

优化超声-盐溶辅助法艾纳香油提取工艺

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摘要: 艾纳香油不仅被广泛应用于中药产业, 其还是一种潜在的杀虫剂和防腐剂, 水蒸气蒸馏法是一种高效、低成本获取艾纳香油的方法。本研究通过正交实验设计、优化超声时间、料液比、盐溶辅助和提取时间来比较艾纳香油的提取率。艾纳香油最佳提取率为 0.42%, 最佳条件组合条件为超声时间为 30 min、液料比为 1:0.6、氯化钠质量分数为 9.0%、提取时间为 4.0 h。通过 GC-MS 法分析艾纳香油的化学成分, 鉴定出 48 中成分 (96.00%)。艾纳香油的主要成分为左旋龙脑 (43.55%)、樟脑 (9.54%)、1-辛烯-3-醇 (8.31%)、石竹烯 (6.51%)、 β -蒎烯 (5.20%)。

关键词: 艾纳香油; 艾纳香; 超声-盐溶辅助水蒸馏; 提取工艺; 正交实验; 化学成分

中图分类号: TQ35

文献标识码: A

DOI: 10.16333/j.1001-6880.2016.S.028

Optimization of Ultrasonic and Salting-out-assisted Extraction of Nǎ lǒng Oil from *Blumea balsamifera* (L.) DC. (Asteraceae)

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Abstract: Nǎ lǒng [*Blumea. balsamifera* (L.) DC.] oil is one of the essential oil that has a wide variety of application in Chinese traditional medicine manufacturing industry, and also has a potential to be used as an insect repellent and antiseptic product in future. Nǎ lǒng oil is obtained using a steam distillation where an aromatic chemical is extracted from *B. balsamifera* (L.) DC. leaves by steam and condensed which provide relatively high yield of product and low cost of running the process. The aim of this study was to improve the yield of Nǎ lǒng oil extraction by using ultrasonic-assisted, salting-out-assisted and optimizing the extraction condition. With the orthogonal test design, the ultrasonic-assisted time, the ratio of material to solvent, the concentration of sodium chloride and the time of extraction were compared to observe the effect on the extraction of volatile oil from Nǎ lǒng plants. The best Nǎ lǒng oil extraction rate by ultrasonic-assisted salting-out hydrodistillation method was 0.42%, and the optimization combinations of best yield were when the ultrasonic-assisted time was 30 min, the ratio of material to water was 1:0.6, the concentration of sodium chloride was 9.0% and extraction time was 4.0 h. The chemical compositions of Nǎ lǒng oil were also analyzed by GC-MS, and 48 components (96.00%) were identified. The dominant components in the Nǎ lǒng oil were borneol (43.55%), camphor (9.54%), 1-Octen-3-ol (8.31%), caryophyllene (6.51%) and β -Pinene (5.20%).

Key words: Nǎ lǒng oil; *Blumea balsamifera* (L.) DC.; ultrasonic-assisted salting-out hydrodistillation; extraction process; orthogonal test; chemical compositions

Introduction

Aromatic plants have been known about for a very long

time and owing to their aromatic and antiseptic properties, they are used as spices and natural food preservatives, aromatic constituents in the perfume industry. Also the plants and essential oil from the plants are widely used for aromatherapy and other different medical purposes [1-5]. Nǎ lǒng (*Blumea balsamifera* (L.)

收稿日期: 2016-01-13 接受日期: 2016-03-23

基金项目: 海南省科技园区建设经费项目; 中央级公益性科研院所基本科研业务费专项 (1630032015020); 国家自然科学基金 (81374065)

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DC., Asteraceae) is one of such plants widely used in South Asia and Southeast Asia^[6]. In Chinese folk medicine, the leaves can be used to cure rheumatism, spondylitis and headache caused by colds^[7], some national people resided in Hainan, Guizhou and Guangxi used the leaves as bathing adjuvant or supplement agent when women newly-born or infant children, the decoction of the roots are used against fever and stomach pain^[8]. In Philippines, the plant is used as diuretic in hypertension and for dissolution of kidney stones^[9]. In other countries of South Asia and Southeast Asia, the leaves of Nä löng also prescribed in the treatment of coryza, fever, influenza, cough and dyspepsia in other countries in South Asia and Southeast Asia^[10-11]. In our continued efforts at identifying the biological activity and pharmacological activity of Nä löng, the leaves also were reported to have antifungal, antibacterial, antifebrile and anodyne activities^[12,13].

Nä löng is also high in essential oil, a lot of chemicals such as *l*-borneol, *d*-camphor, cineol, limonene, palmitic, myristic acids; sesquiterpene alcohol, dimethyl ether, pyrocatechic tannin and other fragrance or flavour constituents were determined from essential oil with GC-mass or other methods^[10,11,14]. The essential oil of Nä löng also had widely use in china folk medicine manufacturing industry, a lot of swallow treating pills or sprays used Nä löng essential oil as efficacy components or basis ingredients, such as “yanlishuang”, “jinhoujian”, “yantejia”, “wanjinxiang” and etc. In our survey of Nä löng essential oil processing technology, we discovered the common methods used currently for the isolation of essential oils from plants are steam distillation, losses of some volatile compounds, low extraction efficiency, degradation of unsaturated compounds through thermal or hydrolytic effects, and high energy waste are common phenomenon may be encountered with these extraction methods. Recently, more efficient extraction methods, such as ultrasonic-assisted extraction, microwave-assisted extraction and supercritical fluid extraction, have been used for the isolation of essential oil from various aromatic plants [15-20], but still now had no reported about ultrasonic-assisted extraction method or salting-out hydrodistillation used in

Nä löng essential oil extraction. The purpose of this research was to determine the optimum conditions of in order to get higher yield of Nä löng oil with ultrasonic and salting-out-assisted method, so with the orthogonal test design, parameters of ultrasonic-assisted time, ratio of material to solvent, concentration of sodium chloride and the time of extraction were compared to observe the effect on the extraction of volatile oil from Nä löng plants, and also the chemical compositions of Nä löng oil were also analyzed by GC-FID and GC-MS.

Materials and Methods

Materials and reagents

Dried Nä löng leaves were obtained from traditional medicinal plant germplasm of south china, Hainan Province, China. The plant was authenticated by Prof. Zhu-nian Wang, voucher specimen was deposited in the Herbarium of tropical crops genetic resources institute (PZS001256), Chinese academy of tropical agricultural sciences, China.

The material of Nä löng were ground to powder using an electric grinder and passed through a 40-mesh sieve. Powder with moisture content of 10% was sealed in a plastic bag and stored at 4 °C in a refrigerator during the experiments. Sodium chloride was purchased from the Shanghai Hushi Chemical Company (Shanghai, China).

Ultrasonic-assisted procedure

An ultrasonicator model SB3200 (Shanghai Branson Ultrasonic Co. Ltd., China) was used in ultrasonic extraction procedure. To begin with, about 100.0 g of Nä löng leaves powder was placed in a distillation flask and mixed with 500.00 mL water. Then the distillation flask with sample was sonicated with continuous power (200 W) for different times in a water bath in the sonicator, whose depth of immersion is 5 cm. The solution of extracting liquid was used for essential oil extraction.

Salting-out hydrodistillation extraction of essential oil

Different ratio of distilled water and different concentration of sodium chloride (Table 1) was then added into the distillation flask, and a volatile oil distillation appa-

ratus was set according to the Chinese pharmacopoeia [7]. The mixture was distilled for 6.0-10.0 h (Table 1). Oil was collected from the condenser dried over anhydrous sodium sulfate.

Optimization of Nä löng oil Extraction with orthogonal design

First, the effects of four independent variables including ultrasonic-assisted time (A, min), ratio of material to solvent (B), concentration of sodium chloride (C, %)

and the time of extraction (D, h) were compared with single factor experimental data.

Based on the preliminary study, four independent variables considered to be the most important parameters in the ultrasonic-assisted salting-out hydrodistillation including ultrasonic-assisted time, ratio of material to solvent, concentration of sodium chloride and extraction time were selected, and their ranges were determined (Table 1).

Table 1 Factors and levels of orthogonal design

Level	Factors			
	Ultrasonic-assisted time (A, min)	Ratio of material to water (B)	Concentration of sodium chloride (C, %)	Extraction time (D, h)
1	10	1:6	3.0	2
2	20	1:8	6.0	4
3	30	1:10	9.0	6

Analysis of Nä löng oil by using GC-FID and GC-MS

The capillary GC-FID analysis was performed using an GC-2010 Plus Network System (Shimadzu), equipped with a FID (supplied with air and hydrogen of high purity) and a split inlet. The chromatographic column used for the analysis was DB-5 capillary column (30 m × 0.25 mm; 0.25 μm film thickness). Helium was used as carrier gas, at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230 °C. Two μL essential oil solution in hexane (HPLC grade) was injected and analyzed with the column held initially at 100 °C for 2 min and then increased to 250 °C with a 3 °C/min heating ramp. The identity of each compound was supported by comparing their retention indices (RI) with published values [25-28]. The sample was analyzed twice and the percentage composition of oil was computed from the GC peak areas without using correction factors.

Chemical constituents of extracted Nä löng oil was also analyzed by GC-MS electron impact-ionization method on GC-2010 Plus gas chromatograph (Shimadzu) coupled to a GC-MS QP 2010A Mass Spectrometer (Shimadzu); fused silica capillary column (30 m × 0.25 mm; 0.25 μm film thickness), coated with DB-5 (J&W); column temperature 100 °C (2 min) to 250

°C at the rate of 3 °C/min; carrier gas, helium at constant pressure of 90 Kpa. Acquisition parameters full scan; scan range 40 - 350 amu. The essential oil constituents were identified by comparing the mass spectra from NIST Library (NIST-147 & NIST-27).

Identification of components

Retention indices of all the components were determined by Kovats method using n-alkanes (C₆-C₃₂) as standards. Identification of individual components was made by comparison of their retention times with those of available analytical standards (*l*-borneol, camphor, caryophyllene), and by computer search, matching mass spectral data with those held in Nist and Wiley library of mass spectra and literature comparison [10, 11, 14, 25, 26, 27, 28].

Results and Discussion

Single factor analysis

To determine the single factor experiment condition, the effects of five parameters with four independent variables were compared. First, the ultrasonic-assisted time at different levels with 0, 10, 20, 30 min and 50 min were compared, and other three independent variables such the ratio of material to water with 1:6, concentration of sodium chloride with 0 % and extraction time with 6.0 h were carried out. Second, the ratio of materi-

al to water with 1:4, 1:6, 1:8, 1:10 and 1:15 were compared, and other three independent variables such the ultrasonic-assisted time with 30 min, concentration of sodium chloride with 0% and extraction time with 6.0 h were carried out. Third, the concentration of sodium chloride with 0%, 3.0%, 6.0%, 9.0% and 12.0% were compared, and other three independent variables such the ultrasonic-assisted time with 30 min, the ratio of material to water with 1:8 and extraction time with 6.0 h were carried out. Last, the extraction time with 2.0, 4.0, 6.0, 8.0 h and 10.0 h were compared, and other three independent variables such the ultrasonic-assisted time with 30 min, the ratio of material to water with 1:8 and the concentration of sodium chloride with 6.0% were carried out.

The results of single factor analysis were showed by Fig 1. From Fig. 1A., the effect of ultrasonic-assisted time on Nä löng oil extraction yields were compared by single factor analysis, and the Nä löng oil extraction yields

started to increase with increasing ultrasonic-assisted time and reached a maximum at the ultrasonic-assisted time of 30 mins, followed by a decrease with further increase in ultrasonic-assisted time. Therefore, the optimal ultrasonic-assisted time was 10 ~ 30 mins; The effect of ratio of material to water on Nä löng oil extraction yields were showed by Fig 1B., and also the oil extraction yields started to increase with increasing ratio of material to water, followed by a decrease with further increase, and then the optimal ratio of material to water, was 1:6 ~ 1:10; The effect of concentration of sodium chloride and the time of extraction on Nä löng oil extraction yields were showed by Fig 1C. and Fig 1D., and the optimal parameters of concentration of sodium chloride and the time of extraction were 3.0% ~ 9.0% and 4.0 ~ 8.0 h. Further then, an orthogonal design with ultrasonic-assisted time, ratio of material to solvent, concentration of sodium chloride and extraction time were compared.

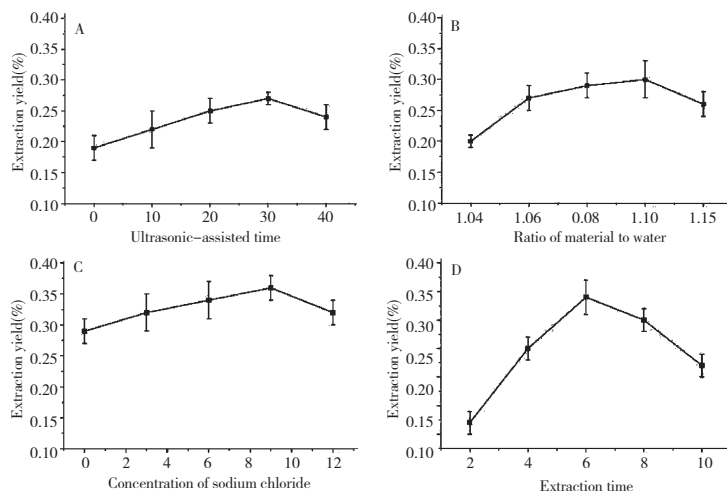


Fig. 1 Effect of ultrasonic-assisted time (A, min), ratio of material to solvent (B), concentration of sodium chloride (C, %) and the time of extraction (D, h) on the extraction yield of essential oil from *B. balsamifera* ($n = 3$)

Extraction of Nä löng oil by orthogonal design

The orthogonal design had been applied successfully to extract biological active components from various materials^[18,21,22,23,24]. In the present investigation, the orthogonal four-factor-three level design was used to optimize extraction conditions, including the ultrasonic-assisted time, the ratio of of material to solvent, the concentration of sodium chloride and the time of extraction. In this design, the power of ultrasonic-assisted was

fixed to 200 W. The total essential oil yield in the extraction supernatant solution was used as evaluation index. The design of orthogonal test and the results are shown in Table 2. The optimization results indicated that the best Nä löng oil extraction rate was reached when the ultrasonic-assisted time was 30 min, the ratio of material to water was 1:06, the concentration of sodium chloride was 9.0% and extraction time was 4.0 h. Under the optimization hydrolysis conditions, the total

essential oil extraction rate reached 0.42%.

The results of the range analysis (Figure 2) showed the ultrasonic-assisted time and the concentration of sodium chloride were the first effective factor and the best Nă lǒng oil extraction condition combination by ultrasonic-assisted salting-out hydrodistillation method was the treatment of T₇ (Table 2, and the rate of volatile oil yield is 0.42%), and the Optimization combinations were the ultrasonic-assisted time was 30 min (A₂, Tables 1 and 2), the ratio of material to water was 1:06 (B₁, Tables 1 and 2), the concentration of sodium chloride was 9.0% (C₃, Tables 1 and 2) and extraction time was 4.0 h (D₂, Tables 1 and 2). Also, it was

found that the ratio of material to water from 1:06 to 1:10 had little effect on the extraction of Nă lǒng oil. For this reason, at view of low solvent, low ratio of material to water might be the best choice for the hydrolysis process.

Analysis of Nă lǒng oil and its chemical constituents

Gas chromatography-mass spectrometry (GC-MS) had become a basic tool for analysis and Characterisation of essential oils [25-28]. The chemicals compositions of *B. balsamifera* (L.) DC. essential oil had been reported by Morallo-Rejesus [14] and Amornchai et al. [10,11], but little report in China.

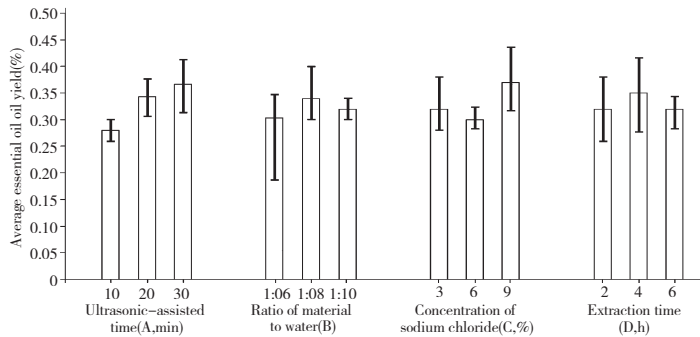


Fig. 2 The range analysis of ultrasonic-assisted salting-out hydrodistillation method orthogonal test (vertical bars were standard errors, $n = 3$)

Table 2 The control factors and levels in Nă lǒng oil Extraction by ultrasonic-assisted salting-out hydrodistillation method

Treatment	Factors				results
	Ultrasonic-assisted time (A, min)	Ratio of material to water (B)	Concentration of sodium chloride (C, %)	Extraction time (D, h)	Volatile oil yield (%)
T ₁	10 (A ₁)	1:6 (B ₁)	3.0 (C ₁)	2 (D ₁)	0.26 ± 0.02
T ₂	10	1:8 (B ₂)	6.0 (C ₂)	4 (D ₂)	0.28 ± 0.03
T ₃	10	1:10 (B ₃)	9.0 (C ₃)	6 (D ₃)	0.30 ± 0.02
T ₄	20 (A ₂)	1:6	6.0	6	0.31 ± 0.01
T ₅	20	1:8	9.0	2	0.38 ± 0.03
T ₆	20	1:10	3.0	4	0.34 ± 0.02
T ₇	30 (A ₃)	1:6	9.0	4	0.42 ± 0.03
T ₈	30	1:8	3.0	6	0.36 ± 0.03
T ₉	30	1:10	6.0	2	0.32 ± 0.05
K ₁	0.280	0.330	0.320	0.320	
K ₂	0.343	0.340	0.303	0.347	
K ₃	0.367	0.320	0.367	0.323	
R	0.087	0.020	0.064	0.027	

Notes: 1) T₁-T₉ are the abbreviations of various extracting treatments; 2) K_i = \sum the amount of target compounds at factors of A, B, C or D; 3) R = $\max\{K_i\} - \min\{K_i\}$.

Table 3 Chemical constituents of Nä löng oil

No.	Compounds	Formula	RT/min	Relative content (%)	RI	No.	Compounds	Formula	RT/min	Relative content (%)	RI
1	(<i>E</i>)-2- Hexenal	C ₆ H ₁₀ O	5.810	0.04	814	25	4-isopropylbenzaldehyde	C ₁₀ H ₁₂ O	26.925	0.10	1230
2	(<i>Z</i>)-3-hexen-1-ol	C ₆ H ₁₂ O	5.915	0.11	868	26	Perillaldehyde	C ₁₀ H ₁₄ O	29.160	0.13	1207
3	(<i>E</i>)-2- Hexen-1-ol	C ₆ H ₁₂ O	6.255	0.08	868	27	Bornyl acetate	C ₁₂ H ₂ O ₂	30.065	0.41	1277
4	Hexyl alcohol	C ₆ H ₁₄ O	6.330	0.21	860	28	2,2,8-Trimethyltricyclo[6.2.2.0 ^{1,6}]dodec-5-ene	C ₁₅ H ₂₄	32.485	2.93	1416
5	butyl acrylate	C ₇ H ₁₂ O ₂	7.305	0.26	874	29	γ-Maaliene	C ₁₅ H ₂₄	33.655	2.14	1443
6	α-Pinene	C ₁₀ H ₁₆	8.835	2.04	948	30	dehydro-aromadendrene	C ₁₅ H ₂₂	34.525	0.11	1396
7	Camphene	C ₁₀ H ₁₆	9.485	1.50	943	31	α-gurjunene	C ₁₅ H ₂₄	37.865	0.22	1419
8	β-Pinene	C ₁₀ H ₁₆	10.840	5.20	943	32	β-caryophyllene	C ₁₅ H ₂₄	38.470	6.51	1494
9	1-Octen-3-ol	C ₁₀ H ₁₆ O	11.045	8.31	969	33	2-tert-butyl-1,4-dimethoxybenzene	C ₁₂ H ₁₈ O	38.960	3.07	1386
10	3-Octanone	C ₈ H ₁₆ O	11.430	0.21	952	34	α-humulene	C ₁₅ H ₂₄	40.505	1.54	1579
11	β-Myrcene	C ₁₀ H ₁₆	11.665	0.10	958	35	alloaromadendrene	C ₁₅ H ₂₄	42.340	0.78	1386
12	3-Octanol	C ₈ H ₁₈ O	11.905	1.36	979	36	aromadendrene	C ₁₅ H ₂₄	44.225	0.12	1386
13	β-Pinene	C ₁₀ H ₁₄	13.420	0.24	1042	37	γ-cadinene	C ₁₅ H ₂₄	44.230	0.15	1435
14	d-Limonene	C ₁₀ H ₁₆	13.655	0.39	1018	38	δ-cadinene	C ₁₅ H ₂₄	44.830	0.20	1469
15	Eucalyptol	C ₁₀ H ₁₈ O	13.840	0.06	1059	39	Palustrol	C ₁₅ H ₂₆ O	47.250	0.06	1530
16	(<i>E</i>)-3,7-dimethylocta-1,3,6-triene	C ₁₀ H ₁₆	14.295	0.19	976	40	Caryophyllene oxide	C ₁₅ H ₂₄ O	48.110	0.89	1507
17	β-Ocimene	C ₁₀ H ₁₆	14.890	0.76	976	41	Guaiol	C ₁₅ H ₂₆ O	49.085	0.41	1614
18	β-Linalool	C ₁₀ H ₁₈ O	18.040	1.76	1082	42	Ledol	C ₁₅ H ₂₆ O	49.275	0.12	1530
19	Hotrienol	C ₁₀ H ₁₆ O	18.315	0.28	1072	43	Rosifoliol	C ₁₅ H ₂₆ O	49.555	0.20	1598
20	Chrysanthenone	C ₁₀ H ₁₄ O	19.500	0.22	1119	44	γ-eudesmol	C ₁₅ H ₂₆ O	50.200	0.59	1626
21	Camphor	C ₁₀ H ₁₆ O	20.660	9.54	1121	45	Alloaromadendrene oxide	C ₁₅ H ₂₄ O	51.145	0.34	1462
22	Borneol	C ₁₀ H ₁₈ O	22.185	43.55	1138	46	β-Eudesmol	C ₁₅ H ₂₆ O	51.890	0.39	1593
23	1-Terpinen-4-ol	C ₁₀ H ₁₈ O	22.905	0.09	1137	47	Juniper camphor	C ₁₅ H ₂₆ O	52.145	0.48	1647
24	α-terpineol	C ₁₀ H ₁₈ O	23.810	0.24	1143	48	Brevifolin	C ₁₀ H ₁₂ O ₄	53.050	1.40	1628

The chemical compositions of Nä löng oil were also analyzed by GC-MS, and 48 components (96.00%) were identified, Forty-six components were found in the leaf oil (Table 3.). Same as the *B. balsamifera* essential oils in Thailand [10-11] and Bangladesh [14], the main chemical compositions of *B. balsamifera* essential oils are borneol, camphor, caryophyllene, caryophyllene oxide, guaiol and etc. And also some different chemical compositions were first discovered from Nä löng oil such as 1-Octen-3-ol, 3-Octanol, 2-tert-butyl-1,4-dimethoxybenzene, brevifolin, and etc. How cause the difference, the reason maybe multiple, maybe those difference were caused by the same plant but from different areas or the difference of extraction method, and the definite reason need more materials from different areas

and more retest.

This is the first report of Nä löng oil in China. Thus, our data suggested that the oils offered new possibilities for the isolation of natural borneol and caryophyllene. Those may give valuable information for chemotaxonomic studies on *Blumea* species and their relationships in the family *Asteraceae*.

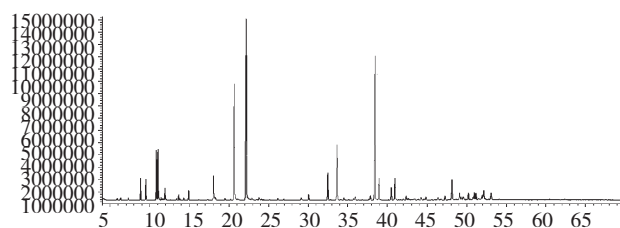


Fig. 3 GC-MS chromatogram of Nä löng oil obtained with the method described previously

Acknowledgments

This work was supported by the grants of Hainan science and technology park infrastructure projects, central public-interest scientific Institution basal research Fund (No. 1630032015020) and the National Natural Science Foundation of China (No. 81374065).

References

- 1 Aburjarai T, Natsheh FM. Plants used in cosmetics. *Phytotherapy Res*, 2003, 17 :987-1000.
- 2 Ajose FO. Some Nigerian plants of dermatologic importance. *Int J Dermatol*, 2007, 46(1) :48-55.
- 3 Fisher K, Phillips C. Potential antimicrobial uses of essential oils in food; is citrus the answer. *Trends Food Sci Tech*, 2008, 19:156-164.
- 4 Natsch A, Wasescha M. Fragrance raw materials and essential oils can reduce prostaglandin E2 formation in keratinocytes and reconstituted human epidermis. *Int J Cosmet Sci*, 2007, 29:369-376.
- 5 Buchbauer G. In Handbook of essential oils//Science, Technology and Application, K. H. C. Baser, G. Buchbauer (eds). London: CRC Press, Taylor and Francis. 2010. 235-280.
- 6 Perry LM, Metzger J. Medicinal plants of East and Southeast Asia. //attributed properties and uses. Cambridge: Massachusetts Institute of Technology, MA. 1980. 87.
- 7 Chinese Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China. Beijing: China Medical Science Press, 2010. Vol I, 56.
- 8 Santos AC, Santos GH, Obligacion MB, et al. Philippines plants and their contained natural products//Biological and pharmacological literature survey. Research Library and Information Division of Metro Manila (eds), Manila: National Research Council of the Philippines National Relief Charities press, 1981. 2121.
- 9 Jia MR, Li XW. Ethnography of China [M]. Beijing: Chinese medical science and technology press, 2005. 98.
- 10 Amornchai T, Sriubolmas N, De-Eknamkul W, et al. Chemical composition and antimicrobial activity of the essential oil of Thai *Blumea lacera*. Proc. Chulalongkorn University 80th Anniversary Research Conference, Bangkok, Thailand.
- 11 Amornchai T, De-Eknamkul W, Ruangrunsi N. Essential oil composition of Thai *Blumea balsamifera*. Proc. First Indochina Conference on Pharmaceutical Sciences, Bangkok. 1997.
- 12 Ragasa CY, Kristin CC, Rideout AL, et al. Antifungal metabolites from *Blumea balsamifera*. *Nat Prod Res*, 2005, 19:231-237.
- 13 Asolkar LV, Kakkar KK, Chakre OJ. Second Supplement to Glossary of Indian Medicinal Plants with active principles. Part-1 (A-K), CSIR, New Delhi. 1992.
- 14 Morallo-Rejesus MB, Maini HA, Hsawa K, et al. Insecticidal actions of several plants to *Callosobruchus chinensis* L. // Bruchids and legumes. Economics, Ecology and Coevolution. Fujii K., et al. (eds). Okayama: Springer Netherlands press, 1990. 91-100.
- 15 Hao JY, Han W, Huang SD, et al. Microwave-assisted extraction of artemisinin from *Artemisia annua* L. . *Sep Purific Technol*, 2002, 28:191-196
- 16 Braga Mara EM, Ehelerta P, Ming LC, et al. Supercritical fluid extraction from *Lippia alba*: global yields, kinetic data, and extract chemical composition. *J Supercritical Fluids*, 2005, 34:149-156.
- 17 Japón-Luján R, Luque-Rodríguez JM, Luque de Castro MD. Multivariate optimisation of the microwave-assisted extraction of oleuropein and related biophenols from olive leaves. *Anal Bioanal Chem*, 2006, 385:753-759.
- 18 Xiao JB, Chen JW, Xu M. Supercritical fluid CO₂ extraction of essential oil from *Marchantia convoluta*: global yields and extract chemical composition. *Electronic J Biotechnol*, 2007, 10:141-148
- 19 Hui T, Jo IH, Choi YH. Optimization of ultrasonic-assisted extraction of phenolic compounds from Chinese quince (*Chaenomeles sinensis*) by response surface methodology. *J Korean Soc Appl Biol Chem*, 2010, 53:618-625.
- 20 Yu XC, Sun DL, Li XS. Ultrasound-enhanced supercritical CO₂ extraction of masson pine wood essential oil. *Asian J Chem*, 2012, 24:409-412.
- 21 He GD, Xiong HP, Chen QH et al. Optimization of conditions for supercritical fluid extraction of flavonoids from hops (*Humulus lupulus* L.). *J Zhejiang Univ Sci B*, 2005, 6:999-1004.
- 22 Li Z, Pan QH, Cui XY, et al. Optimization on anthocyanins extraction from wine grape skins using orthogonal test Design. *Food Sci Biotechnol*, 2010, 19:1047-1053.
- 23 Wang BH, Lin LP, Lu L, et al. Optimization of β -carotene production by a newly isolated *Serratia marcescens* strain. *Electronic J Biotechnol*, 2012, 15:1075-1082.
- 24 Wu JC, Huang GR, Cheng J. Optimization of enzymatic hydrolysis for extraction of flavonoids from *Apium graveolens* L. stalks by entropy weight method. *J Food Agric Environ*, 2012, 10:182-185.
- 25 Marriott PJ, Shellie R, Cornwell C. Gas chromatographic technologies for the analysis of essential oils. *J Chromatogr A*, 2001, 936:1-22.