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Research Article

ANTIBACTERIAL EFFICACY OF CRUDE EXTRACTS OF *TRICHODERMA* SPP. ISOLATED FROM MANGROVE RHIZOSPHERE

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ABSTRACT

The present study was carried out to determine the antibacterial efficacy of crude extracts of *trichoderma* spp. fungi isolated from mangrove rhizosphere soil. Four different strains of *trichoderma* spp. were isolated and identified from marine mangrove rhizosphere soil. Crude extracts of these fungi were prepared. The antibacterial efficacy was determined by agar disc diffusion method against three different bacterial strains viz., *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), and *Staphylococcus aureus* (ATCC 25923) according to Kirby-Bauer disc diffusion method. Good inhibitory activity was observed in the crude extracts of *Trichoderma harzianum(1)*, *Trichoderma viridae* followed by *Trichoderma harzianum(2)* and *Trichoderma inhamatum*. All the *Trichoderma* spp. inhibit Gram positive bacteria *Staphylococcus aureus* followed by the Gram negative bacteria *E.coli* and minimum inhibition was noted in *Pseudomonas aeruginosa*.The secondary metabolites of the marine mangrove fungi belonging to *Trichoderma* spp. can be used as a potential antibacterial agent in the increasing scenario of antibiotic drug resistance.

Keywords: antibacterial, *Trichoderma* spp., secondary metabolites, drug resistance

INTRODUCTION

Ocean covers almost 70% of the earth's surface and nowadays wide range of pharmaceutical medicines are discovered from marine sources. Marine habitat represents an enormous resource for the discovery of potential therapeutic agents. Over the last several decades, numerous compounds have been found in marine organisms with interesting pharmaceutical activities[1]. Harsh and diverse marine environments lead to the growth of wide range organisms with potential chemical diversity. These microbes can provide a valuable novel compounds for the development of bioactive products[2].The beneficial effects of mangrove plants have been mentioned in folklore medicine. Extensive research on mangrove metabolites have sprung up in the last two decades that establishes the mangroves as a source of novel compounds having good biological activity[3].

Marine microbes represent a potential source for commercially important bioactive compounds and their bioremediation capabilities are also remarkable [4]. Numerous novel compounds have been isolated from marine organisms and many of these have been reported during the past 30 to 40 years to have biological activities, some of which are of interest from the point of view of potential drug development[5].This highlights the importance of marine microorganisms as a prospective source of natural products [6]. The need for new and safe bioactive compounds to provide comfort in all aspect of human's life is ever increasing.

Synthetic antibiotics and drugs are extending antibiotic resistant microbes; however it is essential to investigate a new way to treat infectious diseases[7].The ocean represents a rich resource for the novel compounds with great potential as pharmaceuticals, nutritional supplements, cosmetics, agri-chemicals, enzymes and antibiotics. It harbours antagonistic microbial populations which forms potential secondary metabolites that efficiently shows antibacterial activity [8].Marine fungi produce diverse range of biologically active secondary metabolites which can be obtained from various sources of marine products like sponges, fishes, mangroves, grasses and algae.[9]

MATERIALS AND METHODS

Collection method

Rhizosphere soil of Mangrove plant *Avicennia marina* and *Rhizophora mucronata* samples were collected and kept in a sterile plastic petri plates (Hi-media) and brought to the laboratory immediately. It was collected during three seasons namely, In Summer –April to June, Pre-monsoon July to September and Monsoon during October to December. Based on microscopic and cultural characteristics, *Trichoderma spp.* were identified and used for the further analysis.

Preparation of crude extracts

Preparation of crude extracts of mangrove rhizosphere fungi *Trichoderma spp.* and extraction of active components were done by the following method .[10] Mass scale fermentation of required fungi were transfered into sterile Erlenmeyer flask (1L) containing 100 ml of distilled water and 100 g of rice. Then the culture was incubated at room temperature for 30 days. After 30 days of incubation, 250 ml of ethylacetate were added to the culture and left overnight. The contents were filtered under vacuum using Buchner funnel, for optimal extraction of fungal biomass. The extraction was repeated up to three times with

ethylacetate until the colour faded. All the filtrates were combined and washed with distilled water. The aqueous and ethylacetate phases are left in a separation funnel until complete separation of the two immiscible liquid phases is achieved. The dry residue obtained from EtOAc extracts were partitioned between *n*-hexane and 90% methanol in the ratio of 1:1 (vol/vol) (~150 ml each).Separation of two immiscible liquid phase is achieved and methanol phase was dried under vacuum (~200 mbar) using rotary evaporator at 40°C.Finally solid residue were obtained and used for further purification.

Microorganisms used for test

The test cultures for general antibacterial activity were done using *Pseudomonas aeruginosa (ATCC 27853), Escherichia coli (ATCC 25922), and Staphylococcus aureus (ATCC 25923).* All the bacterial strain used for this study are ATCC strain (American Typed Culture Strains).

Antibacterial activity of crude extracts on standard bacteria

Antibacterial activity was carried out by by Kirby–Bauer agar disc diffusion method. All the extracts were screened for their antibacterial activities against the bacterial strains. Five sets of dilutions viz., 5, 25, 50, 100, and 250 µg/ml of *Trichoderma* crude extract were added to sterile filter paper discs and dried. Mueller-Hinton agar plates were prepared and seeded with the corresponding bacterial strains. Corresponding antibiotics for tested bacteria were used as the positive control. Sterile discs with distilled water were used as the negative control. The zone of inhibition was measured after 24 hrs of incubation [11].

Table 1 Antibacterial activity of *Trichoderma harzianum* (1)

Antibacterial activity (zone of inhibition) in mm	Crude concentration in µg/ml				
Test-microorganisms	5 µg	25 µg	50 µg	100 µg	250 µg
Pseudomonas aeruginosa (ATCC 27853)	-	11mm	13mm	16mm	18mm
Escherichia coli (ATCC 25922)	-	14mm	15mm	17mm	19mm
Staphylococcus aureus (ATCC 25923)	-	13mm	15mm	16mm	20mm

Table 2 Antibacterial activity of *Trichoderma viridae*

Antibacterial activity (zone of inhibition) in mm	Crude concentration in µg/ml				
Test microorganisms	5 µg	25 µg	50 µg	100 µg	250 µg
Pseudomonas aeruginosa (ATCC 27853)	-	10mm	13mm	14mm	16mm
Escherichia coli (ATCC 25922)	-	12mm	13mm	13mm	18mm
Staphylococcus aureus (ATCC 25923)	-	12mm	14mm	15mm	20mm

Table 3 Antibacterial activity of *Trichoderma inhamatum*

Antibacterial activity (zone of inhibition) in mm	Crude concentration in µg/ml				
Test-microorganisms	5 µg	25 µg	50 µg	100 µg	250 µg
Pseudomonas aeruginosa (ATCC 27853)	-	10mm	08mm	08mm	08mm
Escherichia coli(ATCC 25922)	-	12mm	11mm	13mm	16mm
Staphylococcus aureus (ATCC 25923)	-	12mm	12mm	15mm	18mm

Table 4 Antibacterial activity of *Trichoderma harzianum* (2)

Antibacterial activity (zone of inhibition) in mm	Crude concentration in µg/ml				
Test-microorganisms	5 µg	25 µg	50 µg	100 µg	250 µg
Pseudomonas aeruginosa (ATCC 27853)	-	10mm	12mm	13mm	16mm
Escherichia coli (ATCC 25922)	-	11mm	11mm	15mm	18mm
Staphylococcus aureus (ATCC 25923)	-	12mm	12mm	16mm	19mm

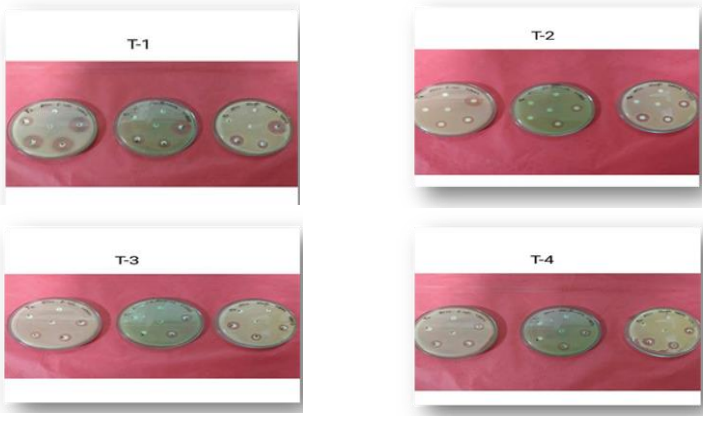

Figure 1: Antibacterial activity of *Trichoderma Harzianum(1)* T1, *Trichoderma Viridae* T2, *Trichoderma Inhamatum* T3 *And Trichoderma Harzianum(2)*T4 on 3 ATCC strains

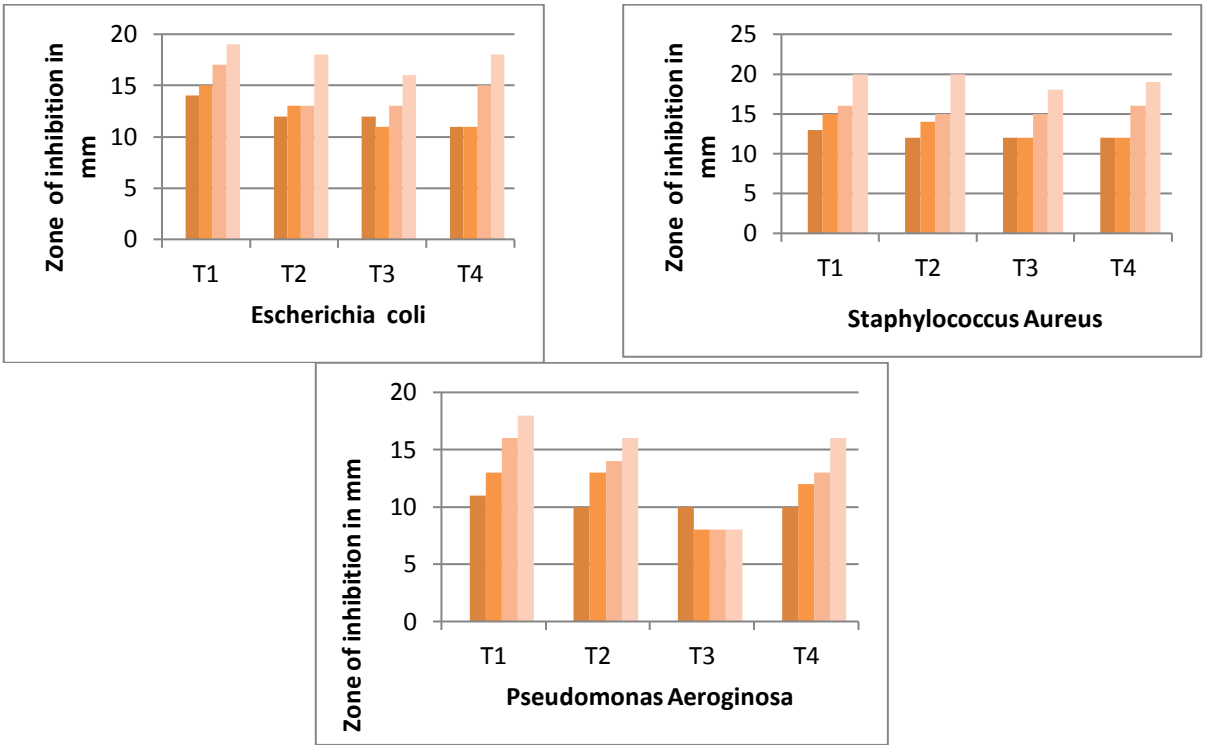

Figure 2: Chart depicting the Antibacterial activity of *Trichoderma Harzianum(1)* T1, *Trichoderma Viridae* T2, *Trichoderma Inhamatum* T3 *And Trichoderma Harzianum(2)*T4 on 3 ATCC strains

RESULTS & DISCUSSION

Marine environment associated fungi producing secondary metabolites are exploited for medical, agricultural and industrial purposes. Microbial production of drugs from marine related fungi will be of immediate interest than pharmacological active components from medicinal plants.

It's been reported that more than 105 marine fungi were used for the isolation of anti microbrial compounds. The sponges, corals and mangroves associated fungi were expected to synthesize unique secondary metabolites. In addition, 20 compounds with promising antimicrobial activities were derived from fungi isolated from marine sediments.This study indicates that marine sediments act as a reservoir for fungal isoaltion[12]. In our present study, new source of mangrove rhizosphere soil fungus was taken for the bioactive compound production. Trichodermaquinone was isolated from *T. aureoviride* and exhibited antibacterial activity against MRSA[13]. Compound Trichodermaketone A was obtained from *T. koningii* and exhibited synergistic antifungal activity against *C. albicans* [14] Likewise the ethanol crude extract of Trichoderma spp. show inhibitory activity against pathogenic bacteria. The secondary metabolites with antibiotic activities that are produced by *T. harzianum* are classified in different groups based on volatile compounds and are associated with the antibacterial activity [15]. In this study, volatile compounds act as anti-bacterial activity against Gram positive as well as Gram negative bacteria. Peptaibol, Trichodermin and Cyclosporin A are the common drug obtained from *Trichoderma spp.* which are commonly used as antibiotics, antifungal agents and immunosuppressants.

Hence this present study focused to identify the bioactive compound from *Trichoderma spp*. Four rhizosphere fungi *Trichoderma spp.* were selected based on the antimicrobial significance for testing antibacterial activity. The crude metabolites exhibited strong to moderate antimicrobial activity against all the test pathogens. Good inhibitory activity was observed in the crude extracts of *Trichoderma harzianum(1)*, *Trichoderma viridae* followed by *Trichoderma harzianum(2)* and *Trichoderma inhamatum*. All the *Trichoderma* spp. inhibit Gram positive bacteria *Staphylococcus aureus* followed by the Gram negative bacteria *Escherichia coli* and minimum inhibition was noted in *Pseudomonas aeruginosa*.

CONCLUSION

In conclusion, the study emphasizes that novel microbes like *Trichoderma spp*. from different environmental sources such as the mangrove rhizosphere produce unique secondary metabolites which can be harnessed to develop drug molecules to curb the crisis of multidrug resistance in bacteria and therefore can be used for the treatment of infectious diseases.

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