MOLECULES AND MEDICAL FUNCTION OF DIOSPYROS LOTUS L.

by

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Original scientific paper https://doi.org/10.2298/TSCI190529042Y

The potential use of organic solvent extracts of Diospyros lotus L. was analyzed by using FT-IR, GC-MS, and MTT. The results showed that the Diospyros lotus L. extracts contained many kinds of bioactive organic ingredients with antibacterial, antitumor, and anti-inflammatory activities. Meanwhile the extracts have significant effect on HEGP2 and SGC-7091, which can be used as anticancer medicine. Therefore, it provides a scientific foundation for potential application prospects in the fields of biomedicine, skin care products and other fields.

Key words: Diospyros lotus L., FI-RT, GC-MS, MTT

Introduction

Diospyros lotus L., with common names date-plum, Caucasian persimmon, or lilac persimmon, is a widely cultivated species of the genus *Diospyros*, native to subtropical southwest Asia and southeast Europe [1-5]. Its English name derives from the small fruit for the taste reminiscent of both plums and dates. It was known to the ancient Greeks as *God's fruit*, referring to the taste of this fruit which is reminiscent of both plums and dates. This species is one candidate for the *lotus tree* mentioned in *The Odyssey*.

Diospyros lotus L. is one of the oldest plants in cultivation and mainly grows in the lower and middle mountain zones among the valley, mountainous region and slope or beside of the forest edge [6, 7]. In China, *Diospyros lotus* L. are distributed throughout Shandong, Henan, Hebei, Shanxi, Gansu, Zhejiang, Anhui, Jiangxi, Hunan, Hubei, Sichuan, Yunnan and Tibet provinces. The *Diospyros lotus* L. is the deciduous tree and the important fruit yielding species of genus *Diospyros*. The fruit is used usually as a sedative, astringent, nutritive, antiseptic, antidiabetic, antitumor, laxative, antipyretic and for the treatment of constipation [8]. The fruit extract has a high amount of total phenolic and flavonoid contents and showed the strong antioxidant and free-radical scavenging activity [9-16]. Meanwhile fruits are used for the treatment of diarrhea, dry coughs and hypertension for the less side effects and used traditionally to treat favism in Iran though it is not scientifically proven [17].

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The timber of *Diospyros lotus* L. is rare dark wood and used as fine furniture and stationery for beautiful texture and color. The timber chemical properties are an important aspect for wood properties and utilization [18, 19]. Some of these ingredients are important raw materials for many sectors such as chemical industry, medicine and light chemical industry, and have certain economic value [20]. This paper selects the timber of *Diospyros lotus* L. for the study. The organic solvent extracts were analyzed by FT-IR, GC-MS. Meanwhile, MTT was used for anti-cancer effect. Then biological activities ingredients were identified and prospect of resource utilization was prospected.

Material and methods

Experimental materials

Samples were collected from Xixia forest in Henan Province. Samples were processed from fresh material into powder and oven-dried to absolute dryness at 60 °C. Two kinds of extracts were named as W1 and W2 which were extracted by ethanol, ethanol/benzene (1:2) respectively [21].

Experimental methods

The FT-IR analysis

The FT-IR spectrum of the samples were obtained on a FT-IR spectrophotometer (IR100) using a KBr disk containing 1.00% finely ground samples [22]. The scanning of each extracts was collected at a spectral resolution of 4 cm⁻¹ and the spectral range was 500- -4000 cm^{-1} [23].

The GC-MS analysis

The two extracts were analyzed using a GC-MS (Agilent GC-MS 7890B 5977A). Column HP-5MS (30 m \times 250 µm \times 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 20:1. The temperature program of the GC starts at 50 °C, rises to 250 °C at a rate of 8 °C/min, and then rises to 300 °C at a rate of 5 °C/min. The MS program scan 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.

The MTT analysis

To validate the results obtained by using the proposed complementary techniques we carried out, with the same samples, the conventional MTT colorimetric assay. This procedure ensures that the signal obtained is due to formazan and allows for comparisons of sensitivity and dynamic range of each technique [24].

Briefly, cells were treated with an isopropanol-10% Triton X-100 solution, collected the solubilized formazan and transferred triplicate aliquots to a 96-well standard plate (NUNC). We measured formazan production by spectrophotometry using the SOFTmax Pro microplate reader (Molecular Devices) equipped with the SOFT max Pro software package (v.4.7). Absorbance was collected at $\lambda = 570$ nm, subtracting the background measured at $\lambda = 690$ nm.

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Results and discussion

Analysis of FT-IR

Figure 1 showed the infrared spectra of two extracts of *Diospyros lotus* L., the absorption peak band at 3362 cm⁻¹ is the O-H stretching vibration, and band at 2924 cm⁻¹ is C-H stretching vibration, C=C stretched at 1635 cm⁻¹, C-H stretched at 1459 cm⁻¹, 1273 cm⁻¹ 1048 cm⁻¹, and 821 cm⁻¹ via C-C stretching vibration, C-O stretching vibration, and C-H bending vibration [25-28]. The 1601 cm⁻¹ and 1511 cm⁻¹ is the aromatic carbon skeleton vibration, represent the aromatic region of lignin. Except for different infrared absorption intensities, two spectra showed exactly similar spectra.

The values of the transmission intensities of W1 were higheer than W2, which indicated that the phenolic compounds were extracted by organic solvent. The phenolic compounds are the main components that affect the color of the wood, which further explains the reasons for the lighter color of the wood after the organic sol-vent extraction experiment.

Comparison of two extraction with different solvents, the infrared spectra has different degrees of change in infrared transmittance. As shown in fig. 1, the absorption peaks of the *Diospyros lotus* L. extract are mainly concentrated in the wave segments of 3700-3000 cm⁻¹, 3000-2800 cm⁻¹ and 1690-970 cm⁻¹. The main chemical components are phenols, alcohols, ethers, fatty acids, hydrocarbons and aromatic compounds. And the characteristic absorption peaks are reduced, indicating that phenols, alcohols, ethers, fatty acids, ethers, fatty acids, hydrocarbons and aromatic compounds are partially extracted [29-31].

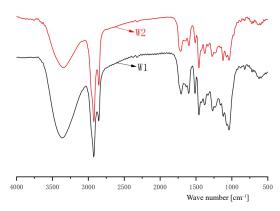


Figure 1. The FT-IR spectra of samples W1 and W2

Analysis of GC–MS

The total ion chromatograms of two kinds of extractives of ethanol, ethanol/benzene were shown in figs. 2 and 3, which were analyzed by GC-MS.

According to the results of GC-MS analysis, 20 peaks were detected in W1, and 18 chemical constituents were identified. The results show that the more components are: 1,4-Dimethyl-

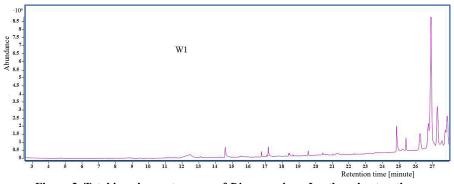


Figure 2. Total ion chromatograms of Diospyros lotus L. ethanol extractives

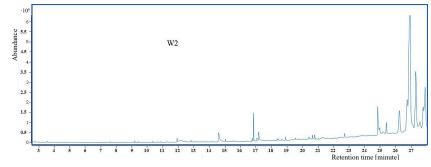


Figure 3. Total ion chromatograms of *Diospyros lotus* L. ethanol/benzene extractives

8-isopropylidenetricyclo[5.3.0.0(4,10)]decane (45.31%), .beta.-Amyrin (17.89%), Lup-20(29)en-3-one (8.56%), Androst-1-en-3-one, 4,4-dimethyl-, (5.alpha.)-(4.87%), 9-Octadecenamide, (Z)-(4.66%), Ribitol (4.42%), .beta.-Amyrone (3.72%), (E)-4-(3-Hydroxyprop-1-en-1-yl)-2methoxyphenol (2.43%), Squalene (1.61%), trans-Sinapyl alcohol (1.58%), Lupeol (1.54%), .alpha.-Tocopheryl acetate (0.80%), (2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one (0.77%), Butyl citrate (0.41%), Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1R-(1.alpha.,2.alpha.,3.beta.,6. alpha.)]-(0.39%), 1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-(0.27%), n-Hexadecanoic acid (0.54%), 9,12-Octadecadienoic acid (Z,Z)-(0.24%).

According to the results of GC-MS analysis, 20 peaks were detected in W2, and 18 chemical constituents were identified. The results show that the more components are: 1,4-Dimethyl-8-isopropylidenetricyclo[5.3.0.0(4,10)]decane (48.42%), .beta.-Amyrin (11.72%), .beta.-Amyrone (9.73%), Lup-20(29)-en-3-one (8.96%), Androst-1-en-3-one, 4,4-dimethyl-, (5.alpha.)-(5.53%), 9-Octadecenamide, (Z)-(3.78%), (2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one (2.33%), (E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol (2.06%), n-Hexadecanoic acid (0.29%), Dibutyl phthalate (1.87%), Ethyl 9-tetradecenoate (0.38%), trans-Sinapyl alcohol (1.39%), trans-Geranylgeraniol (1.17%), .alpha.-Tocopheryl acetate (0.67%), Lupeol (0.65%), Ribitol (0.38%), Butyl 9-tetradecenoate (0.34%).

The results showed that eighteen kinds of components were identified from the W1 and W2 samples. Obviously, the representative compound are 1,4-Dimethyl-8-isopropylidenetr icyclo[5.3.0.0(4,10)]decane, .beta.-Amyrin, Lup-20(29)-en-3-one, Androst-1-en-3-one, 4,4-dimethyl-, (5.alpha.)-, 9-Octadecenamide, (Z)- and .beta.-Amyrone. The representative compound contents of W1 and W2 is 85.7% and 88.14% of the total content, respectively. The identified compounds can be classified into alkanes, phenols, acids, alcohols, ketones, squalene, and so on. The extracts contain the Lup-20(29)-en-3-one, trans-Geranylgeraniol and Lupeol. They are the active ingredient of tumor suppression, prevention, which can reduce risk of cancer, especially breast and prostate cancer. 9,12-Octadecadienoic acid (Z, Z)- has the function of lowering cholesterol and lipid in human blood, which is mainly used for atherosclerosis, coronary heart disease, cerebrovascular disease. Squalene is the active ingredient antibacterial anti-inflammatory and has the effect on hypertension hepatocirrhosis, diabetes, brochitis and other diseases. D1-.alpha.-Tocopherol can eliminate the deposition of lipofuscin in cells, improve normal function of cells, slow down the senescence process of cells, promote the synthesis of protein renewal and so on [32-35]. Ribitol is an important intermediate in the synthesis of

antihepatitis drug Silybin. The other compounds can be used in the industry of food, chemical product, medicine, textile, and the like.

Analysis of MTT

The MTT method is a simple, rapid, economical, sensitive and accurate method, which is among the most widely used screening methods to evaluate cell viability and proliferation. It requires little material for detection and quantification of antioxidant activity in plant extracts. It is a good screening approach of plant extracts for antioxidant capacity before a detailed study of antioxidant mechanisms is conducted [33-48].

As the fig. 4 showed, the W1 has a significant anticancer effect on HEGP2 and SGC-7091. The mortality was 40.98% and 25.16%, respectively. The special effect of the extract on HEGP2 cells was the most obvious. Some ingredients in the wood of *Diospyros lotus* L. can effectively prevent and cure cancer, which can be used for medical industry.

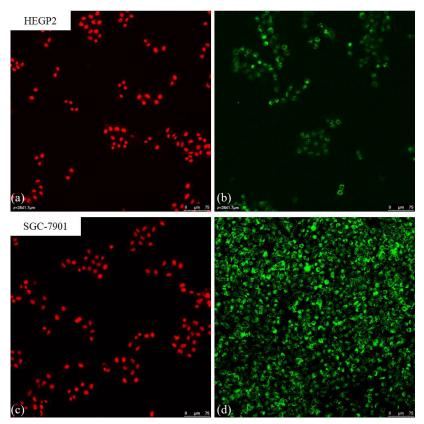


Figure 4. The MTT anti-cancer effect of W1 sample; (a) and (b) were the dead nuclear and live nuclear of HEGP2, respectively, (c) and (d) were the dead nuclear and live nuclear of SGC-7091, respectively

Conclusion

Based on the previous research, the extracts of the *Diospyros lotus* L. wood with ethanol and ethanol/benzene, respectively, have similar infrared spectra. The absorption peaks of the extracts are mainly concentrated in the wave segments of 3700-3000 cm⁻¹, 3000-2800 cm⁻¹ and 1690-970 cm⁻¹. The main chemical components are phenols, alcohols, ethers, fatty acids, Hydrocarbons and aromatic compounds. A total of 20 peaks were isolated and 18 compounds were identified, respectively, by GC-MS gas chromatographic analysis of W1 and W2. The MTT test results showed that the extracts ingredients have can effectively prevent and cure cancer.

It can be seen that *Diospyros lotus* L. has antibacterial, antitumor, anti-inflammatory, and antioxidant, which provides scientific basis for the comprehensive utilization of high-grade resources for applications such as medicine, cosmetics, skin care products, and so on. Energy use provides a good foundation.

Acknowledgment

This research was supported by the Planned Science and Technology Project of Hunan Province, China (No. 2016SK2089; No. 2016RS2011), Major scientific and technological achievements transformation projects of strategic emerging industries in Hunan Province (2016GK4045), Academician reserve personnel training plan of lift engineering technical personnel of Hunan Science and Technology Association (2017TJ-Y10).

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