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BIOMEDICAL MOLECULAR CHARACTERISTICS OF YBSJ EXTRACTIVES FROM *ILLICIUM VERUM* FRUIT

Wanxi Peng, Zhi Lin, Junbo Chang, Fangliang Gu, Xiangwei Zhu
Central South University of Forestry and Technology, School of Materials Science and Engineering, Changsha, China
Correspondence to: Wanxi Peng
E-mail: pengwanxi@163.com

ABSTRACT

Illicium verum, which is an expensive medicinal plant, contains many valuable active ingredients. However, the rich extractives in Illicium verum biomass are wasted to a large extent due to the inefficient extraction and separation processes. In order to further utilize the resources, four extractives were obtained by the four-stage extractions, and then the active moleculars of YBSJ extractives were identified and analyzed by GC-MS. The results showed that the release rate of Illicium verum fruits increased during drying. The first-stage extractives were acetic acid (48.67%), anethole (26.19%), etc; the second-stage extractives were anethole (80.34%), benzene, cyclohexyl- (19.66%); the third-stage extractives were toluene (26.86%), octane(3.53%), etc; and the fourth -stage extractives were anethole (58.67%), undecane (18.49%), etc. All four of the extractives of the Illicium verum fruits had a majority of their retention times between 10 and 20 min. Furthermore, the four extractives were suitable to extract anethole, whereas only the fourth-stage extraction was suitable to extract hydrocarbons. Thus, the YBSJ extractives of Illicium verum fruits was rich in bioactive components that could be used in biomedicines, rare spices, and high-grade cosmetics and skin care products.

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Introduction

Traditional Chinese medicine devised a unique system to diagnose and cure illnesses through clinical practice, observation and continual education, and has consequently made significant contributions to the treatment of different diseases and the protection of human health (12). There are currently about 12 000 different kinds of medicinal plants in China, and some of the se plants have been used to develop new drugs and lead compounds with novel structures that are highly efficient and non-toxic (19). Some natural medicines have been found in poisonous plants. According to historical records, more than 900 species of poisonous plants have been recorded in China, and in some cases toxic components have been found to coexist with the active ingredients. Furthermore, the toxic components have been identified as the active ingredient in some cases, and suitable doses of these materials could be used to treat a number of severe diseases (11). Many structurally novel and strongly active compounds such as camptothecin, vincristine and taxol were isolated from poisonous plants, including the acuminata, periwinkle and yew tree, respectively (17). Traditional Chinese medicines have traditionally been collected from herbaceous materials, but the growing demand for these medicines means that the herbaceous sources are no longer capable of providing enough material. A large number of plantations have been established to provide resources for woody medicines, and industries involved in the extraction of woody medicines recently emerged with significant ties to the pharmaceutical, fine chemical, agricultural and forestry sectors.

Illicium verum is well known throughout the world for its medicinal and economical benefits, and the wood of this plant has been used for its fragrance and vermifuge effects, as well as being used for the construction of sculptures, furniture, and timbers for interior decoration (22). *Illicium verum* fruits have been used in medicine in China for a long time, and their use was first documented in 1505 in a book entitled "Herbal Essentials Collection", where it was reported to heal fistula and cholera. In 1769, Huang Gongxiu reported that *Illicium verum* could remove heavy and inveterate colds. In the book „Herbal positive", it was reported that *Illicium verum* could be used to treat diseases of the mouth and teeth, as well as being able to detoxify and descend qi. Wang Fu discovered that *Illicium verum* could be used to exert reinforcing effect on the kidney, relieve depressed liver, and heal beriberi. Given the variety of different therapeutic applications of *Illicium verum*, this material is still used today in many traditional Chinese medicines. The fruits of *Illicium verum* are antibacterial, diuretic, carminative, stimulatory, odontalgic and stomachic (1). Furthermore, *Illicium verum* has been prescribed as an digestive aid to nursing mothers to promote breast-milk production, as well as being used as a breath freshener, as sleeping aide, and for its anti-bacterial and anti-fungal effects against asthma, bronchitis and dry coughs (16, 22). The essential oil of *Illicium verum* contains 75-90% anethole, which possess an estrogenic effect, and has been usefully applied to provide relief from rheumatism and lower back pain (20, 22). Research towards identifying the pharmaceutical ingredients of *Illicium verum* have been underway since 1948, and Kouno

et al. reported the isolation and identification of neolignans and a phenylpropanoid glucoside from *Illicium* defengpi (9). Thomas et al. studied novel seco-prezizaane sesquiterpenes from North American IIIicium species in 1999 (18), whereas Chinese and Japanese scholars have jointly studied the chemical composition of *Illicium verum* biomass (8, 18, 23, 24). Chinese scientists in particular have performed extensive research towards the development of effective extraction technologies, the identification of the active ingredients and the use of the *Illicium verum* biomass (2, 6, 7, 8, 14, 15, 26). The main biologically active ingredients to have been determined to date were volatile and fatty oils, proteins, and resins. In 2005, *Illicium verum* biomass was used in the production of Tamiflu, and two independent reports appeared in the literature demonstrating that the key intermediate in the synthesis of Tamiflu (i.e., shikimic acid) could be extracted from *Illicium verum* (5, 10). Unfortunately, however, the rich extractives of the *Illicium verum* biomass have traditionally been wasted because of inefficient extraction and separation processes. The planted area of *Illicium verum* in China is now currently greater than 3.3×10^5 ha, and provides a 1.25×10^4 ton yield of *Illicium verum* fruits, as well as more than 700 ton of anise oil. China is already the world's largest producer of *Illicium verum*, with cultivation in the Guangxi province accounting for approximately 90% of the total output. Therefore, the four extractives were obtained by the four-stages extraction technique, and subsequently analyzed and identified the active molecules from the four extractives using GC-MS with the aim of further utilizing the high quality resources contained in *Illicium verum* biomass.

Materials and Methods

Materials

The *Illicium verum* fruits were provided by the Guangxi Academy of Forestry, and were collected in August 2012 from the Nanning Forest Farm, Guangxi province, P. R. China. The fresh fruits were air-dried indoors and subsequently sieved through about 40 mesh powder AS200 Sieving Instrument. Benzene, methanol, ether, petroleum ether and ethanol were purchased at a chromatographic grade and used without further purification. The quantitative filter paper, cotton bag and cotton were all extracted with a mixture of benzene/ethanol for 12 h. The benzene/ethanol solution was made-up as a 1:4 mixture (v/v), the methanol/ethanol solution was made-up as a 1:3 mixture (v/v), the ether/ethanol solution was made-up as a 1:9 (v/v) mixture, and the petroleum ether/ethanol solution was made-up as a 1:1 mixture (v/v).

Four-steage extractives preparation

Eight pieces of the sieved powder were weighed out (about 10 g with a 1.0 mg accuracy) and parcelled in a cotton bag tied with a cotton thread and signed. The four-stages of extraction were then carried out using large-calibre Soxhlet with 800 mL of solvent which were ether/ethanol solution, benzene/ethanol solution, petroleum ether/ethanol and methanol/ethanol, respectively. 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 obtained extraction solutions 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 extractives.

GC-MS analysis

The above extractives were analyzed using an online linked gas chromatograph-mass spectrometer (GC-MS). The GC/MS analyses were carried out on an Aglient 6890N+5975C GC-MSTM system (Aglient Co., Ltd, USA) linked to a mass selective detector. An elastic quartz capillary column DB-5MS (30m×250μm×0.25μm) coated with a neutral phase (Hewlett-Packard-5 cross-linked 5% phenyl methyl silicone) was used. The carrier gas was helium and the injection port temperature was 250°C. The temperature program for the GC started at 50°C and increased at the rate of 8°C/min to 250°C, and then at a rate of 5°C/min until it reached 300°C, followed by a split injection at a ratio of 15:1. The MS program scanned over the molecular weight (m/z) range of 35 to 335 atomic mass units (AMU), 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 ion source and quadrupole temperatures were set at 230°C and 150°C, respectively.

Volatilization of the *Illicium verum* fruits

Sixty pieces of the sieved powders were weighed out (about 5g with an accuracy of 1.0 mg) and parcelled in a cotton bag tied with a cotton thread, and signed. The samples were dried in an automatic electric heating drying oven with drying times of 1, 2, 3, 4, and 5 h at drying temperatures of 60, 80, 100, and 120ºC, respectively. The release rates of the volatile components from *Illicium verum* fruits were subsequently calculated.

Results and Discussion

Volatilization characteristics of *Illicium verum* fruits

The release rates of the *Illicium verum* fruits have been listed in **Table 1**.

TABLE 1

Release rates of the volatile components from *Illicium verum* fruits [%]

Drying time [h]	Drying temperature [ºC]			
	60	80	100	120
1	2.53	6.05	6.97	10.71
2	4.25	7.55	7.96	12.58
3	4.85	8.71	8.20	13.46
4	5.27	9.22	9.97	14.40
5	5.83	9.65	11.01	14.49

Based on the results in **Table 1**, the analysis of variance revealed that the drying temperature and drying time had a significant effect on the release rates of the *Illicium verum* fruits at the level of 0.01 ($F_{temperature}$=747>$F_{0.01}$(3.12)=5.95; F_{time}=99.4>$F_{0.01}$(4.12)=5.41). As the drying temperature and drying time increased, the release rates of the *Illicium verum* fruits increased (as seen in **Table 1**), and the release rates were 4.55, 8.24, 8.82 and 13.13% at 60, 80, 100 and 120ºC, and 6.57, 8.09, 8.81, 9.72 and 10.25% at 1, 2, 3, 4 and 5 h, respectively. Thus, the release rate of the *Illicium verum* fruits increased during drying.

Components of *Illicium verum* YBSJ extractives

According to the above extraction method, the four extractives (ether/ethanol, benzene/ethanol, petroleum ether/ethanol, and methanol/ethanol) were respectively obtained. The total ion chromatograms of these four extractives by GC/MS are shown in **Fig. 1**. The relative content of each component was counted by area normalization. Subsequent analysis of the MS data using the NIST standard MS map by computer, as well as open-published books and papers (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 13, 14, 15, 16, 18, 20 21, 22, 23, 24, 25, 26, 27), allowed for the individual components to be identified.

According to GC/MS result, 107 components were identified on 127 peaks of YY02 extractives from *Illicium verum* fruit. The result showed that the main components were acetic acid (48.67%), anethole (26.19%), benzaldehyde, 4-methoxy- (1.82%), benzeneacetic acid,.alpha.-hydroxy-3-methoxy-,methyl ester (1.63%), linoleic acid ethyl ester (1.60%), benzenemethanol, 4-methoxy-.alpha.-(2-nitrocyclopentyl)-, [1.alpha.(r*),2.alpha.]- (1.46%), hexadecanoic acid, ethyl ester (1.20%), ethyl oleate (1.11%), dehydroabietic acid (1.06%), 1,6-octadien-3-ol, 3,7-dimethyl- (1.05%), ethanone, 2-hydroxy-1,2-bis(4-methoxyphenyl)- (1.00%), d-limonene (0.76%), 2-propanone, 1-(4-methoxyphenyl)- (0.68%), and so on. Others were cyclohexanol, 2-[2-pyridyl]-, octadecanoic acid, ethyl ester, 1-(cyclopropylmethyl)-4-(methyloxy)benzene, catechol, benzene, 1-fluoro-4-(2-nitro-2-propenyl)-, caryophyllene, n-hexadecanoic acid, 5,6,7,8-tetrahydroquinoxaline, trans-4-methoxycinnamaldehyde, 1-adamantyl m-tolyloxyacetate, 1,3,6,10-dodecatetraene, 3,7,11-trimethyl-, (z,e)-, 1-oxaspiro[4.5]dec-3-en-6-ol, 6,10,10-trimethyl-, acetate, 4(7h)-pyrazolo[3,4-d][1,2,3]-triazinone, 3,4-dihydro-, bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-, eucalyptol, 2-cyclohexen-1-one, 2,4,4-trimethyl-3-(3-oxo-1-butenyl)-, 4-((1e)-3-hydroxy-1-propenyl)-2-methoxyphenol, benzeneacetic acid, .alpha.-(acetyloxy)-4-methoxy-, methyl ester, propanoic acid, 3-tert-butyl-4-hydroxyanisole, (4-ethoxy-3-methoxy-benzyl)-(1-phenyl-cyclopentylmethyl)-amine, 2h-1-benzopyran, 3,4-dihydro-, di-sec-butyl phthalate, 2-furancarboxaldehyde, 5-(2-hydroxy-2-phenylacetyl)-, dimethylhydrazone, other isomer, cyclohexene, 4-methylene-1-(1-methylethyl)-, phenol, 4-(1,1-dimethylpropyl)-, benzeneacetic acid, .alpha.-(acetyloxy)-3-methoxy-, methyl ester, 1,2-cyclohexanediol, 1-methyl-4-(1-methylethenyl)-, propanoic acid, 3-hydroxy-3-(4'-methoxyphenyl)-, t-butyl ester, benzenethiol, o-isopropyl-, trans-.alpha.-bergamotene, .alpha.-terpineol, 4'-methoxybutyrophenone, benzoic acid, 2-methoxy-, methyl ester, ethane, 1,2-bis(1-phenylcyclopropyl)-, benzenepropanoic acid, 4-methoxy-.beta.-oxo-, methyl ester, 4-(borane-dimethylamino)pyridine, 1,3-dithiolane-2-acetic acid, 2-methyl-, methyl ester, stigmast-4-en-3-one, (-)-cis-myrtanyl acetate, benzoic acid, 4-methoxy-, (e,z)-.alpha.-farnesene, benzamide, n-(3-nitrophenyl)-4-methoxy-, benzofuran, 2,3-dihydro-, (3-methoxyphenyl) methanol, 1-methylpropyl ether, cis, 6-octadecenoic acid, trimethylsilyl ester, 4-methoxyphenylpropane-2-ol, cis-.beta.-farnesene, 1-phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a-

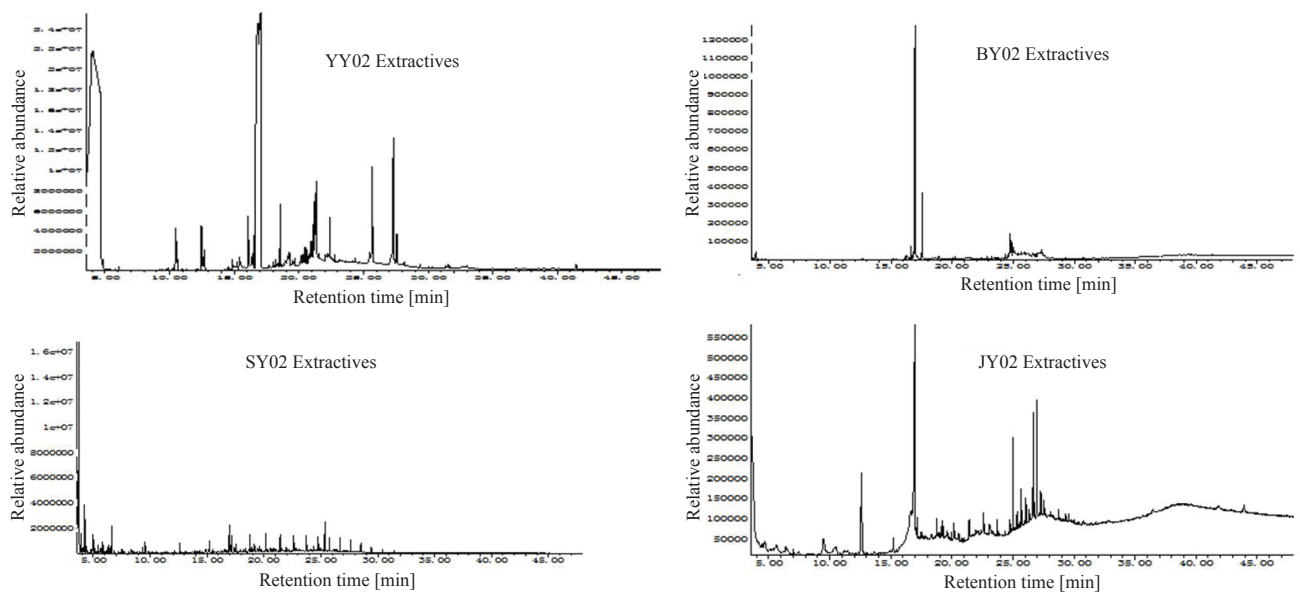

Fig. 1. Total ion chromatogram of four extractives from *Illicium verum* Fruit by GC/MS

octahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1s-(1.alpha.,4a.alpha.,10a.beta.)]-, estragole, d-glucose, 3-o-methyl-, diethyl mercaptal, monoethylmalonate monoamide, benzoic acid, 4-methoxy-, methyl ester, phenol, 2-methyl-6-(2-propenyl)-, terpinen-4-ol, 2h-pyran-2-one, 5,6-dihydro-, formic acid, 2,6-dimethoxyphenyl ester, phenol, 2(1h)-pyrimidinone, 5-methyl-, methyleugenol, 2-carene, .gamma.-sitosterol, urethane, naphthalene, 1,1'-(1,2-ethanediyl)bis[decahydro-, 4-isopropylbenzenethiol, s-methyl-, heptadecanoic acid, ethyl ester, 1,3-benzodioxole, 5-propyl-, ethanone, 2-(5-furan-2-yl-[1,3,4]oxadiazol-2-ylsulfanyl)-1-(2,4,6-trimethylphenyl)-, .gamma.-terpinene, benzamide, 4-methoxy-n-[4-(1-methylcyclopropyl)phenyl]-, bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-, 3-carene, 4-pentenoic acid, 2-acetyl-4-methyl-, methyl ester, 5-methyl-2-trimethylsilyloxy-acetophenone, 4h-1-benzopyran-4-one, 5-hydroxy-7-methoxy-2-phenyl-, (1s)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene, .beta.-ocimene, quinoline, 2,3,4,4a,5,6-hexahydro-7-methoxy-, 2-furanmethanol, 5-ethenyltetrahydro-.alpha.,.alpha.,5-trimethyl-, cis-, .beta.-myrcene, pyridine-3-carboxamide, oxime, n-(2-trifluoromethylphenyl)-, 1,2,5-oxadiazol-3-amine, 4-(4-methoxyphenoxy)-, 1h-indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester,(+)-3-carene, cis-linaloloxide, 1h-dibenzo[a,i]fluorene, eicosahydro-, methanamine, n-(diphenylethenylidene)-, squalene, trans-4,4'-dimethoxychalcone, 2-ethylacridine, (2e)-4-(4-hydroxy-3-methoxyphenyl)-2-butanone oxime, ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate, tricyclo[5.4.3.0(1,8)]tetradecan-6-one, 4-ethenyl-3-hydroxy-2,4,7,14-tetramethyl, hydrazine, propyl-.

Two components were identified on 2 peaks of BY02 extractives from *Illicium verum* fruit. The result showed that the components were anethole (80.34%), benzene, cyclohexyl- (19.66%).

One hundred and eighteen components were identified on 139 peaks of SY02 extractives from *Illicium verum* fruit. The result showed that the main components were toluene (26.86%), octane (3.53%), tridecane (2.40 %), nonane (2.31%), hexadecane (2.24%), p-xylene (2.23%), anethole (2.20%), heptadecane (1.96%), dibutyl phthalate (1.91%), naphthalene, 1,6,7-trimethyl- (1.54%), tetradecane (1.49%), octadecane (1.35%), pentadecane (1.32%), cyclohexane, ethyl- (1.25%), cyclohexane, 1,3-dimethyl-, cis- (1.16%), nonadecane (1.12%), cyclohexane, 1,4-dimethyl-, trans- (1.11%), heneicosane (1.05%), phenanthrene, 2-methyl- (1.04%), cyclohexane, 1,1,3-trimethyl- (1.04 %), dodecane (1.02%), undecane (1.01%), and so on. Others were decane, naphthalene, 2,3,6-trimethyl-, benzene, 1,2,3-trimethyl-, 2-pentene, 5-bromo-2,3-dimethyl-, eicosane, naphthalene, 1,6-dimethyl-, naphthalene, 2,7-dimethyl-, benzene, cyclohexyl-, pentadecane, 2,6,10-trimethyl-, naphthalene, 2,6-dimethyl-, naphthalene, 1,2,3,4-tetrahydro-1,4-dimethyl-, cyclopentane, butyl-, naphthalene, 1,2,3,4-tetrahydro-5-methyl-, naphthalene, 1,3-dimethyl-, 1,2-benzenedicarboxylic acid, butyl 8-methylnonyl ester, cyclohexane, 1,2,4-trimethyl-, docosane, cyclohexane, 1,2-dimethyl-, trans-, hexadecane, 2-methyl-, benzene, 1-ethyl-2-methyl-, naphthalene, 1,2,3,4-tetramethyl-, naphthalene, 1,4,6-trimethyl-, naphthalene, 2-methyl-, exadecane, 2,6,10,14-tetramethyl-, dodecane, 2,6,10-trimethyl-, tricosane, sulfurous acid, 2-ethylhexyl tetradecyl ester, naphthalene, benzene, (1-methyl-1-butenyl)-, cyclopentane, 1-ethyl-2-methyl-, bicyclo[3.2.1]octane-6-carboxylic acid, 2-oxo-, methyl ester, (6s)-, 1-isopropenylnaphthalene, tridecane, 2-methyl-, octane, 2,6-dimethyl-, ethylbenzene, cyclohexane, propyl-, 1,1'-biphenyl, 4-methyl-, benzene, 2-ethyl-1,4-dimethyl-, octane, 2-methyl-, cycloheptane, methyl-, tetracosane, dimethylsulfoxonium formylmethylide, benzene, (1,2-dimethyl-1-propenyl)-, decahydro-4,4,8,9,10-pentamethylnaphthalene, benzene, pentamethyl-, benzene, 2,4-diethyl-1-methyl-, p-cymene, heptane, 2,6-dimethyl-, 1h-indene, 2,3-dihydro-4-methyl-, heptadecane, 3-methyl-, phenanthrene, 2,5-dimethyl-, 4,6,8-trimethylazulene, benzene, 1-ethyl-2,4-dimethyl-, naphthalene, 1,2,3,4-tetrahydro-1,6,8-trimethyl-, naphthalene, 1,2,3,4-tetrahydro-6-methyl-, 1-ethyl-4-methylcyclohexane, nonane, 2-methyl-, undecane, 2-methyl-, 9h-fluorene, 2-methyl-, undecane, 2,6-dimethyl-, benzene, 1,2,3,5-tetramethyl-, benzene, (1-methyl-1-propenyl)-, (z)-, phenanthrene, 2,3,5-trimethyl-, dodecane, 2-methyl-, phenol, 4-chloro-2,6-bis(1,1-dimethylethyl)-, 1h-indene, 2,3-dihydro-4,7-dimethyl-, phenanthrene, 1-methyl-, benzene, 1,1'-(1-fluoro-1,2-ethenediyl)bis-, (e)-, 1,1'-biphenyl, 2-methyl-, pentadecane, 2-methyl-, cyclohexane, butyl-, cyclohexane, 1,2,3-trimethyl-, (1.alpha.,2.beta.,3.alpha.)-, di-p-tolylacetylene, bicyclo[4.2.1]nona-2,4,7-triene, 7-ethyl- dodecane, 4-methyl- 4-octene, 2,6-dimethyl-, [s-(e)]-, trans-1,2-diethyl cyclopentane, octane, 4-methyl-, benzene, 1,2,3,4-tetramethyl-, 2-methylbicyclo[3.2.1]octane, decane, 2-methyl-, benzene, 4-chloro-2-fluoro-1-methoxy-, heptane, 3-ethyl-2-methyl-, phenanthrene, 3,6-dimethyl-, hexacosane, naphthalene, 1,2,3,4-tetrahydro-2-methyl-, 1h-indene, 2,3-dihydro-4,6-dimethyl-, tridecane, 3-methyl-, decane, 4-methyl-, benzenecarbodithioic acid, butyl ester, benzene, propyl-, naphthalene, 1-propyl-, benzene, 1-methyl-3-propyl-, undecane, 3-methyl-, cyclohexane, 1-methyl-2-pentyl-, heptane, 2,4-dimethyl-.

Six components were identified on 7 peaks of JY02 extractives from *Illicium verum* fruit. The result showed that the components were anethole (58.67%), undecane (18.49%), methyl stearate (7.75%), 9-octadecenoic acid (z)-, methyl ester (6.58%), hexadecanoic acid, methyl ester (5.58%), 9,12-octadecadienoic acid, methyl ester, (e,e)- (2.92%).

Molecular distribution of YBSJ extractives from *Illicium verum*

The results of the GC-MS analysis showed that the molecular distribution of the YBSJ extractives from *Illicium verum* was the richest in terms of the number of components present after the first-stage of the extraction with the ether/ethanol system, with compounds such as acetic acid (48.67%),

anethole (26.19%) being identified in the extractives. The relative hydrocarbon, alcohol (phenol alcohols), aldehyde/ketone, ether, and acid/ester contents, as well as other unknown compounds, accounted for 6.38%, 5.39%, 2.60%, 26.51%, 56.62% and 2.5% of the ether/ethanol extractives, respectively. The components from the second-stage extraction with the benzene/ethanol system were anethole (80.34%), benzene, cyclohexyl- (19.66%). The relative hydrocarbon, alcohol (phenol alcohols), aldehyde/ketone, ether, and acid/ester contents, as well as the other unknown compounds, accounted for 19.66%, 0.00%, 0.00%, 80.34%, 0.00% and 0.00% of benzene/ethanol extractives, respectively. The most abundant components from the third-stage extraction with the petroleum ether/ethanol system were toluene (26.86%), octane (3.53%). The relative hydrocarbon, alcohol (phenol alcohols), aldehyde/ketone, ether, and acid/ester contents, as well as the other unknown compounds, accounted for 93.44%, 0.26%, 0.00%, 2.20%,1.82% and 2.54% of petroleum ether/ethanol extractives, respectively. The most abundant components from the fourth -stage extraction with the methanol/ethanol system were anethole (58.67%), undecane (18.49%). The relative hydrocarbon, alcohol (phenol alcohols), aldehyde/ketone, ether, and acid/ester contents, as well as the other unknown compounds, accounted for 18.49%, 0.00%, 0.00%, 58.67%, 15.09% and 7.75% of methanol/ethanol extractives, respectively. Taken together, the results suggested that the four extraction systems were suitable for the extraction of anethole, and that the fourth-stage extraction was better suited to the extraction of hydrocarbons.

The retention times of the different component from the different extractions of *Illicium verum* showed a particular trend. For the first-stage extraction, the molecules with retention times of ≤10, ≤20, ≤30, ≤40 min and ≥ 40min were 49.09%, 34.31%, 15.72%, 0.76% and 0.12%, respectively. For the second-stage extraction, the molecules with retention times of ≤10, ≤20 min were 0.00% and 100.00%, respectively. For the third -stage extraction, the molecules with retention times of ≤10, ≤20, ≤30, ≤40 min and ≥ 40min were 50.11%, 24.60%, 24.81%, 0.47% and 0.00%, respectively. For the fourth-stage extraction, the molecules with retention times of ≤10, ≤20, ≤30 min were 0.00%, 77.16% and 22.84%, respectively. These results showed that most of their components from the four extractives of the *Illicium verum* biomass had retention times between 10-20 min.

Biomedical Resource utilization of YBSJ extractives from *Illicium verum*

Illicium verum is a medicinal woody plant, and its extractives and derivatives could be used as novel lead compounds to create new drugs. Research in this area has shown that there were many rare biomedical components in the YBSJ extractives of *Illicium verum* biomass. Given its officinal value, 9,12-octadecadienoic acid ester has been identified as the main medical component of dried worms, and has diuretic, swelling and detoxification properties (3). Anethole has been widely used as a flavoring substance, and is present in the essential oil derived from guarana which has been reported to cause psychoactive effects. Furthermore, this material has shown potent antimicrobial properties, against bacteria, yeast, and fungi (22). *In vitro*, anethole has shown anthelmintic activity towards the eggs and larvae of the sheep gastrointestinal nematode *Haemonchus contortus*, and nematicidal activity against the plant nematode *Meloidogyne javanica in vitro* and in pots of cucumber seedlings (22). Caryophyllene is the one of the aroma components of the *Illicium verum* fruit, and has been used as a flavoring of cloves, pepper, nutmeg, citrus and herbs (13). D-Limonene is a natural monoterpenoid which has been widely used in the perfume industry. This compound also exhibits efficacy as an anti-oxidation, anti-inflammatory, cholagogic and litholytic agent. D-Limonene can also be used for the prevention and treatment of cancers of the colon, breast, gut, lung, skin, liver, and pancreas induced by chemical carcinogens (4). *cis*-β-Farnesene is the main active ingredient of Chinese dalbergia and has shown efficacy to quicken the blood and treat qi, analgesia and hemostasis. Dehydroabietic acid possesses anti-fungal and hemostasis properties. α-Terpineol can be used as a disinfectant, antioxidant, and medicine. Squalene, which can be used to resist fatigue and strengthen the body's resistance, as well as to protect the liver and improve human immunity, has been used in a number of nutraceutical and pharmaceutical products (27). [1S-(1α,4aα,10aβ)]-1,2,3,4,4a,9,10,10a-Octahydro-1,4a-dimethyl-7-(1-methylethyl)-1-phenanthrenecarboxylic acid is the active ingredient in a number of skin care products and can be used to heal facial peeling (21). Stigmast-4-en-3-one, stigmasta-4,6,22-trien-3β-ol and γ-sitosterol are the physiological active of several natural medicines, and have been used as growth hormones in plants and animals, as well as being used as anti-inflammatory, antipyretic, and anti-ulcer treatments. Furthermore, γ-sitosterol has been used for the treatment of cervical cancer and skin cancer, and is one of the major active ingredients of hair perfume, shampoo, cream and other cosmetics products that are used for the moisturization of dry skin and keratinization, and to inhibit the formation of corns, and improve skin texture (25). These medicines have effectively enhanced the economic value of *Illicium verum* fruit extractives for the future.

Conclusions

For volatilization of *Illicium verum* fruits, the drying temperature and drying time had a significant effect on the release rates of *Illicium verum* fruits at the level of 0.01. And the release rate of *Illicium verum* fruits increased during drying.

The YY02, BY02, SY02, and JY02 extractives of *Illicium verum* fruit gave the following number of components: 107, 2, 118 and 6, respectively, that could be identified by GC-MS. The first-stage extractives were acetic acid (48.67%), anethole (26.19%), etc. The second-stage extractives were anethole (80.34%), benzene, cyclohexyl- (19.66%). The third-stage extractives were toluene (26.86%), octane (3.53%), etc. And

the fourth-stage extractives were anethole (58.67%), undecane (18.49%), etc. All four of the extractives of *Illicium verum* fruits had their majority of their retention times between 10 and 20 min. Furthermore, the four extractives were suitable for the extraction of anethole, whereas only the fourth-stage extraction was suitable for the extraction of hydrocarbons. Thus, the functional analytical result suggested that the YBSJ extractives of *Illicium verum* fruits was rich in bioactive components that could be used in biomedicines, rare spices, and high-grade cosmetics and skin care products.

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