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Original article

Biological analysis on extractives of bayberry fresh flesh by GC–MS

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ABSTRACT

Bayberry has been largely planted in China, and the waste of fresh flesh of bayberry was still abandoned. Therefore, the extractives of fresh flesh of bayberry were studied to further utilize the bio-resources. Through the Foss method, the result shown that ketone, aldehyde, ester and acid compounds were accounted for 1.30, 92.61, 0.54 and 6.09% of the extractives which were extracted from fresh flesh of bayberry by methanol solvents. Aldehyde, bicyclic sesquiterpenes, acid, ester and alcohol compounds accounted for 53.74, 9.95, 28.49, 6.79 and 1.05% of the extractives which were extracted from fresh flesh of bayberry by ethanol solvents. Ketone, aldehyde, carbohydrate, acid and ester compounds accounted for 4.77, 77.95, 12.06, 4.77 and 0.44% of the extractives which were extracted from fresh flesh of bayberry by ethyl acetate solvents. The extractives of fresh flesh of bayberry were rich in rare drug and biomedical activities and the ethanol is more better to extract the fresh flesh of bayberry.

1. Introduction

Bayberry (*Myrica rubra* Sieb. et Zucc.) is a characteristic fruit and which is widespread in China (Chen et al., 2004; Zhu et al., 2013). The mature fruit is beautiful, sweet and delicious, which is also beneficial for treating inflammation, cough and anxiety (Ying et al., 2013). Its outputs are about 400,000 tons every year (Xie et al., 2011a; Herrera et al., 2017). In addition, the fresh fruits are also processed into juice or wine (Zhang et al., 2012). The tree and fruits has been used in traditional medicine for more than 2000 years in China (Chen et al., 2004; Miao et al., 1987). The south of China is main area of cultivation such as Zhejiang province, Hunan province (Chen et al., 2004). The total cultivated area for fruit has exceed 200000 ha in China (Xie et al., 2011b; Issaka and Ashraf, 2017). The revenue from selling red bayberry fruit has become an important source of family income for who are living in mountainous regions due to its good economic benefits. However, bayberry is often wasted because it is difficult to save which resulting in a serious waste of resources and environmental pollution. Especially, the effective functional ingredients of bayberry have not been fully utilized, and the extractives of bayberry has a great potential value for application.

Currently, traditional solvent extraction is an effective process to extract the bayberry (Sun et al., 2012; Yamasaki et al., 2011; Sardar et al., 2017). Water soluble red pigment was nontoxicity which was extracted from the fruit of *Myrica rubra* Sieb et Zuce. by ion exchange chromatography, and this natural colorant is potentially useful in the food industry (Gao et al., 2000; Sharma et al., 2017). Kang found terpenes was predominant and its concentration represented over 89.9% of the overall compounds, and alcohols, aldehydes, ketones, esters, acids, and others were typically present in lesser amounts, and who used Liquid-liquid extraction to extract the volatile compounds (Kang et al., 2012). Geng et al. researched the antimicrobial characteristics of the ethanolic extracts of bayberry (*Myrica rubra* Sieb. et Zucc) and the results indicated that the extract showed obvious antimicrobial actions to Staphylococcus aureus, Salmonella typhi, Streptococcus haemolyc, Shigella dysenteriae, especially to Staphylococcus aureus and Streptococcus haemolyc (Kang et al., 2012; Sultana et al., 2017). In short, the researchers' current research is focused on some molecules. However, there are less researches to use a variety of extraction methods to study the components of fresh flesh of bayberry. Therefore, it is necessary to use a variety of extractants to extract the functional components of bayberry to further utilize bayberry resources.

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2. Materials and methods

2.1. Materials

Fresh bayberry was collected from the Hunan Yipin Oriental Biological Technology Co., Ltd, Hunnan Province, China. The fresh flesh was cut down from bayberry and kept at −3 °C in vacuum. Methanol, ethanol and ethyl acetate were purchased from Hunan Huihong Reagent CO., Ltd, Hunnan Province, China, which were prepared for the subsequent experiments and that were all chromatographic grade. Cotton bag and cotton thread were extracted in methanol, ethanol and ethyl acetate solution for 12 h, respectively. The rotary evaporator was purchased from Gongyi Yuhua Instrument CO., Ltd, Henan Province, China. The anhydrous sodium sulfate was purchased from Tianjin Kemiou Chemical Reagent Co., Ltd, Tianjin, China. GC–MS was purchased from Agilent Technologies, Inc., America.

2.2. Experiment methods

2.2.1. Extraction

Weighed three copies of fresh flesh of bayberry, each was about 60 g (0.1 mg accuracy) and then parceled into the cotton bag and tied by cotton thread, and signed. Extraction was carried out in 300 ml methanol, ethanol and ethyl acetate solvents by the Foss method for 6 h at a temperature of 60, 70 and 70 °C, respectively. After extraction, the methanol, ethanol and ethyl acetate was removed by a rotary evaporator, respectively. And dried with anhydrous sodium sulfate, the resulting extractives was stored at −3 °C. We named three kinds of extractives as Y1, Y2 and Y3 samples which were extracted by methanol, ethanol and ethyl acetate, respectively.

GC/MS determination: GC condition: quartz capillary column is 30 mm × 0.25 mm × 0.25 μm, the temperature of column is 120 °C, program warming is 5 °C/min, the temperature of the inlet is 250 °C, column flow is 1.0 ml/min, pre-column pressure is 100 kPa, split ratio is 10:1, and carrier gas is high helium.

MS condition: ionization mode is EI, the electron energy is 70 Ev, the temperature of transmission line is 250 °C, the temperature of ion source is 230–250 °C, the temperature of quadrupole is 150–200 °C, quality range is 10–550 M/Z, use the wiley7 n.1 standard spectrum and computer search qualitative.

3. Results

The total ion chromatograms of three kinds of extractives were shown in Fig. 1, which were analyzed by GC–MS.

The spectrum of each peak is retrieved by using a computer and wiley7n.1 standard spectrum, according to the laws of the mass spectrum cracking to checking, and peak area normalization method is used to calculate the content of each component, specific results are shown in Tables 1–3.

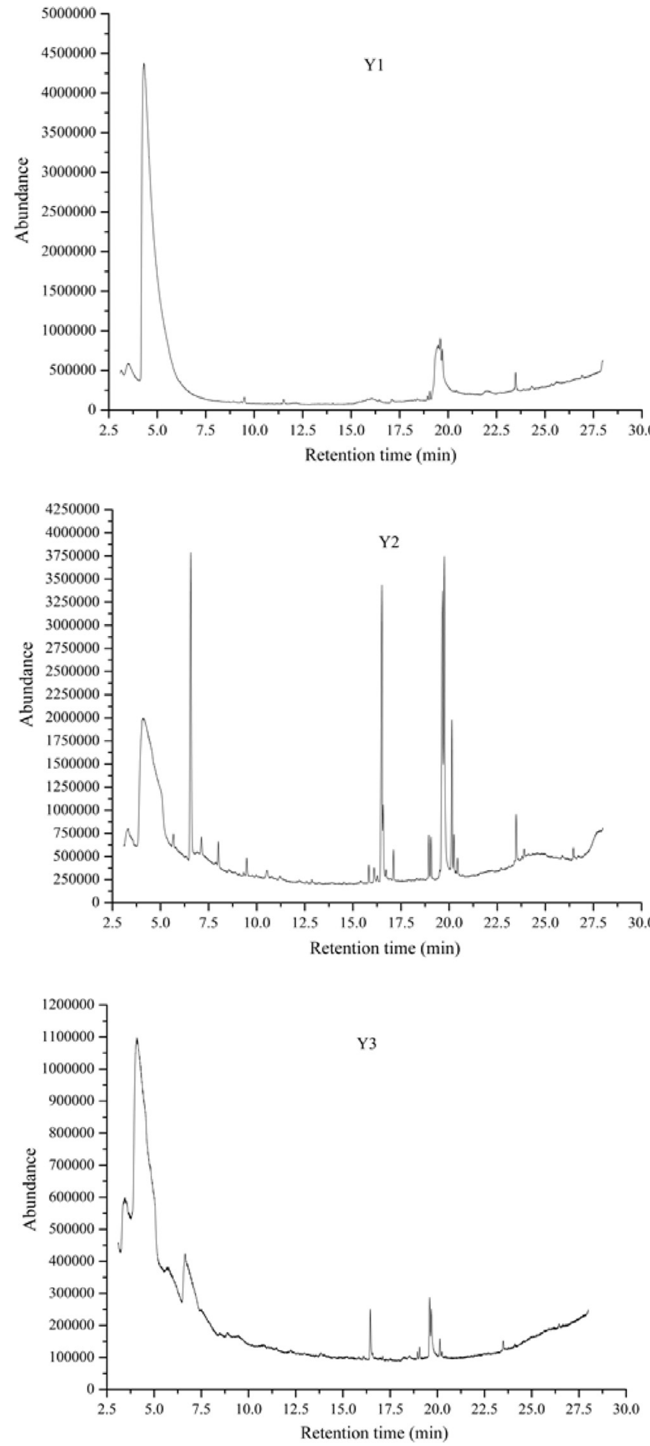

Fig. 1. Total ion chromatograms of the fresh flesh of *Myrica rubra* which were extracted by methanol, ethanol and ethyl acetate, respectively.

4. Discussion

As can be seen from Table 1, the Y1 samples were identified five kinds of components totally, including one kind of ketone compound, one kind of aldehyde compounds, one kind of ester compounds and two kinds of acid compounds which accounted for 1.30, 92.61, 0.54 and 6.09% of the Y1 samples. Obviously, the representative compound is 5-Hydroxymethylfurfural.

Table 2 showed that Y2 samples were identified ten kinds of components totally, including one kind of aldehyde compounds, a class of bicyclic sesquiterpenoids, three kinds of acid compounds, four kinds of ester compounds and one kind of alcohol compounds which accounted for 53.74, 9.95, 28.49, 6.79 and 1.05% of the Y2 samples. And the representative compound is also 5-Hydroxymethylfurfural.

According to the Table 3, Y3 samples were identified seven kinds of components totally, including one kind of ketone compounds, one kind of aldehyde compounds, one kind of carbohydrate compounds, three kinds of acid compounds and one kind of ester compounds which accounted for 4.77, 77.95, 12.06, 4.77 and 0.44% of the Y3 samples. And 5-Hydroxymethylfurfural is a representative compound.

Table 1
GC–MS analysis of Y1 sample.

No.	Retention time (min)	Peak area (%)	Component
1	3.503	1.30	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
2	4.308	92.61	5-Hydroxymethylfurfural
3	19.488	3.34	9,12-Octadecadienoic acid (Z,Z)-
4	19.59	1.07	9,12-Octadecadienoic acid (Z,Z)-
5	19.704	0.68	9,12-Octadecadienoic acid (Z,Z)-
6	22.048	0.54	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-
7	23.48	0.46	Oleic Acid

Table 2
GC–MS analysis of Y2 sample.

No.	Retention time (min)	Peak area (%)	Component
1	4.097	53.74	5-Hydroxymethylfurfural
2	6.571	9.95	Caryophyllene
3	16.506	8.16	*n*-Hexadecanoic acid
4	16.57	1.62	1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester
5	18.947	0.97	8,11-Octadecadienoic acid, methyl ester
6	19.061	0.94	10-Octadecenoic acid, methyl ester
7	19.65	10.08	9,12-Octadecadienoic acid (Z,Z)-
8	19.752	10.25	cis-Vaccenic acid
9	20.141	3.26	Linoleic acid ethyl ester
10	23.48	1.05	1-Heptatriacotanol

Table 3
GC–MS analysis of Y3 sample.

No.	Retention time (min)	Peak area (%)	Component
1	3.46	4.77	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
2	4.081	77.95	5-Hydroxymethylfurfural
3	6.636	12.06	Melezitose
4	16.446	1.83	n-Hexadecanoic acid
5	19.59	1.75	9,12-Octadecadienoic acid (Z,Z)-
6	19.682	1.19	cis-Vaccenic acid
7	20.131	0.44	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-

Consequently, three different methods to extract showed that the 5-Hydroxymethylfurfural is a representative compound in fresh flesh of bayberry, and there are most of the species of acid compounds in fresh flesh of bayberry. Especially, 5-Hydroxymethylfurfural has a good application value, which could be used as raw material for organic synthesis, also used in synthetic resins, varnishes, pesticides, coatings and others (Kang et al., 2012; Chheda et al., 2007; Gao, et al., 2017), some acid compounds of fresh flesh of bayberry such as caryophyllene could be flavoring agent which has anti-inflammatory, anxious, anti-cytotoxicity, expectorant and other characteristics and melezitose also can be hydrolyzed to glucose and loose sugar which has a wide range of uses in biomedicine (Chheda et al., 2007; Michalczyk et al., 2015; Belliardo et al., 1979). In addition, the ethanol could extract more kinds of compounds from the fresh flesh of bayberry. Thus, there are many potential values of extractives of fresh flesh of bayberry and the extract way is also critical.

5. Conclusion

In this study, the fresh flesh of bayberry was extracted by methanol, ethanol and ethyl acetate, and the effect of extraction of ethanol is better than methanol and ethyl acetate. In the fresh flesh of bayberry, there are thirteen kinds of compounds, and the 5-Hydroxymethylfurfural occupy a large part which has a good application value, which could be used as raw material for organic synthesis, also used in synthetic resins, varnishes, pesticides, coatings and others. What's more, the fresh flesh of bayberry extractives, which was drug and medical activities, including caryophyllene, melezitose and others. According to the relative content, the extractives of fresh flesh of bayberry were rich in rare drug and biomedical activities and the ethanol is more better to extract the fresh flesh of bayberry.

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