Original Article

Antimicrobial activity of *Symphorema polyandrum* Wight. Seeds

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Abstract

Symphorema polyandrum Wight. seeds are reported for its use in the management of snake bite, scorpion stings and associated skin ailments. The alcoholic extracts of its seed was screened for *in vitro* antimicrobial activity following agar diffusion method and compared with Gentamycin, Cefpodoxime, Streptomycin as standard antibacterial and Amphotericin B, Fluconazole and Clotrimazole as standard antifungal agents. For antibacterial assay *B. subtilis, S. aureus, S. epidermidis, E. coli, K. pneumonia , S. typhi* and for antifungal activity on *A. flavus* and *C. albicans* as test organisms in different concentrations (25µg/ml, 50µg/ml, 100µg/ml and 250µg/ml), were evaluated following standard procedures. It was observed that the seed extract is effective against all six bacteria and two fungal strains, when compared to standard drugs. The zone of inhibition for antibacterial activity of the test drug, against 6 strains of bacterias was found between 11 to 15 mm and 12 to 17 mm for two fungal strains.

Keywords: Antimicrobial activity, *Symphorema polyandrum,*

Introduction

Symphorema polyandrum Wight. belonging to family Verbenaceae, commonly known by tribal people as 'Badichang or Mahasindhu' grows in different parts of Odisha, Chatisgargh and Madhay Pradesh. Different parts of *S. polyandrum* Wight., has been reported for various ethnopharmacological uses viz. snake bite, scorpion stings, cat bite, mad dog bite and mosquito bite [1,2,3]. Microbes are having important role in spreading various local or systemic infections [4]. Ayurveda advocates the management of various infectious diseases with herbal remedies. The antibiotic resistance has become a global concern [5]. Recent literature survey shows that its seeds though reported for its ethno medicinal claim for management of different aliments but have not been evaluated for its antimicrobial activities. Hence the present study was designed to assess the antimicrobial activity of its seed.

Materials and methods

Collection of plant material

S. polyandrum Wight. was identified by studying its morphological characters with the help of various floras [6,7,8]. A voucher specimen (herbarium) of the sample has been preserved in the institute Pharmacognosy laboratory (vide no. 6059.) Its mature seeds were in the month of May June 2012; shade dried and was coarsely powdered to mesh 60# and kept in airtight glass jar bottle for future use.

Preparation of extract:

1g of *S. polyandrum* seed powder was extracted with methanol by sonicating it for 10 min and then keeping it overnight. Next day after filtration, methanol evaporated, then by taking weight of residue, 4 different concentrations 25 µg/ml, 50 µg/ml, 100µg/ml, 250µg/ml of the sample, were prepared. These are used for determination of

antimicrobial activity and coded as SP.

Determination of microbial load for plant material

Microbial load of the test sample was done by total viable aerobic count method [9,10]. To 500 mg, accurately weighed sample, 1-2 drops of Tween80 and a homogeneous suspension was prepared by slowly adding 5 ml of sterile buffered sodium chloride peptone (SBSCP) solution of pH 7.0. This suspension was diluted 10^{-1} onwards as required in sterile dilution blanks (SBSCP). One ml each from these aliquots was added to sterile melted and cooled top agar (Soyabean casein digest agar, for fungal count Potato dextrose agar medium used) tubes. These tubes were poured to sterile petridish and allowed to solidify. These plates were incubated at 30-35^{0}C for 48 hours. The numbers of colonies were counted and the results were expressed in Cfu/g.

$$\text{Cfu / g} = \frac{\text{Number of average colonies}}{\text{Dilution X Volume plated}}$$

Antimicrobial activity of plant materials

Culture conditions: The antimicrobial efficacy of these plant materials was tested on 6 different strains, 3 Gram positive bacteria namely *Bacillus subtilis (NCIM 2063) Staphylococcus aureus (NCIM 2079) & Staphylococcus epidermidis (NCIM 2439);* 3 Gram negative bacteria namely *Escherichia coli* (NCIM 2065), *Klebsiella pneumoniae* (NCIM 2719) and *Salmonella typhi* (NCIM 2501) as well as 2 fungal strains namely *Aspergillus flavus* (NCIM 1028) and *Candida albicans* (NCIM 3471). All cultures were obtained from National Chemical Laboratory, Pune. 24 hours old cultures of all these organisms were inoculated in sterile broths and incubated till 0.5 Mcfarland standard turbidity obtained, and then used for assay. The antimicrobial activity of methanol extracts of SP seed was studied in different concentrations (25 µg/ml, 50 µg/ml, 100µg/ml, 250 µg/ml) against six pathogenic bacteria and two fungal strains. 0.3 ml of different extracts as well as Streptomycin, Fluconazole and Clotrimazole standards were added in the Agar well for the assay; while Gentamycin, Cepodoxime and Amphotericin B standard antibiotics discs were used [11].

Antimicrobial assay

Sterile soybean casein digest agar (25 ml per plate) used for antibacterial activity and sterile sabouraud agar (25ml per plate) used for antifungal activity. 20 ml sterile medium was poured aseptically in sterile plates and allowed to solidify. Then 0.5 ml of culture was inoculated in 5 ml sterile, melted, cooled medium and poured them on solidified agar plates aseptically. After solidification of medium, wells were made with the help of cup borer and 0.3 ml of sample was inoculated. Three Standard drug solutions were added in the well and other standard antibiotic discs were directly placed on agar surface and pressed with the help of sterile forceps aseptically. For diffusion purpose, plates were placed in refrigerator for 20-25 mins. Then plates were incubated at 37^0 C for 24 hrs except sabouraud agar plates and plates containing *K. pneumoniae* organism, they were incubated at 30^0 C for 24-48 hrs. After incubation, zone of inhibition was measured with Himedia antibiotic zone scale- c [12].

Pathogen study

Same extracts were used as for anti-microbial activity assay, these extracts were transferred to specialized mediums given below and incubated at their optimum temperature for growth, then after incubation plates were observed and results were concluded [13] .

Selective differential mediums according to pathogens:

- *Pseudomonas aeruginosa* - Citrimide agar
- *Salmonella typhi* - TSI agar slant, XLD agar
- *Escherichia coli*- EMB agar
- *Staphylococcus aureus* - Mannitol salt agar

Result and discussion:

Microbial load:

The observations of the microbial load of *S. polyandrum* seed showed that the tasted samples, when collected from their natural sources, are either free or within prescribed limit of the microbes [14]. When the samples were tested for bacterial contents,

all the samples were found free of common pathogens and bacterial and fungal count was under permissible limit (Table 1).

Antimicrobial activity:

The antimicrobial activity of methanol extracts of SP seed was studied in different concentrations (25µg/ml, 50µg/ml, 100µg/ml, 250µg/ml) against six pathogenic bacterial strains (Table 2) (three Gram positive *B.subtilis* NCIM 2063, *S.aureus* NCIM 2079 & *S. epidermidis NCIM 2439*; three Gram negative (*E. coli* NCIM 2065, *K. pneumoniae NCIM* 2719 and *S. typhi* NCIM 2501) and two fungal strains (Table 3) (*S. flavus* NCIM 1028 and *C.albicans* NCIM 3471). Antibacterial and antifungal potential of extracts were assessed in terms of zone of inhibition (ZOI). The result showed that the extracts of all samples were found to be effective against all the microbes tested. The antibacterial and antifungal activity of the SP increased linearly with increase in concentration of extracts (µg/ml). As compared with standard drugs, the results revealed that in the extracts for all six bacterial activity, were around equally sensitive and for fungal activity *C. albicans* showed good result as compare to *S. flavus*. The growth inhibition zone measured ranged from 11-15 mm for all the sensitive bacteria, and ranged from12-19 mm for fungal strains (Table 2 and 3). The inhibitory effect of SP showed in mm at 25, 50, 100, 250µg/ml were against -

B.subtilis 11, 12, 13, 14.5;
S.aureus 11, 11.5, 13, 14.5;
S. Epidermidis 11, 11.5, 12, 14.5;
E. coli 0, 11.5, 12, 13.5;
K. pneumoniae 11, 12, 13, 14;
S. typhi 11, 12, 12.5, 14.5;
and two fungal strains *S. flavus* 12, 12.5, 14, 16 and *C.albicans* 14, 15, 16, 17 respectively.

Conclusion:

The zone of inhibition for antibacterial activity of the test drug, against 6 bacterial strains was found between 11 to 15 mm and 12 to 17 mm for two fungal strains. However, further studies would be necessary at different concentration and by different extraction media to increase the efficacy of the test drug.

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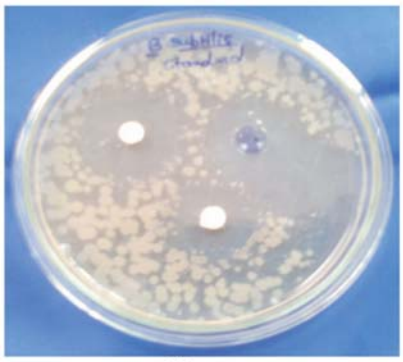
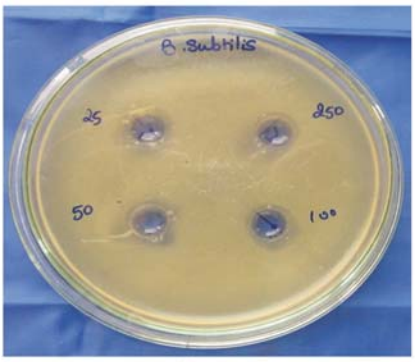


Fig. a



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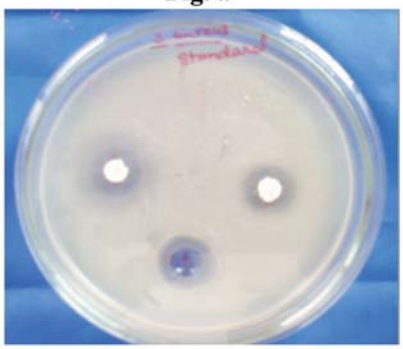

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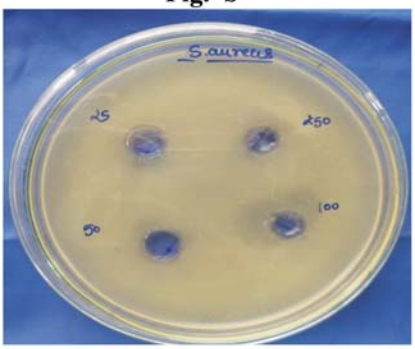

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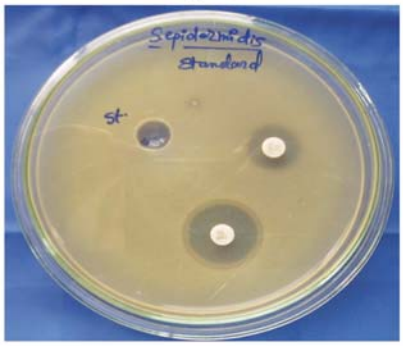

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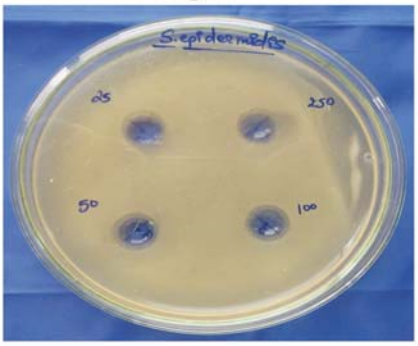

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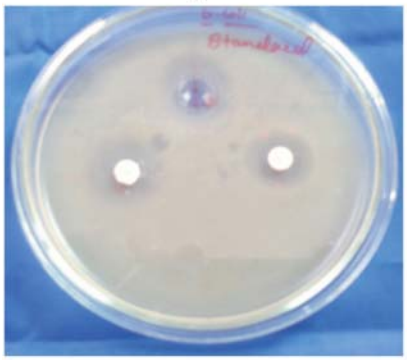


Fig. g

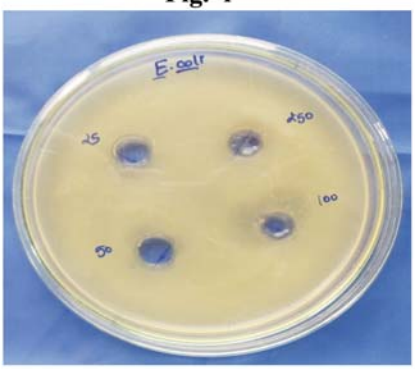


Fig. h

PLATE - 1

Photographs of antimicrobial study of *S. polyandrum* Wight.seeds

Fig. a: Showing zone of inhibition by standard against *B. subtilis*
Fig. b: Showing zone of inhibition by SP against *B. subtilis*
Fig. c: Showing zone of inhibition by standard against *S. aureus*
Fig. d: Showing zone of inhibition by SP against *S. aureus*
Fig. e: Showing zone of inhibition by standard against *S. epidermidis*
Fig. f: Showing zone of inhibition by SP against *S. epidermidis*
Fig. g: Showing zone of inhibition by standard against *E. coli*
Fig. h: Showing zone of inhibition by SP against *E. coli*
SP - *Symphorema polyandrum* Wight. seed

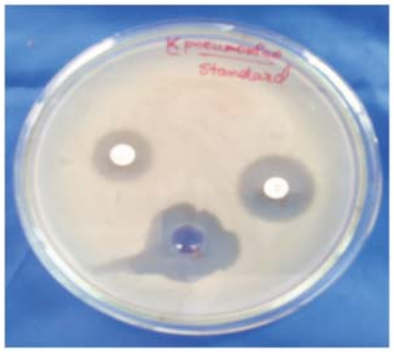
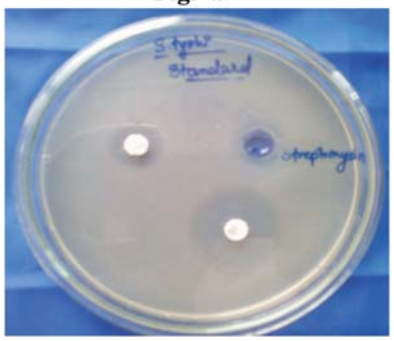


Fig. a

Fig. b



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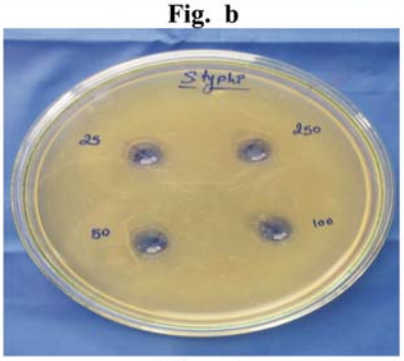

Fig. d

Fig. e

Fig. f

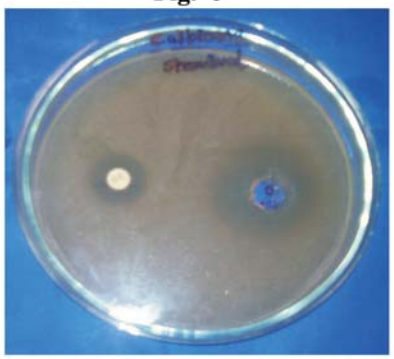

Fig. g

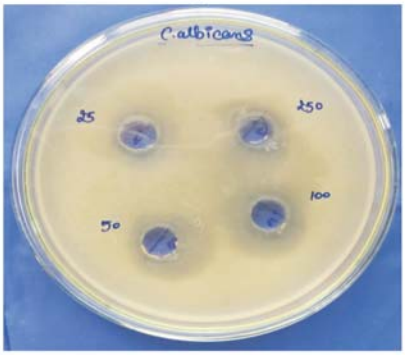

Fig. h

Plate 2

Photographs of antimicrobial and antifungal study of *S. polyandrum* Wight. seeds

Fig. a: Showing zone of inhibition by standard against *K. pneumoniae*
Fig. b: Showing zone of inhibition by SP against *K. **pneumoniae***
Fig. c: Showing zone of inhibition by standard against ***S. typhi***
Fig. d: Showing zone of inhibition by SP against ***S. typhi***
Fig. e: Showing zone of inhibition by standard against *A. flavus*
Fig. f: Showing zone of inhibition by SP against *A. flavus*
Fig. g: Showing zone of inhibition by standard against *C. albicans*
Fig. h: Showing zone of inhibition by SP against *C. albicans*