Original Article

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Biochemistry Section

Efficacy of Aqueous and Methanolic Extracts of *Rheum Spiciformis* against Pathogenic Bacterial and Fungal Strains

KHALID BASHIR DAR[1], AASHIQ HUSSAIN BHAT[2], SHAJRUL AMIN[3], SUHAIL ANEES[4], AKBAR MASOOD[5], MOHAMMED IQBAL ZARGAR[6], SHOWKAT AHMAD GANIE[7]

ABSTRACT

Introduction: *Rheum spiciformis* is a newly identified edible medicinal plant of genus *Rheum*. The plant is used to treat various diseases on traditional levels in Kashmir Valley, India.

Aim: To evaluate the phytochemical screening, antibacterial and antifungal potential of aqueous and methanolic extracts of *Rheum spiciformis*, a traditionally used edible medicinal plant.

Materials and Methods: Methanolic and aqueous extracts of *Rheum spiciformis* were tested for their antimicrobial activities against six bacterial strains namely *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Escherichia coli* and four fungal strains *Penicillium chrysogenum*, *Aspergillus fumigatus*, *Candida albicans* and *Saccharomyces cerevisiae.* The susceptibility of microbial strains to the two extracts was determined using agar well diffusion method. Phytochemical screening was carried out by using various standard procedures.

Results: Methanolic extract showed potent antimicrobial activity as compared to aqueous extract at the concentrations of 10, 30, 50, 80 and 100mg/ml. The most susceptible bacterial strains were *Staphylococcus aureus* with zone of inhibition (25±0.10mm), *Klebsiella pneumonia* (23±0.25mm), *Proteus vulgaris* (22±0.10mm) at the concentration of 100mg/ml. Aqueous extracts at the higher concentration were found effective against *Proteus vulgaris* and *Bacillus subtilis* with zone of inhibition (17±0.24mm) and (17±0.10mm), respectively. Among fungal strains the most susceptible were *Aspergillus fumigatus* (21±0.10mm), *Saccharomyces cerevisiae* (20±0.20mm) and *Penicillium Chrysogenum* (17±0.15mm) at the concentration of 100mg/ml methanol extract. The zone of inhibition for aqueous extract against fungal strains ranged between 14±0.13mm to 16±0.19mm at the highest concentration of plant extract. Phytochemical analysis revealed the presence of various secondary metabolites like flavonoids, saponins, volatile oils, phenols, steroids, terpenoids and alkaloids.

Conclusion: Our results indicate that this plant has enough potential to serve as an excellent candidate for obtaining antimicrobial compounds to combat bacterial and fungal infections.

Keywords: Antimicrobial activity, Extracts, Phytochemicals

INTRODUCTION

Pathogenic microbes have always been a matter of serious concern for human health by causing various infections and diseases. From times human beings have become victim of pathogenic diseases such as cholera, typhoid, chicken pox, syphilis, malaria, candidiasis, aspergillosis, cryptococcosis and shigellosis. In fact the diseases caused by infectious microbes are one of the leading causes of morbidity and mortality especially in developing countries [1,2]. Before the era of antibiotics, people on all parts of the world used traditional herbal medicine to protect themselves from the attack of microbial infection. With the discovery of antibiotics in 20th century, the use of synthetic or semi synthetic antimicrobials increased. However with the passage of time, the overuse of these drugs led to the development of Multiple Drug Resistant (MDR) microbes, which are no longer treatable with the currently available commercial drugs. Several mechanisms such as target site modification, metabolic inactivation and expression of the efflux pumps have been proposed to contribute the development of MDR strains of microbes [3]. Time has come to search for alternative sources of antimicrobial drugs otherwise the increasing trend of antimicrobial resistance will engulf the whole world and kill millions. Medicinal plants are the best sources for the development of futuristic antimicrobial therapeutics and are rich in compounds like alkaloids, phenols, flavonoids, terpenoids, naphthoquinones and essential oils which possess potent antimicrobial properties [4]. The present work has been carried out to evaluate the antibacterial and antifungal potential of *Rheum spiciformis,* a plant that belongs to the family polygonaceae and is locally named as "*Pamb challan*". It is an herbaceous plant, growing from fleshy roots. The plant has somewhat triangular shaped large leaves with long, fleshy petioles. The herb is edible and used traditionally as purgative [5]. This is the first study to report the antibacterial and antifungal properties of *Rheum spiciformis.*

MATERIALS AND METHODS

Plant Collection and Extraction

Preliminary phytochemical screening and *in vitro* antibacterial and antifungal activity of *Rheum spiciformis* extracts were evaluated by using various pathogenic bacterial and fungal strains. This study was conducted from the month of February 2015 to July 2015. The rhizome of *Rheum spiciformis* was collected from higher reaches of Kashmir valley, India and identified by the Centre of Plant Taxonomy, Department of Botany, University of Kashmir, and authenticated by Akhter Hussain Malik (Curator, Centre for Plant Taxonomy, University of Kashmir). A reference specimen has been retained in the herbarium of the Department of Botany at the University of Kashmir under reference number 2320-KASH. The plant material (rhizome) was dried in the shade at 30±2°C. Then, 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 methanol and water for 48h using a Soxhlet extractor (60-80°C). The extract was then concentrated with the help of rotary evaporator under reduced pressure and the solid extract was stored in refrigerator for further use.

Test Micro-organisms

Bacterial and fungal strains were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh (India), with specific microbial type culture collection (MTCC) numbers. Six bacterial strains, including two Gram positive bacteria, namely *Staphylococcus aureus* (MTCC-2940), *Bacillus subtilis* (MTCC-441) and four Gram negative bacteria, namely *Proteus vulgaris* (MTCC-426), *Klebsiella pneumoniae* (MTCC-139), *Escherichia coli* (MTCC-739) and *Pseudomonas aeruginosa* (MTCC-424), were employed for antibacterial assay. Four fungal strains, *Candida albicans* (MTCC-227), *Saccharomyces cerevisiae* (MTCC-170), *Aspergillus fumigatus* (MTCC-1811) and *Penicillium chrysogenum* (MTCC-947) were used for antifungal assay. Bacterial and fungal strains were maintained by subculturing them on Mueller Hinton Agar (MHB) and Sabouraud Dextrose Agar (SDA), respectively after every 15 days and then stored at 4ºC. Gentamycin (discs) and Nystatin (powder) was obtained from EOS Laboratories, India and served as positive controls for antibacterial and antifungal assays respectively. A 10% Dimethylsulfoxide (DMSO) was used as negative control.

Antibacterial Assay

Antibacterial activity of aqueous and methanolic extract was evaluated by agar well diffusion method as described by Irshad [6] with some modifications. Standardized inoculum (100µl) of (0.5 Mc Farland) of each test bacterium was inoculated on molten Mueller Hinton Agar, homogenised and then poured into sterile petri plates to yield a uniform depth of 4mm. The petriplates were allowed to solidify inside the laminar hood. Sterile cork borers of 5mm in diameter were used to make uniform and equidistant wells into each petriplate. Plant extracts at the concentration of (10mg/ml, 30mg/ml, 50mg/ml, 80mg/ml and 100mg/ml) were loaded into different peripheral wells. Gentamycin (10µg/disc) disc was placed at the centre of each petriplate and served as positive control, while as 10% Dimethylsulfoxide served as negative control in a separate petri plate. The petri plates were then incubated at 37ºC for 18 to 24 hours in an incubator. The plates were then observed for the zones of inhibition. Antibacterial potential was evaluated by measuring the diameters of zones of inhibition in millimeters (mm) with the help of a standard measuring scale.

Antifungal Assay

Antifungal activity of the aqueous and methanolic extracts was also performed by the method of agar well diffusion as described by Ahmad [7] with some modifications. A 100µl of standardized inoculum (0.5 Mc Farland) of each test fungi were inoculated on sterile molten Sabouraud Dextrose Agar, homogenised and poured into a sterile petri plate to yield a uniform depth of 4mm. The petriplates were allowed to solidify inside the laminar hood. Sterile cork borers of 5mm in diameter were used to make five wells at periphery and one well at centre of each petriplate. A 100µl of each concentration (10mg/ml, 30mg/ml, 50mg/ml, 80mg/ml and 100mg/ml) of plant extract were loaded into five different peripheral wells. A 100µl of standard antibiotic Nystatin (0.5mg/ml) was loaded into the central well while as 10% Dimethylsulfoxide alone was used as negative control in a separate petri plate. The plates were then incubated at 32ºC for 24 to 36 hours. After incubation period, the plates were observed for the zones of inhibition. Antifungal potential was evaluated by measuring inhibition zone diameters in millimeters (mm) with the help of standard measuring scale.

Phytochemical Analysis

Preliminary phytochemical profiling of different extracts of *Rheum spiciformis* was determined according to the method of Harborne [8].

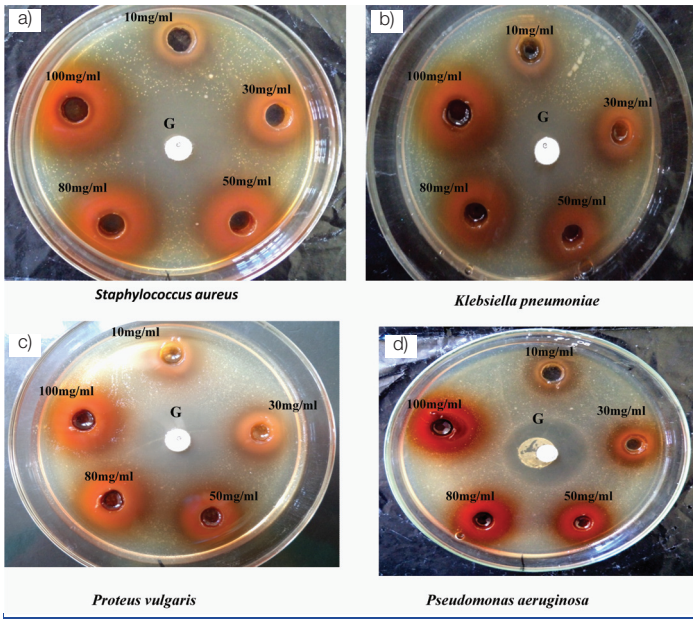
STATISTICAL ANALYSIS

The values are expressed as mean ± standard deviation (SD). The results were evaluated by using the SPSS (version 12.0) and evaluated by one way ANOVA followed by Bonferroni t-test.

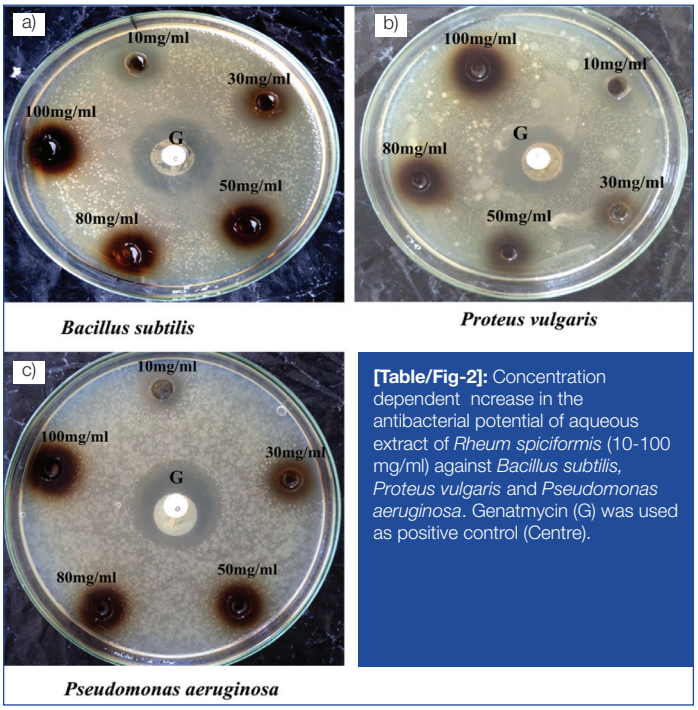
RESULTS

Antibacterial Activity

Out of the two extracts used, methanolic extract showed maximum activity against all the tested bacterial strains with the zone of inhibition equal to 25±0.10mm (*Staphylococcus aureus*), 23±0.25mm (*Klebsiella pneumoniae*), 22±0.10mm (*Proteus vulgaris*), 20±0.12mm (*Pseudomonas aeruginosa*) [Table/Fig-1], 19±0.14mm (*Bacillus subtilis*) and 16±0.30mm (*Escherichia coli*) at the concentration of 100mg/ml. The aqueous extract also showed considerable activity with the zones of inhibition 17±0.10mm against *Bacillus subtilis* and *Proteus vulgaris*, 15±0.20mm against *Pseudomonas aeruginosa* [Table/Fig-2] and14±0.23mm against *Klebsiella pneumoniae, Staphylococcus aureus* and 14±0.26



[Table/Fig-1]: Concentration dependent increase in the antibacterial potential of methanolic extract of *Rheum spiciformis* (10-100 mg/ml) against *Staphylococcus aureus, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa*. Genatmycin (G) was used as positive control (Centre).



[Table/Fig-2]: Concentration dependent ncrease in the antibacterial potential of aqueous extract of *Rheum spiciformis* (10-100 mg/ml) against *Bacillus subtilis, Proteus vulgaris* and *Pseudomonas aeruginosa*. Genatmycin (G) was used as positive control (Centre).

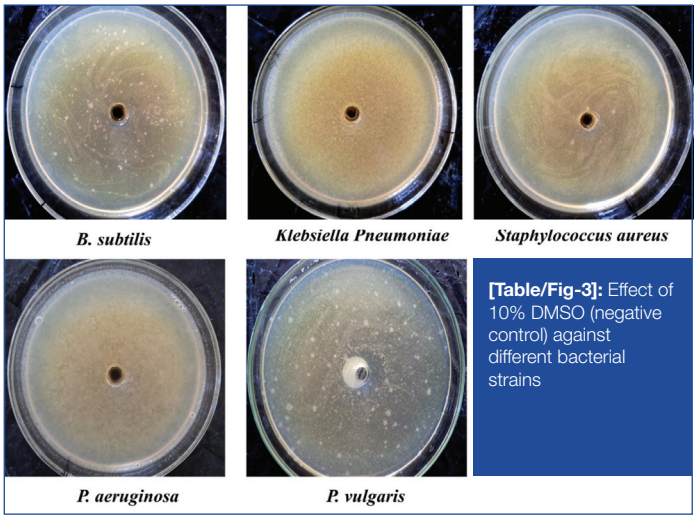
against *Escherichia coli* at the concentration of 100mg/ml. DMSO (10%) was used as negative control [Table/Fig-3]. The results were compared to positive control (Gentamycin), which showed the zone of inhibition of 25±1.10mm against *Klebsiella pneumoniae, Bacillus subtilis, Proteus vulgaris* and *Pseudomonas aeruginosa,* 27±1.23mm against *Staphylococcus aureus* respectively and 20±1.29mm against *Escherichia coli* as shown in [Table/Fig-4].

Antifungal Activity

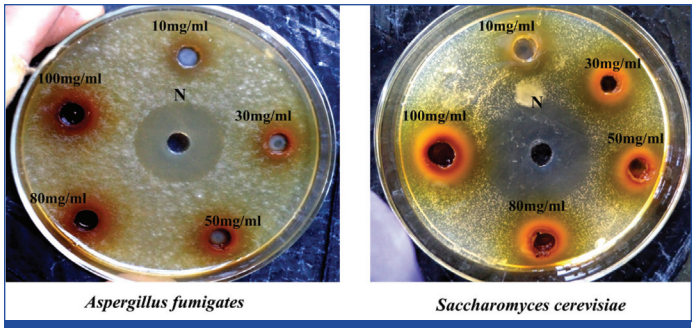
As far as the antifungal activity is concerned methanolic extract showed maximum activity against all the tested fungal strains with the zones of inhibition equal to 21±0.10mm (*Aspergillus fumigatus*), 20±0.20mm (*Saccharomyces cerevisiae*) [Table/Fig-5], 16±0.19mm (*Candida albicans*), 17±0.15mm (*Penicillium chrysogenum*), at the concentration of 100mg/ml. The aqueous extract showed the inhibition zone of 15±0.15mm against *Aspergillus fumigatus* and 15±0.23mm against *Candida albicans* [Table/Fig-6], 14±0.13mm against *Penicillium chrysogenum* and 16±0.19mm against *Saccharomyces cerevisiae,* at the concentration of 100mg/ml. 10% DMSO (negative control) showed no activity against any of the tested fungal strains [Table/Fig-7]. The results were compared to positive control (Nystatin) which showed the zones of inhibition equal to 30±1.39mm against *Candida albicans,* 30±1.48mm against *Saccharomyces cerevisiae,* 25±0.98mm against *Penicillium chrysogenum* and 27±1.29mm against *Aspergillus fumigatus* as shown in [Table/Fig-8].

Phytochemical Analysis

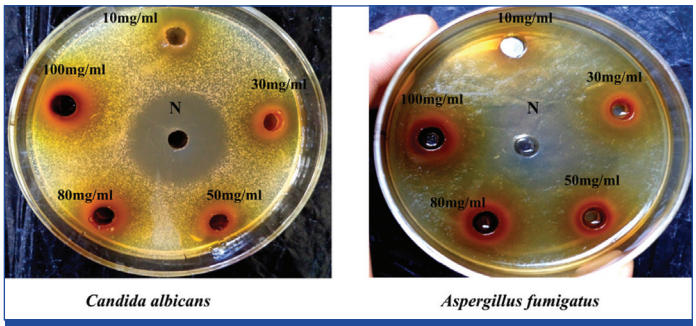
Analysis of phytochemical composition of *Rheum spiciformis* revealed that the plant is rich in all the tested phytochemicals.



[Table/Fig-3]: Effect of 10% DMSO (negative control) against different bacterial strains



[Table/Fig-5]: Concentration dependent increase in the antifungal potential of methanolic extract of *Rheum spiciformis* (10-100 mg/ml) against *Aspergillus fumigatus* and *Saccharomyces cerevisiae*. Nystatin (N) was used as positive control (Centre).



[Table/Fig-6]: Concentration dependent increase in the antifungal potential of aqueous extract of *Rheum spiciformis* (10-100 mg/ml) against *Aspergillus fumigatus* and *Candida albicans*. Nystatin (N) was used as positive control (Centre).

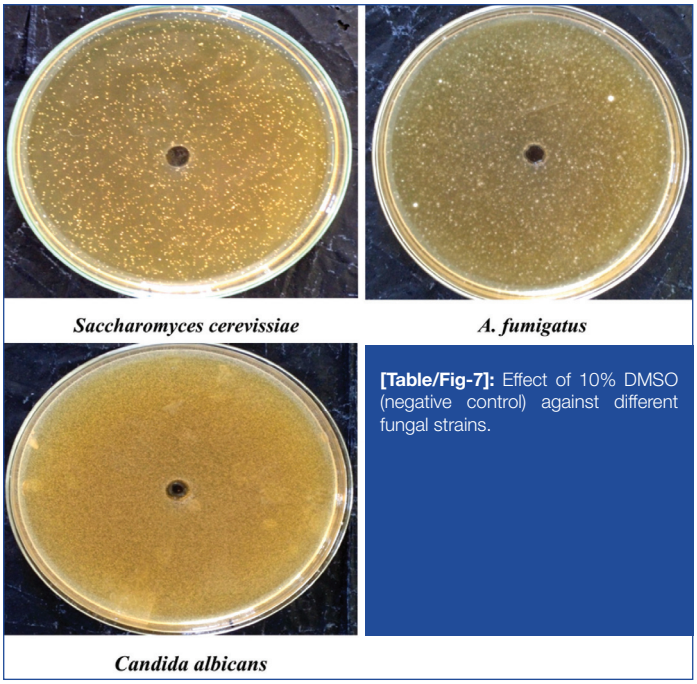
Flavonoids, phenols, phlobtannins, saponins, tannins and volatile oils were present in both the aqueous and methanolic extracts. Terpenoids, steroid, cardenolides and cardiac glycosides were present only in aqueous extract, while as alkaloids and anthraquinones were detected only in methanolic extract as shown in [Table/Fig-9].

DISCUSSION

The excessive use of antibiotics has led to the growing problem of antimicrobial resistance [9]. There is desperate need for the development of alternate antimicrobial drugs. Medicinal plants are the greatest sources on this planet for obtaining antimicrobial compounds [10]. The current study aimed at evaluating the antibacterial and antifungal potential of *Rheum spiciformis*. The methanolic extract showed the zone of inhibition ranging between 16-25mm against bacterial strains and 16-21mm against fungal strains at the maximum concentration (100mg/ml). The aqueous

		Concentration of plant extract					
Bacterial strain	Solvent	10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml	Gentamycin (10µg/disc)
Klebsiella pneumoniae	Aqueous	11±0.27	11±0.25	13±0.44	14±0.59	14±0.23	25±1.22
	Methanol	14±0.28	18±0.26	20±0.11	22±0.22	23±0.25	
Escherichia coli	Aqueous	12±0.58	12±0.26	13±0.38	13±0.28	14±0.26	20±1.29
	Methanol	12±0.21	12±0.15	13±0.20	14±0.28	16±0.30	
Proteus vulgaris	Aqueous	11±0.23	14±0.28	15±0.21	16±0.36	17±0.24	25±1.10
	Methanol	16±0.26	17±0.27	18±0.33	20±0.14	22±0.10	
Staphylococcus aureus	Aqueous	11±0.29	12±0.21	12±0.20	13±0.22	14±0.23	27±1.23
	Methanol	15±0.25	18±0.12	21±0.20	23±0.13	25±0.10	
Pseudomonas aeruginosa	Aqueous	11±0.24	12±0.31	12±0.21	13±0.18	15±0.20	25±1.58
	Methanol	13±0.14	15±0.28	17±0.38	19±0.23	20±0.12	
Bacillus subtilis	Aqueous	13±0.22	14±0.19	15±0.15	16±0.21	17±0.10	25±1.88
	Methanol	13±0.22	15±0.29	17±0.27	18±0.13	19±0.14	

[Table/Fig-4]: Zones of inhibition (in millimeter) of aqueous and methanolic extract of *Rheum spiciformis* against bacterial strains. Each value represents the mean±SD of three independent experiments and evaluated by one way ANOVA followed by the Bonferroni t-test.



[Table/Fig-7]: Effect of 10% DMSO (negative control) against different fungal strains.

reported that the type of solvent used has an important role in determining the activity of the extract [13-16]. This is due to the difference in the relative solubility of different phytochemicals in the solvents with different polarities. The study also reveals that the plant is more potent against bacterial strains as compared to fungal strains. These results are in agreement with that of an early study [17,18]. The systemic phytochemical analysis of plant extracts is an important strategy to find the new lead compounds of therapeutic value [19]. Phytochemical analysis of *Rheum spiciformis* revealed that the plant is rich in compounds like flavonoids, phlobtannins, saponins, steroids, steroids, alkaloids, anthraquinones, tannins, phenols, terpenoids, cardiac glycosides and cardenolides, which could be responsible for its antibacterial and antifungal properties. Phytochemicals exert antimicrobial effect via diverse mechanisms like intercalation of DNA, destruction of cell membrane, inactivation of microbial adhesions and enzymes [20].

LIMITATION

The limitations of the present study include the use of crude extracts and needs further investigations for the isolation of pure antimicrobial compounds and testing their selective toxicity and efficacy using *in vitro* and *in vivo* experiments.

		Concentration of plant extract					
Fungal strain	Solvent	10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml	Nystatin (0.5mg/ml)
Aspergillus fumigatus	Aqueous	11±0.26	12±0.28	13±0.31	14±0.28	15±0.15	27±1.29
	Methanol	11±0.28	13±0.31	15±0.28	18±0.25	21±0.10	
Candida albicans	Aqueous	9±0.12	12±0.33	13±0.35	14±0.20	15±0.23	30±1.39
	Methanol	11±0.19	13±0.23	14±0.25	15±0.32	16±0.19	
Penicillium chrysogenum	Aqueous	9±0.25	12±0.32	13±0.22	14±0.12	14±0.13	25±0.98
	Methanol	11±0.23	13±0.31	15±0.25	16±0.26	17±0.15	
Saccharomyces cerevisiae	Aqueous	8±0.21	12±0.31	14±0.17	15±0.22	16±0.19	30±1.48
	Methanol	14±0.33	15±0.23	16±0.29	18±0.25	20±0.20	

[Table/Fig-8]: Zones of inhibition (in millimeter) of aqueous and methanolic extract of *Rheum spiciformis* against fungal strains. Each value represents the mean±SD of three independent experiments and evaluated by one way ANOVA followed by the Bonferroni t-test.

Phytochemicals	Aqueous extract	Methanol extract
Alkaloids	-	+
Anthraquinones	-	+
Cardiac glycosides	+	-
Cardenolides	+	-
Flavonoids	+	+
Phenols	+	+
Phlobtannins	+	+
Saponins	+	+
Steroids	+	-
Tannins	+	+
Terpenoids	+	-
Volatile oils	+	+

[Table/Fig-9]: Preliminary phytochemical screening of aqueous and methanolic extracts of *Rheum spiciformis*
+ (present), - (absent)

extract exhibited the zone of inhibition ranging between 14-17mm against bacterial strains and 14-16mm against fungal strains at the maximum concentration (100mg/ml). *Staphylococcus aureus, Klebsiella pneumoniae* and *Proteus vulgaris* were the most susceptible bacterial strains while *Aspergillus fumigatus* and *Penicillium chrysogenum* were the most susceptible fungal strains. In general, methanolic extract was found to be more potent than aqueous extracts, against both the bacterial and fungal strains, which is in agreement with the results obtained by previous studies [11,12]. Besides, various studies have

CONCLUSION

The current research has proved that *Rheum spiciformis* contains compounds with antibacterial and antifungal potential. There is need for the isolation and structure elucidation of such compounds which could be the alternate futuristic drugs to combat the growing trend of antimicrobial resistance.

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PARTICULARS OF CONTRIBUTORS:
1. M.Phil Student, Department of Clinical Biochemistry, Faculty of Biological Sciences, University of Kashmir, Jammu and Kashmir, India.
2. PhD Student, Department of Clinical Biochemistry, Faculty of Biological Sciences, University of Kashmir, Jammu and Kashmir, India.
3. Associate Professor, Department of Biochemistry, Faculty of Biological Sciences, University of Kashmir, Jammu and Kashmir, India.
4. M.Phil Student, Department of Clinical Biochemistry, Faculty of Biological Sciences, University of Kashmir, Jammu and Kashmir, India.
5. Professor and Dean, Department of Biochemistry, Faculty of Biological Sciences, University of Kashmir, Jammu and Kashmir, India.
6. Associate Professor, Department of Pharmaceutical sciences, Faculty of Applied Sciences, University of Kashmir, Jammu and Kashmir, India.
7. Assistant Professor, Department of Clinical Biochemistry, Faculty of Biological Sciences, University of Kashmir, Jammu and Kashmir, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Showkat Ahmad Ganie,
Assistant Professor, Department of Clinical Biochemistry, Faculty of Biological Sciences,
University of Kashmir, Jammu and Kashmir-190006, India.
E-mail: showkat_ganie786@yahoo.com

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