CHARACTERIZATION OF BIOACTIVE AND BIOENERGY COMPONENTS FROM FRESH WALNUT (*JUGLANS REGIA*) LEAF

by

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Walnut (Juglans regia) is an important economic tree specie, and has unique aroma in its leaf. In order to investigate the bioactive and bioenergy characteristic of compounds in ethanol and benzene extracts from fresh walnut leaf by gas chromatography/mass spectrometry (GC-MS). The results indicated that ethanol and benzene extracst fresh walnut leaf contain abundant alcohols, aromatic hydrocarbons, quinones, phenols, aldehydes, acids, ketones, alkanes, esterscompounds, especially including dl-.alpha.-Tocopherol, juglone, squalene andlupeol, which are important bioactive components. The functional analytic result suggested that compounds from fresh walnut leaf extracts can be developed into raw materials for industries of biofuel, biomedicine, cosmetic, spices and food additive. The ethanol extract of fresh walnut leaf is more rich in biomedicine and food additives components, such as ketones, alkanes, aldehydes, amines, and acides compounds. The benzene extract of fresh walnut leaf is more rich in biofuels, spices, and cosmetic components, such as phenols, aromatic hydrocarbons, olefines and quinones compounds.

Key words: *walnut, leaf, extract, bioenergy, bioactive components, volatile organic compounds*

Introduction

Plants contian abundant bioactive components [1, 2], which have been proved to be used in many fields [3]. Especially, some plants extracts can be used as traditional medicines to treat various diseases [4]. Many medicinal plants are also excellent sources of pharmaceutical ingredients [5]. Lots of plant extracts have anti-inflammatory, analgesic, antibacterial, antioxidant and anti-tumor activity, such as *Ginkgo biloba* leaf extracts having obvious effect on treating coronary heart disease, angina pectoris and hyperlipidemia [6, 7]. *Ginkgo biloba leaf* extract has the effects of inhibiting fungi, anti-allergy, unblocking blood vessels, improving brain function, delaying brain aging, enhancing memory, treating alzheimer's disease. Branches, barks and fruits of *Broussonetia papyrifera* have strong antioxidant activity [8, 9]. It has been clearly demonstrated that some plant extracts can also be used as bioenergy resources [10, 11]. In addition, some plant extracts can also be used in high value-added industries, such as spices, cosmetics and food additives.

Walnut (*Juglans regia*) belongs to the genus *Juglans* of the *Juglandaceae* family, which is widely planted all over the world [12-14]. Walnut is famouse conomic tree specie

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[15-17]. The walnut fruits are a highly nutritious nut [18-20], which are rich in oil composed of unsaturated fatty acids. Unsaturated fatty acids can reduce blood lipids, prevent and treat vascular sclerosis, inhibit the growth of tumors, and enhance the immunity of the body. So, unsaturated fatty acids plays an important role in human health. But the insufficient law of utilization of walnut leaf, a large number of walnut leaf are wasted, resulting in environmental pollution. In order to solve the problem, in this paper the bioactive components of volatile organic compounds in fresh walnut leaf extract was identified to provide reference value for further utilizing volatile organic compounds in fresh walnut leaf extract.

Materials and methods

Materials

As shown in fig.1, fresh walnut leaf was collected from Henan agricultural university campus, henan Province, China.

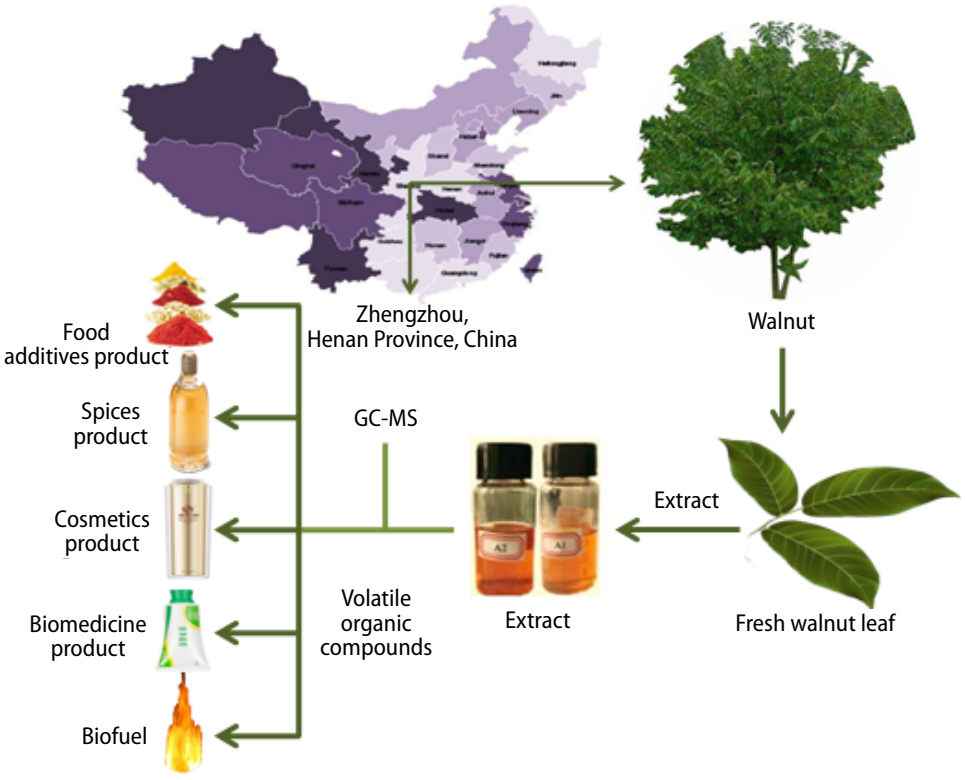

Figure 1. Experimental process

Methods

Extraction

The fresh walnut leaf was put in a liquid nitrogen tank, which was bring back to the laboratory for grinding into powder. Two copies of the fresh walnut leaf was weighed, each was about 15 g (0.1 mg accuracy). Extraction was carried out in 300 ml ethanol and benzene solvents. Ultrasound for 5 minute, using Soxhlet extractor method for 3 hours, temperature of 79 and 80

°C, respectively. After extraction, the ethanol, benzene were removed via rotary evaporation and dried with anhydrous sodium sulfate, and the resulting extractives were stored at 4 °C.

The FT-IR analysis

The resulting extractives of fresh walnut leaf extract was used for FT-IR analysis. The FT-IR spectra of the samples were obtained on a FT-IR spectrophotometer (IR 100) using KBr discs, containing 1.00 % finely ground sample.

The GC-MS analysis

The GC-MS determination: GC: Column HP-5MS (30 m × 250 μm × 0.25 μm), elastic quartz capillary column, the carrier gas used for high purity helium, flow rate of 1 mL/min. The split ratio is 50:1.The temperature program of the GC starts at 50 °C, rises to 250 °C at a rate of 10 °C/min, and then rises to 280 °C at a rate of 5 °C/min. MS: program scans mass range of 30-600 amu, ionization voltage of 70 eV, ionization current of 150 μA electron ionization (EI). The ion source and the quadrupole temperature were set at 230 °C and 150 °C, respectively.

Results and analysis

The GC-MS analysis of the fresh walnut leaf extract

In this study, FT-IR spectra of the fresh walnut leaf ethanol and benzene extract was performed to characterize the chemical structure and groups. As shown in fig. 2, FT-IR of ethanol and benzene extract from fresh walnut leaf shows similar spectral patterns except for different infrared absorption intensities. For the fresh walnut leaf ethanol and benzene extract, a wide absorption peak at 3350 cm^{-1} and 3420 cm^{-1} was caused by stretching the vibration of aromatic and aliphatic O−H groups. In addition, the absorption peaks at 2920 and 2852 cm^{-1} were caused by stretching the vibration of the C−H stretching vibration of CH_3, $-CH_2-$ and −CH− groups. The absorption bands at 2920 and 2915 cm^{-1} are C−H stretching vibration of alkanes. The absorption peak appears 1730-1445 cm^{-1}, which is attributed to the C=O bond stretching vibration. This observation suggests the presence of a compound containing a carbonyl group, such as an aldehydes, ketones, acids and esters. Moreover, a wide absorption peak at 1030 cm^{-1} was caused by stretching the vibration of the C−O−O stretching vibration of CH_3, $-CH_2-$ and −CH− groups. Compared all the absorption peaks of the fresh walnut leaf ethanol and benzene extract with each other, the absorption peaks of ethanol extract were obviously stronger than benzene extract. This indicated that ethanol could extract more compounds than benzene, especially aromatic, aliphatic, alkanes, aldehydes, ketones, and acids compounds, which were abundant in fresh walnut leaf ethanol extract.

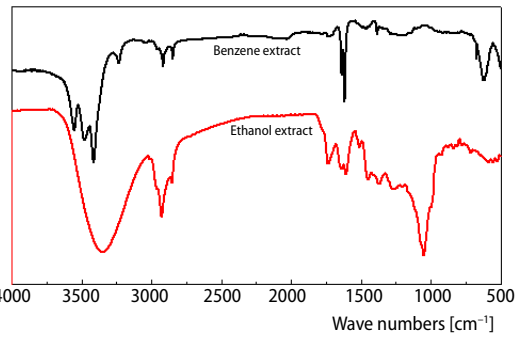

Figure 2. The FT-IR spectra of fresh walnut leaf ethanol and benzene extract

The GC-MS analysis of the fresh walnut leaf extract

To investigate volatile organic compounds in the fresh walnut leaf ethanol extract, GC-MS analysis was carried out. As shown in fig. 3(a), a total of 97 volatile organic compounds were identified from 104 peaks in fresh walnut leaf ethanol extract. According to the functional

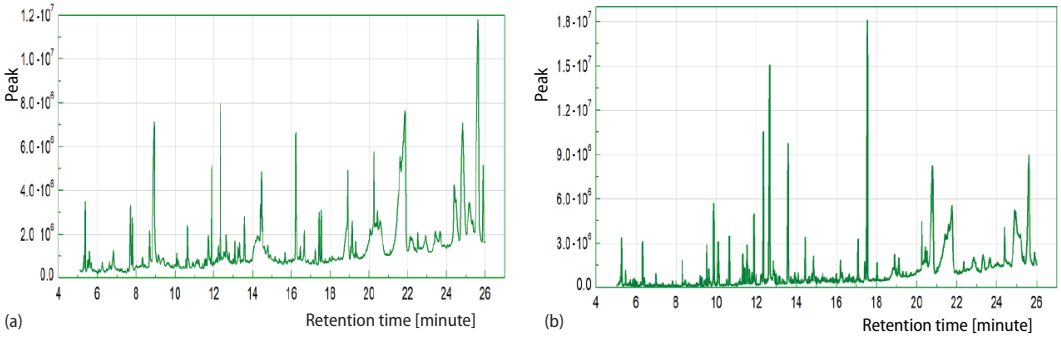

Figure 3. (a) Total ion chromatogram of fresh walnut leaf ethanol extract via GC-MS; (b) total ion chromatogram of fresh walnut leaf benzene extract via GC-MS

group; these compounds were divided into different types of chemical groups. As shown in fig. 4(a), there were mainly 11 types of compounds for fresh walnut leaf ethanol extract, which contains ketones compounds (28.68%), alcohols compounds (14.03%), phenols compounds (11.80%), alkanes compounds (9.47%), esters compounds (7.24%), aldehydes compounds (6.35%), amines compounds (4.68%), aromatic hydrocarbons compounds (3.72%), olefins compounds (2.03%) and quinones compounds (0.70%).

Figure 3(a) shows that the ketones compounds mainly include 3(2H)-isothiazolone, 2-octyl- (0.28%). Among them, 3(2H) - isothiazolone, 2-octyl - is a low toxicity, high efficiency, broad-spectrum antifungal agent, which has a strong killing effect on fungi and can achieve ideal antifungal effect. The alcohols compounds mainly include lupeol (4.05%), phytol (3.55%), and tripentaerythritol (0.38%). Among them, lupeol has anti-oxidation. Lupeol wound healing pharmacological effects, which shows anti-cancer activity in pancreatic cancer, breast cancer, prostate cancer, melanoma and other tumors [21]. Phytol is the basic raw material for the production of vitamin K1 and vitamin E [22]. The phenols compounds mainly include dl-.alpha.-tocopherol (10.94%) and eugenol (0.80%). Among them, dl-.alpha.-tocopherolhas the activity of protecting cells from oxidative damage [23]. The alkanes compounds mainly include non-acosane (7.1%). The esters compounds mainly include phenol, 2-methoxy-4-(2-propenyl)-, acetate (1.30%), ethanedioic acid, diethyl ester (1.37%). The aldehydes compounds mainly include5-Hydroxymethylfurfural (6.35%). The acids compounds mainly include 2-Furancarboxylic acid (0.81%), formic acid, TMS derivative (0.75%). The olefins compounds mainly include squalene (1.56%). Squalene can be used as a nutritional supplement to improve liver function and tissue activity. In addition, squalene also has the function of oxygen up take by red blood cells [24, 25].

As shown in fig. 4(b), the functional analytic result suggested that volatile organic compounds from fresh walnut leaf ethanol extract can be developed into raw materials for industries of biomedicine (32.41%), biofuel (1.86%), chemical materials (6.42%), spices (5.94%) and food additive (3.70%).

As shown in fig. 3(b), a total of 66 volatile organic compounds were identified from 74 peaks in fresh walnut leaf benzene extract. As shown in fig. 4(c), there were mainly 10 types of compounds for fresh walnut leaf ethanol extract, which contains ketones compounds (23.96%), alcohols compounds (14.13%), phenols compounds (20.79%), aromatic hydrocarbons compounds (9.87%), esters compounds (9.18%), alkanes compounds (7.71%), quinones compounds (7.34%), olefins compounds (2.58%), acids compounds (1.37%) and aldehydes compounds (0.76%).

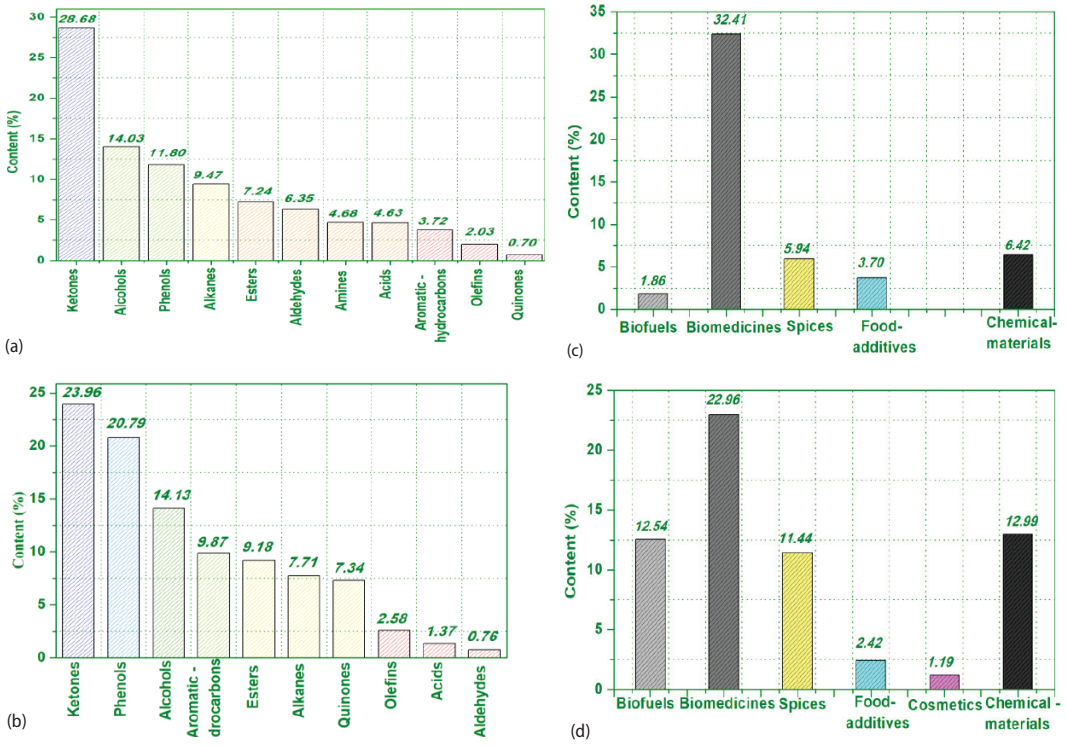

Figure 4. (a) Comparison of different types in volatile organic compounds from fresh walnut leaf ethanol extracts, (b) comparison of different functional uses in volatile organic compounds from fresh walnut leaf ethanol extract, (c) comparison of different types in volatile organic compounds from fresh walnut leaf benzene extract, and (d) comparison of different functional uses in volatile organic compounds from fresh walnut leaf benzene extract

Figure 3(b) shows that the ketones compounds mainly include dihydrofuran-2-one, 4-(3,4-dimethoxybenzyl)-3-(4-hydroxy-3-methoxybenzyl) (16.84%), 7-methoxy-1-tetralone (3.82%). The alcohols compounds mainly include gamma.-sitosterol (10.22%), phytol (1.14%), and n-tetracosanol-1 (1.43%). The phenols compounds mainly include.delta.-tocophero (9.91%) and dl-.alpha.-tocopherol (8.33%). Among them, dl-.alpha.-tocopherolhas the activity of protecting cells from oxidative damage [26]. The alkanes compounds mainly include do-decane (1.22%) and heneicosane (0.82%). The esters compounds include dibutyl phthalate (7.48%), hexadecanoic acid (0.95%) and methyl ester (0.23%). Among them, dibutyl phthalate is a flammable liquid; which is mainly used as plasticizer for nitrocellulose, acetic acid fibre and polyvinyl chloride [27]. The aldehydes compounds mainly include octadecanal (0.33%). The acids compounds mainly include oxiraneoctanoic acid, 3-octyl-, methyl ester, cis- (0.85%) and n-hexadecanoic acid (0.43%). The olefins compounds mainly include cis-.beta.-farnesene (1.52%). The quinones compounds mainly include juglone (7.73%). Juglone has hemostatic and antimicrobial activities [28], which can treat eczema, psoriasis and psoriasis. In addition, Juglone is also used as spices [29-32].

As shown in fig. 4(d), the functional analytic result suggested that volatile organic compounds from fresh walnut leaf benzene extract can be developed into raw materials for

industries of biomedicine (22.96%), biofuel (12.54%), chemical (12.99%), spices (11.44%) and food additive (2.42%) and cosmetics (1.19%).

As shown in figs. 5(a) and 4(b) volatile organic compounds in the fresh walnut leaf ethanol extract is richer in ketones compounds, alkane compounds, aldehydes compounds, amines compounds and acids compounds. Fresh walnut leaf ethanol extract can be developed into raw materials for industries of biomedicine and food additives are richer. Volatile organic compounds in the fresh walnut leaf benzene extract are richer in phenols compounds, esters compounds, aromatic hydrocarbons compounds, olefins compounds and quinones compouds are richer, which can be developed into raw materials for industries of biofuel, chemical, spices and cosmetics are richer.

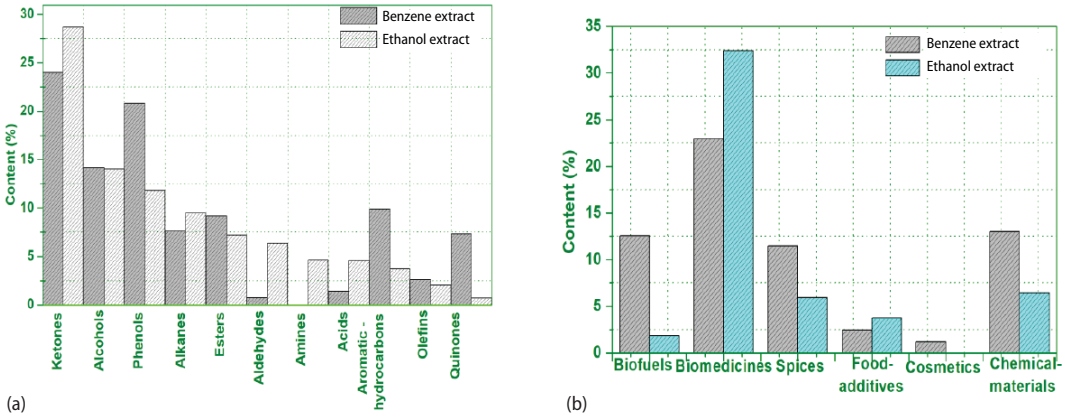

Figure 5. (a) Comparison of different types in volatile organic compounds from fresh walnut leaf benzene and ethanol extract, (b) comparison of different functional uses in volatile organic compounds from fresh walnut leaf benzene and ethanol extract

Conclusion and discussion

In this work, fresh walnut leaf was extracted to gain natural bioactive components, and the components were analyzed. We demonstrated that.

- The volatile organic compounds functional analytic result indicated that the fresh walnut leaf extract was rich in natural bioactive components.
- Volatile organic compounds in the fresh walnut leaf extract mainly contain ketones compounds, alcohls compounds, phenols compounds, alkanes compounds, esters compounds, aldehydes compounds, amines compounds, acids compouds, aromatic hydrocarbons compounds, olefins compounds and quinones compouds.

The fresh walnut leaf extract contains a large number of substances that can be widely used in biofuel, biomedicine, chemical materials industry, and food additives,spicesand cosmetics industries.

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