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RESEARCH ARTICLE

ENDOPHYTIC ACTINOBACTERIA FROM THREE ETHNOMEDICINAL PLANT SPECIES OF SOUTHERN WESTERN GHATS, INDIA EXHIBIT ANTIOXIDATIVE AND ANTIBACTERIAL POTENTIALS

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ABSTRACT

Objective: Isolation and molecular identification of actinobacterial endophytes from *Zingiber nimmonii* (J. Graham) Dalzell., *Polygonum chinense* L., and *Justicia wynaadensis* Heyne, important ethnomedicinal plant species of the 'Western Ghats', a hotspot location in southern India and characterization of secondary metabolites for the antibacterial activity by bio-autography and Gas Chromatography - Mass Spectrometry (GC-MS) techniques. **Methods:** The endophytic actinobacteria were isolated from the plant parts and identified by sequencing of the 16S rRNA genes. The secondary metabolites produced by the strains in the ISP-1medium were extracted with ethyl acetate and the crude dry extracts were evaluated for the total phenolic, flavonoid, antioxidant capacities and antibacterial potentials. **Results:** 14 endophytic actinobacteria belonging to five taxa were identified from three plant species as *Corynebacterium*, *Curtobacterium*, *Arthrobacter*, *Streptomyces* and *Nocardiopsis*. The total phenolic content of the actinobacterial extracts ranged from 5.2±0.1 to 24.4±0.08 mg gallic acid equivalent (GAE) /g dry extract. Flavonoid content was detected in three extracts (3.2±0.1 to 5.6±0.3 mg catechin equivalents (CE)/g dry extract). The radical scavenging activity (IC_{50} values) of actinobacterial extracts varied from 489.4 ± 2.3 µg/mL to 1968.3±112.0 µg/mL. Antibacterial activity was detected in all the extracts against six test bacterial strains *viz*., *Bacillus subtilis* (MTCC 121) and *Staphylococcus aureus* (MTCC 7443), *Pseudomonas aeruginosa* (MTCC 7093), *Escherichia coli* (MTCC 729), *Enterobacter aerogenes* (MTCC 111) and *Klebsiella pneumoniae* (MTCC 661). Eight extracts among the 14 showed inhibition zones ranging from 7.2±0.1 to 28.8±0.2 mm diameter against four of the six test pathogenic bacteria. *S. indiaensis* isolated from *Z. nimmonii* exhibited highest inhibition zones against all test pathogenic strains (14.2±0.1 to 28.8±0.2 mm dia) with the Minimum Inhibitory Concentrations (MIC's) ranging from 0.02 to 0.16 mg/mL. Characterization of *Streptomyces indiaensis* extract by thin layer chromatography and GC-MS revealed the presence of eight aliphatic and volatile organic compounds. **Conclusions**: Three ethnomedicinal plant species from Western Ghats harbor diverse actinobacterial taxa with bioactivities.

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INTRODUCTION

The Gram-positive members of the novel phylum Actinobacteria are present in myriad ecosystems on the earth. Actinobacteria form associations with plants from litter degrading, symbiotic, and endophytic to pathogenic types. These associations often have benefited scientists to introspect the potentials of strains and their secondary metabolites for industrial and agricultural applications (Nalini and Prakash, 2020).

The endophytes are microorganisms comprising fungi, actinobacteria and yeasts reside within the plant tissue without any apparent infections (Bacon and White, 2000). Endophytic actinobacteria known previously as actinomycetes, were isolated from forage, agricultural crops and fruit plants with antagonistic effects on the plant pathogens. The diversity of actinobacteria differs in plant species and tissue types (Golinska *et al*., 2015).

It has also been well recognized that medicinal plants are promising source for the isolation of potent endophytic microorganisms as they also plays a substantial role in the development of medicinal property of the plants (Yu *et al*., 2010). About 68% of antibacterial compounds and 34% of products used in cancer therapy are either natural products or their derivatives (Newman and Cragg, 2007). The world's biodiversity estimates indicate that our planet's forests are in grave danger (Bryant *et al*., 1997). The Western Ghats of southern India is a rich area in floristic composition as well as the concentration of endemic taxa (Nayar, 1996). It is one among the 34 hotspots recognized by Conservation International and yet harbors 2,180 endemic plant species representing 0.7% of the global endemic plants (Myers *et al*., 2000). Traditionally, natural forests of the region have been the source of nearly 500 medicinal plants of which some are used for traditional and folk medicinal practices (Joy *et al*., 2001). Many plant species with potent ethnomedicinal values are found growing in natural conditions from this region.

In this study, we selected three medicinal plants with enormous ethno medicinal properties growing in natural populations in the Talacauvery sub cluster of Western Ghats, an area with semi evergreen forest types. These plants were previously subjected to endophytic fungal isolations and their antioxidative and antibacterial potentials are reported (Das *et al*., 2017a, b; Das *et al*., 2018; 2020). *Zingiber nimmonii* (J. Graham) Dalzell., is an endemic species of the Western Ghats. Besides, *Zingiber* species are cultivated in the tropics for their medicinal properties. The bioactivity potential of the plant is well established as sources antimicrobial properties (Sabulal *et al*., 2006; Finose and Gopalakrishnan., 2015). *Polygonum chinense* L., is common in Asia and is a source of antimicrobial compounds Maharajan *et al*., 2012). In local health traditions, the decoction of stem and roots mixed with jaggery and fed to women after child birth. *Justicia wynaadensis* Heyne. (Acanthaceae) locally known as 'Maddhuthoppu'is an endemic plant, naturally occurring in the rain forests of Western Ghats, East Nilgiris and South Malabar Hills in South India (Gamble, 1928). The juice of the plant has ethnomedicinal properties and is used locally to cure asthma, boost immunity, as anthelmintic and anti-diabetic (Lingaraju *et al*., 2013). *J. wynaadenesis* also has cellular cholesterol lowering properties (Subbiah and Norman, 2002). A deep purple colored extract obtained through the decoction of the plant is consumed during the first week of august yearly as a sweet dish by the local community. Owing to the traditional uses of these plants in providing health benefits, they were selected for the isolation of actinobacterial endophytes from the Talacauvery subcluster of Western Ghats and characterization of secondary metabolites of actinobacteria responsible for the bioactive potential by chromatographic, bioautography and mass spectra techniques.

MATERIALS AND METHODS

Chemicals: DPPH (1, 1-diphenyl-2- picrylhydrazyl), was purchased from Sigma Aldrich (St. Louis, MO, USA). Folin-Ciocalteu's reagent was purchased from SRL Pvt. Ltd. (Mumbai, India). Sodium hypochlorite, potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride, sodium nitrite, aluminum chloride, potassium persulphate, general chemicals and solvents were of analytical grade. Triple distilled water was used wherever necessary. Antibiotic disk and Mueller-Hinton medium were purchased from Hi-media (Mumbai, India).

Collection of plant samples: Three ethnomedicinal plant species viz., Z. *nimmonii*, *P. chinense* and *J. wynaadensis* were collected from natural habitats of Talacauvery sub cluster (012°17' to 012°27' N and 075°26' to 075°33'E) of Western Ghats, in Kodagu district, Karnataka state, India. The roots, rhizomes, leafy stem, leaves and inflorescences of *Z. nimmonii* were collected during November 2011. The stem and leaves of *P. chinense* and *J. wynaadensis* was collected during June and August, 2012 respectively. The plants were identified taxonomically with the help of flora (Gamble, 1928). Herbarium specimens have been preserved and deposited in the Department of Studies in Botany, University of Mysore. The plant parts were collected, kept in zip-lock polythene bags and brought to the laboratory and processed for the isolation within 24 h of collection.

Isolation of endophytic actinomycetes from plant parts: The actinobacterial isolation was carried out under aseptic conditions according to Akshatha *et al*. (2016). The plant parts were subjected to surface sterilization in 70% (v/v) ethanol for 1 min followed by sodium hypochlorite (3.5%) for 3 min and washed 3-4 times in sterile distilled water. Two hundred dried fragments measuring 1.0 cm × 0.1 cm × 0.1 cm pieces from plant parts were placed on the Actinomycete Isolation Agar (AIA, 21.7 g/L, Himedia, Mumbai, India) amended with 1% glycerin and cyclohexamide (50 mg/L). The plates were sealed with Clean wrap™ cling film and incubated at 28± 2^0C with 12 hours of light and dark cycles for 8-10 weeks. The actinobacteria that emerged on the fragments were cultured in AIA slants at 28±2 °C for 10-15 days and maintained as pure cultures at 4°C for further use.

Identification of the endophytic actinobacteria

Mycelia and spore morphology analysis: The morphology of actinobacterial mycelia and spores was studied by scanning electron microscope (SEM, S-3400N, Hitachi, Japan). The specimens were prepared by the method of Amano and Gyohbu (2000). The samples were mounted on a tungsten filament and sputter coated with gold and palladium for 15 min. The specimens were observed and photographed at 10K to 20K magnifications.

Molecular identification of endophytic actinobacteria: The endophytic actinobacteria were identified through the sequencing analysis of 16S rDNA using a set of universal primers 27F and 1492R. The isolates were grown in ISP-1 (International Streptomyces Project- 1; 8 g/L, Himedia, Mumbai, India) medium for 21 days at 28±2 ºC. The mycelium was collected for DNA extraction by centrifugation. DNA isolation and purification was done by employing DNA isolation kit RKT 24 (Chromous Biotech Pvt Ltd., Bangalore). The quantification of DNA was done by Nanodrop method (Thermo Scientific Nanodrop 2000/2000c, Bangalore, India). The proper sized DNA was then amplified by PCR amplification using PCR kit PCR 08A (Chromous Biotech Pvt Ltd. Bangalore). The amplification conditions consisted of an initial denaturation step at 95 °C for 2 min, followed by 30 cycles of 94 °C for 1 min, 58 °C for 2 min, 72 °C for 2 min and final extension at 72 °C for 8 min. The amplified actinobacterial products were subjected to sequencing at Chromous Biotech Pvt. Ltd., Bangalore, India. The endophyte

sequences were aligned with the reference sequences using the BLAST algorithm and submitted to the NCBI GenBank nucleotide collection.

Fermentation and extraction of metabolites: The pure cultures of endophytic actinobacteria were inoculated into 500 ml of ISP-1 medium in duplicates. The fermentation broth of each endophyte was extracted three times with ethyl acetate at room temperature and further concentrated by a Rotary flash evaporator (Superfit Model, PBU-6D, India). The crude dry extracts obtained stored in glass vials, until use.

Determination of total phenolic content: The total phenolic content of the fungal extracts was assessed according to the Folin–Ciocalteau (FC) method of Liu *et al.* (2007) with some modifications. The total phenolic content of the extracts was expressed as mg of gallic acid equivalent (GAE)/g of the extract.

Determination of total flavonoid content: The total flavonoid was determined according to the method of Barros *et al*. (2007). The content of flavonoid was calculated using calibration curve of catechin and the results were expressed as mg of catechin equivalent (CE)/g of the extract.

Determination of antioxidant capacity

DPPH radical scavenging activity: The quenching ability of DPPH was measured according to the procedure of Liu *et al.* (2007) with some modifications. The scavenging activity was expressed as IC_{50} (µg/ml) with Ascorbic acid as the standard. The scavenging ability of the DPPH radical was calculated by the formula: % scavenging = $[(A_{control} - A_{sample})/A_{control}]\times100$.

Reducing power assay: The reducing power was measured by the method of Oyaizu (1986) with some modifications. The activity was expressed as mg ascorbic acid (AA)/g dry extract.

Detection of antibacterial activity

Test organisms: Two Gram-positive bacteria *viz*., *B. subtilis* (MTCC 121), *S. aureus* (MTCC 7443), and four Gram-negative bacteria *viz*. *P. aeruginosa* (MTCC 7093), *E. coli* (MTCC 729), *E. aerogenes* (MTCC 111), *K. pneumoniae* (MTCC 661) were used. The test bacteria were procured from the Department of Studies in Microbiology, University of Mysore, Karnataka, India.

Antibacterial activity: The inhibitory effect of the endophytic actinobacterial extracts was tested by disk diffusion method (Bauer *et al.*, 1966). The crude extract of endophytic actinomycetes was dissolved in dimethyl sulfoxide (DMSO) and tested on Mueller-Hinton agar medium seeded with the test bacterium at 250 µg per disk (5mm diameter, Whatman no. 1) concentration. Streptomycin (10 µg/ disk) was the positive control and DMSO was the negative control. The test plates were incubated for 24 h at 35 ± 2 ºC and the inhibition zone was measured.

Determination of minimal inhibitory and minimum bactericidal concentration: The minimal inhibitory concentration (MIC) was determined by modified broth dilution method (Xu *et al*., 2008), using sterile 96-well microplate (Tarsons, Kolkata, India). The wells were filled with reaction mixture containing 90 µl bacterial suspensions (10^6 CFU/ ml) and 10 µl of test sample with different concentrations (2 mg/ml to 0.02 mg/ml). The culture medium with 1% DMSO was used as the negative control and streptomycin sulphate (0.4 mg/ml to 0.01 mg/ml) was the positive control. The microplates were incubated for 24 h at 35 ± 2 ºC. 10 µl of the indicator 3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (0.5 mg/ ml phosphate buffer saline) was added After the incubation to visualize the microbial growth. The lowest sample concentration at which no blue color appeared was determined as MIC. Wells containing MIC concentration and above was inoculated onto agar medium to check cell viability. The lowest concentration with no viable cells was determined as minimum bactericidal concentration (MBC).

Thin layer chromatography (TLC) and bio-autography: The ethyl-acetate extract of endophytic actinobacteria with antibacterial activity was subjected to TLC for the separation of active compound. A mixture of chloroform: ethyl acetate: formic acid (20:16:4) was the solvent systems used. The developed chromatograms were subjected to agar overlay bio-autography method (Rahalison *et al*., 1991).

GC-MS analysis of antibacterial compounds: GC-MS (GC-17A with QP5000 MS, Shimadzu Corp., Kyoto, Japan) was used to analyse the volatile and aliphatic compounds. A SPB-1 column (30 m × 0.32 mm with film thickness 0.25 µm; Sigma-Aldrich, St. Louis, MO, USA) was used and 2 µl sample dissolved in acetone was injected with split ratio 20:1. The analysis carried out with oven temperature programmed at 50 ºC (hold 3 min) and raised to 260 ºC at a rate of 5 ºC/min. The injection port temperature was 250 ºC, transfer temperature was 200 ºC and ion source temperature was 180 ºC. The carrier gas Helium was used at a flow rate of 1ml/min. The instrument was calibrated to scan range *m/z* 40-400. The compounds were identified by matching their mass spectral fragmentation patterns with the NIST-MS library.

Data and statistical analysis: The colonization of actinobacteria was expressed as percentage colonization and calculated by the formula: % colonization = $[F_{col}/F_t] \times100$, where F_{col} is the total number of fragments colonized by the actinobacteria; Ft is the total number of fragments plated (Carroll and Carroll, 1998). The relative frequency (RF) was calculated by the following formula: % RF = $[I_0/I_t] \times100$, where, I_0 is the number of isolates of one species; I_t is the total number of isolates (Huang *et al*., 2007). Data reported as mean ± standard deviation (SD) of three independent replicates. Comparison among means were analysed with one-way ANOVA and Tukey-Kramer multiple comparisons tests using Graph Pad InStat 3.0. Any two data were considered statistically significant at $p < 0.05$ and denoted with different superscripts.

RESULTS

Isolation of endophytic actinobacteria: A total of 122 endophytic actinobacteria were isolated from 1,700 plant fragments of three medicinal plants. The highest number of isolates were obtained from the rhizome of *Z. nimmonii* (13.5%) followed by stem of *P. chinense* (11.5%) and stem of *J. wynaadensis* (10.5%). From the root of *Z. nimmonii* 2.5% isolates were obtained while, the leafy stem and inflorescence parts were devoid of any isolates.

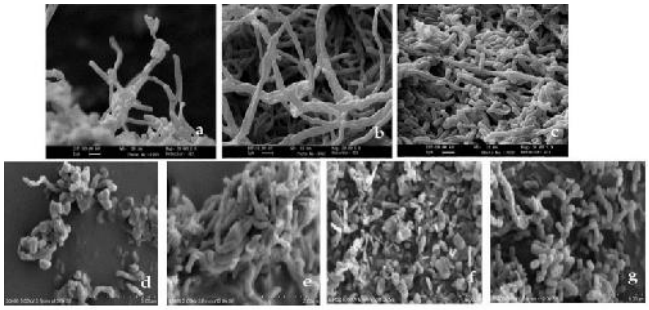
Table 1 Percent colonization of endophytic actinobacteria isolated from three medicinal plants

Plant name	Plant part	% Colonization
Zingiber nimmonii (J. Graham) Dalzell.	Leaf	9.0
	Rhizome	13.5
	Root	2.5
	Leafy stem	--
	Inflorescence	--
Polygonum chinense L.	Leaf	5.5
	Stem	11.5
Justicia wynaadensis Heyne.	Leaf	8.5
	Stem	10.5

'–' Indicates the absence of the endophytic actinobacteria in the plant part

The percent colonization of actinomycetes on different plant parts of selected host species is given in Table 1.

Identification of endophytic actinobacteria: A total of 10 taxa belonged to five genera such as *Streptomyces, Nocardiopsis, Arthrobacter*, *Curtobacterium* and *Corynebacterium*. Their colony characteristics on AIA plates, genbank accession numbers and percent similarity are depicted in Table 2. *Streptomyces* was isolated from all three host plants. *Arthrobacter* sp. was isolated from two hosts *viz*., Z. *nimmonii* and *J. wynaadensis*. *Curtobacterium* and *Corynebacterium* were isolated from *Z. nimmonii* while, *Nocardiopsis* was recovered from *P. chinense*. Five species of *Streptomyces* and two species of *Nocardiopsis* were identified. The morphology of different genera as viewed under SEM is provided in Fig. 1. The isolates showed 98-100% similarity to their assigned taxa. The %RF of the actinobacterial strains is represented in Fig. 2. *S. clavuligerus* showed highest %RF (24.6) followed by *Streptomyces* sp. (20.0). The lowest %RF was recorded for *S. coelicolor* (3.3). The most recurrent genus found was *Streptomyces* which comprised 59% of all strains.



a-c. *Streptomyces* spp. (x20K) d. *Curtobacterium* sp. (x10K); e. *Nocardiopsis* sp. (x15K); f. *Corynebacterium* sp (x10 K); g. *Arthrobacter* sp. (x10K)

Fig. 1. SEM images of endophytic actinobacterial genera isolated from the medicinal plants

Total phenolic and flavonoid content of endophytic actinobacteria: The total phenolic content (TPC) of the actinobacterial extracts ranged from 5.2±0.1 to 24.4±0.08 mg GAE/g dry extract (Fig. 3). High TPC of 24.4±0.08 mg GAE/g dry extract was detected in *Arthrobacter* sp. extract from *Z. nimmonii*. *Nocardiopsis alba* from *P. chinense* and *Streptomyces coelicolor* from *Z. nimmonii* exhibited lower phenolic contents (5.2±0.1 mg GAE/g dry extract and 5.3±0.2 mg GAE/g dry extract, respectively). Flavonoid content was detected in three endophytic strains. The total flavonoid content (TFC) ranged from 3.2±0.1 to 5.6±0.3 mg CE/g dry extract (Fig. 3).

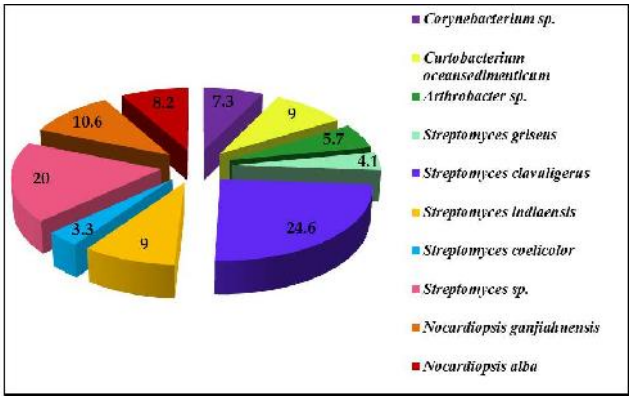


Fig. 2. Percent relative frequency (%RF) of endophytic actinobacterial isolations

Determination of antioxidant capacity of endophytic actinobacteria

DPPH radical scavenging capacity: The IC_{50} value of the actinobacterial extracts presented as 50% scavenging capacity (IC_{50}) varied from 489.4 ± 2.3 µg/ml to 1968.3±112.0 µg/ml (Table 3). The results indicated that *Arthrobacter* sp. isolated from *Z. nimmonii* had high scavenging capacity (489.4±2.3 µg/ml). No significant difference (p<0.05) was recorded between *Streptomyces clavuligerus* extracts isolated from *Z. nimmonii* and *P. chinense*, whereas *S. clavuligerus* isolated from *J. wynaadensis* contained significant difference (p<0.05) in the scavenging capacity. *Streptomyces griseus* from *P. chinense* exhibited least capacity (1968.3±112.0 µg/ml). The scavenging capacity of *Streptomyces indiaensis* (isolated from *Z. nimmonii*), *Nocardiopsis ganjiahuensis*, and *S. griseus* (isolated from *P. chinense*) was found to be very low.

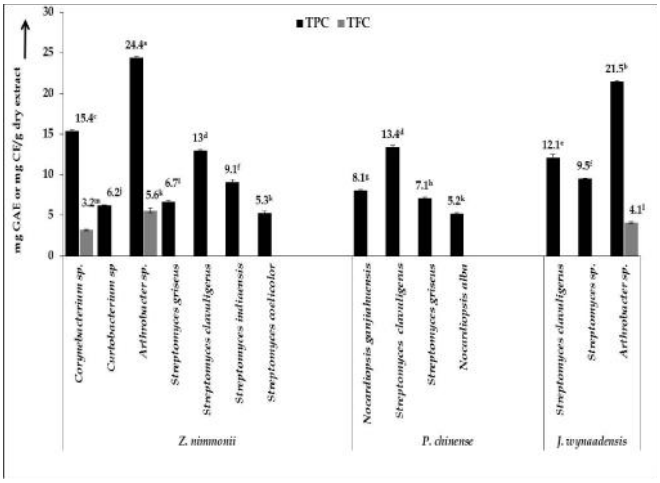


Fig. 3. Total phenolic and flavonoid content of endophytic actinobacteria isolated from three medicinal plants

Reducing power assay: The reducing power of endophytic extracts is represented in terms of ascorbic acid equivalent in Table 3. The values of reducing power assay ranged from 9.3±0.3 to 27.3±0.2 mg AAE/g dry extract. *Arthrobacter* sp. isolated from *Z. nimmonii* displayed high activity (27.3±0.2 mg AAE/g dry extract). *N. alba* from *P. chinense* and *S. coelicolor* from *Z. nimmonii* exhibited lower reducing capacities. Data are reported as mean ± SD of three independent analyses (n=3). Mean with the different superscript within a column are significantly different (p<0.05) by one-way ANOVA test; AAE- Ascorbic acid equivalent.

Table 3. Antioxidant capacity of actinobacterial endophytes isolated from three ethnomedicinal plants

Host plant	Actinobacterial strains	DPPH radical scavenging capacity (IC_{50} µg/ml)	Reducing Power (mg AAE/g dry extract)
Z. nimmonii	*Arthrobacter* sp.	489.4 ± 2.3[b]	27.3 ± 0.2[a]
	Corynebacterium sp.	913.6 ± 11.6[d]	20.4 ± 0.2[c]
	S. clavuligerus	1185 ± 21.5[e]	17.5± 0.06[d]
	S. indiaensis	1554.5 ± 15.2[g]	14.9 ± 0.1[f]
	C. oceanosedimentum	—	12.5 ± 0.2[h]
	S. griseus	—	11.9 ± 0.4[i]
	S. coelicolor	—	9.5 ± 0.2[j]
P. chinense	*S. clavuligerus*	934.1 ± 17.3[d]	17.7 ± 0.2[d]
	N. ganjiahuensis	1890.7 ± 124.0[h]	13.6 ± 0.1[g]
	S. griseus	1968.3±112.0[i]	12.3±0.5[h]
	N. alba	—	9.3±0.3[j]
J. wynaadensis	*Arthrobacter* sp.	554.7± 27.3[c]	24.0±0.2[b]
	S. clavuligerus	1210± 38.0[ef]	16±0.2[e]
	Streptomyces sp.	1313± 87.2[f]	15.2±0.3[f]
	Ascorbic acid	7.7±0.02[a]	

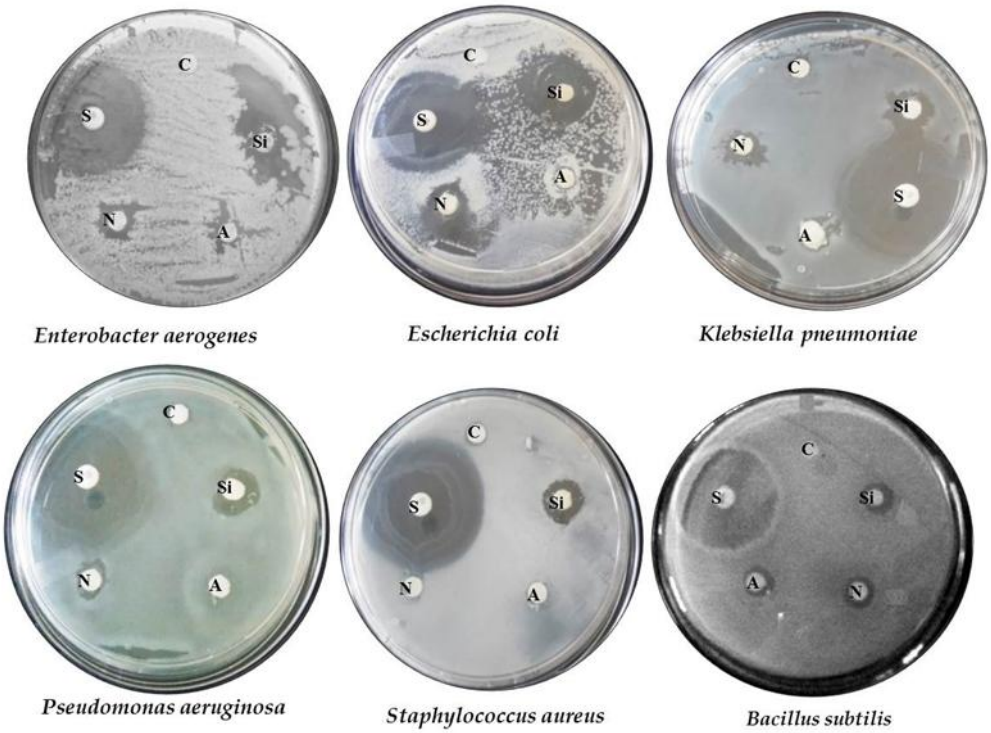


Fig. 4. Inhibition zones formed by endophytic actinobacterial extracts (250 µg extract/ disk)

Table 4. Antibacterial activity of actinobacterial endophytes against six pathogenic bacteria

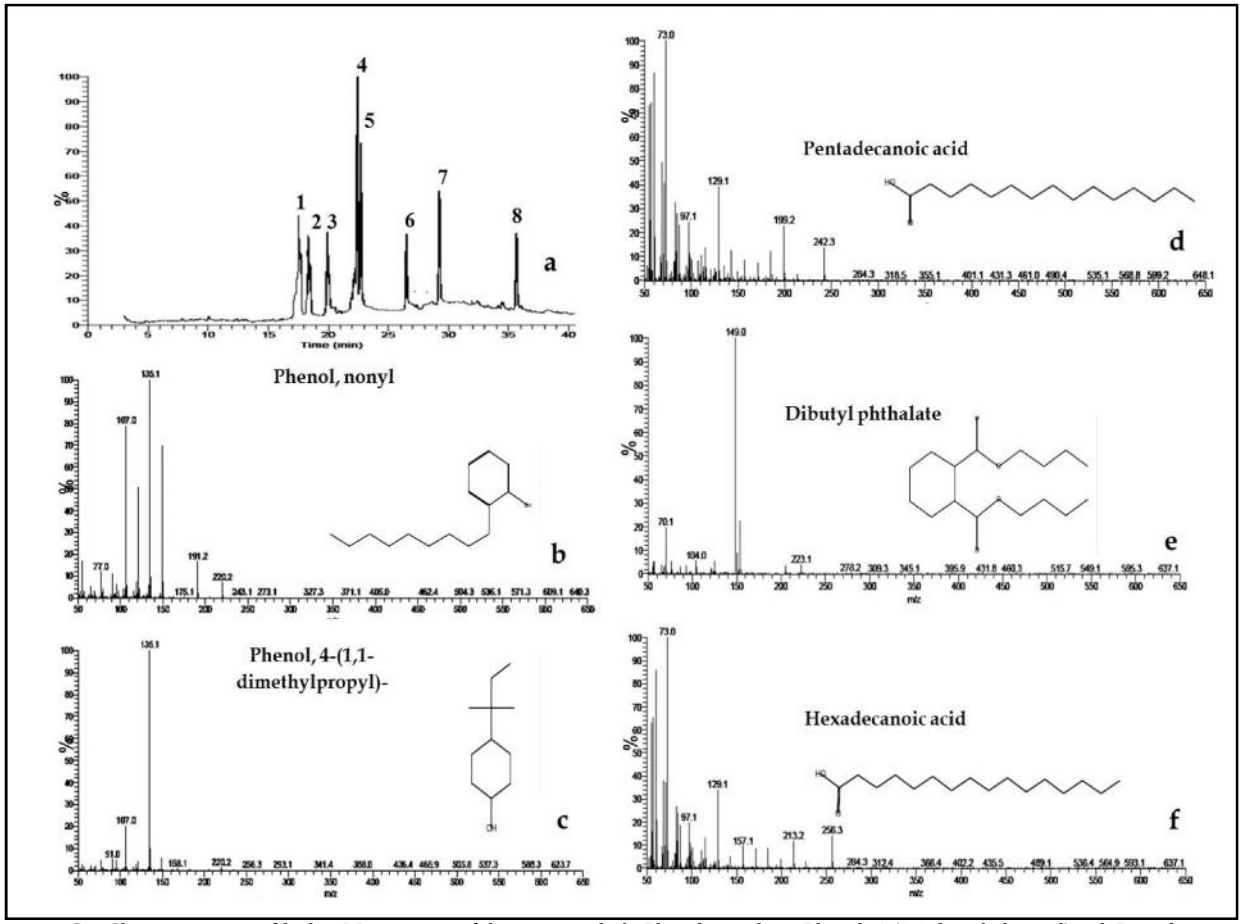
Host plant	Actinobacterial strains extract	Gram positive		Gram negative			
		Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Enterobacteraerogenes*	*Klebsiella pneumoniae*
Z. nimmonii	*Arthrobacter* sp.	-	-	9.2±0.2	11.1±0.5	10.3±0.4	8.1±0.1
	Corynebacterium sp.	-	-	-	9.9±0.3	9.2±0.2	-
	S. clavuligerus	10.0±0.2	-	7.2±0.1	12.1±0.3	10.5±0.5	-
	S. indiaensis	17.0±0.2	17.5±0.5	14.2±0.1	28.8±0.2	29.4±0.1	16.3±0.3
	C. oceanosedimentum	-	-	8.2±0.2	10.5±0.5	-	-
	S. griseus	11.1±0.1	-	9.5	13.3±0.1	12.9±0.1	7.2±0.4
	S. coelicolor	11.5±0.5	-	10.1±0.2	12.0±0.2	7.8±0.2	-
P. chinense	*S. clavuligerus*	9.2±0.1	-	7.5±0.3	10.2±0.3	9.7±0.2	-
	N. ganjiahuensis	-	-	8.0±0.1	-	-	-
	S. griseus	8.8±0.4	-	8.5±0.5	10.2±0.4	9.0±0.1	-
	N. alba	12.1±0.2	-	12.5±0.8	13.3±0.3	10.1±0.5	11.2±0.2
J. wynaadensis	*Arthrobacter* sp.	8.5±0.2	-	-	9.2±0.1	10.5±0.3	-
	S. clavuligerus	14.2±0.3	-	12.4±0.1	18.5±0.5	17.3±0.5	14.3±0.2
	Streptomyces sp.	12.3±0.2	-	13.1±0.1	12.9±0.1	9.2±0.2	-
	Streptomycin*	32±0.1	22.0±0.1	31.5±0.5	33±0.3	30±0.2	20±0.1

Table 5. Minimal inhibitory (MIC mg/ml) concentration and minimum bactericidal concentration (MBC mg/ml) of actinobacterial endophytes

Host plant	Endophytic actinobacterial strains extract	Test bacterial strains											
		P. aeruginosa		*B. subtilis*		*S. aureus*		*E. coli*		*E. aerogenes*		*K. pneumoniae*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Z. nimmonii	*Arthrobacter* sp.	1.0	1.2	-	-	-	-	0.6	0.8	0.8	0.8	1.4	1.6
	Corynebacterium sp.	2.0	-	1.8	2.0	-	-	0.8	1.0	1.0	1.2	-	-
	S. clavuligerus	1.6	1.8	0.8	1.0	2.0	-	0.4	0.6	0.6	0.8	1.6	1.8
	S. indiaensis	0.16	0.18	0.1	0.12	0.1	0.12	0.02	0.04	0.02	0.04	0.14	0.16
	C. oceanosedimentum	1.4	1.6	-	-	-	-	0.8	1.0	2.0	-	-	-
	S. griseus	1.0	1.2	0.4	0.6	-	-	0.2	0.4	0.2	0.4	1.8	1.8
	S. coelicolor	0.8	1.0	0.6	0.8	-	-	0.4	0.6	1.4	1.6	2.0	-
P. chinense	*S. clavuligerus*	1.4	1.6	1.0	1.2	2.0	-	0.8	1.0	1.0	1.0	1.8	2.0
	N. ganjiahuensis	1.4	1.6	-	-	-	-	1.6	1.8	2.0	-	-	-
	S. griseus	1.2	1.2	1.0	1.2	-	-	0.8	0.8	1.0	1.2	-	-
	N. alba	0.2	0.4	0.2	0.4	-	-	0.18	0.2	0.8	1.0	0.6	0.8
J. wynaadensis	*Arthrobacter* sp.	1.2	1.4	1.8	2.0	-	-	0.6	0.8	1.0	1.2	-	-
	S. clavuligerus	0.2	0.4	0.16	0.18	1.6	1.8	0.08	0.1	0.1	0.12	0.16	0.16
	Streptomyces sp.	0.18	0.2	0.2	0.4	-	-	0.18	0.2	1.0	1.2	1.8	2.0
	Streptomycin sulphate	0.02	0.02	0.02	0.04	0.02	0.04	0.04	0.06	0.04	0.06	0.04	0.04

Table 6. Identification of antibacterial compounds from *S. indiaensis* extract by GC-MS

Peak	Retention Time (min)	Molecular weight	Chemical formula	Name of the compound	% Area
1	17.53	220	$C_{15}H_{24}O$	Phenol, nonyl	11.28
2	18.32	164	$C_{11}H_{16}O$	Phenol, 4-(1,1-dimethylpropyl)-	10.34
3	19.92	242	$C_{15}H_{30}O_2$	Pentadecanoic acid	5.82
4	22.43	278	$C_{16}H_{22}NO_4$	Dibutyl phthalate	13.10
5	22.71	256	$C_{16}H_{32}O_2$	Hexadecanoic acid (palmitic acid)	7.99
6	26.50	284	$C_{18}H_{36}O_2$	Octadecanoic acid (stearic acid)	4.28
7	29.24	244	$C_{14}H_{16}N_2O_2$	Pyrrolo[1,2-a]pyrazine-1,4dione, hexahydro-3-(phenylmethyl)-	6.50
8	36.34	410	$C_{30}H_{50}$	Squalene	1.12



a. Gas Chromatogram profile, b-i. Mass spectra of the compounds, b. Phenol, nonyl c. Phenol, 4-(1,1-dimethylpropyl)- d. Pentadecanoic acid e. Dibutyl phthalate f. Hexadecanoic acid g. Octadecanoic acid h. Pyrrolo[1,2-a]pyrazine-1,4dione, hexahydro-3-(phenylmethyl)- i. Squalene.

Fig. 5. GC-MS of the TLC fraction from *S. indiaensis* extract

Antibacterial activity of endophytic actinobacteria: The antibacterial activity of strains was tested against six test pathogenic bacteria and the appeared area of inhibition zone is presented in Table 4. The strains were further tested for MIC and MBC. The results depicted in Table 5 indicate that *S. indiaensis* isolated from *Z. nimmonii* exhibited highest inhibition zone against all the pathogens followed by *S. clavuligerus* strain from *J. wynaadensis*. The inhibition zone formed by *S. indiaensis* is represented in Fig. 4. *S. aureus* was found to be susceptible only to *S. indiaensis* extract. All actinobacterial extracts assayed showed inhibitory activity against *E. coli* and *E. aerogenes* within the concentration range of 1.6 to 0.02 mg/ml and 2 to 0.02 mg/ml respectively. *S. indiaensis* showed significant antibacterial activity against all the test pathogenic strains. Therefore, the extract of *S. indiaensis* was further characterized for the identification of antibacterial compounds by TLC and bio-autography techniques.

TLC and bio-autography: The extract of *S. indiaensis* was characterized for the presence of antibacterial compounds by TLC and bio-autography methods. This analysis aided direct visualization of the presence of active compound as inhibition zone at *Rf* - 0.78 on the chromatogram. The compound at the *Rf*- 0.78 was collected carefully and further characterized by GC-MS analysis.

GC-MS analysis of antibacterial compounds: GC-MS of the TLC fraction of *S. indiaensis* extract revealed eight major peaks corresponding to the presence of (1) Phenol, nonyl; (2) Phenol, 4-(1,1-dimethylpropyl)-; (3) Pentadecanoic acid; (4) Dibutyl phthalate; (5) Hexadecanoic acid (palmitic acid); (6) Octadecanoic acid (stearic acid); (7) Pyrrolo[1,2-a]pyrazine-1,4dione, hexahydro-3-(phenylmethyl)- and (8) Squalene (Table6). Dibutyl phthalate (13.10%); phenol, nonyl (11.28%) and phenol, 4-(1,1-dimethylpropyl)- (10.34%) were found as major compounds. The chromatogram of gas chromatography with mass spectra is represented in Fig. 5.

DISCUSSION

The Western Ghats, extending along the west coast of India comprises one of the 34 biodiversity hotspots as recognized by the Conservation International (Myers *et al*., 2000). The plant species selected for endophytic isolations have utmost ethno medicinal value and therefore 14 taxa were isolated. *Z. nimmonii* contained highest diversity of isolated strains with *Streptomyces* as the dominant genus. *Streptomyces* has also been reported from the root parts of *Z. officinale* (Taechowisan and Lumyong, 2003; Taechowisan *et al*., 2012) and *Polygonum cuspidatum* (Sun *et al*., 2013; Wang *et al*., 2016). The rhizome of *Z. nimmonii* and stem parts of *P. chinense* and *J. wynaadensis* contained more endophytic actinobacteria than other parts. Molecular identification of the actinobacterial isolates by partial sequencing of 16S rRNA revealed the presence of five genera in four orders of class I. Actinobacteria *viz., Streptomycetales, Streptosporangiales, Corynebacteriales* and *Micrococcales* (Ludwig *et al*., 2012). In this study, *Nocardiopsis ganjiahuensis, S. clavuligerus, S. coelicolor* and *S. indiaensis* are first time reported as endophytic actinobacteria. A recent study of actinobacterial communities in the roots of herbaceous species of tropical deciduous forest in Meghalaya, north-eastern India encompassing the mega diversity hotspot region, reported 22 different genera of actinobacteria, indicating enormous seasonal diversity in their occurrence (Barman and Dhar, 2020). In the present study, three endophytic actinobacterial strains were positive for flavonoid content, while all the strains contained phenolics. There is very less literature published on the total phenolic and flavonoid contents of endophytic actinobacteria. Akshatha *et al*. (2016) reported the phenolic content of endophytic actinomycetes isolated from the selected medicinal plants of Western Ghats. According to their study, *Streptomyces globosus* and *Arthrobacter* sp., extracts displayed high phenolic content (83.47±1.52 µg GAE/g extract and 72.2±2.10 µg GAE/g extract respectively), whereas in the present study, *Arthrobacter* sp., extract was also found to contain high phenolic content (24.4±0.08 mg GAE/g dry extract) amongst all actinomycetes strains. Christhudas *et al*. (2013) reported the phenolic content of *Streptomyces* sp., isolated from *Datura stramonium* as 176 mg catechol equivalent/g extract. *Arthrobacter* sp., isolated from *Z. nimmonii* displayed higher TPC and antioxidant capacity than the strain isolated from *J. wynaadensis. S. clavuligerus* strains were isolated from all three hosts. Their TPC ranged between 12.1±0.4 to 13.4±0.2 mg GAE/g dry extract among the hosts and are significantly different (p<0.05). It is well recognized that biological and environmental factors defines the endophytic populations in plants (Adams and Kloepper, 2002). Though a single endophytic species may form relationships with more than one plant species but demonstrates a preference for one particular host (Cohen, 2004 and 2006).

The widespread use of antimicrobial agents for therapeutic purposes frequently in human, veterinary or agricultural purposes were facilitating the survival and extent of resistant pathogens. The search for new natural therapeutic agents by continuous screening of secondary metabolites produced from potential microbial taxa which reduce the chance of drug resistance of pathogenic micro flora and help to conserve precious medicinal plants are one most viable solution in present scenario (Gabreseleema *et al*., 2003). Such research requires the systematic screening of producers of antimicrobial products from unexplored sources and endophytes are one such source. *Zingiber* is known for its traditional medicinal uses. To the best of our knowledge there are no reports available on the antibacterial potential of actinobacteria from *Z. nimmonii*. Sabulal *et al*. (2006) reported the antibacterial activity of *Z. nimmonii* rhizomatous oil, with inhibition zones ranging from 11.3±0.6 mm to 7.7±0.6 mm, against *B. subtilis, S. aureus, E. coli* and *K. pneumoniae*. In the present study, *S. indiaensis* isolated from *Z. nimmonii* showed strong inhibition properties against these bacterial strains with the inhibition zones between 16.3±0.3 mm to 28.8±0.2 mm. These evidences substantiate endophytes as alternative sources of plant medicines. Taechowisan *et al*. (2013) reported the inhibitory activity of crude extract of endophytic S*treptomyces* sp., against *S. aureus* (MIC- 32 µg/ml), *B. subtilis* MIC- 32 µg/ml), *E. coli* (MIC- 512 µg/ml) and *P. aeruginosa* (MIC- 512 µg/ml). The present study also indicated strong antibacterial activity of *S. indiaensis* against these bacteria (Table 5). *P. chinense* is traditionally used to treat various diseases. Huang *et al.* (2008) reported plant extracts to exhibiting strong antibacterial activity against *Bacillus cereus* (MIC- 2.50 mg/ml), *Listeria monocytogenes* (MIC-5.00 mg/ml) and *S. aureus* (MIC-5.00 mg/ml). Maharajan *et al*. (2012) reported *P. chinense* extract to possess strong antimicrobial activity against *B. subtilis, S. aureus, P. aeruginosa* and *E. coli*.

The endophytic actinobacteria isolated in his study showed inhibitory activity against both Gram-positive and Gram-negative test bacteria (Tables 4 and 5). There are no reports available on the isolation of endophytic actinobacteria from *J. wynaadensis*. Hence, our study is the first report. Since endophytic *S. indiaensis* extract showed strong activity against all the test pathogens, the active compounds of extracts were isolated through bioautography method and identified through GC-MS. Phthalic acid ester, a plasticizer compound was identified in the extract of *S. indiaensis*. Phthalic acid ester reported in the present study was found to be antimicrobial by previous researchers (Barakat and Baltagy, 2015). In addition, Al-Bari *et al*. (2006) isolated a phthalate derivative from *Streptomyces bangladeshiensis* and reported it to be potent against *B. subtilis* and *S. aureus*. Two major compounds i.e. nonyl phenol and phenol, 4-1,1-dimethylpropyl)-, identified from *S. indiaensis* extract are alkylphenols. These are used in manufacture of antioxidants, detergents, emulsifiers and solubilizers (Soares *et al*., 2008). Phenol, 4-(1,1-dimethylpropyl) is commercially used as active ingredient in various disinfectants (Paul and McCue, 2001). Three saturated fatty acids were identified in the extract *viz*., pentadecanoic, palmitic and stearic acid. Fatty acids have gained attention as potential therapeutic antimicrobial agents. Fatty acids have broad spectrum of activity, due to microorganisms are deficient in resistance mechanisms of actions against these compounds (Desbois and Lawlor, 2013). Palmitic acid and stearic acid are reported as antimicrobial compounds in the leaf extract of *Jatropha curcas (Rahman et al*., 2014). Radha Krishnan *et al*. (2016) reported palmitic acid as active antimicrobial compounds in the extract of *Canthium parviflorum* leaves. The compound was found active against *E. coli*, *S. aureus* and *B. subtilis*. Stearic acid is also reported to have antiviral and anti-inflammatory activities (Khalifat *et al*., 2000).

Pyrrolo[1,2-a]pyrazine-1,4dione, hexahydro-3-(phenylmethyl)- detected in the extract is a pyrrolizidine. Pyrrolizidines are natural occurring complex heterocyclic compounds. These compounds are known to exhibit a broad spectrum bioactivity including antimicrobial, anticancer and antioxidant activities (Wang *et al*., 2013). Pyrrolo[1,2-a]pyrazine-1,4dione derivative was also reported in *Streptomyces cavouresis* KU-V39. Another compound squalene, identified in the extract is a dehydrotriterpenic hydrocarbon. This compound is reported to possess antimicrobial as well as antioxidant capacity (Popa *et al*., 2015). Due to its antibacterial properties, squalene is used for the treatment of burns (Ogawa and Doi, 1996). Hence, the findings presented in the paper can be useful in the battle against infectious diseases and in the search for novel compounds which are safe for human use.

CONCLUSION

To the best of our knowledge, this is the first attempt to explore the treasure of actinobacterial endophytes from three medicinal species *viz*., *Z. nimmonii*, *P. chinense* and *J. wynaadensis* from the Western Ghats. Four isolated strains *viz*., *N. ganjiahuensis*, *S. clavuligerus*, *S. coelicolor* and *S. indiaensis* are first time reported as actinobacterial endophytes. The present study has revealed the presence of various antibacterial compounds in the endophytic extracts of *S. indiaensis*. The present study encourages the exploration of these endophytes and their potential uses.

Conflicts of interest: The authors declare no conflicts of interest is involved in the study

Glossary of Abbreviations

SEM- Scanning electron Microscope
µg/ml- microgram per mililitre
TLC- Thin layer chromatography
rpm- rotation per minute
AIA- Actinomyces agar medium
ISP2- International Streptomyces Project type-2
g/l- gram per litre
mg/ml- milligram per mililitre
M- molar
v/v- volume by volume
nm- nanometer
ANOVA- Analysis of variance

REFERENCES

Nalini MS., Prakash HS. 2020. Actinobacteria: diversity, plant interactions and biotechnology applications. In: Yadav AN. et al. (eds.), Plant Microbiomes for Sustainable Agriculture, Sustainable Development and Biodiversity. Springer Nature Switzerland AG, p. 199-244, ISBN 978-3-030-38452-4,https://doi.org/10.1007/978-3-030-38453-1_7.

Bacon CW., White JF. 2000. Microbial Endophytes. Marcel Dekker, New York, p. 341– 388.

Golinska P., Wypij M., Agarkar G., Rathod D., Dahm H., Rai M. 2015. Endophytic actinobacteria of medicinal plants: diversity and bioactivity. *Antonie van Leeuwenhoek.* 108(2): 267–289.

Yu H., Zhang L., Li L., Zheng C., Guo L., Li W., Sun P., Qin L. 2010. Recent developments and future prospects of antimicrobial metabolites produced by endophytes. *Microbiol. Res*. 165: 437-449.

Newman DJ., Cragg GM. 2007. Natural products as sources of new drugs over the last 25 years. *J. Nat. Prod.* 70(3): 461-477.

Bryant D., Nielson D., Tangley T. 1997. The last frontier forests: ecosystems and economics on the edge. World Resources Institute, Washington, DC.

Nayar MP. 1996. Hot spots of endemic plants of India, Nepal and Bhutan. Thiruvananthapuram: Tropical Botanic Garden and Research Institute, India.

Myers N., Mittermeier RA., Mittermeier CG., De Fonseca GAB., Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403: 853–858.

Joy PP., Thomas J., Mathew S., Skaria BP. 2001. Scope of medicinal, aromatic and dye yielding crops in Kerala. In: National seminar cum workshop on “Scope of medicinal, aromatic and dye yielding crops in dry land agriculture.” Central Research Institute for Dryland Agriculture, Hyderabad, India; 11–13 December.

Das M., Prakash HS., Nalini MS. 2017a. Antioxidative properties of phenolic compounds isolated from the fungal endophytes of *Zingiber nimmonii* (J. Graham) Dalzell. *Front. Biol*. 12(2): 151–162.

Das M., Prakash H.S., Nalini MS. 2017b. Antioxidative and antibacterial potentials of fungal endophytes from *Justicia wynaadensis* Heyne: an ethnomedicinal rain

forest species of Western Ghats. *Asian J. Pharm. Clin. Res*.10(6): 203–209.

Das M., Prakash HS., Nalini MS. 2018. Bioactive sesquiterpene, plasticizer, and phenols from the fungal endophytes of *Polygonum chinense* L. *Ann. Microbiol* .68: 595–609.

Das M., Prakash HS., Nalini MS. 2020. Antibacterial metabolites from *Bipolaris specifera*, an endophytic fungus from the endemic medicinal plant, *Zingiber nimmonii* (J. Graham) Dalzell. 3 *Biotech*.10: 317, https://doi.org/10.1007/s13205-020-02307-0.

Sabulal B., Dan M., John JA., Kurup R., Pradeep NS., Valsamma RK., George V. 2006. Caryophyllene-rich rhizome oil of *Zingiber nimmonii* from South India: chemical characterization and antimicrobial activity. *Phytochemistry*. 67(22): 2469–2473.

Finose A., Gopalakrishnan VK. 2014. Antioxidant potential of *Zingiber nimmonii* (J. Graham) Dalzell. *International Journal of Pharmacy and Pharmaceutical Sciences*.6(6): 50-52.

Maharajan M., Rajendran A., Thomas B., Aravindhan V. 2012. Antibacterial and antifungal activities of *Polygonum chinense* Linn. *Asian J. Plant Sci. Res.* 2(5): 577-580.

Gamble JS. 1928. Flora of the Presidency of Madras. Vol. II and III, Bishen Singh Mahendra Pal Singh, Dehra Dun, India, p.755-756.

Lingaraju DP., Sudarshana MS., Rajashekar N. 2013. Ethnopharmacological survey of traditional medicinal plants in tribal areas of Kodagu district, Karnataka, India. *J. Pharm. Res.* 6: 284-297.

Subbiah MTR., Norman EJ. 2002. Rain forest plant extract with cellular cholesterol lowering properties. United States Patent. 6: 365-411.

Akshatha JV., Prakash HS., Nalini MS. 2016. Actinomycete endophytes from the ethnomedicinal plants of southern India: antioxidant activity and characterization studies. *J. Biol. Act. Prod. Nat.* 6(2): 150-165.

Amano S., Gyhbu Y. 2000. Preparation of specimens for scanning electron microscopy using a freeze-dry method. In: Identification Manual of Actinomycetes (The Society for Actinomycetes Japan, ed.), Business Center for Academic Societies Japan, Tokyo, p. 35.

Liu X., Dong M., Chen X., Jiang M., Lv X., Yan G. 2007. Antioxidant activity and phenolics of an endophytic *Xylaria* sp. from *Ginkgo biloba*. *Food Chem*. 105: 548-554.

Barros L., Ferreira MJ., Queirós B., Ferreira CFR., Baptista P. 2007. Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chem*. 103(2): 413–419.

Oyaizu M. 1986. Studies on product of browning reaction prepared from glucose amine. *J. Nutr.* 44: 307–315.

Bauer AW., Kirby WM., Sherries JC., Turck M. 1966. Antibiotics susceptibility testing by the standardized single disk method. *Am. J. Clin. Pathol.* 45: 493–496.

Xu L., Zhou L., Li J., Li X., Wang J. 2008. Fungal endophytes from *Dioscorea zingiberensis* and their antimicrobial activity. *Lett. Appl. Microbiol*. 46: 68-72.

Rahalison L., Hamburger M., Hostettmann K., Monod M., Frenk E. 1991. A bioautographic agar overlay method for the detection of antifungal compounds from higher plants. *Phytochem. Anal*. 2: 199-203.

Carroll GC., Carroll FE. 1978. Studies on the incidence of coniferous needle endophytes in the Pacific Northwest. *Can. J. Bot*. 56: 3034-3043.

Huang WY., Cai Y-Z., Hyde KD., Corke H., Sun M. 2007. Endophytic fungi from *Nerium oleander* L. (Apocynaceae): main constituents and antioxidant activity. *World J. Microbiol. Biotechnol.* 23: 1253–1263.

Taechowisan T., Lumyong S. 2003. Activity of endophytic actinomycetes from roots of *Zingiber officinale* and *Alpinia galanga* against phytopathogenic fungi. *Ann. Microbiol*. 53(3): 291-298.

Taechowisan T, Chanaphat S, Ruensamran W, Phutdhawong WS. 2012. Anti-inflammatory effect of 3-methylcarbazoles on raw 264.7 cells stimulated with lps, polyinosinic-polycytidylic acid and pam3csk. *Adv. Microbiol.* 2: 98-103.

Sun H., He Y., Xiao Q., Ye R., Tian Y. 2013. Isolation, characterization, and antimicrobial activity of endophytic bacteria from *Polygonum cuspidatum*. *Afr. J. Microbiol. Res.* 7(16): 1496-1504.

Wang L, Peng Q, Long X-F, Zhang S, Zeng Z-G, Tian Y-Q. 2016. Comparative analysis of chemical constituents, antimicrobial and antioxidant activities of ethylacetate extracts of *Polygonum cuspidatum* and its endophytic actinomycete, *Streptomyces* sp. A0916. *Chin. J. Nat. Medicines* 14(2): 0117-0123.

Ludwig W., Euzeby J., Whitman WB. 2012. Taxonomic outline of the phylum actinobacteria. In: Goodfellow M., Kämpfer PR., Busse H-J., Trujillo ME., Suzuki K., Ludwig W., Whitman WB. (eds.), Bergey's Manual® of Systematic Bacteriology, Vol 5. The Actinobacteria Part A, Springer, p.29-35.

Barman D., Dkhar MS. 2020. Seasonal variation influence endophytic actinobacterial communities of medicinal plants from tropical deciduous forest of Meghalaya and characterization of their plant-growth promoting potentials. *Curr. Microbiol*. 77: 1689-1698.

Christhudas NIVS., Praveenkumar P., Agastian P. 2013. In vitro α-glucosidase inhibition and antioxidative potential of an endophyte species (*Streptomyces* sp. Loyola UGC) isolated from *Datura stramonium* L. *Curr. Microbiol.* 67: 69-76.

Adams PD., Kloepper JW. 2002. Effect of host genotype on indigenous bacterial endophytes of cotton (*Gossypium hirsutum* L.). *Plant Soil*. 240: 181-189.

Cohen SD. 2004. Endophytic-host selectivity of *Discula umbrinella* on *Quercus alba* and *Quercus rubra* characterized by infection, pathogenicity and mycelial compatibility. *Eur. J. Plant Pathol.* 110: 713-721.

Cohen S.D. 2006. Host selectivity and genetic variation of *Discula umbrinella* isolates from two oak species: Analyses of intergenic spacer region sequences of ribosomal DNA. *Microbial Ecol.* 52: 463-469.

Gebreselema G., Feleke M., Samuel S., Nagappan RJ. 2013. Isolation of potential antibiotic producing actinomycetes from water and sediments of lake Tana, Ethiopia. *Asian Pac J. Trop. Biomed.* 3(6): 426-435.

Taechowisan T., Chanaphat S., Ruensamran W., Phutdhawong WS. 2013. Antibacterial activity of Decursin from *Streptomyces* sp. GMT-8; an endophyte in *Zingiber officinale* Rosc. *J. Appl. Pharm. Sci*. 3: 74-78.

Huang W-Y., Cai Y-Z., Xing J., Corke H., Sun M. 2008. Comparative analysis of bioactivities of four *Polygonum* species. *Planta Med*. 74: 43-49.

Barakat KM., Beltagy EA. 2015. Bioactive phthalate from marine *Streptomyces ruber* EKH2 against virulent fish pathogens. *Egypt. J. Aquat. Res*. 41(1): 49–56.

Al-Bari MAA., Sayeed MA., Rahman MS., Mossadik MA. 2006. Characterization and antimicrobial activities of a phthalic acid derivative produced by *Streptomyces bangladeshiensis*, a novel species collected in Bangladesh. *Res. J. Med. Sci.* 1: 77–81.

Soares A., Guieysse B., Jefferson B., Cartmell E. 2008. Nonylphenol in the environment: a critical review on occurrence, fate, toxicity and treatment in wastewaters. *Environ. Int*. 34(7): 1033–1049.

Paul AG., McCue KA. 2001. Phenolic compounds. In: Disinfection sterilization and preservation. Block, SS. (ed.), Philadelphia, USA, Vol 5, p. 255-284.

Desbois AP., Lawlor KC. 2013. Antibacterial activity of long-chain polyunsaturated fatty acids against *Propionibacterium acnes* and *Staphylococcus aureus*. *Mar. Drugs* 11: 4544-4557.

Rahman MM., Ahmad SH., Mohamed MTM., AbRahman MZ. 2014. Antimicrobial compounds from leaf extracts of *Jatropha curcas*, *Psidium guajava*, and *Andrographis paniculata*. *Sci. World J*. http://dx.doi.org/ 10.1155/ 2014/635240.

Radha Krishnan K., James F., Mohan A. 2016. Isolation and characterization of n-hexadecanoic acid from *Canthium parviflorum* leaves. *J. Chem. Pharm. Res*. 8(8): 614-617.

Khalil MH., Marcelletti JF., Katz LR., Katz DH., Pope LE. 2000. Topical application of docosanol- or stearic acid-containing creams reduces severity of phenol burn wounds in mice. *Contact Derm*. 43(2): 79-81.

Wang C., Wang Z., Qiao X., Li Z., Li F., Chen M., Wang Y., Huang Y., Cui H. 2013. Antifungal activity of volatile organic compounds from *Streptomyces alboflavus* TD-1. FEMS *Microbiol. Lett.* 341: 45–51 doi: 10.1111/1574-6968.12088.

Popa O., Bsbeanu NE., Popa I., Nits S., Dinu-Pârvu CE. 2015. Methods for obtaining and determination of squalene from natural sources. *BioMed Res. Int.* 2015: http://dx.doi.org/10.1155/2015/367202.

Ogawa Y., Doi H. 1996. The use of squalene, squalane or mixtures thereof for preparing a cooling composition for the local treatment of burns. European Patent Office *457193A2*.

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