JSR Publications

Available Online

J. Sci. Res. **1** (3), 662-666 (2009)

JOURNAL OF SCIENTIFIC RESEARCH

www.banglajol.info/index.php/JSR

Short Communication

Antimicrobial Activity of *Phoebe Lanceolata* and *Stephania Glabra:* Preliminary Screening Studies

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Received 4 May 2009, accepted in revised form 15 August 2009

Abstract

Ethanolic extracts of *Phoebe lanceolata* stem bark and *Stephania glabra* tubers were evaluated for their antibacterial and antifungal activities against five bacterial species, *Staphylococcus aureus* (along with ten hospital strains), *Staphylococcus mutans*, *Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae* and six fungal species *Aspergillus niger, Aspergillus fumigatus, Penicillum citranum, Microsporum gypseum, Microsporum canis, Trichophyton rubrum,* obtained from different culture media. The plants/parts extracts were found active against most of the tested microorganism with MIC range of 50-100µg/ml. The MIC was taken at the lower concentration where inhibition ceased. Novobiocin (15 µg/ml) and erythromycin (15 µg/ml) were used as positive controls for bacterial and fungus species respectively.

*Keywords:*Antimicrobial activity; *Streptococcus mutans*; *Klebsiella pneumonia*; *Microsporum gypseum*; Nutrient and Sabouraud's dextrose agar medium.

DOI: 10.3329/jsr.v1i3.2423 J. Sci. Res. **1** (3), 662-666 (2009)

1. Introduction

The herbal medicines have recently gained fame and scientific attention. The plants have been used for the treatment of different ailments worldwide. Plant kingdom is known to be a potential source of antimicrobial drugs. Researchers have been interested to isolate bioactive constituents from such plants for antimicrobial purposes because many of the pathogenic microorganisms have built resistance against antibiotics [1]. Therefore, the search for new antimicrobial agents has become increasingly important.

Phoebe lanceolata (Lauraceae) and *Stephania glabra* (Menispermaceae) are common plants of central Himalaya. These plants have been used in the traditional system of

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medicine in India for treatment of various diseases. Aqueous extract of berries from *P. lanceolata* is important remedy for wounds and sore and *S. glabra* tubers has been used in psycho-disorders, tuberculosis, asthma, diabetes, dysentery and fever [2]. These plants genus are rich in alkaloidal constituents [3-5]. Recently we have isolated various alkaloids from these plant species [6-10]. *n*-hexane, acetone and ethanol extracts of these plants were evaluated for antimicrobial activities against the test microorganisms to determine the MIC and IZD. *n*-hexane and acetone extracts showed poor activity against most of the test microorganisms. Herein, we report the activity with ethanolic extracts only.

2. Materials and Method

2.1. *Plant material*

P. lanceolata (stem bark) was collected from Kartikswami, District Chamoli whereas *S. glabra* (tuber) was collected from Chaka, District Tehri, during the mid summer. The plants were identified from Taxonomic Laboratory, Department of Botany, H.N.B.G.U. Srinagar Garhwal, voucher specimen (v. No. GUH-17598 and 17600 respectively) of the plants were deposited in the departmental herbarium.

2.2. *Preparation of extract*

Air dried and coarsely powdered parts of the plants were extracted with 95% EtOH for 18 h at 50°C and fractionated with n-hexane, acetone and ethanol using soxhlet apparatus separately.

2.3. *Microorganisms and culture media*

Five bacterial species *Staphylococcus aureus* (methecillin resistant), *S. mutans*, *S. epidermidis, Escherichia coli* and *Klebsiella pneumoniae* and six fungus pathogens *Aspergillus niger, A. fumigatus, Penicillum citranum, Microsporum gypseum, M. canis* and *Trichophyton rubrum* including ten hospital strains of *S. aureus* obtained from different culture media were bio-assayed for inhibition zone diameter (IZD) and minimum inhibitory concentration (MIC) determination using novobiocin (15 µg/mL; Merck) as a positive control for bacterial species and erythromycin (15 µg/mL; Alembic) for fungus pathogens [8]. All tested bacterial species were grown in nutrient agar medium (peptone-5 g, beef extract-3 g, NaCl-5 g, agar-agar-20 g and distilled water-1000 ml at pH-7.0) and nutrient broth medium (same contents as for nutrient agar medium excluded agar-agar) whereas all fungal isolates were cultured on Sabouraud's dextrose agar medium (peptone-10 g, dextrose-40 g, agar-agar-20 g, distilled water-1000 ml at pH-5.6) and dextrose broth medium (same contents as for Sabouraud's dextrose agar medium excluded agar-agar) [11-13]. These cultures were obtained from the Standard Culture Collection Center of Institute for Microbial Technology (IMTECH) and maintained in the Microbiology Laboratory, S.B.S. (PG) Institute of Biomedical Science and Research, Balawala,

Dehradun. Ten more strains of *Staphylococcus aureus* were obtained from different hospital infections used as test microorganisms by pathologists.

2.4. *Antimicrobial assay*

Evaluation of antimicrobial activity of the plant extracts was carried out by agar disc diffusion method [11-15]. The extracts were dissolved in 5 per cent DMSO (Merck) and then in sterile water, to reach a final concentration of 100 µg/ml and sterilized by filtration (0.22 µm millipore filter). The concentrations at 25, 50 and 100 µg/ml were taken in each case. The discs (6 mm diameter) were saturated with 10 µl of the extracts at a concentration of 25, 50 and 100 µg/ml and placed on the inoculated agar of 10^6 cfu/ml [12,13]. The inoculated plates were incubated at 37°C for 24 h for bacterial growth (Nutrient broth) and 27°C for 5 days for fungal isolates (Sabouraud broth). MIC was determined as the least concentration of extract inhibiting the growth of the test organisms during 24 h [14]. After 24 h of 37°C and 48 h of 25°C for bacteria and fungi inoculation, the inhibition zone surrounding the discs by the diffusion of compounds was measured in mm diameter [15]. The results having large IZD with low MIC were only considered whereas small IZD with high MIC were omitted.

3. Result and Discussion

n-hexane, acetone and ethanol extracts were evaluated for antimicrobial activities against the test organisms to determine the MIC and IZD. The ethanolic extracts showed most significant results against all test microorganisms. Table 1 showed the results with bacterial strains whereas Table 2 showed the results with test fungus pathogens compared with positive controls. Extract from *S. glabra* was found most active since it showed positive results with all test microorganisms. The maximum inhibition zone was found against two bacterial strains *S. mutans* and *S. epidermidis* with MIC of 50 µg/ml, the extract was also found active against the hospital strains of *S. aureus*. Most of the fungus pathogens including *T. rubrum, M. gypseum* and *M. canis* were inhibited by the extract with MIC of 25 µg/ml. The extracts showed significant inhibition zone against bacteria and fungus species in comparison of positive control (Tables 1, 2).

Table 1. IZD and MIC of plant extracts against bacterial strains.

EX	Inhibition zone diameter (IZD) in mm														
	Sa	Ec	Sm	Se	Kp	H3	H4	H5	H7	H8	H10	H11	H15	H23	H25
SG	14	6	19	20	8	24	20	25	24	20	28	18	21	20	15
PL	13	-	14	15	-	20	15	13	13	11	17	10	15	14	16
NV	21	17	22	15	12	23	19	26	18	18	25	19	23	20	18
	Minimum inhibitory concentration (MIC) in µg/ml														
SG	100	100	50	50	100	25	50	25	25	50	25	100	50	100	100
PL	100	-	100	50	-	50	100	100	100	100	50	25	100	100	50

Abbrev: (EX) extract; (-) no inhibition zone; (NV) Novobiocin; (SG) *S. glabra*; (PL) *P. lanceolata*; (Sa) *Staphylococcus aureus*; (Ec) *Escherichia coli*; (Sm) *Streptococcus mutans*; (Se) *Staphylococcus epidermidis*; (Kp) *Klebsiella pneumonia*; (H) Hospital strains of *S. aureus.*

The extract from *P. lanceolata* was found active against three bacterial species of *Streptococcus* and four fungus pathogens having maximum zone of inhibition against *T. rubrum* (23) and *M. gypseum* (22). *K. pneumoniae* and *E. coli* were almost unaffected by the sample drugs except *S. glabra*, which showed less potent activity against these bacterial species.

Table 2. IZD (mm) and MIC (µg/ml) of plant extracts against fungus pathogens.

EX	An		Af		Pc		Tr		Mg		Mc	
	IZD	MIC	IZD	MIC	IZD	MIC	IZD	MIC	IZD	MIC	IZD	MIC
SG	15	100	21	50	19	50	35	25	28	25	29	25
PL	-	-	12	100	14	100	23	50	22	50	-	-
ER	23		18		22		20		15		18	

Abbrev: (ER) Erythromycin; (An) *Aspergillus niger*; (Af) *Aspergillus fumigatus*; (Pc) *Penicillum citranum*; (Tr) *Trichophyton rubrum*; (Mg) *Microsporum gypseum;* (Mc) *Microsporum canis.*

Crude extracts of the plants are generally contains a mixture of active and non-active constituents. The MICs of less than 100 mg/l for pure and individual compounds may be suggestive of good anti-microbial activity. The low MIC shown by these extracts against tested organisms revealed that they can be used as an alternative to standard antibiotics in the treatment of infections caused by these microorganisms [16] and since most of the microorganisms are developing resistance against the known antibiotics, therefore, these can be the best therapeutics option as antimicrobial agents. Usually, the extract having large IZD with low MIC can be recognized as more potent drug than that of small IZD and high MIC.

The overall results showed that *S. aureus* and its ten hospital strains were inhibited by both sample drugs. These plant extracts can be used in the treatment of boils, sores and wounds, since *S. aureus* has been implicated as causative agents of these ailments [17].

Since ethanolic extracts of these plants were found active against most of the test microorganisms, therefore the classes of polar compounds (i.e. their glycosides) are responsible for the activity. TLC analysis showed that flavonoids, steroids, alkaloids and their glycosides are the major constituents of these plants.

In conclusion the extracts obtained from *S. glabra* and *P. lanceolata* inhibited most of the test microorganisms with significant values of IZD. The plant extracts either in crude form or pure constituents responsible for the activity can demonstrate promising antimicrobial potential.

Acknowledgement

The work was supported by UGC New Delhi, India; Grant No. 33-282/2007(SR). Our sincere thanks to Miss. N. Bamola, S.B.S. (PG) Institute of Biomedical Science and Research, Balawala, for carried out the antimicrobial work.

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