

DIVERSE BIOACTIVE COMPONENTS FROM *GINKGO BILOBA* FRUIT

by

**Xinya GAO^a, Qiang JIAO^b, Bingqian ZHOU^a, Qimei LIU^{a*},
and Dangquan ZHANG^{a*}**

^a School of Forestry, Henan Agricultural University, Zhengzhou, China

^b Henan Province Port Institute for Food Inspection and Testing, Zhengzhou, China

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The fruit of Ginkgo biloba is widely concerned because of its high economic and medical value. In this study, efficient extraction methods were used to identify the bioactive components in the fruit of Ginkgo biloba. The bioactive constituents of Ginkgo biloba fruit were identified and the uses of these bioactive components were discussed. There are more than 160 kinds of chemical extracts in Ginkgo biloba fruit. It mainly contains flavonoids, terpenes, phenols, alkaloids, polyisopentene, quinine acid, linoleic acid, monoxalic acid, ginkgol, ginkgosterone and other substances. It contains a variety of substances, and has many applications in anti-bacterial, anti-tumor, lipid-lowering and other aspects. The results suggested that Ginkgo biloba fruit has great potential in chemical raw materials, biological health care, biomedical treatment, aroma and bioenergy.

Key words: *Ginkgo biloba*, fruit, bioactive components

Introduction

Ginkgo biloba, a deciduous tree, is the oldest surviving gymnosperm. It is suitable for growing in subtropical monsoon area and has good hydrothermal conditions. It is widely distributed in many countries and regions around the world.

The branch of *Ginkgo biloba* tree is flat, the leaves are fan-shaped, the crown of the tree shows orderly elliptic, and the leaves can become golden yellow in autumn, which make *Ginkgo biloba* of great ornamental value. *Ginkgo biloba* leaves and *Ginkgo biloba* fruits are important export products with high economic value [1]. *Ginkgo biloba* fruit is rich in nutrition and is an edible fruit with health care function. The rich vitamins and carotene in the fruit play a certain role in delaying aging, promoting the metabolism of our skin, and whitening effect [2]. In the meantime, *Ginkgo biloba* fruit also has very good officinal value and is an important medical raw material that prevents and cure hypertension and heart disease. Elderly people often eat *Ginkgo biloba* fruit can better prevent cardiovascular disease. *Ginkgo biloba* fruit also has the function of regulating respiratory organs, and its water-soluble components in the outer seed skin have good antitussive and expectorant effects. Because of containing *Ginkgo biloba* acid, it has stronger inhibitory effect against tuberculosis bacilli and skin fungus which can be used to treat tuberculosis and skin disease [3]. *Ginkgo Biloba* exocarp extract also has inhibition rate of 88-100% on apple anthrax and other 11 plant bacteria, which has a very important

* Corresponding author, e-mail: liuqimei@163.com, zhangdangquan@163.com

impact on plant disease control. *Ginkgo biloba* fruit also has the effect on lowering blood fat, anti-tumor and inhibiting the growth activity of cancer cells.

Due to the high nutritritional value and important medical value of *Ginkgo biloba* fruit, our study used modern detection methods and experimental techniques to extract and determine the composition of *Ginkgo biloba* fruit, and explored its potential research value [4]. Figure 1 is the experimental process in our research.

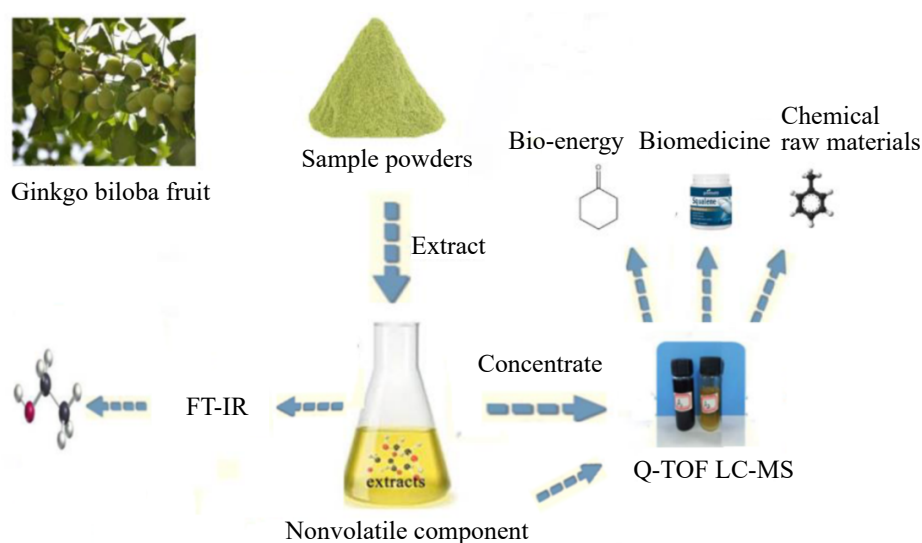


Figure 1. The experimental process in this research

Materials and methods

Experimental materials

The immature fruit of *Ginkgo biloba* were collected from the Henan Agricultural University. The *Ginkgo biloba* fruit were dried in a constant temperature oven at 35 °C. The samples were smashed into powder by using a FZ102 disintegrator suitable for plant (Tanjing Taisite Ins. Corp., China), in succession, 200 mesh powders were sieved out [5].

Methods

Extraction by two solvents

The immature fruit of *Ginkgo biloba* were extracted by ethanol and benzene, respectively, with the solid-liquid ratio of 1:20. After immersing for 12 hours at room temperature, the mixed samples were fully extracted by automatic FOSS Soxhlet Extracted apparatus (Agilent, USA) at 78 °C, 80 °C for 4 hours, and then filtrated fast with filter paper immersed in ethanol and benzene, respectively for 24 hours. The filtrated extraction was evaporated at 40-45 °C under 0.01 MPa vacuum, and concentrated to 20 mL, then transferred to a sealed reagent bottle [6]. Concentrated extracts were kept in 4 °C refrigerator for the subsequent determination [7].

Analysis of group changes during extraction by FT-IR

The powders of *Ginkgo biloba* fruit, and their extracted residues were dried at 100 °C for 4 hours. And then put them in the dry container with desiccant to prevent moisture absorption, so as not to affect the detection [8]. A certain amount of potassium bromide were ground

and sieved out using AS200 Sieving Instrument (USA), put in the dry pot, then keep in the muffle furnace (with SX-2.5-10 box-type control resistance furnace control box) at 150 °C for 5 hours, after that, removed under a heating lamp cover. Take 200 mg of potassium bromide to an agate mortar with a smooth surface, and 0.5-2 mg of the sample was mixed fast and completely with potassium bromide in the mortar, and then placed in the tablet press for tableting [9]. As a liquid solution, the extracts were directly placed in the tablet press for tableting. The pressed samples were tested in a Fourier transform infrared spectroscopy from 4000 cm^{-1} -400 cm^{-1} .

High-performance liquid chromatography- QTOF – mass spectrometry

High-performance liquid chromatography-Mass spectrometry: Agilent 1290 Infinity chromatograph [10]. Chromatographic column: Thermo Scientific Acclaim RSLC C18 100 × 2.1 mm (2.2 μm , 120 Å). Mobile phase: A phase (0.1% FA, 99.9% H_2O); B phase (0.1% FA, 99.9% ACN). Mass spectrometry: Mass Spectrometry Type: Compact High-resolution Mass Spectrometer. Ion source: ESI source. Scan mode: positive ion mode scan. Scanning range: m/z 100-1000 [11].

Results

Regulation of the changes in chemical groups in *Ginkgo biloba* fruit extracts

According to the relationship between infrared spectra of organic compounds and functional groups, the infrared spectra of two kinds of extracts of *Ginkgo biloba* fruit were analyzed.

By analyzing the infrared spectrum of ethanol extract, it was found that there were three distinct peaks in the characteristic frequency of ethanol extract [12]. Among them, the absorption peak at 3387 cm^{-1} was the strongest, which was caused by O-H stretching vibration, indicating the presence of alcohols and phenols. The resulting fig. 2 shows that there is an absorption peak at 1641 cm^{-1} , which is the result of the stretching vibration of NH_2 at the absorption peak. There is an absorption peak at 1055 cm^{-1} , which is caused by C-N stretching vibration. By analyzing the infrared spectrum of benzene extract, the characteristic frequency of benzene extract has three obvious peaks. An absorption peak is observed near 2925 cm^{-1} , which is caused by the CH_3 - stretching vibration of the absorption peak.

The general trend of infrared spectra of extracts from the two *Ginkgo biloba* fruits was similar. The adsorption strength of ethanol extract near 3387 cm^{-1} was higher than that of benzene extract, which indicated that the content of alcohol and phenolic compounds in ethanol extract was higher than that of benzene extract. The adsorption strength of benzene extract near the absorption peak of 1650 cm^{-1} was higher

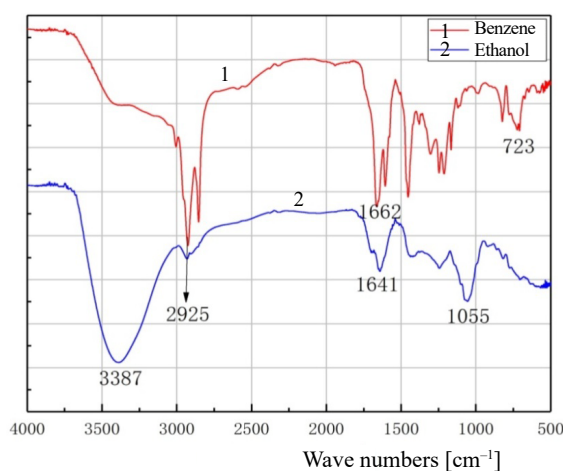


Figure 2. Group changes of the two extracts *Ginkgo biloba* fruit via FT-IR

than that of ethanol, indicating that the content of amine compounds in benzene extract was higher than that of ethanol [13].

Rich and diverse bioenergy and bioactive compounds in *Ginkgo biloba* nut extracts via QTOF-HPLC-MS

By HPLC-QTOF-MS of the ethanol extract from *Ginkgo biloba* fruit, ethanol extracts were found to have good chromatographic separation effect and abundant spectrum peaks [14]. A total of 166 corresponding substances were detected, fig. 3. Some useful components were also found, among which alantolactone has good insect repellent and antibacterial effects, and has exciting and inhibiting effects on cardiovascular diseases, fig. 4. Meanwhile, alantolactone also has a variety of anti-tumor effects. Protopanaxatriol has the effect of preventing or inhibiting the growth activity of cancer cells [15]. Vitamin K₂ can treat and prevent osteoporosis [16]. Vitamin K₂ makes bone protein, which is combined with calcium to form bone, increasing bone density and preventing fractures. It can prevent liver cirrhosis from progressing to liver cancer.

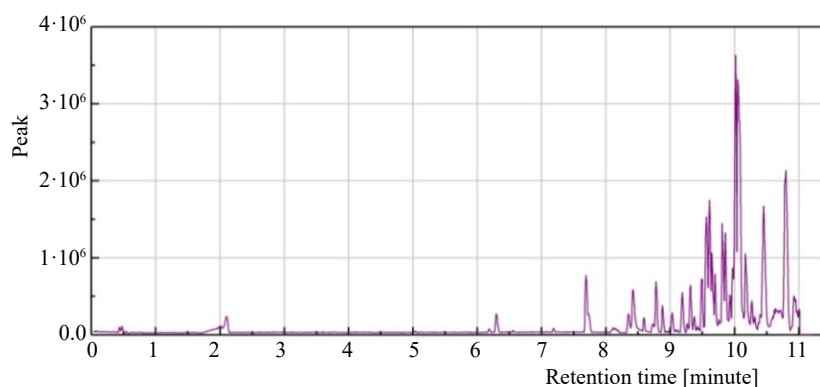


Figure 3. Ion chromatogram of ethanol extract from *Ginkgo biloba* fruit

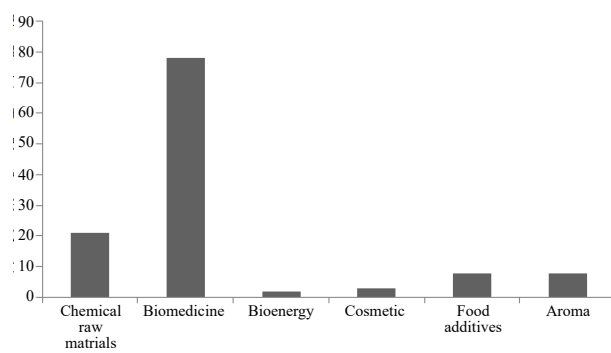


Figure 4. Classification of compounds in ethanol extracts

It can diuretic, strengthen the liver's detoxification function, and reduce blood pressure. Arbutin is used in medicine as a diuretic and an anti-infective agent for urinary system [17]. At the same time, arbutin is also an ideal whitening agent for whitening cosmetics, which is added in many whitening and maintenance products. Effectively whiten and remove freckles, gradually fade and remove skin freckles, chloasma, melanosis, acne and senile spots. High safety,

no stimulation, sensitization and other side effects, with good compatibility with cosmetics components, anti uv radiation stability. It can eliminate redness and swelling, promote wound healing, remove scars, and inhibit the formation of dandruff. Protostemonine has anti-inflammatory, antibacterial, anti-allergy and antitussive effects, and has significant inhibitory effect on asthma. It is of great value in the treatment of clinical asthma, providing a new direction for

the search for anti-asthma drugs. Quinic acid can enhance bile secretion, reduce fat, detoxify the liver and prevent fatty liver [18].

By HPLC-QTOF-MS, the acetone extracts from *Ginkgo biloba* fruit were found to have a good chromatographic separation effect and abundant spectrum peaks. A total of 164 corresponding substances were detected, fig. 5. Some useful components were also found, among which Ginkgolic acid [19] has antibacterial effect and can inhibit the growth of mycobacterium tuberculosis in vitro, fig. 6. Praeruptorin E can dispel phlegm, dilate blood vessels and lower blood pressure [20]. Bavachinin has the effect of anti-angiogenic activity, anti-osteoporosis, inhibiting tumor and inhibiting tumor angiogenesis [21]. Ginkgolide B is the most potent platelet activator ever discovered and is clinically used to treat clots, acute pancreatitis, cardiovascular disease, and aneurysms [22]. Ginkgolide B has a prophylactic effect on infected nerve cells, antioxidants, and anti-aging. Linderolactone has a chilling effect on the stomach and kidneys and can effectively remove pain in some parts of the body [23-25]. Protopanaxatriol prevents and inhibits the growth and activity of cancer cells. Alantolactone has a variety of anti-tumor effects, at the same time alantolactone has a good insect repellent effect, antibacterial effect, cardiovascular excitement and inhibition. Ethyl alpha-linolenate has the effect of treating hyperlipidemia [26]. Sophoflavescenol can inhibit the activity of some tumor cells. Nodakenin has the function of clearing away heat and eliminating phlegm [27]. Hyodeoxycholic acid is used to reduce the concentration of cholesterol in the blood and has the effect of lowering blood lipids. Hupehenine has the effect of lowering blood lipid [28]. Protostemonine has anti-inflammatory, anti-bacterial, anti-allergic, cough action [29]. Muscone can reduce swelling and relieve pain and promote blood circulation for removing obstruction in collaterals [30]. Muscone

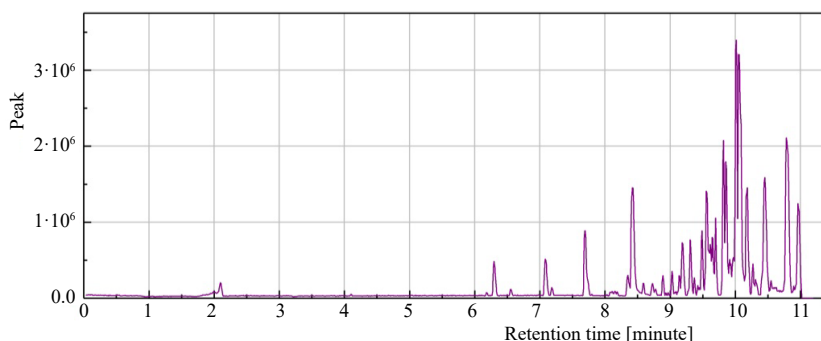


Figure 5. Ion chromatogram of benzene extract from *Ginkgo biloba* fruit

has anticoagulant, anti-inflammatory, anti-bacterial, anti-ulcer, anti-tumor and other effects. A small dose can stimulate the central nervous system, while a large dose can inhibit it.

The two extracts contained a large number of chemical raw materials and biomedicines. Bioenergy and cosmetic contents were the lowest in the two extracts, at less than 5%. The content of food additives and aroma was about 10% in the two extracts,

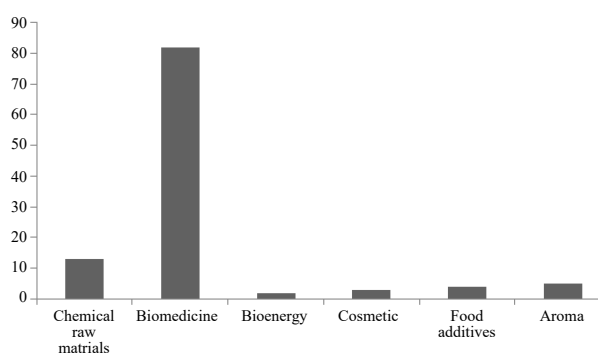


Figure 6. Classification of compounds in benzene extracts

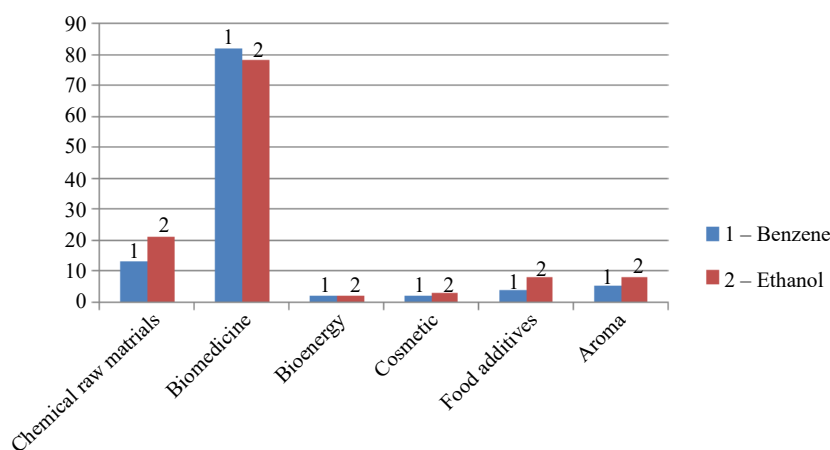


Figure 7. Classification by use of compounds

fig. 7. The biomedicine extracts contained in them have good effects on anti-tumor, lowering blood pressure and anti-cancer.

Conclusion and discussion

Ginkgo biloba fruit benzene extract and ethanol extract composition similar, mainly contains chemical raw materials, biomedical components, spices and so on. The most abundant ginkgolic acid has antibacterial effects, but it has potential sensitization, mutagenesis and strong cytotoxicity. Ginkgolide B has a strong physiological activity. It is the strongest platelet activating factor antagonist ever found and can be used clinically to treat thrombus, acute pancreatitis and cardiovascular disease. Ginkgolide B also be used in the treatment of metastatic cancer and has a protective effect on damaged neurons. At the same time, it has the function of anti-oxidation and delaying senescence. It has a good prospect of medical application. *Ginkgo biloba* P. E. has a magical effect on the treatment of coronary heart disease, angina pectoris, cerebral arteriosclerosis, senile dementia, hypertension and other diseases. It can be used in pharmaceutical, health care products, daily necessities, cosmetics and other fields. Bilobol has many physiological activities such as antibacterial, antiparasitic and antitumor. *Ginkgo biloba* fruit extract also contains a variety of anti - tumor substances, such as sophoflavescenol, Alantolactone, bavachinin and so on. Protopanaxatriol have the effect of preventing or inhibiting the growth and activity of cancer cells. In addition, *Ginkgo biloba* fruit also contains a large number of anti-blood pressures, lowering blood lipids and other medical care substances.

Through the analysis of *Ginkgo biloba* fruit extract, the information of various substances contained in *Ginkgo biloba* fruit was obtained. These substances can be widely used in biomedicine, chemical raw materials, medical care and other fields, and have a good development prospect.

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