Study on New Extraction Technology and Chemical Composition of Litsea Cubeba Essential Oil

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Abstract: Evaluated with the yield of litsea cubeba essential oil, water extraction technology of essential oil from the seed of *Litsea Cubeba (Lour.)* Pers. assisted by microwave and ultrasonic is studied for the first time in this paper. The optimal water extraction technology of litsea cubeba essential oil assisted by pure microwave is as follows: extracting time 40 minutes, microwave power 650 W, solid-liquid ratio 1:4 (g/ml). Average yield of litsea cubeba essential oil is up to 10.287% (g/g) under the above optimal technology. The optimal water extraction technology of litsea cubeba essential oil assisted by microwave together with ultrasonic is as follows: extracting time 8 minutes, microwave power 600 W, solid-liquid ratio 1:7 (g/ml), extracting temperature 85°C. Average yield of litsea cubeba essential oil is up to 14.188% under the above optimal technology. Microwave and ultrasonic can effectively enhance the extraction process of litsea cubeba essential oil.

Chemical composition of litsea cubeba essential oil, including its components and the concent of each determined component, is analized by Gas Chromatography Mass Spectrometry (GC-MS).

44 compounds in the oil extracted by water assisted by pure microwave are seperated and 31 of which are identified as Bicyclo-[3,1,1]hept-2-ene, 2,6,6-trimethyl-,(±)-; α-pinene; 5-Hepten-2-one,6-methyl-; β-pinene; Bicyclo-[3,1,0]hexan-2-ol, 2-methyl-5-(1-methylethy)-(1α,2α,5α)-; 1-Methyl-4-(1-methylethenyl)-,acetate; Camphene; 1,5,5- trimethyl -6- methyl ene-cyclohexene; Linalool; Bicyclo-[2,2,1]heptane-2,5-diol, 1,7,7-trimethyl-,(2-endo, 5-exol)-; 5-Caranol,trans,trans-(+)-; Isoborneol; Bicyclo [3,1,1]hept-3-en -2-ol,4,6,6-trimethyl-,[1s-(1α,2β,5α)]-; geranialdehyde; Estragole; 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde; Ethanol,2-(9,12-octadecadienyloxy)-,(z,z)-; 1-Heptatriacotanol; Caryophyllene; α-Caryophyllene; n-Decanoic acid; Caryophyllene oxide; Bicyclo[4,4,0]dec-2-ene-4-ol,2-methyl-9-(prop-1-en-3-ol-2-yl)-; Dodecanoic acid; Retinol; Fenretinide; 5-(7α-lsopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-penta-2,4-dien-l-ol; Podocarp-7-en-3-one, 1,3β -methyl-1,3-vinyl-; 4,8,13-Cyclotetradeca-triene-1,3-diol,1,5,9-trimethyl-12-(1-methylethyl)-; 1-Heptatriacotanol; 5-(7a-lsopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-penta-2,4-doen-1-ol.

48 compounds in the oil extracted by water assisted by microwave together with ultrasonic are seperated and 31 of which are identified as Bicyclo-[3,1,1]hept-2-ene, 2,6,6-trimethyl-,(±)-; α-pinene; 5-Hepten-2-one,6-methyl-; β-pinene; 1-Methyl-4-(1-methylethenyl)-,acetate; Camphene; 1,5,5- trimethyl -6- methyl -ene-cyclohexene; Linalool; α-Caryophyllene; Isoborneol; 5,8,11-Heptadecatrien-1-ol; α-terpineol; Bicyclo [3,1,1] he pt-3-en-2-ol,4,6,6-trimethyl-,[1s-(1α,2β,5α)]-; geranialdehyde; Estragole; Ethanol,2-(9,12-octadecadienyloxy)-,(z,z)-; 1-Heptatriacotanol; Caryophyllene; α-Caryophyllene; n-Decanoic acid; Butylated Hydroxytoluene; Caryophyllene oxide; Bicyclo[4,4,0]dec-2-ene-4-ol,2-methyl-9-(prop-1-en-3-ol-2-yl)-; 3-Cyclohexen-4-ol-1-one,3-tridecanoyl-; Dodecanoic acid; Retinol; Fenretinide; 5-(7α-lsopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-penta-2,4-dien-l-ol; Podocarp-7-en-3-one, 1,3β-methyl-1,3-vinyl-; 10,12,14-Nonacosatriynoic acid; 1-Heptatriacotano.

Keywords: Extraction technology, chemical composition, microwave, ultrasonic, litsea cubeba essential oil, yield, concent.

1. INTRODUCTION

Litsea cubeba (Lour.) Pers. is one of the specific perfume plants in China and distributes widely in more than 15 provinces including Hunan, Guangdong, Hubei, etc. [1, 2]. Litsea cubeba oil is essential oil mainly extracted from the seed of *Litsea cubeba (Lour.)* Pers. For a long time, China acts as both the biggest origine and the biggest exporting country of litsea cubeba oil all over the world. Litsea cubeba oil made in China is always welcomed by world market because its high concent of geranialdehyde, whose special value lies in that it can be used as raw material in preparing ionone type of perfumes, and that it possesses many biological activities such as antibacterial activity, antioxidation, antiasthmatic effect, antianaphylaxis effect and anthelmintic activity, etc. [2-4].

At present, the processing mode of *Litsea cubeba (Lour.)* Pers. in China presents many disadvantages in lagging technology, small scale, limited varieties of products and low quality of products, which blocks the highly valuable utilization of *Litsea cubeba (Lour.)* Pers.

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This paper focuses on feasible extraction technology with high yield of litsea cubeba oil. Two kinds of means, microwave and ultrasonic are applied to enhance the extraction process for the first time. Meanwhile, chemical composition of litsea cubeba essential oil, including its components and the concent of each determined component, is studied. The research results can provide basis for the highly efficient ulilization of *Litsea Cubeba (Lour.)* Pers.

2. MATERIAL AND METHOD

2.1. Experimental Material

The seed of *Litsea Cubeba (Lour.)* Pers. were collected in 2009 from the outskirts of Zhangjiajie, which were broken to pieces, dried at 60°C and sifted successively. The voucher specimen is deposited at the College of Material Science and Engineering of the Central South University of Forestry and Technology (20091020).

2.2. Extraction of Litsea Cubeba Essential Oil from the Seed of *Litsea Cubeba (Lour.)* Pers

30 g of seed powder of *Litsea Cubeba (Lour.)* Pers. is soaked in distilled water at predetermined solid-liquid ratio for 4 hrs, then extracted for a certain time at a certain power of microwave in the extraction apparatus. After finishing extraction, the extract is centrifugal separated at 3000 r/min. The upper clear liquid is extracted with petroleum ether. The essential oil is weighed accurately after removing the petroleum ether. The yield of litsea cubeba essential oil is calculated according to the formula (1) below:

Yield of litsea cubeba oil (%) = mass of litsea cubeba oil (g)/mass of material (g) (1)

2.3. Analysis of the Chemical Composition of Cubeba Essential Oil by GC-MS

2.3.1. Gas Chromatography (GC) Conditions

TR-1 silica capillary column (30 m×0.32 mm×0.25 μm); carrier gas: He, flow rate 1 ml/min, splitless (split ratio 50:1), constant presure mode; injector temperature: 250°C; programmed oven temperature: Initial temperature 50°C and hold time 3 minutes, Ramp1:from 50°C to 115°C at 2°C/min and hold time 1 minute, Ramp2: from 115°C to 120°C at 1°C/min and hold time 1 minute, Ramp3: from 120°C to 128°C at 3°C/min and hold time 1 minute, Ramp4: from 128°C to 130°C at 0.2°C/min and hold time 1 minute, Ramp5: from 130°C to 145°C at 10°C/min and hold time 1 minute, Ramp6: from 145°C to 170°C at 1.5°C/min and hold time 5 minutes.

2.3.2. Mass Spectrometry (MS) Conditions

DSQ Single Quadrupole Mass Spectrometer, Electron Impact Ionization Source (EI) with an ionization voltage of 70 eV and electron multiplier voltage of 1262 V, ion source temperature 250°C, scan mass range: 50~650.

2.3.3. Qualitative and Quantitative Analysis Method

Identification of the components is based on the comparison of the mass spectrum of each compound with that of known compounds searched in the combining computer database and assisted by some physical and chemical analytic methods. Quantification is elaborated as the percentage contribution of each compound to the total amount present after calculated by peak area normalization.

3. RESULT AND DISCUSSION

3.1. Water Extraction of Litsea Cubeba Essential Oil Assisted by Pure Microwave

3.1.1. The Optimal Extraction Technology

On the basis of the results of single factor experiments, orthogonal experiment of $L_9(3^3)$ is carried out to optimize the water extraction technology of litsea cubeba essential oil assisted by pure microwave. The experimental results are shown in Table **1**.

Table 1. Results of Orthogonal Experiment

No.	Factors			Yield of Litsea Cubeba Oil (%)
	Extracting Time [Minute]	Microwave Power [Watt]	Solid-Liquid Ratio [g/ml]	
1	20	500	1:4	9.328
2	20	650	1:5	8.842
3	20	800	1:6	8.617
4	30	500	1:5	9.115
5	30	650	1:6	10.157
6	30	800	1:4	9.538
7	40	500	1:6	9.101
8	40	650	1:4	9.871
9	40	800	1:5	10.037
K_1	8.929	9.181	9.579	
K_2	9.603	9.623	9.331	
K_3	9.670	9.397	9.292	
R	0.741	0.442	0.287	

The results of orthogonal experiment of $L_9(3^3)$ show that the optimal water extraction technology of litsea cubeba essential oil assisted by pure microwave is as follows: extracting time 40 minutes, microwave power 650 W, solid-liquid ratio 1:4 (g/ml). The R value reveals that extracting time is the most important influencing factor in the water extraction process of litsea cubeba essential oil assisted by pure microwave. Verified experiments are carried out for three times and the average yield of litsea cubeba essential oil is up to 10.287% (g/g) under the above optimal technology.

3.1.2. Chemical Composition of Litsea Cubeba Essential Oil Obtained Through Water Extraction Assisted by Pure Microwave

The components and their concent in the litsea cubeba essential oil is determined by GC-MS analysis. 44 compounds are seperated and identified separately. Total ion chromatogram of components 31 of which are in litsea cubeba essential oil obtained through water extraction assisted by pure microwave is shown in Fig. (**1**). and the name together with concent of each determined compound are listed in Table **2**.

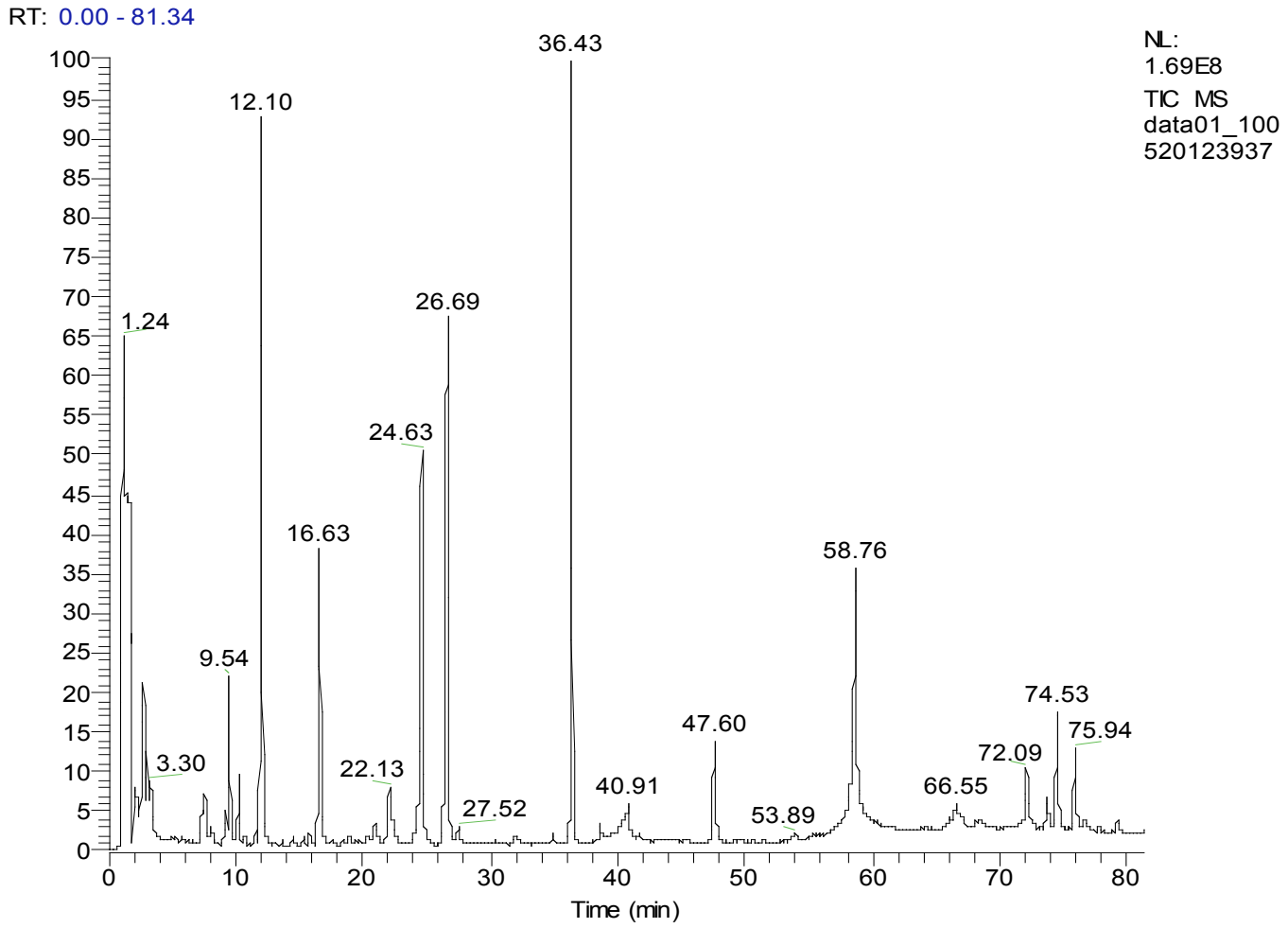

Fig. (1). Total ion chromatogram of components in litsea cubeba essential oil obtained through water extraction assisted by pure microwave.

3.2. Water Extraction of Litsea Cubeba Essential Oil Assisted by Microwave Together with Ultrasonic

3.2.1. The Optimal Extraction Technology

On the basis of the results of single factor experiments, orthogonal experiment of $L_{16}(4^4)$ is carried out to optimize the water extraction technology of litsea cubeba essential oil assisted by microwave together with ultrasonic. The experimental results are shown in Table **3**.

The results of orthogonal experiment of $L_{16}(4^4)$ show that the optimal water extraction technology of litsea cubeba essential oil assisted by microwave together with ultrasonic is as follows: extracting time 8 minutes, microwave power 600 W, solid-liquid ratio 1:7 (g/ml), extracting temperature 85°C. The R value reveals that extracting temperature is the most important influencing factor in the water extraction process of litsea cubeba essential oil assisted by microwave together with ultrasonic. Verified experiments are carried out for three times and the average yield of litsea cubeba essential oil is up to 14.188% (g/g) under the above optimal technology.

3.2.2. Chemical Composition of Litsea Cubeba Essential Oil Obtained Through Water Extraction Assisted by Microwave Together with Ultrasonic

The components and their concent in the litsea cubeba essential oil is determined by GC-MS analysis. 48 compounds are seperated and identified separately. Total ion chromatogram of components 31 of which are in litsea cubeba essential oil obtained through water extraction assisted by microwave together with ultrasonic is shown in Fig. (**2**). and the name together with concent of each determined compound are listed in Table **4**.

3.3. Discussion

The reported yield of litsea cubeba essential oil by traditional steam distillation method was 4~6% [5], which is far lower than that obtained in existence of microwave and ultrasonic. The higher yield of litsea cubeba essential oil can be obtain in this research because that the penetrating power of microwave accelerates the process of target substances' diffusion from interior to exterior of material cells, and the cavitation effect of ultrasonic further improves the breakage

Table 2. Chemical Composition of Litsea Cubeba Essential Oil Obtained Through Water Extraction Assisted by Pure Microwave

No.	Retention Time (Min)	Relative Concent (%)	Formula	Molecular Weight	Name of Compound
1	2.2	0.02286	------		
2	2.77	0.07565	------		
3	5.05	0.00181	------		
4	5.32	0.00154	------		
5	5.81	0.00266	------		
6	7.62	0.01651	$C_{10}H_{16}$	136	Bicyclo-[3,1,1]hept-2-ene, 2,6,6-trimethyl-,(±)-
7	8.09	0.00259	$C_{10}H_{16}$	136	α-pinene
8	9.54	0.03253	$C_8H_{14}O$	126	5-Hepten-2-one,6-methyl-
9	10.27	0.01065	$C_{10}H_{16}$	136	β-pinene
10	10.76	0.00162	$C_8H_{18}O$	154	Bicyclo-[3,1,0]hexan-2-ol, 2-methyl-5-(1-methylethy)-(1α,2α,5α)-
11	12.1	0.1355	$C_{12}H_{20}O_2$	196	1-Methyl-4-(1-methylethenyl)-,acetate
12	14.51	0.00194	$C_{10}H_{18}O_2$	170	Camphene
13	15.34	0.00213	$C_{10}H_{16}$	136	1,5,5- trimethyl -6- methyl ene-cyclohexene
14	15.79	0.00316	------		
15	16.63	0.06479	$C_{10}H_{18}O$	154	Linalool
16	18.46	0.00152	$C_{10}H_{18}O_2$	170	Bicyclo-[2,2,1]heptane-2,5-diol, 1,7,7-trimethyl-,(2-endo, 5-exol)-
17	19.03	0.0025	$C_{10}H_{18}O$	154	5-Caranol,trans,trans-(+)-
18	20.39	0.00334	$C_{10}H_{18}O$	154	Isoborneol
19	21.01	0.00777	------		
20	22.13	0.02312	------		
21	24.63	0.08431	$C_{10}H_{16}O$	152	Bicyclo [3,1,1]hept-3-en -2-ol,4,6,6-trimethyl-,[1s-(1α,2β,5α)]-
22	26.69	0.1391	$C_{10}H_{16}O$	152	geranialdehyde
23	27.52	0.00396	$C_{10}H_{12}O$	148	Estragole
24	29.42	0.00161	------		
25	30.4	0.00143	$C_{23}H_{32}O$	324	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde
26	31.99	0.0044	$C_{20}H_{38}O_2$	310	Ethanol,2-(9,12-octadecadienyloxy)-,(z,z)-
27	34.9	0.00219	$C_{37}H_{76}O$	536	1-Heptatriacotanol
28	36.43	0.1052	$C_{15}H_{24}$	204	Caryophyllene
29	38.63	0.00396	$C_{15}H_{24}$	204	α-Caryophyllene
30	40.91	0.01267	$C_{10}H_{20}O_2$	172	n-Decanoic acid
31	47.6	0.02666	$C_{15}H_{24}O$	220	Caryophyllene oxide
32	49.67	0.00158	------		
33	53.89	0.00388	$C_{15}H_{24}O_2$	236	Bicyclo[4,4,0]dec-2-ene-4-ol,2-methyl-9-(prop-1-en-3-ol-2-yl)-
34	58.76	0.08044	$C_{12}H_{24}O_2$	200	Dodecanoic acid
35	66.14	0.0043	------		
36	66.55	0.0122	------		
37	68.45	0.00555	------		
38	72.09	0.02152	$C_{20}H_{30}O$	286	Retinol
39	73.7	0.00904	$C_{26}H_{33}NO_2$	391	Fenretinide
40	74.53	0.02853	$C_{20}H_{32}O$	288	5-(7α-lsopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-penta-2,4-dien-l-ol
41	75.94	0.02125	$C_{20}H_{30}O$	286	Podocarp-7-en-3-one, 1,3β -methyl-1,3-vinyl-
42	76.72	0.00489	$C_{20}H_{32}O_2$	306	4,8,13-Cyclotetradecatriene-1,3-diol,1,5,9-trimethyl-12-(1-methylethyl)-
43	77.82	0.00176	$C_{37}H_{76}O$	536	1-Heptatriacotanol
44	79.24	0.00538	$C_{20}H_{32}O$	288	5-(7a-lsopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-penta-2,4-doen-1-ol

Table 3. Results of Orthogonal Experiment

No.	Factors				Yield of Litsea Cubeba Essential Oil [%]
	Extracting Time [Minute]	Solid-Liquid Ratio [g/ml]	Extracting Temperature [°C]	Microwave Power [Watt]	
1	2	1:4	80	300	8.397
2	2	1:5	85	450	8.110
3	2	1:6	90	600	9.154
4	2	1:7	95	750	6.253
5	4	1:4	85	600	8.789
6	4	1:5	80	750	7.478
7	4	1:6	95	300	5.946
8	4	1:7	90	450	8.925
9	6	1:4	90	750	8.902
10	6	1:5	95	600	6.909
11	6	1:6	80	450	8.603
12	6	1:7	85	300	10.682
13	8	1:4	95	450	8.945
14	8	1:5	90	300	9.143
15	8	1:6	85	750	9.550
16	8	1:7	80	600	9.972
K_1	7.978	8.758	8.613	8.542	
K_2	7.785	7.910	9.283	8.646	
K_3	8.774	8.313	9.031	8.706	
K_4	9.402	8.958	7.013	8.046	
R	1.617	1.048	2.270	0.660	

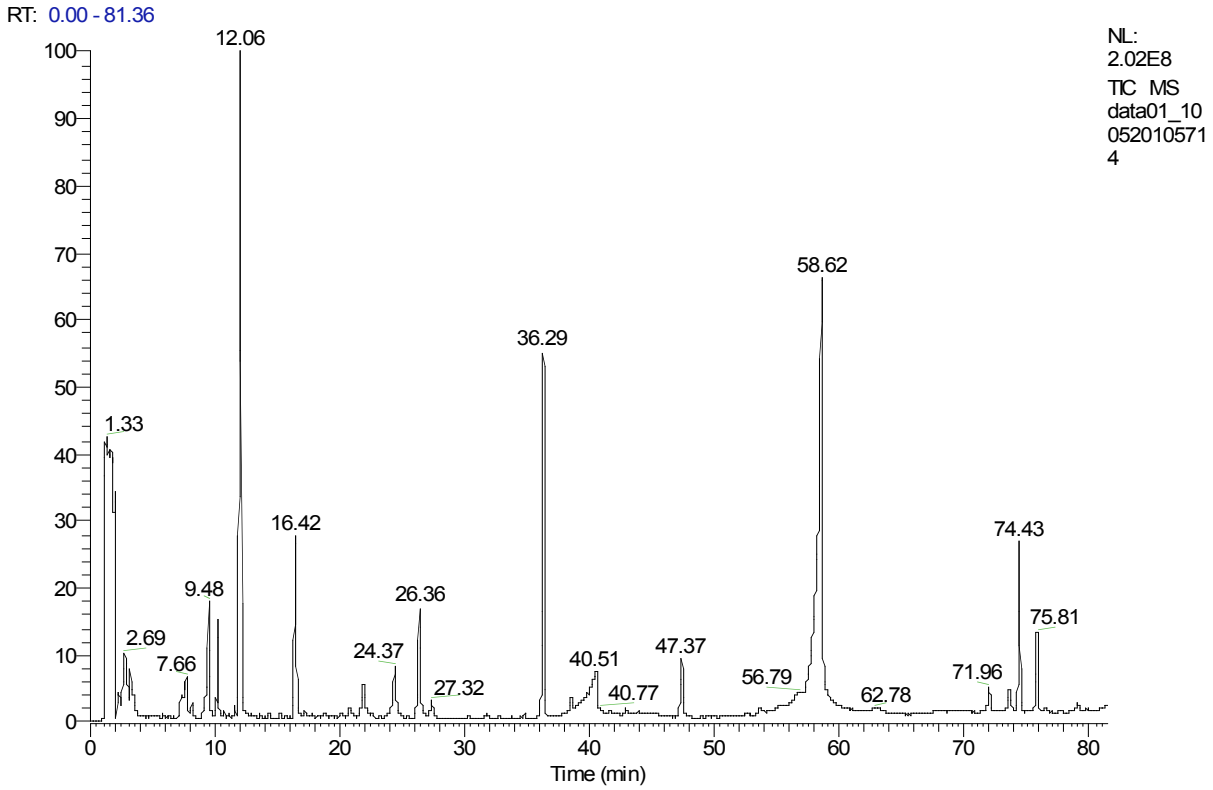

Fig. (2). Total ion chromatogram of components in litsea cubeba essential oil obtained through water extraction assisted by microwave together with ultrasonic.

Table 4. Chemical Composition of Litsea Cubeba Essential Oil Obtained Through Water Extraction Assisted by Microwave Together with Ultrasonic

No.	Retention Time (Min)	Relative Concent (%)	Formula	Molecular Weight	Name of Compound
1	2.28	0.01245	------		
2	2.69	0.04269	------		
3	3.18	0.01815	------		
4	5.81	0.001402	------		
5	7.66	0.02683	$C_{10}H_{16}$	136	Bicyclo-[3,1,1]hept-2-ene, 2,6,6-trimethyl-,(±)-
6	8.19	0.005301	$C_{10}H_{16}$	136	α-pinene
7	9.48	0.03844	$C_8H_{14}O$	126	5-Hepten-2-one,6-methyl-
8	10.23	0.01801	$C_{10}H_{16}$	136	β-pinene
9	12.06	0.1807	$C_{12}H_{20}O_2$	196	1-Methyl-4-(1-methylethenyl)-,acetate
10	14.32	0.002309	$C_{10}H_{18}O_2$	170	Camphene
11	15.22	0.001707	$C_{10}H_{16}$	136	1,5,5- trimethyl -6- methyl -ene-cyclohexene
12	16.42	0.06179	$C_{10}H_{18}O$	154	Linalool
13	17.16	0.002522	$C_{15}H_{24}$	204	α-Caryophyllene
14	18.89	0.002002	------		
15	20.17	0.002272	$C_{10}H_{18}O$	154	Isoborneol
16	20.78	0.003221	$C_{17}H_{30}O$	250	5,8,11-Heptadecatrien-1-ol
17	21.84	0.01996	$C_{10}H_{18}O$	154	α-terpineol
18	24.37	0.02667	$C_{10}H_{16}O$	152	Bicyclo [3,1,1] he pt-3-en-2-ol,4,6,6-trimethyl-, [1s-(1α,2β,5α)]-
19	26.36	0.04069	$C_{10}H_{16}O$	152	geranialdehyde
20	27.32	0.004642	$C_{10}H_{12}O$	148	Estragole
21	30.26	0.001863	------		
22	31.7	0.001133	$C_{20}H_{38}O_2$	310	Ethanol,2-(9,12-octadecadienyloxy)-,(z,z)-
23	34.8	0.002361	$C_{37}H_{76}O$	536	1-Heptatriacotanol
24	36.29	0.08161	$C_{15}H_{24}$	204	Caryophyllene
25	38.51	0.002304	$C_{15}H_{24}$	204	α-Caryophyllene
26	40.57	0.02455	$C_{10}H_{20}O_2$	172	n-Decanoic acid
27	42.87	0.002585	$C_{15}H_{24}O$	220	Butylated Hydroxytoluene
28	43.87	0.002728	------		
29	44.64	0.002485	------		
30	45.09	0.002561	------		
31	47.37	0.02396	$C_{15}H_{24}O$	220	Caryophyllene oxide
32	52.67	0.001525	------		
33	53.63	0.003264	$C_{15}H_{24}O_2$	236	Bicyclo[4,4,0]dec-2-ene-4-ol,2-methyl-9-(prop-1-en-3-ol-2-yl)-
34	55.32	0.001479	$C_{19}H_{32}O_3$	308	3-Cyclohexen-4-ol-1-one,3-tridecanoyl-
35	56.64	0.004416	------		
36	58.62	0.2257	$C_{12}H_{24}O_2$	200	Dodecanoic acid
37	62.78	0.003546	------		
38	63.29	0.004072	------		
39	67.53	0.002272	------		
40	71.96	0.007973	$C_{20}H_{30}O$	286	Retinol
41	73.57	0.00874	$C_{26}H_{33}NO_2$	391	Fenretinide
42	74.43	0.0413	$C_{20}H_{32}O$	288	5-(7α-lsopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-penta-2,4-dien-l-ol
43	75.28	0.001443	------		
44	75.81	0.02493	$C_{20}H_{30}O$	286	Podocarp-7-en-3-one, 13β -methyl-13-vinyl-
45	76.55	0.002341	$C_{29}H_{46}O_2$	426	10,12,14-Nonacosatriynoic acid
46	77.67	0.001412	------		
47	79.04	0.003353	$C_{37}H_{76}O$	536	1-Heptatriacotano
48	79.91	0.002336	------		

effect of the material cell wall. Microwave and ultrasonic effectively enhance the extraction process of litsea cubeba essential oil.

4. CONCLUSIONS

Two kinds of optimal water extraction technology of litsea cubeba essential oil assisted by pure microwave and microwave together with ultrasonic from the seed of *Litsea Cubeba (Lour.)* Pers. are obtained for the first time.

The yield of litsea cubeba essential oil when assisted by microwave and ultrasonicis is far higher than that obtained by traditional steam distillation method. Microwave and ultrasonic can effectively enhance the extraction process of litsea cubeba essential oil.

By GC-MS analysis, 44 compounds in the litsea cubeba essential oil extracted by water assisted by pure microwave and 48 compounds in the litsea cubeba essential oil extracted by water assisted by microwave together with ultrasonic are seperated and of which 31 compounds are identified separately. The concent of each seperated component is determined.

The research results can provide basis for the highly efficient ulilization of *Litsea Cubeba (Lour.)* Pers.

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