BIOENERGY AND BIOACTIVE COMPONENTS IN LEAVES OF *TOONA SINENSIS*

by

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Toona sinensis is an important tree for urban greening construction. Toona sinensis sprouts are widely used because of their high medicinal and edible value. However, the functions and effective components of mature Toona sinensis leaves have not been fully exploited and utilized. In order to explore the value of Toona sinensis leaves in bioactive and bioenergy utilization, Toona sinensis leaves were extracted with benzene and ethanol, and then the components in extracts were analyzed by FT-IR and GC-MS. The results showed that benzene and ethanol extracts from Toona sinensis leaves mainly contained phenols, hydrocarbons, esters, alcohols, acids and other bioactive components. These bioactive components can be used in the fields of bioenergy, biomedicine, chemical row materials, food additives and spices. Among them, the content of bioactive components from benzene extract was higher than that from ethanol extract. These findings indicate that Toona sinensis leaves contain a large number of bioactive components which can be used in bioenergy and other fields, and have broad prospects for development.

Key words: *bioenergy, Toona sinensis leaves, bioactive component, resources, GC-MS, FT-IR*

Introduction

Toona sinensis, known as a famous *tree vegetables*, is widely distributed throughout China [1]. *Toona sinensis* is light-loving, shade-tolerant and suitable for growing in wet soil areas. *Toona sinensis* is rich in protein, fat, sugar, vitamins, crude fiber, calcium, phosphorus and iron. *Toona sinensis* is a treasure from all part of its body [2, 3]. *Toona sinensis* sprouts, rich in nutrition and unique in taste, have good curative effects on hair loss, cold, stomach pain, dysentery, lung heat and cough, etc. *Toona sinensis* seeds can be used to regulate kidney and assist in the treatment of chronic pharyngitis. *Toona sinensis* root can help remove dampness and heat [4]. *Toona sinensis* wood is kind of excellent wood for furniture, interior decoration and shipbuilding [5, 6]. At present, the research on the components of *Toona sinensis* leaves mostly focuses on its pharmacological functions, while the research on other energy utilization is not deep enough.

Therefore, in order to fully tap the potential utilization value of *Toona sinensis* leaves and investigate its bioactive components, the *Toona sinensis* leaves were extracted by organic solvent extraction method, and the active components of *Toona sinensis* leaves

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were analyzed by FT-IR and GC-MS. It provides a basis for the further research on energy utilization of *Toona sinensis* leaves.

Materials and methods

Experimental materials

Toona sinensis leaves selected in the experiment were collected from the plantation of Henan Agricultural University in June. The samples were washed and dried at 40 °C in the oven, crushed, screened with 200 meshes, sealed and stored at dark [7].

Methods

Extraction by two solvents

As shown in fig. 1 extraction of *Toona sinensis* leaves with organic solvent benzene and ethanol. Ratio of solid to liquid: 1:30. Soak the mixture at room temperature for 12 hours. Full extraction was carried out at 79 °C and 80 °C. The extraction time was 3 hours. The mixed samples were filtered by a filter device. The filtered extract was evaporated rotationally in a vacuum of 0.01 MPa at 40-45 °C. Concentrate to 20 mL and seal in reagent bottle. The extract residue was dried at 25-30 °C. Finally, all extracts and residues were placed in a refrigerator at 4 °C for further detection [8, 9].

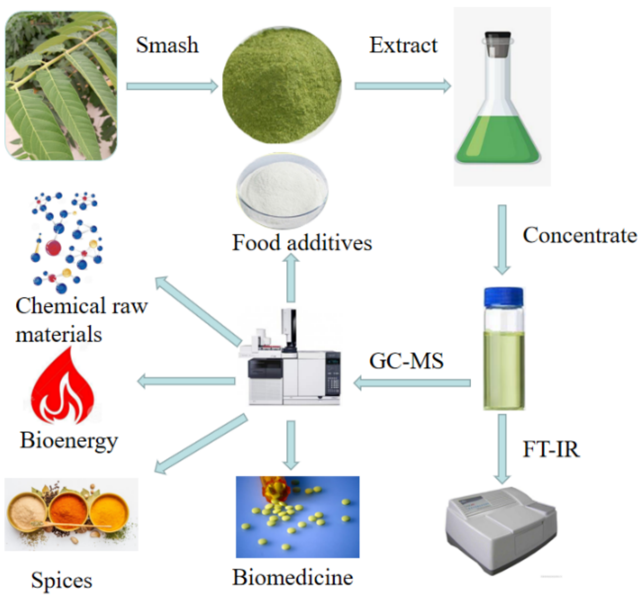

Figure 1. Flow chart of experiment

The FT-IR analysis

Infrared spectrum analysis of the original powder of 200 mesh screened *Toona sinensis* leaves and the extract extracted by organic solvent. The 0.1 g of raw powder and 1 g of dried potassium bromide powder were added to agate mortar. The mixture is ground thoroughly and then put into the tablet press for pressing [10], and subsequently quantified with FT-IR (Nicolet is 5) spectroscopy from 4000-500 cm^{-1}.

The GC-MS analysis

The GC: Column HP-5 MS (30 mm × 0.25 mm × 0.25 μm). Elastic quartz capillary column with high purity helium as carrier gas, flow rate: 1 mL/min, shunt ratio: 5:1, gas chromatography temperature is set to start at 50 °C, then rises to 250 °C at the rate of 10 °C/min, and finally rises to 280 °C at the rate of 5 °C/min [11].

The MS: Programmed scanning quality range is controlled at 30-600 amu, ionization voltage: 70 ev, ionization current: 150 μA electron ionization (EI). The ion source is set at 230 °C and the quadrupole temperature is 150 °C [12, 13].

Results

Group changes during extraction

Extract *Toona sinensis* leaves powder by benzene and ethanol. Infrared liquid detection and solid detection were carried out for extract liquid and original powder respectively. As shown in fig. 2(a), according to the relationship between infrared spectra of organic compounds and functional groups, the infrared spectra of raw powder and extract of *Toona sinensis* leaves were analyzed. A group of peaks appeared near 3330 cm^{-1}, which was caused by O-H stretching vibration, indicating the presence of hydroxyl groups [14]. A group of peaks appeared near 2920 cm^{-1}, which was caused by C-H stretching vibration [15]. The absorption peaks near 1650 cm^{-1} are caused by the C=C. The absorption peaks at 1060 cm^{-1} was caused by the stretching vibration of C=O [16]. As shown in fig. 2(b), the absorption peaks of *Toona sinensis* leaves were mainly concentrated in 3660-3000cm^{-1}, 3000-2500cm^{-1}, 2000-1340cm^{-1}, and 1340-500cm^{-1}. The results show that the main chemical components of *Toona sinensis* leaves are phenols, hydrocarbons, esters, alcohols and acids.

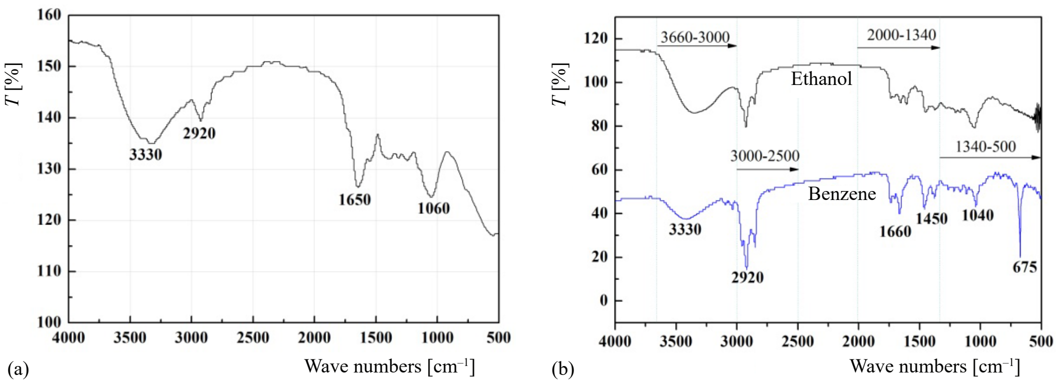

Figure 2. (a) The FT-IR spectra of the original powder, (b) the FT-IR spectra of extracts benzene and ethanol

Comparison of volatile components in two extracts

The volatile components of benzene extracts from *Toona sinensis* leaves were analyzed by GC-MS. As shown in fig. 3(a), a total of 73 components were found in 87 peaks, which mainly contained esters, hydrocarbons, phenols, alcohols, acids, *etc*. Vitamin E (5.11%), ursolic aldehyde (1.74%), 2, 4-Di-tert-butylphenol (1.64%), di-p-tolyl-Methane (1.55%) and 2, 2'-methylenebis [6-(1, 1-dimethylethyl) -4-methyl phenol (1.52%) were the main volatile components of benzene extracts from *Toona sinensis* leaves.

Benzene extract from *Toona sinensis* leaves contains a large number of volatile components, which can be used in different resource utilization directions. For example, a large number of bioactive ingredients can be used in bioenergy. Dimethyl phthalate, Mesitylene are flammable liquids and may be used as biofuels [17-19]. Hexanedioic acid, bis (2-ethylhexyl) esterare used as cold-resistant plasticizers for various resins, especially for polyvinyl chloride and vinyl chloride copolymers. They can also be used as raw materials and plasticizers for aviation greases. The 2, 4-Di-tert-butylphenol can be used as an intermediate of antioxidant, stabilizer and ultraviolet absorbent. There are abundant active ingredients which can be used in biomedicine. Vitamin E is used for the prevention of habitual abortion and threatened abortion, as well as for the treatment of infertility and infant nutritional giant cell anemia [20, 21].

The 2,5-bis(1,1-dimethylethyl)-1,4-Benzenediolcan be used as medical supplies. Lupeol has anti-tumor, anti-inflammatory and antioxidant effects, and can be used in the treatment of leukemia, pancreatic cancer, breast cancer, prostate cancer, melanoma, liver cancer and other tumors [22]. It can also be used in the prevention of acute anti-host disease after bone marrow transplantation. It plays an important role in medical treatment. Some bioactive ingredients can be used in the spices and food additives industry. Diphenyl methane can be used as a substitute for fragrant leaf oil [23, 24]. It is suitable for making soap essence and perfume. Benzene acetaldehyde can be used as food spices and daily necessities [25].

The volatile components of ethanol extracts from *Toona sinensis* leaves were analyzed by GC-MS. As shown in fig. 3(b), a total of 102 components were found in 122 peaks, including esters, hydrocarbons, phenols, alcohols, acids, *etc*. The volatile components of benzene extracts from *Toona sinensis* leaves were beta-sitosterol acetate (2.82%), tetraethyl silicate (1.37%), 2,6-bis (1,1-dimethylethyl) - phenol (1.36%) and tributylacetyl citrate (1.32%).

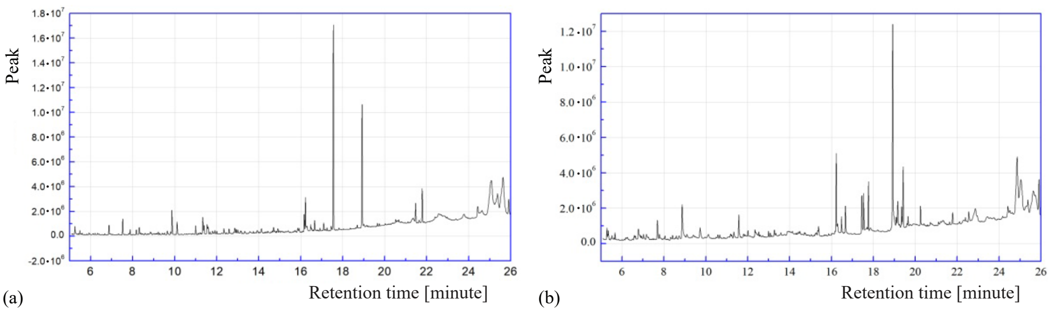

Figure 3. (a) Ion chromatography of volatile components in benzene extracts from *Toona sinensis* leaves, (b) ion chromatography of volatile components in ethanol extracts from *Toona sinensis* leaves

Ethanol extract from *Toona sinensis* leaves contains a large number of active ingredients, which can be used in different resource utilization directions. For example, it contains a large number of active ingredients which can be used in the field of bioenergy. Tetraethyl silicate, dibutyl phthalate, 1, 2, 3-propanetriol, 1-acetate are flammable liquids and may be used as biofuels [26-28]. The 2,2'-methylenebis 6-(1, 1-dimethylethyl)-4-methyl-phenol is widely used as antioxidant in natural rubber, rubber, latex, other synthetic materials and petroleum products. Some active ingredients can be used in the biomedical industry, 9,12-octadecadienoic acid (z, z)- can be used as lipid-lowering drugs. Ethyl hydrogen malonatecan be used as pharmaceutical intermediates. Ethanedioic acid, diethyl esteris mainly used in the pharmaceutical industry, as solvent, dye intermediates, and the synthesis of paints and drugs. Myristic acid is used to manufacture emulsifier, waterproof agent, curing agent, PVC heat stabilizer and plasticizer, *etc*. It is also the raw material of perfume and medicine. Squalene, as a nutritional supplement, has the effect of improving liver function and tissue activity [29-31]. Some bioactive components were found in benzene extracts which can be used in spices and food additives. Hexadecanoic acid, ethyl ester for perfume flavor, Octadecanoic acid, ethyl ester are mainly used for the preparation of bacon flavor [32-34]. Tetradecanoic acid is the raw material of spices.

Figure 4 shows that the benzene extract from *Toona sinensis* leaves contains higher active ingredients which can be used in biomedicine, chemical raw materials, food additives, spices and bioenergy. The effect of benzene extract from *Toona sinensis* leaves is better than that of ethanol extract from *Toona sinensis* leaves. The content of benzene extract from *Toona sinensis* leaves which can be used as bioenergy was significantly higher than that of ethanol extract from *Toona sinensis* leaves.

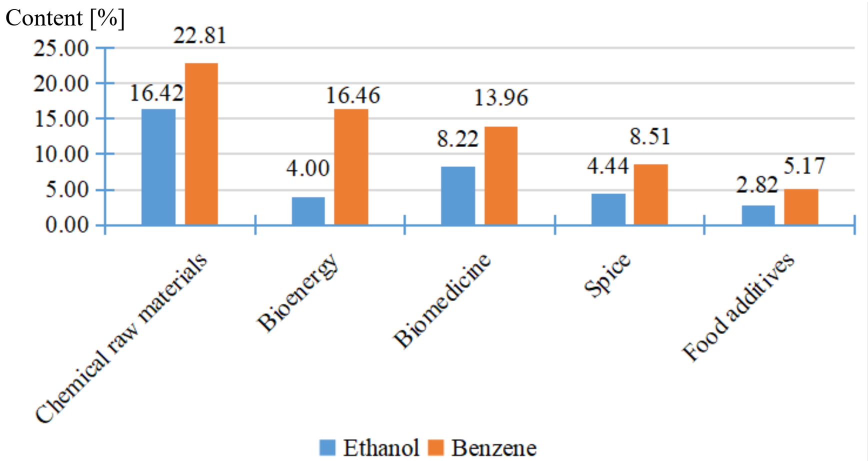

Figure 4. Comparison of functional categories of the *Toona Sinensis* leaves extracts by GC-MS

Figure 5 shows that alchols, phenols and hydrocarbons are higher in the volatile components of benzene extract from *Toona sinensis* leaves, while acids and esters are higher in the volatile components of ethanol extract from *Toona sinensis* leaves.

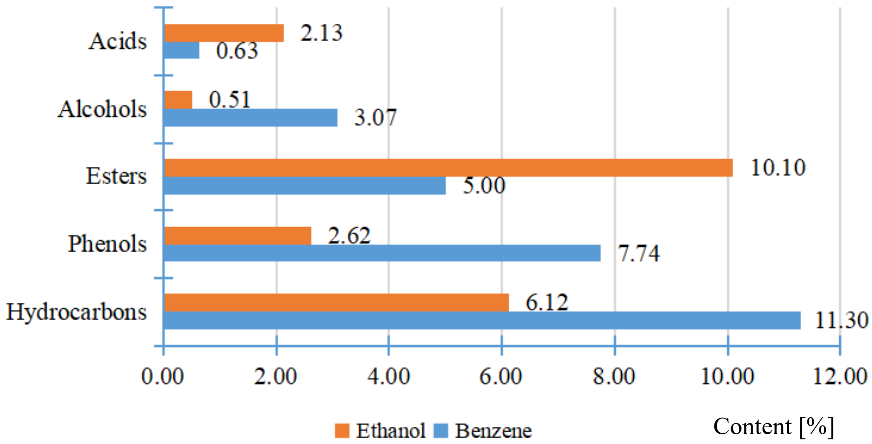

Figure 5. Different types of compounds of the extraction products of *Toona sinensis* leaves

Conclusions

The FT-IR analysis showed that the original powder and two extracts of *Toona sinensis* leaves changed in different degrees after infrared irradiation. The main chemical components of *Toona sinensis* leaves were esters, hydrocarbons, phenols, alcohols, and acids.These findings indicate that there are a large number of bioactive components in *Toona sinensis* leaves.

The GC-MS analytic results showed that 73 components were found in benzene extracts from *Toona sinensis* leaves and 102 components were found in ethanol extracts from *Toona sinensis* leaves. By consulting the literature and related information, a large number of bioactive components found in these two extracts can be used in different industries. Vitamin

E is an important antioxidant, which can be used for the prevention of habitual abortion and threatened abortion, as well as for the treatment of infertility and infant nutritional giant cell anemia. Squalene is used in the development of biochemistry and pharmaco chemistry, as stationary liquid for chromatographic analysis, organic coloring materials, rubber, spices, surfactants, *etc*. Dibutyl phthalate is a flammable liquid. It is mainly used as plasticizer for nitrocellulose, acetic acid fibre, polyvinyl chloride, *etc.* It can also be used to make paint, adhesives, artificial leather, printing ink, safety glass, fabric lubricant, *etc.*

Through functional classification and comparison of the volatile components of the two extracts, it was found that the benzene extracts from *Toona sinensis* leaves could be used in biomedicine, chemical rowmaterials, food additives, spices and bioenergy. The extraction effect of benzene extracts from *Toona sinensis* leaves was higher than that of ethanol extracts from Toona sinensis leaves. The content of bioenergy is the most obvious.

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