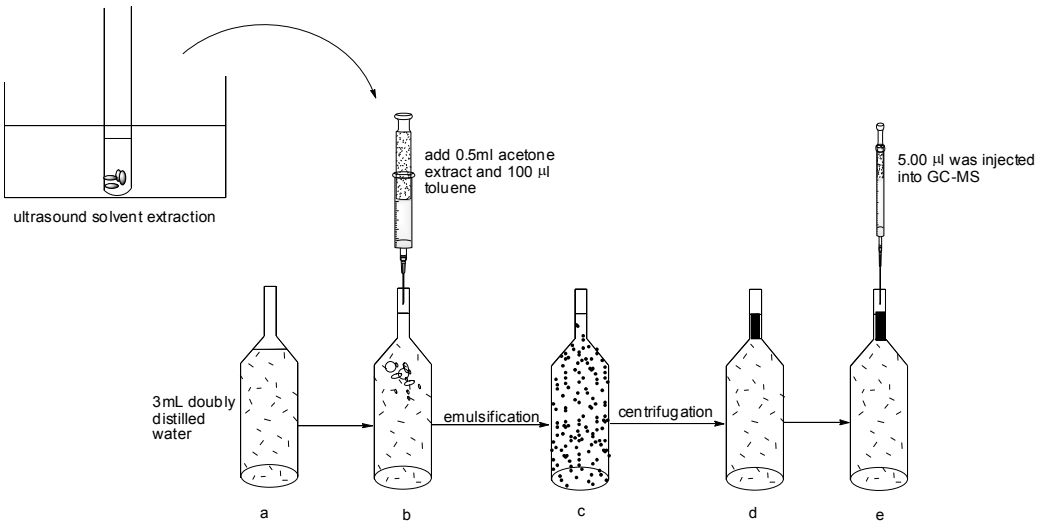

Fig. 1 Schematic of UAE-DLLME: (a) 3mL doubly distilled water was added to a self-made glass vial; (b) 0.5mL acetone extract and 100μl toluene were rapidly injected into the vial; (c) the DLLME process; (d) the cloudy solution was separated into two phases after centrifugation (the upper phase was the toluene extracting); (e) 5.0μl organic phase was collected and injected for GC-MS analysis.

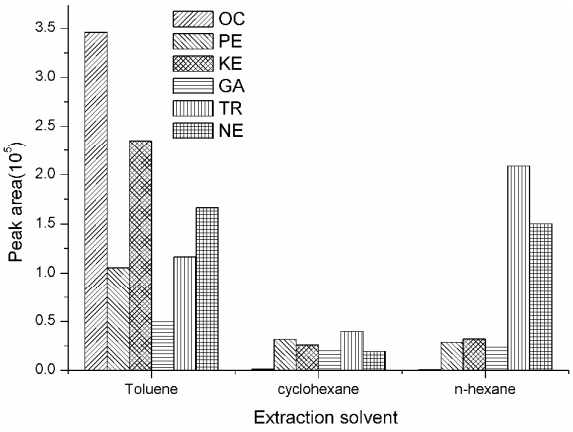

Fig. 2 Effect of the extraction solvent on the extraction efficiency of *β-cis*-Ocimene (OC), Phenethyl acetate (PE), Ketole (KE), Geranyl acetate (GA), Tridecanal (TR), *trans*-Nerolidol (NE) from *Edgeworthia chrysantha* Lindl.

(UAE conditions: extraction solvent: 1 mL methanol; ultrasonic extraction time: 10 min. DLLME conditions: 3mL doubly distilled water; dispersive solvent: 0.5mL methanol; the volume of extraction solvent: 100μl; directly emulsion; centrifugation time: 5 min.)

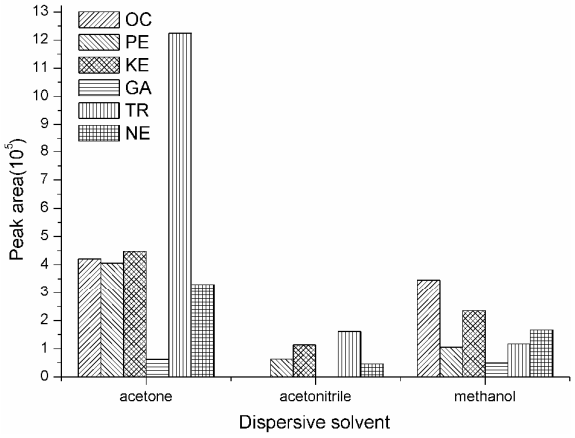

Fig. 3 Effect of the dispersive solvent on the extraction efficiency of *β-cis*-Ocimene (OC), Phenethyl acetate (PE), Ketole (KE), Geranyl acetate (GA), Tridecanal (TR), *trans*-Nerolidol (NE) from *Edgeworthia chrysantha* Lindl.

(UAE conditions: the volume of extraction solvent: 1 mL; ultrasonic extraction time: 10 min. DLLME conditions: 3mL doubly distilled water; the volume of dispersive solvent: 0.5mL; extraction solvent: 100μl toluene; directly emulsion; centrifugation time: 5 min.)

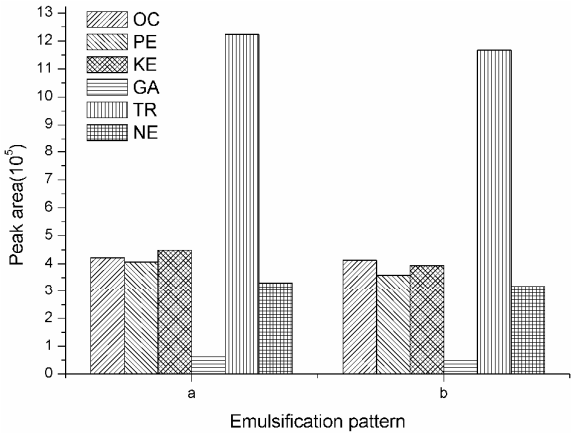

Fig. 4 Effect of the process of emulsion formation ((a) directly emulsion,(b) ultrasound assisted emulsion) on the extraction efficiency of *β-cis*-Ocimene (OC), Phenethyl acetate (PE), Ketole (KE), Geranyl acetate (GA), Tridecanal (TR), *trans*-Nerolidol (NE) from *Edgeworthia chrysantha* Lindl.

(UAE conditions: extraction solvent: 1 mL acetone; ultrasonic extraction time: 10 min. DLLME conditions: 3mL doubly distilled water; dispersive solvent: 0.5mL acetone; extraction solvent: 100μl toluene; centrifugation time: 5 min.)

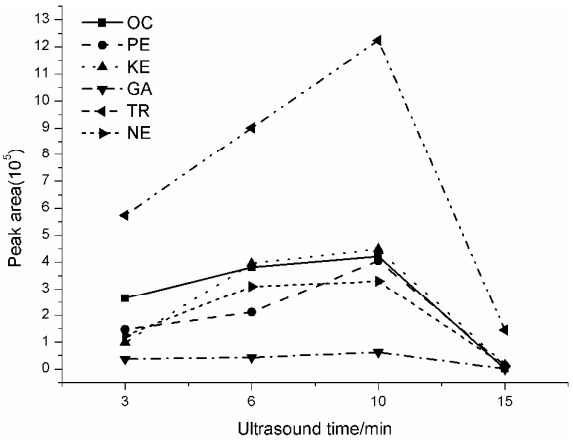

Fig. 5 Effect of the ultrasound time on the extraction efficiency of *β-cis*-Ocimene (OC), Phenethyl acetate (PE), Ketole (KE), Geranyl acetate (GA), Tridecanal (TR), *trans*-Nerolidol (NE) from *Edgeworthia chrysantha* Lindl.

(UAE conditions: extraction solvent: 1 mL acetone. DLLME conditions: 3mL doubly distilled water; dispersive solvent: 0.5mL acetone; extraction solvent: 100μl toluene; directly emulsion; centrifugation time: 5 min.)

Table 1 Identification of main chemical components in essential oils extracted by UAE-DLLME from four different flowering stages of *Edgeworthia chrysantha* Lindl.

NO.	Retention time(min)	Compound	Calculated RI (Literature RI)	Formula	Mw	Mass spectra (m/z)	Identification method	Relative content (%)			
								A	B	C	D
1	17.16	Benzaldehyde	960（963）	C_7H_6O	108	105（100）,77（65）,51（27）	MS	1.13	1.42	2.69	4.91
2	20.34	2-ethyl-1-Hexanol	1023(1028)	$C_8H_{18}O$	130	67（100）,93（55）,94（43）	RI, MS	0.51	0.81	0.66	2.10
3	20.54	Benzyl Alcohol	1027(1032)	C_7H_8O	108	79(100)，77(68)，108(58)	RI, MS	-	0.43	1.19	-
4	21.03	Benzeneacetaldehyde	1039(1045)	C_8H_8O	120	91(100)，92(37)，65(32)	RI, MS	-	1.15	2.82	-
5	21.19	*β-cis*-Ocimene	1042（1040）	$C_{10}H_{16}$	136	93(100)，91(87)，77(57)	RI, MS	-	3.60	2.63	-
6	22.08	Benzoyl chloride	1059	C_7H_5ClO	140	105（100），77（60），51（23）	MS	-	0.59	-	-
7	23.06	*α*-Cumyl alcohol	1079	$C_9H_{12}O$	136	43（100），121（90），77（19）	MS	0.73	0.63	0.35	2.47
8	23.45	Methyl benzoate	1087（1093）	$C_8H_8O_2$	136	105（100），77（64），136（24）	RI, MS	-	-	0.66	-
9	24.10	Nonanal	1100（1100）	$C_9H_{18}O$	142	41（100），57（85），67（76）	RI, MS	3.66	2.17	2.10	3.98
10	24.26	2,2-Dimethyl-3,4-octadienal	1104（1098）	$C_{10}H_{16}O$	152	123（100），95（65），55（27）	RI, MS	1.71	1.24	1.35	2.39
11	26.86	Benzyl acetate	1158（1161）	$C_9H_{10}O_2$	150	108（100），91（53），79（43）	MS	0.69	0.67	1.13	2.11
12	28.20	Methyl salicylate	1187（1191）	$C_8H_8O_3$	152	120（100），92（73），152（72）	RI, MS	-	-	1.33	-
13	28.32	*trans*-3-Dodecene	1189	$C_{12}H_{24}$	168	55（100），69（87），41（75）	MS	2.46	1.05	1.56	4.88
14	28.75	4,6-dimethyl-Undecane,	1199	$C_{13}H_{28}$	184	57（100），71（79），43（64）	MS	2.12	1.32	1.24	3.17
15	29.07	*cis*-3-Decen-1-ol	1205	$C_{10}H_{20}O$	156	67（100），41（88），55（79）	MS	8.17	3.83	3.18	4.57
16	31.05	Lemonol	1249（1256）	$C_{10}H_{18}O$	154	69（100），41（80），67（42）	RI, MS	-	0.35	0.52	-
17	31.19	Phenethyl acetate	1252（1249）	$C_{10}H_{12}O_2$	164	104（100），43（38），78（22）	RI, MS	0.69	2.20	2.01	-
18	32.84	Indole	1287（1286）	C_8H_7N	117	117（100），90（51），89（50）	RI, MS	-	-	4.48	-
19	35.62	1,1,6-Trimethyl-1,2-dihydronaphthalene	1350（1354）	$C_{13}H_{16}$	172	157（100），142（31），172（28）	RI, MS	-	0.63	0.35	-
20	36.27	n-Decanoic acid	1365（1366）	$C_{10}H_{20}O_2$	172	129（100），73（73），60（72）	RI, MS	2.24	1.20	1.74	3.30
21	36.81	Geranyl acetate	1377（1382）	$C_{12}H_{20}O_2$	196	69（100），41（59），43（51）	RI, MS	-	1.06	0.33	-
22	37.34	*cis*-Jasmone	1390（1396）	$C_{11}H_{16}O$	164	79（100），122（86），77（72）	RI, MS	-	0.70	1.84	-
23	38.01	*α*-Longifolene	1405 (1404)	$C_{15}H_{24}$	204	161（100），91（69），105（58）	RI, MS	1.24	1.05	0.33	0.10

24	39.13	9-Oxononanoic acid methyl ester	1432	$C_{10}H_{18}O_3$	186	55（100）83（94）87（70）	MS	-	-	0.98	1.16
25	39.95	*β*-Farnesene	1451（1456）	$C_{15}H_{24}$	204	69（100），41（76），93（73）	RI, MS	-	-	1.99	-
26	40.21	*β*-Ionene	1458	$C_{13}H_{20}O$	192	177（100），135（56），220（55）	MS	1.74	-	-	0.76
27	40.32	4-methyl-Tetradecane,	1460（1460）	$C_{15}H_{32}$	212	71（100），57（87），85（59）	RI, MS	4.11	4.21	4.15	2.12
28	41.38	Hexyl octyl ether	1486	$C_{14}H_{30}O$	214	71（100），57（90），85（67）	MS	3.34	4.39	1.37	2.88
29	41.51	*α*-Farnesene	1489	$C_{15}H_{24}$	204	93(100)，119(99)，91(76)	MS	-	-	0.50	-
30	42.37	Tridecanal	1510（1513）	$C_{13}H_{26}O$	198	67（100），82（88），81（79）	RI, MS	16.21	12.13	8.85	8.99
31	42.82	Dihydroactinidiolide	1522	$C_{11}H_{16}O_2$	180	111（100），137（86），109（66）	MS	-	2.94	1.56	-
32	43.21	Unknown				71（100），57（90），85（65）		2.75	2.81	0.47	2.23
33	44.29	*trans*-Nerolidol	1559（1564）	$C_{15}H_{26}O$	222	93(100)，69(91)，41(83)	RI, MS	-	1.11	1.95	-
34	47.28	1,4-Dimethylindanyl acetate	1637	$C_{13}H_{16}O_2$	204	147（100），162（90），43（28）	MS	-	-	1.89	3.08
35	50.15	*trans*-Farnesol	1715（1718）	$C_{15}H_{26}O$	222	67（100），82（90），81（88）	MS	14.37	13.45	7.86	7.11
36	60.73	9-propyl-Acridine	(2088)	$C_{16}H_{15}N$	221	221（100），192（41），193（12）	MS	-	-	1.65	2.74
37	66.57	Unknown				82（100），67（91），96（84）		3.43	3.27	4.70	3.11
38	67.68	Unknown				71（100），57（98），85（82）		1.82	4.46	3.82	3.04
39	69.45	Unknown				57（100），82（93），96（87）		5.25	4.27	3.20	3.84
40	72.19	Unknown				82（100），67（89），81（88）		6.13	7.16	5.88	3.80
41	74.85	10,13-Eicosadienoic acid, methyl ester	(2292)	$C_{21}H_{38}O_2$	322	82（100），96（88），81（81）	MS	4.08	3.93	3.64	6.19
42	77.43	Unknown				82（100），96（88），81（81）		6.91	4.97	7.13	12.60
43	79.80	Unknown				394（100），288（57），350（44）		4.50	1.74	2.27	1.58
44	80.64	Unknown				71（100），85（95），57（81）		-	3.08	1.63	0.80

(A) flower buds; (B) the initial flowering stage;(C)the full flowering stage; (D)the final flowering stage