ACTIVE COMPONENTS IN LEAVES OF *RHUS CHINENSIS MILL*

by

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Rhus chinensis Mill is a high-quality eco-economic resource for potential sustainable development. To analyze the chemical constituents of extracts from the leaves of Rhus chinensis Mill for resource values, the following analytical methods were performed: Fourier transform infrared (FT-IR) spectrum, gas chromatography-mass spectrometry (GC-MS), thermogravimetry, and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). The results showed that the leaves of Rhus chinensis Mill were rich in volatile substances that could be exploited and used.

Key words: *Rhus chinensis Mill, FT-IR spectrum, GC-MS, thermogravimetric analysis, Py-GC-MS*

Introduction

Rhus chinensis Mill. is a tree species in China that can be used as raw material for pharmaceutical and industrial dyes. Because of its light-loving properties, strong adaptability to climate and soil, fast growth, drought resistance, and strong tillering ability (production of multiple stems), it is an important tree species for afforestation and landscape greening. It is also a useful plant for the recovery of waste land (such as cinder dump of burning lime). Its tender stems and leaves can be eaten as wild vegetable and feed for pigs in mountainous areas. Its flowers are the source of high-quality honey and powder in early autumn. *Rhus chinensis Mill.* is a Chinese traditional medicine used to treat different types of solid tumors [1-4].

The purpose of our research is to explore the active ingredients of *Rhus chinensis Mill.*, in order to improve the current utilization rate and to determine further applications.

Methodology

Test materials

Rhus chinensis Mill was provided by Luanchuan Forest Farm, in Luanchuan County, Henan Province. The following reagents were all of chromatographic purity: ethanol, benzene, methanol, ether, and distilled water. The filter paper was qualitative.

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Test methods

Sample preparation

Fresh leaves of *Rhus chinensis Mill* were picked, freeze-dried, crushed, and stored in self-sealed bags for later use. Leaf powder was sifted through 200 meshes and weighed into four parts of 10 g/portion. The leaf powder was extracted with ethanol (80℃), methanol (60 ℃), ether (30 ℃), and benzene/ethanol (1:1, 65 ℃). Each solvent volume was 300 ml. Extraction for 5 hours. The extracts concentrated to 20 ml by rotary evaporator. Then they were each filtered.

The FT-IR analysis

The 1 ml of each extract was dripped into ground KBr powder, which was dried and pressed, and the FT-IR spectra of the extracted samples were obtained with a FT-IR spectrophotometer (Thermo Fisher Scientific iS10) using KBr discs containing a 1.00% finely ground sample [5-9].

The GC-MS analysis

GC: The elastic quartz capillary column HP-5MS measured 30 m × 250 μm × × 0.25 μm). The carrier gas was high purity helium, with a flow rate of 1 mL/min. The split ratio was 50:1. The temperature of the GC began at 50 ℃, rose to 250 ℃ at a rate of 10 ℃/min, and then rose to 280 ℃ at a rate of 5 ℃/min.

MS: The program scan mass range was 30 amu-600 amu, with ionization voltage of 70 eV and ionization current of 150 μA electron ionization (EI). The ion source and the quadrupole temperature were set at 230℃ and 150℃, respectively [10-14].

The Py-GC-MS analysis

The KBr powder was analyzed via thermal cracking-GC-MS (Frontier Lab-Agilent 7890 B-5977 B). The carrier gas was high purity helium, the pyrolysis temperature was 850 ℃, and the pyrolysis time was 30 seconds. The pyrolysis product transfer line and the injection valve temperature were set to 300 ℃. The capillary TR-5MS column measured 30 m × × 0.25 mm × 0.25 μm, with a shunt mode, split ratio of 50:1, shunt rate of 50 mL/min. The temperature of the GC program started at 40 ℃ for 2 minute, increased to 120 ℃ at a rate of 5 ℃/min, and then increased to 200 ℃ at a rate of 10 ℃/min for 2 minute. Ion source (EI) temperature was 230 ℃, with scanning range of 30-600 amu [15, 16].

Thermogravimetric analysis

Samples of different parts of *Meliosma cuneifolia* were subject to thermogravimetric analysis (TGA Q50 V20.8 Build 34). The equilibrium gas was nitrogen, with a release rate of 40 ml/min. The temperature program of TG began at 30 ℃, rose to 850 ℃ at the rate of 10 ℃/min, 20 ℃/min, and 40 ℃/min, respectively, and kept constant temperature at 850 ℃ for 5 minute [15, 17-19].

Results and analysis

The FT-IR analysis

According to the relationship between the infrared spectra of organic compounds and functional groups, the infrared spectra of the leaves of *Rhus chinensis Mill* revealed a wider and stronger absorption peak near 3400 cm^{-1}, which was caused by the O-H of the absorption

peak, indicating possible alcohols or phenols, fig. 1. Two absorption peaks at 3000-2750 cm^{-1}, caused by the saturated C-H bond, appeared near 2960 cm^{-1} and 2876 cm^{-1}, which are caused by the stretching vibration of CH_3- and -CH_2-bonds, respectively. There was a strong absorption peak near 1650 cm^{-1}, which is caused by the C=O bond. Therefore, the existence of carbonyl compounds such as aldehydes, ketones, and carboxylic acids can be indicated. Near 1450 cm^{-1}, there are many overlapping peaks, which are caused by the skeleton vibration of aromatic compounds. Two absorption peaks between 1300 and 1030 cm^{-1} are caused by the C-O-C group. The absorption peaks of the extracts from the leaves of *Rhus chinensis Mill* were mainly concentrated in the bands of 3500-2750 cm^{-1} and 1800-1000 cm^{-1}, fig. 1.

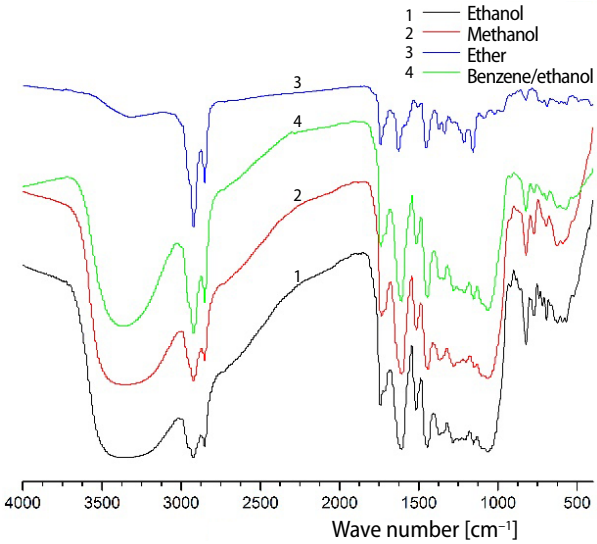

Figure 1. Infrared spectra of extracts of the leaves of *Rhus chinensis Mill*

The GC-MS analysis

The GC-MS revealed 22 peaks for the ethanol extract of the leaves of *Rhus chinensis Mill.*, among which 4H-1-benzopyran-4-one, 2,3-dihydro-5,7-dihydroxy-2-phenyl, (S)-, pinosylvin, 2-propen-1-one, and (E)-1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl were the most abundant, fig. 2. Twenty-three peaks were detected in the methanol extract, of which 4H-1-benzopyran-4-one, 2,3-dihydro-5,7-dihydroxy-2-phenyl-, (S)-, phenol, 3-pentadecyl-, and eucalyptol were more abundant. Thirty-four peaks were detected in the ether extract, of which 13-docosenamide, (Z)-, phenol, 3-pentadecyl- and gamma-sitosterol were the most abundant. Thirty-three peaks were detected in the benzene/ethanol (1:1) extract from the leaves of *Rhus chinensis Mill.*, among which 9-octadecenamide, (Z)-, 1,4-benzenediol, 2,5-bis(1,1-dimethylethyl)- and 2-propen-1-one, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl-, (E)- were more abundant, fig. 2.

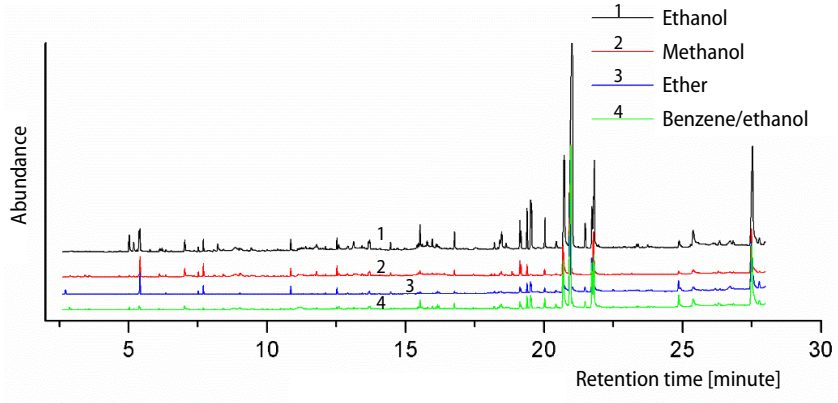

Figure 2. The GC-MS ion spectra of extract of the leaves of *Rhus chinensis Mill*

The GC-MS analysis is able to classify *Rhus chinensis Mill* compounds as alkanes, phenols, alcohols, acids, and esters, which are widely used in high value-added industries such as medicine and chemical industry. For example, palmitoleic acid is used as a lipid hormone to regulate systemic metabolism, by affecting the formation of fat, leading to obesity. Palmitoleic acid can regulate the accumulation of esters in plasma of diseases such as atherosclerosis and fatty liver [20]. In addition, palmitoleic acid, as a monounsaturated fatty acid, has very good resistance to low temperatures and oxidation, and is suitable for the production of high-quality biodiesel [21].

The (Z)9-Octadecenamide can be used as smoothing agent, antistatic agent, demolding agent, dispersant of polyolefin and PVC plastics, additive in printing inks. The acetate of L-.alpha- -terpineol has the fragrance of citron and lavender, and is used for the preparation of essence in soaps. It is also used in medicine, pesticide, plastics, ink, instrument and telecommunication industry. It is an excellent solvent for color on glassware.

The E-4-Methoxy-6-styryl-2H-pyran-2-one has sedative, hypnotic, antifungal, and antithrombotic effects. Eucalyptol is widely used in medicine, and also for the preparation of toothpaste, essence, cough sugar, and artificial mint [22, 23].

The N-Hexadecanoic acid is used as precipitant, chemical reagent, and waterproof agent, as raw materials for the manufacture of other food additives, and for candles, soaps, greases, softeners, and synthetic detergents [24].

The Py-GC-MS analysis

The Py-GC-MS, fig. 3, revealed 159 peaks in the ethanol extract of the leaves of *Rhus chinensis Mill.*, among which cyclopropyl carbinol, 4-penten-1-ol, and 1,3,5-cycloheptatriene were the most abundant.

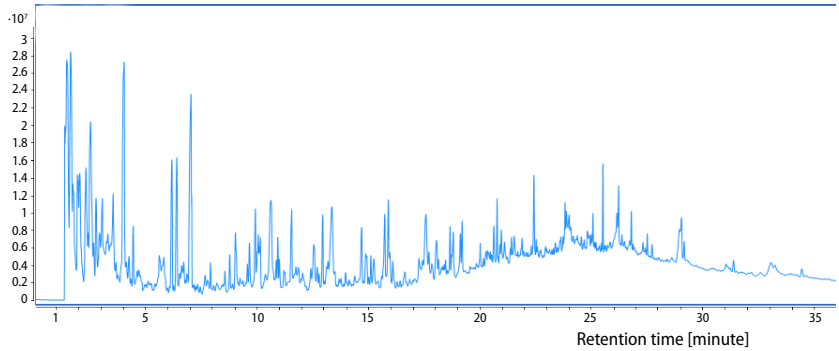

Figure 3. Total ion content of the leaves of *Rhus chinensis Mill.* by Py-GC-MS

These compounds are widely used in high value-added industries such as medicine and the chemical industry. For example, ethylbenzene is mainly used in the petrochemical industry as an intermediate in the production of styrene. It is also used as an intermediate of synmycin in medicine, and as a diluent and organic synthetic solvent for nitro spraying paint [25-27]. When mixed with ethanol and ethyl acetate, it forms a good solvent for cellulose ether. P-cresol is the material for making antioxidants and rubber antioxidants. It is also a significant basic material for the production of medicines and dyes [28]. The 3-Methyl-phenol is mainly used in pesticides, medicine, perfume, resin plasticizer, film, antioxidant, and reagents [29-31]. Cyclopropyl carbinol is an intermediate used in chemical laboratory research and development of organic compounds and pharmaceuticals [32-34].

Thermogravimetry analysis

Thermogravimetric analysis reflects the thermal stability of substances. The weight change of the sample was measured by thermogravimetric analyzer, and the curves of TGA and DTG were obtained.

The thermogravimetric curve can be separated into three stages. The first stage is the initial temperature to 200 ℃. The curve declines rapidly, and the weight loss rate is about 9%. It is the stage of water evaporation of the sample. The second stage is from 200 ℃ to 450 ℃. At this stage, the curve declines rapidly and the sample loses weight sharply, which is the most important stage of weight loss. The weight loss rate is about 55%. In the third stage, the curve from 450 ℃ to 850 ℃ declined smoothly, and the weight loss rate was about 17%. Figure 4 reveals two distinct peaks in DTG curve between 50 ℃ to 150 ℃ and 250 ℃ to 400 ℃. The weight loss rate of bark powder is about 81% [35-40].

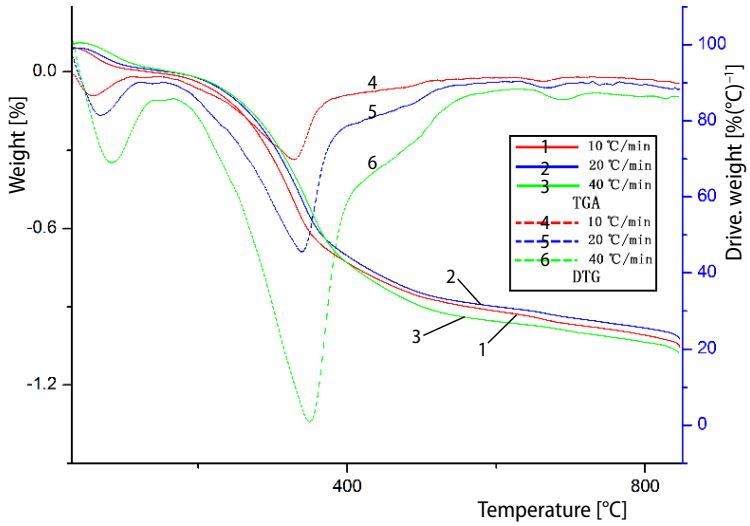

Figure 4. The TG chart of different heating rates of the leaves of *Rhus chinensis Mill*

Conclusions

According to FT-IR analysis, the absorption peaks of the extracts from the leaves of *Rhus chinensis Mill.* were mainly concentrated in the bands of 3500-2750 cm^{-1} and 1800-1000 cm^{-1}. In GC-MS, 22, 23, 34, and 33 compounds were detected from the extracts by ethanol, methanol, ether, and benzene/ethanol (1:1), respectively, from the leaves of *Rhus chinensis Mill.* Py-GC/MS of *Rhus chinensis Mill's* leaves indicated that there are many substances that can be widely used in high value-added industries.

Thermogravimetry analysis revealed a loss of thermogravimetry of *Rhus chinensis Mill's* leaves was about 81%. The DTG curves of *Rhus chinensis Mill's* leaves had two distinct peaks between 50 ℃ and 150 ℃ and 250 ℃ to 400 ℃, indicating that leaves of *Rhus chinensis Mill.* are rich in volatile substances, which can be widely used in medicine, industry, and food.

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