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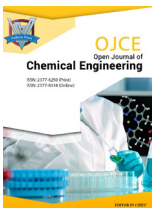

ARTICLE

AROMA CHARACTERISTICS OF *OSMANTHUS FRAGRANS LEAVES*

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ABSTRACT

Osmanthus fragrans, also known as guihua, native to China, is one of the top ten traditional flowers in China. O. fragrans is an ornamental and practical garden tree species with an integrated set of greening, beautification, purification, and perfuming. Recent studies on the development and utilization of O. fragrans's products have been mainly molecular structure analysis of petal extracts and their medicinal value. There are few studies on the utilization of leaves. This article provides a systematic chemical basis for the high-grade development and utilization of O. fragrans leaves. We describe the component differences and functional characteristics of various solvent extracts, and analyze the law of thermal weight loss and change of surface microstructure of O. fragrans leaves. Osmanthus fragrans leaves are rich in phytol, n-hexadecanoic acid, and squalene in three organic solvent extracts: petroleum ether, ethanol, and benzene/ethanol. Phytol is often used in the manufacture of vitamins, chemicals, emulsifiers, antioxidants, and nutritional supplements. Squalene is a bioactive substance with many physiological functions, such as increasing superoxide dismutase (SOD) activity in vivo, enhancing immunity, and anti-aging and antitumor properties. Among the three extracts, we also found a variety of substances with relatively low content and good medicinal value, which includes antioxidant, anti-inflammatory, radiation damage, anticancer, antibacterial, antihelmintic, antidepressant, and neurological effects, the oil content in the leaves of O. fragrans is also not low. The total content trend of the extract's petroleum ether, ethanol, and benzene / ethanol is from high to low, and the kinds of extracted substances are also from many to less, indicating that petroleum ether extract has the best development prospect. FTIR further demonstrated that O. fragrans leaves contain esters, phenols, aldehydes, carboxylic acids, and alkenes. In addition, organic solvent extraction did not change components of O. fragrans leaves significantly. Thermogravimetric analysis showed that the thermal weight changes of O. fragrans leaves were divided into four stages, with the rate of mass loss ranging Third stage, fourth stage, second stage, and first stage. At three critical temperature turning points during TG treatment, the mass of O. fragrans leaves changed significantly, which may be caused by pyrolysis of macromolecules into small molecules. Scanning electron microscope observation revealed that the powder of O. fragrans leaves is not suitable for use as absorbent materials, but is more suitable for making gel and chemical pulp.

KEYWORDS

Osmanthus fragrans; molecular characteristics; volatile organic component; extractives.

1. INTRODUCTION

Osmanthus fragrans is one of the top ten traditional ornamental flowers in China. A long period of planting, natural hybridization, and artificial breeding has produced more than thirty different varieties, which are an integrated set of greening, beautification, purification, and perfuming. Because of its adaptability, the species is widely distributed in China in the subtropical areas. South of the Yangtze River, the distribution of wild sweet Osmanthus is almost the same except in Yunnan and Sichuan. Concentrated areas are Fujian, Jiangxi, Hunan, southern Zhejiang, northern Guangdong, northern Guizhou, northern Guangxi, and the East Guangxi, with the southern part of Zhejiang, Fujian, Jiangxi, and Hunan being the modern distribution centers. In addition, it is also distributed in India, Nepal, and Cambodia. Osmanthus fragrans is also a medicinal herb. Records from ancient China describe use of the flowers, fruit, and roots of Osmanthus fragrans to treat cough, stomachache, and rheumatism.

Recent studies of medical applications of the Osmanthus fragrans petal are extensive [1]. Such uses include kaempferol extracts to treat allergic bronchitis [2], verbascoside to inhibit cancer cell growth [3], quercetin to inhibit cell proliferation, and isopropyl and rut in to inhibit free radical activity [4-7]. The structure analysis of specific compounds in petal extracts has also made great progress.

Additional studies of the processing of Osmanthus fragrans as food

and its effects on other food materials [8] aim to find a suitable way of adding Osmanthus fragrans to recipes so as to maintain people's health. There is also research on the neuroprotective effect of sweet scented Osmanthus aroma.

Compared to the study of petals, there has been no systematic and comprehensive analysis of the utilization of branches and leaves of Osmanthus fragrans [9]. This article discusses the volatile organic components of Osmanthus fragrans leaves that were extracted by solvents (ethanol, benzene/ethanol, and petroleum ether) unanalyzed gas chromatography / mass spectrometry (GC/MS), Fourier transform infrared spectroscopy (FTIR), and thermogravimetry (TG). The systematic characterization of volatile organic components of Osmanthus fragrans and pyrolyzates may lay a foundation for the uses of Osmanthus fragrans leaves.

2. MATERIALS AND METHODS

2.1 Materials and reagents

The variety of Osmanthus fragrans jingui was provided by the Biotechnology Laboratory of Central South University of Forestry and Technology. The samples were dried at 40°C and smashed into powder with a FZ102 Disintegrator suitable for plants (TanjingTaisite Ins. Corp., China). Then, 200 mesh powders were sieved out through AS200 Sieving Instrument (USA). All reagents were purchased from the Sigma Chemical Company (USA) unless noted otherwise [10-12].

2.2 Methods

2.2.1 Osmanthus fragrans leaf extraction by three solvents

Leaves of Osmanthus fragrans were separately extracted with ethanol, benzene/ethanol (2:1), and petroleum ether, the mixed solutions were fully extracted by an automatic FOSS Soxhlet Extracted apparatus (Agilent, USA) at 70°C for 5 h, and then filtrated with filter paper immersed in ethanol for 24 h. The filtrated extraction was evaporated at 45°C under a vacuum of 0.01 MPa and concentrated to 20 mL, transferred to a sealed reagent bottle, and dried at 50°C. Both the concentrated extraction and the residues were keptat 4°C for until the following determinations [13-16].

2.2.2 Volatile organic components analysis by GC/MS

1.0 mL concentrated extractives were aspirated into a 1.5 mL centrifuge tube, mixed with an appropriate amount of anhydrous sodium sulfate at room temperature for 2 h. After centrifugation at 1200 rpm for 2 min, 700 μL of supernatant was removed into a reagent bottle for analysis by GC/MS. GC temperature program consisted of start temperature at 50°C held for 1 min, followed by a temperature ramp of 5°C min^{-1} to 150°C held for 10 min, then 8°C min^{-1} to 250°C held for 2 min, while the volatiles of the extract (1 μL) were released in a split mode (ratio of 1:10) to a capillary column (30 m×250 μm×0.25 μm); the carrier gas was high purity He (99.999%), with a column pressure 57.4 KPa; vaporization chamber temperature was 280°C. The program of MS was scanned over the 35-600 AMU (m/z), with an ionizing voltage of 70 eV and an ionization current of 150 μA of electron ionization (EI). The flow velocity of helium was 1.2 mL/min. The temperature of Ion source was 230°C, and the temperature of quadropole was 200°C [17-21].

2.2.3 Osmanthus fragrans leaf thermostability by TG

Thermogravimetric analysis (TGA) was conducted using a thermogravimetric analyzer (TGA Q50 V20.8 Build 34, USA) with a sensitivity of 0.1 μg, using an empty crucible clamp as a reference. Furnace temperature ranges from 30°C to 600°C. An accurate mass of Osmanthus fragrans leaf sample (6606.00 μg) was placed in a TG crucible. The temperature program of TGA started at 30°C, and increased to 600°C at 10°C/min. The carrier gas is high purity nitrogen, with a flow rate of 40 mL/min [22-25].

2.2.4 Osmanthus fragrans leaves and extracted residue analysis by FTIR

Fourier transform infrared spectroscopy (FTIR) is widely applied in the identification of chemical bond and functional groups of various compounds [26-28]. Osmanthus fragrans leaf samples and its three kinds of extracted residues were dried at 100°C for 4h, in succession, placed in a dry container with desiccant to prevent moisture absorption, which will reduce adverse influence on posterior FTIR. A certain amount of potassium bromide was ground and sieved using an AS200 Sieving Instrument (USA), placed in a dry pot, then kept in the muffle furnace (with SX-2.5-10 box-type control resistance furnace control box) at 150°C for 5 h, finally covered by a heating lamp. (0.5-2) mg of the sample was mixed with 200mg potassium bromide fast and completely in the mortar with a smooth surface, and then tableted in the tablet press. The pressed sample was tested in a FTIR (SHIMADZU, IRAffinity-1) from 4000 cm^{-1}–400 cm^{-1} [29-30].

2.2.5 Osmanthus fragrans leaf powder and extracted residue analysis by SEM

Osmanthus fragrans leaf samples and the extracted residues were baked at 70°C for 12 h. A small amount of sample were spread onto double-backed cellophane tape attached to a stub before coating with gold-palladium at room temperature 32°C for 1 min. Scanning electron micrographs of the samples were taken with a JEOL scanning electron microscope (JSM-6380LV) at an acceleration voltage of 15.00 kV, a distance of 100 μm, and particles were imaged between 200× and 2000× nominal magnification.

2.3 Statistical analysis

The graphs were prepared with Origin 8.5 and Microsoft Office Excel 2007; tables were prepared with Microsoft Office Word 2007.

3. RESULTS AND ANALYSIS

3.1 VOC characteristics of extractives from Osmanthus fragrans leaves

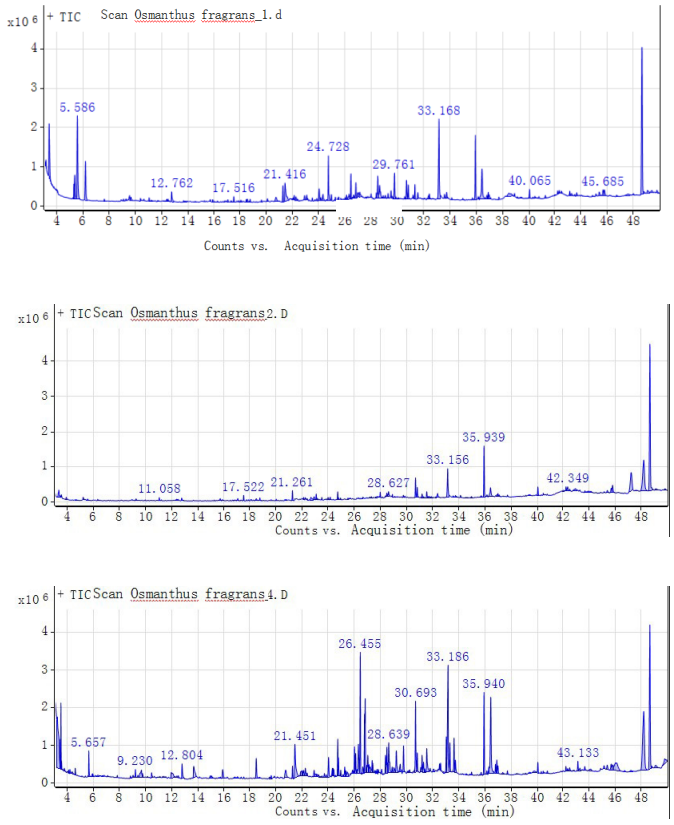


Figure 1: GC-MS chromatograms of three Osmanthus fragrans leaf extracts.

51, 32, and 17 volatile organic components were detected from three Osmanthus fragrans leaf extracts by solvents (ethanol, petroleum ether, and benzene/ethanol (2:1). Each of the three extracts of volatile organic components from Osmanthus fragrans leaves has two identical peaks with large relative area and one specific peak in GC/MS chromatograms (Figure 1). At the time of 33.16 min, is n-hexadecanoicacidis present with the relative contents of 6.1%, 5.22%, and 9.26% in the three extracts,

respectively, and is most soluble in benzene/ethanol ether (Table 1). At 35.94 min, phytol is present with the relative contents 3.01%, 6.02%, and 4.87%, respectively (Table 1). Compared with extractions by ethanol and benzene/ethanol, the phytol extraction is greatest by petroleum ether. The richest component is squalene, which appears at 48.65 min, with the relative contents 8.12%, 27.43% and 17.97% in the three extracts (Table1), respectively, and is also greatest in benzene/ethanol ether. In addition to the three components with higher content, there is another component which has a higher peak but cannot be extracted by benzene/ethanol ether. It is Vitamin E (48.484 min), with the relative contents 7.52% and 13.14% (Table 1), respectively.

Other components with medicinal value found in extracts of the three solvents were: β-caryophyllene (21.255 min), p-hydroxyphenylethanol (21.416 min), α-caryophyllene (22.122 min),d-mannose (22.671 min), 2,4-di-tert-butylphenol (23.511 min), homovanillyl alcohol (24.021 min), β-guaiene (26.312 min), β-eudesmol (26.787 min), 4-hydroxy-3-methoxycinnamaldehyde (28.556 min), coniferyl alcohol (28.633 min), neophytadiene (30.69 min), 6,10,14-trimethylpentadecan-2-one(30.823 min),β-sitosterol(38.498 min),erythrodiol (42.35 min), and γ-sitosterol (46.00 min). Their common feature is that the content is less than 3% and has considerable medicinal value [31-37], such as antioxidant, anti-inflammatory, radiation damage, anti-cancer, antibacterial, antihelmintic, antidepressant, and neurological regulation [38-39] Also present were: 1-methoxy-2-propanol (4.619 min), Nonanal (12.489 min), phenethyl alcohol (12.804min), 2-hydroxy-5-methylacetophenone (18.49 min), 2-methylundecane (18.768 min), (E)-2-tridecen-1-ol (20.015 min), hexadecane (23.083 min), and methyl arachidonate (25.458 min). In common with tobacco leaves, they have toxicity and irritation properties [40-45]. Basically, they are aromatic compounds, or an intermediate in the synthesis of aromatic compounds.

The alcohol extract contained linolenic acid (4.98%), oleic acid (0.34%), ethyl oleate (0.58%), and linoleic acid (0.22%), while 5.34% of linoleic acid could be obtained in the phenethyl alcohol extract.

A considerable variety of other compounds were present in the extracts. The physiological activity or pharmacological toxicity of these compounds is still unrevealed (Table 1). After comparing the three extracts, we found that ethanol was better for extraction of alcohols and phenols. Petroleum ether performed well in extractions, whatever number or quantity. The extraction performance of phenethyl alcohol was poor.

Table 1: Variety of other compounds in the extracts.

No.	Retention time (min)	Compounds	Relative content (%)		
			Ethanol	Petroleum ether	Phenyl ethanol
1	3.521	1-Butanol, 3-methyl-	1.41		
2	3.556	Cyclohexane, 1,2-dimethyl-, trans-		0.31	
3	3.93	1-Octanol, 2,7-dimethyl-		0.29	
4	4.114	1-Pentanol, 5-methoxy-	0.08		
5	4.209	(S)-(+)-1,2-Propanediol	0.13		
6	4.381	2-Pentanol	0.16		
7	4.619	2-Propanol, 1-methoxy-	0.2		
8	4.666	1-Undecene, 5-methyl-		0.17	
9	5.23	4-Penten-1-ol, 3-methyl-		0.47	
10	5.657	4-Hexen-1-ol	0.76		
11	6.346	Nonane		0.17	
12	9.284	2-Octen-1-ol, (E)-		0.31	
13	11.06	Octane, 2,4,6-trimethyl-		0.35	
14	12.35	1-Octyn-3-ol, 4-ethyl-		0.16	
15	12.41	1-Undecene, 4-methyl-		0.16	
16	12.49	Nonanal		0.15	
17	12.8	Phenylethyl Alcohol	0.77		
18	13.7	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	1.06		
19	16.83	Ether, 6-methylheptyl vinyl		0.15	
20	17.07	1-Octanol, 2-butyl-		0.4	
21	17.33	.beta. -D-Glucopyranose, 4-O-. beta. -D-galactopyranosyl-	0.06		
22	17.35	3-Trifluoroacetoxydodecane		0.14	
23	17.52	Decane, 2,3,5,8-tetramethyl-		0.59	
24	18.49	Ethanone, 1-(2-hydroxy-5-methylphenyl)-	0.97		
25	18.77	Undecane, 2-methyl-		0.32	
26	20.02	2-Tridecen-1-ol, (E)-		0.17	
27	21.26	Caryophyllene	0.45	1.13	1.24
28	21.42	Benzeneethanol, 4-hydroxy-	2.7		3.58
29	22.12	Humulene		0.2	

30	22.39	2,5-Octadecadiynoic acid, methyl ester	0.1			
31	22.67	d-Mannose	0.47	1.04		
32	23.08	Hexadecane		0.65		
33	23.26	12,15-Octadecadiynoic acid, methyl ester	0.13			
34	23.51	2,4-Di-tert-butylphenol		0.23		
35	24.02	Homovanillyl alcohol	1.07			1.39
36	24.16	Disulfide, di-tert-dodecyl		0.34		
37	24.73	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	1.25	0.87		3.75
38	25.27	cis-Z-. alpha. -Bisabolene epoxide	0.46			
39	25.35	Ledene oxide-(II)	0.23			
40	25.46	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	0.35			
41	25.86	Dodecanoic acid, 3-hydroxy-	0.16			
42	26.02	Cyclohexanemethanol, 4-ethenyl-. alpha.,. alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1. alpha.,3. alpha.,4. beta.)]-	1.02			
43	26.1	2-((2S,4aR)-4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalen-2-yl) propan-2-ol				0.35
44	26.11	6-(3-Isopropenylcycloprop-1-enyl)-6-methylhept-3-en-2-one	0.7			
45	26.22	2,5-Octadecadiynoic acid, methyl ester	0.18			
46	26.31	. beta. -Guaiene	1.51			
47	26.45	Cyclohexanemethanol, 4-ethenyl-. alpha.,. alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1. alpha.,3. alpha.,4. beta.)]-				3.5
48	26.46	Guaiol	7.14			
49	26.66	5-Methoxy-2,2,6-trimethyl-1-(3-methyl-buta-1,3-dienyl)-7-oxa-bicyclo [4.1.0] heptane	0.3			
50	26.79	2-Naphthalenemethanol, decahydro-. alpha.,. alpha.,4a-trimethyl-8-methylene-, [2R-(2. alpha.,4a. alpha.,8a. beta.)]-	2.03			
51	27.23	2,2,6-Trimethyl-1-(3-methylbuta-1,3-dienyl)-7-oxabicyclic [4.1.0] heptan-3-ol	0.4			
52	27.42	(1aR,4aS,8aS)-4a,8,8-Trimethyl-1,1a,4,4a,5,6,7,8-	0.27			
53	28.56	Coniferyl aldehyde	0.39			
54	28.63	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-	2.03			2.26
55	28.9	2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H-chromen-4a-yl hydroperoxide	0.23			
56	29.22	Cyclohexane, 1,2,3,4-bis(epoxy)-2,6,6-trimethyl-1-(pent-2-en-4-one-2-yl)-	1.27			
57	29.52	2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H-chromen-4a-yl hydroperoxide	0.46			
58	30.68	Phytol, acetate	2.97			1.68
59	30.69	Neophytadiene		2.33		
60	30.82	2-Pentadecanone, 6,10,14-trimethyl-		1.22		1.15
61	31.19	2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H-chromen-4a-yl hydroperoxide	0.65			
62	31.3	3-Isopropyl-6,7-dimethyltricyclo [4.4.0.0(2,8)]decane-9,10-	0.53			
63	31.32	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester				1.08

64	31.55	2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H-chromen-4a-yl hydroperoxide	1.38		
65	32.53	(4aS,7R)-7-(2-Hydroxypropan-2-yl)-1,4a-dimethyl--4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one	0.38		
66	33.05	Cyclohexane, 1,2,3,4-bis(epoxy)-2,6,6-trimethyl-1-(pent-2--en-4-one-2-yl)-	1.42		
67	33.16	n-Hexadecanoic acid	6.1	5.22	9.26
68	35.94	Phytol	3.01	6.02	4.87
69	36.32	9,12-Octadecadienoic acid (Z, Z)-	0.22		5.34
70	36.44	7-Methyl-Z-tetradecen-1-ol acetate		2.37	
71	36.46	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	4.98		
72	36.81	Oleic Acid	0.34		
73	36.92	Ethyl Oleate	0.58		
74	38.5	. beta. -Sitosterol			1.77
75	40.07	7-Methyl-Z-tetradecen-1-ol acetate		1.07	0.74
76	42.02	Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3. beta.)-		0.26	
77	42.35	Olean-12-ene-3, 28-diol, (3. beta.)-			0.26
78	45.8	1H-2,8a-Methanocyclopenta[a] cyclopropa[e]cyclodecen-11-one, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl-, [1S-(1.alpha.,1a.alpha.,2.alpha.,5.beta.,5a.beta.,6.beta.,8a.alpha.,9.alpha.,10a.alpha.)]-	0.41		
79	46	. gamma. -Sitosterol	1.98		
80	48.18	Vitamin E	7.52	13.1	
81	48.65	Squalene	8.12	27.4	18

3.2 Volatility characteristics of Osmanthus fragrans leaves

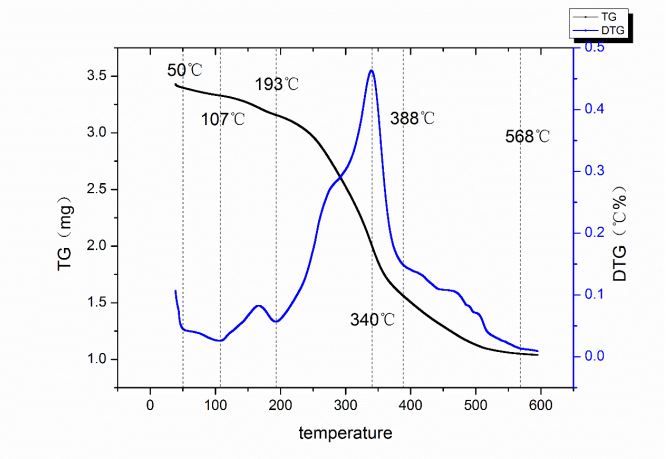


Figure 2: TG Curve and DTG curve.

There are four obvious stages of thermal loss for Osmanthus fragrans leaves during 30°C-600°C thermogravimetric (TG) analysis (Figure2). The loss in the first stage is from the initial temperature of 37°C to 107°C, with the mass loss of 0.09893 g. This part of the mass loss should be the evaporation of water and a small amount of freed volatile matter.

The second stage starts at 107°C, the mass has been reduced by 0.171321 g grams when the temperature arrives at 193°C, which is caused by further shrinkage between the molecules or by pyrolysis of some macromolecules into small molecules with better volatility.

The mass in the third stage from 193°C to 388°C reduces significantly, with the loss of 1.593552 g. During 30°C-600 °C TG treatment, there is an obvious peak observed in the corresponding DTG curve, which reaches its maximum at 340°C, showing that the rate of Osmanthus fragrans leaves mass loss is the fastest in this temperature range.

The fourth stage begins with a gentle mass loss period from 388°C until the pyrolysis of Osmanthus fragrans leaves is completed at 568°C, when the mass loss is about 0.514364 g. According to the DTG curve, the rate of mass loss is ranged: third stage, fourth stage, second stage, and first stage. Moreover, there are three critical temperature turning points during TG treatment. At the three temperature points, the mass of the Osmanthus fragrans leaves changes significantly, which may be caused by chemical changes, such as pyrolysis of macromolecules into small molecules [46]. Therefore, these temperature points can provide a theoretical basis for heating treatment of Osmanthus fragrans leaves.

3.3 Chemical group changes of Osmanthus fragrans leaves and residues

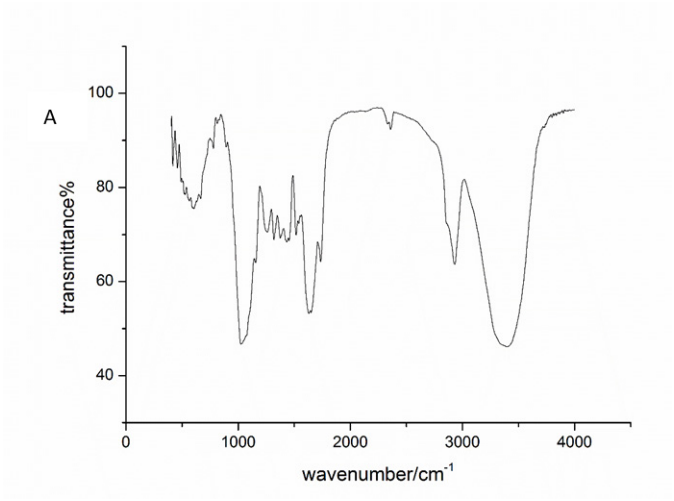
Fourier transform infrared spectroscopy (FTIR) is a rapid detection technique with high sensitivity and is suitable for the identification of chemical bonds and functional groups of various compounds.

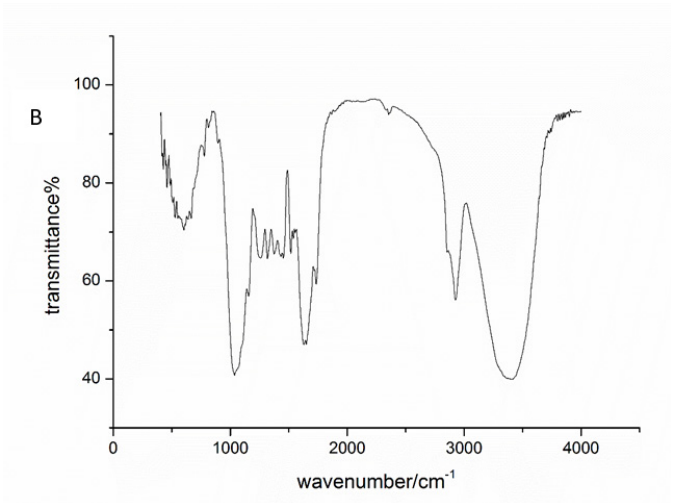
Figure 3 shows the change of the chemical groups of the residue after various extraction methods. It can be seen that the overall trend of infrared absorption peaks of Osmanthus fragrans and its extracted residues has not changed significantly, only slight differences are

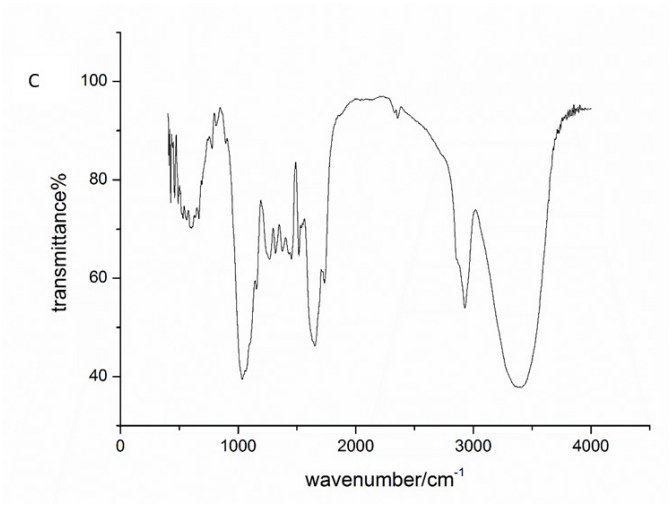
observed.

The FTIR absorption peaks mainly occurred between 1800cm^{-1} and 1000cm^{-1} in the range from 4000cm^{-1} to 400cm^{-1}. There is a strong absorption peak between 700 cm^{-1} and 900cm^{-1} (Figure 3A), belonging to the vibrational peaks of aromatic compounds. The peak at 1000 cm^{-1} and 1640 cm^{-1} corresponds to the C = C bond of the olefin compounds, and the peak at 3011 cm^{-1} is caused by C = C-H stretching. Indeed, we can find a large number of olefins compounds from GCMS results. The peak at 1190 cm^{-1} and 1300 cm^{-1} corresponds to the C-O bond, the peak at 1345 cm^{-1} corresponds to the C=O bond of the aldehyde compounds, and the peaks at 1400 cm^{-1} and 1550 cm^{-1} correspond to the –COO- bond of the carboxylic compounds. The peak at 1717 cm^{-1} corresponds to the C=O bond of esters compounds. The peak at 1490 cm^{-1} corresponds to the -CH2- bond, the peak at 2950 cm^{-1} corresponds to the -CH3 bond, and the peak at 3400 is caused by NH stretching. However, we did not find any nitrogen compounds, indicating that some nitrogen compounds have been extracted by the three organic solvents.

Most groups in the chemical structure of volatile organic components of Osmanthus fragrans leaves detected by gas chromatography/mass spectrometry (GC/MS) are consistent with the FTIR results, further indicating that the Osmanthus fragrans leaves contain esters, phenols, aldehydes, carboxylic acids, and alkenes. In addition, it can be concluded that organic solvent extraction does not make compound groups of Osmanthus fragrans leaves significantly changed.







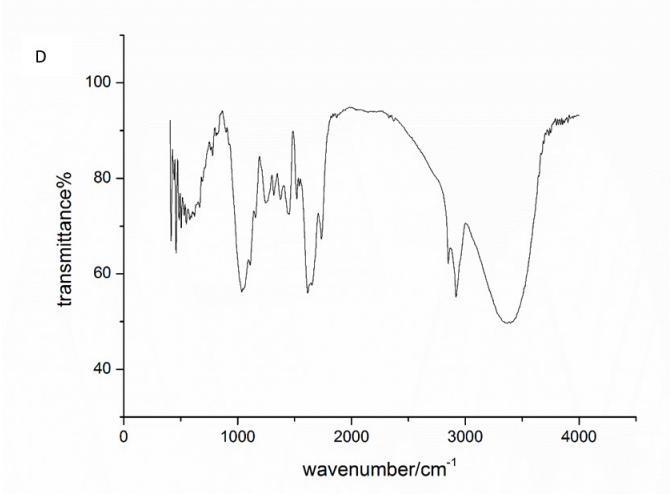
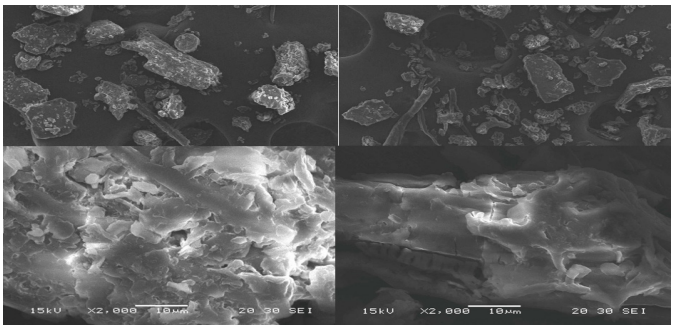


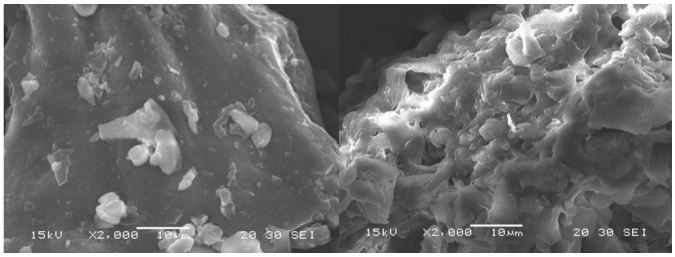
Figure 3: A, B and C are Infrared spectra of the residue of Osmanthus fragrans leaves which are extracted by ethanol, petroleum ether, and benzene/ ethanol, respectively. D is the original sample.

3.4 Micromorphology of powder and extraction residue

Scanning electron microscopy (SEM) is a microscopic morphological characterization between transmission electron microscopy and light microscopy; the material properties of the sample surface material can be directly imaged. The powder of Osmanthus fragrans leaves and three kinds of extraction residues were all scanned under × 200 and × 2000 multiples, and the site selected that can represent the whole microscopic morphological characteristics to observe and photograph. Each sample was scanned at SEM × 200 and × 2000, respectively, and two representative scanning pictures were selected (Figure 4).



A1 (1)A2 (1)



B1 (1)B2 (1)

Figure 4: Scanning electron microscopy (SEM).

Figure 4 (A1) SEM×200powder of Osmanthus fragrans leaves; (A2) SEM×200residue of Osmanthusfragransleaves; (B1) SEM×2000powder of Osmanthusfragransleaves; (B2) SEM×2000residueof Osmanthus fragrans leaves.

Under the SEM × 200-fold, the powder of Osmanthus fragrans leaves exhibited a variety of different microscopic shapes, including rectangular and polygonal lobes and lumps. The sizes of the granules were different and the surface was rough. Under the SEM × 200-fold, most of the extracted residue showed a lump, a small amount of hook like, the sizes of the particles are not much different, the difference is not obvious, but the surface is relatively smoother.

Under SEM × 2000 times, the leaves of Osmanthus fragrans powder can be divided into two kinds, one is smooth and distinct, the other is rough, the crushing is obvious, and all kinds of tiny particles are stuck together. The shapes and sizes of micro particles are irregular. Under SEM × 2000 time, the surface extraction residue can be clearly divided into two kinds: one is the smooth surface that is stuck on a few pieces, another looks like many small particles melted after mutual adhesion, particle shape is spherical or ellipsoidal, and the sizes of micro particles are basically the same.

4. CONCLUSION AND DISCUSSION

Plant alcohol and squalene can be obtained from the leaves of Osmanthus fragrans in three different solvents, and vitamin E can be obtained by extraction with ethanol and petroleum ether. The content of these substances is relatively large, and has excellent practical value. Plant alcohol is commonly used as the basic material for the production of vitamins K1 and E. It is also used in many types of cosmetics and W/O emulsifiers. It has a good antioxidant effect and can also be used as a food emulsifier, antioxidant, and nutritional supplement [47-48]. Squalene was first found in shark liver oil. It is a nontoxic biologically active substance that can prevent and cure diseases. It is a valuable and scarce nutrient drug [49-51]. Vitamin E is also a compound with excellent medicinal value. Previous experiments showed that the extraction of Osmanthus fragrans leaves with petroleum ether contained 27.43% squalene, 13.14% vitamin E, and 6.02% phytol. This shows that the Osmanthus fragrans leaves are a very promising raw material for the production of medical products. A variety of medicinal materials, such as β-sitosterol and β-caryophyllene, were found in previous studies, but their content was relatively low in the extracts. Perhaps there is a better extraction method. From the solvent point of view, the total content of petroleum ether extract, ethanol extract, benzene / ethanol extract is from high to low, and the types of extracted materials are also from many to less, which shows that petroleum ether extract has the best prospects for development.

On the other hand, the extraction of Osmanthus fragrans leaves with three solvents can be obtained 3.01% (alcohol), 6.02% (petroleum ether), and 4.87% (benzene ethanol) of palmitic acid separately 4.98% linolenic acid can also be found in alcohol extracts and 5.34% of linoleic acid can be obtained in phenethyl alcohol extract. Although the fatty acids content in the extract does not represent the fatty acids content in the leaves, the extraction of fatty acids from Osmanthus fragrans leaves is still feasible. Compared with the leaves, the fruit has the advantage of being collected many times in a year, if want to extract fatty acids from the leaves, Osmanthus fragrans leaves can be an excellent basic material.

There are also small amounts of chemicals that are common in tobacco. Because the content is relatively low, the possibility of processing Osmanthus fragrans leaves into cigarettes is also very low. Osmanthus fragrans leaves donot have obvious toxicity and irritation. This is good news for the safety of Osmanthus fragrans leaves and the treatment of the leaves of Osmanthus fragrans after extraction.

The results of FTIR further indicate that the Osmanthus fragrans leaves contain esters, phenols, aldehydes, carboxylic acids, and alkenes. In addition, it can be concluded that organic solvent extraction does not make compound groups of Osmanthus fragrans leaves significantly changed. In the future, we can optimize the extraction method on this basis to further study the processing and utilization of Osmanthus fragrans leaves.

The powder and residue of Osmanthus fragrans leaves were observed by electron microscope. Scanning pictures taken from 200 times showed that the fine powder of Osmanthus fragrans leaves easily fractured and blocked the pores of its surface. This means that Osmanthus fragrans leaves are not suitable for processing into adsorption materials. As seen inthe scanned photo of 2000 magnification, the powder of Osmanthus fragrans leaves results in surface rupture after extraction, which means it has a brittle fracture surface; and there is no obvious sense of hierarchy but adhesions, which means that after similar treatment, can be prepared into glue or chemical pulp.

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REFERENCES

[1] Zhou, F., Zhao, Y., Li, M., Xu, T., Zhang, L., Lu, B., Wu, X., Ge, Z. 2017. Degradation of phenylethanoid glycosides in Osmanthus fragrans lour. Flowers and its effect on anti-hypoxia activity.Scientific Reports, 7 (1), 10068-10068.

[2] Hung, C.Y., Huang, F.L., Shi, L.S., Ka, S.M., Wang, J.Y., Tsai, Y.C., Hung, T.J, Ye, Y.L. 2013. The ethanol extract of Osmanthus fragrans flowers reduces oxidative stress and allergic airway inflammation in an animal model. Evidence-based Complementary and Alternative Medicine, 304290-304290.

[3] Jin, S., Park, H.J., Oh, Y.N., Kwon, H.J., Kim, J.H., Choi, Y.H., Kim, B.W. 2015. Anti-cancer Activity of Osmanthus matsumuranus Extract by Inducing G2/M Arrest and Apoptosis in Human Hepatocellular Carcinoma Hep G2 Cells. Journal of cancer prevention, 20 (4), 241-249.

[4] Lee, H.H., Lin, C.T., Yang, L.L. 2007. Neuroprotection and free radical scavenging effects of Osmanthusfragrans. Journal of Biomedical Science, 14 (6), 819-827.

[5] Xiong, L., Mao, S., Lu, B., Yang, J., Zhou, F., Hu, Y., Jiang, Y., Shen, C., Zhao, Y. 2016. Osmanthus fragrans Flower Extract and Acteoside Protect Against d-Galactose-Induced Aging in an ICR Mouse Model. Journal of Medicinal Food, 19 (1), 54-61.

[6] Hung, C.Y., Tsai, Y.C., Li, K.Y. 2012. Phenolic antioxidants isolated from the flowers of Osmanthusfragrans. Molecules, 17 (9), 10724-10737.

[7] Jiang, Y., Mao, S., Huang, W., Lu, B., Cai, Z., Zhou, F., Li, M., Lou, T., Zhao Y. 2016. Phenylethanoid glycoside profiles and antioxidant activities of Osmanthus fragrans lour. Journal of Agricultural and Food Chemistry, 64 (12), 2459-2466.

[8] Mao, S., Wang, K., Lei, Y., Yao, S., Lu, B., Huang, W. 2017.Antioxidant synergistic effects of Osmanthus fragrans flowers with green tea and their major contributed antioxidant compounds. Scientific Reports, 7 (1), 46501-46501.

[9] Machida, K., Sakamoto, S., Kikuchi, M. 2008. Structure elucidation and nmr spectral assignments of four neolignan glycosides with enantiometricaglycones from Osmanthus ilicifolius. Magnetic Resonance in Chemistry, 46 (10), 990-994.

[10] Li, D.L., Ge, S.B., Peng, W.X., Wu, Q.D., Wu, J.G. 2017. Chemical structure characteristics of wood/lignin composites during mold pressing. Polymer Composites, 38 (5)955–965.

[11] Li, D.L., Wang, L.S., Peng, W.X., Ge, S.B., YuzoFuruta. 2017. Chemical Structure of Hemicellulosic Polymers Isolated from Bamboo Bio-Composite during Mold Pressing. Polymer Composites, 38 (9), 2009–2015.

[12] GE, S.B., LIU, Z.L., Peng, W.X. 2017. Characteristics of activated carbon remove sulfur particles against smog. Saudi Journal of Biological Sciences, 24 (6), 1370-1374.

[13] Li, A.N., Li, S., Li, Y., Xu, D.P., Li, H.B. 2016. Optimization of ultrasound-assisted extraction of natural antioxidants from the Osmanthus fragrans flower. Molecules, 21 (2).

[14] Yin, R.J., Huang, X.F., Kong, L.Y. 2010. Constituents from the heartwoods of Osmanthus armatus. Natural Product Research, 24 (10), 948-952.

[15] Peng, W.X., Li, D.L., Zhang, M.L., Ge, S.B., Mo, B., Li, S.S., Makoto Ohkoshi. 2017. Characteristics of Antibacterial Molecular Activities in Poplar wood Extractives. Saudi Journal of Biological Sciences, 24 (2), 399–404.

[16] Hui Ouyang, Kun Hou, Shengbo Ge, Heping Deng & Wan Xi Peng. 2017. Antimicrobial Activities of Flavonoids Against Bamboo-destroying Fungi and Molds. Toxicological & Environmental Chemistry, 99 (5-6), 892-899.

[17] Cai, X., Mai, R.Z., Zou, J.J., Zhang, H.Y., Zeng, X.L., Zheng, R.R., Wang, C.Y. 2014. Analysis of aroma-active compounds in three sweet osmanthus (Osmanthus fragrans) cultivars by GC-olfactometry and GC-MS. Journal of Zhejiang University-SCIENCE B, 15 (7), 63.

[18] Hu, C.D., Liang, Y.Z., Guo, F.Q., Li, X.R., Wang, W. P. 2010. Determination of essential oil composition from Osmanthus fragrans tea by gc-ms combined with a chemometric resolution method. Molecules, 15 (5), 3683-3693.

[19] Zhou, F., Zhao, Y., Li, M., Xu, T., Zhang, L., Lu, B., Wu, X., Ge, Z. 2017. Degradation of phenylethanoid glycosides in Osmanthusfragranslour Flowers and its effect on anti-hypoxia activity Scientific Reports, 7 (1), 10068-10068.

[20] Wang, L., Wesemann, S., Krenn, L., Ladurner, A., Heiss, E.H., Dirsch, V.M., Atanasov, A.G. 2017. Erythrodiol, an olive oil constituent, increases the half-life of abca1 and enhances cholesterol efflux from thp-1-derived macrophages. Frontiers in Pharmacology, 375-375.

[21] Wu, Z.J., Ma, X.L., Fang, D.M., Qi, H.Y., Ren, W.J., Zhang, G.L. 2009. Analysis of caffeic acid derivatives from Osmanthus yunnanensis using electrospray ionization quadrupole time-of-flight mass spectrometry. European Journal of Mass Spectrometry, 15 (3), 415-429.

[22] Dominguez, M.T., Madejon, P., Madejon, E., Diaz, M.J. 2017. Novel energy crops for mediterranean contaminated lands: Valorization of dittrichiaviscosa and silybummarianum biomass by pyrolysis. Chemosphere, 968-976.

[23] Akram, M., Kim, K.A., Kim, E.S., Shin, Y.J., Noh, D., Kim, E., Kim, J.H., Majid, A., Chang, S.Y., Kim, J.K., Bae, O.N. 2016. Selective inhibition of JAK2/STAT1 signaling and iNOS expression mediates the anti-inflammatory effects of coniferyl aldehyde. Chemico-Biological Interactions, 102-110.

[24] Peng, W.X., Wang, L.S., Mirzaee, M., Ahmadi, H., Esfahani, M.J., Fremaux, S. 2017b. Hydrogen and syngas production by catalytic biomass gasification. Energy Conversion and Management, 135, 270–273.

[25] Peng, W.X., Ge, S.B., Abdol, G.E., HikmatHisoriev, M.J. 2017. Syngas production by catalytic co-gasification of coal-biomass blends in a circulating fluidized bed gasifier. Journal of Cleaner Production, 168, 1513-1517.

[26] Khatua, S., Acharya, K. 2017. Alkaline extractive crude polysaccharide from russulasenecis possesses antioxidant potential and stimulates innate immunity response. Journal of Pharmacy and Pharmacology, 69 (12), 1817-1828.

[27] Savchenko, D., Vorlicek, V., Kalabukhova, E., Sitnikov, A., Vasin, A., Kysil, D., Sevostianov, S., Tertykh, V., Nazarov A. 2017. Infrared, raman and magnetic resonance spectroscopic study of sio2: Cnanopowders Nanoscale Research Letters, 12 (1), 292-292.

[28] Yeter, O. 2017. Identification of the Synthetic Cannabinoid 1-(4-cyanobutyl)-N- (2-phenylpropan-2-yl)-1H-indazole-3-carboxamide (CUMYL-4CN-BINACA) in Plant Material and Quantification in Post-Mortem Blood Samples. Journal of Analytical Toxicology, 41 (9), 720-728.

[29] Machida, K., Sakamoto, S., Kikuchi, M. 2009. Two new neolignan glycosides from leaves of Osmanthus heterophyllus. Journal of Natural Medicines, 63 (2), 227-231.

[30] Sakamoto, S., Machida, K., Kikuchi, M. 2008. Lignan glycosides from the leaves of Osmanthus heterophyllus. Journal of Natural Medicines, 62 (3), 362-363.

[31] Sirikhansaeng, P., Tanee, T., Sudmoon, R., Chaveerach, A. 2017. Major Phytochemical as -Sitosterol Disclosing and Toxicity Testing in Lagerstroemia Species. Evid Based Complement Alternat Med, 7209851.

[32] Govindarajan, M., Benelli, G. 2016. Alpha-humulene and beta-elemene from syzygiumzeylanicum (myrtaceae) essential oil: Highly effective and eco-friendly larvicides against anopheles subpictus, aedesalbopictus, and culex tritaeniorhynchus (diptera: Culicidae). Parasitology Research, 115 (7).

[33] Alberti, T.B., Barbosa, W.L., Vieira, J.L., Raposo, N.R., Dutra, R. C., 2017. (-)-beta-caryophyllene, a cb2 receptor-selective phytocannabinoid, suppresses motor paralysis and neuroinflammation in a murine model of multiple sclerosis. International Journal of Molecular sciences, 18 (4), 691.

[34] Baldissera, M.D., Souza, C.F., Grando, T.H., Stefani, L.M., Monteiro, S. G. 2017. Beta-caryophyllene reduces atherogenic index and coronary risk index in hypercholesterolemic rats: The involvement of cardiac oxidative damage. Chemico-biological interactions.

[35] Chen, X.W., Chen, Y.J., Wang, J.M., Guo, J., Yin, S.W., Yang, X.Q. 2017. Tunable volatile release from organogel-emulsions based on the self-assembly of beta-sitosterol and gamma-oryzanol. Food Chemistry, 221 (PT.2), 1491-1498.

[36] Wang, Y., Fu, J., Zhang, C., Zhao, H. 2016. HPLC-DAD-ESI-MS Analysis of Flavonoids from Leaves of Different Cultivars of Sweet Osmanthus Molecules, 21 (9).

[37] SelestinoNeta, M.C., Vittorazzi, C., Guimaraes, A.C., Martins, J.D., Fronza, M., Endringer, D.C., Scherer R. 2017.Effects of beta-caryophyllene and murrayapaniculata essential oil in the murine hepatoma cells and in the bacteria and fungi 24-h time-kill curve studies. Pharmaceutical Biology, 55 (1), 190-197.

[38] Karamac, M., Koleva, L., Kancheva, V.D., Amarowicz, R. 2017. The Structure–Antioxidant Activity Relationship of Ferulates Molecules, 22 (4).

[39] Sanchez-Quesada, C., Lopez-Biedma, A., Gaforio J.J. 2015. The differential localization of a methyl group confers a different anti-breast cancer activity to two triterpenes present in olives. Food & Function, 6 (1), 248-255.

[40] Padmavathi, A.R., Bakkiyaraj, D., Thajuddin, N., Pandian, S.K. 2015. Effect of 2, 4-di-tert-butylphenol on growth and biofilm formation by an opportunistic fungus candida albicans. Biofouling, 31 (7), 565-574.

[41] Ramos Alvarenga, R.F., Wan, B., Inui, T., Franzblau, S.G., Pauli, G.F., Jaki, B.U. 2014. Airborne antituberculosis activity of eucalyptus citriodora essential oil. Journal of Natural Products, 77 (3), 603-610.

[42] Ambroz, M., Matouskova, P., Skarka, A., Zajdlova, M., Zakova, K., Skalova, L. 2017. The effects of selected sesquiterpenes from myricarubra essential oil on the efficacy of doxorubicin in sensitive and resistant cancer cell lines Molecules, 22 (6), 1021.

[43] Zeng, X., Liu, C., Zheng, R., Cai, X., Luo, J., Zou, J., Wang, C. 2016. Emission and accumulation of monoterpene and the key terpene synthase (tps) associated with monoterpene biosynthesis in OsmanthusfragransLour. Frontiers in Plant Science, 1232-1232.

[44] Lu, B., Li, M., Zhou, F., Huang, W., Jiang, Y., Mao, S., Zhao, Y., Lou, T. 2016. The Osmanthus fragrans flower phenylethanoid glycoside-rich extract: Acute and subchronic toxicity studies. Journal of Ethnopharmacology, 205-212.

[45] Fu, J., Hou, D., Zhang, C., Bao, Z., Zhao, H., Hu, S. 2017. The emission of the floral scent of four Osmanthus fragrans cultivars in response to different temperatures Molecules, 22 (3).

[46] Zhou, F., Peng, J., Zhao, Y., Huang, W., Jiang, Y., Li, M., Wu, X., Lu, B. 2017. Varietal classification and antioxidant activity prediction of Osmanthusfragranslour. Flowers using uplc-pda/qtof-ms and multivariable analysis. Food Chemistry, 490-497.

[47] Islam, M.T., Streck, L., De Alencar, M.V., Cardoso Silva, S.W., Da Conceicao Machado K., Da Conceicao Machado K., Gomes Junior A.L., Paz M.F., Da Mata A.M, De Castro E.S.J.M., Da Costa Junior J.S., LinsRolim H.M., Da Silva-Junior A.A., De CarvalhoMelo-Cavalcante A.A. 2017. Evaluation of toxic, cytotoxic and genotoxic effects of phytol and its nanoemulsion. Chemosphere, 93-101.

[48] Lee, B.J., Kim, H.Y., Lim, S.R., Huang, L., Choi, H.K. 2017. Discrimination and prediction of cultivation age and parts of panax ginseng by fourier-transform infrared spectroscopy combined with multivariate statistical analysis. PLOS ONE, 12 (10).

[49] Hien, H.T.M., Ha, N.C., Thom, L.T., Hong, D.D. 2017. Squalene promotes cholesterol homeostasis in macrophage and hepatocyte cells via activation of liver x receptor (lxr) alpha and beta. Biotechnol Lett, 39 (8), 1101-1107.

[50] Katselou, M.G., Matralis, A.N., Kourounakis, A.P. 2017. Developing potential agents against atherosclerosis: Design, synthesis and pharmacological evaluation of novel dual inhibitors of oxidative stress and squalene synthase activity. European Journal of Medicinal Chemistry, 748-760.

[51] Sobot, D., Mura, S., Yesylevskyy, S.O., Dalbin, L., Cayre, F., Bort, G., Mougin, J., Desmaele, D., Lepetre-Mouelhi, S., Pieters, G., Andreiuk, B., Klymchenko, A.S., Paul, J.L., Ramseyer, C., Couvreur, P. 2017. Conjugation of squalene to gemcitabine as unique approach exploiting endogenous lipoproteins for drug delivery Nature Communications, 8 (1), 15678-15678.