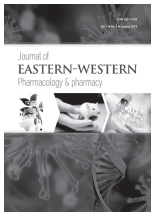

Original Article

https://doi.org/10.64269/jewpp.v6i1.4216

Journal of
EASTERN-WESTERN
Pharmacology & pharmacy

Quantitative analysis of bioactive compounds in the aqueous extract of *Ribes diacanthum* Pall.

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KEYWORDS

Ribes diancanthum Pall, Aqueous extract, Total flavonoids, Total phenolics

Received: 2024/11/19 Accepted: 2024/12/26
Revised: 2024/12/15 Published: 2025/01/30
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ABSTRACT

Over the past 30 years, herbal medicines have been used globally, with over 80% of people turning to plant-based remedies for health-related needs. *Ribes diacanthum* Pall., known in Mongolia as "Tekhiin Sheeg," is a shrub belonging to the *Saxifragaceae* family and commonly found in rocky mountain slopes, river valleys, and sand-rich areas near willow and poplar groves. In traditional Mongolian medicine, the aerial parts of this plant are used as a Aqueous extract to treat kidney and urinary tract inflammations, retention, and swelling. This quantifies specific bioactive compounds in the aqueous extracts prepared from different parts of *Ribes diacanthum* Pall., including the aerial parts. Aqueous extracts were obtained from the fruit (Sample 1), leaves (Sample 2), and stems (Sample 3) of *Ribes diacanthum Pall* using partial maceration. Total flavonoid and total phenolic contents in each sample were measured using spectrophotometry. The highest total flavonoid content was found in Sample 1 at 0.0032 ± 0.00058%, while the highest total phenolic content was in Sample 1 at 0.059 ± 0.0047%. Our study reveals that the aerial parts contain the highest total flavonoid concentration, while the fruit has the highest total phenolic concentration, indicating their potential as valuable medicinal components.

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INTRODUCTION

Medicinal plants have been used for centuries to treat and prevent various diseases and continue to be utilized today. The use of plant-based medicines has increased over the past 30 years, with more than 80% of people worldwide using plant-based medicines for health-related reasons[1]. The *Ribes diacanthum* Pall is a shrub belonging to the Saxifragaceae family, found in various regions of Mongolia, including Khentii, Khangai, Mongol Daguur, Ikh Khiyangan, and Dornod Mongolia. It typically grows in areas with stony slopes, hillsides, cliff and, debris, and along the banks of rivers and in sandy soils with willow and poplar thickets. Historically, Mongolian traditional medicine has used infusions prepared from the upper parts of the plant to treat kidney and urinary tract infections, urinary retention, and swelling[2].Researchers, including Bayarmaa.B[3] and others, have discovered that the ethanolic extract of the *Ribes diancanthum* Pall contains a significant amount of flavonoids. At the same time, Akhtolkhin.T[4] and his team have demonstrated that aqueous extracts of the plant's upper parts exhibit anti-inflammatory effects in experimental animals with cisplatin-induced kidney inflammation. In this study, aqueous extracts will be prepared from the aerial parts (fruits, leaves, stems) and the total phenolics and flavonoid contents in them will be determined.

MATERIALS AND METHODS

Sample preparation

From August to September 2023, the aerial parts (fruits, leaves, and stems) of *Ribes dianthu*s Pall. were collected from Tsagaan Nuur sum in Selenge province, Mongolia. The plants were cleaned and air-dried under shade.

Preparation of aqueous extract[5]

A 50 g sample from each dried organ of *Ribes diacanthum* Pall was weighed, and 500 ml of distilled water (1:10 ratio) was added. The mixture was allowed to soak for 30 min and then infused for 1 hour. After filtration, the residue was 400 ml of distilled water (1:8 ratio) for 1 hour, followed by filtration. The remaining residue was then soaked in 300 ml of distilled water (1:6 ratio) for 40 min and filtered again. The filtered extracts were combined and concentrated using a vacuum rotary evaporator until a final volume of 50 ml was reached. All samples were placed in a glass bottle and stored at 4°C until further analyses.

Determination of total phenolics[6]

The total phenolic contents in each extract were determined using the Folin-Ciocalteu reagent according to the specified method. **Preparation of Sample Solution:** 5 ml of the aqueous extract was transferred into a 25 ml volumetric flask. 2 ml of distilled water and 20 ml of 70% ethanol were added, and the mixture was ultrasonicated for 15 minutes. The solution was then diluted to the volume with 70% ethanol and filtered. Subsequently, 2 ml of the filtered solution was transferred into a 25 ml volumetric flask, 9 ml of distilled water was added, followed by 1 ml of Folin-Ciocalteu reagent, and the mixture was thoroughly mixed. After 15 minutes, 10 ml of 7% sodium carbonate solution was added, and the solution was diluted to the volume with distilled water. The mixture was then allowed to stand for 90 minutes at room temperature in darkness.

Preparation of Standard Solution: 0.01 g (accurate to 0.0001 g) of gallic acid was weighed and dissolved in a 100 ml volumetric flask with 70 ml of 70% ethanol, then diluted to the volume with 70% ethanol. 1 ml of this standard solution was transferred into a 25 ml volumetric flask, 9 ml of distilled water was added, followed by 1 ml of Folin-Ciocalteu reagent, and the mixture was thoroughly mixed. After 15 minutes, 10 ml of 7% sodium carbonate solution was added, and the solution was diluted to the volume with distilled water. The mixture was then allowed to stand for 90 minutes at room temperature in darkness.

Preparation of Blank Solution: 1 ml of 70% ethanol was added to a 25 ml volumetric flask, followed by 9 ml of distilled water. 1 ml of Folin-Ciocalteu reagent was then added, and the mixture was thoroughly mixed. After 15 minutes, 10 ml of 7% sodium carbonate solution was added. The solution was diluted to the volume with distilled water and allowed to stand for 90 minutes at room temperature in darkness.

Procedure: A spectrophotometer was used to measure the absorbance of the sample and standard solutions relative to the blank solution at a wavelength of 750 nm.

Determination of total flavonoids[7]

Preparation of Sample Solution: 5 ml of the aqueous extract was measured and transferred into a 25 ml volumetric flask. 2 ml of distilled water and 20 ml of 70% ethanol were added, and the mixture was ultrasonicated for 15 minutes. The solution was diluted to the volume with 70% ethanol and filtered. 5 ml of the filtered solution was transferred into a 25 ml volumetric flask, and 10 ml of 70% ethanol, 0.5 ml of 30% acetic acid solution, and 2 ml of 2% aluminum chloride solution dissolved in 70% ethanol were added. The solution was diluted to the volume with 70% ethanol and allowed to stand in darkness for 30 minutes.

Preparation of Standard Solution: 0.01 g (±0.0001 g) of apigenin was weighed and transferred into a 50 ml volumetric flask. 30 ml of 70% ethanol was added, and the mixture was ultrasonicated for 15 minutes to ensure complete dissolution. The solution was diluted to the volume with 70% ethanol. 1 ml of this standard solution was transferred into a 25 ml volumetric flask, and 10 ml of 70% ethanol, 0.5 ml of 30% acetic acid solution, and 2 ml of 2% aluminum chloride solution dissolved in 70% ethanol were added. The solution was diluted to the volume with 70% ethanol and allowed to stand in darkness for 30 minutes.

Preparation of Reference Solution 1: 1 ml of the standard solution was taken and transferred into a 25 ml volumetric flask. 10 ml of 70% ethanol and 0.5 ml of 30% acetic acid solution were added. The solution was diluted to the volume with 70% ethanol.

Preparation of Reference Solution 2: 5 ml of the filtered sample solution was taken and transferred into a 25 ml volumetric flask. 10 ml of 70% ethanol and 0.5 ml of 30% acetic acid solution were added. The solution was diluted to the volume with 70% ethanol.

Procedure

After 30 minutes, the absorbance of the standard solution and sample solution was measured at 385 nm using a spectrophotometer, comparing the standard solution with Reference Solution 1 and the sample solution with Reference Solution 2.

RESULTS

1. **Aqueous extracts obtained from the aerial part of *Ribes diacanthum* Pall.**

Aqueous extracts were obtained from the fruits, leaves, and stems of *Ribes diacanthum Pall*. collected from the Tsaagan Nuur sum of Selenge province, using the maceration method with water, reported in Figure 1.

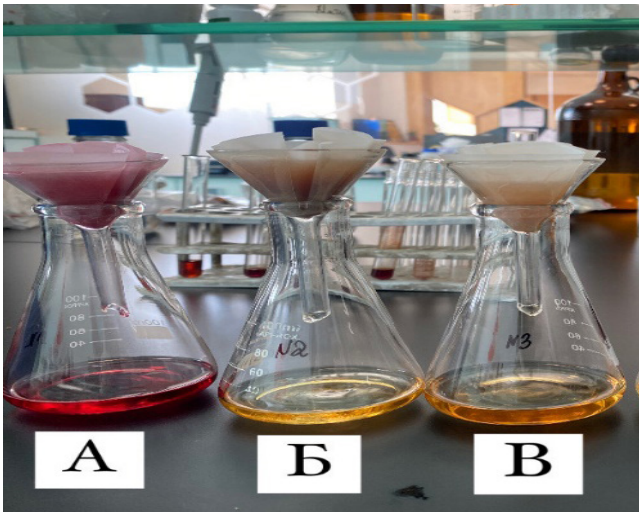

Figure 1. Aqueous extracts.

N1-F. Aqueous extract prepared from the fruits of *Ribes diacanthum* Pall.; N2-L. Aqueous extract prepared from the leaves of *Ribes diacanthum* Pall.; N3-S. Aqueous extract prepared from the stems of *Ribes dianthus* Pall.The three samples obtained were evaluated based on visual appearance and odor as quality parameters, and the results are reported in Table 1.

Table 1. Quality parameters of aqueous extracts from *Ribes diacanthum* Pall.

Quality parameterww	N1-F	N2-L	N3-S
Appearance	Reddish-purple liquid	Pale yellowish wliquid	Light orange liquid
Odour	Faint distinctive herbal aroma	Faint distinctive herbal aroma	Faint distinctive herbal aroma

N1-F. Aqueous extract prepared from the fruits of *Ribes diacanthum* Pall.; N2-L. Aqueous extract prepared from the leaves of *Ribes diacanthum* Pall.; N3-S. Aqueous extract prepared from the stems of *Ribes dianthus* Pall.

1.1. ***Total phenolics and total flavonoids content***

Calibration curve

The calibration curve was drawn with five doses at gallic acid concentrations from 0.165 to 0.872 mg/ml. The regression equations were calculated as y=ax+b, where y and x correspond to the absorbance and regression equation. The correlation coefficient was y = 4.5517x–0.079, R^2= 0.9967.

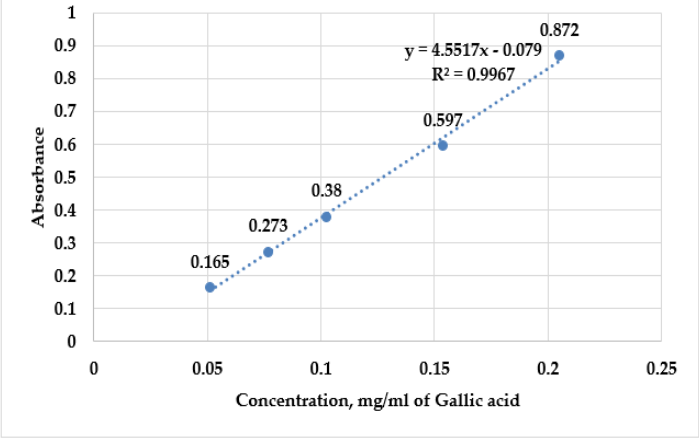

Figure 2. Calibration curve of Gallic acid

The calibration curve was drawn with five doses at apigenin concentrations from 0.06 to 0.426 µg/ml. The regression equations were calculated as y=ax+b, where y and x correspond to the absorbance. The regression equation and correlation coefficient were as follows: y = 0.0302x–0.0137, R^2 = 0.9996.

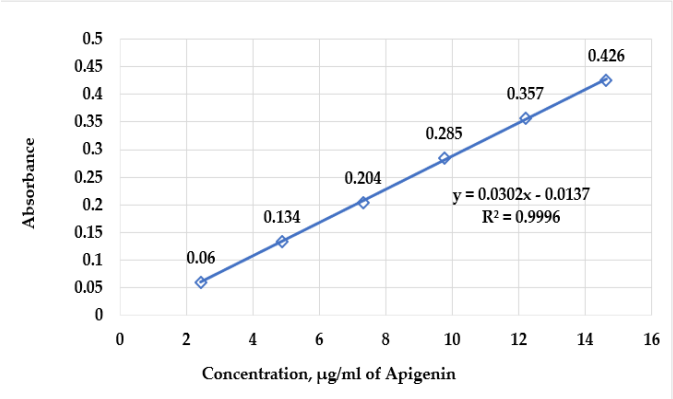

Figure 3. Calibration curve of Apigenin

Table 2. Total phenolics and flavonoid contents of aqueous extracts from *Ribes diacanthum* Pall.

Sample	Total phenolics (Gallic acid, %)	Total flavonoids (Apigenin, %)
N1-F	0.059±0.00047	0.0023±0.00012
N2-L	0.032±0.00047	0.0032±0.00058
N3-S	0.046±0.002	0.011±0.00058

N1-F. Aqueous extract prepared from the fruits of *Ribes diacanthum Pall.*; N2-L. Aqueous extract prepared from the leaves of *Ribes diacanthum Pall.*; N3-S. Aqueous extract prepared from the stems of *Ribes diacanthum Pall.*

DISCUSSION

According to the research of E. Sansarkhuyag et al., the flavonoid content in the leaves was 18.16±0.18, while in the branches it was 9.18±0.16, which is higher than the results obtained in our study[8].

B. Bayarmaa et al. investigated the aerial parts of *Ribes diacanthum Pall.* and extracted them with solvents such as methanol, ethanol, distilled water, and ethyl acetate. Their study revealed that the ethanol extract contained the highest amount of total flavonoids and total phenolic compounds[3].

T. Akhtolkhyn et al. demonstrated, in pharmacological research, that an aqueous extract of the aerial parts of *Ribes diacanthum Pall.* exhibited anti-inflammatory effects and antioxidant activity in experimental animals with induced kidney inflammation[9].

The anti-inflammatory effects of flavonoids are closely associated with their antioxidant properties, as they increase the glutathione levels in kidney tissues. Therefore, flavonoids are considered critical agents in treating kidney damage caused by toxicity from certain chemical substances[10].

In our study, the highest total flavonoid content was found in Sample 1 (fruit), while the highest total phenolic content was also found in Sample 1 (fruit), indicating the potential for selecting raw materials in future research.

CONCLUSION

Five different aqueous extracts were prepared from the fruits, leaves, branches, and aerial parts of Ribes diacanthum Pall. The highest concentration of total phenolics was found fruits and total flavonoids was found leaves of *Ribes diacanthum Pall.* The highest total phenolics and flavonoids contents in friut and leaves aqueous extracts were 0.059, and 0.0032%, respectively.

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