ACTIVE CONSTITUENTS OF *KOELREUTERIA PANICULATA* ROOT

by

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Original scientific paper
https://doi.org/10.2298/TSCI190517046W

In this paper, Koelreuteria paniculata roots was taken as the research object, and its active substances were analyzed. First, Koelreuteria paniculata roots was crushed, dried, extracted, filtered, and concentrated by rotary evaporation. Then, the active substances of Koelreuteria paniculata roots were extracted with ethanol, benzene/ethanol, and methanol. Finally, Koelreuter was detected by GC-MS, FT-IR, TD-GC-MS, and TG analyses. Data analysis revealed that Koelreuteria paniculata roots contains oleic acid, linoleic acid, Lupinol, retinal, and other active substances that are of great value to medical, chemical, and food industries.

Key words: *Koelreuteria paniculata, GC-MS, linoleic acid, FT-IR analysis, TG analysis*

Introduction

Koelreuteria paniculata is a plant of the genus *Koelreuteria Paniculata*, which belongs to the family *Sarcandraceae*. *Koelreuteria paniculata* is a deciduous tree or shrub with bark that is thick and grayish-brown to grayish-black, leaves that are opposite and oval; nuts that grow on branches in cymes, and fruits that have oval-shaped petals with acuminate apexes and reticulated exteriors [1].

Koelreuteria paniculata is light-loving, cold-tolerant, and drought-tolerant. It has the characteristics of easy cultivation, few pests and diseases, and strong adaptability. It grows in limestone-weathered calcium-based soils. In China, *Koelreuteria paniculata* sprouts late in spring, falls early in autumn, and grows slowly in the lower reaches of the Yellow River and Yangtze River basins. It is often used in urban greening and courtyard ornamentation. Its wood can only be used to make small utensils, and its seeds can be used to extract industrial oil [2-4].

Experiment mode

Experimental preparation

Sample: *Koelreuteria paniculata* was collected from Luanchuan County, Luoyang City (111.6E, 33.8N).

Reagents: C_2H_6O, H_2O, C_6H_6, and CH_3OH are all chromatographically pure.

Main instruments

Our main instruments included a plant crusher (model: FW-400), GC-MS (model: Agilent 7890B-5977A), Fourier transform infrared spectroscopy, an electronic constant tem-

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perature water bath pot (model: dzkw-4), and a circulating water multi-purpose vacuum pump (model: SHZ-D).

Test method

Koelreuteria paniculata roots were separated from the trunk and their bark was removed. The roots were cut into 3-5 cm lumps, then crushed with a crusher. The freshly crushed samples were divided into three parts averaging 10 g each then dried in a constant temperature box until they had a constant weight.

Three fresh samples were put into three flasks. The 300 ml each of ethanol, benzene/ethanol (volume ratio 1:1), and methanol were added. Components of the *Koelreuteria paniculata* roots were extracted in constant temperature water bath pots at 80 °C, 68 °C, and 64 °C.

The extracts achieved by the three solvents above were filtered, steam-rotated, and concentrated to 20 ml. In the process of experiment, to keep the environment dry and pollution-free, the instrument should be cleaned and cleaned before and after each step.

The GC-MS detection method

GC: Chromatographic column HP-5 MS. A small amount of samples were placed in the middle of the quartz capillary column, and high purity helium was used as carrier. The separation ratio was 50:1 and the flow rate was 1 ml/min. The detection temperature of gas chromatography starts to rise from 50-280 °C.

MS: Ionization voltage 70 ev, ionization current 150 muA, electron ionization (EI), program scanning mass range 30 amu-600 amu, the ion source temperature is set to 230°C and the quadrupole temperature is set to 150 °C [5-7].

The FT-IR analysis

A small amount of potassium bromide was ground in a KBR disc then tableted [5-7]. The extract sample was dripped onto the tablet to ensure full absorption. The FT-IR spectrum of the sample was obtained via FT-IR spectrophotometer (Thermo Fisher Scientific IS10) [8-11].

The TD-GC-MS method

The initial temperature is 30 °C (retained for 1 minute), then rises to 100 °C (retained for 5 minutes) at a rate of 10 °C/min, then rises to 200 °C (not retained) at a rate of 10 °C/min. The transmission line temperature is 230 °C [12]. The initial temperature is –30 °C (1 min), then rises to 230 °C (1 minute) at the rate of 10 °C (1 minute). The GC-MS Instrument: Agilent Gas Chromatography-Mass Spectrometry (GC7890B/MS5977B).

- GC parameters:
  - splitless
  - chromatographic column HP-5 MS
- Column temperature program:
  - starting at 30 °C, without retention, then rising to 100 °C at 10 °C/min rate, without retention, then rising to 250 °C at 8 °C/min rate, without retention, and then rising to 280 °C at 5 °C/min for 2 min [13, 14].
- MS parameters:
  - Ion source temperature: 230 °C
  - Four-stage bar temperature: 150 °C
  - Scanning starting and ending points: 30-600
  - Standard mass spectrometry library retrieved by analytical software: NIST17.L [14, 15]

The TG analysis

Koelreuteria paniculata root samples after freeze-drying were analyzed by thermogravimetric analysis. The equilibrium gas used in the experiment is nitrogen at a rate of 100 ml/min. The experimental samples were heated at three rates of 10 °C/min, 25 °C/min and 50 °C/min, from 30 °C to 850 °C and kept for 5 minutes [16-18].

Analysis of experimental data

The GC-MS result analysis

The GC-MS analysis revealed, fig. 1, that the total ionogram of the ethanol extract from *Koelreuteria paniculata* roots had 49 peaks and contained 33 active substances. The main components of the ethanol extract from *Koelreuteria paniculata* roots were Ribitol (18.22%), and gamma-Sitosterol Ribitol (5.37%).

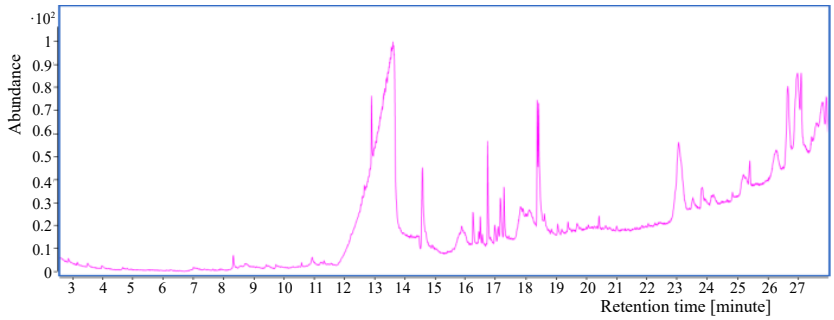

Figure 1. Total ion flow diagram of the ethanol extract from *Koelreuteria paniculata* root

The GC-MS analysis, fig. 2, revealed that the total ionogram of the benzene/ethanol extract from *Koelreuteria paniculata* roots had 46 peaks and contained 29 active substances. The main components of the benzene/ethanol extract from *Koelreuteria paniculata* roots were Stigmast-4-en-3-one (15.86%), 9,12-Octadecanoic acid (Z, Z) -(13.54%), Hexadecanoic acid (4.72%), Lupeo. L (3.96%) and Lactose (1.23%).

The GC-MS analysis, fig. 3, showed that the total ionogram of the methanol extracts from *Koelreuteria paniculata* roots had 28 peaks and contained 23 active substances. The main

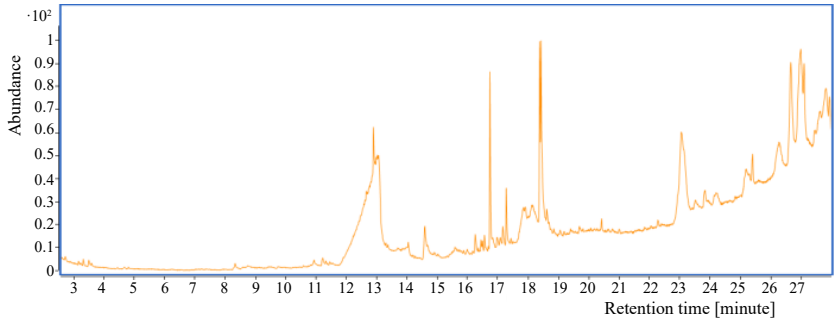

Figure 2. Total ion flow diagram of benzene/ethanol extracts from *Koelreuteria paniculata* root

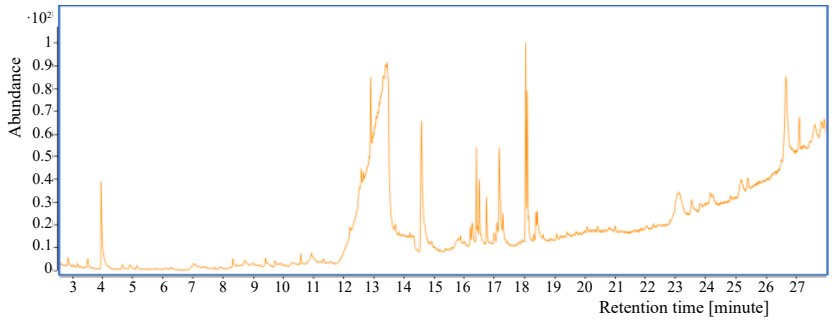

Figure 3. Total ion flow diagram of methanol extract from *Koelreuteria paniculata* root

components of the methanol extract from *Koelreuteria paniculata* roots were Ribitol (59.35%), gamma. -Sitosterol (5.47%), Methyl 9-cis, 11-trans-octadecadienoate (2.99%), and 6-pentylpiperidin-2-one (2.58%).

Under the GC-MS experimental scheme, the extracts of *Koelreuteria paniculata* roots from three different solvents contained the following main substances. First, linoleic acid is a kind of unsaturated fatty acid. linoleic acid can reduce blood lipids, blood pressure, and soften blood vessels. It can promote human microcirculation. Linoleic acid can avoid the deposition of serum cholesterol in blood vessel walls and has the reputation of being a *scavenger of blood vessel* [19-26]. Second, Lupinol is a triterpene, which has the functions of antioxidation, anti-inflammation, promoting skin healing in animal experiments, and so on. It can inhibit breast cancer, prostate cancer, and melanoma in mice [21-24]. Third, retinal aldehyde is an important signal transduction molecule in retinal sensory cells. It is widely used in the study of the retina and the dermatosis of experimental myopia [27, 28].

Analysis of FT-IR results

The FT-IR results, fig. 4, showed that there were peaks at wavenumber 3300--3500 cm^{-1}, indicating that the extract of *Koelreuteria paniculata* roots contained -OH bond, which was strong and sharp [8]. According to existing studies, the extract may contain free hydrogen bonds of alcohols and phenols; there were peaks at wavenumber 2850-3000 cm^{-1}, indicating that *Koelreuteria paniculata* roots contained -OH bond. There are C-H bonds in the *Koelreuteria paniculata* roots extract, which have strong absorption intensity and double peaks. According to existing studies, the extract may contain $-CH_2$ alkanes. There are peaks at wavenumbers 1600 cm^{-1}, 1500 cm^{-1}, and 1200 cm^{-1}, indicating that the extract of *Koelreuteria paniculata* roots contains C=C and C-O bonds, and that the peaks are strong and wide. Studies have shown that the extract may contain ketones, acids, and esters [11].

Analysis of the results of TD-GC-MS

The TD-GC-MS results, fig. 5, showed that there were 150 peaks in the *Koelreuteria paniculata* roots thermal desorption ion map, which contained 81 active substances. The main components of *Koelreuteria paniculata* roots were Oleic acid (10.72%), Acetic acid (7.85%), 2-Hydroxy-2-methylhept-6-en-3-one (4.72%), Tiglic acid (4.40%), Dodecanoic acid (3.76%), Farnesol isomer a (3.10%), Phthalic acid, butyl undecyl ester (2.57%), and Undec-10-ynoic acid, decyl ester (2.60%).

Koelreuteria paniculata roots contains the following important substances: First, oleic acid is an unsaturated fatty acid, which is the fatty acid that makes up the essential oil.

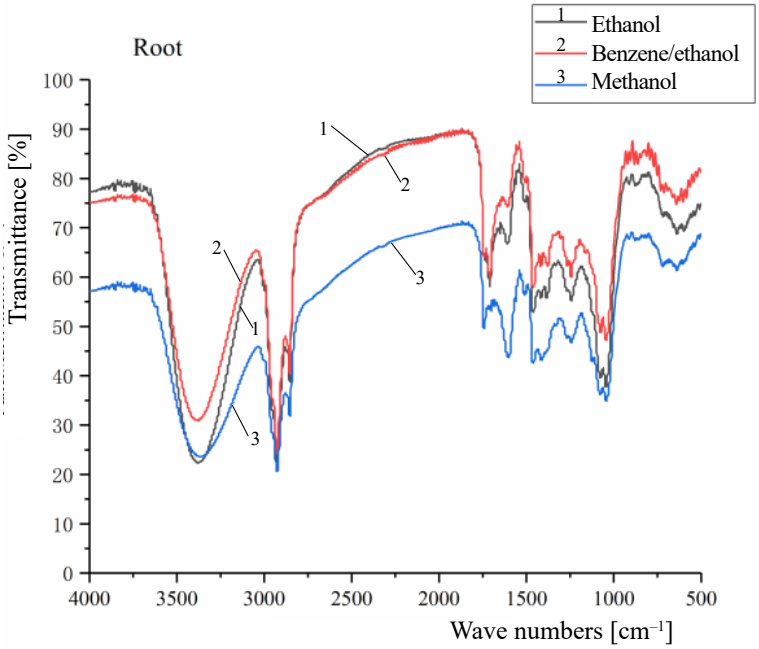

Figure. 4 Infrared spectra of three reagent extracts from *Koelreuteria paniculata* root

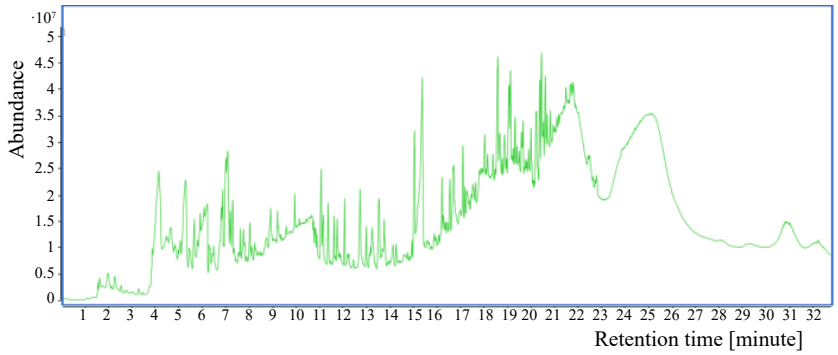

Figure. 5 *Koelreuteria paniculata* root thermal desorption ion diagram

Oleic acid has cis-trans isomers. Natural oleic acid is cis-structure (trans-structure cannot be absorbed by the human body), which has certain effects on softening blood vessels. Eating edible oil with high oleic acid content is beneficial to human health. Second, glycerol has weak acidity and can interact with alkaline hydroxides. Glycerol can lubricate and stimulate the intestinal wall, soften stool, and help with dehydration for general brain edema rescue, chapped skin, and peeling in spring and winter. Glycerol is also widely used in textiles, food, paper making, metal processing, paint, daily chemicals, medicine, tobacco, and many other fields [27-30].

Analysis of TG results

The TG results, fig. 6, showed that *Koelreuteria paniculata* roots has undergone three stages of weightlessness, and the three heating modes are basically the same.

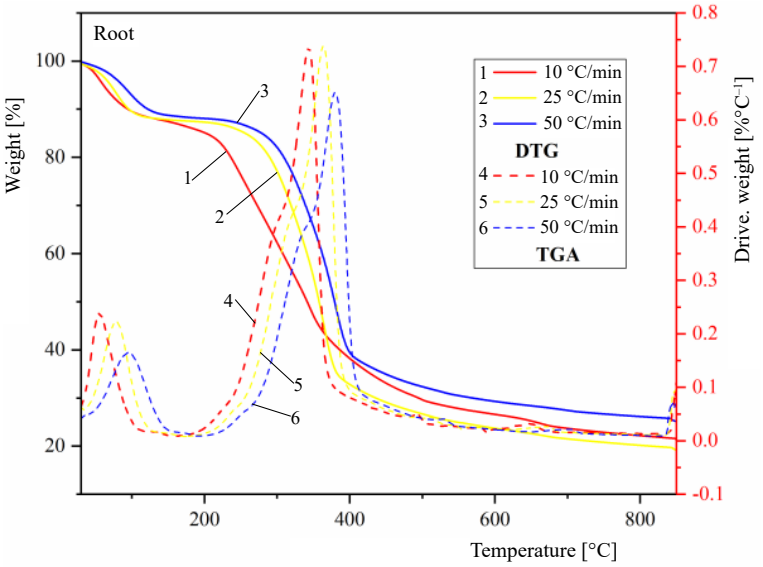

Figure. 6 Thermogravimetric analysis of *Koelreuteria paniculata* roots at 10, 25, and 50 °C/min

First is the dehydration stage, mainly consisting of the loss of bound water and free water. The weight-loss rate is about 15%, which is completed at 100-200 °C. Second is the thermal weight loss stage, mainly the decomposition of lignin, hemicellulose and cellulose. This is the main stage of pyrolysis. The weight-loss rate is about 40%, and the mass decreases sharply from 200 °C to 500 °C. Third is the carbonization stage, in which the weight-loss rate is about 10%, and the temperature range is 500-700 °C. After the third stage of the sample, the sample quality basically does not change.

At three different heating rates, the weight-loss rate of the samples reached two peaks. The first peak ranges from 50-100°C, and the second peak ranges from 300-400 °C. When the sample is no longer weightless, what remains is about 25% of the sample before weightlessness.

Conclusions

Analysis of the above results reveals that *Koelreuteria paniculata* roots contains a large number of compounds, and important compounds can be detected in different schemes.

Under the GC-MS experimental scheme, *Koelreuteria paniculata* roots contained several main substances in three different solvents. First, Linoleic acid can promote human microcirculation, reduceing to blood lipid and blood pressure. Second, Lupinol has anti-oxidation, anti-inflammatory, and skin healing effects in animal experiments, and it has certain inhibitory effects on breast cancer, prostate cancer, and melanoma in mice. Third, Flavoxaldehyde is an important signal transduction molecule in the development of the eyeball. It is widely used in the study of the retina and the dermatosis of experimental myopia.

The FT-IR results showed that the samples had peaks at wavenumbers 3300-3500 cm^{-1}, 2850-3000 cm^{-1} and 1200-1600 cm^{-1}, which indicated that the extract of *Koelreuteria paniculata* roots contained C-O bond, C-H bond, -OH bond and C=C bond with strong and sharp peaks. According to existing studies, the extracts may contain free hydrogen bonds of alcohols and phenols, $-CH_2$, alkanes, ketones, acids, and esters.

The TD-GC-MS results showed that *Koelreuteria paniculata* roots contained the following important substances: First, oleic acid is the fatty acid that makes up the essential oil, and it has certain effects on softening blood vessels. Second, glycerol can lubricate and stimulate the intestinal wall, soften stool, and help with dehydration, which is used for general brain edema rescue, chapped skin, and peeling in spring and winter. Glycerol is also widely used in textiles, food, paper making, metal processing, paint, daily chemicals, medicine, tobacco, and many other fields.

The TG results showed that the weightlessness of the sample was divided into three stages, and that the weightlessness rate had two extremes. The weightlessness of the sample after weightlessness was about 25% of that of the sample before weightlessness.

Koelreuteria paniculata roots contains a large number of compounds that can be used to help human beings. They can be used widely in medical, chemical, food, and other industries, so that *Koelreuteria paniculata* can give rise to economic benefits.

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Paper submitted: May 17, 2019
Paper revised: August 24, 2019
Paper accepted: September 5, 2019