

Research Article



Antimicrobial Activity of Methanolic and Aqueous Extracts of *Rheum emodi* and *Podophyllum hexandrum*

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Accepted on: 02-11-2014; Finalized on: 31-12-2014.

ABSTRACT

In the present study two extracts, methanolic and aqueous, of each of the two ethnomedicinal plants, *Rheum emodi* and *Podophyllum hexandrum*, were prepared and checked for their antimicrobial activity against the bacteria (*Pseudomonas aeruginosa* MTCC 3541 and *Bacillus megaterium* MTCC 1684) and the fungi (*Fusarium solani* MTCC 3871 and *Aspergillus flavus* MTCC 3784). To check the antimicrobial activities, Disc diffusion assay was used. Both *Rheum emodi* and *Podophyllum hexandrum* inhibited the bacteria and the fungal strains with zone of inhibition ranging between 0.9 to 1.8 cm. MIC was determined to be in the range of 0.4 to 1.5 mg/ml.

Keywords: Antibacterial activity, plant extracts, *Rheum emodi*, *Podophyllum hexandrum*, *Bacillus megaterium*, *Pseudomonas aeruginosa*

INTRODUCTION

The valley of Kashmir has been a hub for medicinal plants. The people there are using these medicinal plants for the cure and prevention of various diseases since ancient times. A total of 937 plant species belonging to 129 families have so far been reported to have a traditional medicinal use by indigenous communities of Jammu and Kashmir.¹ However these days we have new and different medicines for these diseases, which unfortunately are accompanied by various side effects. Subsequently, there is a need to have the active principals of natural origin which can be used for the treatment and/or prevention of diseases/infections with no side effects. Thus, there has been a growing interest in natural plant products as these are more compatible to the human body with little or no toxic side effects. In the present study, we used two plants namely *Rheum emodi* and *Podophyllum hexandrum* to check their antimicrobial potential against Gram positive and Gram negative bacteria and against fungal strains. Both *Rheum emodi* and *Podophyllum hexandrum* are herbaceous perennial plants which have acquired the endangered status. It is due to overexploitation for local medicinal uses. *Rheum emodi* or Himalayan Rhubarb is used for fever, cough, indigestion, constipation, diarrhoea, dysentery, menstrual and liver disorders.² It has also been reported to have protective effect in many inflammatory diseases and oxidative stress related injuries.³

Podophyllum hexandrum or Himalayan Mayapple is used in the treatment of ulcers, hepatic disorders, wounds, cuts, tuberculosis, constipation, mental disorders and as anti-cancerous agents.^{4,5} *Rheum emodi* has anthraquinone and stilbene as the most common constituents which confer upon them anticancer, anti-inflammatory, antioxidant, antimicrobial, antiulcer,

hepatoprotective and nephroprotective activities.^{2,6} On the other hand, the major active constituents of *Podophyllum* are podophylotoxin, quercetin and kampherol which show anticancerous activities, antirheumatic, radioprotective, antimicrobial and antihelminthic properties.⁷⁻⁹ Antimicrobial activity studies of *Rheum emodi* have been done against bacteria like *Bacillus subtilis*, *Bacillus sphaericus*, *Staphylococcus aureus*, *Klebsiella* sp., *Chromobacterium* sp., *Pseudomonas aeruginosa* and *Helicobacter pylori*.^{10,11} So far, antifungal activity of *Rheum emodi* has been done against *Alternaria solani*, *Helminthosporium penniseti*, *Curvularia palliscens*, *Erysiphe cichoracearum*, *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton* sp., *Aspergillus fumigatus*, *A. niger* and *Rhizopus oryzae*.^{10,12,13} While a lot of antimicrobial studies have been done for *Rheum emodi*, very few studies have been done on the antimicrobial activity of *Podophyllum hexandrum*.

The rhizome extract has been reported to inhibit growth of *Candida albicans* and *Aspergillus niger*.¹³ The results of the present study show significant antimicrobial activities of methanolic and aqueous extracts of *Rheum emodi* and *Podophyllum hexandrum* against the bacteria *Pseudomonas aeruginosa* and *Bacillus megaterium* and the fungi *Aspergillus flavus* and *Fusarium solani*.

MATERIALS AND METHODS

Plant Materials

The rhizomes of both the plants *Rheum emodi* & *Podophyllum hexandrum* were collected from a nursery located at Ganderbal, Jammu and Kashmir. These rhizomes were washed thoroughly, dried and then ground to fine powder using pestle and mortar. Soxhlet assembly was set to yield the extracts of both the plants using 15g of powdered plant rhizomes in methanol and water respectively. Thus two extracts one methanolic and



other aqueous were prepared for each of the plant. These extracts were concentrated using the rotary vacuum evaporator and then stored at 4°C.

Test Bacterial Strains

The bacterial strains used were *Bacillus megaterium* MTCC 1684 and *Pseudomonas aeruginosa* MTCC 3541. The fungal strains used were *Fusarium solani* MTCC 3871 and *Aspergillus flavus* MTCC 3784.

These cultures were procured from the Microbial type Culture Collection (MTCC), IMTECH, Chandigarh, India.

Antimicrobial assay

The methanolic and aqueous extracts of both the plants *Rheum emodi* and *Podophyllum hexandrum* were used to check for their antimicrobial activity, using Disc Diffusion method. 100µl active culture of the test organism was spread on the Muller-Hilton Agar plates.

Antibiotic disc (Erythromycin for *Bacillus* and Tetracycline for *Pseudomonas*), filter paper disc impregnated with 50 µl of methanol or water and filter paper disc impregnated with 50 µl of plant extract (methanolic or aqueous extract) were placed at three different positions of the inoculated plates to serve as positive control, negative control and test sample respectively. The plates were incubated at 37°C for 24 hours. Then zone of inhibition was checked and measured.

For antifungal activity tests, fungal plugs (0.4 mm in diameter) were placed in the petridishes with Potato dextrose agar (PDA) media and filter paper discs dipped in the plant extracts were placed at equivalent positions around the fungal plug. Antifungal disc nystatin (30µg/disc) was used as a positive control. Radial growth of mycelia was checked for 7 days.

Samples were screened three times and a mean of the observed values was reported.

Phytochemical screening of crude extracts of *Rheum emodi* and *Podophyllum hexandrum*:

Alkaloids

About 0.2 g of the extracts was warmed with 2% H₂SO₄ for two minutes. It was filtered and few drops of Dragendroff's reagent were added. Orange red precipitate indicates the presence of alkaloids.

Carbohydrates

The test solution was treated with few drops of alcoholic alpha-naphthol. 0.2ml of conc. Sulphuric acid was added slowly through the sides of the test tube. A purple to violet color ring which appears at the junction shows positive result.

Tannins

Small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green

solution indicates the presence of tannins.

Steroids

Two millilitre of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of H₂SO₄. The color changed from violet to blue or green in some samples indicating the presence of steroids.

Flavonoids

Extract of about 0.2 g was dissolved in NaOH and HCl was added. A yellow solution that turns colourless indicates the presence of flavonoids.

Saponins

About 0.2 g of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing of the extracts shows the presence of Saponins.

Terpenoids

About 0.1 g of extract was dissolved in 2 ml of chloroform and then 1 ml of H₂SO₄ was added to it. Reddish brown precipitate indicates the presence of terpenoids.

Amino acids

Three drops of 5% ninhydrin solution were added in 3 ml sample and heated for 10 minutes in boiling water bath. Purple or blue colour solution indicates presence of amino acids.

Phenol

To 0.1 ml extract few drops of ferric chloride was added. Blue or green colour solution shows the presence of phenol.

Phlobatannins

The extract (0.5 g) was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl. Red precipitate shows the presence of phlobatannins.

RESULTS AND DISCUSSION

The antimicrobial activities of *Rheum emodi* and *Podophyllum hexandrum* against the bacteria and fungi used were qualitatively assessed by the presence of inhibition zones. Both *Rheum emodi* and *Podophyllum hexandrum* inhibited *Bacillus megaterium* MTCC 1684 and *Pseudomonas aeruginosa* MTCC 3541 with zone of inhibition ranging between 0.9 to 1.4cm (Table 1 and Table 2). Also both *Rheum emodi* and *Podophyllum hexandrum* inhibited the fungi *Aspergillus* and *Fusarium* with zone of inhibition ranging between 0.9 to 1.8 cm (Table 3). Reports on the antimicrobial activity of *Rheum emodi* against *Bacillus subtilis* and *Pseudomonas aeruginosa* show similar results with zone of inhibition ranging between 0.7 to 1.4 cm.² Although *Podophyllum hexandrum* has received significant attention for its tumour necrotizing properties and is used in the treatment of warty lesions, very few studies have been done for its antimicrobial activity. *Podophyllum hexandrum* and *Rheum emodi* rhizome extracts were



reported to have antifungal activity against pure cultures of clinical isolates of *Aspergillus niger* ATCC 1197 and *Candida albicans* ATCC 10231.¹³ *Podophyllum hexandrum* leaf has been found to be highly effective against *Bacillus subtilis*.⁹ Results obtained in the present study shows that the rhizome extract also has similar effect against *Bacillus* sp. The phytochemical analysis of *Rheum emodi* showed the presence of steroids, flavonoids, saponins, terpenoids, amino acids and phenol. *Podophyllum hexandrum* showed the presence of tannins, steroids, flavonoids, saponins, terpenoids, amino acids and phenol (Table 3). These results are similar with other reports on *Rheum emodi* and *Podophyllum hexandrum*.^{6,13,14} The presence of flavonoids and tannins are indicative of presence of antimicrobial activity.¹⁵

MIC of the plant extracts ranged between 0.4 to 1.5 mg/ml (Table 4). Aqueous extracts of *Rheum emodi* showed MIC values less than methanolic extracts whereas in case of *Podophyllum hexandrum* methanolic extract showed MIC value relatively lower than that of aqueous extract. This may be due to the solubility of the antimicrobial compounds in the respective solvents used. Low MIC values with *Rheum emodi* extracts have been reported against *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes* and *Aspergillus fumigatus* (MIC 0.25 to 2.5 mg/ml).¹² Leaf extract of *Podophyllum hexandrum* was reported to have antimicrobial effect against *Bacillus subtilis* with MIC 8 µg/ml.¹⁶

Table 1: Antibacterial activity of methanolic and aqueous extracts (60mg/ml) of *Podophyllum hexandrum* and *Rheum emodi* on *Bacillus megaterium* and *Pseudomonas aeruginosa*

Test organism	Zone of inhibition (cm)				
	*Antibiotic	<i>Podophyllum hexandrum</i> extract		<i>Rheum emodi</i> extract	
		Methanolic	Aqueous	Methanolic	Aqueous
<i>Bacillus</i>	2.0	0.9	1.0	1.5	1.2
<i>Pseudomonas</i>	1.8	1.1	1.0	1.6	1.0

*Antibiotic: Erythromycin for *Bacillus* and Tetracycline for *Pseudomonas*

Table 2: Antifungal activity of methanolic and aqueous extracts (60 mg/ml) of *Rheum emodi* and *Podophyllum hexandrum* on *Aspergillus flavus* and *Fusarium solani*

Test organism	Positive control	<i>Rheum emodi</i> extract		<i>Podophyllum hexandrum</i> extract	
	Nystatin	Methanolic	Aqueous	Methanolic	Aqueous
<i>Fusarium</i>	2.0	1.8	1.6	0.9	1.0
<i>Aspergillus</i>	1.8	1.5	1.4	1.0	1.06

Table 3: Phytochemical tests

Tests	<i>Rheum emodi</i>		<i>Podophyllum emodi</i>	
	Methanolic	Aqueous	Methanolic	Aqueous
Alkaloids	-	-	-	-
Carbohydrates	-	-	-	-
Tannins	-	-	+	+
Steroids	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Terpenoids	+	+	+	+
Amino acids	-	+	+	+
Phenol	+	+	+	+
Phlobatannins	-	-	-	-

Table 4: MIC of *Rheum emodi* and *Podophyllum hexandrum* against *Bacillus*, *Pseudomonas*, *Aspergillus* and *Fusarium*

Test organism	MIC (mg)			
	<i>Rheum emodi</i>		<i>Podophyllum hexandrum</i>	
	Aqueous extract	Methanolic extract	Aqueous extract	Methanolic extract
<i>Bacillus</i>	0.6	1.5	1.4	0.5
<i>Pseudomonas</i>	0.65	1.25	1.0	0.4
<i>Aspergillus</i>	0.8	1.5	1.2	0.8
<i>Fusarium</i>	0.5	1.2	1.0	0.6

*Results are the mean of triplicate readings



CONCLUSION

It can be concluded from the obtained results that both of these endangered medicinal plants have significant antimicrobial activity against the test bacterial and fungal strains used. The results show that both *Rheum emodi* and *Podophyllum hexandrum* have broad range of antimicrobial activity as they were able to inhibit the growth of both Gram negative and Gram positive bacteria as well as that of fungi. This is in accordance with what is reported in the traditional knowledge of the indigenous people as both the plants are used in the treatment of gastrointestinal infections, respiratory infections, liver and skin infections.

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Source of Support: Nil, Conflict of Interest: None.

