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Evaluation of antagonistic activities of microbes from Vallapattanam and Pappinishery mangrove ecosystems of Kannur district in Kerala, India

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Abstract

Mangrove soil habitat is biologically rich and provides a unique ecological niche to a variety of organisms. The highly productive and diverse microbial community of the mangrove ecosystems have inspired us to study the antagonistic relation among the different microbial forms in the Vallapattanam and Pappinishery mangrove soils. From these mangrove samples a total of 28 bacteria, 22 actinomycetes and 3 fungal forms were isolated. Majority of the bacterial isolates were gram positive rods. 12 actinomycetes showed antagonistic activity towards the bacteria isolated from the same soil samples, with the highest activity shown by A11 and A16 which inhibited all the native bacteria. A16, A18, A24 and C14 demonstrated significant fungicidal activity (inhibitory zones ranging from 16 mm to 39 mm) against the three native fungi, namely, *Aspergillus flavus, Penicillium* and *Trichoderma.* A17 and A24 have shown excellent antibiosis against hospital isolates of *Candida albicans, C. parapsilosis, Trichophyton rubrum, T. mentagrophytes* and *Cryptococcus.* All the three fungal forms demonstrated antibacterial activity as high as 35 mm. These results indicate that the crude extracts of the antagonistic fungal and actinomycete forms possess promising antimicrobial properties and can be studied in details for their novel chemotherapeutic properties.

Key-Words: Mangrove, Actinomycetes, Antagonistic, Fungicidal, Antimicrobial

Introduction

Mangroves are coastal wetland forests mainly comprising of an assemblage of tropical trees and shrubs that inhabit the intertidal zone of tropical and subtropical latitudes. The specific regions where mangrove plants grow are termed as "mangrove ecosystem". It is estimated that mangrove forests cover a total area of about 1, 81,000 km^2 in over one fourth of the world's coastline[1, 2]. Mangrove communities are considered highly productive and contribute significantly to the global carbon cycle. Mangrove ecosystems produce large amount of litter in the form of falling leaves, branches and other debris that after autolysis and microbial breakdown, produce detritus (plant material converted to dead organic matter) which is the most important source of energy for the estuarine food chain. Thus mangroves, by introducing sizable quantities of organic material to the community, play an important and essential role in supporting a wide range of offshore marine organisms in the early stages of development, thereby sustaining coastal fisheries[3].

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Microbes perform various activities in the mangrove ecosystem like photosynthesis[4], nitrogen fixation[5], methanogenesis[6], production of antibiotics and enzymes (arylsuphatase, L-glutaminase, chitinase, L-asparaginase, cellulose, proteasae, phosphatase), etc. which result in the high productivity[7]. Bacteria and fungi constitute to be the major decomposers of the mangrove material and facilitate the cycling of nutrients. Fungi are the primary litter invaders, reaching their peak in the early phases of decomposition[8]. The phylloplane fungi do not attack live leaves and begin to break down the leaf material only after it has been submerged. Bacterial colonies appear shortly after the litter has been colonized by fungi. The bacteria grow quickly and can reach very high densities making them the highest found living forms in the mangrove ecosystems. Among the microbes, the bacterial populations in mangroves are many-fold greater than the fungi and exist as symbionts with plants and animals, saprophytes on dead organic matter, and as parasites on living organisms.

Antibiotics are secondary metabolites generally produced by multi-step biosynthetic pathways starting from intermediates of primary metabolites[9]. These

antibiotics are widely distributed in the nature, where they play an important role in regulating the microbial population of soil, water, sewage, and compost. The mangrove environment is a potent source for the isolation of antibiotic-producing actinomycetes[10]. Approximately 80% of naturally occurring antibiotics, including many of medical importance, have been isolated from actinomycetes, a major group of aerobic, gram-positive soil bacteria that are very widely distributed[11].

The antimicrobial potential of extracellular metabolites of actinomycetes strains against bacterial pathogens is abundant. However, there are less scientific literatures on the antagonistic effects of fungi on bacteria and the antifungal activities of actinomycetes in a mangrove community. Thus the present study aims at exploring the antagonistic activities of the rhizosphere microbial communities from the untapped Vallapattanam and Pappinishery mangrove ecosystems of Kerala, which can be rich sources of useful metabolites.

Material and Methods

Collection of soil sample

The soil samples were sampled from the rhizosphere region of the young *Avicennia officinalis* plants inhabiting the study site located at Valapattanam (11° 56′ 0″ N, 75° 18′ 0″ E) and Pappinishery (11°57′N, 75°21′E) mangrove forest of Kannur district, Kerala during October, 2010. Care was taken to keep the root-sediment ball intact. All the samples were aseptically collected in sterile zip lock covers and transported to the laboratory for further processing.

Determination of sediment temperature

The temperature of the soil at the three different sites was determined by the use of thermometer. The thermometer was inserted into the soil up to depth of 5cm and allowed to stay for 15 min, after which the temperature reading was obtained[12].

Determination of sediment pH

The soil pH values were determined by digital pH meter using standard methods of Watson and Brown[13]. Using this method, 3 g of soil sample was weighed into a beaker containing 3 ml of distilled water, which was stirred for five seconds and allowed to stand for 10 minutes. The electrode of the pH meter was then inserted into the slurry and swirled carefully. The reading was taken thereof and was recorded for each site.

Sample processing

On the arrival of the samples they were kept under refrigeration and dark condition until used. All the three samples were processed within one hour and the pH, texture, colour was recorded. The soil samples were divided into two halves, out of which one half was used for the isolation of bacteria and fungi. The other half of the soil sample was used for the isolation of actinomycetes, wherein the soil sample was air dried aseptically. After a week, sediment samples were incubated at 55˚C for 5 min in order to facilitate the isolation of actinomycetes.

Isolation of bacteria

For the isolation of bacteria from the three different samples collected (Sample A, Sample B and Sample C), one gram soil sample was serially diluted and 100 µl of the diluted samples were spread plated on the nutrient agar plates. The plates were kept in incubator at 37˚C for 24 h for the appearance of the bacterial isolates.

Isolation of fungi

For the isolation of the fungal forms from the three different samples collected (Sample A, Sample B and Sample C), one gram soil sample was serially diluted and 500µl of the diluted samples were spread plated on the potato dextrose agar (PDA, Hi-Media). The plates were kept in incubator at 27˚C for 3 to 5 days until the development of the cottony colonies of fungi.

Isolation of actinomycetes

For the isolation of actinomycetes, the three different samples collected (Sample A, Sample B and Sample C), one gram of the oven dried soil sample was serially diluted and 100 µl of the diluted samples were added from each of the dilutions and spread plated on the starch casein nitrate (SCN) agar medium plates containing (g/l): starch, 10.00; potassium nitrate, 2.00; di - potassium hydrogen phosphate,2.00; sodium chloride, 2.00; casein, 0.30; magnesium sulfate, 0.03; calcium carbonate, 0.02; ferrous sulfate, 0.01; agar, 20.00. Nalidixic acid (20 µg/ml) and cycloheximide (50 µg/ml) were added to the medium in order to retard the growth of bacteria and fungi, respectively. The plates were left in incubator at 30˚C for ten days.

Fermentation

The spore suspensions of the fungal and the actinomycetes isolates were prepared by suspending the spores from a well grown culture slant in 10 ml of sterile distilled water and were used to inoculate 50 ml of potato dextrose broth and SCN broth in 250 ml conical flasks and incubated at 30°C for 7 days on a rotary shaker at 150 rpm.

Preparation of the cell free supernatant (CFS)

Following incubation, the fungal and actinomycetes cultures were centrifuged at 5000 rpm for 20 min at 4˚C to get the CFS. Following centrifugation the pellets were discarded and the supernatants were filtered through 0.2µm filter paper. The pH of the CFSs was adjusted to pH 7 with 1 M NaOH to exclude

antimicrobial effects of organic acids[14]. All the CFSs were stored at 4°C until used.

Partial purification of the antimicrobials

The filtered CFS was made to adsorb on to1.5 g of activated charcoal. This was then filtered using Whatman filter paper and the residue collected was slowly washed with 25 ml of methanol: dil. H_2SO_4 solution (9:1 ratio). The wash was collected in crucibles and acetone was added to it. This was left in hot air oven for 24 h.

Detection of antimicrobial potency of the actinomycetes isolates

Each of the tests in the determination of antimicrobial activity was conducted in triplicates.

Antibacterial activity

The antimicrobial activities of these isolates were studied by the disc diffusion procedure[15, 16]. Sterile cotton swabs were dipped into the cultures of the isolated bacterial forms (previously propagated in nutrient broth for 24 h at 37°C) and inoculated by swabbing over the entire surface of the pre-set Mueller-Hinton agar (MHA) plates. Care was taken to evenly distribute the test pathogens throughout the entire surface of the plates. Within 5 to 15 min after swabbing the test pathogens, sterile filter paper discs were impregnated with 50 µl of the partially purified actinomycetes CFSs, air dried and placed onto the surface of the plates. After 18 to 24 h of incubation at 37°C each plate was examined for the zone of inhibition. The diameters of the inhibitory zones were measured including the diameters of the discs to the nearest whole number. Similar procedure was also followed for the hospital isolates which included bacterial pathogens like *Staphylococcus aureus, S. citreus, Bacillus polymyxa, B. cereus Salmonella typhi, Serratia marcescens, Pseudomonas aeruginosa* and *Proteus mirabilis.*

Antifungal activity towards the native soil isolates

For screening their antifungal activity, spore suspension of the respective fungal isolates was prepared by mixing a loopful of the spores of the in 10 ml of sterile distilled water. A uniform spore suspension was obtained by mixing vigorously, which was measured for absorbance under white light. 1 ml of a spore suspension (10^7 spores/ ml) was swabbed onto the MHA plates and then the above mentioned procedure was followed. The plates were incubated at 27˚C for 5 days and examined for zones of inhibition.

Antifungal activity towards the hospital isolates

Sterile cotton swabs were dipped into the cultures of the hospital isolates of *Candida albicans, C. parapsilosis, Trichophyton rubrum, T. mentagrophytes* and *Cryptococcus* (previously propagated in Brain Heart Infusion (BHI) broth for 48 h at 37°C) and the antimicrobial activity was detected as mentioned above.

Detection of antimicrobial potency of the fungal isolates

Antibacterial activity

Sterile cotton swabs were dipped into the cultures of the isolated bacterial forms (previously propagated in nutrient broth for 24 h at 37°C) and inoculated by swabbing over the entire surface of the pre-set Mueller-Hinton agar plates. Care was taken to evenly distribute the test pathogens throughout the entire surface of the plates. Within 5 to 15 min after swabbing the test pathogens, sterile filter paper discs were impregnated with 50 µl of the partially purified CFSs, air dried and placed onto the surface of the plates. After 18 to 24 h of incubation at 37°C each plate was examined for the zone of inhibition. The diameters of the inhibitory zones were measured including the diameters of the discs to the nearest whole number. Similar procedure was also followed for testing the effect of the antimicrobials on hospital isolates.

Antifungal activity

The spore suspensions of the respective native fungal isolates were swabbed as mentioned above and the hospital isolates of yeasts (following propagation in the BHI broth), were seeded onto the MHA plates as previously mentioned and checked for the effect of antimicrobials after 5 days of growth at 27˚C.

Identification and characterization of the fungal isolates

The fungal isolates were identified based on the growth pattern and the morphological characteristics on PDA plates and the structure of the conidiophores under light microscope[17].

Identification and characterization of the actinomycete isolates

The potent actinomycetes isolates were identified based on the criteria given by Nonomura[18], which includes determination of aerial mass colour, melanoid pigment production, reverse side pigment, soluble pigment production, spore chain morphology and utilization of carbon sources.

Results and Discussion

Mangrove plants have adapted to a unique habitat with muddy saline waters, anaerobic soil, brackish tidal activities and high microbial and faunal competition. Microbial association with mangrove plants confers protection from adverse environmental conditions and allows them to successfully compete with soil borne pathogens[19]. It is well depicted in literatures that due to the microbial action, the rhizospheres of mangrove

plants are a rich source of metabolites that are exploited in pharmaceutical and agricultural industries[20].

Keeping in mind that infectious diseases are still the second leading cause of death worldwide[21], there is a greater need for new antimicrobial agents to combat the emergence of multidrug resistance in common pathogens[22]. Natural products remain to be the most propitious source of antibiotics including about 1000 derived from marine microbes[23]. Antibiotic, generally, refers to antibacterial. However, because the term is loosely defined, it is preferable to specify compounds as being antibacterials, antifungals and antivirals. The ability to produce antibiotics has been found mainly in fungi (group Aspergillales), and in a few other bacteria, especially the actinomycetes.

Isolation of microbial forms

Following plating, a total of 28 bacterial samples were obtained. Based on the Gram staining results, 23 isolates was found to be gram positive rods and 5 isolates were found to be gram negative rods in scattered form. 3 fungal isolates appeared on the PDA plates and 22 actinomycetes were isolated on SCN agar medium.

Identification of fungal isolates

Based on the colony morphology and the arrangement, structure and colour of conidia, conodiospores, F1, F2 and F3 were identified as *Trichoderma sp*, *Penicillium sp* and *Aspergillus flavus* **(Table1)**.

Identification of actinomycete isolates

The potent actinomycetes isolates were identified based on the criteria given by Nonomura[18], which includes determination of aerial mass colour, melanoid pigment production, reverse side pigment, soluble pigment production, spore chain morphology and utilization of carbon sources. Following were the conclusion of the identification: A10 (*Streptomyces orientalis*), A11 *(Streptomyces viridodiastaticus)*, A16 *(Streptomyces antibioticus)*, A18 (*Streptomyces albus*) A21 (*Micromonospora*), A23 (*Streptomyces badius*), C14 (*Streptomyces lavendulae)*, C15 (*Streptomyces aureomonopodiales*), whereas A13, A17, A22 and A24 remained unidentified **(Table 2)**.

Antimicrobial potency of the actinomycete isolates

Of the 22 actinomycetes isolated, 12 (A10, A11, A13, A16, A17, A21- A24, C14 and C15) showed antagonistic activity towards the 23 bacterial and 3 fungal forms. The results demonstrated that the zone of inhibition of the soil bacterial isolates varied between 9 mm to 27 mm **(Table 3)**. This property of the actinomycetes is noteworthy because some of the soil bacteria and fungi are potential plant and animal pathogens including humans. Bacteria by virtue of their proliferic growth outnumbers all the other forms of microbes in the soil habitat and constantly pose threat by developing nutrient depletion for the other forms of life particularly in terms of energy molecules and essential metal ions like iron. Since there had been a high number of actinomycetes forms associated with the antibacterial activity isolated from the same soil it state that these forms of life have evolved over long periods of coexistence and the actinomycetes species have started producing antibacterials to counteract the severe competition from the bacterial forms.

Infectious diseases are the leading health problems with high morbidity and mortality in the developing countries[24]. The development of resistance to multiple drugs is a major problem in the treatment of infectious diseases caused by pathogenic microorganisms. This multidrug resistance is presently an urgent focus of research and new bioactive compounds are necessary to combat these multidrug resistance pathogens.

Of the 12 isolates, isolate A11 and A16 showed significant zone of inhibition against multi drug resistant hospital isolates of *Staphylococcus citreus, S. aureus, Salmonella typhi, Serratia marcescens, Pseudomonas aeruginosa, Bacillus polymyxa, B. cereus* and *Proteus mirabilis* **(Table 4)**. As compared to A16, A11 gave better zone of inhibition towards these hospital isolates. Thus it can be understood that the marine derived antibiotics are more efficient at fighting microbial infections because the terrestrial bacteria have not developed any resistance against them[25].

The actinomycetes found in the marine and coastal ecosystems may be viewed as a rich gene pool possibly containing isolates capable of producing useful metabolites[26]. However, only few attempts have been made out to isolate marine actinomycetes especially for their antifungal activity[27]. In the present study, all the three filamentous fungal isolates showed sensitivity when tested against the antimicrobial compounds produced by the actinomycetes. Though all the 12 actinomycete isolates showed significant fungicidal activity, isolates, A16, A18, A24 and C14 demonstrated zone of clearance ranging between 16 mm to 39 mm, whereas the other actinomycete isolates on an average demonstrated no significantly less antimicrobial activity **(Table 5)**. The antagonistic activity of these actinomycetes to fungal pathogens is usually related to the production of antifungal compounds like polyenes[28] and may be related to chitinase production[29].

The result obtained in our study is significant since to the best of our knowledge no earlier work has concentrated on the use of the phenomenon of

actinomycetes antagonism against a wide variety of yeast pathogens. Previous works have mainly concentrated on the antagonistic effects of actinomycetes on *C. albicans* alone. Isolate A17 and A24 have shown excellent antibiosis against pathogenic yeasts like *C. albicans, C. parapsilosis, T. rubrum, T. mentagrophytes* and *Cryptococcus* with zones of inhibitions between 10 mm to 38 mm **(Table 6)**.

Antimicrobial potency of the fungal isolates

Though investigations on mangrove fungal metabolites with antimicrobial potency are scarce, in our study, all the fungi tested showed antimicrobial activity, emphasizing the potential of fungi of mangrove plants as producers of novel metabolites **(Table 7)**. Earlier, several metabolites of the marine isolate, *Aspergillus niger* showed anti-bacterial and anti-fungal potential[30].

Of the three fugal isolates *A. flavus* showed the highest activity against *S. citreus* (20 mm) and for all the other hospital isolates this fungus demonstrated the antimicrobial activity ranging between 13 mm to 19 mm. Though *Trichoderma* and *Penicillium* showed less antimicrobial activity the results were not very different from that of *A. flavus.* The zones of inhibition of *Trichoderma* are less compared to that of *Penicillium* **(Table 8)**.

Earlier, *A. flavus* was found to inhibit *C. albicans, E. coli* and *S. aureus* by producing inhibition zones of 12mm, 8mm and 12mm, respectively, but had no effect on *P. aeruginosa* [31]. There is not much evidence of antibacterials from *Trichoderma* and they are mainly active against fungal pathogens by virtue of their array of hydrolytic enzymes. However, *Trichoderma viride* showed inhibition zones of 7 mm, 18 mm and 9 mm respectively against *C. albicans, E. coli, P. aeruginosa* [31]. Antimicrobials from *Penicillium* have been mainly active against gram positive bacteria but at higher concentrations these are also found to be active against gram negatives.

Fourteen endophytic fungal strains were isolated from mangrove angiosperm associate, *Acanthus ilicifolius* L. and mangrove fern, *Acrostichum aureum* L. of Nethravathi mangrove, on the southwest coast of India[32]. When screened for anti-microbial activity, none of the fungal extracts inhibited *Trichophyton mentagrophytes*, but *Aspergillus sp*. 2 and *Aspergillus sp*. 3 inhibited *Candida albicans*. All bacteria were inhibited by a sterile fungal isolate MSI 1. *Cumulospora marina* and *Pestalotiopsis sp.* inhibited representatives of both Gram-positive and Gram-negative bacteria. None of the crude extracts were inhibitory against *Cryptococcus albidus.*

The above results indicate that the Vallapattanam and Pappinishery mangrove ecosystems of Kannur district in Kerala, India are rich with actinomycetes and fungi which are diverse in their ability for the production of various antimicrobials. In the lime light of the fast increasing cases of antibiotic resistance by several pathogens, the metabolites of these microbial forms could be further investigated for applications as chemotherapeutic agents.

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Table 1: Macroscopic and microscopic identification of the fungal isolates

Isolates	Colony morphology	Microscopic characteristics	Identification
F1	Green and woolly	Highly branched conidiophores with one or more phialides. Phialides are typically enlarged with single or clustered ellipsoidal conidia.	*Trichoderma*
F2	Blue green and velvety	Simple or branched conidiophores, bearing metulae, brush-like phialides and chains of spherical conidia.	*Penicillium*
F3	Yellow green and powdery	Erect conidiophores composed of long stalks terminating in inflated vesicles. The vesicle produces pale green globose to subglobose conidia.	*Aspergillus flavus*

Table 2: Morphological and physiological characteristics of the actinomycete isolates

	A10	A11	A13	A16	A17	A18	A21	A22	A23	A24	C14	C15
Gram's staining	+	+	+	+	+	+	+	+	+	+	+	+
Aerial mycelia	W	G	G	G	W	Y	-	G	Y	G	P	W
Spore chain morphology	OL	OL	CL	OL	OL	CL	Cluster	OL	Linear	OL	CL	CL
Melanoid pigment	-	-	-	+	+	-	-	+	-	-	-	+
Reverse side pigment	Y	-	-	-	-	Pale brown	-	P	Y	-	-	-
Soluble pigment	-	-	-	Tan	Black	-	Orange	P	Y	-	-	-
Sugar fermentation												
Glucose	+	+	+	+	-	-	+	+	-	+	+	+
Fructose	+	+	-	+	+	+	+	+	-	+	+	+
Mannitol	+	+	-	+	+	+	+	+	-	+	+	+
Xylose	+	+	-	+	+	+	+	+	-	+	-	+
Arabinose	+	+	-	+	+	+	+	+	+	+	+	+
Rhamnose	+	+	-	+	-	-	+	-	-	+	+	+
Inositol	+	+	-	+	+	-	+	+	-	+	-	+

Keys: W, white; G, grey; Y, yellow; P, pink; OL, open loop; CL, closed loop; +, positive

Table 3: Antibacterial activity of the isolated actinomycetes against indigenous bacterial isolates showing zones of inhibition (in mm)

Bacterial isolates	Actinomycete isolates											
	A10	A11	A13	A16	A17	A18	A21	A22	A23	A24	C14	C15
A1	9±0.1*	15±0.1	-	18±0.3	7±0.5	20±0.1	-	9±0.3	15±0.8	9±0.1	13±0.1	12±0.4
A2	8±0.5	17±0.6	-	16±0.6	-	24±0.4	-	7±0.1	13±0.2	9±0.1	14±0.1	13±0.1
A3	12±0.2	21±0.4	-	18±0.2	6±0.6	23±0.9	-	18±0.4	17±0.4	20±0.7	24±0.1	-
A4	7±0.8	15±0.2	-	20±0.6	-	19±0.6	-	18±0.1	10±0.1	10±0.2	10±0.4	12
A5	7±0.3	15±0.5	-	17±0.4	6±0.1	20±0.2	-	15±0.1	15±0.9	9±0.1	18±0.9	13±0.1
A6	9±0.1	22±0.6	-	23±0.3	7±0.3	24±0.5	-	25±0.4	16±0.5	9±0.6	18±0.6	15
B1	-	16±0.2	-	23±0.9	-	23±0.5	-	-	-	8±0.4	20±0.3	16±0.1
B2	8±0.3	22±0.3	-	23±0.1	8±0.1	17±0.9	-	10±0.8	14±0.3	-	13±0.5	-
C1	10±0.5	23±0.1	-	18±0.2	5±0.5	22±0.9	-	12±0.6	12±0.7	7±0.1	15±0.4	14±0.5
C2	-	19±0.4	-	21±0.6	-	24±0.1	-	12±0.2	12±0.9	-	20±0.6	18±0.1
C3	-	17±0.8	-	19±0.9	-	21±0.3	-	18	13±0.1	9±0.5	20±0.9	15±0.6
C4	9±0.9	19±0.1	-	21±0.1	-	18±0.1	-	-	12±0.3	8±0.5	17±0.5	11±0.5
C5	-	21±0.1	14	20±0.4	11	-	-	12±0.4	14±0.8	-	-	-
C6	-	19±0.5	-	19±0.8	-	18±0.6	-	13±0.3	12±0.6	-	-	13±0.6
C7	-	19±0.2	-	19±0.6	-	17±0.2	-	10±0.1	13±0.7	7±0.1	15±0.8	12±0.2

C8	-	15±0.2	-	16±0.9	-	21±0.4	-	12±0.1	13±0.6	10±0.8	21±0.1	14±0.4
C9	-	10±0.3	-	15±0.1	-	20±0.9	-	-	-	8±0.6	10±0.7	13±0.9
N1	8±0.8	19±0.6	-	18±0.1	10±0.5	15±0.6	-	11±0.6	11±0.1	-	-	-
N2	7±0.1	17±0.4	-	18±0.7	7±0.6	18±0.3	-	9±0.3	15±0.2	9±0.43	12±0.1	11±0.4
N3	8±0.4	20±0.4	-	20±0.1	6±0.5	22±0.9	-	8±0.9	15±0.8	9±0.1	16±0.3	18±0.5
N4	7±0.1	20±0.6	-	20±0.3	-	19±0.5	-	10±0.6	18±0.4	6±0.5	20±0.1	18±0.5
N5	8±0.6	19±0.7	-	18±0.6	8±0.9	18±0.1	-	9±0.4	11±0.1	8±0.2	20±0.5	14±0.4
P1	6±0.6	20±0.3	-	17±0.4	7±0.8	25±0.4	-	10±0.6	11±0.2	-	-	12±0.1
P2	-	20±0.1	-	20±0.5	-	22±0.4	-	-	-	10±0.9	11±0.1	11±0.9
K1	-	21±0.1	-	19±0.1	-	-	-	13±0.4	15±0.4	-	-	-
K2	12±0.9	19±0.5	-	18±0.4	-	17±0.1	-	9±0.9	14±0.8	10±0.1	12±0.5	10±0.4
K3	-	19±0.8	-	17±0.8	-	26±0.5	-	-	11±0.2	-	15±0.5	16±0.6
K4	6±0.4	19±0.6	-	14±0.4	-	27±0.4	-	-	15±0.8	-	15±0.5	23±0.5

Key: *, values are mean ± standard deviation

Table 4: Antibacterial activity of actinomycete isolates against bacterial pathogens

Act.no.*	*S.citreus*	*S.aureus*	*S.typhi*	*S.marcescens*	*P.aeruginosa*	*B.polymyxa*	*P.mirabilis*	*B.cereus*
A10	9±0.2[a]	10±0.1	17±0.1	17±0.2	-	9±0.3	15±0.5	11±0.9
A11	32±0.6	18±0.4	-	-	14±0.3	14±0.8	-	15
A13	-	-	-	-	-	-	-	-
A16	25±0.3	15±0.6	16	12±0.3	13±0.4	12±0.6	19±0.2	13±0.5
A17	-	-	-	-	-	-	-	-
A18	11±0.3	15	11±0.5	18±0.1	-	-	-	15±0.6
A21	11±0.1	10±0.1	10	18±0.5	10±0.3	12±0.9	13±0.4	10±0.4
A22	9±0.2	9±0.3	-	-	-	-	-	-
A23	-	-	7±0.3	-	-	9±0.2	7±0.6	-
A24	-	-	-	-	-	-	-	-
C14	9±0.7	10	8±0.4	-	9±0.1	-	7±0.1	9
C15	15±0.8	10±0.5	10±0.6	15±0.2	10±0.1	9±0.3	10±0.6	14±0.6

Key: *, Actinomycete isolate number; [a], values are mean ± standard deviation

Table 5: Antifungal activity of the isolated actinomycetes against indigenous fungal isolates

Act.no.*	*Trichoderma*	*Penicillium*	*A. flavus*
A10	17±0.13[a]	37±0.34	30±0.70
A11	27±0.47	27±0.14	28±0.23
A13	24±0.07	28±0.19	33±0.52
A16	37±0.12	30±0.35	31±0.64
A17	28±0.65	38±0.82	32±0.40
A18	16±0.33	39±0.13	37±0.09
A21	19±0.31	37±0.48	36±0.61
A22	16±0.17	-	37±0.36
A23	23±0.36	31±0.34	21±0.17
A24	35±0.15	35±0.19	34±0.58
C14	37±0.91	37±0.71	32±0.73
C15	22±0.54	22±0.53	30±0.16

Key: *, Actinomycete isolate number; [a], values are mean ± standard deviation

Table 6: Antifungal activity of actinomycete isolates against fungal pathogens

Act.no.*	*C.albicans*	*C.parapsilosis*	*T.rubrum*	*T.mentagrophytes*	*Cryptococcus*
A10	-	12±0.83[a]	-	-	11±0.65
A11	-	10±0.15	-	-	13±0.09
A13	-	10±0.26	-	-	10±0.11
A16	-	14±0.31	-	-	14±0.23
A17	15±0.13	13±0.28	-	23±0.70	30±0.17
A18	-	10±0.42	-	-	14±0.13
A21	-	10±0.17	-	-	18±0.16
A22	-	10±0.23	-	-	12±0.17
A23	-	-	38±0.14	-	-
A24	10±0.61	14±0.07	-	-	23±0.26
C14	-	9±0.14	-	-	20±0.31
C15	-	11±0.38	-	-	11±0.00

Key: *, Actinomycete isolate number; [a], values are mean ± standard deviation

Table 7: Antibacterial activity of the isolated fungal forms against indigenous bacterial isolates

Bacterial isolates	*Trichoderma*	*Penicillium*	*A. flavus*
A1	22±0.14[a]	28±0.64	21±0.82
A2	10±0.64	16±0.31	15±0.28
A3	12±0.13	16±0.44	25±0.59
A4	20±0.91	20±0.88	25±0.61
A5	10±0.09	17±0.15	23±0.26
A6	14±0.61	16±0.26	25±0.46
B1	13±0.86	17±0.33	22±0.18
B2	10±0.16	16±0.11	28±0.13
C1	10±0.11	17±0.58	21±0.15
C2	10±0.83	14±0.14	26±0.47
C3	9±0.24	10±0.64	18±0.87
C4	-	21±0.11	35±0.71
C5	10±0.49	15±0.83	20±0.14
C6	10±0.77	17±0.13	19±0.33
C7	8±0.20	21±0.78	17±0.11
C8	10±0.63	22±0.61	24±0.84
C9	12±0.07	11±0.09	27±0.26
N1	8±0.30	19±0.59	21±0.26
N2	10±0.74	20±0.16	24±0.43
N3	33±0.54	30±0.37	23±0.25
N4	8±0.78	14±0.33	20±0.31
N5	20±0.26	25±0.14	19±0.89
P1	26±0.47	19±0.21	19±0.74
P2	9±0.86	10±0.17	14±0.15
K1	13±0.15	15±0.47	23±0.17
K2	19±0.09	24±0.31	16±0.14
K3	9±0.18	25±0.30	17±0.56
K4	19±0.16	16±0.94	22±0.47

Key: [a], values are mean ± standard deviation

Table 8: Antimicrobial activity of the fungal isolates against bacterial and fungal pathogens

Test pathogens	*Trichoderma*	*Penicillium*	*A. flavus*
S. citreus	12±0.16[a]	13±0.87	20±0.95
S. aureus	8±0.31	13±0.33	19±0.18
S. typhi	10±0.42	13±0.71	17±0.45
S. marcescens	10±0.11	13±0.39	18±0.62
P. aeruginosa	10±0.27	11±0.13	13±0.15
B. polymyxa	10±0.78	10±0.41	14±0.31
B. cereus	10±0.15	10±0.41	13±0.51
P. mirabilis	10±0.20	10±0.65	16±0.67
C. albicans	9±0.19	12±0.22	20±0.45
C. parapsilosis	13±0.77	10±0.58	18±0.56
T. rubrum	14±0.39	13±0.17	17±0.91
T. mentagrophytes	12±0.05	15±0.03	15±0.31
Cryptococcus	11±0.60	11±0.41	17±0.78

Key: [a], values are mean ± standard deviation