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Antibacterial Activity of Twenty Different Endophytic Fungi Isolated from *Calotropis procera* and Time Kill Assay

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Abstract

Background: Development of drug resistance in bacteria is a common and alarming problem worldwide and there is continuous and urgent need of antibacterial agents. Endophytes offer a plethora of secondary metabolites with various biological activities. These secondary metabolites may help the host plant in defense from pathogens and insects, growth stimulators also helps the host plant in stress tolerance. *Calotropis procera* is a well-known medicinal plant, used to cure various health ailments traditionally. So, the endophytic fungi isolated from different tissues (leaf, stem and root) of *C. procera* were evaluated for their antibacterial potential.

Methods: The antibacterial activity of crude ethyl acetate extracts of 20 different endophytic fungi was evaluated by using agar well diffusion assay against total nine bacterial reference strains. Minimum inhibitory concentration was determined by using microbroth dilution method. Time kill assay study was performed against the *Salmonella typhi* bacterial strain by using *Aspergillus nomius* extract.

Results: Out of the total 20 different endophytic fungal strains 7 endophytic fungal extracts showed activity against all tested bacterial strains. The endophytic fungi which belong to *Aspergillus* and *Fusarium* genus exhibited good antibacterial activity. Maximum zone of inhibition (17.33 mm) was shown by extracts of *Aspergillus nomius*, *Fusarium solani*, *Aspergillus oryzae* and *Curvularia hawaiiensis* against *S. typhi*, *S. flexneri*, *S. typhi* and *S. marcescens* respectively. Extracts of *Aspergillus nidulans*, *Curvularia hawaiiensis*, *Chaetomium arcuatum* and *Chaetomium atrobrunneum* also exhibited significant antibacterial activity against the tested bacterial strains. The MIC values were ranged between 15.6 µg/well to 250 µg/well. The endophytic fungal extracts were more efficient against the growth of Gram-positive bacteria as compared to Gram-negative. Time kill assay study against the *S. typhi* showed bacteriostatic effect of *Aspergillus nomius* strain extract at different concentrations.

Conclusion: Several endophytic fungi inhabit the different tissues of *C. procera* have capability of producing bioactive secondary metabolites with significant antibacterial activity. Further isolation and identification of these secondary metabolites may provide a new lead for development of novel drug molecules.

Keywords: Endophytic fungi; *Calotropis procera*; Antibacterial activity; Minimum inhibitory concentration (MIC); Time kill assay; Aspergillus, Fusarium

Introduction

The emergence of resistance in pathogenic bacteria to the commercial antibiotics is a very common and alarming problem around the world. In addition to intrinsic capacity of bacteria to develop resistance against the drugs, many other extrinsic factors favour this situation [1]. These extrinsic factors mainly include the improper and extensive use of antibiotics, immigration, emigration, lack of proper or late diagnosis of infection and others. As a result, there is a continuous need for novel and effective antimicrobial agents. In order to isolates the new drug molecules, endophytic fungi provide a unique niche because these are less evaluated as compare to sand fungi.

Schulz et al. [2] indicates that about 51% of biologically active molecules isolated from endophytic fungi were previously unidentified. So, there has been a tremendous increase in interest to explore the endophytes for their diversity, ecological role, secondary metabolites and bioactivity.

Endophytes are the organisms which are in an imperceptible relationship with the plant in which they reside for their whole lifetime or a part of their life [3]. These include fungi, bacteria and some algae [4]. They colonize all the plants evaluated till now and isolated from almost all the plant parts like leaves, roots, stems, flowers, barks and even from dry seeds [5].

Endophytic fungi have a capacity to produce diverse class of plant associated secondary metabolites with a wide variety of biological activities such as taxol (anticancer) [6], rohitukine (anticancer) [7], antimicrobial agent hypericin [8] and acetylcholinesterase inhibitor huperzine A [9].

Plants used in the traditional medicine have offered a very important habitat to isolate the strains the endophytic fungi which produce new bioactive compounds. Many studies suggested that the medicinal properties of a plant may be due to the metabolites produce by their endophytic microorganisms [10]. Despite this fact, a large number of medicinal plants yet to be studied for the presence of

endophytes and their bioactivity like *Calotropis procera*. *C. procera* is a well-known medicinal plant belongs to family Apocynaceae, native to Asia and tropical Africa. [11].

C. procera has been used in traditional medicinal system with a number of properties like antimicrobial [12], analgesic, anti-inflammatory, antidiabetic, cytotoxic, anticancerous, dyspepsia and hepatoprotective effects [13]. Its root bark is used for treatment of skin related anomalies. The secretion from the root bark is traditionally used for the treatment of skin diseases, enlargements of abdominal viscera and snakebite, and for intestinal worms problems [14]. Previous research showed that procerin, a compound isolated from *C. procera* displayed remarkable antimicrobial, insecticidal, proteolytic activities while proceragenin, calotoxin, hydroxyketone showed significant cytotoxic and anti-helmintic activity [15]. Previous studies reported that certain endophytic fungi produce secondary metabolites especially those that are exclusively synthesized by their host plants [16,17].

The aim of the present work was to obtain crude extracts of fungal endophytes isolated from different tissues of *C. procera* and investigate their antibacterial activity against the reference strains along with time kill kinetic study.

Materials and Methods

Plant parts were collected, identified and authenticated by comparing the herbarium specimen available in the Department of Genetics, M. D. University, Rohtak (Voucher no. MDU 4602).

Isolation of endophytic fungi

Plants have endophytes as well as epiphytes associated with them. So, for the isolation of endophytic fungi plant tissues were first surface sterilized to remove any associated epiphytes [18]. Healthy tissues of *C. procera* were washed with running tap water. The plant tissues were sterilized by dipping into the 70% ethanol for 1 min followed by immersion in sodium hypochlorite (4% w/v solution of available chlorine, Merck, India) for 4 min; in 75% ethanol for 45 sec. Tissues were washed thrice with the sterile distilled water.

The efficiency of sterilization was checked by incubating last rinse water of the plant tissues on to the Petri dishes containing potato dextrose agar (PDA) medium, pH 5.6 ± 0.2. Sterilized plants tissues were cut into small pieces and incubated over PDA containing Petri dishes supplemented with streptomycin (100 mg/L, Himedia) to prevent the growth of endophytic bacteria. The Petri dishes were incubated at 28 ± 2°C in incubator. Plates were observed continuously for the growth of endophytic fungi. The tips of fungal mycelia were continuously transferred on fresh media till the isolation of pure culture. A total of 20 actively growing endophytic fungi were molecularly identified by using ITS1 and ITS4 primer pair and sequences were deposited in NCBI (data is unpublished yet).

Preparation of crude fungal extracts

For preparation of crude fungal extracts the endophytic fungi were incubated by inoculating fungal mycelia in culture media contained potato dextrose broth (PDB) media for 10-15 days at 28 ± 2°C on an incubator shaker at 140 rpm. Fungal mycelia were separated by using Whatman filter paper. Filtrate was transferred to a big separating funnel to which approximate same volume of ethyl acetate was added. The separating funnel was strongly stirred up and left for an hour.

A clear boundary was formed on behalf of polarity in between upper phase contained secondary metabolites dissolved in ethyl acetate and lower phase contained media. Ethyl acetate phase containing secondary metabolites was collected and concentrated in rotary evaporator. Fungal mycelia obtained after filtration was also subjected for extraction by using cold percolation method with the ethyl acetate. Extracts obtained after evaporation were stored at 40°C until use.

Assessment of antibacterial activity

Tested microorganisms: The crude extracts of endophytic fungi were tested for their antibacterial potential against total 9 American Type Culture Collection (ATCC) reference bacterial strains. Among them two strains were Gram-positive: *Enterococcus faecalis* (ATCC 29212) and *Staphylococcus aureus* (ATCC 259323) and 7 strains were Gram-negative includes *Pseudomonas aeruginosa* (ATCC 27853), *Serratia marcescens* (ATCC 27137), *Shigella flexneri* (ATCC 12022), *Salmonella typhi* (ATCC 13311) *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 43071) and *Klebsiella pneumoniae* (ATCC 700603). Bacteria previously stored at 4°C were freshly streak on nutrient agar medium and incubated at 35°C for 24 hours. After incubation, bacteria were suspended in sterile peptone water to obtained standard inoculums of approximately 5×10^8 CFU/ml equivalents to 0.5 McFarland units.

Antibacterial activity: The antibacterial activity of crude endophytic fungal was determined using the agar well diffusion method as described by Taye et al. [19]. Fresh bacterial inoculums (100 µL) prepared in peptone water were uniformly spread over Petri dishes containing nutrient agar medium using sterile spreader. Wells of 6 mm were made with the help of a sterile borer. Stock solution of different endophytic fungal extracts was prepared at a concentration of 100 mg/ml of DMSO ($1/10^{th}$ diluted). A volume of 40 µl of each extracts was added to each well for all bacterial strains tested. Streptomycin (HiMedia laboratories Pvt. Ltd. India, 10 µg/disc) was used as positive control. DMSO ($1/10^{th}$ diluted) used as negative control. Zone of inhibition of fungal extracts around each well was measured with the help of a standard transparent scale HiAntibiotic ZoneScaleTM-C (HiMedia Laboratories Pvt. Ltd. India).

Minimum inhibitory concentration (MIC): Minimum inhibitory concentration (MIC) is the measure to check the growth of bacterial growth after 24 h of incubation on lowest concentration of an antibacterial agent. MIC values for different extracts of endophytic fungi were determined using microbroth dilution method of Sarker et al. [20] in 96 well microtitre plates. 50 µl of sterile nutrient broth and 50 µl of normal saline were added to each well of microtitre plate followed by the addition of 100 µl of fungal extract in DMSO (stock solution 100 mg/ml dissolved in $1/10^{th}$ diluted DMSO) in the first row of the microtitre plate. Serial dilutions were performed in a way that first row had highest concentration and the last row had lowest concentration of endophytic fungal extracts. A volume of 10 µl of resazurin dye solution (HiMedia laboratories Pvt. Ltd.) was used as indicator (prepared by dissolving 270 mg resazurin in 40 ml of sterile distilled water) was also added to each well. Finally 10 µl of bacterial inoculums with 5×10^8 CFU/ml was added to each well. Each plate had a first column with streptomycin positive control and second column with $1/10^{th}$ diluted DMSO used as negative control. Plates were wrapped in cling film to prevent dehydration of bacteria. The plates were incubated at 37°C for 24 h in an incubator. Change of colour from purple to pink or to colourless indicates the growth of

bacteria. The lowest concentration at which no color change observed was considered as the MIC value of that extract.

Time kill assay: Time kill assay was performed against the *S. typhi* using extract of *Aspergillus nomius* using method of Yadav et al. [21]. *S. typhi* was grown on PDA media on Petri dish. Individual colony of bacterium was isolated from 24 h old culture and suspended in sterile normal saline. Density of bacterial culture was adjusted to a 0.5 McFarland standard. This suspension was diluted 1:10 time in nutrient broth. For this 100 µl of bacterial suspension was added into the 900 µL of nutrient broth. *Aspergillus nomius* extracts were prepared at concentration of 1/2 MIC, MIC and 2 × MIC (1 ml). Above prepared bacterial suspension was added to the extracts and incubated at 28 ± 2°C for 24 h. Positive control includes the bacterial suspension without endophytic fungal extracts and the negative control includes endophytic fungal extracts without bacterial inoculums. A volume of 25 µl of sample was pipette out from each tube and spreaded over freshly prepared nutrient agar Petri dishes at 0, 2, 4, 6, 8 and 24 h. Petri plates were incubated at 28 ± 2°C for 24 h in an incubator. Colonies on individual plates were counted and expressed as number of colony forming units/ml (CFU/ml). The killing rate was determined by plotting logarithm of the viable colony counts (CFU/ml) against time. The percentage reduction in total viable count of CFU was counted by using the formula:

$$Percentage\ reduction = \frac{Initial\ count - count\ at\ x\ \text{interval}}{initial\ count} \times 100$$

Statistical analysis

All experiments were performed in triplicates. Results are represented as mean ± standard deviation (SD).

Name of fungus strains	*E. coli*	*K. pneumonia*	*S. aureus*	*P. aeruginosa*	*S. typhi*	*S. marcescens*	*S. flexneri*	*E. Faecalis*	*P. mirabilis*
Chaetomium arcuatum	-	14 ± 1.00	15 ± 1.00	-	17 ± 1.00	17 ± 1.00	17 ± 1.00	14.66 ± 0.58	-
Chaetomium sp.	12 ± 1.00	13.66 ± 0.58	13.66 ± 0.58	-	16 ± 1.00	16 ± 1.00	16 ± 1.00	15 ± 1.00	14.66 ± 0.58
Chaetomium atrobrunneum	11 ± 1.00	16 ± 1.00	16 ± 1.00	-	17 ± 1.00	-	16 ± 1.00	15 ± 1.00	13.66 ± 1.15
Penicillium crustosum	-	13.66 ± 0.58	14.33 ± 0.58	11.33 ± 0.58	13.66 ± 0.58	13 ± 1.00	14.33 ± 058	15 ± 1.00	12 ± 1.00
Penicillium citrinum	-	11 ± 1.00	15 ± 1.00	12 ± 1.00	12.66 ± 0.58	11.66 ± 0.58	13 ± 1.00	17 ± 1.00	15 ± 1.00
Fusarium chlamydosporum	8.66 ± 0.57	13.66 ± 0.58	16.33 ± 0.58	11 ± 1.00	14 ± 1.00	16 ± 1.00	-	-	-
Fusarium graminearum	11.66 ± 0.58	14 ± 1.00	13 ± 1.00	8.66 ± 1.15	-	15.66 ± 0.58	14.66 ± 0.58	13.66 ± 0.58	14.33 ± 1.15
Fusarium solani	-	-	15.66 ± 0.58	11 ± 1.00	-	16 ± 1.00	17.33 ± 0.58	15.66 ± 0.58	-
Fusarium thapsinum	-	15.66 ± 0.58	14.66 ± 0.58	11 ± 1.00	16 ± 1.00	14.66 ± 0.58	15 ± 1.00	15 ± 1.00	12 ± 1.00
Fusarium delphinoides	-	10 ± 1	13 ± 1	12.33 ± 0.52	14.33 ± 0.58	12.66 ± 0.58	13 ± 1	8 ± 1	-
Aspergillus nomius	11.66 ± 0.33	15 ± 1.00	14.66 ± 0.58	13.66 ± 0.58	17.33 ± 0.52	16 ± 1.00	16 ± 1.00	15 ± 1.00	14.66 ± 1.15
Aspergillus oryzae	12 ± 1.00	16 ± 1.00	14.66 ± 0.58	14 ± 1.00	17.33 ± 0.52	-	14.66 ± 0.58	15 ± 1.00	15 ± 1.00
Aspergillus niger	12.66 ± 0.58	16 ± 1.00	15.66 ± 0.57	14.66 ± 058	15.66 ± 0.58	17 ± 1.00	14.66 ± 0.58	15 ± 1	15 ± 1
Aspergillus terrus	10 ± 1 .00	15 ± 1.00	15 ± 1.00	13 ± 1.00	15 ± 1.00	15 ± 1.00	14.66 ± 0.58	15.66 ± 0.58	12.66 ± 0.58
Aspergillus nidulans	11.33 ± 1.52	17 ± 1.00	14 ± 1.00	13.66 ± 0.58	17 ± 1.00	15 ± 1.00	16.66 ± 0.58	17.33 ± 0.58	15.33 ± 1.15
Candida blankii	10.66 ± 0.58	12 ± 1.00	13 ± 1.00	12 ± 1.00	11 ± 1.00	13 ± 1.00	12.33 ± 0.58	13 ± 1.00	11 ± 1.00
Curvularia hawaiiensis	9 ± 1.00	13.33 ± 0.58	13.67 ± 0.58	9.67 ± 0.58	14.33 ± 0.58	17.33 ± 0.58	14 ± 1.00	14.33 ± 0.58	14.33 ± 0.58
Cochliobolus hawaiiensis	11 ± 1.00	17 ± 1.00	16 ± 1.00	.-	15 ± 1.00	13.66 ± 0.58	13.66 ± 0.58	12.33 ± 0.2	-
Alternaria alternata	8 ± 1.00	15.33 ± 1.52	15 ± 1.00	11.33 ± 0.52	15.66 ± 0.58	14.66 ± 0.58	15 ± 1.00	13 ± 1.00	14 ± 1.00
Mucor circinelloides	-	11 ± 1.00	12 ± 1.00	-	13.33 ± 0.57	13 ± 1.00	12.33 ± 0.57	13.66 ± 0.57	-
Control	32.44 ± 46	29.44 ± 0.44	34.46 ± 0.44	29.46 ± 0.36	32 ± 0.42	33.46 ± 0.44	33.34 ± 0.43	32.58 ± 0.40	31.42 ± 0.48

Table 1: Zone of inhibition of endophytic fungi isolated from *C. procera* against different tested strains in mm.

Results

Antibacterial activity

In the present study, total 20 different endophytic fungi crude ethyl acetate extracts were screened for their antibacterial potential. The antibacterial activity of fungal extracts was determined by measuring zone of inhibition and MIC values against 9 tested bacterial strains. The zones of inhibition against the bacterial strains are represented in Table 1. The diameter of inhibition zones was ranged from 8 mm to 17.33 mm. Out of 20 endophytic fungi, 7 fungi exhibited antibacterial activity against the all tested bacterial strains. 6 endophytic fungi showed activity against 8 tested bacterial strains. Maximum zone of inhibition (17.33 mm) was shown by *Aspergillus nomius*, *Fusarium solani*, *Aspergillus oryzae* and *Curvularia hawaiiensis* against *S. typhi*, *S. flexneri*, *S. typhi* and *S. marcescens* respectively. Extracts of *Aspergillus nidulans*, *Curvularia hawaiiensis*, *Chaetomium arcuatum* and *Chaetomium atrobrunneum* also exhibited significant activity against the tested bacterial strains. Minimum zone of inhibition (8 mm) was exhibited by *Fusarium delphinoides* against *E. faecalis*, *Fusarium chlamydosporum* and *Alternaria alternata* against *E. coli*. The extract of *Aspergillus nomius* was most effective in inhibiting the growth of bacteria. *Mucor circinelloides*, *Fusarium delphinoides* were least active because they showed smaller zone of inhibition comparative to the others. Among the tested bacterial strains, *E. coli* was less susceptible to the fungal extracts and *S. aureus* found to be most susceptible bacterial strain inhibited by all tested endophytic fungal extracts. In context to Gram- positive and Gram-negative bacteria, Gram-positive bacteria were more susceptible to the fungal extracts. The MIC values for the fungal extracts against all the tested bacteria are represented in Table 2. The MIC values ranged in between 15.6 µg/well to 250 µg/well.

Name of fungus strains	*E. coli*	*K. pneumonia*	*S. aureus*	*P. aeruginosa*	*S. typhi*	*S. marcescens*	*S. flexneri*	*E. faecalis*	*P. mirabilis*
Chaetomium arcuatum	250	31.2	31.2	250	15.6	15.6	15.6	31.2	250
Chaetomium sp.	62.5	62.5	62.5	250	15.6	15.6	15.6	31.2	31.2
Chaetomium atrobrunneum	62.5	15.6	15.6	250	15.6	250	15.6	31.2	62.5
Penicillium crustosum	250	62.5	31.2	62.5	31.2	62.5	31.2	31.2	62.5
Penicillium citrinum	250	62.5	31.2	62.5	62.5	31.2	62.5	31.2	31.2
Fusarium chlamydosporum	125	62.5	15.6	62.5	31.2	15.6	250	250	250
Fusarium graminearum	62.5	31.2	62.5	125	250	31.2	31.2	62.5	31.2
Fusarium solani	250	250	31.2	62.5	250	62.5	15.6	31.2	250
Fusarium thapsinum	250	31.2	31.2	62.5	15.6	15.6	31.2	31.2	62.5
Fusarium delphinoides	250	125	62.5	62.5	31.2	62.5	62.5	125	250
Aspergillus nomius	62.5	31.2	31.2	31.2	15.6	15.6	15.6	31.2	31.2
Aspergillus oryzae	62.5	15.6	31.2	31.2	15.6	250	31.2	31.2	31.2
Aspergillus niger	62.5	15.6	31.2	31.2	31.2	31.2	31.2	31.2	31.2
Aspergillus terrus	125	31.2	31.2	62.5	31.2	15.6	31.2	31.2	62.5
Aspergillus nidulans	62.5	15.6	31.2	62.5	15.6	31.2	15.6	15.6	31.2
Candida blankii	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
Curvularia hawaiiensis	125	62.5	62.5	125	31.2	15.6	31.2	31.2	31.2
Cochliobolus hawaiiensis	62.5	15.6	15.6	250	31.2	62.5	62.5	62.5	250
Alternaria alternata	125	31.2	31.2	62.5	31.2	31.2	31.2	62.5	31.2
Mucor circinelloides	250	62.5	62.5	250	62.5	62.5	62.5	62.5	250

Table 2: MIC values of different endophytic fungi against different tested strains in µg.

Time kill assay

Time kill assay was performed over a period of 24 h with the *S. typhi* being exposed to ½MIC (0.78 mg/ml), MIC (1.56 mg/ml) and 2 × MIC values (3.12 mg/ml) of ethyl acetate extract of *Aspergillus nomius*. A graph was plotted between the logarithmic number of CFU/ml and time (Figure 1). Time kill assay showed 91.42% and 95.33% reduction at MIC and 2 × MIC concentration respectively. Maximum reduction at MIC and 2 × MIC concentrations was observed at 6 h of incubation for MIC value and at 24 h for 2 × MIC concentration respectively. The ½MIC value reduced the growth up to 74.35% at 8 h of incubation. From the Figure 1 it was observed that effect of endophytic fungal extract at different concentrations was bacteriostatic.

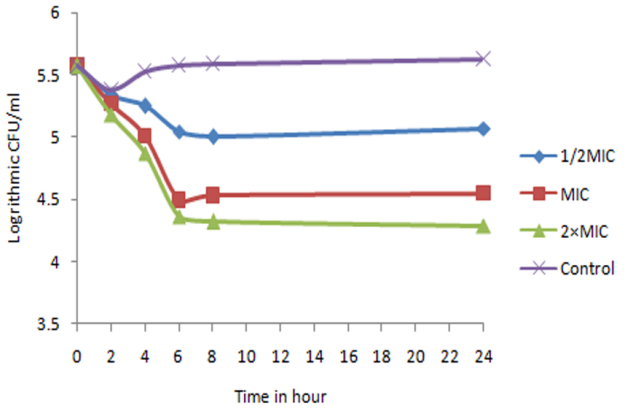

Figure 1: Represents the time kill assay graph of ethyl acetate extract of *Aspergillus nomius* against *S. typhi*.

Discussion

The antibacterial activity of the crude ethyl acetate extracts of various endophytic fungi was evaluated to test their antibacterial potential. Endophytic organism especially fungi have enormous potential to produce large range of bioactive secondary metabolites in order to protect their host plant against pathogens [22]. The antibiotic compounds massariphenone and ergosterol peroxide extracted from *Verticillium* sp. fungi associated with host plant *Rehmannia glutinosa* possess activity against the plant pathogen, *Pyricularia oryzae* P-2b [23]. Endophytic fungi also produce a variety compounds which have plant growth promoting activity [24], nematocidal activity [25] and also produce antifungal and antibacterial molecules [26]. So, the isolation and identification of endophytic fungi is of great importance. Yadav et al., studied that out of total 22 different endophytic fungi isolated from the *E. jambolana* (leaf, petiole and stem), 15 were showed significant antibacterial activities against the tested microorganisms [27]. Aharwal et al. [28] isolated 12 endophytic fungi from *C. procera* viz. *Aspergillus niger*, *Aspergillus tamari*, *Aspergillus japonicus*, *Cladosporium herbarum*, *Alternaria alternata*, *Alternaria tenuissima*, *Drechslera nodulosa*, *Fusarium solani*, *Curvularia pallescens* and *Curvularia lunata*. Out of which two were unidentified and the *Fusarium solani* extract showed most potent activity against *S. epidermidis* and *E. coli*. In the present study we have isolated 20 different fungal species; only three fungal species (*Aspergillus niger*, *Alternaria alternata* and *Fusarium solani*) are common to the above reported study.

Garcia et al. [29] reported that the ethyl acetate solvent system was most efficient method to extract endophytic fungi principle compounds. Highest antibacterial activity was reported in the ethyl acetate extract in comparison to other solvents as observed from previous studies on endophytic fungi species [30,31]. We have also used ethyl acetate as the extraction solvent. The results of this study correlate with the finding of other previous reports on antibacterial activity of endophytes [32,33]. The extracts of endophytic *Aspergillus niger* inhabiting *Eugenia jambolana* [27] and *Aspergillus* sp. isolated from *Justicia adathoda* [34] has been previously reported to displayed significant antimicrobial activities against tested bacteria and fungi strains. In the present study ethyl acetate extracts of *Aspergillus nomius*, *Aspergillus oryzae*, *Aspergillus terrus* and *Fusarium solani* and *Penicillium citrinum* showed significant antibacterial activity. Bugni et al. [35] concluded that *Aspergillus* genus is a major contributor of antimicrobial compound of fungal origin. Kalyanasundaram et al. [36] study showed the antibacterial activity of endophytic fungi against *S. typhi*, *E. coli*, *P. mirabilis*, *Salmonella paratyphi*, *Vibrio cholera*, *Klebsiella oxytoca*, *K. pneumonia* and *S. aureus*. These endophytic fungi may have quite good potential for utilization to inhibit the bacterial growth. In the present study endophytic fungal extracts are more effective against the Gram-positive bacteria as compared to Gram-negative bacteria. This may be due to the reason that the Gram-negative bacteria have an additional outer membrane barrier as compared to the Gram-positive bacteria.

The time kill assay against the *S. typhi* indicates that the extracts of *Aspergillus nomius* were bacteriostatic because no sharp decrease in CFU/ml was observed. Previously Sarbadhikary et al. [37] also reported the bacteriostatic effect of isolated fungi from leaves of *Melastoma malabathricum L.* on *S. aureus* and *E. coli*. In some case during the treatment it is advantageous to have drugs with bacteriostatic effect rather than the bactericidal action [38].

Conclusion

This study indicates that endophytic fungi isolated from different tissues of *C. procera* have pharmaceutical bioactive compounds with antibacterial potential. This may be due to the fact that endophytic microorganisms produce bioactive secondary metabolites. This study serves as first step needed to isolate and identify the pure bioactive compounds which are responsible for antibacterial activity of endophytic fungi.

Conflict of Interest

There is no conflict of interest between authors.

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