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BRIEF COMMUNICATION

Anticandidal activity of medicinal plants and *Pseudomonas aeruginosa* strains of clinical specimens

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This study was designed to investigate the *in vitro* anticandidal activity of some medicinal plants and *Pseudomonas aeruginosa* strains against *Candida* species. The antifungal activity of methanolic extracts of five medicinal plants, namely, *Cinnamomum porrectum, Lippia nudiflora, Cestrum nocturnum, Trachyspermum ammi*, and *Sida carpinifolia* were studied. The medicinal characteristics of these plants were compared with commercially used antibiotics. The antimicrobial assay was done by agar well diffusion and the broth dilution method. Among the plants used, *T. ammi* and *C. nocturnum* were found to be more potent than the others. Twenty *P. aeruginosa* strains were isolated from various clinical specimens. The total inhibitions obtained were found to be 47%, 38%, and 36% in blood agar, whereas in Sabouraud dextrose agar (SDA) the inhibitions were 57%, 48%, and 37%, respectively.
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Introduction

Northeast India has been identified as one of the biodiversity hotspots among the 32 hotspots identified worldwide.[1] The medicinal plants of this area are still to be identified and studied properly. The objective of the present investigation is to screen medicinal plant species and *Pseudomonas aeruginosa* strains isolated from patients at the local civil hospital for antifungal activities against the pathogenic microorganism *Candida* species, which inhabits the gastrointestinal tract, buccopharyngeal cavity, and vulvovaginal tract, is capable of causing serious systemic infections, and is frequently associated with indwelling catheters and immunosuppressive agents.[2] *Candida* species are also benign members of skin and mucosal flora. When host defenses falter, however, *Candida* species initiate invasive growth that can lead to severe diseases.

According to an National Nosocomial Infection Surveillance (NNIS) survey from 1980 to 1990, the most frequently

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isolated nosocomial fungal pathogen was *Candida albicans* (59.77%) followed by other *Candida* species (18.6%). From time immemorial, medicinal and aromatic plants have been used to get relief from various ailments. The widespread belief that green medicine is healthier than synthetic products has revived the interest in natural drugs.

P. aeruginosa is a part of the human microflora of healthy individuals. Because of the ingestion of the organism through the colonization respiratory tract, the bowel is the most likely colonized site. The likelihood of colonization increases with the longevity of hospitalization. Pseudomonads represent the major group of nondifferentiating microorganisms that produce antibiotics. The antibiotic substances are pyocyanin, pyrrolnitrin, and pseudomonic acid. It has been demonstrated that some secondary metabolites, production of phytotoxins, slime production, production of antifungal compounds, produced by pseudomonads, give an obvious selective advantage to these organisms in their natural environment.[3,4]

This study investigated the *in vitro* efficacy of some medicinal plants and *P. aeruginosa* strains against *Candida* species.

Materials and methods

Plant material

Five medicinal plants were chosen for this study. The plant materials (leaves, fruits, and seeds) were collected from Tezpur (border of Northeastern India). The plant materials collected were brought to the laboratory in polythene bags, identified by experts of the institute, and processed. Fresh plant materials were washed under running water, shade dried, homogenized to a fine powder, and processed accordingly.

Microorganisms

Twenty *P. aeruginosa* isolates were isolated from the culture of patients of a local civil hospital. Each single isolate was collected from different patients. The strains were identified by standard bacterial identification methods.[5] *P. aeruginosa* isolates were identified on the basis of their characteristic colony morphologies and the production of diffusible pigments. *P. aeruginosa* strains were identified based on the positive oxidase test, triple sugar ion reaction of alkaline over no change, no growth at 42°C. The strains of *Candida* including *Candida krusei* ATCC 6258, *C. albicans*, and *Candida tropicalis* were the clinical isolates obtained from the School of Tropical Medicine, Kolkata, India. To investigate the *in vitro* anticandidal activity of *P. aeruginosa* strains, the Kerrs method[4] was taken into consideration.

Preparation of the plant extract and test extracts

A total of 5 g of the material was extracted with 150 mL methanol in Soxhlet apparatus. The solvent was evaporated at room temperature and the extracts thus obtained were stored in airtight bottles for further studies. Each extract was dissolved in dimethyl sulfoxide at a concentration of 0.2 mg/mL and was used as working stock. Clotrimazole 1000 μg/mL was used as the standard for comparison.

Technique

The antifungal assay of the plant extracts was evaluated employing an agar well diffusion method.[6] Sterilized Sabouraud dextrose agar (SDA) medium was poured into sterile Petri plates and allowed to solidify. After solidification of the medium, the broth was vortexed and 100 μL of the broth was spread evenly over the surface of the agar plates. A well of 8 mm diameter was made in each plate with a sterile cup borer. Into each well was introduced 100 μL of the test extract and the plates were incubated at 28°C for 24 hours. The experiment was replicated three times. The efficacy was determined by measuring the diameter of the inhibition zone exhibited by the extracts against the test pathogen. The minimal inhibitory concentrations (MICs) of the aqueous plant extract as well as reference drugs against *Candida* strains were determined by the broth dilution method.[7]

Activity index determination

The activity index of the extracts was determined by the following formula provided by Jain and Sharma:[8]

$$\text{Activity index} = \frac{\text{Inhibition zone of extracts}}{\text{Inhibition zone of standard}}.$$

To investigate the *in vitro* anticandidal activity of *P. aeruginosa* strains, the Kerrs method was employed. The isolated *P. aeruginosa* strains were stored at +4°C on the nutrient agar slopes and *Candida* isolates were maintained on SDA plates. The inoculums were prepared by fresh 24 hour plate cultures of each *P. aeruginosa* strain of 10^6 CFU/mL in 0.08% NaCl to test the anticandidal activity. Freshly prepared inoculum (20 μL) was streaked on SDA and blood agar with a width of 1 cm across. Plates were then incubated at 30°C for 24 hours. Further growth was removed using a glass slide. Filter paper disks of 5 cm in diameter were cut, soaked in chloroform, and laid on a metal tray in a safety cabinet. Each plate was then placed face down on the top of a chloroform-containing filter paper disk and was left for 20 minutes so that the microscopic remnants of the culture were killed. The plates were removed from the cabinet, and traces of chloroform were eliminated by exposure to air for few minutes. A fresh 24 hour plate culture of each fungal strain was used to prepare an inoculum of 10^6 CFU/mL. This fungal suspension was streaked onto the chloroform treated medium at right angles to the line of the original medium: plates were then incubated for 24 hours at 30°C. Each of the 20 *P. aeruginosa* strains was tested against each of the three *Candida* strains. Plates were read as follows: total inhibition fungal growth was recorded as (a ± b) and no inhibition of fungal growth was recorded as (−).

Statistical analysis

The Chi-square test was used to compare *in vitro* anticandidal activity on blood agar and on SDA.

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