Separation characteristics and component comparison of woody extracts of *Illicium verum* fruit

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Abstract: *Illicium verum* was a medicinal plant containing many valuable active ingredients. However, the rich extracts from its fruit are invariably wasted for inefficient separation processes. To further utilize these resources, the four extracts were obtained. The results showed that the optimum extraction times for methanol/ethanol, ether/ethanol, benzene/ethanol, and petroleum ether/ethanol extraction were 3, 5, 9, and 5 h for each single extraction, respectively. Among the four methods, the third method was found to be optimum, and gave a maximum yield of 31.63%. It was suggested that the extracts could be used as rare spices, biomedicines, liquid bioenergy, etc.

Keywords: *Illicium verum* fruit, Woody derived biomedicine, bioimmunology extracts, four-stage extraction.

INTRODUCTION

Illicium Verum, commonly called star anise, belongs to *Illicium Linn*, *Magnoliaceae*. It is an evergreen tree growing in hilly and mountain with elevation of 100-2100 m, and widely presents in Hainan, Guangdong, Guangxi, Fujian, Yunnan and other provinces of China as important economic forest with homology of medicine and food (Wang *et al.,* 2005). *Illicium Verum* is a well-known Chinese herb and almost 90% grows in China, which is widely used in food and spice industry. The star anise is planted in China for more than 1,000 years. A literature search disclosed that the people lived in Ningning and Longzhou of Guangxi province of China were convinced to firstly collect, plant and use star anise. It is noted that star anise was first recorded in the medical herbal book written by Sun Simiao (618-907 AC), the famous herbal doctor of China in Tang Dynasty, in which he said that the star anise was first found in Guangxi province of China (Wang *et al.,* 2005; Ning *et al.,* 1983). In recent 20 years, the cultivation in China increased significantly. At the end of 20th century, the star anise was planted in more than 90 counties of Yunnan, Guangxi, Guangdong, Fujian, Anhui, Jiangxi, Hunan and other provinces. The cultivation area reached over 23 millions of hectares, giving 1.25 million tons of dry fruit and 700 tons of seeds oil. Star anise is therefore becomes one of the most important economic forest in South China.

Illicium Verum as herb has long history. And it is determined to show anti-inflammatory and analgesia activities. The etheric extracts of star anise can promote maturation of marrow cells to release into peripheral blood, and has obvious effects on increasing white blood cell, especially on neutrophile granulocyte to cure leucopenia (Wang *et al.,* 2005). While the fruit of star anise has good effects for curing flatulence, diuresis, phlegm and excitation, and is beneficial for stomach (Peng *et al.,* 2007). It is also usually used to cure cold retching and anorexia, cold hernia and celialgia, kidney waist pain, dry or wet beriberi disease. Furthermore, star anise feeding to nursing women can help digestion and stimulate milk secretion. Star anise has the abilities of anti-bacteria and anti-fungi, therefore, it is not only effect to asthma, bronchitis and dry cough (Wang *et al.,* 2005), but also is filled in the products for mouth odor cleaning. The essential oil of star anise, containing anesole, displays good female hormone activity (Wang *et al.,* 2005; Wang *et al.,* 2011). Since 80's in 20th century, Chinese and Japanese scholars have isolated many bioactivity compounds from bark, stem, pericarp and seeds in the flora of *Illicium Linn*. Kouno et al (1993) isolated and identified the compounds of phenylpropanes, lignanoids and icar side E3. Chinese and Japanese scholars also isolated and identified many sesquiterpene loctones from star anise (Yakushijin *et al.,* 1983; Yoshiyasu *et al.,* 1994; Kenichi *et al.,* 1984). Especially, Chinese scholars carried out a series of studies on active components and pharmacological actions of star anise (Jun *et al.,* 2006; Lu *et al.,* 2007; Kang *et al.,* 2008; Lu *et al.,* 2008; Zhao *et al.,* 2006; Fu *et al.,* 2011; Wanxi *et al.,* 2013a; 2013b; 2013c; 2013d), and determined pharmacological actions of trans-anetholes and sikimitoxins. Tamiflu, patented by swiss firm roche, is found to show excellent inhibition against birds flu virus during the outbreak of bird flu, and the literature search suggested that the shikimic acid in star anise is the key intermediate product for Tamiflu (Krämer *et al.,* 2003; Johansson *et al.,* 2005). Zhu-Ping *et al* (2013) studied the synthetic drugs (Wanxi *et al.,* 2011; 2012a; 2012b). Especially, the researchers had analyzed many woody extractives (Wanxi *et al.,* 2009; 2013a; 2013b; 2013c; 2013d; 2014a; 2014b; 2014c; Lansheng *et al.,*

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2014), the pyrolysis products (Wanxi *et al.,* 2012c; Hongchen *et al.,* 2012), and biological active ingredients and wood biomass (Yongchang *et al.,* 2014; Qiu *et al.,* 2014; Le *et al.,* 2014a; 2014b; Lansheng *et al.,* 2014; 2013a; 2013b). However, for the low effective isolation, the extracts of star anise are not full used. In the present work, we took four stage separations and got four kinds of extracts.

MATERIALS AND METHODS

Materials

The *Illicium verum* fruits were were collected from the Nanning Forest zone, Guangxi province, China in August 2012. The fresh fruits were air-dried indoors and subsequently sieved through 40 mesh powder AS200 Sieving Instrument (Retsch Co., Ltd, Germany). Benzene, methanol, ether, petroleum ether and ethanol were purchased in the chromatographic grade and used without further purification. The quantitative filter paper, cotton bag and cotton were all extracted in benzene/ethanol solution for 12 h. The $V_{benzene}/V_{ethanol}$ = 1:4, the $V_{methanol}/V_{ethanol}$ = 1:3, the $V_{ether}/V_{ethanol}$ = 1:9, and the $V_{petroleum\ ether}/V_{ethanol}$ = 1:1 (Wanxi *et al.,* 2013b; 2013c; 2013d).

MATERIALS AND METHODS

Single extraction

Around 80 pieces of the sieved powders were weighed out (about 10 g with 1.0 mg accuracy) and parceled in a cotton bag tied with a cotton thread and signed. The extraction processes were carried out in 800 mL of solvent using a large-calibre Soxhlet extractor. Samples were removed for analysis at different time points (i.e., 1, 3, 5, 7, and 9 hours). The solvents used were benzene/ethanol, petroleum ether/ethanol, methanol/ ethanol and ether/ethanol, respectively. All four of the extractions were conducted at temperatures in the range of 85 to 90°C. Two samples were taken in parallel at each time point. Following extraction, the samples were distilled to dryness and the resulting residues weighed. The amount of material dissolved in the extracts was then calculated (Wanxi *et al.,* 2013b; 2013c; 2013d).

Four-stages extraction

The 32 pieces of the sieved powder were weighed out (about 10 g with 1.0 mg accuracy) and parceled in a cotton bag tied by a cotton thread, and signed. The four-stages extractions were then carried out using a large-calibre Soxhlet extractor with 800 mL of solvent according to Table 1. Two samples were taken in parallel at each time point. The extraction times for the methanol/ethanol, ether/ethanol, benzene/ethanol and petroleum ether/ethanol solutions were 3, 5, 9 and 5 h, respectively, with an extraction temperature in the range of 85 to 90°C being used in all cases. Following the extractions, the four extraction solutions obtained were reduced in volume to 10 mL under vacuum (0.05-0.07 MPa) at 45°C to give the residues from the methanol/ethanol, ether/ethanol, benzene/ethanol and petroleum ether/ethanol extracts. The samples were then baked to absolute dryness and weighed, and the amount of material in the extracts was then calculated (Wanxi *et al.,* 2013b; 2013c; 2013d).

RESULTS

The leaching rates (LR) of the *Illicium verum* extracts in different solvents are shown in Table 2. For each single extraction, the optimum extraction times of the methanol/ethanol, ether/ethanol, benzene/ethanol, petroleum ether/ethanol extractions were 3, 5, 9, and 5 h, respectively. According to the optimum extraction times, the LRs of four-stage extractions have been listed in table 3.

DISCUSSION

Leaching characteristics of Illicium verum extracts in single extraction

During the single extractions, three different factors led to mass loss from the *Illicium verum* fruits, including (1) the *Illicium verum* fruits contained about 20% water (w/w) which was lost through evaporation during the indoor drying of the fruits; (2) the different volatile components in the fruit remained with the fruit material; and (3) the non-volatile components of *Illicium verum* fruits were dissolved. When the extracts were dissolved in water, the LRs were 22.63, 23.50, 26.64, 26.34, and 26.02% following extraction times of 1, 3, 5, 7 and 9 h, respectively. The LR increased with increasing extraction time. The results in table 2, however, revealed that the LRs were different in the methanol/ethanol, ether/ethanol, benzene/ethanol and petroleum ether/ethanol extractions, which provided maximum values of 26.28, 23.68, 25.80 and 24.35%, respectively. Given that the mass of the *Illicium verum* fruits increased in methanol, ethanol, ethyl ether, benzene, and petroleum ether, it became clear that changes in the octagonal cell wall cellulose, hemicellulose, and lignin chemical reactions of the *Illicium verum* fruits varied considerably depending on the solvent. The optimal extraction times for the methanol/ethanol, ether/ethanol, benzene/alcohol, and petroleum ether/ethanol extractions were 3, 5, 9 and 5 h, with LRs of 30.35, 28.46, 28.85, and 25.35%, respectively.

Leaching characteristics of Illicium verum extracts in four-stage extraction

During the four-stage extractions, the masses of the *Illicium verum* fruits were increased as a consequence of the chemical reactions between methanol, ethanol, ethyl ether, benzene, and petroleum ether with the cellulose, hemicellulose, and lignin of the cell walls. At the same

Table 1: Extraction methods used for the four-stage extraction

Stage	First method		Second method		Third method		Fourth method	
	Solvent	No.	Solvent	No.	Solvent	No.	Solvent	No.
1st	Methanol/ethanol	ME01	Ether/ethanol	EE02	Benzene/ethanol	BE03	Pet. ether/ethanol	PE04
2nd	Ether/ethanol	EE01	Benzene/ethanol	BE02	Pet. ether/ethanol	PE03	Methanol/ethanol	ME04
3rd	Benzene/ethanol	BE01	Pet. ether/ethanol	PE02	Methanol/ethanol	ME03	Ether/ethanol	EE04
4th	Pet. ether/ethanol	PE01	Methanol/ethanol	ME02	Ether/ethanol	EE03	Benzene/ethanol	BE04

Table 2: LRs of the different extractions

Extraction time[h]	No.	LR [%]	No.	LR [%]	No.	LR [%]	No.	LR [%]
1	ME1	24.16	EE1	18.55	BE1	25.14	PE1	22.67
3	ME3	30.35	EE3	19.59	BE3	19.37	PE3	24.70
5	ME5	25.25	EE5	28.46	BE5	27.50	PE5	25.35
7	ME7	27.28	EE7	26.25	BE7	28.12	PE7	23.71
9	ME9	24.35	EE9	25.57	BE9	28.85	PE9	25.31

Table 3 LRs of the four-stage extractions

No.	LR [%]	No.	LR [%]	No.	LR [%]	No.	LR [%]
ME01	29.16	EE02	28.10	BE03	29.55	PE04	28.13
EE01	28.89	BE02	26.83	PE03	30.98	ME04	30.95
BE01	28.37	PE02	27.01	ME03	31.63	EE04	30.69
PE01	29.20	ME02	27.97	EE03	30.58	BE04	30.85

time, the masses were also reduced by the extraction processes and as a consequence of water leaching out from the fruits. With these increases and reductions in the weight of the *Illicium verum* fruits, the LRs varied considerably. During the sequential methanol/ethanol –ether/ethanol–benzene/ethanol–petroleum ether/ethanol extraction, the maximum LR was 29.20%, whereas during the sequential ether/ethanol–benzene/ethanol–petroleum ether/ethanol–methanol/ethanol extraction, the maximum LR was 28.10%. During the sequential benzene/ ethanol-petroleum ether/ethanol–methanol/ethanol–ether/ ethanol extraction, the maximum LR was 31.63%, whereas during the petroleum ether/ethanol -methanol/ ethanol-ether/ethanol-benzene/ethanol extraction, the maximum LR was 30.95%. Based on these LRs, the sequential benzene/ethanol–petroleum ether/ethanol–methanol/ethanol–ether/ethanol extraction PEstem was selected as the optimum extraction method.

Component comparison of woody extracts from Illicium verum fruit

The extracts of *Illicium verum* fruit were anethole, benzene, cyclohexyl-, 2-hydroxy-2-(4-methoxy-phenyl)-*N*-methyl-acetamide, benzaldehyde, 4-methoxy-, undecane, decane, dodecane, tridecane, benzaldehyde, 4-methoxy-, nonane, 2(1H)-pyridinone, 1,4,6-trimethyl-, mesitylene, naphthalene, *p*-xylene, benzene, 1,2,3-trimethyl- and so on (Wanxi *et al.,* 2013b; 2013c; 2013d; Wang *et al.,* 2005; Ning *et al.,* 1983; Wang *et al.,* 2011; Kouno *et al.,* 1993; Yakushijin *et al.,* 1983; Yoshiyasu *et al.,* 1994; Kenichi *et al.,* 1984; Lu *et al.,* 2007; Zhao *et al.,* 2006).

There were many rare biomedical components in the extracts of *Illicium verum* biomass. For example, pentadecane heneicosanoic and nonadecene were could be used for the treatment of pneumonia, coughs, hemoptysis, vaginal discharge, and the swelling of the limbs, as a topical treatment for bruises, and scalding burns (Fan *et al.,* 2008). Anethole has been widely used as a flavoring substance, and was present in the essential oil derived from guarana which has been reported to cause psychoactive effects. Furthermore, it has shown potent antimicrobial properties, against bacteria, yeast, and fungi (Wang *et al.,* 2005). Caryophyllene was the one of the aroma components of *Illicium verum* fruit, and was used as a flavoring of cloves, pepper, nutmeg, citrus and herbs (Li *et al.,* 2007). Eucalyptol could be used to reduce inflammation and pain, and destroy leukemia cells in vitro, and as a mouthwash and cough suppressant (Camurça-Vasconcelos *et al.,* 2007; Oka *et al.,* 2000). Squalene could be used to resist fatigue and strengthen the body's resistance so as to protect the liver and improving human immunity (Kim *et al.,* 2012). Stigmast-4-en-3-one, stigmasta-4,6,22-trien-3β-ol and γ-sitosterol were the physiological active of several natural medicines, and was one of the major active ingredients of hair perfume, shampoo, cream and other cosmetics products that were used for the moisturization of dry skin and keratinization, and to inhibit the formation

of corns, and improve skin texture (Zhang, 2005). These medicines have effectively enhanced the economic value of *Illicium verum* fruit extracts for the future.

CONCLUSIONS

For each single extraction, the optimum extraction times of the methanol/ethanol, ether/ethanol, benzene/ethanol, and petroleum ether/ethanol extraction systems were 3, 5, 9, and 5 h, respectively, and their LRs were 30.3, 28.46, 28.85 and 25.35%, respectively. Of the four extraction methods used in the four-stage extraction, the benzene/ethanol-petroleum ether/ethanol-methanol/ ethanol-ether/ethanol extraction method gave the best results with a maximum value of 31.63%.

It suggested that the extracts of the *Illicium verum* biomass was rich in bioactive components that could be used in rare spices, biomedicines, high-grade cosmetics and skin care products. What's more, the by-products could also be used as a source of liquid bioenergy. However, there were some toxic compounds in the BS ME extracts of the *Illicium verum* biomass.

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