Ekoloji 28(108): 135-139 (2019)

LETTER TO THE EDITOR

Diverse Functional Components from *Robinia Pseudoacacia* Flowers by High-efficient Extraction

Xuanxuan Wu[1#], Tao Yang[2#], Zanpei Zhang[1#], Jinghua Ma[1#], Qingzhi Ma[1#*], Tongqiang Chen[2], Wenyang Xu[2], Yu Meng[1], Yuanyuan Chen[1*], Dangquan Zhang[1*]

[1]College of Forestry, Henan Agricultural University, Zhengzhou 450002, China

[2]Hunan Institute of Food Quality Supervision and Research, Changsha 410117, China

[#] These authors contributed equally to this work.

[*]Corresponding author: cyuan091@163.com (Y Chen), zhangdangquan@163.com (D Zhang).

Robinia pseudoacacia is one of the main tree species for greening in Henan Province, while R. pseudoacacia flowers (RPFs) are not enough utilized except edibility. The active ingredients in solution extracts of Robinia Pseudoacacia flowers (RPFs) were detected and analyzed. The results for the RPFs characteristic groups indicated that there are organic compounds and contain double bonds in RPFs, which may contain saturated hydrocarbons and unsaturated hydrocarbons. The results on non-volatile substances and volatile substances in RPFs indicated that the extracts of acetone, benzene and ethanol are rich in active substances. The findings suggested that RPFs have great potential in high-efficient extraction resourcing for biomedicine and chemical materials.

I Introduction

Robinia pseudoacacia were introduced to Asia, Europe and other regions, and it has been widely cultivated in the world. The height of the R. pseudoacacia can reach 25 meters, which can form a good garden landscape. At the same time, due to its low environmental requirements and certain anti-pollution ability, it has been planted in many industrial areas (Liu et al. 2015, Shen et al. 2017).

The *R. pseudoacacia* flowers (RPFs) contain oleic acid, enamel and flavonoids and it is an excellent honey plant. The production of RPFs increased dramatically and cannot be utilized, which has caused great waste (Eriksson et al. 2018, Kumar et al. 2019). In this study, in order to understand the effective active ingredients and relative contents of RPFs, the characteristic groups of RPFs were detected by Fourier Transform infrared spectroscopy (FTIR). In order to know the composition of volatile substances in RPFs, the RPFs raw powder was extracted with three solutions of benzene, acetone and ethanol, and the extracts of the three solutions were detected by Gas Chromatography-Mass Spectrometer (GC-MS/MS) (Cheng et al. 2018). At the same time, quadrupole time of flight-liquid chromatography-mass spectrometry (QTOF-LC-MS) helps analyze highly polar, hardly volatile, thermally unstable compounds that cannot be analyzed by gas chromatography-mass spectrometry (GC-MS/MS) (Chen et al. 2015, Zheng et al. 2015).

II Materials and Methods

Materials

The RPFs were picked large quantity from *Robinia pseudoacacia* (Wu et al. 2014, Zhao et al. 2016). In mid-April The RPFs were cleaned and placed at room temperature (25°C) for 24 h.

Methods

Extraction. RPFs powder were separately extracted by ethanol, benzene, or acetone with an automatic FOSS Sox let Extracted apparatus (Agilent, USA) for 3-5 h at 80°C, 60°C, and 56°C, respectively, then extraction liquids were evaporated between 45°C-50°C under 0.01 MPa vacuum, and ultimately concentrated to 10 mL.

FT-IR analysis. Previously prepared and dried RPFs powder was baked under an infrared lamp to reduce the absorption of moisture in the air and ensure the dryness of the sample (Lakra et al. 2015; Lam et al, 2019).

GC-MS/MS determination. the GC conditions in a quartz capillary column (30 mm × 0.25 mm × 0.25 μm) were start at 50°C, no retention, reach 130°C at a continual heating rate of 5 °C/min, heat to 180°C at 2 °C/min, and then immediately switch to a heating rate of 30 °C/min to.

QTOF-LC-MS. column ACQUITY UPLC® BEH C18 1.7 μm × 2.1 × 50 mm, capillary (kV) achieve a temperature of 300°C for 5 min (Henninot et al. 2015, Kayacelebi et al. 2014)

III Results

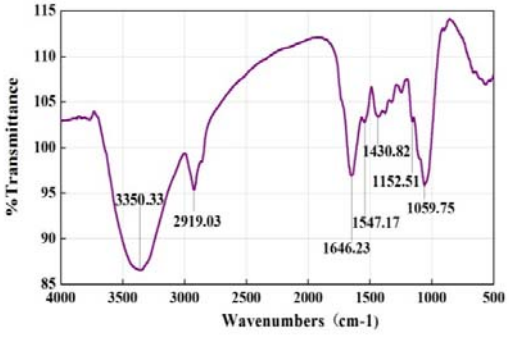
(1) Group change characteristics of RPFs extracts

Fourier transform infrared (FTIR) spectroscopy is a high-sensitivity rapid detection technology that can quickly and efficiently identify chemical bonds and functional groups of many kinds of compounds, and many scientific studies use it to make preliminary determinations of substances (Gupta et al. 2015). The extract was obtained by distillation, filtration and concentration of three solvents of benzene, acetone and ethanol, and then the extract and the original solid powder were detected by FT-IR, in order to reveal the change of chemical groups in the extract (Meng et al. 2015).

Seven distinct peaks were detected in the original powder of RPFs (Figure 1). Significant absorption peaks appeared in the range of 4000-2500 cm^{-1}, indicating the presence of X-H bond. Due to the small amount of water in the sample, it is concluded that the absorption peak appearing around 3350 cm^{-1} may be caused by the stretching vibration of the O-H bond (Steinborn et al. 2016). The absorption peak appearing at 2919 cm^{-1} may be caused by the stretching vibration of the C-H bond.

In summary, it can be concluded that organic compounds are present in the RPFs and saturated hydrocarbons may be present. In addition, by analyzing the infrared spectrum of the three solution extracts, it was found that ethanol as the extract has the best effect on the original powder extraction of RPFs.

a)



b)

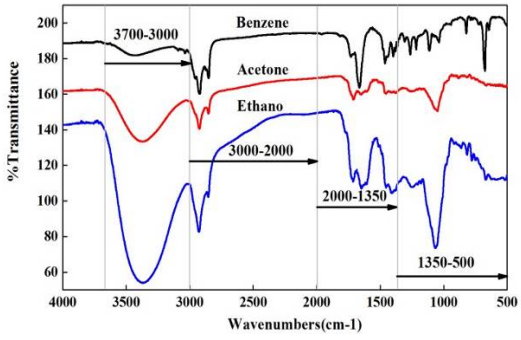


Figure 1: Figure a) is an infrared spectrum of the RPFs raw powder, and Figure b) is an infrared spectrum of the three solution extracts. From the Figure a, we can see the organic compounds contained in the RPFs. The infrared spectrum of the three solution extracts (Figure b) shows that ethanol as the extract has the best effect on the original powder of RPFs.

(2) Volatile components in extracts of RPFs

There are many volatile components shows a variety of bioenergy and biologically active. The extract was originally used for liquid bioenergy development and the remainder of bioactive component resources. Figure 2 a shows the percentage of benzene solution extracts ingredients in chemical raw materials, bioenergy, biomedicine, spices, food additives, and cosmetics. 1-Hexanol, 2-ethyl- is an important chemical raw material, in addition to being used in the production of plasticizers. (Singh and Bukowska 2015, Yuan et al. 2016).

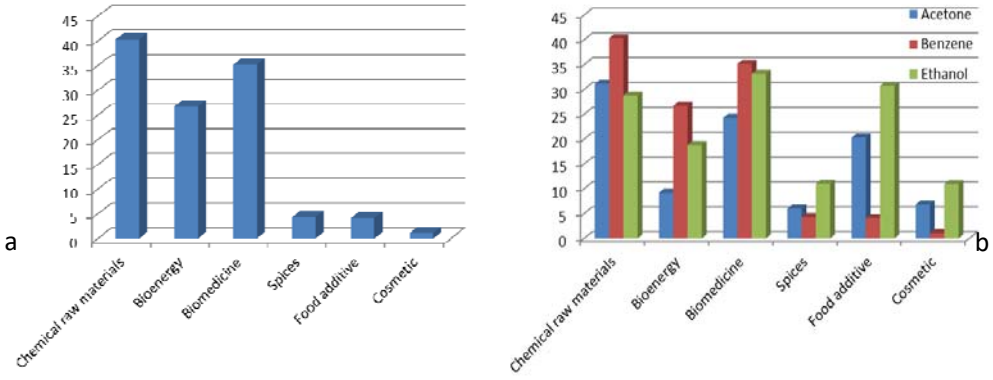


Figure 2: a) The functional classification of benzene solution extract. b) Comparison of the functional classification of substances in different solution extracts.

(3) Non-volatile components in extracts of RPFs

There are many non-volatile components detected in the three different solvent extracts of *Robinia pseudoacacia* via QTOF-LC-MS. As can be seen from the Figure 2 b, the chemical raw materials, bioenergy and biomedicine extracted from the benzene solution are the most. And from ethanol extracts, the most spices, food additives and cosmetic raw materials can be obtained (Hao et al. 2015, Cai et al. 2016). By comparison, it can be found that in the three extraction solutions, the extraction effect of acetone is the worst overall. In the production process, we can consider abandoning acetone as the extraction solution.

Many active substances were detected in the benzene solution extract. And these substances mainly can be used for the production of biomedicine, spices, chemical raw materials, food additives and cosmetics. The main ingredients of biomedicine are: Arachidonic acid, Vitamin K2 and Ethyl linoleate (He et al. 2015).

There are many chemicals includes ginkgolic acid, rhynchophylline and vitexin. Ginkgolic acid has an antibacterial effect and it inhibits the growth of Mycobacterium tuberculosis in vitro (Lu et al. 2014, Tsikas et al. 2014). Rhynchophylline has antihypertensive effect on renal hypertension, and clinical trials have certain curative effect on patients with stage I and II hypertension. Emodin has a significant diuretic effect and it has anti-tumor effect on renal medulla Na+-K+-ATPase. At a certain concentration, emodin can reduce the maximum growth density of human lung cancer A-549 cells and decrease the division index.

IV Conclusion

There exist many active ingredients in the extract of RPFs that can be used as biomedicine, chemical raw materials, cosmetics, spices, food additives, etc. (Ryu et al. 2015). Although the types of substances are diverse, the content of a substance is very small. What's more, the cost of separating these active ingredients separately is high, which has become a major obstacle to how to apply the active ingredients in the hedgehog RPFs to actual production. However, we can think about the problem from another perspective. If we can separate the active ingredients of a certain species and the substances that inhibit this effect, it may be able to meet the actual demand while reducing the certain production costs. Therefore, in the following period, we will make further experiments and explorations on how to effectively use the ingredients in the RPFs, and we also hope that all of us can find a solution to the problem as soon as possible.

Acknowledgements

This project was supported by the talent project (Dangquan Zhang) of Henan Agriculture University, China, and the Project of Henan Provincial Science Research, China (192102110174).

References

Anjos O, Campos MG (2015) Application of FTIR-ATR spectroscopy to the quantification of sugar in honey. Food Chem 169:218-223.

Brian M, Agustín D (2014) Surface Functionalization of Zirconium Phosphate Nanoplatelets for the Design of Polymer Fillers. ACS Appl. Mater. Interfaces 6 (1), 585–592.

Bukowska B, Michałowicz J (2015) The effect of catechol on human peripheral blood mononuclear cells (in vitro study). Environ Toxicol Pharmacol 39 (1):187-193.

Cai J, Lin C, Ma J (2014) Determination of rhynchophylline in rat plasma by liquid chromatography mass spectrometry and its application. J Chromatogr Sci 52 (7):661-665.

Chen L, Wang Z (2018) Gene Cloning and Gene Expression Characteristics of Alcohol Dehydrogenase in Osmanthus fragrans var. semperfloren. Emirates Journal of Food and Agriculture 30 (10): 820-827

Cheng X, Yang T (2018) New method for effective identification of adulterated Camellia oil basing on Camellia oleifera-specific DNA. Arab J Chem 11 (6): 815-826

Choi SY, Gong G (2015) Extreme furfural tolerance of a soil bacterium Enterobacter cloacae GGT036. J Biotechnol 193: 11-3.

Eriksson M, Ghosh R, Hansson E, Basnet S, Lagerkvist C (2018) Environmental consequences of introducing genetically modified soy feed in Sweden. Journal of Cleaner Production 176:46-53.

Hao X, Xie P, Zhu YG, Taghavi S, Wei GH (2015) Copper tolerance mechanisms of Mesorhizobium amorphae and its role in aiding phytostabilization by Robinia pseudoacacia in copper contaminated soil. Environ Sci Technol 49 (4):2328-2340.

He H, Qin J (2018) Effects of exogenous 6-BA and NAA on growth and contents of medicinal ingredient of Phellodendron chinense seedlings. Saudi J Biol Sci 25 (6): 1189–1195

Kayacelebi AA, Beckmann B (2014) GC-MS and GC-MS/MS measurement of the cardiovascular risk factor homoarginine in biological samples. Amino Acids 46 (9):2205-2217.

Kruve A, Rebane R, Kipper K (2015) Tutorial review on validation of liquid chromatography-mass spectrometry methods: part II. Anal Chim Acta 870:8-28.

Kumar V, Sharma A, Kaur P, Kumar R, Keshavarzi A, Bhardwaj R, Thukral AK (2019) Assessment of soil properties from catchment areas of ravi and beas rivers: a review. Geology, Ecology, and Landscapes

3(2):149-157.

Kusano M, Zaitsu K (2015) Positional isomer differentiation of synthetic cannabinoid JWH-081 by GC-MS/MS. J Mass Spectrom 50 (3):586-591.

Lam SS, Mahari WAW, Ma NL, Azwar E, Kwon EE, Peng WX, Chong CT, Liu ZL, Park YK (2019) Microwave pyrolysis valorization of used baby diaper. Chemosphere 230: 294-302

Liu L, Cheng X (2018) Systematic Characterization of Volatile Organic Components and Pyrolyzates from Camellia oleifera Seed Cake for Developing High Value-added Products. Arabian Journal of Chemistry 11 (6): 802-814

Lu N, Xu Z (2014) Proteomic analysis of etiolated juvenile tetraploid Robinia pseudoacacia branches during different cutting periods. Int J Mol Sci 15 (4):6674-6688.

Meng X, Li L., Ye Q, Voort F (2015) Fourier Transform Infrared (FTIR) Spectroscopy as a Utilitarian Tool for the Routine Determination of Acidity in Ester-Based Oils. J Agric Food Chem 63 (37):8333-8338.

Naushad M, Khan MR (2015) Removal of BrO_3^- from drinking water samples using newly developed agricultural waste-based activated carbon and its determination by ultra-performance liquid chromatography-mass spectrometry. Environ Sci Pollut Res Int 22 (20):15853-15865.

Ryu J H, Hong S (2017) Bio-inspired adhesive catechol-conjugated chitosan for biomedical applications: A mini review. Acta Biomater 27:101-115.

Shen Y, Mi W, Zhang Z (2017) A positioning lockholes of container corner castings method based on image recognition. Polish Maritime Research 24(SI):95-101.

Singh DK, Sahu A (2015) Study of the forced degradation behavior of prasugrel hydrochloride by liquid chromatography with mass spectrometry and liquid chromatography with NMR detection and prediction of the toxicity of the characterized degradation products. J Sep Sci 38 (17):2995-3005.

Steinborn A, Alder L (2016) Determination of Glyphosate Levels in Breast Milk Samples from Germany by LC-MS/MS and GC-MS/MS. J Agric Food Chem 64 (6):1414-1421.

Xu K, He G, Qin J (2018) High-efficient extraction of principal medicinal components from fresh Phellodendron bark (cortex phellodendri). Saudi Journal of Biological Sciences 25 (4):811-815

Zhao YH, Jia X (2016) Growth under elevated air temperature alters secondary metabolites in Robinia pseudoacacia L. seedlings in Cd- and Pb-contaminated soils. Sci Total Environ 565:586-594.