ANTIMICROBIAL ACTIVITY OF CHEMOCHEMICALS OF ROSES OF SALACIA MACROSPERMA

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ABSTRACT: The ethanolic extract of roots of Salacia macrosperma (Hippocrataceae) exhibited significant in vitro antimicrobial activity. The ethanolic extract was fractionated with different solvents and all the fractions were screened for their antimicrobial spectrum against eight gram-positive, five gram-negative and ten fungal strains. Chloroform fraction followed by benzene fraction of ethanolic extract showed significant antimicrobial effect against all the microorganisms tested. The dose-dependent activity of fractions was evaluated against selected microbial strains and compared with appropriate standards.

INTRODUCTION

The leaves and roots of Salacia macrosperma were reported to possess hypoglycemic activity (1). A compound, presetimerin was isolated from the bark and reported to possess in-vitro activity against large number of gram – positive cocci (2). In the present paper, the antimicrobial spectrum of non – polar and polar fraction of ethanolic extract of roots of S. macrosperma was established and dose – dependent activity against selected strains has been reported.

MATERIALS AND METHODS

The powdered roots of S.macroserma were extracted with ethanol (95%) by double maceration. The extract was concentrated in vacuo using rotary flash evaporator. The alcoholic extract was dissolved in ethanol (95%) and adjusted to 70% v/v alcoholic strength with distilled water, and was successively extracted with benzene, chloroform, ethyl acetate, and butanol. The alcoholic phase remained after fractionation was concentrated in vacuo, dried in a desiccator and extracted with methanol. The remaining purified residue fraction (RPR) was also used for present investigations.

Preparation of Test Samples. The test samples of ethyl acetate, butanol, ethanol and RPR fractions of ethanolic extract of roots of S.macroserma were prepared in sterile distilled water (10 mg/ml). For the preparations of test samples of benzene and chloroform fractions of alcoholic extract, about 100 mg of accurately weighed fraction was dissolved in one ml of alcohol (95%) and diluted to 10 ml with sterile distilled water (10mg/ml). Further dilutions of test solutions as required, were made with sterile distilled water.

Standard Solutions The standard antibiotics, benzylic pencillin and streptomycin sulphate, were dissolved in sterile distilled water to give 10 mg/ml of stock solutions. Different
concentrations of standard solutions were prepared by diluting the stock solution with sterile distilled water. The standard stock solution of nystatin was prepared in buffered 70% propanol (10mg/mL). The buffer was prepared by mixing 7 volumes of n-propanol and 3 volumes of 0.056M phosphate buffer of pH7. Further dilutions, as required, were made with buffered 70% propanol.

**Inoculum** Inoculum was prepared by transferring a loopful of stock culture to a 250 ml Erlenmeyer flask containing 80ml of nutrient both in case of bacteria and Sabouraud’s broth in case of fungi. The inoculum flask were incubated at 37°C in case of bacteria for 18 hours and at 25°C in case of fungi for 25 hours, and used in the study.

**Preparation of Plates** The nutrient agar medium and Sabouraud’s agar medium were sterilized by autoclaving at 121°C for 15 minutes. The Petri-dishes and pipettes plugged with cotton were sterilized in an oven at 150°C for one hour. About 25 ml of the molten agar medium was poured in each sterilized Petri dish (diameter 10cm), under asceptic conditions. About 0.5 ml of inoculums broth of different strains of bacteria and fungi were added to the respective petr-dishes. The contents of Petri-dishes were mixed thoroughly by rotary motion. The medium containing inoculums was allowed to solidify at room temperature. After solidification of medium, four cups (Diameter 8mm) were made in each petr-dish with sterile borer at equal distances.

**Measurement of Activity** (3) Accurately measured 0.1 ml of fractions and standard solutions were added to cups and labeled accordingly. The Petri-dishes were kept undisturbed fun a cool place for one hour to allow the diffusion of solutions into the medium. The Petri-dishes were then kept for incubation at 37°C for 24 hours in case of bacteria and at 25°C for 48 hours in face of fungi. After the incubation period, the presence of a zone of inhibition surrounding the cups indicated antimicrobial activity. The diameter of zone of inhibition was recorded with antibiotic zone reader. For the determination of bactericidal fungicidal or bacteriostatic fungistatic activity, the Petri-dishes were left undisturbed for 3 days at respective temperatures. The observations were made for presence or absence of microbial growth in zone of inhibition. The experiments were performed in triplicate.
Fig. 1: Dose Dependent Antimicrobial Activity of Chloroform Fraction of Alcohol Extract of *S. macrosperma*
Fig. 2 Dose Dependent Antimicrobial Activity of Benzene Fraction of Alcohol Extract of *S. macrosperma*
RESULTS AND DISCUSSION

Of all the fractions of alcoholic extract of S. macrosperma screened for antimicrobial activity, chloroform and benzene fractions showed considerable antimicrobial activity, which was in few cases comparable with that of standard drugs (Table 1). Anti fungal effect of both the fractions was most effective as an antimicrobial agent. Both the fractions were found to possess bacteriostatic and fungistatic activity. Ethyl acetate, Butanol, methanol and RPR fractions did not exhibit any significant antimicrobial activity.

The dose-dependent activity of chloroform and benzene fractions was carried on Bacillus subtilis, Escherechia coli and Aspergillus niger (Fig.1 and 2). The antimicrobial activity of both the fractions increased with increased concentrations of fractions. However, no linear relationship was observed, which may be because of inability of active principles to diffuse faster with increase in concentration. It was observed that the overall activity with increased concentration of test samples was more pronounced against fungi (A. niger), as compared to other microorganisms tested.

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TABLE – 1

Antimicrobial Evaluation of Benzene and Chloroform Fractions of Alcoholic Extract of S. macrosperma (Diameter of cup 8 mm)

| S. Micro organism          | Diameter of zone of inhibition (mm)* | Brentzyl pencillin (200 ug/ml) |
|---------------------------|-------------------------------------|-------------------------------|
|                           | Benzene fraction (1 mg/ml) | Chloroform fraction (1 mg/ml) | Standard drug           |
| Gram – positive bacteria  |          |                              |                              |
| 1. Staphylococcus auteus  | 11.1     | 12.5                         | 21.0                        |
| 2. Staphylococcus albus   | 12.0     | 14.5                         | 15.5                        |
| 3. Bacillus cereus        | 12.2     | 11.0                         | 14.5                        |
| 4. Bacillus subtilis      | 12.0     | 12.8                         | 21.0                        |
| 5. Sarcine lute           | 14.0     | 15.8                         | 16.0                        |
| 6. Lactobacillus casei    | 10.1     | 10.3                         | 17.4                        |
| 7. Bacillus pumulis       | 10.3     | 14.1                         | 17.5                        |
|     | Gram-negative bacteri          |                |                |
|-----|-------------------------------|----------------|----------------|
| 8.  | *Streptococcus faecalis*      | 12.3           | 14.0           |
|     |                               |                | Streptomycin sulphate (200 ug/ml) |
|     | 1. *Fusarium devorans*        | 10.1           | 11.3           |
|     | 2. *Escherichia Coli*         | 12.3           | 12.8           |
|     | 3. *Klebsiella aerogens*      | 12.8           | 15.0           |
|     | 4. *Proteus vulgaris*         | 13.0           | 15.1           |
|     | 5. *Salmonella typhi*         | 12.2           | 12.6           |
|     |                               |                | Nystatin (200 ug/ml) |
|     |                               |                |                |
| Fungi|                               |                |                |
|     | 1. *Aspergillus niger*        | 12.2           | 14.0           |
|     | 2. *Aspergillus flavus*       | 12.2           | 14.1           |
|     | 3. *Acremonium terricola*     | 12.3           | 11.9           |
|     | 4. *Culvularia lunata*        | 12.0           | 15.7           |
|     | 5. *Drechslera specifier*     | 11.0           | 15.3           |
|     | 6. *Fusarium solani*          | 14.0           | 17.5           |
|     | 7. *Fusarium oxysporum*       | 12.5           | 15.6           |
|     | 8. *Lasiodipoldia theobroma*  | 10.5           | 11.5           |
|     | 9. *Trichoderma viride*       | 13.2           | 14.1           |
|     | 10. *Pencillium viridicatum*  | 14.1           | 14.0           |
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