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In vitro evaluation of antioxidant and antimicrobial activities of the root of *Rheum tanguticum*

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Abstract
Rheum tanguticum, belonging to the family Polygonaceae, is one of the most important Traditional Chinese Medicines (TCM) which have been used for centuries. The roots, stems, and rhizomes have been reported to contain active pharmacological components that are of biological importance. The rhizomes and roots of *Rheum* species have been one of the most important and abundant ingredients used in TCM and have been used in the prevention of many diseases since prehistory. It has been reported to possess various pharmacological activities such as anticancer, antifungal, antiviral, antibacterial, purgative, hepatoprotective, anti-inflammatory, anti-platelet aggregation, anticoagulant, etc. An *In vitro* antioxidant activity of the DPPH Radical Scavenging Activity of ethanol, methanol, ethyl acetate, and water extracts of the root of *R. tanguticum* was found to be concentration-dependent and had an IC_{50} of 17.327 µg/mL, 18.848 µg/mL 19.769 µg/mL, and 49.639 µg/mL respectively as compared to the standard, L-ascorbic acid of 7.4 µg/mL. The crude extract was highest in methanol (42.25%), followed by ethanol (30.59%), water (25.55%), and ethyl acetate (10.40%) extracts. The antimicrobial activity of the root of *R. tanguticum* of ethanol, methanol, ethyl acetate, and water extracts was determined using the paper disc and agar well diffusion methods and exhibited a range of activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Pseudomonas solanacarum*, and *Xanthomonas oryzae* as compared to the positive control, neomycin. The high phenolic content, antioxidant and antimicrobial activities exhibited by the root of *R. tanguticum* is an indication of the widely used of the plant as one of the main ingredients in TCM and could be used as a prototype in developing natural cost-effective antibiotics against a wide range of pathogenic microorganisms and oxidative-related diseases.

Keywords: rhubarb, *Rheum tanguticum*, antimicrobial activity, antioxidant activity, DPPH

Introduction

The medicinal herb, *Rheum tanguticum*, belonging to the family Polygonaceae, is one of the three genuine species of rhubarb (*R. palmatum*, *R. officinale*, and *R. tanguticum*) in the Chinese Pharmacopeia. The rhizomes and roots of the plant have been employed by pharmaceutical industries for the manufacturing of various drugs for many years due to the presence of varied pharmacological properties. Rhubarbs are reported to possess purgative, clearing heat-fire, removing toxic materials from the body, and cooling and promoting blood circulation effects (Matsuda *et al*., 2001)[21]. It is endemic to China, and other parts of Asia and Europe**.**

Free radicals, in the form of reactive oxygen and nitrogen species, are an integral part of normal physiology. An over-production of these reactive species can occur due to oxidative stress brought about by the imbalance of the bodily antioxidant defense system and free radical formation. These reactive species can react with biomolecules, causing cellular injury and death. This may lead to the development of chronic diseases such as cancers and those that involve the cardio- and cerebrovascular systems (Wong, Leong, & Koh, 2006). Antioxidants are agents that can augment cellular defenses and help to prevent oxidative damage to cellular components (Halliwell, 1989) [10]. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have been widely used as antioxidants in the food industry and have been responsible for liver damage and carcinogenesis (Grice, 1986, 1988) [8, 9]. One solution to this problem is to supplement the diet with antioxidant compounds that are contained in natural plant sources (Knekt, Jarvinen, Reunanen, & Maatela, 1996) [17]. The goal of antioxidants in fighting diseases and as a booster of good health has been given the necessary recognition. The call for foods containing antioxidants is increasing quickly since people are actualizing the goal of diets that are antioxidants rich to stay healthy. Antioxidants are viewed and valued as a major class in the nutraceutical industries as they help to increase food span by eliminating lipid peroxidation, thereby helping to keep such foods fresh for a longer period. 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 antibiotics from plant sources has gained much attention over the past decade to identify and screen compounds that can replace synthetic antimicrobial drugs. 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. Plants have been the major force behind the manufacturing of traditional medicines throughout the world for thousands of years and continue to provide new remedies to humankind; a lot of efforts have therefore commenced on using new experimental procedures to identify natural antioxidants from plants. Several authors have reviewed the beneficial uses of these plant species (Matkowski & Piotrowska, 2006; Scartezzini & Speroni, 2000) [20, 27]. The rhizomes of *Rheum palmatum* L., R. *tanguticum* Maxim., *R. officinale* Baill., *R. coreanum* Nakai, and *R. undulatum* L., have been reported to possess antioxidant activity (Matsuda *et al*., 2001) [21]. The rhizomes and roots of Rheum species have also been one of the most important and abundant ingredients used in TCM and have been used in the prevention of many diseases since prehistory. This varied pharmacological importance is attributed to the presence of the many bioactive components found in the plant (Aburjai, 2000) [1]. Rheum species have reported to possess anticancer (Chan, Chang, Koonchanok, & Geahlen, 1993) [4], purgative (Feng *et al*., 2008) [6], hepatoprotective (Huang, Siying, Yongsui, & Wangyun, 1998) [12], anti-inflammatory (Mi Kyoung, Kang, Lee, Kim, & Lee, 2006) [23], anti-platelet aggregation, and anticoagulant (Jiachen, Wu, Dong, Li, & Gao, 2018) [13], anti-tumour (Zheng & Zhang, 1993) [35], antibacterial, antifungal activities. In this study, we examined the *In vitro* antioxidant and antibacterial activities of different extracts of the root of *R. tanguticum*.

Materials and Methods

Collection and identification of plant material

Rheum tanguticum roots were collected from Ruoegai County, Aba Tibetan Autonomous Prefecture, Sichuan Province, China, in May 2021 by Prof. Lin Ma of the Department of Plant Biology, School of Life Sciences and Engineering, Southwest University of Science and Technology, China. The root of the plant was then washed thoroughly with running water to remove any debris and dried at room temperature for 21 days. The plant material was pulverized in a plant grinder into fine particles. The sample was stored in a clean rubber glass and stored in a cool dry place for further analysis.

Chemicals

DPPH (1, 1-diphenyl-2-picrylhydrazyl), neomycin, Folin-Ciocalteu reagent, pyrogallic acid, sodium carbonate, Dimethyl sulfoxide (DMSO).

Instruments

Rotary evaporator (RE-52AA, Shanghai Yarong Biochemical Instrument Factory), High-Pressure Steam Sterilizer (MLS-3751L-PC, SANYO Techno Solutions Tottori Co, Ltd,). Single Double-sided Purifying Workbench (SW-CJ-15, Suzhou Purification Equipment Co, Ltd), Precision constant temperature incubator (BPH-9162, Shanghai Yihang Scientific instrument Co, Ltd), UV-VIS spectrophotometer (UV-800, METASH Shanghai Yuanshi Instrument Co, Ltd).

Test organisms

The test strains, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Pseudomonas solanacarum*, and *Xanthomonas oryzae* were provided by Prof. Ma Lin at the laboratory of microbiology, Southwest University of Science and Technology, China. These bacterial cultures were maintained on BA agar.

Total polyphenol content determination

The total polyphenol content was determined with the Folin-Ciocalteu reagent using pyrogallic acid as standard. In a 10ml volumetric flask, 4ml of deionized water was mixed with 0.4ml of the sample, followed by the addition of 0.4ml of Folin-Ciocalteu reagent. The resulting solution was shaken and kept for 5 mins. 4ml of 7% (w/v) Na_2CO_3 solution was added into the reaction mixture, shaken, and the volumetric flask was brought to the mark with deionized water. The absorbance was measured at 730nm using deionized water as blank after incubating the sample in the dark for 90 mins (Koczka, Stefanovits-Bányai, & Ombódi, 2018; Musci & Yao, 2017).

Total polyphenol standard curve: Known concentrations of pyrogallic acid [0.02, 0.04, 0.06, 0.08, 0.10, 0.12mg/ml] were prepared using ethanol. The total polyphenol content of these samples was determined by adding 4ml of deionized water to 0.4ml of the samples in a 10ml volumetric flask. The resulting solution was mixed followed immediately by the addition of 0.4ml of Folin-Ciocalteu Reagent (FCR), shaken, and kept for 5mins. 4ml of 7% Na_2CO_3 was later added and deionized water was added to the 10ml mark. The absorbance was measured at 730nm using deionized water as blank after incubating for 90 mins.

DPPH Radical Scavenging Activity determination

With a slight modification to Blois *et al*. (Blois, 1958) [3], 2mL of the sample (10, 15, 20, 25, 50 µg/mL) was mixed with 3 mL of 0.004% DPPH solution 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. The DPPH RSA was determined using the formula; (Osei, Lin, Wei, Yueweng, & Wang, 2021) [25].

$$\text{DPPH RSA } \% = 1 - \frac{Abs\,(DPPH + Sample) - Abs\,(\text{Sample} + \text{Ethanol})}{Abs\,(DPPH + Ethanol)} \times 100\%$$

Antimicrobial activity determination

The antibacterial activity was determined by using the Kirby-Bauer's paper disc and the agar well diffusion methods (Fiebelkorn, Crawford, McElmeel, & Jorgensen, 2003) [7]. These methods are both sensitive, practical, and widely accepted methods for the determination of the efficacy of plant extracts against a wide range of microorganisms. Both methods measure the zone of no bacterial growth (inhibition) after a known concentration of plant extracts is tested against a bacterial. The size of the zone of inhibition determines the susceptibility or resistance of the antibiotic used.

Test with paper disc diffusion method

In Petri dishes (diameter 90 mm) filled with bacterial growth medium and seeded with 100 µg of the test organism, a 6mm sterile filter disc was impregnated with test concentration, and allowed to dry. The disc was gently applied to the top of the medium using forceps. The zones of growth inhibition around the discs were measured after 24 h of incubation at 37°C. Each microorganism was tested in triplicate and the solvent, 5 % DMSO, was used as a negative control, while 50 µg/mL neomycin was used as a positive control.

Test with agar well diffusion method

Similar to the procedures employed in the disk diffusion method, the agar plate surface was inoculated by spreading 100 µg of the bacterial inoculum over the entire agar surface. A 6 mm well was then punctured in the agar medium with a cork borer and 100 µg of 5 mg/mL of the plant extract was poured into the well. The plates were incubated at 37℃ for 24 hours. The zone of inhibition was then measured and compared to the positive control (50 µg/mL neomycin)

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 % ($p < 0.05$).

Results and discussions

Crude extract yield and IC_{50} of methanol, ethanol, ethyl acetate, and water extracts

The extraction efficiency and phytochemical compositions have been reported to be affected by several factors including; the chemical nature of phytochemicals present, the extraction method used, sample particle size, as well as the solvent, used (Stalikas, 2007) [30]. The most important parameters of a solvent that is considered to have a greater influence on the extraction yield are its polarity and pH, temperature and extraction time of the extraction technique, and the composition of the sample to be extracted. The highest crude extract yield was recorded in methanol (42.25%), followed by ethanol (30.39 %), water (25.55 %), and the least was recorded in ethyl acetate (10.40 %).

Table 1: Crude extract yield and IC_{50} of four solvents

Solvent extract	IC_{50} (µg/mL)	Crude extract yield (%)
Methanol	18.848	42.25
Ethanol	17.327	30.39
Ethyl acetate	19.769	10.40
Water	49.639	25.55
L-Ascorbic acid	7.4	

***In vitro* antioxidant activity assay**

The DPPH Radical Scavenging Activity (RSA) of the methanol, ethanol, and ethyl acetate extracts of the root of *R. tanguticum* were compared to the standard, L-ascorbic acid, as shown in figure 1. According to the results, the DPPH RSA of the extracts was concentration-dependent and had a stronger scavenging activity. The inhibition concentration (IC_{50}) which is an indication of the effectiveness of the sample was found to be highest in ethanol extract (17.327 µg/mL), followed by methanol extract (18.848 µg/mL) ethyl acetate extract (19.769 µg/mL) and the least was recorded in water extract (49.639 µg/mL) as compared to the standard, L-ascorbic acid (7.4 µg/mL). Distilled water extract had the lowest antioxidant activity as compared to the three organic solvents because it has been proven that the antioxidant activity of plant extracts is mainly ascribed to the concentration of the phenolic compounds present in the plants, and water has been reported to be a poor solvent to extract most of these phenolics, hence its low antioxidant activity (Heim, Tagliaferro, & Bobilya, 2002) [11]. Rheum species including *R. tanguticum* have been reported to contain a high amount of antioxidant activities (Alkaya, Seyhan, & Ozturk, 2019; Osei *et al*., 2021) [2, 25]. Antioxidants found in plants have been reported to prevent a lot of diseases found in humans (Liangli *et al*., 2002) [19]. The roots and stem of *R. ribes* have been found to possess high antioxidant activity of 93.1% and 84.1% respectively (Öztürk, Aydoğmuş-Öztürk, Duru, & Topçu, 2007) [26]. *R. rhabarbarum*, and *R. franzenbachii* have also been reported to exhibit considerably higher antioxidant activity (Kalisz *et al*., 2020; Wang *et al*., 2014) [15, 31]. The stem of the Tibetan rhubarb, *R. tanguticum*, has been investigated to possess antioxidant activity (Osei *et al*., 2021) [25].

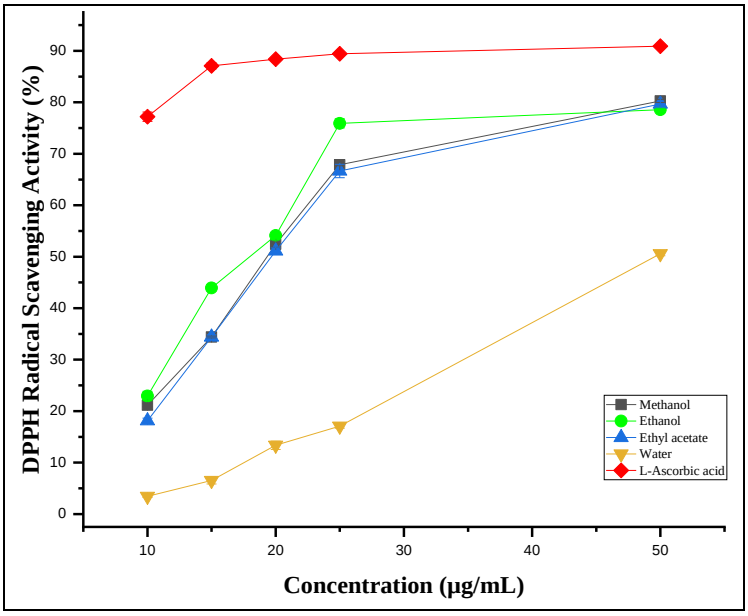

Fig 1: DPPH Radical Scavenging Activity of methanol, ethanol, ethyl acetate, and water extracts of R. Tanguticum

Antioxidants that are found in living cells in a small quantity, cannot stop the damaging effects associated with free radicals in the body (Simic, 1988) [28]. Therefore, there is the need to supplement the antioxidant levels in the body by intake of natural antioxidants that are abundant in plants, fruits, and vegetables. Antioxidants are therefore considered to be vital for human cell growth and their sustainability (Stähelin, Gey, & Brubacher, 1989) [29].

Antimicrobial activity

The antimicrobial activity was determined using the two most widely accepted and reliable methods, the paper disc and agar well diffusion methods. The bacterial, *Pseudomonas aeruginosa, Staphylococcus aureus, Pseudomonas solanacarum, and Xanthomonas oryzae*, were tested against four solvents extracts (methanol, ethanol, ethyl acetate, and water) of the root of *R. tanguticum* as shown in table 1. All the solvent extracts were able to inhibit the growth of the tested bacterial at a concentration of 5 mg/mL. The mean zones of inhibition obtained from the paper disc were between 8.16 and 21.5 mm as compared to 18.3 and 25.3 mm of the positive control. The agar well diffusion method also had a mean zone of inhibition between 11.3 and 24.0 mm as compared to 16.8 and 28.6 mm of the neomycin. The zones of inhibition from the organic solvents were bigger than the distilled water extract. With the paper disc method, methanol extract was more effective in inhibiting the growth of *P. solanacarum, S. aureus, P. aeruginosa,* and *X. oryzae* than the other solvent extracts. With the agar, well diffusion method, methanol, ethanol, and ethyl acetate extracts exhibited a higher zone of inhibition than that of water. Water has been reported by several authors to be a poor solvent to extract phenolic compounds that contribute to the pharmacological importance of plants (Heim *et al*., 2002) [11] and hence the obtained results. Rhubarb and its associated species have been reported to possess a wide variety of active compounds that contribute to the plant's high efficacy and it's been widely used as one of the main ingredients in TCM for years. These compounds found in Rhubarb species have been reported to be lethal against both gram-positive and negative bacteria (Jong-Chol *et al*., 1987; Xiao, He, & Wang, 1984) [14, 33].

Table 2: The zone of inhibition of four solvent extracts (methanol, ethanol, ethyl acetate, and water) against four tested bacterial (*Pseudomonas solanacarum, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Xanthomonas oryzae*)

Organism	***Pseudomonas solanacarum***			***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Xanthomonas oryzae***
Zone of inhibition (mm) of solvent extracts	Methanol	Paper disc	14.0±3.7	19.1±3.1	21.5±3.6	10.0±0.8
		Agar well	15.0±2.9	24.1±0.2	24.0±0.8	15.0±0.8
	Ethanol	Paper disc	10.0±0.8	17.5±3.9	20.3±2.3	10.0±0.8
		Agar well	15.0±0.8	21.1±0.8	22.8±0.6	14.8±0.2
	Ethyl acetate	Paper disc	12.3±1.2	16.5±0.8	18.0±1.6	12.0±0.8
		Agar well	16.0±0.8	23.1±1.2	21.8±1.0	17.1±1.8
	Water	Paper disc	8.3±0.4	17.5±1.8	14.0±0.8	8.16±0.6
		Agar well	12.1±1.0	19.5±0.7	22.1±1.0	11.3±0.4
	Neomycin (Control)	Paper disc	18.3±0.9	25.3±1.2	24.3±0.9	23.1±0.8
		Agar well	16.8±3.7	25.6±1.8	24.6±0.4	28.6±1.2

The concentration of extracts was 5 mg/mL and 50 µg/mL of neomycin (positive control); Methanol, Ethanol, Ethyl acetate, and Water extracts were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Pseudomonas solanacarum, and Xanthomonas oryzae*, 5% DMSO solution as a negative control. Rhubarb species have been employed in TCM since prehistory (You-Ping, 1998) [34] due to their therapeutic properties in the treatment of many ailments. Rheum species are still widely used for various purposes globally (McDougall, Dobson, & Jordan-Mahy, 2010) [22]. The pharmacological importance of *Rheum* species including *R. tanguticum* is principally attributed to the existence of varied active components such as anthraquinones, anthocyanins, flavones, stilbenes, naphthalene, and chromones that are stored in their roots and rhizomes (KASHIWADA, Nonaka, & Nishioka, 1986) [16]. Rheum species have been reported to be effective in fighting a wide spectrum of pathogens including bacterial and fungi (Cyong *et al*., 1987; Osei *et al*., 2021) [5, 21].

Conclusion

Plants have shown over the past few years as a good derivative source for many biological agents, as they yield a wide spectrum of secondary metabolites as an innate defence against pathogen attacks. It is therefore important to screen their products for the remedy of various ailments. The use of plant metabolites to fight infections has been in existence for a long time. Plants owe their pharmacological attributes to the many secondary metabolites they produce, which are employed in medicines, nutraceuticals, and agriculture. Several plant species are under intensive scientific investigations to identify and screen their active compounds, paving for the development of cost-effective and less toxic antimicrobial drugs.

This study shows that the root of *R. tanguticum* has both antioxidant and antibacterial effects and can be exploited further for the isolation of the active constituents that can be used for the treatment of diseases caused by pathogenic microorganisms.

Conflict of interest

The authors declare no conflict of interest.

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