

Comparison on FTIR Spectrum and Thermal Analysis for Four Types of Rehmannia Glutinosa and Their Extracts

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Abstract

Rehmannia glutinosa (*R. glutinosa*) is a plant material and widely used clinically in China. Due to different processing methods and various healing effects, there are four types of *R. glutinosas* (RR, RRP, RRC, and RRPC) and their corresponding extracts for convenient use. As complicated mixture systems, chemical components of *R. glutinosa* are very difficult to identify and discriminate. In order to effectively control its quality during processing, Fourier transform infrared spectroscopy (FTIR), second derivative spectrum, and thermal analysis were used in this study. It was found that the major active ingredients were retained in the extracts when extracted from their corresponded *R. glutinosa* and subtle differences could be distinguished by the second derivative spectrum. FTIR spectrum, second derivative spectrum, and thermal analysis are valid method to validate and identify plant material, traditional Chinese medicine material, as well as their corresponding extracts.

1 Introduction

Rehmannia glutinosa (also known as *R. glutinosa*) is an edible plant material and a traditional Chinese medicine with extraordinary pharmaceutical value and a long history of medicinal use in China. Studies have shown that the main active ingredients of *R. glutinosa* are polysaccharides and catalpol. *R. glutinosa* polysaccharides can boost immunity, act as antitumor agents, regulate blood lipid concentrations, and replenish blood. Additionally, *R. glutinosa* polysaccharides have hypoglycemic effects, reduce triglyceride and cholesterol levels in animal models, and considerably reduce glucose metabolism abnormalities in rats with chronic stress on a high-fat diet. *R. glutinosa* polysaccharides can also promote the formation of serum circulating hemolysin and hemolytic antibody plaques in normal and CTX-induced immunosuppressed mice. *R. glutinosa* polysaccharides induce anti-anxiety effects by enhancing serum lysozyme activity¹⁻⁸ Also, *R. glutinosa* polysaccharides have substantial effects on diabetic nephropathy⁹, and they can improve cholinergic function and reduce D-galactose-induced inflammatory cytokines in aging mice¹⁰. Numerous Chinese researchers have isolated polysaccharides from *R. glutinosa* and conducted extensive studies on its chemical structures and pharmacologic actions¹¹⁻²². The nature and efficacy of *R. glutinosa* change after it is processed. Typically, there are four types of preparations for *R. glutinosa*, which are Radix Rehmanniae (RR), Radix Rehmanniae Praeparata (RRP), Radix Rehmanniae Charcoal (RRC), and Radix Rehmanniae Charcoal Praeparata (RRPC) according to application of traditional Chinese medicine. RR is obtained by processing and roasting of fresh *R. glutinosa* and its nature is cold. RRP is obtained from RR through steaming and drying, and its nature is warm^{1,2}. RRC and RRPC are obtained from RR and RRP, respectively, by the same processing, their surfaces are partially carbonized. RRC has the effect of cooling blood and stopping bleeding. RRPC is mainly used on blood-suppressing and hemostasis, and is often used to treat uterine bleeding or debilitating bleeding¹⁰. Therefore, different processed products of *R. glutinosa* have different medicinal uses. Liu's research showed that the contents of catalpol in different *R. glutinosa* products were listed as the following order: RR > RRP > RRC > RRCP, indicating that the processed method has an important influence on *R. glutinosa*¹¹.

Traditional Chinese medicine extract has the advantages of free cooking, direct use, safe and hygienic, easy to carry and preserve, and etc. The four types of *R. glutinosa* are often processed into their extracts (RRE,

RRPE, RRCE, and RRPCE). Nowadays, there are considerable study reports on pharmacological effects of *R. glutinosa*¹⁵⁻²³. However, there were relatively less researches on the similarities and differences of RR, RRP, RRC, RRPC and their extracts (RRE, RRPE, RRCE, RRPCE) by means of FTIR spectroscopy and thermal analysis. FTIR spectroscopy has fingerprint characteristics with strong features; it is the main method for drug identification in various pharmacopoeias²⁴. Thermal analysis is also a primary method for quality control of drugs according to its record in pharmacopoeia²⁴⁻²⁸. In this study, the similarities and differences of four types of *R. glutinosa* and their extracts were investigated by FTIR spectroscopy, second derivative spectrum and thermal analysis. The aim of this study is to develop an effective analysis method for studying integrally the main constituents in the complicated mixture systems such as plant materials and their corresponding extracts.

2 Experiments

2.1 Materials

Four types of *R. glutinosa* (RR, RRP, RRC, and RRPC) were obtained from Jiaozuo, Henan Province, China. Four types of *R. glutinosa* extracts (RRE, RRPE, RRCE, and RRPCE) were purchased from Jiangsu Jiangyin Tianjiang Pharmaceutical Co. Ltd (Jiangyin, China). Their pictures were as follows:

2.2 Methods

2.2.1 FTIR spectral analysis

The sample of four types of *R. glutinosa* and their extracts were vacuum dried (110°C, 2h), ground into powder, and sieved with a 80-mesh screen. The powder (0.002 g) was thoroughly mixed with the KBr powder (0.2 g) and pressed into a pellet for measurement by a Fourier transform infrared (FTIR) spectrophotometer (Nicolet iS-1063001, Thermo Fisher Scientific (China) Co. Ltd.) in the range of 400-4000 cm^{-1} with a resolution of 4 cm^{-1} . An average of 16 scans was used for each sample. In this study, the second derivative spectra of FTIR spectrograms were analyzed by Omnic 8.3 software and SPSS 22.0 software.

2.2.2 Similarity:

Correlation coefficient is a common method evaluating the similarity for Infrared spectroscopy and other fingerprints of traditional Chinese medicine. In this study, the correlation coefficient was obtained using the calculation function of origin software, which indicates the similarity of two infrared spectra matching each other. The similarity was evaluated by the correlation coefficient of four types of *R. glutinosa* and their extracts. The correlation coefficient formula is as follows:

2.2.3 Precision experiment

A sample of RR was taken for infrared spectral analysis by measuring five times continuously. The similarity value (correlation coefficient) was calculated by SPSS 22.0. The similarity values were 0.999970, 0.999997, 0.999997, 0.999997, and 0.999951, respectively. All data were shown as relative standard deviation (RSD=0.02‰), respectively. The high similarity demonstrated the instrument with a high precision.

$$p = \frac{\sum_{i=1}^n (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^n (x_i - \bar{x})^2} \sqrt{\sum_{i=1}^n (y_i - \bar{y})^2}}$$

2.2.4 Stability experiment

A sample of RR was taken for infrared spectral analysis by measuring five times every hour. The similarity value (correlation coefficient) was calculated by SPSS 22.0. The similarity values were 0.999993, 0.999996, 0.999994, 0.999994, and 0.999977, respectively. All data were shown as relative standard deviation (RSD=0.008%). The high similarity indicated that the samples had a high stability.

2.2.5 Reproducibility experiment

Five samples of RR were prepared in parallel, and the infrared spectra of the samples were determined, respectively. The similarity value (correlation coefficient) was calculated by SPSS 22.0. The similarity values were 0.987412, 0.986810, 0.999997, 0.994340, 0.994825, and 0.9985, respectively. All data were shown as relative standard deviation (RSD=0.55%). The high similarity indicated that the proposed method had a high reproducibility.

2.2.6 Thermal analysis

Thermal analysis experiments of the samples of four types of *R. glutinosa* and their extracts were carried out under nitrogen atmosphere in a thermal analyzer (NETZSCH STA449 F3 Jupiter, Hexico Scientific Instruments (Shanghai) Co., Ltd.). The TG (Thermal Gravity Analysis) curves were conducted at heating rates in the range of 20 °C/min from room temperature to 800°C in a nitrogen atmosphere (20 ml/min). The sample amount used was between 10 and 15 mg per specimen, and the collected data were used for further analysis.

3 Results And Discussion

3.1 FTIR spectra analysis of four types of *R. glutinosa* and their extracts

The chemical components of *R. glutinosa* mainly include polysaccharides, oligosaccharides, iridoid glycosides (catalpol was the main element of iridoid glycosides in *R. glutinosa*), amino acids, and etc. These were material basis for the pharmacological effects of *R. glutinosa*. Fig. 2 illustrated the FTIR spectra of four types of *R. glutinosa* and their extracts. Detailed peak positions and assignments of the samples were summarized in Tab. 1. As shown in Fig. 2 and tab 1, FTIR spectra of four types of *R. glutinosa* and their extracts showed higher similarities and typical absorption peaks of polysaccharides, oligosaccharides, catalpol, amino acids, and etc. There were 7 groups of typical common peaks. These absorption peaks were as follows. 1) -OH stretching vibration of the hydroxide radical from *R. glutinosa* polysaccharides, oligosaccharides, and catalpol molecules was near 3418 cm⁻¹. 2) -CH stretching vibration of methyl and methylene from polysaccharides and oligosaccharides was near 2926 cm⁻¹. 3) C=O stretching vibration of polysaccharides and amide I band produced by bending vibration of -NH group in amino acid molecules was

near 1636 cm^{-1} . 4) the peak of 1419 cm^{-1} was attributed by the variable angle vibration of C-H from polysaccharides and oligosaccharides. 5) C-O stretching vibration of glycoside molecules was near 1263 cm^{-1} . 6) the region of 1145-1025 cm^{-1} corresponded to ring vibrations overlapped with stretching vibrations of (C-OH) side groups and (C-O-C) glycosidic bend vibration of polysaccharides and oligosaccharides. 7) C-O stretching vibration of carbohydrates and glycosides was near 586 cm^{-1} . In a nutshell, there were many common features in FTIR spectra of four types of *R. glutinosa* and their extracts. These indicated that the chemical components of four types of *R. glutinosa* and their extracts were basically the same.

Table 1
Peak positions of FTIR of four types of *R. glutinosa* and their extracts

sample	Wave number / cm^{-1}											
RR	3418	2926	1636	1518	1419	1263	1145	1070	-	-	-	598
RRP	3421	2929	1636	-	1419	1260	1145	1075	-	871	799	586
RRC	3431	2926	1636	-	1419	1238	1149	1072	-	-	-	529
RRPC	3426	2924	1636	-	1419	1259	1150	1075	-	-	-	586
RRE	3407	2926	1617	-	1419	1230	1150	1075	1026	899	772	578
RRPE	3384	2924	1636	-	1419	1239	1151	1077	1025	867	774	578
PPCE	3407	2926	1635	-	1418	1240	1150	1078	1025	868	770	578
RRPCE	3387	2927	1635	-	1413	1237	1151	1078	1025	867	766	578

As shown in Fig. 2a, the intensities of absorption peaks of RRP were stronger than those of RR at around 3418 cm^{-1} and 2926 cm^{-1} , and the absorption peaks were observed at 871 cm^{-1} and 799 cm^{-1} for RRP. This was due to RRP was obtained from RR through processing, steaming, and drying. When RR was processed and processed into RRP, polysaccharides and oligosaccharides were converted to monosaccharides (galactose, fructose, and glucose). According to the literature, monosaccharides made RRP "sweet as sugar", fructose could react with amino acids to form melanin, which made RRP "black as lacquer", and the monosaccharide content of RRP was more than twice of RR^{1,22}, which was the reason of RRP to turn black. Due to the presence of -OH and -CH in the galactose, fructose, and glucose molecules, the intensities of absorption peaks in 3418 cm^{-1} and 2926 cm^{-1} of RRP were stronger than those of RR. It could also be seen from Fig. 2a that the intensities of absorption peaks near 3418 cm^{-1} , 2926 cm^{-1} , 1636 cm^{-1} , and 1263 cm^{-1} were significantly reduced in RRC and RRPC, which indicated lower levels of polysaccharides, oligosaccharides, catalpol, amino acids, and etc. The reason was that RRC and RRPC were obtained from RR and RRP, respectively. Partial carbonization occurred during processing, which reduced the intensities of the characteristic peaks of polysaccharides, oligosaccharides, and catalpol hydroxyl groups. The order of the intensities of absorption peaks of four types of *R. glutinosa* were RRP> RR> RRPC> RRC near 3418 cm^{-1} and 2926 cm^{-1} .

As shown in Fig. 2b, the intensities of absorption peaks of RRPE were slightly stronger than those of RRE at around 3407 cm^{-1} and 2926 cm^{-1} , while RRCE and RRPCE were slightly lower. The peak intensities at around

3418 cm^{-1} and 2926 cm^{-1} of four types of extracts exhibited the same order as corresponding *R. glutinosa*, which were RRPE> RRE> RRPCE> RRCE. Therefore, it could be concluded that RRE and RR, RRPE and RRP, RRCE and RRC, and RRPCE and RRPC share similar chemical components, respectively. The absorption peaks were observed at around 1025 cm^{-1} , 867 cm^{-1} , and 774 cm^{-1} for four types of *R. glutinosa* extracts while no obvious corresponding peaks were seen for four types of *R. glutinosa* except RRP at 871 cm^{-1} and 799 cm^{-1} . The reason might be that four types of extracts were extracted from corresponding *R. glutinosa* respectively, and a small amount of malt dextrin were added during extraction, which could cause absorption peaks at around 1025 cm^{-1} , 867 cm^{-1} , and 774 cm^{-1} . It could also be seen from Tab. 1 that the absorption peaks moved towards low wavenumber near 3418 cm^{-1} and 1260 cm^{-1} for four types of *R. glutinosa* extracts, and these might be caused by malt dextrin. In addition, the absorption peaks disappeared at 1518 cm^{-1} , which mainly corresponded to amide I band and amide II in RRE. Thus, it could be deduced that the relative content of protein and plant acid in RRE were lower than those in RR. The characteristic absorption peaks of excipient malt dextrin were at around 1025 cm^{-1} , 867 cm^{-1} , and 774 cm^{-1} .

3.2 Comparative analysis of FTIR spectra of four types of *R. glutinosa* and their extracts

Figure 3 presented a comparison of FTIR spectra and relative intensity of absorption peaks of extracts with those of corresponding *R. glutinosa* (relative intensity of absorption peaks was the ratio of the intensities of 5 groups of characteristic peaks to those of the maximum peak at around 3400 cm^{-1} respectively). As shown in Figure 3, the main absorption peak positions and shapes of four types of extracts were quite similar with those of corresponding *R. glutinosa*. Comparison between two spectra in Figure 3a-3d revealed that the main specific peak positions and shapes were fairly similar to each other for RR and RRE, as well as for RRP and RRPE, RRC and RRCE, and RRPC and RRPCE, respectively, which demonstrated that four types of *R. glutinosa* extracts and corresponding *R. glutinosa* had similar chemical compositions. However, the differences were also observed that some absorption peak intensities and absorption peak width of the extracts were wider than those of *R. glutinosa* at around 3418 cm^{-1} . The reason was caused by adding malt dextrin.

It could also be seen from Fig. 3 that the relative intensity of peaks showed differences at around 1636 cm^{-1} , 1419 cm^{-1} , 1240 cm^{-1} , 1150 cm^{-1} and 1075 cm^{-1} for four types of *R. glutinosa* and their extracts (i.e. RR and RRE, RRP and RRPE, RRC and RRCE, RRPC and RRPCE). For example, the relative intensity of peak near 1636 cm^{-1} of RR was higher than that of RRE, and slight differences near 1419 cm^{-1} and 1240 cm^{-1} , whereas the relative intensity of peaks near 1150 cm^{-1} , and 1075 cm^{-1} of RR were lower than those of RRE. The peak of 1636 cm^{-1} was produced by C=O stretching vibration of polysaccharides and amide I band by bending vibration of -NH group in amino acid molecules, It showed that when RR was extracted into RRE, the content of polysaccharides and the amino acids were reduced. The peak of 1419 cm^{-1} was attributed by the variable angle vibration of C-H from polysaccharides and oligosaccharides and the peak of 1150 cm^{-1} was mainly generated by polysaccharide with hydroxide radical (C-OH), the region of 1145-1025 cm^{-1} corresponded to ring vibrations overlapped with stretching vibrations of (C-OH) side groups and (C-O-C) glycosidic bend vibration of polysaccharides and oligosaccharides and C-O stretching absorption peaks were identified at 899 cm^{-1} and 772 cm^{-1} for glycosides and carbohydrates, respectively. Therefore, glycosides and carbohydrates

characteristic peaks were more prominent for RRE, and Similarly, RRP and RPE, RRC and RCE, RRPC and RRPCE had the same characteristics as the above analysis. In short, the characteristic peaks of glycosides and carbohydrates were more obvious for four types of extracts.

3.3 Second derivative spectra of four types of *R. glutinosa* and their extracts

Generally, the second derivative infrared spectrum could greatly enhance the spectral resolution and amplify tiny differences in FTIR spectrum. Since *R. glutinosa* and their extracts were a mixture of various active components, some absorption peaks were overlapped in FTIR spectra. The second derivative infrared spectroscopy with higher resolution could be applied to further analyze tiny differences of *R. glutinosa* and their extracts. As illustrated in Fig. 4, a number of differences invisible in FTIR spectra became clearer, especially in the range from 1200 to 500 cm^{-1} , which mainly reflected the absorption of C-O and C-OH vibration of polysaccharides, oligosaccharides, and monosaccharides. There manifested as multiple “saw-tooth” peaks in the range of 1200 - 500 cm^{-1} wave bands with significant differences in peak number and peak intensities for four types of and their extracts. The peak intensities in the range of 900 - 770 cm^{-1} were higher in RRP and RRPE (the absorption peak at 830 cm^{-1} and 778 cm^{-1} were caused by α -glycosidic bond). Since RRP was processed by RR, polysaccharides and oligosaccharides of RR were converted into monosaccharides (galactose, fructose, and glucose). RRPE were extracts from RRP. Therefore, RRP and RRPE had greater contents of monosaccharide (fructose and glucose) and their characteristic peaks of monosaccharides were more obvious, by which they could be easily distinguished from others. RRC and RRPC were obtained from RR and RRP, respectively, and with partial carbonization, lower contents of polysaccharides, oligosaccharides, and monosaccharide were in RRC and RRPC, lower numbers of “saw-tooth” absorption peaks were in RRC and RRPC, and fewer characteristic peaks of polysaccharides, oligosaccharides, and monosaccharides could distinguish RRC and RRPC with other *R. glutinosa* and extracts. There were slightly higher contents of glycosides and carbohydrates in four types of extracts due to the addition of malt dextrin excipients during extraction. Thus, slightly higher numbers of “saw-tooth” absorption peaks were in four types of extracts. For example, some peak intensities of RRCE and RRPCE in the range of 1200 - 700 cm^{-1} were slightly higher than those of RRC and RRPC. Absorption peaks could also been seen at around 1150 cm^{-1} and peak intensities were different and conformed to the above analyses in four types of *R. glutinosa* and their extracts. It could be seen that second derivative spectra amplify the differences and reveal the potentially characteristic FTIR absorption bands, as well as enhance the spectral resolution and obtain more new information for distinguishing similar complicated samples. Therefore, it could be concluded that the second derivative infrared spectrum could distinguish four types of *R. glutinosa* and their extracts.

3.4 The similarity of FTIR of four types of *R. glutinosa* and their extracts

The correlation coefficient could indicate the similarity of two infrared spectra matching each other. Tab 2 showed significant similarity of FTIR spectra between the extracts and corresponding *R. glutinosa*, and the similarities were 0.951, 0.963, 0.960, and 0.954 for RRE and RR, RRPE and RRP, PPCE and RRC, and RRPCE

and RRPC, respectively. Among them, RRPE and RRP exhibited the highest similarity (0.963). In addition, the chemical structures of the extracts were similar to those of the corresponding *R. glutinosa*, which further proved that four types of extracts had multiple identical chemical components with their corresponding *R. glutinosa* and retained active ingredients from raw medicinal materials. The minor differences observed between the extracts and corresponding *R. glutinosa* may be caused by small amounts of components that were not completely extracted and the addition of malt dextrin excipients during extraction.

Table 2
The similarity of FTIR of four types of *R. glutinosa* and their extracts

	RR	RRP	RRC	RRCP
RRE	0.951	0.963	0.960	0.954
RRPE				
PPCE				
RRPCE				

3.5 Comparison of thermal analysis of four types of *R. glutinosa* and their extracts

Figure 5 showed a comparison of TG and DTG curves of four types of *R. glutinosa* and their extracts. It could be seen that TG and DTG curves of four types of extracts were very similar to those of corresponding *R. glutinosa*, and the peak positions and shapes of characteristic temperature in DTG curve of four types of extracts were also very similar to those of corresponding *R. glutinosa*, and the same time the TG and DTG curves of RR and RR were similar, while RRC and RRPC were more similar. This proved that four types of *R. glutinosa* extracts retained the effective ingredients of their raw materials. This also proved that the active ingredients of RR and RRP were similar, while PPC and RRPC were more similar, which was consistent with the above spectral analysis. It could be seen from DTG curve in Figure 5 that peak 1 and 3 of RR, RRP and their extracts were not obvious while peak 2 was sharp and larger, indicating a lower rate of mass loss in the first and third stage and a higher rate of mass loss in the second stage (about 190°C – 350°C). The reason could be that RR and its extract were rich in polysaccharides, catalpol and a variety of amino acids, and these organic biomasses, especially polysaccharides would be decomposed into monosaccharides when heated. Monosaccharides would be dehydrated to caramel at 190°C to 220°C. Caramel was subjected to further heating to form carbon dioxide and carbon monoxide at high temperature, resulting in a large mass loss. Thus, RR and its extract had a greater mass loss in the second stage. The same reason was for RRP and its extract. Peak 1 in DTG curves of RRC and RRPC were larger than those of RR and RRP, which indicated that RRC, RRPC had higher mass loss rates in the second stage, at the same time, the mass loss rate of RRC and RRPC were slightly higher than those of RR and RRP in the first stage. The reasons were that RRC and RRPC processed by RR and RRP respectively and they were partially carbonized, and these might caused them to absorbed water easily, RRC and RRPCs had slightly higher mass loss rate in the first stage ($\leq 190^\circ\text{C}$) due to the removal of water vapor molecules. RRCE and RRPCE have low water absorption due to processing into extracts.

4 Conclusions

Based on systematical analysis of four types *R. glutinosa* and their extracts using FTIR, second derivative spectrum, and thermal analysis, it can be concluded that these methods could support large numbers of microscope structure information and entire rules of chemical constituents in medicinal materials. By using microscope fingerprint characters of FTIR spectrum, second derivative spectrum, and thermal analysis, the constituents in the extracts can be tested accurately, instantly, and effectively and the quality of medicinal materials can be validated. Thus, FTIR spectrum, second derivative spectrum, and thermal analysis reflecting objectively the panorama of chemical constituents in complex system is the most credible method to validate and identify the mix-substance systems, such as traditional Chinese medicine, herbal medicine, as well as their corresponding extracts.

Declarations

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Figures

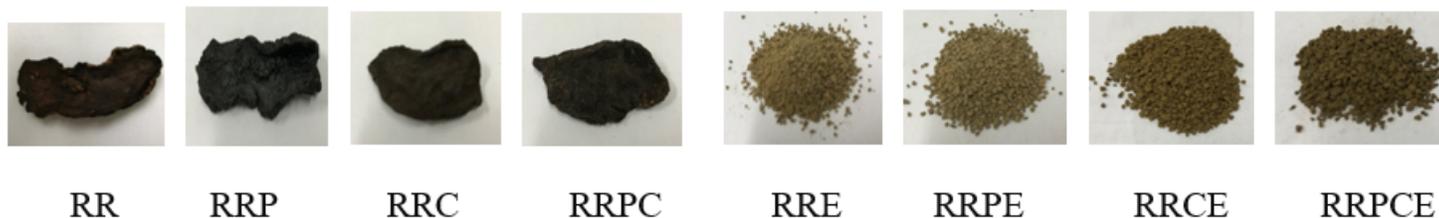
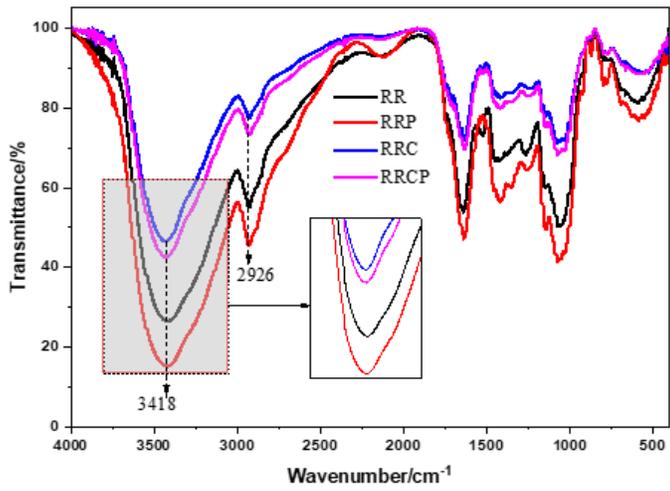
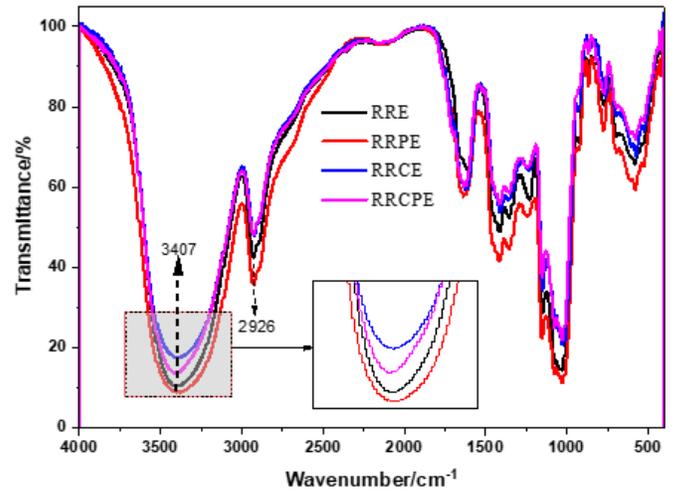


Figure 1

Four types of *R. glutinosa* and their extracts



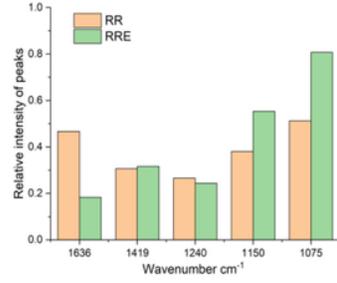
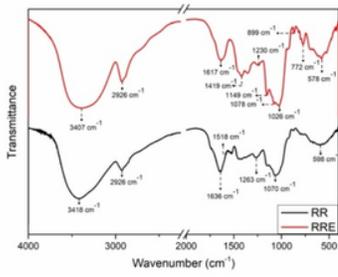
a RR, RRP, RRC, and RRCP



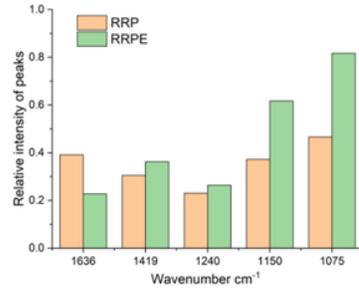
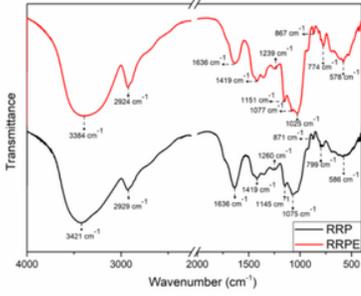
b RRE, RRPE, RRCE, and RRPCE

Figure 2

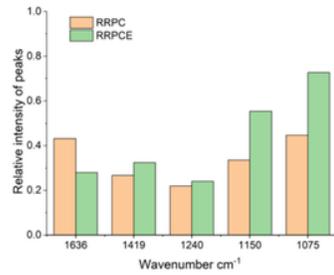
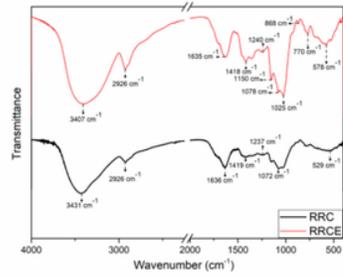
FTIR spectra of four types of *R. glutinosa* and their extracts



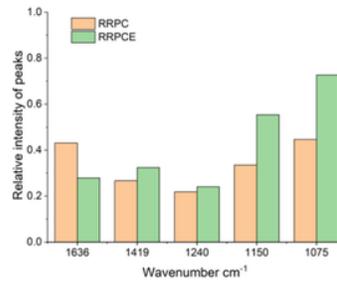
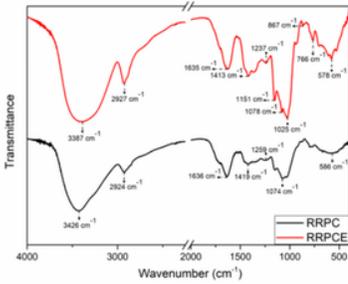
a RR and RRE



b RRP and RRPE



c RRC and RRCE



d RRCP and RRPCE

Figure 3

Comparison of FTIR spectra and the relative intensity of absorption peaks of extracts and their corresponding *R. glutinosa*

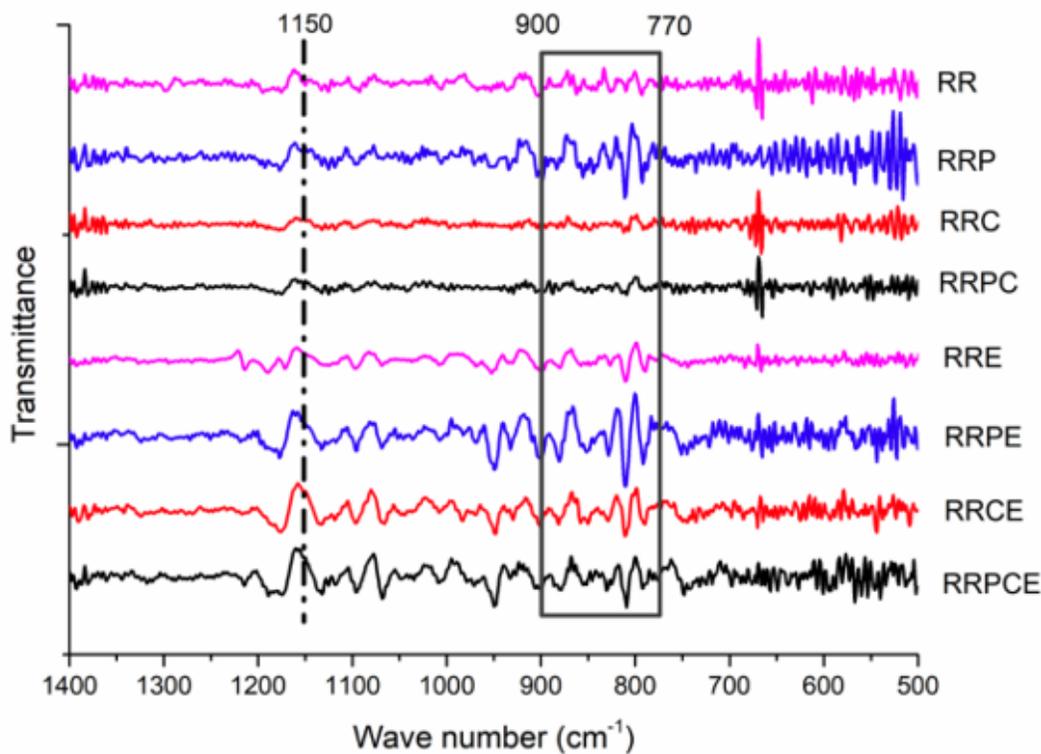
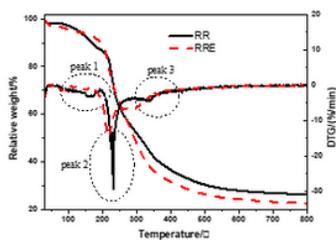
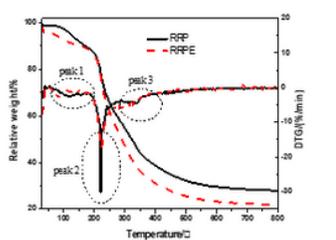


Figure 4

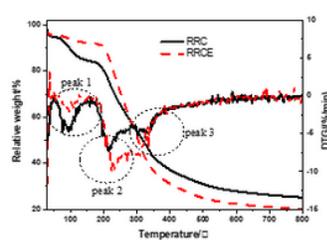
Second derivative spectra of four types of *R. glutinosa* and their extracts



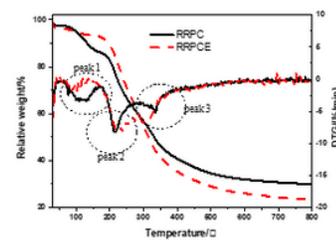
a RR and RRE



a RRP and RRPE



c RRC and RRCE



d RRPC and RRPCE

Figure 5

TG & DSC curves of four types of *R. glutinosa* and their extracts.