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Research Article

ANTIFUNGAL ACTIVITY OF MANGROVE MEDICINAL PLANTS AGAINST *Candida albicans* AND *Candida glabrata*

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Abstract

Ten mangrove medicinal plants *viz*., *Avicennia marina*, *Rhizophora mucuronata*, *Rhizophora mangle*, *Asparagus officinalis*, *Ceriops decandra*, *Aegiceras corniculatum*, *Acanthus ilicifolius*, *Bruguiera cylindrica*, *Rhizophora apiculata* and *Xylocarpus grantum* were collected from mangrove forest of Pichavaram, Tamil Nadu, India. The antifungal activity of mangrove plant extracts (300 mg/ml) against *Candida albicans* and *Candida glabrata* was determined by Disc diffusion method. The methanol extract of *Ceriops decandra* showed maximum mean zone of inhibition against *Candida albicans* and *Candida glabrata* followed by *Avicennia marina, Rhizophora mucronata*, *Aegiceras corniculatum, Rhizophora apiculata, Rhizophora mangle*, *Acanthus ilicifolius*, *Asparagus officinalis, Xylocarpus grantum* and *Bruguiera cylindrica***.** The hexane crude extract of mangrove plants showed minimum zone of inhibition against *Candida albicans* and *Candida glabrata*. The DMSO was used as a blind control and the antifungal agent Fluconazole (300 mg/ml) was used as a positive control. Minimum inhibitory concentration (MIC) of the mangrove plant extracts against *Candida albicans* and *Candida glabrata* was tested in Mueller Hinton broth by Broth macro dilution method. The MIC of mangrove plants against *Candida albicans* and *Candida glabrata* was ranged between 20 mg/ml to 640 mg/ml.

Keywords: Mangrove medicinal plants, *Candida albicans*, *Candida glabrata* and Antifungal activity.

Introduction

Microorganisms have potential to cause human diseases. Most of the time viruses, bacteria and fungi act as major pathogenic organisms. The discovery of antibiotics in the early twentieth century provided an increasingly important tool to combat bacterial diseases. As antibiotics are increasingly used and misused, the bacterial strains become resistant to antibiotics rapidly. Therefore, screening of antibacterial activity of medicinal plants is very important since vast number of medicinal plants have been used for centuries as remedies for human diseases. Among them extracts from different parts of mangroves and mangrove associates are widely used throughout the world (Abeysinghe *et al*., 2003).

The marine world offers an extremely rich resource for important compounds of structurally novel and biologically active metabolites. It also represents a great challenge which requires inputs from various scientific areas to bring the marine chemical diversity up to its therapeutic potential. So far, many chemically unique compounds of marine origin, with different biological activities, have been isolated and a number of them are under investigation or development (Faulkner, 2000; Da Rocha, 2001).

Natural products and related drugs are used to treat 87% of all categorized human diseases including bacterial infection, cancer and immunological disorders (Newman and Cragg, 2007). About 25% of prescribed drugs in the world originate from plants and over 3000 species of plants have been reported to have anticancer properties (Graham, 2000). About 80% of the

populations in developing countries rely on traditional plant based medicines for their primary health care needs. India has a rich and prestigious heritage of mangrove forest oriented medicines among the South Asian countries. However, the majority of these plants have not yet undergone chemical, pharmacological and toxicological studies to investigate their bioactive compounds (Singh, 2009). Traditional records and ecological diversity indicate that Indian plants represent an exciting resource for possible lead structures in drug design. Numerous studies have been carried out on various natural products screening their antimicrobial activity which can protect the human body against pathogens. Besides small molecules from medicinal chemistry, natural products are still major sources (Nita *et al*., 2002, Ates *et al*., 2003; Bhattacharjee *et al*., 2006; Parekh and Chanda, 2006).

Mangroves can exist under wide ranges of salinities, tidal amplitudes, winds, and temperatures, muddy and anaerobic soil conditions. This variable habitat conditions make them very rich in biodiversity. Mangroves are still play an important role to many people who live along tropical shorelines. They have been managed in some countries as a sustained yield forest crop. Recently their ecological, environmental and socio-economic importance has also been emphasized (Bandaranayake. 1998). As mentioned earlier, Mangroves exist under stressful conditions such as violent environments, high concentration of moisture, high and low tides of water, and abundant living microorganisms and insects. Due to this special growth environment, mangroves produce diverse group of metabolic substances with wide range of biological activities such as antiviral, antibacterial, antifungal and insecticidal. To date, only about 200 bioactive metabolites have been recorded from the mangroves of tropical and sub-tropical populations (Bandaranayake 2002). Large number of mangrove plant species has been used in traditional medicine. Different extracts from mangroves and mangrove associated species were shown to demonstrate diverse effects against human, animal and plant pathogens. However, very limited investigations have been carried out to identify the metabolite agents that possible to be responsible for their bioactivities. The medicinal properties of mangrove and mangrove associated plants represent a wide domain for several biological applications that need to be explored.

Mangrove plants have been used in the folklore medicines and extracts from mangrove species have proven activity against human animal and plant pathogens. Secondary metabolites like alkaloids, phenolics, steroids, terpenoids have been characterized from mangroves and have toxicological pharmacological and ecological importance (Babdaranayake, 2002). Mangrove plant and their products have been extensively used in traditional plants medicine. These plants are well known to have diverse natural products with great pharmaceutical importance and also exhibiting antimicrobial, antiviral, antilarval and antiinsecticidal activity (Kumar *et al*., 2011).

Ceriops decandra species is rare with a restricted distribution. It has an area of occupancy estimated to be less than 4,500 km^2. It is threatened by habitat loss from coastal development throughout its range. Although, exact population reduction is unknown, it is estimated to be between 12 - 26% over a twenty year period (1980 - 2000), and it is therefore listed as near threatened. However, with more information to estimate population reduction over a period of three generation lengths (120 years) declines would likely be much higher, and this species may likely qualify for a threatened category. Recent research has shown that the range of *Ceriops decandra* is restricted to the east coast of India and Bangladesh, Southwestern Thailand and Western part of the Malay Peninsula.

Materials and Methods

Collection of *Ceriops decandra* leaves

The leaves of mangrove medicinal plants *viz*., *viz*., *Avicennia marina*, *Rhizophora mucuronata*, *Rhizophora mangle*, *Asparagus officinalis*, *Ceriops decandra*, *Aegiceras corniculatum*, *Acanthus ilicifolius*, *Bruguiera cylindrica*, *Rhizophora apiculata* and *Xylocarpus grantum* were collected from mangrove forest of Pichavaram, Tamil Nadu, India and the collected plants were used for the present investigation.

Collection of fungal cultures

Candida albicans (MTCC) and *Candida glabrata* (MTCC) isolates were used in this present study. The fungal cultures were procured from Microbial Type Culture Collection (MTCC), Chandigarh, India.

Disc Preparation

Six mm (6 mm) diameter discs were prepared using sterile Whatman No.1 filter paper. The mangrove medicinal plants extracts (300 mg/ml) obtained using solvents (methanol, acetone, chloroform, hexane and ethyl acetate) were mixed with 1ml of 5% Dimethyl sulfoxide (DMSO). The discs were impregnated with 20 μl of different solvent extracts of mangrove plants to check their antifungal activity. The Flucanozole (300 mg/ml) was used as positive control and the 5% DMSO was used as a blind control.

Antifungal assay

The antifungal activity of mangrove medicinal plants extracts were determined by Disc diffusion method proposed by Bauer *et al.* (1966). Petriplates were prepared by pouring 20 ml of Sabouraud's dextrose agar and allowed to solidify for the use in susceptibility test against bacteria. Plates were dried and 0.1 ml of standardized inoculum suspension was poured and uniformly spreaded. The excess inoculum was drained and the plates were allowed to dry for five minutes. After drying, the discs with extract were placed on the surface of the plate with sterile forceps and gently pressed to ensure contact with the agar surface. The Fluconazole (300 mg/ml) was used as positive control and the 5% DMSO was used as a blind control in these assays. The plates were incubated at 28°C for 48 hours (yeasts) and 72 hours (molds). The zone of inhibition was observed and measured in millimeters. Each assay in these experiments was repeated three times for concordance.

Minimum inhibitory concentration for fungi

Minimum inhibitory concentration (MIC) of the mangrove medicinal plants extracts against fungal isolates was tested in Sabouraud's dextrose broth by Broth macro dilution method (Ericsson and Sherri, 1971). The mangrove plant extracts were dissolved in 5% DMSO to obtain 128 µg/ml stock solutions. 0.5 ml of stock solution was incorporated into 0.5 ml of Sabouraud's dextrose broth for fungi to get a concentration of 20, 40, 80, 160, 320 and 640 mg/ml for mangrove plants extracts and 50 µl of standardized suspension of the test organism was transferred on to each tube. The control tube contained only organisms and devoid of mangrove plant extracts. The culture tubes were incubated at 28^{o}C for 48 hours (yeasts) and 72 hours (moulds). The lowest concentration, which did not show any growth of tested organism after macroscopic evaluation was determined as minimum inhibitory concentration (MIC).

Results and Discussion

The antifungal activity of selected mangrove leaf extracts were tested against *Candida albicans* and the results were presented in Table - 1. The methanol extract of *Ceriops decandra* (300 mg/ml) showed maximum mean zone of inhibition against *Candida albicans* (14 ± 0.8 mm) followed by *Avicennia marina* (14 ± 0.6 mm)*, Rhizophora mucronata* (14 ± 0.4 mm), *Aegiceras corniculatum* (13 ± 0.5 mm), *Rhizophora apiculata* (12 ± 0.5 mm)*, Rhizophora mangle* (11 ± 0.7 mm), *Acanthus ilicifolius* (11 ± 0.6 mm), *Asparagus officinalis* (11 ± 0.5 mm)*, Xylocarpus grantum* (11 ± 0.4 mm) *and Bruguiera cylindrica* (10 ± 0.3 mm) at 300 mg/ml**.** The hexane extract of *Ceriops decandra* showed minimum zone of inhibition against *Candida albicans* at 300 mg/ml when compared to the other solvent extracts. No zone of inhibition was seen in DMSO blind control and the positive control Fluconazole (300 mg) showed 16 ± 0.7 mm inhibition zone against *Candida albicans*.

The minimum inhibitory concentration (MIC) of mangrove plants against *Candida albicans* was ranged between 20 mg/ml to 640 mg/ml and the results were showed in Table - 2. The methanol extract of *Ceriops decandra, Avicennia marina* and *Rhizophora mucronata* showed best MIC at 20 mg/ml against *Candida albicans*. *Aegiceras corniculatum, Rhizophora apiculata, Rhizophora mangle, Acanthus ilicifolius, Asparagus officinalis* and *Xylocarpus grantum* showed best MIC at 40 mg/ml. *Bruguiera cylindrical* showed best MIC at 80 mg/ml.

Varahalarao Vadlapudi and Chandrasekhar Naidu (2009) compared the antimicrobial activities of hexane, chloroform and methanol extracts of *Ceriops decandra* using Disk diffusion assay. The methanol extracts of *Ceriops decandra* showed prominent antimicrobial activities, While chloroform and hexane extracts show very less or no antimicrobial activity.

The antifungal activity of selected mangrove leaf extracts were studied against *Candida glabrata* and the results were tabulated in Table - 3. The methanol extract of *Ceriops decandra* showed maximum mean zone of inhibition against *Candida glabrata* (15 ± 0.5 mm) followed by *Avicennia marina* (14 ± 0.5 mm)*, Rhizophora mucronata* (13 ± 0.5 mm), *Aegiceras corniculatum* (12 ± 0.6 mm), *Rhizophora apiculata* (12 ± 0.6 mm)*, Rhizophora mangle* (11 ± 0.7 mm), *Acanthus ilicifolius* (11 ± 0.6 mm), *Asparagus officinalis* (11 ± 0.5 mm)*, Xylocarpus grantum* (11 ± 0.3 mm) and *Bruguiera cylindrica* (8 ± 0.2 mm) at 300 mg/ml**.** The hexane extract of *Ceriops decandra* showed minimum zone of inhibition against *Candida glabrata* at 300 mg/ml when compared to the other solvent extracts. No zone of inhibition was seen in DMSO blind control and the positive control Fluconazole (300 mg) showed zone of inhibition was 17 ± 0.6 mm against the *Candida glabrata.* The minimum inhibitory concentration (MIC) of mangrove plants against *Candida albicans* was ranged between 20 mg/ml to 640 mg/ml and the results were showed in Table - 4. The methanol extract of *Ceriops decandra* showed best MIC at 20 mg/ml against *Candida glabrata*. *Avicennia marina, Rhizophora mucronata, Aegiceras corniculatum, Rhizophora apiculata, Rhizophora mangle, Acanthus ilicifolius, Asparagus officinalis* and *Xylocarpus grantum* showed best MIC at 40 mg/ml. *Bruguiera cylindrical* showed best MIC at 80 mg/ml.

Chandrasekaran *et al*. (2009) studied the antibacterial activity of aqueous and methanol extracts of

Table - 1: Antifungal activity of mangrove medicinal plants against *Candida albicans*

Mangrove leaf extracts (300 mg/ml) and Zone of inhibition (mm)						
Name of the plants	**Hexane**	**Chloroform**	**Ethyl acetate**	**Acetone**	**Methanol**	**Positive Control ***
Avicennia marina	9±0.5	10±0.3	12±0.5	13±0.3	14±0.6	16±0.7
Rhizophora mucronata	11±0.7	11±07	10±0.7	12±0.6	14±0.4	
Ceriops decandra	11±0.7	12±0.7	12±0.4	14±0.4	14±0.8	
Rhizophora mangle	8±0.6	10±0.6	9±0.6	10±0.6	11±0.7	
Asparagus officinalis	8±0.6	8±0.5	7±0.6	10±0.3	11±0.5	
Aegiceras corniculatum	8±0.3	7±0.5	9±0.3	11±0.3	13±0.5	
Acanthus ilicifolius	8±0.3	8±0.5	9±0.3	10±0.5	11±0.6	
Bruguiera cylindrica	9±0.5	10±0.6	8±0.7	8±0.4	10±0.3	
Rhizophora apiculata	7±0.4	9±0.3	9±0.6	11±0.5	12±0.5	
Xylocarpus grantum	8±0.5	8±0.4	10±0.4	11±0.4	11±0.4	

Table - 2: Minimum inhibitory concentration of medicinal plants against *Candida albicans*

Minimum inhibitory concentration (mg/ml)						
Name of the plants	**Hexane**	**Chloroform**	**Ethyl acetate**	**Acetone**	**Methanol**	**Positive Control ***
Avicennia marina	160	80	80	40	20	20
Rhizophora mucronata	160	80	80	40	20	20
Ceriops decandra	160	80	80	40	20	20
Rhizophora mangle	160	160	80	80	40	20
Asparagus officinalis	320	160	80	40	40	20
Aegiceras corniculatum	320	160	80	40	40	20
Acanthus ilicifolius	160	160	80	80	40	20
Bruguiera cylindrica	320	160	160	80	80	20
Rhizophora apiculata	160	80	80	40	40	20
Xylocarpus grantum	320	160	80	80	40	20

Table - 3: Antifungal activity of mangrove medicinal plants against *Candida glabrata*

Mangrove leaf extracts (300 mg/ml) and Zone of inhibition (mm)						
Name of the plants	**Hexane**	**Chloroform**	**Ethyl acetate**	**Acetone**	**Methanol**	**Positive Control ***
Avicennia marina	10±0.4	10±0.3	11±0.6	13±0.6	14±0.5	17±0.6
Rhizophora mucronata	10±0.4	10±0.6	11±0.6	12±0.7	13±0.5	
Ceriops decandra	11±0.3	9±0.5	9±0.4	13±0.6	15±0.5	
Rhizophora mangle	8±0.6	9±0.5	9±0.6	8±0.6	11±0.7	
Asparagus officinalis	9±0.5	9±0.5	9±0.6	10±0.4	11±0.5	
Aegiceras corniculatum	7±0.4	7±0.3	10±0.5	10±0.3	12±0.6	
Acanthus ilicifolius	8±0.6	9±0.3	10±0.3	10±0.5	11±0.6	
Bruguiera cylindrica	10±0.5	10±0.6	9±0.3	11±0.6	8±0.2	
Rhizophora apiculata	8±0.7	8±0.6	8±0.8	10±0.8	12±0.6	
Xylocarpus grantum	7±0.7	9±0.3	9±0.5	11±0.6	11±0.3	

Table - 4: Minimum inhibitory concentration of medicinal plants against *Candida glabrata*

Minimum inhibitory concentration (mg/ml)						
Name of the plants	**Hexane**	**Chloroform**	**Ethyl acetate**	**Acetone**	**Methanol**	**Positive Control ***
Avicennia marina	160	160	80	80	40	20
Rhizophora mucronata	160	160	80	80	40	20
Ceriops decandra	160	80	80	40	20	20
Rhizophora mangle	320	160	160	80	40	20
Asparagus officinalis	320	160	160	80	40	20
Aegiceras corniculatum	320	160	80	80	40	20
Acanthus ilicifolius	320	160	80	80	40	20
Bruguiera cylindrica	320	160	160	80	80	20
Rhizophora apiculata	320	160	80	80	40	20
Xylocarpus grantum	320	160	160	80	40	20

leaves/shoots of five salt marsh halophytes and six mangroves against Methicillin resistant, clinical isolates of *Staphylococcus aureus*. There was a clear comparability between the salt marsh halophytes and mangroves in their antibacterial action. The mangrove plants possessed higher antibacterial potency than the salt marsh halophytes. The highest activity was recorded with the methanol extract of *Excoecaria agallocha* followed by the methanol extracts of *Aegiceras corniculatum*, *Lumnitzera racemosa* and *Ceriops decandra*. The minimum inhibitory concentration (MIC) values ranged from 0.125 to 4 mg/mL and 1 to 16 mg/ml for methanol and aqueous extracts.

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