Research Article**Diversity of fungal endophyte of *Ananus comosus* L. var. kew from Unokoti district, Tripura with bioactive potential of *Neopestalotiopsis piceana*****Sanchita Bhattacharya^{1,2*}, Sanjit Debnath², Panna Das³, Ajay Krishna Saha²**¹Department of Botany, M.B.B. College, Agartala-799004, Tripura West, India²Mycology and Plant Pathology Laboratory, Department of Botany, Tripura University, Suryamaninagar-799022, Tripura, India³Microbiology Laboratory, Department of Botany, Tripura University, Suryamaninagar-799022, Tripura, India

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Abstract

Background: Diversity of fungal communities may differ between and within organs and tissues of an individual plant. Plants as an important source of biologically active compounds and thus are used in traditional healing practices. Some fungi, isolated from the host plant are also co-producer of active metabolites. **Objective:** Present study documented the distribution of fungal endophytes in green leaf and root explants from *Ananus comosus* var. kew in fruiting and non-fruiting season. **Materials and methods:** Three Experimental sites were randomly selected for sampling of explants from Unokoti District, Tripura. Isolation and identification of fungal endophytes were done. Cultivation and extraction of *Neopestalotiopsis piceana* (isolated from both leaf and root) was done for determination antioxidant and antibacterial activity. Malt extract broth (MEB) and Potato dextrose broth (PDB) were used as growth media and ethyl acetate and methanol were used as extraction solvent. **Results:** Total twenty four endophytic fungal strains and four non-sporulating forms were isolated. Highest relative frequency was recorded in *Pseudopestalotiopsis theae* from leaf, *Trichoderma asperellum* from root and *Neopestalotiopsis piceana* from both leaf and root endophytes. Lowest EC₅₀ was recorded in case of ethyl acetate extract of mycelial mat harvested from PDB. Maximum inhibition zone was obtained for ethyl acetate extracted mycelial mat harvested from PDB growth medium against *Staphylococcus aureus*. **Conclusion:** Growth media and type of extraction solvent affect the secondary metabolite production in fungal strain. This is the first report of *N. piceana*, isolated from *A. comosus* of Tripura and hence its bioactive potential was evaluated.

Key words: Antimicrobial activity, broth extract, DPPH activity, mycelial extract

Introduction

Endophytes are microorganisms that live within the plant tissues of roots, stems, and or leaves, without causing any overt symptoms of disease (Petrini, 1991). Diversity of endophytic fungi depends on various factors which influence their compositions within the host plants. The fungal communities may differ between and within organs and tissues of an individual tree (Kumar and Hyde, 2004), in the different seasons of the year (Osono, 2008), or in same host plants from different regions (Fisher et al., 1994). Leaves and roots are considered as most dynamic interfaces between plants and their environment. Fungi inhabiting the biologically active tissues may

share characteristics, allowing them to grow and survive in a constantly-changing biochemical environment and changing gene expression of host tissues with their growth and age (Arnold, 2007). Endophytic fungi were reported to be associated with tropical epiphytic plants belong to the family Bromeliaceae (Dreyfuss and Petrini, 1984). Plants as an important source of biologically active compounds are utilized in natural products research. Many plant species which are used in traditional healing practices also studied for their pharmacological properties. Some fungi isolated from their host medicinal plants reported to be involved in the co-production of active metabolites (Alvin et al., 2014). Endophytic fungi isolated from plants used for traditional medicine produce new bioactive compounds and thus medicinal properties of a plant may be due to the metabolites produced by their endophytic microorganisms (Kusari et al., 2013).

Endophytic fungi reported to have potent antimicrobial and

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antioxidant activities (Basu Sarbadhikary and Mandal, 2017; Bhagobaty and Joshi, 2012; Jayanthi et al., 2011; Prabukumar et al., 2015) and produce diverse classes of antimicrobial compounds such as alkaloids, peptides, steroids, terpenoids, phenols, aliphatic compounds, quinones, flavonoids etc. (Mousa and Raizada, 2013). The metabolites from endophytic fungi are quantitatively superior to the host metabolites as they can be mass multiplied and are more economical to produce (Gupta and Chaturvedi, 2017).

Subtropical agro-climatic conditions of Tripura state favours Pineapple (*Ananas comosus* L.) cultivation practices. Queen and Kew are the main cultivars. Pineapple has been used in folk medicine. The pineapple fruit extracts using different solvents screened for phytochemicals revealed the presence of different secondary metabolites such as flavonoids, phenolics etc. and pineapple fruit being rich in polyphenols may be a source of antioxidant along with antibacterial properties (Kaushik and Kundu, 2018). Phytochemicals extracted for quantitative screening from pineapple peel contained alkaloids, oxalate, tannins, phytate and glycosides etc. and *A. comosus* peel have varying degrees of antimicrobial activity against *E. coli* and *S. aureus* (Dabesor et al., 2017). Therefore, diversity of endophytic fungal association of the host plant from different sites and explants types needs to be assessed.

Materials and methods

Collection of explants

Three experimental sites were randomly selected for sampling of explants from Unokoti district which were Darchawi, Kumarghat (N24°08.277' E092°03.313') East Betchara, (N24°06.917' E092°01.364') and Chinibagan, Kailashahar, (N24°18.544' E092°04.316'). Green leaves and roots were collected from healthy plants during fruiting season (FS) and non-fruiting season (NFS).

Isolation of endophytic fungi

Isolation of fungal endophytes was done according to the standard protocol (Schulz et al., 1998; Strobel and Daisy, 2003) with slight modification. Four to five segments of plant tissues were placed on potato dextrose agar (PDA) plate supplemented with streptomycin (100 µg/ml), and incubated in a BOD incubator for 21 days at 25± 2°C. In order to ensure proper surface sterilization, the sterilization protocol was validated using leaf imprint method (Schulz et al., 1998). Hyphal tips from fungus growing out from the samples were subsequently transferred onto fresh PDA plates to isolate pure colony.

Identification of endophytes

The microscopic identification of the isolates was carried out by lacto phenol staining technique. On the basis of macroscopic and microscopic characteristics, the fungus was identified with the

help of standard manuals. Identification of selected fungal strain for bioactive potential was authenticated by Molecular identification (ITS sequence of rDNA) by Agharkar Research Institute (NFCCI, Pune, India). The rDNA sequence of fungal strain was submitted to NCBI Gen Bank database and the accession number (KU258050) was obtained. Voucher specimen was deposited to NFCCI, Pune (accession number -3719). Relative frequency (RF) of isolated fungi was determined. Diversity indices were calculated using PAST.

Cultivation and extraction of fungal metabolites

Neopestalotiopsis piceana was isolated from both leaf and root and hence selected for bioactivity assessment. Selected endophytic fungal isolates of *Neopestalotiopsis piceana* were further inoculated into 250 ml Erlenmeyer flasks containing 100 ml of Potato Dextrose Broth (PDB) or Malt Extract Broth (MEB) and incubated at 25±2°C for 20 days under stationary conditions. After completion of incubation, the broth cultures from each type of medium were filtered to separate the mycelia and filtrate. For solvent optimization two different solvents i.e. ethyl acetate and methanol were used. Cultured mycelia were dried at 40°C, then extracts were prepared with two types of solvents and extraction was repeated for three times. The separated culture broths were also extracted with ethyl acetate and methanol. Equal volume of the cell free culture filtrate and solvent was taken in a separating funnel and shaken vigorously for 10 min and kept for 5 mins till the two clear immiscible layers were formed. The upper layer of solvent containing the extracted compounds was separated. The samples were extracted separately with two solvents. Fractions collected after extractions were composited. All the Ethyl acetate and Methanolic extract were concentrated separately under reduced pressure conditions in Rotary evaporator (Rotavap: PBV-7D) to yield the final extract. The bioactive potential of extracted component from *N. piceana* was assessed. All the extracts obtained from tested fungal species were stored in dark at 4°C before being used for the *in vitro* antioxidant and antimicrobial activity.

Antioxidant assay

DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging activity

Radical scavenging activities of fungal extracts were measured by slightly modified method (Miliauskas et al., 2004). Methanolic stock solutions of extracts were prepared and various concentrations of extracts were obtained by serial dilution and than 0.5 ml of methanolic solution of DPPH (1mM) was added to the extract solutions. The reaction mixture was shaken and allowed to

stand at room temperature for 30 mins. Absorbance was read at 570 nm using methanol as blank reference in spectrophotometer (Eppendorf AG 22331Hamburg). Ascorbic acid was used as control. DPPH scavenging activity (%) of the standard and extracts were determined by the formula:

$$\text{Percentage of inhibition} = [(A_{\text{Blank}} - A_{\text{Sample}}) / A_{\text{Blank}}] \times 100$$

Where, A_{Blank} and A_{Sample} denotes the absorbance of control and test compound, respectively.

EC_{50} value (mg/ml) is the effective concentration at which DPPH radicals were scavenged by 50% and the value was obtained by interpolation from linear regression analysis. All assays were performed in triplicate in three independent and separate experiments. The data were presented as mean $\pm$ standard deviations (SD) from three independent analyzes.

Antibacterial activity

The antimicrobial activity of mycelial extracts and culture broth were determined by paper disc diffusion method with slight modification (Bauer et al., 1966) against *Staphylococcus aureus* (MTCC-96), *Escherichia coli* (MTCC-40). The test organisms were procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India. To evaluate the antibacterial activity, bacteria were grown in Muller Hinton Broth medium (Himedia) for 24 h, adjusted at a concentration of 10^6 cells/ml. 100 μ l of bacterial suspension was spread on Petri dishes containing Muller Hinton Agar medium. In each petridish, four disks of sterile filter paper (6 mm diameter) were placed and inoculated with 20 μ l of extracts. Streptomycin and extraction solvents were used as positive and negative controls, respectively. The dishes were incubated at 37°C in BOD for 24 h. The antibacterial activity was evaluated by the formation of inhibition zones and each experiment was done in triplicate sets.

Statistical analysis

For each one of the mushroom species, the assays were carried out in triplicate form. The results were expressed as mean values $\pm$ standard deviations (SD) and the significance was tested. The average data recorded for each replica were subjected to one way ANOVA followed by Tukey's Least Significant Differences (LSD).

Results and discussion

Twenty four endophytic fungal strains and four non-sporulating forms were isolated from the green leaf and root of the host plants. Sterile or non-sporulating forms were isolated as endophytes from different host plants (Frohlich et al., 2000), which supported the present findings. Among isolated fungal endophytes six were isolated only from root, thirteen strains were isolated from leaf explants only and five endophytes were isolated from both leaf and root explants. Considering season wise occurrence of endophytes, it was observed that among leaf

endophytes, five endophytes were isolated during fruiting season, five in NFS and three were isolated during both FS and NFS. However, for root endophytes one fungal strain was isolated during FS and also in NFS but, four fungal endophytes was isolated from samples of both FS and NFS. Among fungal strain isolated from both types of explants, one was isolated during fruiting season; two strains were obtained in samples of NFS as well as samples of both the seasons (Table 1). Tissue specific distribution and seasonal occurrence of fungal endophytes may be correlated with the findings that the type of plant tissue, variations in chemical composition and anatomy of host tissue colonized by endophyte has impact on the abundance and community composition of endophytic fungi (Sanchez-Azofeifa et al., 2012; Gond et al., 2007).

Among endophytes isolated only from leaf, highest relative frequency was recorded in case of *Pseudopestalotiopsis theae* (23.07) isolated in NFS from site III. Whereas, for only root endophytes highest relative frequency was observed in *Trichoderma asperellum* (26.92) in FS from site II. But for endophytes isolated from both the explants, *Neopestalotiopsis piceana* exhibited highest relative frequency in FS i.e for root (29.41) from site I and leaf from site III (23.43) respectively. The colonization of endophytes varies in different plant parts. The results correlate with earlier finding and might be affected by substrate, secondary metabolites and the physiological state of the host plants (Meenatchi et al., 2016).

Considering only fruiting season for only leaf endophytes maximum relative frequency was observed in *Cladosporium cladosporioides* (19.69) from site I but for only root endophytes of non fruiting season *T. asperellum* exhibited maximum relative frequency from both site I and III (21.42). In case of endophytes isolated from both the explants in NFS, maximum value was obtained for *Penicillium sclerotiorum* (13.84) from root of site III and *N. piceana* (25.45) from leaf of site II (Table 1). The differences in endophytic fungal colonization in FS and NFS observed in present study may be due to the fact that colonization of endophytic fungi is dependent on both environmental conditions and intrinsic factors i.e. bioactive composition of host plant (Gupta and Chaturvedi, 2017). Similarly, the difference in species composition of endophytes during FS and NFS when host plants physiological states may differ can be correlated with earlier findings which stated that the number and species composition of endophytes is influenced by physiological factors (Islam et al., 2010).

C. lunata, *F. oxysporum*, *Taleromyces* sp. and *Pestalotiopsis*

sp. were isolated as endophytic fungi in the present study which were also isolated from medicinal herbs of south India as endophytes. Similarly *Cladosporium cladosporioides*, *Nigrospora oryzae*, *Rhizoctonia solani*, and *Aspergillus candidus* were found to be associated with *Ananus comosus* var. kew plant as endophytic fungi also reported as endophytes from herbaceous medicinal plants of South India (Krishnamurthy et al., 2008). In present study, some phylloplane fungi were isolated as endophytes. This might be due to that, some phylloplane fungi such as *C. lunata* reported to be capable of penetrating the superficial layers of leaf (Rajagopal et al., 2010) and adopt endophytic mode of life in adverse environmental conditions

(Cabral et al., 1993). *F. oxysporum*, *R. solani*, *C. cladosporioides*, *Neopestalotiopsis* sp. etc. were a plant pathogen to certain plant species was isolated as endophyte of *Ananus comosus* var. kew supported the fact that a pathogen may spend part of the life in an endophytic state (Hyde et al., 1998). Fungi such as *Paecilomyces variotii* was isolated as endophyte is a nematophagous fungus and can be used for biological control (Bhattacharyya et al., 2017).

Highest value of dominance was obtained for root explant samples in FS from site I. Highest Simpson and Shannon index value were obtained in leaf explants collected from

Table 1. Relative frequency of isolated endophytic fungal strains from different parts of *A. comosus* of three different sites

Sl. No.	Name of the fungi	Laboratory Accession number	Site I				Site II				Site III			
			FS*		NFS*		FS		NFS		FS		NFS	
			Leaf (RF*)	Root (RF)	Leaf (RF)	Root (RF)	Leaf (RF)	Root (RF)	Leaf (RF)	Root (RF)	Leaf (RF)	Root (RF)	Leaf (RF)	Root (RF)
1	<i>Aspergillus japonicus</i>	MPL/A/4	0	0	2.94	0	0	0	4.47	0	0	0	0	0
2	<i>Taleromyces stollii</i>	MPL/A/5	0	0	7.35	0	0	0	0	0	0	0	0	10.71
3	<i>Acremonium strictum</i>	MPL/A/7a	0	0	0	0	0	0	8.95	0	0	0	0	0
4	<i>Aspergillus fumigatus</i>	MPL/A/8	0	0	10.29	0	0	0	10.44	0	0	0	7.69	0
5	<i>Aspergillus sydowii</i>	MPL/A/10	13.63	0	10.29	0	10.6	0	10.44	0	15.62	0	12.3	0
6	<i>Penicillium sclerotiorum</i>	MPL/A/12	6.06	0	13.23	10.71	7.57	0	13.43	0	7.81	16.66	13.84	7.14
7	<i>Pseudopestalotiopsis theae</i>	MPL/A/14	0	0	20.58	0	0	0	19.4	0	0	0	23.07	0
8	<i>Nemania bipapillata</i>	MPL/A/15	3.03	0	13.23	0	7.57	0	10.44	0	7.81	0	9.23	0
9	<i>Cladosporium cladosporioides</i>	MPL/A/19	19.69	0	0	0	18.18	0	0	0	9.37	0	0	0
10	<i>Neopestalotiopsis piceana</i>	MPL/A/20	13.63	29.41	4.41	0	13.63	15.38	5.97	25.45	23.43	12.96	7.69	12.5
11	<i>Fusarium sacchari</i>	MPL/A/21	10.6	0	0	0	7.57	0	0	0	9.37	0	6.15	0
12	<i>Trichoderma asperellum</i>	MPL/A/23	0	19.6	0	21.42	0	26.92	0	16.36	0	24.07	0	21.42
13	<i>Rhizoctonia solani</i>	MPL/A/25	0	17.64	0	0	0	7.69	0	10.9	0	9.25	0	10.71
14	<i>Penicillium citrinum</i>	MPL/A/27	0	0	0	0	4.54	0	0	0	0	0	0	0
15	Nonsporulating dematiaceous and hyaline form I	MPL/A/28	0	9.8	2.94	7.14	0	7.69	0	0	0	3.7	0	0
16	<i>Aspergillus candidus</i>	MPL/A/30	0	0	7.35	10.71	0	0	0	14.54	0	0	0	12.5
17	Non sporulating hyaline form II	MPL/A/31	3.03	0	0	0	3.03	0	0	0	6.25	0	0	0
18	<i>Aspergillus fischeri</i>	MPL/A/32	0	9.8	0	14.28	0	11.53	0	0	0	9.25	0	8.92
19	<i>Fusarium sp</i>	MPL/A/33	0	0	0	14.28	0	0	0	9.09	0	0	0	0
20	<i>Fusarium sp. aff. F.oxysporum</i>	MPL/A/34	10.6	0	0	0	4.54	0	0	0	0	0	0	0
21	<i>Khuskia oryzae / Nigrospora oryzae</i>	MPL/A/35	10.6	0	0	0	13.63	0	0	0	7.81	0	0	0
22	Non sporulating hyaline form III	MPL/A/36	0	0	2.94	0	4.54	0	4.47	0	6.25	0	4.61	0
23	<i>Aspergillus ornatus gr.</i>	MPL/A/37	6.06	0	0	0	4.54	15.38	0	0	6.25	0	0	0
24	<i>Pestalotiopsis versicolor</i>	MPL/A/39	0	0	0	0	0	0	0	0	0	11.11	0	0
25	<i>Curvularia lunata</i>	MPL/A/40	0	0	4.41	0	0	0	5.97	0	0	0	9.23	0
26	<i>Paecilomyces variotii</i>	MPL/A/41	0	13.72	0	14.28	0	15.38	0	14.54	0	12.96	0	8.92
27	Non sporulating dematiaceous and hyaline form II	MPL/A/43	0	0	0	7.14	0	0	0	9.09	0	0	0	7.14
28	<i>Pithomyces charatum</i>	MPL/A/47	3.03	0	0	0	0	0	5.97	0	0	0	6.15	0

*Note: FS: Fruiting Season; NFS: Non-Fruiting Season; RF: Relative Frequency

Table 2. Diversity indices of endophytic fungi in fruiting and non fruiting seasons of different sites

Diversity indices	Site I: FS*: Leaf	Site I: FS: Root	Site I: NFS*: Leaf	Site I: NFS: Root	Site II: FS: Leaf	Site II: FS: Root	Site II: NFS: Leaf	Site II: NFS: Root	Site III: FS: Leaf	Site III: FS: Root	Site III: NFS: Leaf	Site III: NFS: Root
Taxa_S	11	6	12	8	12	7	11	7	10	8	10	9
Dominance_D	0.1198	0.1942	0.1159	0.1403	0.1079	0.1687	0.1112	0.1623	0.1269	0.1502	0.1262	0.1263
Simpson_1-D	0.8802	0.8058	0.8841	0.8597	0.8921	0.8313	0.8888	0.8377	0.8731	0.8498	0.8738	0.8737
Shannon_H	2.235	1.713	2.299	2.02	2.346	1.861	2.295	1.883	2.191	1.978	2.19	2.137
Evenness_c^H/S	0.8497	0.9246	0.8305	0.942	0.8699	0.9185	0.902	0.939	0.8947	0.9034	0.8931	0.9415

*Note: FS: Fruiting Season; NFS: Non-Fruiting Season; RF: Relative Frequency

Figure 1. DPPH radical scavenging activity of ascorbic acid and tested fungal extracts

site II in FS. However, highest evenness value was observed in case of root samples from site I in NFS (Table 2). Diversity indices revealed that root samples had higher dominance and evenness value in comparison to leaf irrespective of seasons. Whereas, higher Shannon index value were obtained for leaf rather than root. Explant wise differences in endophytic colonization and diversity indices recorded in the present study correspond with the earlier findings which stated that leaves, petiole, stem and roots of a single plant often differ greatly in the dominant members of their endophytic communities (Gazis et al., 2010).

Antioxidant assay

Free radical scavenging effects of extracts were tested using DPPH as radical scavenger. As antioxidant donate proton to the DPPH, the absorption of reaction mixture was decreased. The extracted samples were tested against this radical at different

concentrations. The scavenging effects of the test samples were compared with the standard Ascorbic acid. The fungal extracts against DPPH radical exhibited a maximum percentage of inhibition at maximum concentration of 16 mg/ml. Maximum percentage of inhibition was recorded inr Ethyl acetate extract of mycelial mat harvested from PDB medium (Figure 1).

Lowest EC_{50} was recorded in case of ethyl acetate extract of mycelial mat harvested from PDB. For extracts of culture filtrate lowest EC_{50} value was also obtained in case of ethyl acetate extracts of culture filtrate obtained from PDB as growth medium. Ascorbic acid, which was used as a standard, had superior EC_{50} (0.072 mg/ml) value in comparison to all the tested samples. Extent of antioxidant activity of extracts of endophytic fungi isolated from *Ananus comosus* depends on type of growth media and extraction solvents used (Bhattacharya et al., 2018).

Table 3. EC₅₀ value of mycelial mat and culture filtrate of *N. piceana* in different growth medium and extracted in different solvent

PDB				MEB			
Mycelial Mat		Culture filtrate		Mycelial Mat		Culture filtrate	
Ethyl Acetate	Methanol	Ethyl Acetate	Methanol	Ethyl Acetate	Methanol	Ethyl Acetate	Methanol
162.51	420.26	618.2	1520.7	248.48	249.49	1700.8	979.39

Table 4. Antibacterial activity of *N. piceana* against gram-positive and gram-negative bacteria

Bacterial strains	MEB				PDB			
	Mycelial Mat		Culture filtrate		Mycelial Mat		Culture filtrate	
	Ethyl Acetate	Methanol	Ethyl Acetate	Methanol	Ethyl Acetate	Methanol	Ethyl Acetate	Methanol
<i>S. aureus</i>	14.667±0.471	10.333±0.816	10.667±0.943	0	20.333±0.471	13.667±0.471	9.333±0.471	8.667±0.471
<i>E. coli</i>	12.333±0.816	7.667±0.471	10±0.816	0	17.667±0.471	11.667±0.471	0	8.333±0.471

Antibacterial activity

Maximum inhibition zone was observed against *S. aureus* for mycelial mat extracted with ethyl acetate and harvested from PDB. Mycelial mat harvested from PDB and extracted from ethyl acetate also induce maximum inhibition zone against *E. coli*. Considering methanolic extract of mycelial mat PDB was more effective growth medium than MEB in respect of antibacterial activity. Among culture filtrate ethyl acetate extract of filtrate harvested from MEB was more effective against two tested bacterial strains. However, methanolic extracts of culture filtrate obtained from MEB medium did not show any activity against two bacterial strain as well as ethyl acetate extracts of culture filtrate from PDB medium showed no activity against *E. coli* (Table 4).

Highest antibacterial activity was reported in the ethyl acetate extract of endophytic fungi in comparison to other solvents (Verma et al., 2009) which correspond with present findings. The variation in inhibition zone diameter recorded in different experimental sets may be due to different bioactive compounds produced by endophytes or because of different concentration of the same compound (Mahapatra and Banerjee, 2010). Differences among the antimicrobial potency of the tested extract against bacterial strains might be due to either presence of unidentified active principle in the extracts in high concentration or probably in smaller amounts or the purification of the screened crude extracts could yield more potent compounds (Idris et al., 2013).

Media dependent differences were observed in antibacterial activity and antioxidant activity of tested fungal strain which correlated with earlier findings that stated, medium composition, incubation temperature, etc has impact on the amount and kinds of compounds that are produced by an endophytic fungus

(Strobel et al., 2004). Several compounds with different extent of solubility in different solvents constitute the metabolites and thus varied degrees of bioactivities were observed for different test extracts. Therefore, the problem of less extraction and activity of compound can be minimized by appropriate selection of solvent (Goutam et al., 2014). The phytochemicals present in the endophytes can be potential source for development of synthetic drugs (Sadananda et al., 2011).

Conclusion

Endophytic fungi represent an unexplored biodiversity which varied between associated host plants. The isolation and identification of these endophytes may provide the selection of suitable strains with bioactive potentials. Growth media and type of extraction solvent affect the secondary metabolite production in endophytic fungi. The *N. piceana* associated with the host plant and its bioactive potential was done for the first time. Crude extract from endophytic fungal strain *N. piceana* was evaluated for antioxidant and antimicrobial activity which exhibited differential bioactive potential. But isolation and purification of active components from extract might yield better bioactivity against free radical and tested bacterial strain. Optimization of growth parameters such as temperature, pH, incubation time etc may enhance the metabolite production.

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Conflict of interest statement

The authors declare that they have no competing interests.

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