CHEMICAL CONSTITUENTS AND MEDICAL FUNCTION OF LEAVES OF *DIOSPYROS LOTUS* L.

by

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The potential use of leaves of Diospyros lotus L. was analyzed by using FT-IR, GC-MS and MTT through organic solvent extracts. The results showed that the leaves extracts of Diospyros lotus L. contains many kinds of bioactive organic ingredients with antibacterial, antitumor, and anti-inflammatory activities. Meanwhile the extracts have significant effect on HEGP2, SGC-7091, and K562. Therefore, it provides a scientific foundation for comprehensive application prospects in the fields of biomedicine, food, skin care products and other fields and turning waste into treasure.

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

Introduction

Plants are the richest sources which included bioactive compounds. All kinds of residual product of plants have different biological activities and play a very important role for maintaining human health and treatment of disease [1-5]. So many plants were used as traditional Chinese Medicine and have many unexpected effects that western medicine cannot achieved [6-8]. It is well known that *Diospyros lotus* L. is one of the plants with specific selectivity [9]. *Diospyros lotus* L. is a species of tree in the genus *Diospyros* and the family *Ebenaceae* and widely distributed in China. It has been cultivated in several countries for its edible fruits and used as traditional medicines for various medicinal purposes [10-17]. *Diospyros lotus* L. has more economic importance with yielding edible fruits and valuable timbers. Its timber was still famous and precious for furniture and wood products. Its leaves are approved as Chinese Medicine for the treatment of stroke and apoplexy syndrome in China or used as a hypotensive drug in Japan [18]. Furthermore, it has been used as herb tea and the treatment of hypertension in patients for long time in Korea [19-21]. However, many researches focused on the fruits and timer, little studies have been done for comprehensive use of the persimmon leaves [22-24]. The objective of the present study was to investigate the constituents of the leaves extracts of *Diospyros lotus* L. by FT-IR, GC-MS, and MTT analysis. Then biological activities ingredients

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were identified and prospect of resource utilization was prospected [25-27]. It is helpful for turning leaves waste into treasure.

Material and methods

Experimental materials

Samples were collected from Xixia forest area in Henan Province. Samples were processed from fresh material into powder and oven-dried to absolute dryness at 60 °C. Three kinds of extracts were named as L1, L2 and L3 samples which were extracted by ethanol, ethanol/benzene (1:2) and methanol respectively [28, 29].

Experimental methods

The FT-IR analysis

The FT-IR spectrum of the sample was obtained on a FT-IR spectrophotometer (IR100) using a KBr disk containing 1.00% finely ground samples. The scanning of each extracts was collected at a spectral resolution of 4 cm^{-1} and the spectral range was 500-4000 cm^{-1} [30].

The GC-MS analysis

The three extracts were analyzed by using a gas chromatography-mass spectrometer (Agilent GC-MS 7890B 5977A). 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 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

Cells are treated with an isopropanol-10% Triton X-100 solution, collected the solubilized formazan and transferred triplicate aliquots to a 96-well standard plate (NUNC). Formazan production was measured by spectrophotometry using the SOFTmax Pro microplate reader (Molecular Devices) equipped with the SOFTmax Pro software package (v.4.7). Absorbance was collected at $\lambda = 570$ nm, subtracting the background measured at $\lambda = 690$ nm [31].

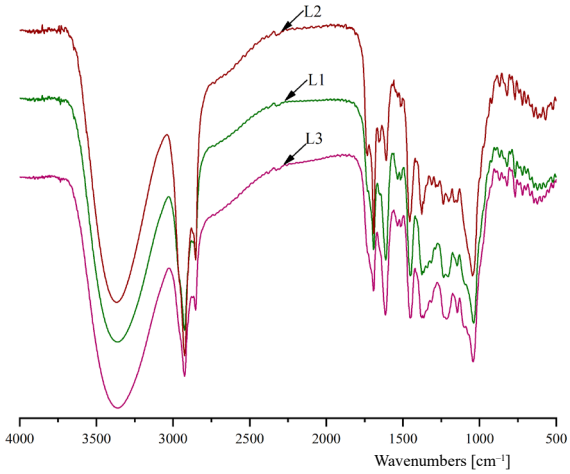

Figure 1. The FT-IR spectra of samples L1, L2, and L3

Results and discussion

Analysis of FT-IR

As the fig. 1 showed that the infrared spectra of three kinds of extracts of *Diospyros lotus* L. was similar. The O-H stretching vibration are at 3361 cm^{-1}, 3366 cm^{-1} and 3357 cm^{-1}. The C-H and -CH_2- stretching vibration are at 2925 cm^{-1} and 2852 cm^{-1}, respectively. Conjugated C=O stretching at 1692 cm^{-1} and aromatic CH stretching at 1650-1430 cm^{-1}. 1613 cm^{-1} and 1515

cm^{-1} is the aromatic carbon skeleton vibration. The absorption peaks of leaves extracts of the *Diospyros lotus* L. 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 alcohols, acids, phenols and aromatic compounds [32-36]. The three absorption peaks have slight difference in infrared, which indicated that the main chemical components are basically identical.

Analysis of GC-MS

The total ion chromatograms of three kinds of extractives of ethanol, ethanol/benzene and methanol were shown in figs. 2-4, which were analyzed by GC-MS.

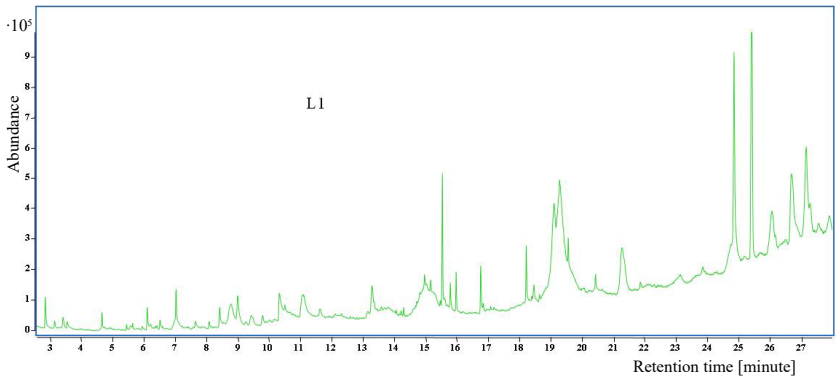

Figure 2. Total ion chromatograms of L1

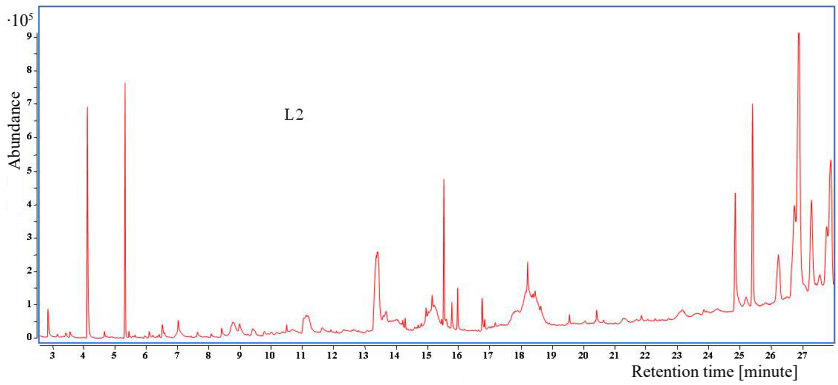

Figure 3. Total ion chromatograms of L2

According to the results of GC-MS analysis, 60 peaks were detected in L1, and 34 chemical constituents were identified. The components are: Glutinol(20.5%), 9-Octadecenamide, (Z)- (10.14%), Squalene (7.71%), 2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-, [3R-(3.alpha.,5a.alpha.,9.alpha.,9a.alpha.)]- (7.53%), Melezitose (7.04%), Lupeol (6.72%), .gamma.-Sitosterol(6.42%), Vitamin E(6.22%), 3-n-Butylthiolane (3.96%), 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R-[R*,R*-(E)]]- (3.64%), Lactose (2.97%), Ethylene glycol butyl ether, trimethylsilyl ether (2.14%), .beta.-Amyrin (1.94%), 2-Butyloxycarbonyloxy-1,1,10-trimethyl-6,9-epidioxydecalin (1.58%), 1,2,3-Benzenetriol (1.53%), 2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol (1.43%), n-Hexadecanoic acid (1.14%), (2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-

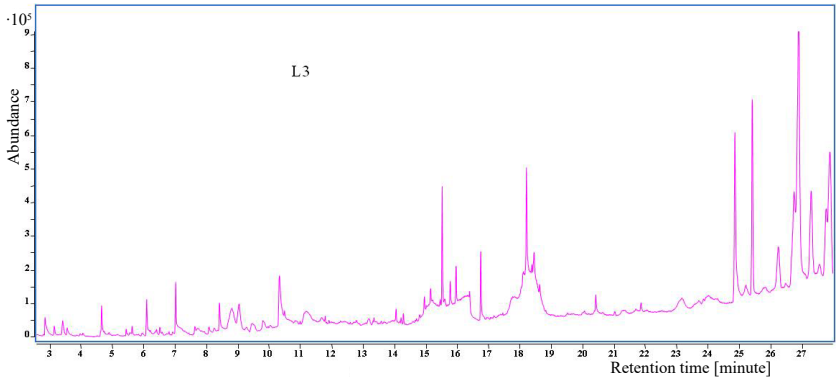

Figure 4. Total ion chromatograms of L3

3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one (0.94%), N,N-Dimethyl-O-(1-methyl-butyl)-hydroxylamine (0.91%), Decanoic acid, 3-hydroxy-, methyl ester (0.68%), Thymine (0.66%), 9,12-Octadecadienoic acid (Z,Z)- (0.64%), Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1R-(1.alpha.,2.alpha.,3.beta.,6.alpha.)]- (0.63%), Bi-1-cyclohexen-1-yl, 3,3,3',3',5,5,5',5'-octamethyl- (0.47%), Undec-10-ynoic acid, propyl ester (0.45%), 2,4-Dimethyl-5-methylthiopent-4-en-2-ol (0.41%), 5-Pregnene, 3-acetoxy-20-[(N-acetyl-4-methylpyrrolidin-2-yl)carbonyl]- (0.29%), p-Toluidine, N-methyl-N-nitroso- (0.24%), Uridine, 5-methoxy- (0.23%), Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl- (0.22%), 2-Hexenoic acid, 5-hydroxy-3,4,4-trimethyl-, (E)- (0.17%), 9-Decenoic acid (0.17%), Silane, 1,3-heptadiynyltrimethyl- (0.14%), .beta.-D-Glucopyranose, 1-thio-,1-[N-hydroxy-5-(methylthio)pentanimidate] (0.13%).

According to the results of GC-MS analysis, 45 peaks were detected in L2, and 32 chemical constituents were identified. The components are: 1,4-Dimethyl-8-isopropylidenetri cyclo[5.3.0.0(4,10)]decane (19.17%), .beta.-Amyrin (10.64%), Lupeol (8.88%), .beta.-D-Glucopyranoside, methyl (8.42%), Lup-20(29)-en-3-one (7.88%), Squalene (5.58%), Lactose (5.12%), Triethylamine (4.54%), 9-Octadecenamide, (Z)- (4.21%), Heptane, 1,1'-oxybis- (3.61%), Androst-1-en-3-one, 4,4-dimethyl-, (5.alpha.)- (3.05%), .beta.-Amyrone (2.98%), .alpha.-d-6,3-Furanose, methyl-.beta.-d-glucohexodialdo-1,4-furanoside (2.68%), Neophytadiene (2.14%), (2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one (1.53%), Ethylene glycol butyl ether, trimethylsilyl ether (1.43%), .beta.-D-Glucopyranose, 1-thio-,1-[N-hydroxy-5-(methylthio)pentanimidate] (0.96%), 2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-, [3R-(3.alpha.,5a.alpha.,9.alpha.,9a.alpha.)]- (0.85%), 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R-[R*,R*-(E)]]- (0.72%), N,N-Dimethyl-O-(1-methyl-butyl)-hydroxylamine (0.71%), n-Hexadecanoic acid (0.59%), .alpha.-Amyrin (0.56%), Melezitose (0.53%), Vitamin E (0.48%), d-Glycero-l-gluco-heptose (0.48%), Galactoseptanoside, methyl 2,3,4,5-tetra-O-methyl-, .alpha.-D- (0.46%), Inositol, 1-deoxy- (0.41%), 17-Octadecynoic acid (0.39%), Decanoic acid, 3-hydroxy-, methyl ester (0.31%), Silane, 1,3-heptadiynyltrimethyl- (0.28%), 2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol (0.22%), Mannosamine (0.20%).

According to the results of GC-MS analysis, 44 peaks were detected in L3, and 29 chemical constituents were identified. The components are: 1H-3a,7-Methanoazulen-6-ol, octahydro-3,6,8,8-tetramethyl-, acetate, [3R-(3.alpha.,3a.beta.,6.alpha.,7.beta.,8a.alpha.)]- (20.63%), .beta.-Amyrin (12.09%), Lup-20(29)-en-3-one (8.77%), 13-Docosenamide, (Z)- (6.72%), Squalene (5.84%), (2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-

2-yl)octahydronaphthalen-1(2H)-one (5.78%), Lupeol (4.15%), 3-n-Butylthiolane (4.14%), Melezitose (3.66%), .beta.-Amyrone (3.61%), D-Galactose (3.30%), 1,2,3-Benzenetriol (3.15%), Farnesyl bromide (2.96%), Lactose (2.52%), 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R-[R*,R*-(E)]]- (2.11%), n-Hexadecanoic acid (1.54%), Decanoic acid, 3-hydroxy-, methyl ester (1.25%), 2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyl] cyclohexan-1-perhydrol (1.19%), D-chiro-Inositol, 3-O-(2-amino-4-((carboxyiminomethyl) amino)-2,3,4,6-tetradeoxy-.alpha.-D-arabino-hexopyranosyl)- (1.15%), Thymine (1.04%), .alpha.-Amyrin (0.96%), 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (0.78%), N,N-Dimethyl-O-(1-methyl-butyl)-hydroxylamine (0.76%), Epi-Inositol (0.57%), Thiophene, tetrahydro-2-methyl- (0.55%), d-Glucosamine (0.30%), d-Glycero-l-gluco-heptose (0.28%), Maltose (0.23%), [1,1'-Bicyclohexyl]-2-one (0.23%).

The identified compounds can be classified into alcohols, acids, phenols, squalene, and so on. The extracts contain the Lup-20(29)-en-3-one and Lupeol, which are the active ingredient of tumor suppression and prevention and can reduced risk of cancer, especially breast and prostate cancer [37-39]. The .beta.-Amyrin can inhibit the synthesis of cholesterol and triglycerides and prevent effectively obesity, atherosclerosis and type 2 diabetes. Squalene is the active ingredient antibacterial anti-inflammatory and has the effect on hypertension hepatocirrhosis, diabetes, brochitis and other diseases. Meanwhile their content was higher in the three extracts. Lactose is widely used in making baby food, candy and other food industries. The n-Hexadecanoic acid is important raw material of soap, candles, lubricants, softeners and synthetic detergents. Triethylamine is a key intermediate in the synthesis of AZT, DDT and related drugs, which was extracted only by ethanol/benzene. Glutinol is a compound with known anti-inflammatory activity which was only extracted by ethanol and higher content. Vitamin E is used for the prevention of habitual abortion and threatened abortion caused by vitamin E deficiency as well as for protecting cells and tissues from lipoperoxidative damage induced by free radicals [40]. The other compounds can be used in the industry of food, chemical product, medicine, and the like. The results also showed that the representative compounds contents with more than 3% accounted for more 80% of the total extracted components respectively. Meanwhile it was found that 11 components of the three extracts were the same, and all of them were more than one third of the total extract content by comparison.

Analysis of MTT

Sample L1 (extracted by ethanol) was selected for MTT experiment [41-46]. The results showed that the L1 has a strong anticancer effect on HEGP2, SGC-7091, and K562. The killing rate was 44.97%, 40.47%, and 28.29% respectively. The antitumor effect on HEGP2 cells was the most obvious. Some ingredients in the leaves of *Diospyros lotus* L. can effectively prevent and cure cancer, which can be used for Chinese medical industry.

Conclusion

The main chemical constituents of the leaves extracts of the *Diospyros lotus* L. are alcohols, acids, phenols, ethers and aromatic compounds. The biological activities ingredients have antibacterial, anti-inflammatory, antitumor and antioxidant, which can be used in medicine, food, skin care products. Furthermore, the leaves extracts could be used as Chinese traditional medicine directly.

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