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Immunology Molecular Characteristics of BSJY Extractives from *Illicium verum* Biomass

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Illicium verum, which has been planted more than 3.3×105 ha, is the expensive medicinal plant in south China. However, rich extractives in _Illicium verum_ biomass were severely wasted for the inefficient extraction and separation processes. In order to further utilize the biomedical resources, the four extractives were obtained by JYBS extraction, and the immunology moleculars of JYBS extractives were investigated and analyzed by GC-MS. The result showed that the first-stage extractives contained 89 components including anethole(54.24%), 2-hydroxy-2-(4-methoxy-phenyl)-n-methyl-acetamide(4.81%), etc; the second-stage extractives had 25 components including anethole(85.17%), d-limonene(1.90%), etc; the third-stage extractives contained 81 components including cyclohexyl-benzene(16.58%), 1-ethyl-1,2,3,4-tetrahydro-naphthalene(10.47%), etc; and the fourth-stage extractives contained 98 components including undecane(7.58%), decane(6.48%), etc. And The JYBS extractives of _Illicium verum_ biomass had a main retention time between 10-20 min. what's more, the JYBS extractives contained many biomedical and immunology moleculars, such as anethole, stigmasta-4,6,22-trien-3β-ol,γ-sitosterol,squalene, and so on. So the functional analytical results suggested that the JYBS extractives of _Illicium verum_ had huge potential in immune and other biomedicines.

Key words: *Illicium verum*; JYBS extractives; GC-MS; Biomedical molecular, Immunology molecular.

Illicium verum, which originated in northeast Vietnam and southwest China, is an evergreen tree. *Illicium verum* fruits have been used as a traditional flavors for a long time. Meanwhile, *Illicium verum* was also used in medicine for more than 500 years. *Illicium verum* has been documented in the book "Herbal Essentials Collection" in 1505's because it was found to heal many fistula and cholera. HUANG Gongxiu found that *Illicium verum* could remove heavy cold and inveterate cold in 1769. The book "Herbal positive" recorded that *Illicium verum* could remove teeth mouth disease, detoxify and descend qi. WANG Fu discovered that *Illicium verum* had the effects of reinforcing kidney, reliering the depressed liver, and healing beriberi. Thus, *Illicium verum* is used in many traditional Chinese medicine today. *Illicium verum* fruits are antibacterial, diuretic, carminative, stimulant, odontalgic and stomachic[1]. *Illicium verum* was prescribed as an digestive aid which could make the nursing mothers to promote breast-milk production, had the anti-bacterial and anti-fungal effection of asthma, bronchitis and dry cough, refreshed the breath, and ensured a good sleep[2,3]. Its essential oil contained 75%-90% anethole and

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had the observed estrogenic effect, and was useful in providing relief from rheumatism and lower back pain[3,4]. Since 1948, the pharmaceutical ingredients of *Illicium verum* began research and analysis. In 1993, Kouno et al reported neolignans and a phenylpropanoid glucoside from Illicium defengpi[5]. Thomas *et al.,* studied novel seco-prezizaane sesquiterpenes from North American IIlicium species in 1999[6]. Chinese and Japanese scholars jointly studied some chemical compositions of *Illicium verum* biomass[6-9]. Especially, Chinese scientists have done extensive research on the extraction technology, the active ingredient identification and utilization of *Illicium verum* biomass[9-15]. Currently, the main biological active ingredients were determined, and were volatile oil, fatty oil, protein, resin and so on. In 2005, *Illicium verum* biomass was use in the production of Tamiflu. The Independent reported news that the medicinal ingredient of Tamiflu was shikimic acid which were mostly extracted from *Illicium verum*[16,17]. It maked *Illicium verum* world famous botanical anti-bird plant which has been planted more than 3.3×10^5 ha. However, rich extractives in *Illicium verum* biomass were severely wasted for the inefficient extraction and separation processes. the four extractives were obtained by JYBS extraction, and then the immunology moleculars of JYBS extractives were investigated and analyzed by GC-MS in order to further utilize the biomedical resources.

MATERIALS AND METHODS

Materials

The *Illicium verum* fruits were collected from Nanning Forest Farm, Guangxi province, P. R. China. The fresh fruits were dried in the indoor air, and About 40 mesh powder was sieved out using AS200 Sieving Instrument (Made in America). Benzene, methanol, ether, petroleum ether and ethanol (chromatographic grade) were prepared for the subsequent experiments. Quantitative filter paper, cotton bag and cotton were all extracted in benzene-ethanol solution for 12 h. The benzene-ethanol solution was mixed according to $V_{ethanol}$ / $V_{benzene}$ 4 double. The methanol-ethanol solution was mixed according to $V_{ethanol}$ /$V_{methanol}$ 3 double. The ether-ethanol solution was mixed according to $V_{ethanol}$ /V_{ether} 9 double. And the petroleum ether-ethanol solution was mixed according to $V_{ethanol}$ / $V_{petroleum\ ether}$ 1 double.

Methods

Weighed 8 pieces of the above powders, each was 10g (1.0mg accuracy), and finally parceled by using the cotton bag and tied by using cotton thread, and signed. Extraction was gradually carried out by large-caliber Soxhlet and extracted in 800 ml methanol-ethanol, ether-ethanol, benzene-ethanol and petroleum ether-ethanol solution, respectively. Parallel sample number was 2. Extraction time of methanol-ethanol, ether-ethanol, benzene-ethanol and petroleum ether-ethanol solution was 3h, 5h, 9h and 5h, respectively. Extraction temperature was 85-90ºC. After extraction, the four extractives solutions obtained and dried to 10ml under the condition of 45ºC and vacuum 0.05-0.07Mpa. The methanol-ethanol, ether-ethanol, benzene-ethanol and petroleum ether-ethanol extractives were obtained, respectively.

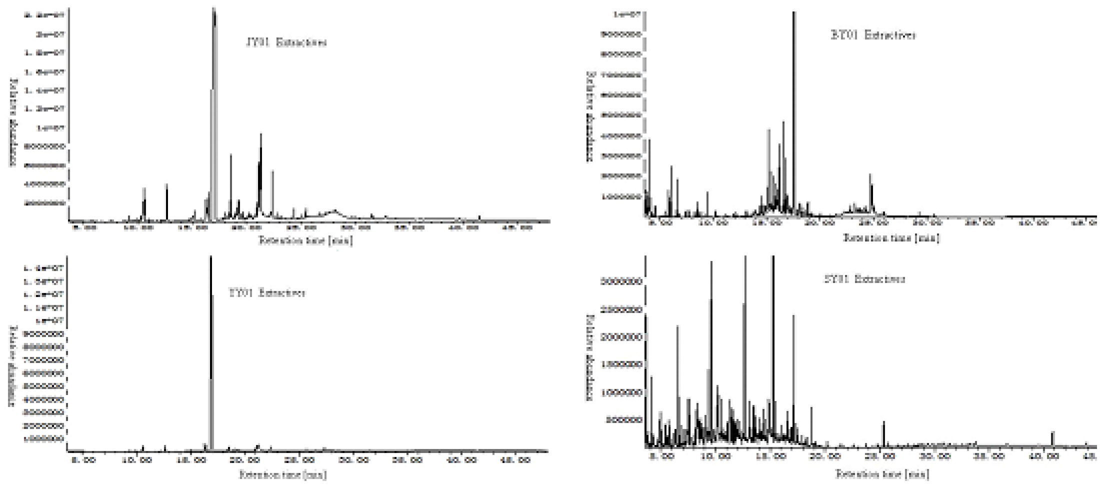
GC-MS analysis

The above extractives were analyzed by online linked gas chromatograph/ mass spectrometer (GC-MS), respectively. The GC/MS analysis was carried out on a Aglient 6890N+5975C GC-MSTM (Aglient Co., Ltd, USA), which was linked to a mass selective detector. An elastic quartz capillary column DB-5MS (30m× 250um×0.25um) 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 of GC began at 50°C and increased at the rate of 8°C /min until 250°C, 5°C / min until 300°C was reached, followed by a split injection at ratio of 15:1. The program of MS was scanned over the 35-335AMU (m/z) respectively, with an ionizing voltage of 70eV and an ionization current of 150?A of electron ionization (EI). The flow velocity of helium was 1.2ml/min. Ion source temperature: 230°C, quadropole temperature 150°C.

RESULTS AND DISCUSSION

Components of JYBS extractives from *Illicium verum*

During JYBS extraction, four extractives (methanol-ethanol, ether-ethanol, benzene-ethanol and petroleum ether-ethanol) were obtained respectively. The total ion chromatograms of four



solvent extractives by GC/MS were shown in Fig.1. Relative content of each component was counted by area normalization. Analyzing the MS data, the NIST standard MS map by computer, open-published books and papers[10-27], then components and their contents were identified.

According to GC/MS result, 89 components were identified on 105 peaks of JY01 extractives from *Illicium verum* fruit. The result showed that the main components were anethole(54.24%), 2-hydroxy-2-(4-methoxy-phenyl)-n-methyl-acetamide(4.81%), 3-methyl-4-(4-methoxyphenyl)-1-buten-4-ol (4.64%), 4-methoxy-benzaldehyde(4.17%), 3,7-dimethyl-1,6-octadien-3-ol(3.18%), trans-4-methoxycinnam-aldehyde (3.07%), d-limonene(2.70%), 1-(4-methoxyphenyl)-2-propanone (2.27%), 1-(3-methyl-2-butenoxy)-4-(1-propenyl)benzene(1.62%), [1s-(1α,4aα,10aβ)]-1,2,3,4,4a,9, 10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-1-phenanthrenecarboxylic acid (1.09%), 2,6-dimethyl-6-(4-methyl-3-pentenyl)-bicyclo[3.1.1]hept-2-ene(1.07%), dehydroabietic acid(0.84%), eucalyptol(0.77%), caryophyllene (0.71%), α-terpineol(0.54%), n-(1-adamantyl) methyl-4-methoxy-benzenesulfonamide(0.53%), 2-(1-hydroxyisoamyl)-1-methoxybenzene(0.52%), 4'-methoxybutyro -phenone (0.51%), and so on. Others were 4-methoxy-Benzeneacetic acid, 1-methyl-4-(1-methylethenyl)-1,2-Cyclohexanediol, n-Hexadecanoic acid, 4-methoxyphenylpropane-2-ol, 4-Hydroxy-2-methylacetophenone, 2-[2-pyridyl]-Cyclohexanol, (Z,E)-3,7,11-trimethyl-1,3,6,10-Dodecatetraene, Terpinen-4-ol, α-Farnesene, Estragole, 2-Cyclohexen-1-one, N-(3-nitrophenyl)-4-methoxy-Benzamide, Catechol, 1,1a,6,6a-tetrahydro-Cycloprop[a]indene, Stigmast-4-en-3-one, 4-ethoxy-3-hydroxy-Benzaldehyde, β-Bisabolene, Phthalic acid isobutyl undecyl ester, 2-methyl-5-(1-methylethyl)-Bicyclo[3.1.0]hex-2-ene, 1-(3-ethylcyclobutyl)-Ethanone, 3-methyl-2-Butenal, 2,3-dihydro-Benzofuran, 2-(2-Acetyl-1-methylcyclopropyl) thiophene, L-α-Terpineol, 1'-acetyl-1',3,4,4',5,5',6,6'-octahydro-2,3'-Bipyridine, 2-methyl-6-(2-propenyl)-Phenol, α-ethyl-4-methoxy-Benzenemethanol, 5-(2-hydroxy-2-phenylacetyl)-2-Furancarboxaldehyde dimethylhydrazone, trimethylphenoxy-Silane, 1,2-Cyclohexanedione, ?-hydroxy-4-methoxy-Benzeneacetic acid, 2,5,6-trimethyl-1,3,6-Heptatriene, (2R,4S,6R)-(-)-2,4,6-trimethyl-Nonanoic acid methyl ester, α-Cadinol, 4-methoxy-Benzoic acid methyl ester, 1-methoxy-4-(1-methylpropyl)-Benzene, 1,2-dimethoxy-4-(1-propenyl)-Benzene, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, β-Myrcene, 3-(1,1-dimethylethyl)-4-methoxy-Phenol, Phenol, Hexadecanoic acid methyl ester, Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate, 6,8-Dimethyl-5-oxo-2,3,5,8-tetrahydroimidazo [1,2-a]pyrimidine, 3-Carene, 1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene, (Z)-3,7-dimethyl-1,3,6-Octatriene, (-)-cis-Myrtanyl acetate, γ-Terpinene, 5-propyl-1,3-Benzodioxole, 3-butyl-Pyridine 1-

oxide, (Z,Z)-9,12-Octadecadienoic acid methyl ester, (E)-8-Octadecenoic acid methyl ester, γ-Sitosterol, Anisaldehyde dimethyl acetal, 3-Methoxyacetophenone, Methyl stearate, Dibutyl phthalate, cis-Linaloloxide, 1,3,3-trimethyl-2-Oxabicyclo[2.2.2]octan-6-ol, N-(4-methoxyphenyl)-4-Methoxybenzenamide, (4-Methoxy-phenyl)-(2-nitrocyclohexyl)-methanol, Squalene, Benzo(a)pyrene 4,5-oxide, 5,6,7,8-Tetrahydroindolizine, 4-(1,1-dimethylethyl)-Benzenemethanol, 2,4-Dimethoxybenzyl alcohol, 3,7,11,15-tetramethyl-, acetate, (E,E,E)-2,6,10,14-Hexadecatetraen-1-ol, 2-Carene, 2-(N,N',N'-Trimethylhydrazino)-1,3-benzothiazole, 1-methyl-4-(1-methylethylidene)-Cyclohexene.

25 components were identified on 27 peaks of YY01 extractives from *Illicium verum* fruit. The result showed that the components were anethole(85.17%), d-limonene(1.90%), 2-hydroxy-2-(4-methoxy-phenyl)-n-methyl-acetamide(1.90%), 4-methoxy-benzaldehyde(1.12%), 3,7-dimethyl-1,6-octadien-3-ol(1.00%), 1-(4-methoxyphenyl)-2-propanone(0.95%), 4-methoxy-benzaldehyde (0.88%), trans-4-methoxycinnamaldehyde(0.87%), 2-hydroxy-2-(4-methoxy-phenyl)-n-methyl-acetamide(0.80%), 3,7-dimethyl-1,6-octadien-3-ol(0.57%), dodecane(0.52%), eucalyptol(0.49%), 2,6-dimethyl-6-(4-methyl-3-pentenyl)-bicyclo[3.1.1]hept-2-ene(0.48%), 2-[2-pyridyl]-cyclohexan -ol (0.47%), [1r-(1r*,4z,9s*)]-4,11,11-trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene (0.44%), 4-methoxy-benzaldehyde(0.40%), trans-4-methoxycinnamaldehyde(0.35%), caryophy -llene (0.30%), 2,3-dihydro-2-methyl-benzofuran(0.28%), ethyl oleate(0.27%), hexadecanoic acid ethyl ester (0.24%), 9,12-octadecadienoic acid ethyl ester(0.20%), octadecanoic acid ethyl ester(0.14%), l-?-terpineol(0.13%), isopropoxycarbamic acid ethyl ester(0.11%).

81 components were identified on 95 peaks of BY01 extractives from *Illicium verum* fruit. The result showed that the main components were cyclohexyl-benzene(16.58%), 1-ethyl-1,2,3,4-tetrahydro-naphthalene(10.47%), 2,7-bis(1,1-dimethylethyl)-naphthalene(7.65%), (3-methylcyclo -pentyl)-benzene(7.22%), 1,3-dimethyl-benzene(2.89%), 7-butyl-bicyclo [4.2.0]octa-1,3,5-triene (2.84%), 1-methoxy-4-methyl-2-(1-methylethyl)-benzene(2.82%), 1-chloro-tricyclo[4.3.1.1(3,8)] undecane(2.49%), cis-3-methyl-endo-tricyclo[5.2.1.0(2.6)]decane(2.42%), toluene(2.24%), (1-propylpentyl)-benzene(1.96%), anethole(1.66%), n-methyl-2-[(phenylmethyl) amino]-acetamide (1.45%), 1,2,3,4-tetrahydro-1,4-dimethyl-naphthalene(1.43%), 1,2,3-trimethyl-benzene(1.36%), p-xylene(1.35%), phthalic acid 2,4-dimethylpent-3-yl isobutyl ester(1.26%), (1-ethylbutyl)-benzene (1.22%), 1-ethyl-3-methyl-benzene(1.15%), α-ethyl-benzeneacetic acid (1.06%), naphthalene (1.06%), 1,1'-hexylidenebis-benzene(1.05%), 3,4,4a,5,6,7-hexahydro-6-methyl-1(2h)-naphthalen -one (1.02%), ethylbenzene (0.95%), 2-benzylamino-n-phenyl-acetamide (0.95%), 2-methyl-heptane (0.89%), 2,3-dihydro-1,4,7-trimethyl-1h-indene(0.89%), (4-methylphenyl) methanol 1-methyl -propyl ether(0.89%), and so on. Others were 2,7-dimethyl-naphthalene, bis(3,5-dimethylphenyl)-diazene, 4-methyl-4-phenyl-2-pentanone, 4,4'-dimethoxy-2,2'-dimethylbiphenyl, 4,8-diethyl-1,5-dimethyl-dicyclopenta[a,d]benzene, 1-(1,1-dimethylethyl)-4-(methoxymethyl)-benzene, 1-isopropyl-3-tert-butylbenzene, 5-ethyl-1,2,3,4-tetrahydro-naphthalene, cyclohexene 3-(tert-butyl) peroxide, (1-methylheptyl)-benzene, (1-methylethylidene)-cyclobutane, 2-hexyl-3-methylene -4-chromanone, trans-1,3-dichloro-cyclohexane, trans-1-(1-butenyl)-4-methoxy-benzene, 5-methyl-nonane, 4-(diethylamino) benzonitrile, (1,1-dimethyldecyl)-benzene, (1-methylpenta-1,3-dienyl) benzene, 2-ethyl-1,4-dimethyl-benzene, 1,2,4-trimethyl-cyclopentane, hexamethyl-dewar benzene, (e)-2,3-dibromo-2-butene-1,4-diol, 1,2,3-trimethyl-cyclopentane, 4-stilbene carboxaldehyde, 4-methyl-heptane, o-cymene, 1,2,3,4-tetrahydro-2-methyl-naphthalene, ethyl-cyclopentane, (2,4-dimethylcyclopentyl)-benzene, (2,2-dimethylcyclopentyl)-benzene, octane, 2,3,6,7-tetrahydro-3a,6-methano-3ah-indene, (1s)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene, 9-methyl-s-octahydroanthrace -ne, 1-phenyl-cyclohexene, mesitylene, 3,4'-diisopropylbiphenyl, 2-(phenylthio)acetonitrile, 1,1,4,4-tetramethyl-2,6-bis(methylene)-cyclohexane, (1-methylethyl)-benzene, 1,2,3,4-tetramethyl-benzene, 1,2,4,5-tetramethyl-benzene, biphenyl, 1,1'-ethylidenebis-benzene, propyl-benzene, nonane, chloro-benzene, hexanedioic acid bis(2-ethylhexyl) ester, phthalic acid di(2-propylpentyl) ester, tert-butyl-benzene, 1,3-dimethyl-cyclohexane, 1-methyl-3-propyl-benzene, 1,4-dimethyl-naphthalene.

98 components were identified on 103 peaks of SY01 extractives from *Illicium verum* fruit. The result showed that the main components were undecane (7.58%), decane(6.48%), dodecane (4.88%), tridecane(3.28%), nonane(3.08%), o-cymene(2.68%), 1,2,3-trimethyl-benzene(2.23%), naphthalene(2.09%), trans-decahydro-naphthalene (1.99%), 3-methyl-decane(1.89%), 1,2,4-trimethyl-benzene(1.70%), 4-methyl-decane(1.67%), 2-methyl-trans-decalin(1.64%), 3,7-dimethyl-decane(1.62%), 1,2,3,4-tetramethyl-benzene(1.57%), 2-methyl-decane(1.53%), 1-ethyl-2,4-dimethyl-benzene(1.51%), 3-methyl-heptane(1.48%), 2,6-dimethyl-octane(1.47%), butyl-cyclohexane(1.41%), 1-ethyl-3-methyl-benzene(1.36%), decahydro-2-methyl-naphthalene (1.33%), 2-methyl-undecane(1.28%), 2-methyl-nonane(1.21%), cis-decahydro-naphthalene (1.19%), pentyl-cyclohexane(1.14%), (1α,2β,3α)-1,2,3-trimethyl-cyclohexane(1.08%), [s-(e)]-4-octene, 2,6-dimethyl-(1.08%), p-xylene(1.08%), octane(1.04%), 6-methyl-undecane(1.02%), 1-methyl-2-propyl-benzene(1.01%), 2,6-dimethyl-undecane(1.01%), propyl-cyclohexane(1.00%),and so on. others were 1-ethyl-1-methyl-cyclohexane, 2,3,7-trimethyl-octane, 4-methyl-undecane, 1-ethyl-2-methyl-benzene, 3-ethyl-2-methyl-heptane, p-cymene, (3-methyl-2-butenyl)-benzene, cis,trans-1,6-dimethylspiro[4.5]decane, mesitylene, o-xylene, propyl-benzene, 1-octadecyne, 2-butyl-1,1,3-trimethyl-cyclohexane, cyclodecane, tetradecane, 2-methyl-naphthalene, ethyl-cyclohexane, sulfurous acid cyclohexylmethyl undecyl ester, 1-ethyl-2-methyl-cyclohexane, d-limonene, 1,1,3-trimethyl-cyclohexane, 3-(3-hydroxy-1-propenyl)-cyclopentanone, 2-methyl-dodecane, 3-methyl-nonane, (4-methylpentyl)-cyclohexane, cis-2-methyl-3-(1-oxopropyl)-cyclopentanone, hexyl-cyclopentane, stigmasta-4,6,22-trien-3?-ol, 4-methyl-dodecane, anethole, 1,2-diethyl-benzene, 1,2-dimethyl-cyclooctene, 1-pentyl-2-propyl-cyclopentane, 4-propyl-cyclohexene, cis, cis-3-ethylbicyclo[4.4.0]decane, 1-ethyl-2,2,6-trimethylcyclohexane, cis-octahydro-1h-indene, dibutyl phthalate, 1-ethyl-4-methylcyclohexane, 3-ethyl-2,5-dimethyl-3-hexene, (z)- (1-methyl-1-propenyl)-benzene, (-)-trans-pinane, 1,4-diethyl-2-methyl-benzene, 2,3-dihydro-1,1,5-trimethyl-1h-indene, cyclotridecane, (r)-(-)-3-methyl-2-butanol, 3,4-dimethylcumene, heptylcyclohexane, (1s,3r)-(+)-m-menthane, cyclohexyl-benzene, ethylbenzene, 1,2,3,4-tetrahydro-5-methyl-naphthalene, 1-ethenyl-4-ethyl-benzene, 2-methyl-octane, 2,5-dimethyl-octane, cis-1,3-dimethyl-cyclohexane, 2,6,10-trimethyl-dodecane, 2-methyl-tridecane, trans-1,2-dimethyl-cyclohexane, 2,6-dimethyl-heptane, 4-methyl-octane, pentadecane.

Molecular distribution of JYBS extractives from *Illicium verum*

The GC-MS analysis results shown the molecular distribution of JYBS extractives from *Illicium verum*. the richest components of first-stage extractives(methanol-ethanol extractives) were anethole(54.24%), 2-hydroxy-2-(4-methoxy-phenyl)-n-methyl-acetamide(4.81%), etc. Relative content of hydrocarbons, alcohols (phenol alcohols), aldehydes/ketones, ethers, acid / esters and other compounds accounted for 17.74%, 12.6%, 3.5%, 54.69%, 3.86% and 7.58% of methanol-ethanol extractives, respectively. The richest components of second -stage extractives(ether-ethanol extractives) were anethole(85.17%), d-limonene(1.90%), etc. Relative content of hydrocarbons, alcohols (phenol alcohols), aldehydes/ketones, ethers, acid / esters and other compounds accounted for 7.54%, 2.66%, 0.95%, 85.17%, 0.69% and 2.99% of ether-ethanol extractives, respectively. The richest components of third-stage extractives(benzene-ethanol extractives) were cyclohexyl-benzene(16.58%), 1-ethyl-1,2,3,4-tetrahydro-naphthalene(10.47%), etc. Relative content of hydrocarbons, alcohols (phenol alcohols), aldehydes/ketones, ethers, acid / esters and other compounds accounted for 88.97%, 0.41%, 1.38%, 1.66%, 3.55% and 4.03% of benzene-ethanol extractives, respectively. The richest components of fourth-stage extractives(petroleum ether-ethanol extractives) were undecane(7.58%), decane(6.48%), etc. Relative content of hydrocarbons, alcohols (phenol alcohols), aldehydes/ketones, ethers, acid / esters and other compounds accounted for 96.25%, 0.92%, 0.59%, 0.52%, 0.00% and 1.72% of petroleum ether-ethanol, respectively. The results suggested that first-stage and second-stage extractives were suitable to extract anethole, and third-stage and fourth-stage extractives were suitable to extract hydrocarbons.

The retention time of each stage extractives of *Illicium verum* showed a particular rule. Among the first-stage extractives, the molecules with the retention time of ≤10min, ≤20 min, ≤30 min, ≤40 min and ≥40min were 1.08%, 75.41%, 22.10%, 1.08% and 0.29%, respectively. Among the second-stage extractives, the molecules with the retention time of ≤10min, ?20 min, ≤30 min, ≤40 min and ≥40min were 0.11%, 94.35%, 5.52%, 0.00% and 0.00%, respectively. Among the third-stage extractives, the molecules with the retention time of ≤10min, ≤20 min, ≤30 min, ≤40 min and ≥40min were 15.02%, 67.38%, 17.40%, 0.16% and 0.00%, respectively. Among the fourth-stage extractives, the molecules with the retention time of ≤10min, ≤20 min, ≤30 min, ≤40 min and ≥40min were 37.71%, 62.11%, 0.59%, 0.00% and 0.56%, respectively. The results showed that the four extractives of *Illicium verum* biomass had a main retention time between 10-20 min.

Resource utilization of JYBS extractives from *Illicium verum*

There were many biomedical components in the JYBS extractives of *Illicium verum* biomass. Because of its officinal value, α-cedrene and β-cedrene were used for the raw materials of advanced odorants[18]. Squalene could resist fatigue and strengthen the body's resistance, protect liver, and improve human immunity. squalene was used in nutraceutical and pharmaceutical industries[19]. α-Cadinol, which acted as anti-fungal and as hepatoprotective, was proposed as a possible remedy for drug-resistant tuberculosis[20-22]. Anethole was widely used as a flavoring substance, present in the essential oil from guarana which was alleged to have a psychoactive effect, and had potent antimicrobial properties, against bacteria, yeast, and fungi[23,24]. *In vitro*, anethole had antihelmintic action on 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[24-26]. Eucalyptol was a colorless natural organic compound which had a fresh camphor-like smell and a spicy, cooling taste. Eucalyptol could reduce inflammation and pain, kill leukaemia cells in vitro, treat mouthwash and cough suppressant, lower headache on bending, frontal headache, sensitivity of pressure points of trigeminal nerve, nasal obstruction, impairment of general condition, and rhinological secretion[24-27]. Especially, stigmasta-4,6,22-trien-3β-ol and γ-sitosterol could reduce serum cholesterol and had effect on atherosclerotic lesion development[26]. According to the relative content of biomedicine components, the JYBS extractives of *Illicium verum* biomass was suitable for the extraction of anethole.

CONCLUSIONS

The 89, 25, 81 and 98 components were identified on the peaks of JY01, YY01, BY01 and SY01 extractives from *Illicium verum* fruit, respectively. The richest components of first-stage extractives were anethole(54.24%), 2-hydroxy-2-(4-methoxy-phenyl)-n-methyl-acetamide(4.81%), etc. The richest components of second -stage extractives were anethole(85.17%), d-limonene(1.90%), etc. The richest components of third-stage extractiveswere cyclohexyl-benzene(16.58%), 1-ethyl-1,2,3,4-tetrahydro-naphthalene(10.47%), etc. The richest components of fourth-stage extractives were undecane(7.58%), decane(6.48%), etc. And the four extractives of *Illicium verum* biomass had a main retention time between 10-20 min. what's more, the first-stage and second-stage extractives were suitable to extract anethole, and the third-stage and fourth-stage extractives were suitable to extract hydrocarbons.

The functional analytical result suggested that the JYBS extractives of *Illicium verum* biomass contained rich pharmaceutical components which had huge potential in biological medicine, especially including anethole, stigmasta-4,6,22-trien-3?-ol, ?-sitosterol, and so on.

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