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Antimicrobial and Phytochemical Screening of *Boswellia serrata* Roxb., *Rhus mysorensis* Heyne, *Strychnos potatorum* Linn. F. and *Schefflera stellata* Gaertn.

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ABSTRACT

Boswellia serrata (Roxb.), *Rhus mysorensis* (Heyne), *Strychnos potatorum* (Linn.F.) and *Schefflera stellata* plants were collected from different localities of Mysore. Antimicrobial activity of the methanolic extracts of the plant parts was evaluated against *Staphylococcus aureus*, *Salmonella typhi*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas axonopodis* pv. *malvacearum*, *Bacillus cereus* and *Micrococcus* sp. by paper disc diffusion assay. The methanolic extract of the plants were qualitatively screened for phytochemicals using standard procedures which revealed the presence of various important bioactive chemical entities. The methanolic extracts of leaves and fruits of *Rhus mysorensis*, leaves and flowers of *Boswellia serrata* and leaves of *Schefflera stellata* have exhibited significant broad spectrum antimicrobial activity. Further work is being carried out to isolate and identify the active constituents of the plants responsible for antimicrobial activity.

Keywords: antibacterial, bioactive, disc diffusion, ethnobotanical, phytochemicals

INTRODUCTION

Plants have been used for centuries in traditional medicine as they contain components of therapeutic values. According to World Health Organization (WHO), more than 80% of the world's population relies on traditional medicines for health care needs. Plants are natural sources of antimicrobial agents. They contain a wide range of metabolites that can be extracted from them and used to treat infectious and chronic diseases. The emerging problem of multi drug resistance in pathogens to the existing drugs has made it essential to search for novel antimicrobial agents. Thus, this has led researchers to investigate the antimicrobial activity of plants. A lot of work has been done with the objective of knowing the different antimicrobial chemical constituents of medicinal plants and using them for treating microbial infections (Duraipandiyan *et al.* 2006; Kaushik and Goyal 2008).

Boswellia serrata (Family: Burseraceae) is a deciduous middle-sized tree, which is mostly concentrated in tropical parts of Asia and Africa. The oleo gum resin of *B. serrata* is used in various Unani and Ayurvedic preparations. It is reported to be useful in the treatment of bronchitis, asthma, cough, bad throat and various intestinal problems. It is a diaphoretic and astringent prescribed in various syphilitic and pulmonary diseases. It acts as both internal and external stimulant, expectorant, diuretic and stomachic. The gum is also prescribed in cases of jaundice, diarrhoea, dysentery, dyspepsia and hemorrhoids. It is also recommended in weak and unhealthy kind of ulceration (Aman and Balu 2009). The plant also possesses potent analgesic and anti-inflammatory activity (Sharma *et al.* 2010).

Rhus mysorensis Heyne. (Family: Anacardiaceae) is a small aromatic shrub used in traditional medicine. The fruits in the tincture of salt are administered to treat dysentery and the leaf decoction is a remedy for itching (Priti and Yadav 2006).

Strychnos potatorum L. f. is a medium sized glabrous deciduous tree. It grows both in the tropic and sub-tropics of north and south-east parts of Africa, Indian peninsula, Sri Lanka and Myanmar. The seed, besides its bark and root is primarily used in the Indian traditional systems of medicine for treating various diseases including microbial infections. It is used in Ayurveda for treating the eye and urinary tract infections; in Unani for gonorrhoea, kidney troubles, leucorrhoea, tuberculosis and venereal diseases. Alkaloids, the prime source of secondary metabolites isolated from several *Strychnos* species are known for their therapeutic importance (Mallikharjuna and Seetharam 2009).

The medicinal use of *Schefflera stellata* Gaertn. (Family: Araliaceae) has not been reported. In the present study, methanolic extracts of the selected plants were evaluated for the antimicrobial activity by means of paper disc diffusion method. Further, preliminary phytochemical screenings of the methanolic extracts were performed.

MATERIALS AND METHODS

Plants

The plants used in the study were collected from Chamundi hills (Mysore, Karnataka, India). The plant specimens were identified by consulting a taxonomist and preserved as herbaria for future reference at the Department of Studies in Microbiology, University of Mysore. The collected plant materials were cleaned, shade dried, powdered coarsely in a blender and then stored in air-tight containers for further use.

Preparation of extracts

A known weight (30 g) of the powdered plant part was extracted with 80% methanol for 24 hours in a Soxhlet apparatus. The obtained extracts were then filtered to remove residual parts of the precipitate. It was then evaporated at room temperature to get a crude dried extract. The dried extracts were weighed to determine the yield. It was stored in a deep freezer at -20°C to prevent the loss of biological activity until used.

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Research Note

Microorganisms

Authentic bacterial strains (*Staphylococcus aureus*, *Salmonella typhi*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas axonopodis* pv. *malvacearum*, *Bacillus cereus* and *Micrococcus*) used in the study were obtained from the stock culture maintained at Department of Studies in Microbiology, University of Mysore. All the microorganisms were maintained at 4°C on nutrient agar slants.

Determination of antimicrobial activity

The antimicrobial activity of the plant extracts was determined by filter paper disc diffusion assay (Bauer *et al.* 1966). Sterile nutrient broth was inoculated with the test organisms under aseptic conditions and incubated for 24 hours at 37°C to obtain actively dividing cells for seeding. After the incubation, the inoculum was adjusted to 10^6 cells/ml and 100 µl of which was spread on the sterile nutrient agar plates under aseptic conditions. Sterile filter paper discs of 5 mm diameter were loaded with 25 µl of the methanolic extracts to yield a final concentration of 1.25 mg/disc. The paper discs were allowed to evaporate and then placed aseptically on the surface of the inoculated agar plates. Standard chloramphenicol discs (30 µg/disc, Himedia Laboratories, Mumbai, India) were used as positive control. Negative control was obtained by loading 25 µl of methanol per disc. Each extract was tested in triplicates. Plates were initially allowed to pre-diffusion for 2 hrs at 4°C and then incubated overnight (18 hrs) at 37°C. At the end of the incubation period the antibacterial activity was evaluated by measuring the diameter of inhibition zones.

Preliminary qualitative phytochemical screening

Qualitative phytochemical analysis of the methanolic extract was carried out using standard procedures to identify alkaloids (Mayer's test), steroids and terpenoids (Liberman-Burchard and Salkowski tests), cardiac glycosides (Keller-Kiliani test), saponins (foam test), flavonoids (Shinoda test), tannins and phenols (Ferric chloride test) as described by Trease and Evans (1989), Sofowara (1993) and Harborne (1993).

Alkaloids were identified by Mayer's reagent test. Here, to 0.5 ml of the extract solution, 2 drops of dilute hydrochloric acid and 0.2 ml of potassium iodide solution were added. Formation of cream precipitate indicated the presence of alkaloids.

Steroids were identified by Libermann-Burchard's test. 2 ml of acetic anhydride was added to 0.5 ml of ethanolic solution of each extract and acidified with 2 ml of concentrated sulphuric acid. The colour change from violet to blue or green indicated the presence of steroids.

Salkowski's test was adopted to identify terpenoids. 5 ml of each extract was mixed with 2 ml of chloroform. To this mixture, 3 ml of concentrated sulphuric acid was carefully added along the sides of the test tube. A reddish brown band in the chloroform layer confirmed the presence of terpenoids.

About 200 mg of the powdered plant samples were boiled in 10 ml of water and then filtered. A few drops of freshly prepared 0.1% ferric chloride were added to 2 ml of the filtrate. The appearance of a blue-black colour confirmed the presence of tannins.

Cardiac glycosides were identified by Keller-Kiliani test. 5 ml of each extract was mixed with 2 ml of glacial acetic acid and a drop of ferric chloride solution. Concentrated sulphuric acid was added along the sides of the test tube. Reddish brown colour at the junction of two liquids and bluish green on upper layer confirmed the presence of cardiac glycosides.

The presence of saponins was detected by foam test. About 200 mg of the powdered sample was boiled with 10 ml of distilled water and filtered. 0.5 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously till frothing. Formation of stable persistent foam indicated the presence of saponins.

For flavonoids, Shinoda's test was adopted. In a test tube, 15 mg of the extract was dissolved in 1ml of ethanol, to which 5-10 drops of dilute hydrochloric acid and 0.5 mg of magnesium turnings were added. The formation of pink, reddish pink or brown colour indicated the presence of flavonoids.

RESULTS AND DISCUSSION

The results of the antimicrobial screening assay of the crude extracts of all plants against the tested strains are shown in **Tables 1** and **2**.

The methanolic extract of the leaves of *Boswellia serrata* at a concentration of 1.25 mg/disc showed significant antimicrobial activity against *X. oryzae* pv. *oryzae*, *X. axanopodis* pv. *malvacearum*, *Staph. aureus* and *S. typhi.* It showed moderate activity against *E. aerogenes*, *P. aeruginosa*, *Micrococcus* sp. and *B. cereus*. The methanolic extract of the flowers of *Boswellia serrata* showed significant antimicrobial activity against *X. oryzae* pv. *oryzae*, *X. axanopodis* pv. *malvacearum*, *Staph. aureus* and *S. typhi*. It exhibited moderate activity against *E. aerogenes* and *P. aeruginosa* and no activity against *Micrococcus* and *B. cereus*. It was reported by Kasali *et al.* (2002) that the essential oil from the bark of *B. serrata* exhibited significant inhibitory activity against *S. aureus*, *E. coli* and *Proteus mirabilis.* Patil *et al.* (2010) also reported the antibacterial effect of stem bark extract of *B. serrata* against *Klebsiella pneumoniae*, *E. coli* and *Bacillus subtilis*. This clearly indicates that the plant's leaves, flowers and bark have antimicrobial activity. The results support the usage of the plant in traditional medicine. The methanolic extract of the flowers and leaves has shown potent antibacterial activity against plant pathogens. Hence, these can be used in the management of plant diseases.

The present work is the first report about the antimicrobial activity of the methanolic extract of the leaves and fruits of *R. mysorensis*. The methanolic extract of leaves of *R. mysorensis* at the concentration of 1.25 mg/disc showed significant antimicrobial activity against *X. oryzae* pv. *oryzae*, *X. axanopodis* pv. *malvacearum*, *Staph. aureus* and *S. typhi.* It exhibited moderate activity against *E. aerogenes*, *P. aeruginosa*, *Micrococcus* sp. and *B. cereus*. The ethyl acetate extract of fruit showed significant antimicrobial activity against *X. oryzae* pv. *oryzae*, *X. axanopodis* pv. *malvacearum*, *Staph. aureus* and *S. typhi*. It has shown moderate activity against *E. aerogenes* and *P. aeruginosa* but no activity against *Micrococcus* sp. and *B. cereus*.

The plant *Strychnos potatorum* is an important medicinal plant used in treating several ailments including microbial infections. Mallikharjuna *et al.* (2009) reported alkaloid fractions isolated from *S. potatorum* seed exhibited antimicrobial properties against some pathogenic Gram-positive, Gram-negative and acid-fast bacteria and fungi. In the present study, the methanolic extract of leaves of *S. potatorum* at the concentration of 1.25 mg/disc showed moderate antimicrobial activity against *E. aerogenes*, but no activity against other test organisms. *E. aerogenes* is known to cause urinary tract infections (Jawetz *et al.* 2007). Therefore, the obtained result justifies the usage of the plant in traditional medicine for treating urinary tract infection.

The use of *Schefflera stellata* in traditional medicine has not been documented. The present study is the first work reporting the antimicrobial activity of the plant *S. stellata.* The methanolic extract of the leaves of *S. stellata* has shown significant antimicrobial activity against *E. aerogenes*, *Staph. aureus*, *X. oryzae* pv. *oryzae*, *X. axanopodis* pv. *malvacearum* and *S. typhi*. The essential oil fractions from *S. stellata* have been reported to possess antibacterial and antifungal activity (Sabulal *et al.* 2008). There was no activity against *P. aeruginosa*, *Micrococcus* sp. and *B. cereus.* The result supports the prospective medicinal value of the plant in treating microbial infections.

All species of plants included in the present study actively inhibited the growth of *S. aureus*. But, they were less effective in inhibiting *P. aeruginosa.*

The overall antimicrobial activity screening of the crude extracts, are indicative of the potential of these herbal drugs as effective medicaments in the treatment of infectious diseases.

The curative properties of medicinal plants are due to the presence of various secondary metabolites. Thus, the

Table 1 Antimicrobial activity of the methanolic extract of *Boswellia serrata* and *Rhus mysorensis*.

Test organisms	Diameter of inhibition zone (in mm)* of the methanolic extracts (1.25 mg/disc).					
	***B. serrata* (leaves)**	***B. serrata* (flowers)**	***R. mysorensis* (leaves)**	***R. mysorensis* (fruits)**	**P**	**N**
Staphylococcus aureus	09	08	08	10	11	-
Salmonella typhi	11	07	11	09	20	-
Enterobacter aerogenes	07	07	09	10	16	-
Pseudomonas aeruginosa	07	-	07	08	10	-
Xanthomonas oryzae pv. *oryzae*	15	08	09	10	18	-
X. axonopodis pv. *malvacearum*	08	08	11	11	10	-
Bacillus cereus	-	-	07	-	10	-
Micrococcus	-	-	07	-	12	-

(-): not active; (P): chloramphenicol 30µg / disc as positive control; (N): methanol 25µl / disc as negative control; *mean of three values with negligible standard deviation

Table 2 Antimicrobial activity of the methanolic extract of *Strychnos potatorum* and *Schefflera stellata*.

Test organisms	Diameter of inhibition zone (in mm)* of the methanolic extracts (1.25 mg/disc).			
	***S. potatorum* (leaves)**	***S. stellata* (leaves)**	**P**	**N**
Staphylococcus aureus	-	07	11	-
Salmonella typhi	-	07	20	-
Enterobacter aerogenes	09	11	16	-
Pseudomonas aeruginosa	-	-	10	-
Xanthomonas oryzae pv. *oryzae*	-	07	18	-
X. axonopodis pv. *malvacearum*	-	09	10	-
Bacillus cereus	-	-	10	-
Micrococcus	-	-	12	-

(-): not active; (P): chloramphenicol 30µg / disc as positive control; (N): methanol 25µl / disc as negative control; *mean of three values with negligible standard deviation

Table 3 Phytochemical screening of methanolic extracts of five plant parts.

Secondary metabolites	Methanolic extract of the plant parts					
	***Boswellia serrata* (leaves)**	***Boswellia serrata* (flowers)**	***Rhus mysorensis* (leaves)**	***Rhus mysorensis* (fruit)**	***Strychnos potatorum* (leaves)**	***Schefflera stellata* (leaves)**
Alkaloids	+	+	+	+	+	+
Sterols	+	+	+	-	-	-
Glycosides	+	+	+	+	+	-
Saponins	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Tannins and phenols	+	+	+	+	-	-

(+): Present; (-): not detected

preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to drug discovery and development. The result of the phytochemical screening of methanolic extract of the plant parts is given in **Table 3**.

The phytochemical screening of methanolic extract of the leaves and flowers of *B. serrata* and the fruit and leaves of *R. mysorensis* revealed the presence of cardiac glycosides, saponins, flavonoids, tannins and phenols. Terpenoids and alkaloids have been reported in the stem bark extract of *B. serrata* in addition to the above phytochemicals (Patil *et al.* 2010). The methanolic extract of *S. potatorum* leaves contained cardiac glycosides, saponins and flavonoids. Only saponins and flavonoids were detected in methanolic extract of *S. stellata*.

Phenolic compounds are very important plant constituents. Because, they exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of peroxides into free radicals (Pokorny 2001). In the present study, total phenolic content was estimated by FC reagent method. The total phenolic content in the methanolic extract of *B. serrata* leaves was 60.5 mg/g of the extract while that of flowers was 56.37 mg/g of the extract. The total phenolic content in the methanolic extract of leaves of *S. potatorum*, *R. mysorensis*, *S. stellata* and fruits of *R. mysorensis* was 57.75, 65.0, 62.5 and 25 mg/g of the extract, respectively.

This study is a preliminary evaluation of antimicrobial activity of the plants and the results obtained are in agreement to a certain degree with the traditional uses of the plants estimated. *S. stellata* has no known ethnobotanical value but has shown potent activity against the tested pathogens. As the plant extracts have shown significant activity towards the plant pathogens, they can also be used as biocontrol agents. The obtained results could form a good basis for selection of these plant species for further investigation in the potential discovery of new natural bioactive compounds.

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