Chemical analysis and bioactivity evaluation of the stem of Rheum tanguticum

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Abstract

Rhubarb (Dahuang in Chinese), belongs to the family Polygonaceae, genus Rheum, has been employed as a traditional Chinese medicinal material for a long time. Rheum officinalis, R. palmatum, R. tanguticum, R. rhaponticum, R. ribes, R. franzembachii, and R. rhabarbarum have been reported to possess many pharmacological active substances that are used to treat diseases. The petioles of R. rhabarbarum, R. ribes, R. rhaponticum are well-known vegetables in some regions of the world. Based on this folk application and active ingredients that may be present in stems, the present study was conducted to analyze the content of nutritional components and active secondary metabolism components, and evaluate the antimicrobial activity and antioxidant activity with prepared solvent extracts.

Methods published by the Association of Official Analytical Chemistry (AOAC) was used to determine the total protein, crude fat, crude fiber, vitamin C, ash, and moisture contents. The results showed that the inflorescence stem of R. tanguticum contained a higher content of crude fiber (89.14%) and vitamin C (16.96%). The content of total protein and crude fat was 8.49% and 1.34% respectively. The results showed that the DPPH Radical Scavenging Activity of the extract had a concentration-dependent effect. The highest activity (83.4%) of the methanol extract sample was recorded at 1.0 mg/mL and the lowest activity, 15.56%, was recorded at 0.2 mg/mL.

0.485 mg/mL was needed to achieve a 50% toxicity. The flavonoid and total phenolic contents of the inflorescence stem of R. tanguticum were found to be 0.022 mg/g and 4.138 mg/g respectively. A concentration of 10 mg/mL successfully inhibited the growth of the pathogenic microorganisms, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus subtilis using the paper disc diffusion method.

In summary, the results of this study confirm that the inflorescence stem of R. tanguticum contains some rich nutritional components and bioactive secondary metabolism components that are beneficial for human health as edible parts or processed products. The results of bioactivity evaluation in this study also showed that the inflorescence stem of R. tanguticum could be developed natural antioxidant and antibacterial agent as a potential resource. This may possibly provide a feasible solution for non-medicinal parts utilization.

Keywords: Rhubarb, Rheum tanguticum, stem, antioxidant, antimicrobial, nutritional

1. Introduction

Malnutrition has been one of the many factors that have contributed to the declining the quality and functioning of life, prolonged hospital stay, and higher health care costs over the past few years [1]. Man has depended on plants for their nutritional purposes since ancient times. Plants supply the body with energy, protein, essential oils, minerals, vitamins, and certain hormone precursors [2]. Several studies carried out in the past years have also revealed the important roles of plants as sources of nutrients and contributors to human dietary requirements [3]. There have also been reports that suggest an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases [4]. Laboratory antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene have been reported to be responsible for a number of diseases [5]. A key solution to this problem is to supplement one’s diet with natural antioxidants [6]. Microorganisms such as viruses, bacteria, and fungi have also over the years act as the major pathogenic microorganisms that have threatened the health and quality of life of mankind around the globe [7]. The diseases and infections caused by these microorganisms are said to be the leading causes of death in most developing countries [8].
The discovery of antibiotics through scientific research in the 21st century has provided an efficient tool for combating these diseases. However, with time, the overuse and misuse of these drugs have led to the development of Multiple Drug Resistant (MDR) microbes. Mechanisms such as target site modifications and metabolic inactivation have been reported to be the cause of MDR strains of microbes [9]. Antibiotic resistance is therefore considered as the greatest threat to patients’ lives.

Medicinal plants have shown to be a good potential source of antimicrobial and antioxidants agents as they produce a wide variety of secondary metabolites. Phytochemicals from plant sources serve as a prototype in developing less toxic and cost-effective medicines against disease-causing microorganisms such as viruses, bacteria, and fungi. These phytochemicals are said to have greater therapeutic importance in treating pathogenic microorganisms than their counterparts that have countless side effects [8]. It has been reported that the leaves, rhizomes, bulbs, barks, roots, and peels of more than 40,000 plant species are used to treat fungal, bacterial, and viral infections [10]. Therefore, scientists are immensely involved in the screening and searching for novel antibiotics from natural origin to identify effective and natural antibiotics as an alternative to the already existing ineffective synthetic drugs [11].

*Rheum tanguticum*, one of the three (*R. palmatum* and *R. officinale*) main genuine species of rhubarb in the Chinese Pharmacopoeia, has been widely used to treat many diseases for more than 200 years. It is mainly found in China and other parts of Europe and Asia. Rhubarb and its associated species are known to possess a wide variety of secondary metabolites such as anthraquinone, anthrones, polyphenols, tannins, flavonoids, phenols, alkaloids, terpenoids, and glycosides. *R. tanguticum*, therefore, derives its pharmacological importance from these metabolites. The extracts from the roots and rhizomes are known to possess different kinds of pharmacological activities, such as antibacterial, anti-inflammatory, antipyretic, anti-cancer, and antioxidant activities [12]. Stilbenes, anthrones, and anthraquinones have been reported to be some of the main chemical components occurring in the roots and rhizomes of *R. tanguticum*, which makes the plant a remarkable antioxidant, anti-inflammatory, anti-allergic, anti-microbial, antitumor, hemostatic, hypercholesterolemic lowering, and cytotoxic properties [13]. The continuous evolutions of antimicrobial resistance due to the misuse and overuse of synthetic antibiotics and re-emerging infections have prompted the search for novel, more lethal, and cost-effective antimicrobial compounds from medicinal plants. The goal of this study is to determine the chemical constituents and to screen the antimicrobial activity from the extract of the stem of *Rheum tanguticum* against antibiotic-resistant bacterial.

2. Materials and Methods
2.1 Collection, identification, and preparation of plant material
*Rheum tanguticum* stems were collected from Ruoeai County, Aba Tibetan Autonomous Prefecture, Sichuan Province, China, during May 2019 and 2020. A portion of fresh stems was used for determining the moisture content. The collected stems of *R. tanguticum* were air-dried under shade for 20 days at room temperature and then dried in an oven at 60 °C for 12 h. The dried stems were then grounded into fine particles using a High-Speed Chinese Medicine grinder, sieved, and stored in a cool dry place. 5g sample was extracted using the ultrasonic extractor (60 °C and 53 KHz) for 3 hr. The sample was then dried at a reduced pressure using the rotary evaporator and stored for further analysis.

2.2 Chemicals
Neomycin, Rutin and gallic acid were purchased from Chengdu Pusi Biotechnology Co., Ltd. Sodium carbonate (Na2CO3), sodium hydroxide (NaOH), potassium hydroxide (KOH), ammonium nitrate (NH4NO3), copper sulfate (CuSO4), potassium sulfate (K2SO4), sodium nitrate (NaNO3), aluminum nitrate [Al(NO3)3], sulfuric acid (H2SO4), DMSO, hydrochloric acid (HCl), ethanol, Methanol, and phosphoric acid, methanol, petroleum ether, n-hexane, glucose, peptone, yeast extract, methyl blue, bromocresol green, 2,6-dichloroindophenol, Folins-Ciocalteu reagent, DPPH (1,1-diphenyl-2-picyrylhydrazyl) were all analytic grade reagents.

2.3 Instruments
Rotary evaporator (RE-52AA, Shanghai Yarong Biochemical Instrument Factory, China), High-Pressure Steam Sterilizer (MLS-375L-PC, SANYO Techno Solutions Tottori Co, Ltd, China). Single Double-sided Purifying Workbench (SW-CJ-15, Suzhou Purification Equipment Co, Ltd, China), Precision constant temperature incubator (BPH-9162, Shanghai Yihang Scientific instrument Co, Ltd, China). UV-VIS spectrophotometer (UV-800, METASH Shanghai Yuanshi Instrument Co, Ltd, China).

2.4 Test organisms
Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus subtilis, provided by the laboratory of Prof. Ma Lin. were used as the test strains for the experiment. These bacterial cultures were maintained on BA agar. All work on the handling of bacterial cultures was performed in a Single Double-sided Purifying Workbench.

2.5 Determination of nutritional components
The nutritional components; ash, moisture, crude fiber, crude fat, protein, vitamin C, and mineral contents, were analysed using methods according to the Association of Official Analytical Chemistry (AOAC) with slight modifications

2.6 Antioxidant activity determination
DPPH radical scavenging assay is one of the simplest and widely used methods to test the ability of compounds to act as hydrogen donors or free radical scavengers. It helps to evaluate the antioxidant activity of foods. In this spectrophotometric method, the stable radical, DPPH (2,2-diphenyl-1-picrylhydrazyl) was then taken against a blank after the time has elapsed.

\[
\text{RSA} \% = 1 - \left( \frac{\text{DPPH} + \text{Sample}}{\text{DPPH} + \text{Methanol}} \right) - \left( \frac{\text{Sample} + \text{Methanol}}{\text{DPPH} + \text{Methanol}} \right) \times 100\%
\]

Five different concentration (0.2, 0.4, 0.6, 0.8, 1.0 mg/mL) were prepared. 3 ml of 0.004% DPPH solution was added to 2 ml of the sample in a flat bottom flask and allowed to stand in the dark for 30 min to scavenge. The absorbance of 517 nm was then taken against a blank after the time has elapsed.

~ 48 ~
2.7 Chemical analysis
2.7.1 Total flavone content
1 g sample was weighed into a conical flask and 30 mL of 70% ethanol was added. The weight of the flask’s content was noticed, and ultrasonic for 2 h. The lost volume was compensated after extraction and filtered. 8 mL of the filtrate was measured into a 100 mL volumetric flask and topped to the mark using 70% ethanol. The content of the flask was shaken well, and its absorbance was taken at 508 nm.
Preparation of standard curve
25 mL of 70% alcohol was added to a measured 6 mg standard rutin to obtain a concentration of 0.24 mg/mL. 0.2, 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 mL of the standard rutin was measured into different beakers. Each was top to 10 mL using 70% alcohol. 10.4 mL sodium nitrate (NaNO3) was first added. After 6 minutes 0.4 mL aluminium nitrate was then added. 6 mins later 0.4 mL sodium hydroxide (NaOH) was added. The absorbance was measured at 508 nm using 70% alcohol as blank after incubating for 15 minutes.

2.7.2 Total phenolic content
1 g sample was weighed into a conical flask. 30 mL of 70% ethanol was then added. The weight of the flask’s content was noticed, and ultrasonic for 2 hr (35 KHz, 46°C). The lost volume was compensated after extraction and filtered. 8 mL of the filtrate was measured into a 100 mL volumetric flask and topped to the mark using 70% ethanol. 1 mL was taken into a 5 ml test tube. 0.15 ml Folin-Ciocalteu reagent was added and shaken for 60 s. 10% sodium carbonate (Na2CO3) was then added and shaken for 30 s. The solution was top to 5 ml using distilled water. The content of the flask was kept in the dark for 100 minutes, and its absorbance was taken at 760 nm against a blank.
Preparation of standard curve 50 mL of 70% alcohol was added to a measured 14 mg standard gallic acid to obtain a concentration of 0.28 mg/mL. 0.2, 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 mL of the standard gallic acid was measured into different beakers. 0.15 mL Folin-Ciocalteu reagent was then added to each sample. The content was shaken for 60 s. 0.20 mL sodium carbonate was then added to the content and shaken for 30 s. The content was kept in the dark for 100 minutes and its absorbance was taken at 760 nm.

2.8 Antimicrobial determination using the paper disk diffusion method
The antibacterial activity was determined by the Kirby-Bauer Disk Diffusion method. It is a simple, practical, reliable, and widely used method for testing the efficacy of antibiotics against specific pathogens. With this method, a highly effective sample (susceptibility) would produce a ring of no bacterial growth, while an ineffective sample (resistant) would show little or no change in the surroundings of the bacterial. The zone of inhibition depends on factors such as the lethality of the sample used as well as the rate of diffusion of the active substance in the sample.

1. Preparation of the bacterial suspension: Bacterial stock cultures were sub-cultured onto BA agar and incubated overnight at 37 °C for 24 hours. Bacterial colonies were then selected from the inoculum plate the next day. The turbidity was then visually compared to that of the prepared control of 0.5 Mc Farland turbidity standard (1.5×108 CFU/mL) using physiological saline.

2. Inoculation of agar plates: The bacteria inoculums (300 µg) were swabbed onto the surface of 20 ml agar medium plates to obtain uniform growth. The inoculated plates were then allowed to dry for 10 minutes.

3. Preparation of antibacterial disks: Sterilized paper disks with 6 mm diameter were soaked into 10 mg/ml of sample solution of the extract for 2 hours so that the disks will absorb the extracts completely. Neomycin (50 µg/mL) and 5% DMSO solution were used as the positive and negative control respectively.

4. Application of disks: Sterilized forceps were used to carry the paper discs onto the inoculated agar plates. After a 24 h incubation period at a temperature of 37°C, the area of no bacteria growth around the paper disc was measured.

3. Statistical analysis
Using SPSS v.20 computer software and Origin 2019 for the charts, data obtained were subjected to One Way Variance Analysis (ANOVA). Different measurements of mean values were made using the Least Significant Difference (LSD). Mean values were rated at an important level of 95% (p<0.05).

4. Results and Discussions
4.1 Nutritional components content
Table 1 shows the results obtained from the proximate analysis of the inflorescence stem of *Rheum tanguticum*. The results showed that the most abundant macronutrient is crude fiber (89.14% ± 0.75), followed by vitamin C (16.96%±0.58), ash content (10.29% ± 0.16), protein (8.48% ± 0.6), and crude fat.
Unfortunately, there is no available information on the nutritional components of the inflorescence stem of *R. tanguticum*. Nevertheless, it has been estimated by Xiong *et al.* in 2003 that 100 g dry petiole of *R. tanguticum* contained 5.84 g protein, 14.86 g crude fiber, 12.91 mg vitamin C. The present study shows that the analysis result of the inflorescence stem of *R. tanguticum* is all higher than those data from the petiole.
The results of the proximate analysis showed that *R. tanguticum* is enriched with protein, crude fiber, crude fat, and vitamin C. This is in support of the findings of Xiong *et al.* that the petioles of *R. tanguticum* have high nutritional attributes, and can be employed in the nutraceuticals industries. The high crude fiber contents (89.14%) in the stem sample show that it is nutritionally potent. It has been reported that the stalks of rhubarb are a potential source of dietary fiber, containing up to 74% Consuming dietary fiber has been reported to reduce cholesterol levels, risk of coronary heart diseases, hypertension, constipation, diabetes, colon and breast cancer.

Table 1: Nutritional contents of the inflorescence stem of *Rheum tanguticum*

| Number | Nutritional content | Mean value |
|--------|---------------------|------------|
| 1      | Ash                 | 10.29±0.16 |
| 2      | Moisture            | 7.68 ±0.19 |
| 3      | Crude fiber         | 89.14±0.75 |
| 4      | Crude fat           | 1.34 ±0.20 |
| 5      | Protein             | 8.49 ±0.60 |
| 6      | Vitamin C           | 16.96±0.58 |

*Mean of 3 replicates*

The high ash content (10.29%), which is an index in determining the mineral content, shows that *R. tanguticum* is a very good source of the mineral element. It has been
reported by Pearson (1976) that plant foods that contain less than 12% of their caloric value from protein are considered to be a poor source of protein. It is therefore empirical to say that the stem of *Rheum tanguticum* does not provide the rich protein content required by the human body [18]. This is also fairly in agreement with an earlier report on proximate compositions of the petiole of *R. tanguticum* (5.84% protein) [15].

### 4.2 Chemical analysis
The flavonoid and total phenolic contents of the inflorescence stem of *R. tanguticum* were found to be 0.022 mg/g and 4.138 mg/g respectively. The biological activities of plants primarily depend on its chemical constituents; including flavones and phenols.

The pharmacological importance of *Rheum* species including *R. tanguticum* is principally attributed to the existence of varied active components such as anthraquinones, anthocyanins, flavonols, stilbenes, phenols, naphthalene, and chromones [19].

Rhubarb species have been employed in TCM since prehistory [20] due to the therapeutic properties in the treatment of many ailments. *Rheum* species are still widely used for various purposes globally [21].

### 4.3 Antioxidant activity
The obtained results of Table 1 were then used to plot a graph of radical scavenging activity (RSA) as the dependent variable against the concentration of the samples as the independent variable (Fig.1), and the effective concentration (IC$_{50}$) was then calculated.

The IC$_{50}$ of a sample is defined as the amount of any active substance needed to achieve 50% of its maximal activity. It shows an indication of the effectiveness of the sample. A lower IC$_{50}$ has higher antioxidant activity and vice versa. The IC$_{50}$ value was then calculated using the graph of % DPPH RSA versus the concentration of the samples as shown in Fig.1.

Ascorbic acid was used as the standard and the different concentrations of the sample were compared to it. The DPPH radical scavenging activity of the sample revealed a 95% effectiveness with an IC$_{50}$ value of 0.485 mg/mL. This shows that a very low concentration of the sample is needed to achieve half of its maximal activity.

![Fig 1: The DPPH RSA of the stem of R. tanguticum](image)

Although, little information was obtained on the antioxidant activity of *R. tanguticum* in the literature, however, *R. tanguticum* was found to downregulate the activities of oxidative mediators which initiate diseases [22]. Nevertheless, the studies on the root and stem of *R. ribes* [23], the rhizomes of *R. nobile* [24], the phenolic constituents from the roots of *R. officinale* [25], and the rhizome of *R. palmatum* [26] have been reported to possess antioxidant activity. The unavailability of antioxidant activity information on the stem of *R. tanguticum* was therefore important.

### 4.4 Antimicrobial activity
All the solvent extracts exhibited a range of antimicrobial activity against the tested organisms at a concentration of 10 mg/mL as shown in Table 2. The mean zones of inhibition obtained were between 6.5 and 11.2 mm as compared to 18.7 and 25.0 mm of the positive control (neomycin). The negative control (5% dimethyl sulphoxide) did not inhibit any of the microorganisms tested. N-hexane extract was more effective in inhibiting the growth of the tested strains as compared to petroleum ether and methanol. The polarities and type of organic solvents, extraction time and temperature, and a number of factors have been reported to have a greater influence on the extraction of plant secondary metabolites that influence the antimicrobial activities of plants’ extracts [27].

The biological activities of plant extracts are primarily due to the presence of several phytochemical compounds such as tannins, saponins, flavonoids, alkaloids, phenols, steroids, terpenoids, phlobatannins, stilbenes etc.

| Organism | Methanol extract | P. ether extract | n-hexane extract | Positive control |
|----------|-----------------|-----------------|-----------------|-----------------|
| E. coli  | 6.5±0.0         | 9.2±2.5         | 11.0±2.0        | 19.3±1.0        |
| B. subtilis | 6.5 ± 0.0      | 10.0 ± 0.9      | 11.2±2.3        | 18.8±0.3        |
| S. aureus | 7.0±0.1        | 8.6±1.2         | 9.5±1.5         | 18.7±0.6        |
| P. aeruginosa | 7.6±0.5       | 9.6±1.2         | 9.6±1.2         | 25.0±5.5        |

The concentration of extracts was 10 mg/mL and 50 μg/mL of neomycin (positive control); petroleum ether; n-hexane, 5% DMSO solution as negative control.

Phytochemical analysis of *R. tanguticum*, have been reported to contain a wide variety of active compounds including anthraquinones and anthrones [19] which have made the plant one of the most important ingredients in the Traditional Chinese Medicines for many years. The obtained result is in fair agreement as to why *Rheum* species, such as *R. tanguticum* *R. officinale* and *R. palmatum*, have been reported by several authors to possess antibacterial activity against a wide spectrum of bacteria, both Gram-positive and Gram-negative microorganisms [28-31].

### 5. Conclusion
The consumption of foods that are rich in antioxidant activity and nutritional components is said to have a positive impact on human lives. The research into antioxidants has rapidly increased over the past decades due to their importance in fighting oxidative-stress related diseases such as inflammation, cancer, skin diseases, heart diseases, eye diseases, etc. that are caused by the oxidation of free radicals in the environment.

Also, the emergence of multi-drug resistant microorganisms
to synthetic drugs has forced scientists to turn their attention to natural sources such as plants. The search for antimicrobials from plant sources has gained much attention over the past decade to identify an antimicrobial from plant sources has gained much attention to natural sources such as plants. The search for antimicrobials from plant sources has gained much attention over the past decade to identify an antimicrobial from plant sources has gained much attention to natural sources such as plants. The search for antimicrobials from plant sources has gained much attention over the past decade to identify an antimicrobial from plant sources has gained much attention to natural sources such as plants.

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