Bioactive and bioenergy ingredients of Rodgersia aesculifolia grown at high altitude

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Rodgersia aesculifolia is a perennial herb widely-distributed in China, and has been used as herbal medicine with long history. Ethanol and diethyl ether extraction were used to explore the bioactive and bioenergy ingredients in leaves and stems fresh Rodgersia aesculifolia and Rodgersia aesculifolia grown at high altitudes in China. The results showed that the Rodgersia aesculifolia grown at high altitude has not only great biomedical value, but also been used as bioenergy materials. In addition, quinic acid and butylated hydroxytoluene were firstly discovered in leaves and stems of fresh Rodgersia aesculifolia grown at high altitudes. As biologically active ingredients, their relative contents are relatively high. All these results have provided a theoretical basis for further utilization of bioactive and bioenergy components from Rodgersia aesculifolia grown at high altitudes.

Key words: Rodgersia aesculifolia, waste utilization, bioenergy, biogasoline

Introduction

Rodgersia aesculifolia is a species of perennial herb in Rodgersia (Saxifragaceae). As a traditional Chinese folk herb, its rhizome is often used as medicinal material [1, 2]. It has the effect of promoting blood circulation and regulating rheumatism, it also has a good effect on the treatment of bruises, irregular menstruation and rheumatoid arthritis. What’s more, it has a significant effect on the treatment of symptoms such as diarrhea, vomiting blood, in the stool and sore.

In recent years, there are a number of literatures which focus on the bacteriostatic and pharmacological activities of Rodgersia aesculifolia. For example, Wang Jian et al. have found that the volatile oil of Rodgersia aesculifolia rhizomes have strong antibacterial activity [3]. The research by Li Tao et al. revealed the change of chemical composition of different solvent extracts of fresh Rodgersia aesculifolia rhizome [4]. However, there is no specific literature of in-depth research on the diversified use of resources in Rodgersia aesculifolia growing at high altitude.

Our research focused on the resources in Rodgersia aesculifolia at high altitude. Ethanol and diethyl ether were used to extract bioactive ingredient in fresh Rodgersia aesculifolia leaves (RALs) and Rodgersia aesculifolia stems (RASs). We hope to find more resources which can be utilized.

Materials and Methods

Materials and reagents

Rodgersia aesculifolia is sampled from the Xiao Qinling Mountains at the altitude of 2,400 meters. Fresh RALs and RASs were separated from the samples. The RALs and RASs were solidified instantly by liquid nitrogen, and then ground into powder in mortar. Then they were placed in the extraction solution at a certain ratio for mixing and extraction.

Methods

Extraction by two solvents

15 g of powdered sample were solved into ethanol and ether respectively. After being placed at 26°C for 8 hours, they were respectively extracted with ethanol and diethyl ether [5]. After 4 hours of extraction, the extract was filtered and the extract was concentrated to 10 mL by using a rotary evaporator at 25-30°C. After all of these were done, all the concentrated extracts are placed in a 4°C medicine cabinet for short-term storage for subsequent sample testing.

FTIR spectroscopy analysis

Infrared spectroscopy was performed using potassium bromide tableting. Take an appropriate amount of KBr in an agate mortar and grind it into a powder (about 2.5 μm in diameter), 140 mg of powdered KBr was placed in a mold for tableting (KBr sheets were colorless and transparent), and then 7 μl of the sample to be tested was dropped into the center of the KBr sheet by a pipette. Then the samples to be tested were dried at 25°C for
infrared detection [6]. When the prepared sample was tested using a Fourier infrared spectroscopy tester (Nicolet IS 10), the instrument parameters were set to the number of acquisitions of 32, the resolution was 4 cm\(^{-1}\), and the data interval was 0.48 cm\(^{-1}\).

**Components analysis by GC-MS**

GC: 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 5:1. The temperature program of the GC starts at 50 °C, rises to 230 °C at a rate of 10 °C/min, and then rises to 250 °C at a rate of 5 °C/min.

MS: program scans mass range of 30amu-600amu, 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.

**Results and Analysis**

**Groups change characteristics of extractions**

In order to obtain information on the chemical bonds and functional groups contained in RALs and RASs, the substances were detected by infrared spectroscopy [7]. The types of compounds that may be contained are judged based on the different absorption frequencies of different chemical bonds and functional groups contained in RALs and RASs.

From the infrared spectrum results of different solution extracts of RALs and RASs (Fig. 1 and Fig. 2), we can see that the functional groups or chemical bond types extracted by them are similar. However, according to the curve of the infrared spectrum, the absorption peak values of the same type of organic matter in different extracts are different. This also shows that the extraction effect of ether and ethanol are different when extracting the same substance. The Infrared spectroscopy results of RALs showed that both the ether and the double bond may be present in the extracts of diethyl ether and ethanol, which indicating that the extract may contain alcohols, aldehydes and ketones. And the Infrared spectroscopy results of RASs indicate that both ether and ethanol extracts may contain hydroxy groups and do not contain triple bonds. It is speculated that they may contain alcohols, ketones, alkanes. And comparing the ethanol and ether extracts, it is not difficult to find that the ethanol extract may contain olefins. Comparing the infrared spectra of RALs and RASs, it can be seen that the
types of substances contained in RALs may be more abundant than that in RASs, that is, the potential energy substances in RALs may be more.

**Volatile compositional diversity in different extracts from RALs and RASs**

In order to know the volatile components contained in different extracts of different parts of *Rodgersia aesculifolia*, we analyzed the volatile substances by using GC-MS/MS [8, 9]. 39 volatiles components were detected in 43 peaks in RALs ethanol extract and 34 volatiles were detected in 39 peaks in the ether extract of RALs. The compounds in ethanol and diethyl ether were classified according to functional groups (Fig. 3 and Fig. 4). It can be seen that the relative content of alcohols in the two extracts is the highest, and the content of phenols is second.

![Fig. 3 Classification of volatile functional groups in RALs ethanol extracts.](image)

2-Myristynoyl pantetheine and benzoic acid, 4-hydroxy-3, 5-dimethoxy- were detected in the extract of RASs ethanol. Benzoic acid, 4-hydroxy-3, 5-dimethoxy-, which has antibacterial, sedative and local anesthetic effects, is consistent with previous studies. Quinic acid was first discovered in the ethanol extract of RASs and the relative content reached 21.23%. Studies have shown that quinic acid has the effect of enhancing bile, reducing fat, detoxifying the liver, and preventing fatty liver [10, 11]. In addition, after analyzing the volatile substances in the ethanol extract of RALs, it is found that effective biopharmaceuticals such as 2-myristynoyl pantetheine, benzoic acid, 4-hydroxy-3,5-dimethoxy- and 9, 12-octadecadienoic acid (z, z)-, what’s more, there is also a substance phenol, 2, 2-methylenebis[6-(1, 1-dimethylethyl)]-4-methyl- that can be used as a phenolic antioxidant and an antioxidant in petroleum products, which is a good bioenergy substance.

Octadecanoic acid and ,gamma.,-sitosterol, were detected in the ether extract of RALs. gamma.,-sitosterol, which have health functions for the human body [12, 13]. In addition, butylated hydroxytoluene was first discovered in the ether extract of RALs. It is an excellent antioxidant additive for various petroleum products [14-16]. It can effectively prevent the acid value or viscosity of lubricating oil and fuel oil from rising, which is an excellent bio-energy material.

From the comparison of the ionograms of the ethanol extract of RALs and the ether extract, it can be seen that the amount of volatile substances in the ethanol extract is more than that in the ether extract (Fig. 5). However, by comparing the two volatile substances, it is found that the relative content of the bio-energy materials contained in the ether extract is relatively high.
Twenty-four volatile components were detected in 26 peaks in the ethanol extract of RASs, and 29 volatiles were detected in 29 peaks in the ether extract of RASs. The volatile compounds in the ethanol and ether extracts were classified according to functional groups (Fig. 6 and Fig. 7). It can be seen that the relative content of sterols in the two extracts are the highest, indicating that the sterols in RASs are the most. The sterols substance in the ethanol extract of RASs is mainly .gamma.-sitosterol, which has health care functions. Raw materials that can be used as biomedicine have been detected in ethanol extracts of RASs, such as .gamma.-sitosterol, vitamin E, clindamycin and n-hexadecanoic acid [17-19]. Vitamin E can be used to regulate menopausal disorders, progressive malnutrition, and it also can prevent coronary heart disease, arteriosclerosis, thrombosis and prevent aging [20]. Of course, the quinic acid was first discovered in the ethanol extract of RASs, with a relative content of about 7.3%. In addition, there are also substances which can be used as bioenergy in the ethanol extract of RASs, such as phenol, 2, 2’-methylenebis [6-(1,1-dimethylethyl)]-4-methyl-.
The sterols substance in the ethanol extract of RASs is mainly \( \gamma \)-sitosterol, which has health care functions. Raw materials that can be used as biomedicine have been detected in ethanol extracts of RASs, such as \( \gamma \)-sitosterol, vitamin E, clindamycin and n-hexadecanoic acid [19]. Vitamin E can be used to regulate menopausal disorders, progressive malnutrition, and it also can prevent coronary heart disease, arteriosclerosis, thrombosis and prevent aging [20]. Of course, the quinic acid was first discovered in the ethanol extract of RASs, with a relative content of about 7.3%. In addition, there are substances that can be used as bioenergy in the ethanol extract of RASs, such as phenol, 2, 2’-methylenebis [6-(1, 1-dimethylethyl)]-4-methyl-. The sterols detected in the ether extract of RASs are mainly composed of \( \gamma \)-sitosterol and stigmasterol, both of which are good health supplements. Stigmasterol is mainly used as a raw material for the synthesis of steroid hormones and vitamin D3, and it is also a raw material for the manufacture of progesterone in medicine [21, 22]. In addition to those substances which could be used as biomedicine, there are substances that can be used as bioenergy, such as dodecane and butylated hydroxytoluene. Butylated hydroxytoluene is the first to be found in the ether extract of RASs. It is not only an excellent antioxidant additive for various petroleum products, but also a food antioxidant and stabilizer in food-grade plastics and packaged foods to delay the rancidity of food. From the comparison of the total ion chromatograms of the volatiles of the ethanol and ether extracts of RASs (Fig. 8), it can be seen that the ether extract of RASs contains more volatile substances than the ethanol extract. Furthermore, the ether extract can be used as bioenergy substances are relatively high, the substances which can be used as biomedicine has a relatively large amount in the ethanol extract.

**Conclusion**

The volatiles in the different extracts of the leaves and stems of *Rodgersia aesculifolia* at high altitudes contain n-hexadecanoic acid and phenol, 2, 2’-methylenebis [6-(1, 1-dimethylethyl)]-4- Methyl-. But there is a
significant difference in their relative content. The relative content of n-hexadecanoic acid in RASs ethanol extract reached 3.7%, which is the highest relative among the four. The relative content of Phenol, 2, 2’-methylenebis [6-(1, 1-dimethylethyl)]-4-methyl- in the RALs ether extract is the highest (7.09%) one. This indicates that there are a large number of n-hexadecanoic acid in RASs, while RALs have a large amount of phenol, 2, 2’-methylenebis [6-(1, 1-dimethylethyl)]-4-methyl-, it further illustrates that RALs do have potential bioenergy materials, which confirms the results of infrared spectroscopy.

Quinic acid and butylated hydroxytoluene were first discovered in Rodgersia aesculifolia at high altitudes. In RALs ethanol extracts and RASs ethanol extracts, quinic acid was first discovered as a biopharmaceutical ingredient, indicating that RALs and RASs at high altitudes contain quinic acid, and ethanol can effectively extract this component. The butylated hydroxytoluene was first found in RALs ether extract and RASs ether extract, with relative contents of 15.09% and 18.50%. This shows that RALs and RASs contain butylated hydroxytoluene, and ether can effectively extract it.

All these results have been provided a theoretical reference for further research on the multi-resource utilization of Rodgersia aesculifolia, especially in the areas of biomedical and bioenergy applications. And in production practice, different methods can be adopted according to different needs in order to make rational use of Rodgersia aesculifolia at high altitude.

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