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Research Article

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Antimicrobial potential of rhizome extract of herb *Rheum emodi* against UTI bacterial strains.

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Abstract

The aim of present study was to investigate the *in-vitro* antibacterial potential of 90% ethanol extract of rhizome of herb *Rheum emodi*. Agarcup-plate method was used to investigate the different concentration of extract against Urinary track infection causing bacteria (*Bacillus subtilis*, *Proteus vulgaris*, *Pseudomonas flouresence*, *Staphylococcus aureus*, *Enterobacter* and *Staphylococcus choni*). Phytochemical screening revealed, the presence of Phenolics, Saponins, and Flavonoids in the 90% ethanol extract. Extract show maximum zone of inhibition 23.59±0.11 in *Bacillus subtilis* at concentration of 100mg/ml. while *Enterobacter*, *S. aureus*, *S. cohni*, *Proteus vulgaris*, *P.f louresence* zone of inhibition 21.98±0.87, 12.92±0.21, 11.98±0.43, 11.99±0.019, 18.89±0.54 respectively. From the present investigation it is revealed that 90% ethanol extract of rhizome have significant potential to inhibit the growth of UTI bacterial strains.

Keywords: Antibacterial, UTI, inhibition, *Rheum emodi*, 90% Ethanol

Introduction

Urinary tract is divided into an upper portion, composed of kidneys, renal pelvis, and ureters and a lower portion made up of urinary bladder and urethra. UTI is an inflammatory response of the urothelium to bacterial invasion .UTI may involve only the lower urinary tract or both the upper and lower tract. UTI are the most frequent bacterial infection in women. [2] The harmful microorganisms can be controlled with drugs and these results in the emergence of multiple drug-resistant bacteria and it has created alarming clinical situations in the treatment of infections. The pharmacological industries have produced a number of new antibiotics; resistance to these drugs by microorganisms has increased. Bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents.[3] Plant-derived medicines have been part of traditional health care in most parts of the world for thousands for year [4]. More than 80% of the population in developing countries depends on plants for their medical needs [5, 6]. In India, medical plants are widely used by all sections of people either directly as folk remedies or in different indigenous medicinal plants and their therapeutic values [7]. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action.[8,9] The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world.[10] Much work has been done on ethnomedicinal plants in India.[11] Plants are rich in a wide variety of secondary metabolites such as tannins,

terpenoids, alkaloids, flavonoids, glycosides, etc., which have been found *in vitro* to have antimicrobial properties [12,13].*Rheum emodi* Wall.ex Meissn, is a leafy perennial herb [14], belongs to the family Polygonaceae. Traditionally it is used to treat pathological ailments like ulcers, bacterial and fungal infections, kidney stone, gout and jaundice [15]also as laxative, tonic, diuretic and to treat fever, cough, indigestion, menstrual disorder since antiquity [16], known to posses Anticancer**,** Antidyslipidemic**,** Antidiabetic**,** Anti Parkinson, Antiplatelet, Anticoagulant, Severe Acute Respiratory Syndrome, Antiulcer, Nephroprotective, Immune-enhancing, Hepatoprotective activity, Antifungal and Antibacterial.[17] However antibacterial activities has been reported earlier in *rheum emodi* [18,19] but no work is done on UTI bacteria till date, so in that context we aim to study the effect of 90% ethanol extract of rhizome against UTI bacteria.

Materials and Methods

Collection and Processing of plant material

The rhizome of *Rheum emodi* were collected from Pabbar valley of Himachal Pradesh in the month of September-October 2017. The collected rhizomes were washed thoroughly under running tap water and then rinsed in distilled water, they were allowed to dry for some time. Then these rhizomes were shade dried for about 5 to 7 weeks and coarse powder was made with the help of a grinder.

Extraction of plant material

The coarse powder of rhizome was extracted sucessively in Soxhlet apparatus using solvents *viz* petroleum ether, chloroform, ethyl acetate and 90% ethanol. The extraction was done for 48 hours in each solvent. The crude extracts thus obtained were then filtered through Whatmann filter paper No. 1. The filtered extracts were concentrated under reduced pressure in a rotary vaccum evaporator. The amount of crude extracts obtained thus weighed and % yield was calculated.

Phytochemical screening

Phytochemical screening of the extracts was carried out according to the standard procedures [20, 21].(Table 1)

Test Organism Used

Bacteria causing urinary tract infection (UTI) in humans *Bacillus subtilis*, *Proteus vulgaris*, *Pseudomonas flouresence*, *Staphylococcus aureus*, *Enterobacter* and *Staphylococcus choni* were used for study all are procured from Pinnacle Biomedical Research Institute (PBRI) Bhopal (M.P.) India.

Antibacterial Assay

90% ethanolic extract of rhizome was evaluated against UTI bacteria. The antibacterial activity was performed using Agar cup-plate method. 24 hours culture of bacterial strains were freshly prepared and spread on to the sterile nutrient agar plates which were prepared by pouring 30ml of the nutrient media into each sterile Petri dish and left until hardened. Inoculation of each bacterium in separate Petri plates was done by spreading using swab which was spread on to the plates uniformly and were incubated at 37°C for 24hr [22]. Four wells were punched into the plates using a sterile cork borer of inner diameter 8.5mm on this solid seeded media [23]. Dried 90% ethanolic extract was dissolved in water to obtain different concentrations (25, 50, 75, 100 mg/ml) and sterilized by filtration through a Whattman filter paper no. 1, and 0.05ml of the different concentrations of extract were added to the respective wells. Allowed to stand for an hour to ensure proper diffusion and there after incubated for 24 hrs at 35^{o}C [24,25]. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity. Antibacterial activity was determined by measurement of zone of inhibition around each well in plate using Zone reader [26]. Measured inhibition zones were recorded as mean diameter in mm [27]. All the readings were taken in triplet.

Results and Discussion

Preliminary Phytochemical screening

After extraction and drying extract, we got 0.052, 0.11, 0.15, 3.2 % yield of petroleum ether, chloroform, ethyl acetate and 90% ethanol extract respectively. Phytochemical screening of the extracts revealed, the presence of Carbohydrates, Glycosides, Phenolic compounds, tannins, Saponins, Steroids, and Flavonoids. (Table 1) the variability in the % yield of extract may be due to solvation of phytoconstituents to their respective polarity of solvent which are responsible for elution of constituents from coarse powder.

Table 1Phytochemical screening of *Rheum emodi* rhizome extracts.

Phytoconstituents	Pet-ether	Chloroform	Ethyl acetate	90% Ethanol
Alkaloids	-	-	-	-
Carbohydrates	+	+	-	-
Glycosides	-	+	+	-
Saponins	-	-	+	+
Phytosterols	-	+	-	-
Phenols	-	-	+	+
Flavonoids	-	-	+	+
Fats	+	-	-	-
Amino acid and Proteins	-	-	-	-

Key: + = present - = absent

Antibacterial Activity

90% ethanolic extract of rhizome was evaluated against UTI bacteria. The results are tabulated in (Figure 1, Table 2 and Graph 1). The extract showed antibacterial activity at 100mg/ml against *Enterobacter*, *S. aureus*, *S. cohni*, *Proteus vulgaris*, *P. flouresence* and *Bacillus subtilis* with zone of inhibition 21.98±0.87, 12.92±0.21, 11.98±0.43, 11.99±0.019, 18.89±0.54, 23.59±0.11respectivly, the strain *Bacillus subtilis* showed maximum zone of inhibition 23.59±0.11 at concentration of 100mg/ml. *B. subtilis* was inhibited by starting concentration of 25mg/ml while for other it was 75mg/ml. The extract is most effective against *B. subtilis*.

The phytochemical screening of 90% ethanol extract have revealed the presence of saponins, phenol and flavonoids. In many studies, plant Saponins are found to be responsible for antibacterial activity [28-31].Various other species of plant extract have been reported to possess antibacterial activity [32],[33]. Many other phytochemical preparations with high flavonoid content have also been reported to exhibit antibacterial activity [34-42]. From the literature studied it is concluded that the antibacterial effect of extract may be due to the presence of these phytochemicals in the *Rheum emodi* rhizome's90% extract.

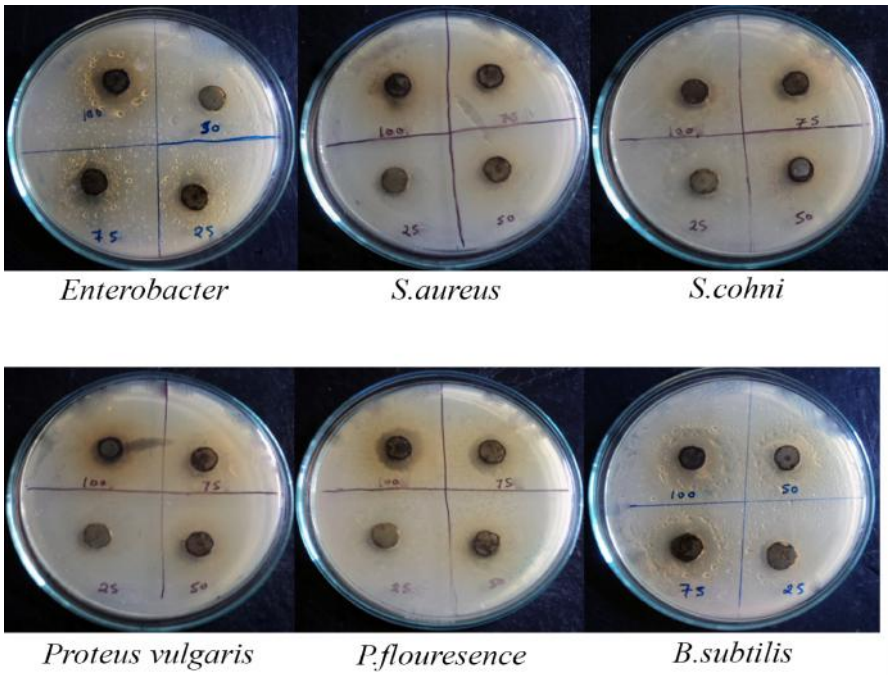
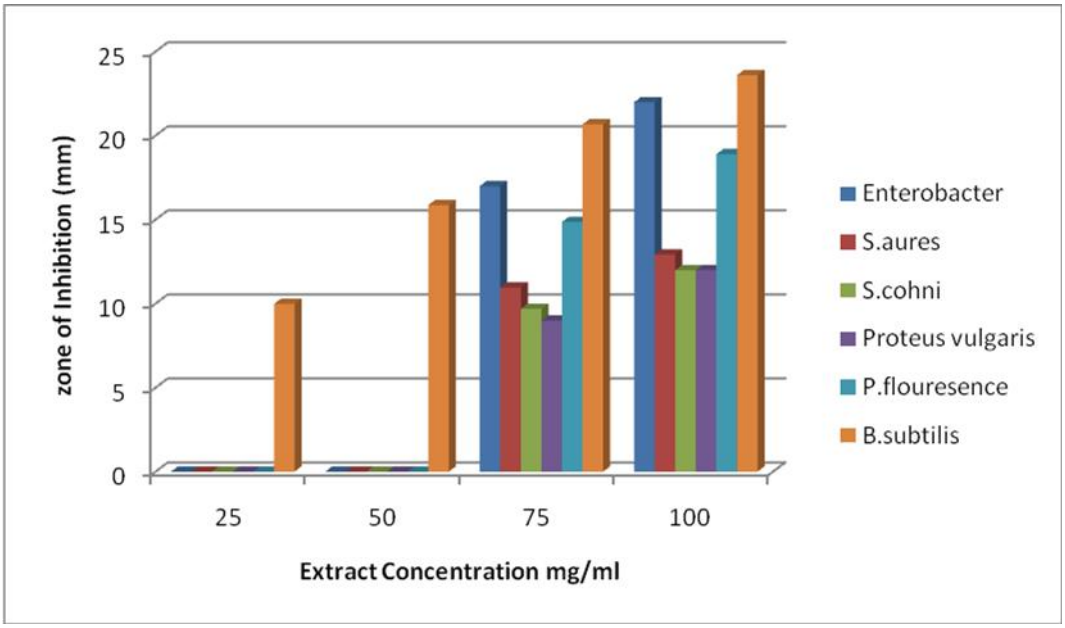


Figure 1 Zone of inhibition of *Rheum emodi* rhizome 90% ethanol extract (25,50,75,100 represents the concentration of extract in mg/ml)

Table 2 Antibacterial activity of *Rheum emodi* rhizome 90% ethanol extract

Name of Bacteria	Zone of Inhibition (mm)			
	25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
Enterobacter	_	_	**16.99±0.36**	**21.98±0.87**
S. aureus	_	_	**10.95±0.87**	**12.92±0.21**
S. cohni	_	_	**9.68±0.43**	**11.98±0.43**
Proteus vulgaris	_	_	**8.99±0.56**	**11.99±0.19**
P. flouresence	_	_	**14.86±0.39**	**18.89±0.54**
B. subtilis	**9.98±0.12**	**15.86±0.87**	**20.67±0.21**	**23.59±0.11**

Values are mean ± SD of three parallel measurements; - = No zone of inhibition



Graph 1 Antibacterial activity of *Rheum emodi* rhizome 90% ethanol extract

Conclusion

The present findings support the applicability of *Rheum emodi* in traditional systems for it's claimed uses like ulcers, kidney stone, gout and jaundice, fungal and bacterial infections. In our study extract is found effective against UTI Bactria and the effect (inhibition) of extract is concentration dependent. Further investigations can be carried out in order to isolate new compounds from the herb and to evaluate the bioactivities as it is necessary to introduce new biologically safe phytochemical compounds which are necessary to suppress the growth of the microorganisms

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