Antibacterial activities of endophytic *Xylaria* sp. strain SR2 from *Araucaria heterophylla* against drug resistant *Staphylococcus aureus* strains

V. RAMESH, S. RAMAKRISHNAN, V. SIVA

J. Mycopathol. Res. 59(4) : *435-441, 2021;*
ISSN 0971-3719

Department of Botany,
University of Calcutta,
Kolkata 700 019, India

J. Mycopathol. Res. **59(4)** : 435-441, 2021; (ISSN 0971-3719)

Antibacterial activities of endophytic *Xylaria* sp. strain SR2 from *Araucaria heterophylla* against drug resistant *Staphylococcus aureus* strains

V. RAMESH[1*], S. RAMAKRISHNAN[1], V. SIVA[2]
[1]Department of Botany, Vivekananda College, Tiruvedakam West -625234, Madurai, Tamil Nadu, India,
[2]PG and Research Department of Microbiology, VHNSN College, Virudhunagar 626 001, Tamil Nadu

Received : 10.8.2021 Accepted : 23.10.2021 Published : 27.12.2021

In the present study, the bioactive endophytic fungal species was isolated from the gymnosperm plants of *Araucaria heterophylla*. The isolated fungal species was identified as *Xylaria* sp. SR2. based on morphological characteristics. The fungal isolate of Xylaria was grown in various range of pH, temperature and culture media for optimizing maximum biomass production in submerged culture conditions. The maximum growth of Xylaria sp. SR2 was observed in PDA and the optimal temperature and pH for the maximum mycelial growth was found to be 30°C and 5.5 respectively. The optimized ethyl acetate extract of cultural filtrate were subjected to antibacterial activities against *Pseudomonas syrirgae* (MTCC 673), *Proteus mirabilis* (MTCC 1429), *Burkholderia glumae* (MTCC 8496), *Moraxella bovis* (MTCC 1775) and drug resistant *Staphylococcus aureus* strains (1-10). The maximum inhibition zone of 23.2 mm and 20.4 mm against *S. aureus* strain 6 and 5 respectively.

Key words: Antibacterial Activity, Drug resistant Bacteria, Endophytic *Xylaria*,

INTRODUCTION

In recent past, emerging of new infectious diseases such as Covid-19, drug resistant microbial diseases, Influenza, SARS, and H1N1 has become the greatest challenges for researchers to safeguard the human health against pathogenic microorganisms. Most of these newly emerged diseases are caused by microorganisms and the causative organisms are increasingly becoming drug resistant over the recent past times (Tauhidur *et al*. 2020, Bhatia and Narain 2010). For treating such infectious diseases, novel bioactive natural compounds from plants as well as microbes could provide the best and significant alternatives as source of potential and promising drugs (Morens *et al.* 2004). Although, plant is the major source of bioactive natural compounds, nevertheless, endophytic fungal species could play a vital role in the search of new novel bioactive natural products (Jalgaonwala *et al*. 2011).

In the last couple of decades, endophytic fungal species are emerged as significant natural resources of bioactive natural products against drug resistant microorganisms like bacteria and fungi, because most of them are occupying millions of peculiar biological niches growing in so many unusual stresses and environmental conditions. There is enormous scope for the discovery and recovery of novel fungal species, genera and biotypes from these environments.

Bioactive natural compounds produced by the endophyticfungal species are promising potential use in safety and human health concerns, eventhough there is still a significant demand of drug industry for synthetic products due to economic and time consuming reasons (Strobel *et al.* 2004).

Endophytic fungal species are to be found in virtually every tissue of plant parts on the earth. They reside in the living tissues of the host plant and do so in a variety of relationships ranging from symbiotic to pathogenic microorganisms (Strobel *et al*. 2004). Endophytic fungal species have been reported from all groups of plants starting from algae to angiosperms and various research groups reported including algae (Zuccaro *et al*. 2008,

*Correspondence: ramesh.vnr09@gmail.com

Suryanarayanan *et al.* 2010) lichens (Suryanarayanan *et al*. 2005) , mosses and angiosperms (Tejesvi *et al*. 2010), and may persist even in aseptically cultured plants also (Lucero *et al*. 2008). Moreover, endophytic fungi are also reported from plants that grow in various environments including tropic (Mohali *et al*. 2005), temperate (Ganley *et al.* 2004), xerophytic (Suryanarayanan *et al.* 2005) and coastal mangroves (Kumaresan and Suryanarayanan 2002). Environment plays an important role on endophyte biodiversity, while the species diversity is dependent upon the nature of the host plant and their ecological location. For example, endophytic fungi in woody plants are highly abundant and diverse, particularly in the tropical areas (Arnold *et al.*2001).

Fungal species of the genus *Xylaria* Hill ex Scharank (Xylariaceae, Asomycetes) both macro and micro fungi, are known to produce diverse classes of bioactive compounds including antifungal multiplolides (Boonphong *et al.* 2001), cytotoxic cytochalasins, acetylcholineesterase inhibitor xyloketals (Lin *et al.* 2001), Xanthones (Healy *et al.* 2004), Xylarighanand Orthosporin (Rong Chen *et al*. 2018), Nigriterpene (Jung *et al*. 2017), Cytochalasin D (Elias Luciana 2018). The present investigation has therefore been designed to study the *in vitro* antibacterial potential of dried fungal extracts of endophytic *Xylaria* sp. SR2 against drug resistant human bacterial pathogens of clinical importance.

MATERIALS AND METHODS

Collection of plant materials

The healthy stem segments of *Araucaria heterophylla* were collected from our holy Gurukula Institute of Vivekananda College, Tiruvedakam west, Madurai, Tamil Nadu during the month of August 2019 (Fig. 1). The stem segments were placed in paper bags after removal of excess moisture. Then the stem samples were stored at 4oC for further use.

Isolation of endophytic fungi

The stem samples collected from *A. heterophylla*were washed thoroughly with distilled water and air dried before they were processed. Endophytic fungi were isolated according to the reported protocol (Petrini 1986) which was slightly modified based on preliminary research. All the stem samples were washed twice in distilled water and then surface sterilized by immersion for 1 min in 70% (v/v) ethanol, 1min in sodium hypochlorite (3% (v/v) available chlorine) and 30 s in 70% (v/v) ethanol and further washed three times in sterilized distilled water for 1 min each time. After surface sterilization, the samples were cut into 5-7 mm pieces and aseptically transferred to Petri plates containing potato dextrose agar (PDA) with 50 ?g/ml of streptomycin to suppress the bacterial growth. These Petri plates were incubated at 30^0C with normal daily light and dark periods. The plates were examined daily for up to one month for the development of fungal colonies growing out from the stem segments. The fungi growing out from the stem tissue were subsequently transferred onto fresh PDA plates without antibiotics.

Microscopic analysis of morphological characterization

The endophytic fungus was grown on PDA at 30°C for 7–9 days, and the formation of conidia was examined under a microscope. Each specimen was examined for morphological characteristics of asci, ascospores, paraphyses and other structures of taxonomic value. Spore dimensions were determined for 50 spores. Lacto phenol cotton blue and distilled water were used as mounting media for microscopy. Dried materials were rehydrated in 3% aqueous KOH. Photography was carried out with a light microscope and binocular microscope attached with computer (COSLAP). The isolated endophytic fungus was identified based on morphological characteristics and standard taxonomic key included colony diameter, texture, color and the dimensions and morphology of hyphae and spore (Ainsworth *et al.* 1973).

Effect of pH on the growth of fungal species

The endophytic was grown in potato dextrose agar plates with the initial pH of 4.0 to 7.0. The culture was incubated for 7 days under static condition. After the incubation, radial growth was measured. The measurements were taken from four different points and the average radial growth was recorded.

Effect of temperature on the growth of fungal species

The endophytic was grown in potato dextrose agar

plates with the initial temperature of 10 to 35^{0}C. The culture was incubated for 7 days under static condition. After the incubation, radial growth was measured. The measurements were taken from four different points and the average radial growth was recorded.

Fermentation and extraction of bioactive compounds

The endophytic fungus was grown on potato dextrose agar (PDA) at 30ºC for 5-7 days depending on growth rate. Six pieces of the grown culture cut from the plate were inoculated into 500 ml Erlenmeyer flasks containing 300 ml potato dextrose broth (PDB) and incubated at 30ºC for 3 s at pH 5.5. After the incubation period, the preparation of extracts from fermented broth was based on our earlier standard protocol (Ramesh *et al.* 2014, Subbulakshmi *et al*. 2012, Arivudainambi *et al*. 2011)

Test Microorganisms

Pseudomonas syringae (MTCC 673), *Proteus mirabilis* (MTCC1429), *Burkholderiaglumae*(MTCC8496), *Moraxella bovis* (MTCC 1775) were purchased from Microbial Type Culture Collection, Chandigarh, India and were used for screening tests. The clinical strains of *Staphylococcus aureus* (1–10) were obtained from Bose Clinical Laboratory and X-ray (Madurai, Tamilnadu, India). *S. aureus* strains were identified by standard biochemical methods (Essers andRadebold 1980, Pourshadi and Klaas 1984).

Antibiotic Susceptibility Test

The Kirby–Bauer disk diffusion test was used to determine the antibiotic resistance of *S. aureus* strains (1–10). The nutrient broth was prepared and well-isolated colonies of the same type from a culture agar plate were inoculated into it. The broth was incubated at 37ºC until the culture equalled 0.5 McFarland standards. A McFarland 0.5 turbidity standard corresponded to an inoculum of 1×108 CFUml-1 (Acarand Goldstein 1996). The remaining procedure was done based on our earlier standard protocol (Ramesh *et al.* 2015, Essers & Radebold 1980)

Antibacterial activity

For preliminary screening, the antibacterial activity was done based on our earlier standard protocol (Ramesh *et al*. 2015, Arivudainambi *et al*. 2011).

Statistical analysis

The triplicate data are expressed as the mean value ± standard error and presented in the form of figures. The error bars are depicted at 5% limit. The overlapping and non-overlapping bars show no significant and significant respectively differences among different treatments.

RESULTS AND DISCUSSION

Totally six endophytic fungal species were isolated from the stem samples of Araucaria sp. at the time of isolation, they were named as SR1, SR2, SR3, SR4, SR5 and SR6 based on morphological characteristics (Fig. 2). Among the six species of fungal endophytes, SR2 was identified as *Xylaria* sp. strain SR2 based on the colony morphology and sporulating structure. Traditionally, identification of fungal species has been based on size, shape of conidia and culture characteristics such as colony colour, growth rate and texture. The remaining five fungal isolates were not able to identify at the genus level due to insufficient spore production. So, they named as fungal isolates of sterilia SR1, SR3, SR4, SR5 & SR6. Among these above fungal isolates, only one endophytic fungal isolates *Xylaria* sp. strain SR2 was selected to further growth optimization and antibacterial screening studies.

In order to obtain large quantities of mycelial biomass, then this could be used for biological activity. The effect of environmental factors such as pH, temperature and culture media were examined and the optimal conditions were determined. The morphological characteristics of the *Xylaria* sp. strain SR2 was observed on PDA after 10 d of growth at 30°C. Growth rate is high, 5.4 - 7.2 cm/week, covering petriplate in 5 - 7 days. Mycelial mat was white at early stage, later it was brown to thick black coloured. Hyphae were thin walled and branched. The morphological characteristics of the endophytic fungal isolate SR2 was observed on PDA after 7 day of growth at 30 ^{0}C. Colonies on PDA was circular, mycelium is raised at first at whitish colour and becoming black with age, sometimes pale grey (Fig. 3a).Asci are 8 spored, uniseriate, cylindrical and stipitate. Ascospores are 17.3 – 17.8 μm broad, 33.1 – 33.8 μm in length. Each ascospore is ellipsoid,

Table 1: Antibacterial activities of ethyl acetate extract of *Xylaria* sp. SR2 against bacterial pathogens

Microorganisms Used	Zone of Inhibition (mm)
Pseudomonas fluroescus (MTCC103)	12.2 ±0.25
Pseudomonas syrirgae (MTCC 673)	11.3 ±0.02
Proteus mirabilis (MTCC 1429)	11.2 ±0.15
Burkholderiaglumae (MTCC 8496)	10.2 ±0.11
Moraxella bovis (MTCC 1775)	10.4 ±0.23

MTCC: Microbial Type Culture Collection

moderate radial growth was observed at pH 5 and 6.0. At pH 8.0, there was no fungal growth (Fig. 4). Earlier reports revealed that many kinds of fungi grow at acidic pH optima (Ramesh *et al.* 2014, Kim *et al.* 2005, Shu and Lung 2004). The radial growth of *Xylaria* sp. Strain SR2 was investigated at various temperatures (10°C to 35°C) in PDA plates at pH 5.5. The radial growth of the mycelium was measured after 7 days of incubation. The influences of temperature on the radial growth of fungal isolates were presented in the Fig 5. The maximum radial growth of 3.5 cm was observed at 30°C in *Xylaria* sp. Strain SR2. Here the influence of the days are directly proportional the radial growth of fungal isolates. The results indicated that the optimum tem-

Table 2: Antibacterial activities of ethyl acetate extract of *Xylaria*sp. SR2 against drug resistant *Staphylococcus aureus* strains

	Zone of inhibition (mm)			
S. aureus	Penicillin (10 uits/mL)	Methicillin (10 µg/mL)	Vancomycin (30 µg/mL)	Ethyl acetate extract of *Xylaria*sp. SR2
Strain 1	12.8 ± 0.15 (R)	10.8 ± 0.14 (R)	16.5 ± 0.08 (S)	18.3±0.21
Strain 2	12.8 ± 0.14 (R)	11.2 ± 0.31 R)	15.4 ± 0.28 S)	19.6±0.11
Strain 3	14.2 ± 0.28 (R)	12.1 ± 0.16 (R)	16.0 ± 0.22 (S)	15.2±0.12
Strain 4	11.5 ± 0.35 (R)	10.2 ± 0.31 (R)	17.2 ± 0.14 (S)	16.1±0.11
Strain 5	10.8 ± 0.14 (R)	8.9 ± 0.22 (R)	18.5 ± 0.28 (S)	20.4±0.06
Strain 6	10.3 ± 0.15 (R)	9.5 ± 0.35 (R)	12.4 ± 0.35 (R)	23.0±0.13
Strain 7	13.8 ± 0.16 (R)	15.5 ± 0.07 (S)	16.9 ± 0.14 (S)	18.4±0.11
Strain 8	10.5 ± 0.23 (R)	8.8 ± 0.14 (R)	13.5 ± 0.21 (R)	17.7±0.13
Strain 9	14.0 ± 0.21 (R)	12.2 ± 0.10 (R)	14.8 ± 0.22 (R)	18.4±0.12
Strain 10	14.9 ± 0.1 (R)	8.9 ± 0.18 (R)	17.4 ± 0.35 (S)	16.6±0.32

R: Resistant, **S:** Sensitive

inequilateral to broad, dark brown, unicellular, smooth, germ slit conspicuous, straight and running full length of spore(Fig 3b). The morphological characters of this fungal isolates was identical with *X. angulosa* (AB274814) growing in soil (Rogers *et al*. 1987). On the other hand, many species of *Xylaria* are actively growing in decaying wood of angiosperms and are known to be saprobic (Rogers *et al*. 2005). The genus *Xylaria* was classified in the family xylariaceae of the class of Pyrenomycetes. The Xylariaceae is a large and relatively well known family, which is the representative of Ascomycete in most countries.

The effect of initial pH on the radial growth of *Xylaria* isolates was studied at pH ranging from 4.0 to 8.0 in PDA medium for 7 d. the maximum radial growths of 4.8. cm was obtained at pH 5.5, whereas, the perature for maximal radial growth was 30°C. This observation was comparable to the growth of many other kinds of fungal species in various regions (Bae *et al.* 2000, Kim *et al*. 2003). Similarly, this present results seem to be consistent with other reports in which the optimal temperature for fungal growth was 20ºC to 30ºC (Lee *et al*., 2004, Lai *et al.* 2014).

To study the effect of culture media on biomass production, three different culture media were used. The fungal isolates were grown in MEB, PDYEB, PDB and basal growth medium of pH 5.5 at 30°C for a period of 10, 20, 30 and 40 day. The maximum biomass production of 1.8 g/l was observed on PDB over a period of 40 d at pH 5.5 & temperatures 30°C (Fig. 6). Likewise, Arivudainambi*et al.* (2011) reported that the maximum biom-

ass of 1.9 g/l produced by the endophytic fungus Collectotrichum gloeos porioides VN1 was studied in PDB at pH 5.5 for a period 28 d. On the contrary, Bilay *et al*. (2000) reported that Ganoderma lucidum had a slow growth rate in PDB medium, whereas there was a significant fungal biomass production in basal medium. Similarly, the maximum fungal biomass production of *Ganoderma* species was observed in basal medium (Roberts 2004).

For the preparation of extract, the endophytic fungus *Xylaria* sp. strain SR2 was grown in PDB at pH

Fig. 1 : *Araucaria heterophylla*.

5.5 for 35 d. After that, the culture filtrate was extracted with various solvents such as hexane, ethyl acetate and methanol. The mycelium was dried and extracted with various solvents. The mycelium extract was also combined with the culture filtrate extract. After extraction the solvents were concentrated by using rotary vacuum evaporator. The dried extracts were dissolved in DMSO and stored at 4^{0}C for studying antibacterial activity.

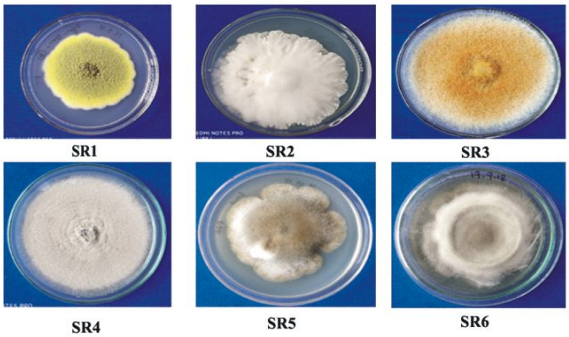

Fig. 2 : Colony morphology of endophytic fungal isolates from *Araucaria heterophylla*

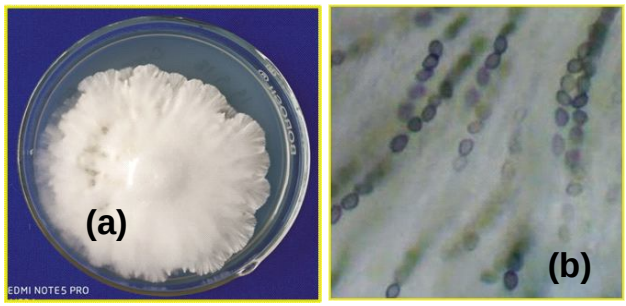

Fig. 3 :(a) Cultural morphology on PDA plate (a) and ascospores (b) of *Xylaria* sp. strain SR2

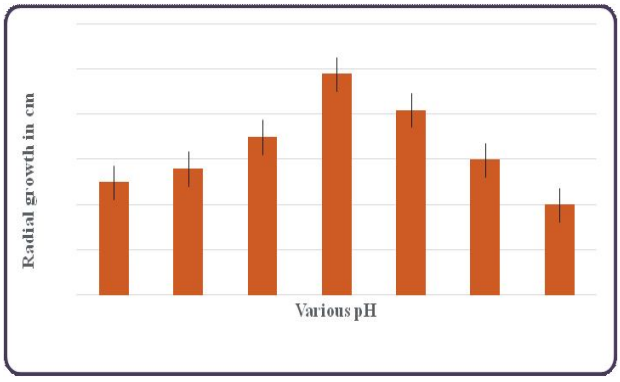

Fig. 4 : Effect of various pH (ranging from4 to 8) on radial growth *Xylaria* sp.Strain SR2

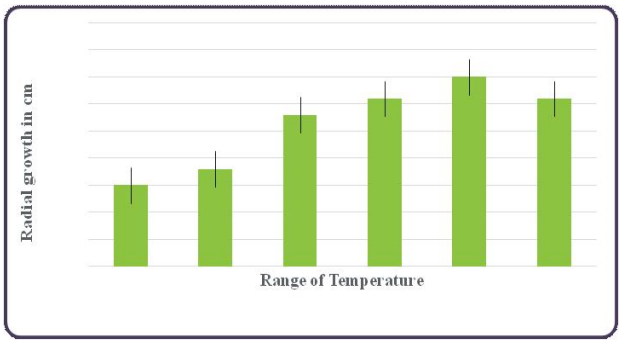

Fig. 5 : Effect of various temperatures (ranging from 10^{0}C to 35^{0}C) radial growth *Xylaria* sp. strain SR2

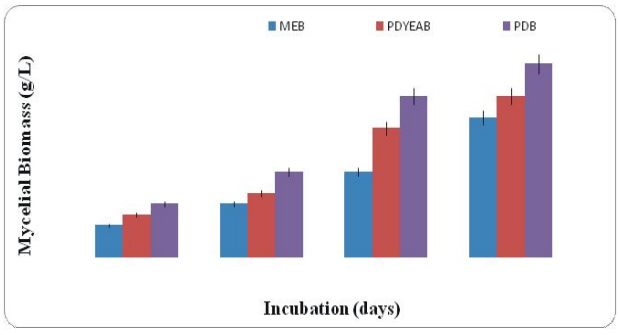

Fig. 6 : Effect of various culture media on fungal biomass production of *Xylaria* sp. strain SR2

The accelerating haunt for new antimicrobial drugs to provide assistance in medical community to combat drug resistance microorganism, the appearance of life-threatening bacteria, and the tremendous increase in the incidence of fungal infections in the world's population. So far, many pharmaceutical, novel and bioactive natural products with unique health enhancing properties have been identified from fungal species and distributed worldwide (Cairney 1999). The antimicrobial activity of the extract of *Xylaria* sp. strain SR2 against five bacterial strains was investigated by agar well method. The results showed that ethyl acetate extract showed significant antibacterial activity against all the tested bacterial pathogens. The Ethyl acetate extract of *Xylaria* sp. strain SR2 produced the maximum inhibition zone of 11.3 mm against *Pseudomonas syrirgae*, 11.2 mm against *Proteus mirabilis*, 10.4 mm against Moraxella bovis and 10.2 mm against *Burkholderia glumae* (Table 1). However, the methanol extract had low inhibitory effect against all the tested organisms. The observed inhibitory effect is in line with the report of Phongpaichit *et al.* (2006) who worked on the antimicrobial metabolites in *Xylaria* sp. Similarly, Ramesh et al. (2012, 2012a, 2015) reported that the crude extract of *Xylaria*spp. exhibited significant antimicrobial activities against human bacterial and fungal pathogens as well as drug resistant bacterial pathogens. Moreover, recently Devaraju *et al.* (2020) studied the antimicrobial potential of culture broth of *Xylaria* sp. FPL-25 exhibited broad-spectrum antimicrobial activity against human bacterial and fungal pathogens by bioactivity guided fractionation using bioautography and chromatography.

Further the ethyl acetate extract was only taken for antibacterial activity against multidrug resistant bacterial strains based on the results of earlier preliminary antimicrobial screening. Among the drug resistant bacteria, methicillin resistant *S. aureus* (MRSA) gained much attention in the last couple of decades (Ramesh *et al*. 2015). Hence, the emergence of the MRSA strain possesses a substantial threat to public health. So we have chosen this multidrug resistant *S. aureus* for further antibacterial bioactivity and obtained from Bose Clinical Laboratory and X-ray (Madurai, Tamilnadu, India). The identification and antibiotic resistant profile of clinical strains of *S. aureus* (1–10) were done and reported earlier (Phongpaichit *et al*. 2006, Ramesh *et al*. 2015, Arivudainambi *et al.* 2011). The ethyl acetate extract showed significant inhibition zone of 23.0 mm and 20.4 mm against *S. aureus* strains 6 and 5, respectively (Table 2). Whereas the moderate inhibition zone of 19.6 mm and 18.4 mm were observed against *S. aureus* strain 2 and 9, respectively. This kind of observation was lined with the activity of macro fungal extract of *Xylaria* spp. (Ramesh *et al*. 2012, 2015). Similarly, Elias Luciana *et al*. (2018) isolated endophytic fungal isolates of *Xylaria* spp. from leaves the of guarana plant. They also reported that the fungistatic activity of that endophytic *Xylaria* spp. against the plant pathogen *Colletotrichum gloeosporioides*. Moreover, Mohd Adnan (2018) also reported that the antibacterial activity of *Xylaria* sp. aginst *Staphylococcus aureus*.

In conclusion, this is the study which is revealing the medicinal importance of endophytic *Xylaria* sp. strain SR2 in terms of antibacterial activity against drug resistant *S. aures*. Therefore, the extract of *Xylaria* sp. strain SR2 may have potential use in the future as an effective antimicrobial solution, in the form of the treatment for *S. aureus* associated infectious diseases. Further results are required, the active chemical nature studies to prove the authenticity of its bacterial activity and its potential use.

ACKNOWLEDGEMENTS

The authors thank the Managing Board of Vivekananda College as well as Virudhunagar Hindu Nadar's Senthikumara Nadar College, Virudhunagar-626 001, Tamilnadu, India, for providing research facilities.

REFERENCES

Acar, J.F., Goldstein, F.W. 1996. Antibiotics in laboratory medicine. In: Lorian V, editor. Disk susceptibility test. 4th ed. Baltimore: Williams & Wilkins, Pa, USA: p.1-51

Ainsworth, G.C., Sparrow, F.K., Sussman, A.S. 1973. The fungi, an advanced treatise. Edition Vol. IV (A), a taxonomic review with keys ascomycetes and fungi imperfecti. Academic Cambridge

Arivudainambi, U.S.E.,Durai Anand, T., Shanmugaiah, V. Karunakaran, C., Rajendran, A. 2011. Novel bioactive metabolites producing endophytic fungus *Colletotrichumgloeosporioides* against multidrug resistant *Staphylococcus aureus*. *FEMS Immunol. Med. Microbiol.* **61**:340-345.

Arnold, A.E. Maynard, Z., Gilbert, G.S.2001. Fungal endophytes in dicotyledonous neotropical Trees: patterns of abundance and diversity. *Mycol. Res*. **105**:1502-1507.

Bae, J.T. Sinha, J. Park, J.P. Song, C.H.,Yun, J.W. 2000. Optimization of submerged culture conditions for exo–biopolymer production by *Paecilimyces japonica*. *J. Microbiol. Biotechnol*. **10**:482-487.

Bhatia, R., Narain, J.P. 2010. The growing challenge of antimicrobial resistance in the South-East Asia Region—are we losing the battle? *Ind. J. Med. Res*.**135**:482-486.

Bilay, V.T. Solomko, E.F., Buchalo, A.S. 2000. Growth of edible and medicinal mushrooms on commercial agar media. In Proc of the 15th Int Congress Sci Cultivation of Edible Fungi. Vol. 1&2 (Griensven LJLDV, ed), AA Balkema, Rotterdam, Maastricht, 757-761

Boonphong, S. Kittakoop, P. Isaka, M. Pittayakhajonwut, D. Tanticharoen, M.,Hebtaranonth, Y. 2001, Multiplolides A and B, new antifungal 10-membered lactones from *Xylaria multiplex*. *J. Nat. Prod*. **64**:965-967.

DevarajuRakshith. DoddahosuruMahadevappaGurudatt. Yashavantha Rao HC. Chandra Mohana, N. Nuthan, B,R, Ramesha, K.P. 2020. Bioactivity-guided isolation of antimicrobial metabolite from *Xylaria* sp. *Process Biochemistry.* **92**:378-385.

Elias Luciana, M. Diana Fortkamp. Sérgio B Sartori. Marília C Ferreira. Luiz H Gomes. João L Azevedo. 2018 The potential of compounds isolated from *Xylaria* spp. as antifungal agents against anthracnose. *Braz. J. Microbiol*. **49**:840-847.

Essers, L., Radebold, K. 1980. Rapid and reliable identi?cation of *Staphylococcus aureus* by a latex agglutination test. *J. Clin. Microbiol*.**12**:641- 643.

Ganley, R.J. Brunsfed, S.J., Newcombe, G. 2004. A community of unknown, endophytic fungi in western white pine. *Proc. Nat1. Acad. Sci. USA***01**:10107-10112.

Healy, P.C. Hocking, A. Tran-Dinh, N. Pitt, J.I. Shivas, R.G. Mitchell, J.K. 2004. Xanthones from a microfungus of the genus *Xylaria*. *Phytochem*. **65**: 2373-2378.

Jalgaonwala, R. Mohite, B., Mahajan, R.A. 2011. Review: natural products from plant associated endophytic fungi. *J. Microbiol.Biotechnol. Res*.**1**: 21-32.

Jung Chun Chang. George Hsiao. Ruo-Kai Lin. Yueh-HsiungKuo. Yu-Min Ju. and Tzong-HueiLeeet. 2017, Bioactive Constituents from the Termite Nest-Derived Medicinal Fungus *Xylaria nigripes*. *J. Nat. Prod.* **80:**38-44.

Kim, H.O. Lim, J.M. Joo, J.H. Kim, S.W. Hwang, H.J. Choi J.W. 2005. Optimization of submerged culture condition for the production of mycelial biomass and exopolysaccharides by *Agrocybecylindracea*. *Bioresour. Technol*. **96**:1175-1182.

Kim, SW. Hwang, H.J. Xu, C.P. Sung, J.M. Choi, J.W., Yun, J.W. 2003. Optimization of submerged culture process for the production of mycelial biomass and exo-polysaccharides by *Cordyceps militaris* C738. *J. Appl. Microbiol*. **94**:120-126.

Kumaresan, V., Suryanarayanan, T.S. 2002. Endophytic assemblages in young, mature and senescent leaves of *Rhizophora apiculata*: evidence for the role of endophytes in mangrove litter degradation. *Fungal Divers*. **9**: 1-91.

Lai, W.H. Salleh, S.M. Wong, E.K. Daud, F. Othman, A.M., Saleh, N.M. 2014. Optimization of submerged culture conditions for the production of mycelial biomass and exopolysaccharides from *Lignosus rhinoceros*. *Sains Malays*. **43**: 73-80.

Lee, B.C. Bae, J.T. Pyo, H.B. Choe, T.B. Kim, S.W, Hwang H.J. 2004. Submerged fermentation conditions for the production of mycelial biomass and exopolysaccharides by the edible basidiomycete *Grifolafrondosa*. *Enzy.Microb. Technol*. **35**:369-76.

Lin, Y. Wu, X. Feng, S. Jiang, G. Luo, J. Zhou, S.L. 2001. Five unique compounds: Xyloketals from mangrove fungus *Xylaria*sp. from the South China Sea Coast. *J. Org. Chem*.**66**: 6252-6256.

Lucero, M.E. Barrow, J.R. Osuna, P. Reyes, I.,Duke, S.E.2008. Enhancing native grass productivity by cocultivating with endophyte-laden calli. *Rangel Ecol. Manag*. **61**:124–130

Mohali, S. Burgess, T.I., Wingfield, M.J. 2005. Diversity and host association of the tropical tree endophyte *Lasiodiplodiatheobromae* revealed using simple sequence repeat markers. *Forest Pathol.* **35**:385-396.

Mohd Adnan. Mitesh Patel. Mandadi Narsimha Reddy.EyadAlsh, amma. 2018, Formulation, evaluation and bioactive potential of *Xylariaprimorskensis* terpenoid nanoparticles from its major compound xylaranic acid. *Sci. Rep*.**8**:1740-1752.

Morens, D.M. Folkers, G.K., Fauci, A.S. 2004. The challenge of emerging and reemerging infectious diseases. *Nature*. **430**: 242-249.

Petrini, O. 1986. Taxonomy of endophytic fungi of aerial planttissues. In: *Microbiology of the Phyllosphere*(Fokkema NJ &vanden Heuvel J, eds), pp. 175–187. Cambridge University Press, New York

Phongpaichit, S. Rungjindamai, N. Rukachaisirikul, V., Sakayaroj, J. 2006. Antimicrobial activity in cultures of endophytic fungi isolated from *Garcinia* species. *FEMS Immunol. Med. Microbiol.* **48**: 367-372.

Pourshadi, M., Klaas, J. 1984. Evaluation of latex agglutination and microtube coagulase tests for detection of *Staphylococcus aureus*. *Diagn.Micr. Infect. Dis*. **2**:287-91

Ramesh, V. Arivudainambi, U.S.E. Thalavaipandian, A. Karunakaran, C., Rajendran A. 2012. Antibacterial activity of wild *Xylaria* sp. Strain R005 (Ascomycetes) against multidrug resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Int. J. Med. Mushr*. **14**: 47-53.

Ramesh, V. Karunakaran, C., Rajendran, A. 2012a.Evaluation of synergistic and antibacterial activity of *Xylariacurta* against drug resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Mycology*. **3**: 252-257.

Ramesh, V. Karunakaran, C., Rajendran, A. 2014. Optimization of submerged culture conditions for mycelial biomass production with enhanced antibacterial activity of the medicinal macro fungus *Xylaria* sp. Strain R006 against drug resistant bacterial pathogens. *Curr. Res. Environ. Appl. Mycol*. **4**: 88-98.

Ramesh, V. Santosh, K. Durai Anand, T. Shanmugaiah, V. Kotamraju, S. Karunakaran, C. 2015. Novel Bioactive wild medicinal mushroom - *Xylaria* sp. R006 (Ascomycetes) against multidrug resistant human bacterial pathogens and human cancer cell lines. *Int J Med Mushr*. **17**:915-1005.

Roberts, L.M. 2004. Australian *Ganoderma* identification, growth and antibacterial properties. PhD thesis. Swinburne University of Technology. Australia

Rogers, J.D, Ju, Y.M., Lehmann, J. 2005. Some *Xylaria* species on termite nests. *Mycologia*. **97**: 914-923.

Rong Chen. Jian-Wei Tang. Xing-Ren Li. Miao Liu. Wen-Ping Ding. Yuan-Fei Zhou 2018. Secondary Metabolites from the Endophytic Fungus *Xylaria* sp. hg1009. *Nat. Prod.Bioprospect*. **8**:121-129

Shu, C.H., Lung, M.Y. 2004. Effect of pH on the production and molecular weight distribution of exopolysaccharide by *Antrodiacamphorata* in batch cultures. *Process Biochem*. **39**:931-937.

Strobel, G. Daisy, B. Castillo, U., Harper, J. 2004. Natural products from endophytic microorganisms. *J. Nat. Prod*. **67**: 257-268.

Subbulakshmi, G.K.,Thalavaipandian, A., Ramesh, V., Bagyalakshmi, Rajendran, A. 2012, Bioactive endophytic fungal isolates of *Biota orientalis* (L) Endl., *Pinus excelsa* Wall. and *Thuja occidentalis* L. *Int. J. Life Sci.***4**: 9-15.

Suryanarayanan, T.S., Venkatachalam, A.,Thirunavukkarasu, N. ,Ravishankar, J.P., Doble, M.. Geetha, V. 2010. Internal mycobiota of marine macroalgae from the Tamilnadu coast: distribution, diversity and biotechnological potential. *Botanica Marina*.**53**:57-468

Suryanarayanan, T.S.,Wittlinger, S.K., Faeth, S.H. 2005. Endophytic fungi associated with cacti in Arizona. *Mycol. Res*.**109**:635-639

Tauhidur R., Nurunnabi, F. S., Dilara, I. S., Lutfun, N., Md. H. Sohrab,SarkerS. D.2020. Antimicrobial activity of endophytic fungi isolated from the mangrove plant *Sonneratia apetala* (Buch. Ham) from the Sundarbans mangrove forest. *Adv.Trad.Med.* **20**: 419-425.

Tejesvi, M.V. ,Ruotsalainen, A.L. ,Markkola, A.M., Pirttilä, A.M. 2010. Root endophytes along a primary succession gradient in northern Finland. *Fungal Divers*. **41**:125-134.

Zuccaro, A., Schoch, C.L.,Spatafora, J.W.,Kohlmeyer, J., Draeger, S.,Mitchell, J. 2008. Detection and identification of fungi associated with the brown seaweed *Fucus serratus*. *Appl. Environ.Microb*. **74**:931-941.