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Phytochemical analysis and antimicrobial activity of *Rheum emodi* (Rhubarb) rhizomes

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Abstract
The present study was under taken to investigate the presence of phytochemical constituents in aqueous and methanol extracts of rhizomes of *Rheum emodi* and to investigate their antibacterial activity. The estimation of phytochemical constituents in methanol proved to be effective solvent for the extraction of secondary metabolites from the rhizomes of *Rheum emodi*. Methanol extract exhibited significantly higher (p<0.05) amounts of secondary metabolites and antibacterial activity against the test organisms at 10mg/ml, 36.7 ± 0.19 and 39.6 ± 0.23 in comparison to standard antibiotics 19.8 ± 0.16 and 19.9 ± 0.21 and aqueous extract and 21.7 ± 0.14 and 18.6 ± 0.15. The methanol extract also exhibited higher zone of inhibition against bacteria from 9-12 mm while aqueous extract showed zone of inhibition from 8-11mm.

Keywords: *Rheum emodi*, phytochemical analysis, extract, zone of inhibition, antimicrobial activity

Introduction
Plants contain a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides, saponins and anthraquinones [1] which confer them a wide variety of pharmacological properties. It is therefore essential to identify the phytochemical constituents from medicinal plants employed in the traditional system for the treatment of various ailments. In addition investigations into antimicrobial activities of medicinal plants will result in the alternate sources of therapeutic agents [2]. *R. emodi* is an important medicinal plant which is extensively used in traditional systems of medicine and has been cultivated over 5000 years for its medicinal properties. Recent studies have showed that *Rheum emodi* possess a wide range of pharmacological activities and have revealed the presence of ninety eight compounds include five flavonoids, twelve anthraquinones, ten stilbenes, sixteen polyphenols and twenty three anthraglycosides and rest others [3]. The Phytochemical constituents from *R. emodi* have been reported to possess antioxidant activity by scavenging free radicals [4], anti-inflammatory activity by attenuating the activity of TNF-α, NFκB, IL-2 and IL-6 [5, 6], anticancer activity by inhibiting the cellular proliferation, induction of apoptosis and prevention of metastasis [7, 8], antidiabetic activity by decreasing the activity of glucose-6-phosphatase, fructose-1,6-diphosphatase, aldolase and increase in the activity of phophohexoisomerase and hexokinase in tissue [9], hepatoprotective activity by restoring the levels of alanine transaminase (ALT), aspartate transaminase (AST), bilirubin, albumin and whole liver homogenate [10]. There are a number of reports about its antifungal, antimicrobial, regulation of blood fat, hepatitis and in the prevention and treatment of Parkinson's disease [11].

Materials and methods
Plant material
The rhizomes of *Rheum emodi* were collected in the month of May - June from Sonamarg area of Kashmir Valley at an altitude of 3000m and got identified at the centre of Plant Taxonomy, Department of Botany, University of Kasmir under voucher no. (Kash-bot/KU/Rh-SB-1746). The plant material (rhizome) was dried in the shade at 30 ± 2 °C. The dried rhizome material was ground into a powder using mortar and pestle and passed through a sieve of 0.3 mm mesh size. The powder obtained was extracted with water and methanol using a Soxhlet extractor (60-80 °C). These extracts were concentrated using the rotary vacuum evaporator and then stored at 4 ^{0}C for future use.

Phytochemical Screening
Chemical tests were conducted on aqueous and methanolic extracts of *Rheum emodi* for qualitative analysis of various phytoconstituents using standard protocols as described by Sofowora 1993, Trease and Evans 1989, Herborne 1973) [12-14].

Quantitative phytochemical estimation:
The alkaloids were estimated spectrophotometrically by the method of Singh and Sah 2006, flavonoids were estimated by the method of Zhishen *et al*., 2010 [15]. Saponins, tannins, phenolic content and sterols were estimated by the methods of Obadoni and Ochuko 2001, Graham 1992, Singleton and Rosi 1965) [16-18].

Test microorganisms
The test organisms were supplied by Department of Microbiology, Government Medical College Srinagar. The bacteria strains used in the study were *Bacillus megaterium* MTCC 1684 and *Psedomonas aeruginosa* MTCC 3541

Preparation of inoculum
Stock cultures were maintained at 4 °C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller-Hinton broth (MHB) and incubated without agitation for 24 h at 37 °C and 25 °C respectively. To 5ml of MHB 0.2 ml of culture was inoculated and incubated till it reached the turbidity equal to that of the standard 0.5 McFarland solution at 600 nm which is equivalent to 10^6– 10^8 CFU/ml.

Antimicrobial assay
Disc diffusion method
Bauer *et al*., 1966 [19] was followed for disc diffusion assay. *In vitro* antimicrobial activity was screened by using Mueller Hinton Agar (MHA) obtained from Himedia. The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 min and 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 min. The different concentrations of extracts (1, 2 and 4 mg/disc) were loaded on 5 mm sterile individual discs. The loaded discs were placed on the surface of medium and the compound was allowed to diffuse for 5 min and the plates were kept for incubation at 37 °C for 24 h. Negative control was prepared using respective solvent. Gentamycin (10 µg/disc) was used as positive control. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate.

Minimum Inhibitory Concentration (MIC) Asssay
The MIC method was applied on extracts that proved their high efficacy against microorganisms by the disk diffusion method [19]. The highest dilution of a plant extract that still retains an inhibitory effect against the growth of a microorganism is known as MIC [20]. Selected plant extracts were subjected to a serial dilution (25 mg/ml to 0.37 mg/ml) using sterile nutrient broth medium as a diluent. In a 96-well titre plate 20 µl of an individual microorganism and 20 µl of selected plant extract were loaded and inoculated at 37^0C for 24 h. The highest dilution of the plant extract that retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism was recorded as the MIC value of the extract. A control experiment was run in parallel to study the impact of the solvent alone (without plant extracts) on growth of test organisms. Methanol was diluted in a similar pattern with sterile nutrient broth followed by inoculation and incubation.

Statistical analysis
All the determinations were carried out in triplicates. The results were expressed as mean ± SE and mean values were plotted in all figures. The level of significance was expressed using Students't-Test. All the analysis was carried out using GraphPad Prism 5 software.

Results and discussions
The rhizomes of plants were extracted using methanol and water by the earlier mentioned method. In the extractive values were found 40.94% and 32.73%, of methanol and aqueous extract of *Rheum emodi* (Table 1). The extracts were subjected to qualitative phytochemical investigation for detection of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, amino acids, terpenoids, tannins, saponins and anthraquinones. The Presence and absence of different phytoconstituents are presented in (Table 2). As per the results depicted in table alkaloids, carbohydrate, tannins, flavonoids, terpenes, anthraquinones, saponins and phenolic compounds were observed to be highly extracted by methanol.

Table 1: Percentage yield of crude extract of *Rheum emodi*

Solvent	Wt. of powdered plant material *R. emodi*	Vol. of solvent	Wt. of extract of *R. emodi*	% yield of extract of *R. emodi*
Aqueous	25 gm	150ml	6 gm	40.94
Methanol	25 gm	150ml	9 gm	32.73

Table 2: Phytochemical Screening of *Rheum emodi*

	Rheum emodi	
Tests	**Methanolic**	**Aqueous**
Alkaloids	+++	++
Carbohydrates	++	+
Tannins	++	+
Steroids	+	+
Flavonoids	+++	++
Terpenes	+++	++
Glycoside	++	+
Terpenoids	++	+
Anthraquinones	+++	++
Saponins	+++	++

Amino acids	-	-
Phenol	++	+
Anthocyanin	+	+

+ = trace amount, ++ = moderately present, +++ = highly present, - = absent

The result obtained from the quantitative estimation of alkaloids, flavonoids, saponins, tannins, phenolics and sterols from methanol and aqueous extracts are depicted in table 3. The quantitative estimation of crude extract of *Rheum emodi* in aqueous and methanol indicates that methanol system was potent in the extraction of alkaloids, flavonoids, phenolics and sterols while aqueous extract was found to be a significant solvent for the extraction of saponins and tannins. The overall results indicate that methanol was the best solvent in the extraction of the phytochemical constituents.

Table 3: Quantitative estimation of phytochemical constituents in Aqueous and Methanol extract

Extract	Alkaloids mg/g	Flavonoids mg/g	Saponins mg/g	Tannins mg/g	Sterols mg/g	Phenolics mg/g
Aqueous Extract	135±0.12	143 ± 0.17	175 ± 0.09	179 ± 0.16	133 ± 0.19	235 ± 0.21
Methanol extract	162±0.19*	185 ± 0.21*	157 ± 0.23*	162 ± 0.17*	149± 0.18*	271±0.27*

Values are mean of three determinations ± standard error (SE)

Antibacterial activity of *Rhum emodi*

The antibacterial activities of *Rheum emodi* against the bacterial strains *Bacillus megaterium* MTCC 1684 (Gram-positive) whose virulent strains can cause anthrax, food poisioning, sepsis, meningitis, soft tissue infection and many more and *Pseudomonas aeruginosa* MTCC 3541 (Gram-negative) which can cause urinary tract infection, gastrointestinal infection, septic shock, pneumonia, skin and soft tissue infections along with standard antibiotics *erythromycin* and *tetracycline* are shown in table 4. The four concentrations 2.5 mg/ml, 5mg/ml, 7.5 mg/ml and 10 mg/ml of the extract were used to study the zone of inhibition (Fig. 1 and 2). The extracts exhibited broad spectrum antimicrobial activity at10 µg/ml by inhibiting the growth of the test microorganisms. The methanolic extract of *Rheum emodi* showed significant antimicrobial activity when compared to aqueous extract. At 10 mg/ml concentration the methanol extracts of *Rheum emodi* showed significant rate of inhibition against the tested microorganisms to the standard antibiotics.

Table 4: Antimicrobial activity of aqueous and methanol extract of *R. emodi*

Microorganism	*R. emodi* A. extract	*R. emodi* M. extract	*Erythromycin*	Tetracyclin
B. megaterium MTCC 1684	21.7±0.14	36.7±0.19	22.9±0.14	19.8±0.16
P. aeruginosa MTCC 3541	18.6±0.15	39.6±0.23	20.7±0.23	19.9±0.21

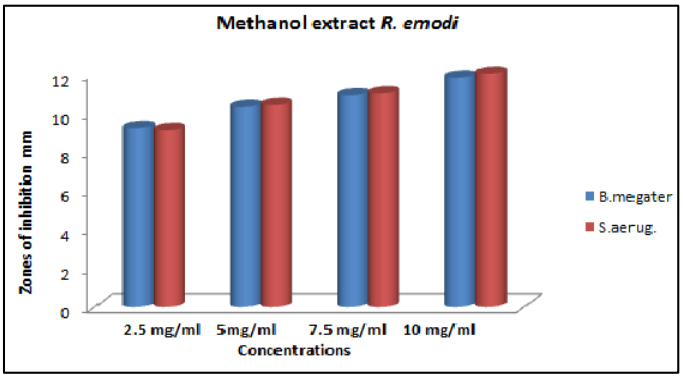

Fig 1: Zones of inhibition (mm) in *Rheum emodi* methanol extract

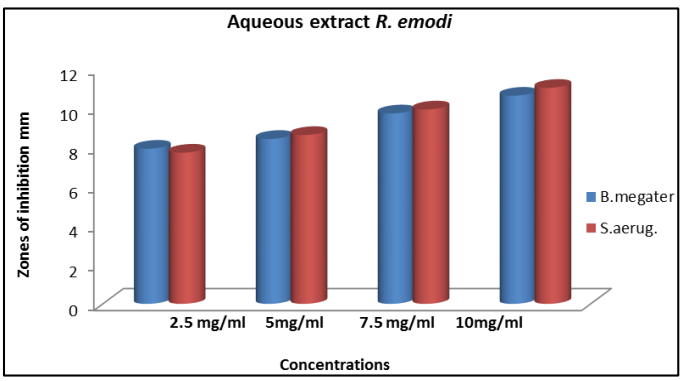

Fig 2: Zones of inhibition (mm) in *Rheum emodi* aqueous extract

Qualitative and quantitative estimation of phytochemical constituents revealed the presence of various phytochemicals present in plant responsible for various pharmacological activities. The study showed the presence of glycosides, flavonoids, saponins, tannins, terpenes, alkaloids, steroids and anthraquinones. The amino acids were found to be absent. Qualitative estimation also revealed the presence of carbohydrates and anthraquinones in *Rheum emodi*. Srinivasarao *et al.*, 2015 [21] also reported the presence of glycosides, flavonoids, terpens, alkaloids, saponins, terpenoids, steroids, carbohydrates, and anthraquinones in *Rheum emodi* extracts however our study contradicts with Srinivasarao *et al*., 2015 [21] in that they reported the presence of amino acids in the *Rheum emodi* extract while in the present study no amino acids were found. Wani *et al*., 2013 [22] Towseef and Salam 2015 [23] also reported the absence of amino acids in the extract of *Rheum emodi* and presence of other phytochemical constituents so the present findings are in concurrence with the findings of Wani *et al*., 2013 [22], and Towseef and Salam 2015 [23].

The antimicrobial activities of aqueous and methanolic extract of *Rheum emodi* against the bacterial strains used were assessed by the presence of inhibition zones. The aqueous extract of *Rheum emodi* at minimum inhibitory concentrations (MICs) of 2.5 mg/ml, 5 mg/ml, 7.5 mg/ml and 10 mg/ml inhibited *Bacillus megaterium* MTCC 1684 and *Pseudomonas aeruginosa* MTCC 3541 with zone of inhibition ranging from 7.5-11mm while methanolic extracts of same concentrations inhibited the growth of bacterial strains ranging from 8-12.3 mm. The antibacterial of *Rheum emodi* has also been reported by many workers [24, 25]. Reports on the antimicrobial activityof *Rheum emodi* against *Bacillus subtilis* and *Pseudomonas aeruginosa* has also been reported by Rehman *et al*., 2014 [26].

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