WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 6, 1315-1329 Research Article ISSN 2278 – 4357

ANTI-MICROBIAL AND ANTI-CANCER ACTIVITY OF *SETOSPHAERIA MONOCERAS*, AN ENDOPHYTIC FUNGUS ASSOCIATED WITH TROPICAL MANGROVE PLANT

Apurva Sawant*, Bernard F. Rodrigues, Yogita Sardessai[1]

Department of Botany, Goa University, Taleigao Plateau, Goa 403 206.

[1]Goa College of Pharmacy, Panjim, Goa 403 001.

Article Received on
09 April 2018,

Revised on 30 April 2018,
Accepted on 20 May 2018,

DOI: 10.20959/wjpps20186-11819

***Corresponding Author**
Apurva Sawant
Department of Botany, Goa University, Taleigao Plateau, Goa 403 206.

ABSTRACT

Endophytes are a promising resource of pharmaceutically and agriculturally valuable secondary metabolites. Screening and isolation of endophytic fungi associated with the predominant mangrove plant *Rhizophora apiculata* from Chorao Island, Goa was undertaken. Eleven fungal strains were isolated, out of which one dominant strain was selected for phenotypic and genotypic identification. Based on 18S DNA gene sequencing, the fungus revealed 100% homology with *Setosphaeria monoceras*, a teleomorph of *Exserohilum monoceras*. GC-MS analysis of crude compounds extracted with ethyl acetate revealed the presence of sulfurous acid, 2-ethylhexyl isohexyl ester, heptacosane, dodecyl pentyl ester, eicosane, 2-methyl and 2-bromotetradecane. Ethyl acetate extracted crude metabolites of this isolate was screened for antimicrobial activity against seven common human pathogenic bacteria *viz.*, *Shigella boydii*, *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enterica*. However, no antifungal activity was noticed against *Aspergillus brasiliensis* and *Candida albicans*. Diameters of inhibition zones measured in the plate assay were significantly high (20-50mm). MIC values were between 3.12 to 6.25 µg/mL. The extract revealed strong bactericidal effect against the test bacterial pathogens. The aliquots on testing against human lung cancer cell line A-549 showed significant anti-cancer activity compared with the activity exhibited by the standard drug cisplatin. This study supports the belief of conferring the potential attributes of endophytic fungi associated to tropical estuarine as *Setosphaeria monoceras* showed impressive bioactivities which may be exploited for novel bioactive compounds. This is the

first antimicrobial study to be undertaken on this relatively novel estuarine endophytic fungal strain from the rich and diverse Chorao mangrove habitat.

KEYWORDS endophytic fungi, fungal extract, GC-MS, cell line.

INTRODUCTION

Endophytes are microorganisms which are associated with broad varieties of plant species, and can invade interior plant tissues without causing any negative effect to the parent body.[1] The term "endophytes" was coined by German botanist Heinrich Friedrich Link in 1809 to define occurrence of bacteria and fungi inside plant tissue.[2] The occurrence of endophytes has been reported for over 400 million years ago.[1] It is estimated that each and every plant species is being colonized by at least two endophytes.[3][4] Over one hundred thousand fungal species have been reported and their numbers keep increasing.[5]

The symbiotic relationship between endophytes and host species has benefited plants in terms of growth promotion and adapting to changing environmental conditions. While residing and reproducing inside the healthy tissue in intimate mutualistic manner, presumable gene recombination or the precursor molecule interaction with the host indicates enhanced biosynthetic capabilities in the endophytes which is why over 80% of the endophyte exhibit positive activity in testing for antimicrobial and biological control.[6][7] Endophytes are chemical synthesizers within plants as they are supposed to be used by host plant species for chemical defense against various stresses.[8] Ecological niches like mangroves deserve exploration as they are bound to have novel microbial species and biotypes particularly endophytes which are relatively less explored.[9]

Due to changing environmental conditions, plants develop adaptive mechanisms in order to acclimatize. Hence it becomes crucial for endophytes to continuously modify its secondary metabolites, in order to penetrate the host plant through its chemotaxic process. To overcome such responses, endophytes secrete certain detoxifying enzymes like cellulase, lactase, xylanase which matches with the plants enzymes to attain colonization. After penetration in the parent host, it is assumed that endophytes undergo three different life stages: neutralism /quiescent stage (at this stage endophyte undergo a latent stage which may be even for lifetime), mutualistic stage (at this stage the host and the endophyte share mutual benefits from each other without any harm), and the antagonistic stage.[10] Such adaptive mechanism has helped endophytes to produce therapeutically important bioactive compounds, exclusive

to those of host plants. It has been stated that the isolation and identification of endophytic mycobiota is crucial to the ethnobotanical profile of the plant, since the medicinal properties of a plant can be due to the endophytes harboured within. This was proved in case of the anticancer drug taxol by isolating taxol producing endophyte *Taxomyces andreanae.*[11]

In the view of the global drug resistance problem, the need to discover novel drug sources particularly antibiotics cannot be over-emphasized.[12] Fungal endophytes have so far yielded alkaloids, terpenoids, quinines, isocoumarin derivatives, flavanoids, phenols, peptides and phenolic acids. They are a prolific source of novel antibiotics, anticancer, antiviral, antioxidant, insecticide, anti-diabetic and immunosuppressant compounds.[13] Pharmacological repertoire of endophytic fungi comprises of diverse bioactive compounds such as antimycotic steroid 22-triene-3b-ol, anticancer cajanol, podophyllotoxin and kaempferol, anti-inflammatory ergo flavin, antioxidant lectin, insecticidal heptelidic acid, immune suppressive sydoxanthone A, B and cytotoxic radicicol.[14]

Isolation and characterization of endophytic fungi and further detailed studies on active secondary metabolites of therapeutic importance might provide an insight in the discoveries of novel groups of chemical compounds across temperate and tropical forests.[15,16]

Goa is a small tropical state at the South-Western part of the India, which houses a very rich and diverse mangrove population. Literature survey indicates that there are very few studies on endophytic mangrove fungi at this location. Therefore, this study was undertaken at Chorao Mangrove Island which houses most of the dominant mangrove species and is a relatively unexplored ecological niche.

MATERIALS AND METHOD

Plant sample collection

Leaves of *R. apiculata* were collected from Mangrove estuarine ecosystem situated at Chorao Island, Goa (Fig. 1). The plant material was brought to the laboratory in zip-lock bags and was further processed within five hours of sample collection.

Fig. 1. *Rhizophora apiculata* plant.

Isolation of Endophytic fungi

Healthy leaves were selected and cut into smaller pieces and surface sterilized using 75% ethanol for 1 minute, 4% NaOCl for 3 minutes, and later rinsed in sterile distilled water.[17] The sample was then inoculated on solidified agar plates amended with antibiotics streptomycin sulphate (0.4 mg/mL) and ampicillin (0.4 mg/mL). Plates were incubated at 25°C and observed for growth after three to four days. The pure fungal cultures were transferred periodically to Potato Dextrose Agar (PDA) plates and stored at 4^0C on slants using the same media.[18]

Identification of Endophytic Fungi

The sporulated fungal isolates were initially identified through microscopic examination using lacto-phenol cotton blue stain. The slides were then photographed by Olympus microscope; after which fungal isolates were identified based on colony characteristics,[19] and confirmed by molecular identification at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerela, India. Fungal isolates were identified based on 18S DNA isolation using NucleoSpin® Plant II Kit (Macherey-Nagel). The fungal DNA was further analyzed by sequencing at the ITS (ITS-1F 5′- TCCGTAGGTGAACCTGCGG -3′ and ITS-4R 5′- TCCTCCGCTTATTGATATGC-3′) and LSU region (LROR 5′-ACCCGCTGAACTTAAGC-3′ and 5′- TCCTGAGGGAAACTTCG-3′) of rDNA. The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems) and sequence alignment and edited by using Geneious Pro v5.1.[20] Later the DNA was subjected to sequencing and it was compared with already existing DNA sequences available at NCBI GenBank (http://www. ncbi.nlm.nih.gov.blast), and phylogenetic tree was constructed using the Maximum Likelihood method to identify the respective fungi.

Extraction of the bioactive metabolites

The endophytic fungal isolate was inoculated in 500 ml Erlenmeyers flasks containing 300 ml PD broth (Potato Dextrose Broth) at pH 6.5. The inoculated flask containing 300 ml PD broth and fungal isolate incubated under stationary conditions at 25^0C for 15 days. The incubated flasks containing the fungal colonies were filtered using three layered Whatman No.1 filter paper. To the filtrate, equal amounts of ethyl acetate were added and mixed well, until the two immiscible layers appear. Upper layer of ethyl acetate fraction containing the fungal metabolites was extracted using sterile pipette under aseptic conditions. Ethyl acetate along with the extracted compounds was kept on a rotary shaker until the solvent evaporated to obtain a concentrated extract. The resultant powder was mixed with DMSO (1mg/ml DMSO) and kept at 4^0C.[21]

Screening for antibacterial activity (Agar cup diffusion assay)

The crude extract was treated for antibacterial activity and was screened against seven human pathogens *viz*., *Klebsiella pneumonia* ATCC 700603, *Escherichia coli* ATCC 8739, *Shigella boydii* ATCC 12030, *Staphylococcus aureus* ATCC 6538, *Candida albicans* 3147 ATCC 10231, *Aspergillus brasiliensis* WLRI 034 and *Salmonella enterica serovar typhimurium* ATCC 14028 (procured from Department of Microbiology, Goa Medical College, Bambolim, Goa), using agar well diffusion method. Mueller-Hinton Agar (MHA) was used as a nutrient supplement for the test pathogens. The young bacterial suspension corresponding to 0.5 McFarland standards was mixed with 20 mL Mueller-Hinton Agar and poured in sterile Petri plates[22]. The solidified plates were bored with 12mm diameter wells. The wells were later filled with 100, 90, 80, 70, 60, 50, 40, 30, 20, 10µl of the crude extract (Table 3). The plates were kept for pre-diffusion at 4^0C for 20 minutes followed by 24 hour incubation at 37^0C. Appropriate positive and negative controls were maintained throughout the study. Each set of experiments was carried out in triplicate.

Determination of Minimum Inhibitory Concentration (MIC)

Inoculum preparation involved two fold serial dilutions using Mueller-Hinton broth (MHB) as medium. Eighteen hours old bacterial suspensions were adjusted to 0.5 McFarland standards in sterile saline to achieve concentrations of 10^7CFU/mL.[23] The MIC determination involved serial dilutions of varying concentrations, followed by incubation period of 24 hours at 37^0C. The lowest concentration at which growth failed to occur was the MIC of the culture. Minimum bactericidal concentration (MBC) determination was done by

culturing aliquots from the MIC tubes on Mueller-Hinton agar medium and incubation for 48 hour at 37^0C. MIC of the crude ethyl acetate extract was determined by serial dilution tube method by measuring the optical density at 600nm using Elico Colorimeter. The lowest concentration at which no bacterial colony was noticed in the plates was considered as MBC of the culture.

Phytochemical screening of the fungal metabolites

The crude extract was tested for the presence of secondary metabolites such as alkaloids, saponins, terpenoids, flavonoids, steroids, phenols, and tannins using standard methods [24].

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis

The GC-MS analysis of fungal crude extract was performed at IIT Bombay. Two µl of each sample was employed for analysis using Elegant Hp 7880 with column of 30 meter length and 0.32 thicknesses. Helium gas was used as carrier gas at constant flow rate of 1ml/ minute. Injector temperature was set at 100^oC. The oven temperature were programmed from 50^oC to 280^oC at 10^oC/ minute to 200^oC then 100^oC/ 3 minutes to 250^oC ending with a 5 minutes isothermal at 280^oC. The sample was injected in split mode as 50:1.[25]

Anticancer activity screening by using A-549 lung cancer cell line

Screening of anticancer activity was performed by using colorimetric assay i.e. by measuring the metabolically active cells (viable cells) that measures the reduction of yellow 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. A-549 human lung cancer cell line was seeded in a 96-well flat-bottom micro plate at a density of approximately 5×10^3cells/well and incubated overnight at 37^0C, 95% humidity and 5% CO_2.[26] Different concentrations of 1000, 500, 250, 125, 62.5, 31.25 µg/mL of the sample were used for the study following incubation period of 24 hours. The cells in well were washed twice with phosphate buffer solution, 20 µL of the MTT staining solution (5mg/ml in phosphate buffer solution) and was incubated at 37^0C. After 4h, 100 µL of di-methyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals, and absorbance was recorded at 570 nm using a micro plate reader.

RESULTS

Isolation and identification of endophytic fungi

Unique symbiotic behaviour was observed in mangrove endophytic fungi that colonize different plant parts. Traditionally endophytes in plant tissues can be detected by two basic

techniques. The first one is direct observation where the fungal structures within the fungal endophyes are observed under light and electron microscope, particularly biotropic fungi which cannot be cultured on standard growth medium. However, this method has taxonomic limitations due to lack of spores. In contrast, cultivation dependent techniques where only internal fungi are isolated and cultivated on nutrient plates after a series of processes to remove all surface organisms are more popular.[27] Some endophytes resist the sporulation within the plant and may remain inactive throughout life. There are different media constituents which are used by researchers across the world such as standard PDA (Potato Dextrose Agar), MEA (malt extract agar) and minimal media with plant tissue or extract.[28] In the present study, PDA medium (Potato dextrose Agar) was used to cultivate the endophytic fungi from mangrove ecosystem by using three step sterilization protocols. A total of eleven endophytic fungi were obtained, out of which one dominant fungal isolate was selected for detailed study. The endophytic fungi were isolated from the leaves of *R. apiculata*, a predominant mangrove species at Chorao Island, Goa. The isolated endophytic fungus (Fig. 2a) was purified and stored at 4^0C followed by study of morphological characteristics (Fig. 2b). Morphotaxonomic treatment coupled with molecular characterization using ITS and LSU primers, confirmed the identification of endophytic isolate M.En01 (mangrove endophyte) as *Setosphaeria monoceras* (Fig. 2 c).

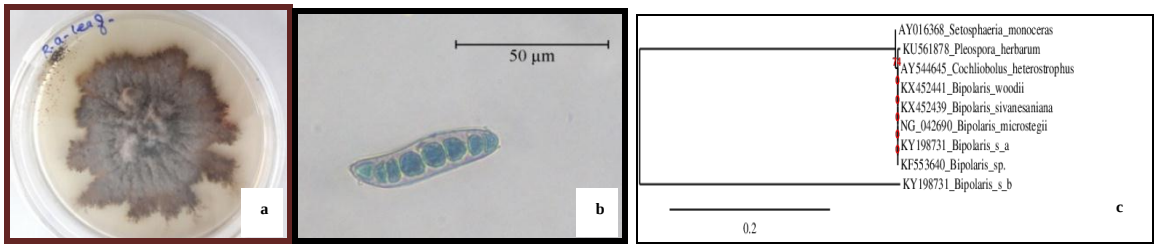

Fig. 2a: The culture of *S. monoceras*; Fig.2b: Single spore of *S. monoceras*; Fig. 2c: Phylogenetic tree.

Mass culture, preliminary phytochemicals and GC-MS analysis

Endophytes are currently being viewed as an excellent source of secondary metabolites and bioactive antimicrobial natural products. They have the ability to produce diverse classes of phytochemicals which have multiple medicinal and pharmaceutical applications; as is evident from worldwide reports on plant endophytes [10][15][29][2]. To study the antimicrobial activity, the fungal colonies were mass cultured, fermented and resultant extract with ethyl acetate as solvent system (Fig. 3a and 3b). This was subjected to preliminary phytochemical analysis which revealed the presence of alkaloids (Table 1). This extract on further analysis revealed the presence of specific groups of secondary metabolites through GC-MS analysis (Table 2).

The identification of the metabolites was done by GC chromatogram and compared the mass value assigned at each mass spectrum of individual secondary metabolite (Fig. 4). The comparison of the mass value with the existing data library confirmed the compounds in the crude fungal extract as sulfurous acid, 2-ethylhexyl isohexyl ester, heptacosane, sulfurous acid, dodecyl pentyl ester, eicosane, 2-methyl and 2-bromotetradecane (Table 2).

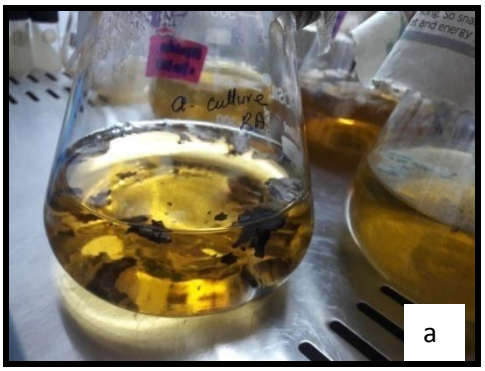

Fig. 3a: Liquid culture of *S. monoceras*;

Fig. 3b: Fermented culture of *S. monoceras* (after 15 days of incubation).

Table 1: Phyto-chemical screening of isolated fungal extract.

Phyto-chemical tests	Results
Alkaloids	+
Flavanoids	-
Phenols	-
Terpenoids	-
Steroids	-
Saponins	-

Legend: + (present) ; - (absent)

Table 2: Compounds identified in ethyl acetate fungal crude extract.

Sr. No.	RT	Name of compound	Category of the Compound	Peak area %	Molecular weight	Molecular formula	Known Bioactive function
1	3.12	-		12.84	-	-	
2	3.71	-		4.04	-	-	
3	4.05	-		1.16	-	-	
4	4.22	-		1.26	-	-	
5	26.70	Sulfurous acid, 2-ethylhexyl isohexyl ester		3.01	278	$C_{14}H_{30}O_3S$	Antidiabetic
6	27.93	Heptacosane	Alkaloid	7.35	380	$C_{27}H_{56}$	Antibacterial
7	29.07	Heptacosane	Alkaloid	11.28	380	$C_{27}H_{56}$	Antibacterial
8	30.13	Sulfurous acid, Dodecyl pentyl ester		15.11	320	$C_{17}H_{36}O_3S$	Antibacterial
9	331.20	Eicosane, 2-methyl	Alkaloid	15.23	296	$C_{21}H_{44}$	Antibacterial
10	32.46	2-Bromotetradecane		14.78	276	$C_{14}H_{29}Br$	Antimicrobial
11	33.90	-		13.9	-	-	

Legend: RT=Retention Time; (-) = Compound not detected.

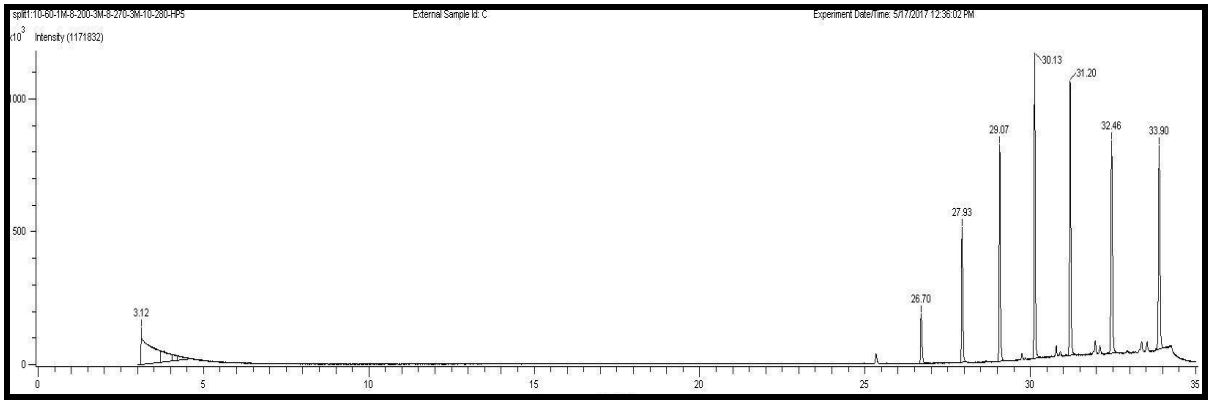

Fig. 4: GC-MS Chromatogram of ethyl acetate extract of *Setosphaeria monoceras*.

Antimicrobial activity of endophytic fungus against human pathogens

The crude ethyl acetate extract of *S. monoceras* exhibits significant antibacterial activity by agar diffusion method (Table 3). On testing, extract inhibits all five bacterial pathogens tested in the study showing fairly large zones of inhibition (Fig.5.a.). The fungal crude extract showed active results even when the crude extract at 10 µL concentration was applied against the human pathogenic bacteria, indicating the efficacy of the crude extract at low concentration against the target pathogens. However, when tested against mold (*Aspergillus brasiliensis*) and yeast (*Candida albicans*) extract did not show inhibitory effect, indicating the lack of antifungal activity. DMSO (Dimethyl sulphoxide) used as negative control showed no zone of inhibition (Fig. 5.a.)

Table 3: Antibacterial activity of *Setosphaeria monoceras* isolated from leaf of *R. apiculata*.

Isolate code	Amount of extract	Average zone of inhibition (mm)				
		K. pneumonia ATCC 700603	*S. aureus* ATCC 6538	*S. Boydii* ATCC 12030	*S. antrica* ATCC 14028	*E. coli* ATCC 8739
M.En.C	100µl	43	44	50	45	43
	90µl	42	42	47	41	43
	80µl	39	42	45	40	34
	70µl	37	41	43	35	32
	60µl	36	35	42	34	32
	40µl	35	33	41	31	30
	30µl	35	34	40	30	30
	20µl	34	32	40	29	30
	10µl	30	30	39	21	29
Control –ve	DMSO	-	-	-	-	-
Control +ve	Streptomycin	39	42	45	46	43

Legend: + =Positive activity; - = No activity; M.En.C: Mangrove Endophyte C

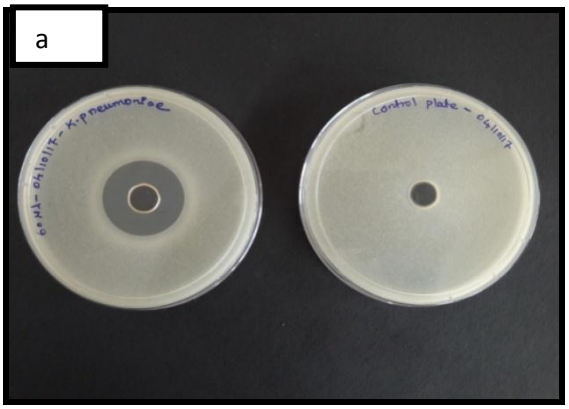

Fig. 5a: Antimicrobial activity of *S. monoceras* against *K. pneumonia*.

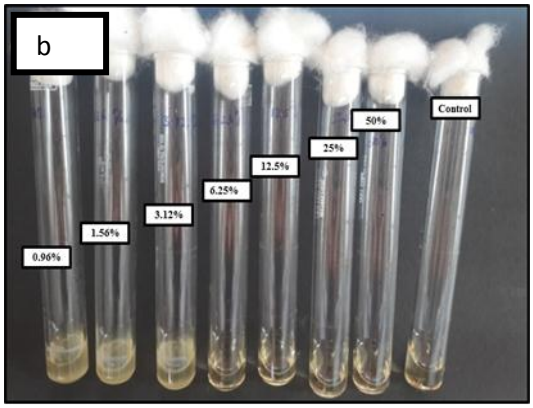

Fig. 5b: MIC of *S. monoceras* culture against *K. pneumonia*.

Minimum inhibitory concentration (MIC) of the endophytic extract against pathogenic bacteria

The ethyl acetate extract of endophytic fungus of *S. monoceras* was tested for MIC. The results revealed significant decrease in growth of pathogenic bacterial organisms in test tubes as observed by decreasing absorbance values at 6.25µg/mL (Fig. 5 b and Fig. 6). The results indicated the MIC value to be between 3.1- 6.25 µg/mL. Further the aliquots from the MIC tubes were sub cultured on Muller Hinton agar medium in order to study MBC of the crude extract. The plates having aliquots from 3.12 to 6.25µg/mL showed no bacterial colony which indicates the strong bactericidal property of the extract.

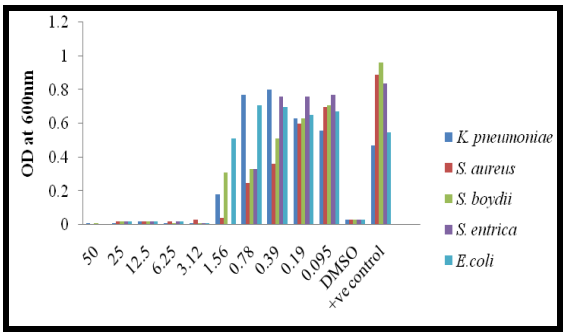

Fig. 6: *S. monoceras* extract against bacterial pathogens using tube dilution method.

Anti-Cancer activity of the Extract by using A-549 lung cancer cell line: Ethyl acetate extract showed significant anti-cancer activity on testing with lung cancer cell line A-549 (Fig. 7 a and Fig. 7 b). The MTT assay was carried out by using different fungal extract concentrations (1000, 500, 250, 125, 62.5, 31.5 µg/mL) which revealed the potency of the extract to kill the cancer cells at varying concentrations (Fig. 7 c). The research provides a basis to further purify, separate and characterize extracts secondary metabolites so that the extract could be used as a effective anticancer drug on lung cancer.

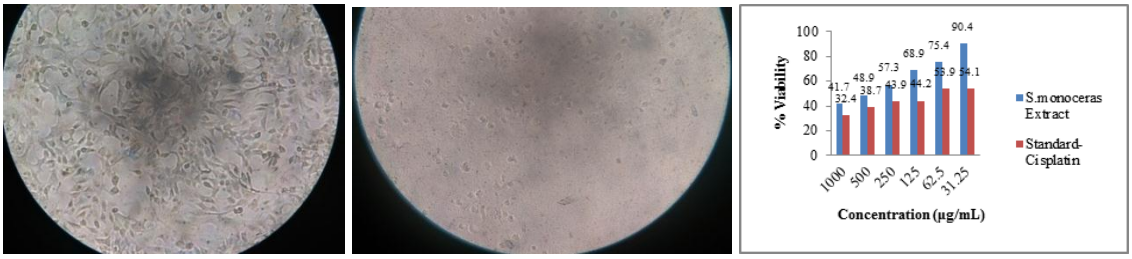

Fig. 7a: A549 untreated cell line; Fig. 7b: A549 treated cell line with *S. monoceras* extract; Fig. 7c: Cell viability of *S. monoceras* extract against A549 cell line.

DISCUSSION

Endophytic fungi are versatile organisms that synthesize active secondary metabolites in order to acclimatize to the host plant and in the process yield bioactive components having pharmaceutical applications. Out of the total medicinal products which are available in the market, 30% are of fungal origin.[15]

Hence, understanding the relationship between host and endophyte will help ideal production of drugs, through manipulation of the standard growth parameters. Due to the evolutionary co-existence between the endophytes and their host, the possible outcome could be mutualism, antagonism and neutralism. Environmental factors of the ecological niche and genetic background of the host plant exert selection pressure on the endophyte. The beneficial interactions include enhancement of growth of host medicinal plants, increase in resistance of host plant to biotic and abiotic stress, and accumulation of secondary metabolites originally produced by medicinal plants. Understanding distribution and population structure pattern of endophytes may provide vital clues for effectively detecting and obtaining bioactive compounds by the host plant in conjunction with its endophytes under selective environmental conditions.[10] The strategy might help to add a particular group of endophytic fungi in host plant and enhance the target medicinal properties of the plant to meet the growing needs of pharmaceutical market. The resultant product will be of natural origin with least side-effects.[30]

Likewise, in case of the endophytic fungus *S. monoceras*, a dominant fungal endophyte obtained from the mangrove host plant *R. apiculata* showed strong activity against human pathogens displaying the impressive inhibition zones that ranged from 20-50 mm. This indicates the synthesis of unique and potent bioactive compounds within the endophytes. The tested human pathogenic bacterial growth was inhibited by the crude fungal extract at a low concentrations (1.56 to 3.12 µg/mL) as shown by MIC studies which also indicated the

bactericidal activity on the tested bacterial pathogenic strains at higher concentrations (3.12 to 6.25 µg/mL). Thereby indicating the antimicrobial and bactericidal potential of the endophyte. The crude fungal extract revealed the presence of strong anti-cancer activity against the human cancer cell line A-549 at the different concentrations indicating the percent viability (Fig. 7) of the tested cancer cell line was significantly impacted by the fungal extract.

Studies carried out in past few years have reported hundreds of products *viz.*, alkaloids, terpenoids, flavonoids, steroids. from endophytic fungi; which are potential antibiotic, anticancer and biological control agents.[29][3] The crude ethyl acetate extract of endophytic fungus *S. monoceras* indicates the presence of different bioactive secondary metabolites such as sulfurous acid, 2-ethylhexyl isohexyl ester, heptacosane, dodecyl pentyl ester, eicosane 2-methyl, and 2-bromotetradecane. These groups of secondary metabolites are reported to have antibacterial, antifungal, anti-diabetic properties.[14][25] Due to amid demand of new and potential bioactive molecules, some recent studies showed that many cryptic secondary metabolites from fungi either may be produced or the production of known one can be enhanced multifold through the treatment of epigenetic modifiers like DNMT (DNA methyl transeferase inhibitors), HDAc (histone deacetylase inhibitors) or both in combination.[31][32]

CONCLUSION

The present study provides a scope for further detailed investigation on the bioactive secondary metabolite yielding potential of this relatively novel estuarine, endophytic fungal strain. *Setosphaeria monoceras* exhibits strong antibacterial activity and anticancer activity. This extract is definitely a promising candidate for further molecular and pharmacological investigations. The ethno-botanical profile of plants like *R. apiculata* which grow in challenging and specialized ecological niches like the estuarine ecosystem is surely enhanced by the symbiotic associations with its endophytes like *S. monoceras.*

ACKNOWLEDGEMENTS

We acknowledge IIT Bombay for GCMS analysis and Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerela, for molecular analysis. We are grateful to Goa Medical College for microbial cultures and the Principal of Goa College of Pharmacy, Panaji for the work facility. Assistance of Prof. V. S. Nadkarni, Chemistry Department, Goa University and Dr. Puja Gawas, Carmel College, Nuvem Goa is gratefully acknowledged.

CONFLICT OF INTEREST

There is no conflict of interest to submit present data.

REFERENCES

1. Xiang, Sun., Liang-Dong, Guo. (2012). Endophytic fungal diversity: review of traditional and molecular techniques. *An International Journal of Fungal Biology*, 3(1): 65-76.
2. Pablo, R.H., Leonard, S., Van, O., Gabriele, B., Anna, M. P., Stephane, C., Andrea, C., Matthias, D., Angela, S. (2015). The Hidden World within Plants: Ecological and Evolutionary Considerations for Defining Functioning of Microbial Endophytes. *Microbiology and Molecular Biology Reviews*, 79(15): 293-320.
3. Wang, K.W., Wang, S.W., W.u B, Wei JG. (2014). Bioctive natural compounds from the mangrove endophytic fungi. *Mini Review in Medical Chemistry*, 14(4): 370-91.
4. Guo, L.D., Huang, G.R. and Wang, Y. (2008). Seasonal and tissue age influences of endophytic fungi from *Pinus tabulaeformis* (Pinaceae) in Dongling Mountain, Beijing. *Journal of Integrative Plant Biology*, 50: 997-1003.
5. Clay, K. and Holah, J. (1999). Fungal endophyte symbiosis and plant diversity in successional fields. *Science*, 285: 1742-1744.
6. Li, Y., Song, Y.C., Liu, J.Y., Ma, Y.M., Tan, R.X. (2005). Anti-*Helicobacter pylori* substances from endophytic fungal culture. *World Journal of Microbiology & Biotechnology*, 21: 553–558.
7. Schulz, B., Boyle, C., Draeger, S., Rommert, A.K., Krohn, K. (2002). Endophytic fungi: a source of novel biologically active secondary metabolites. *Mycological Research*, 106: 996-1004.
8. Wang, Y.T., Lo, H.S., Wang, P.H. (2008). Endophytic fungi from *Taxus mairei* in Taiwan: First report of *Colletotrichum gloeosporioides* as an endophyte of *Taxus mairei*. *Botanical Studies*, 49(1): 39–43.
9. Elsa, L.J., Bhima, B.V.A. (2012). Fungi from mangrove plants: their antimicrobial and anticancer potentials. *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(3): 139-142.
10. Min, Jia., Ling, Chen., Hai-Liang, Xin., Cheng-Jian, Zheng., Khalid, Rahman., Ting, Han., Lu-Ping, Qin. (2016). A friendly relationship between endophytic fungi and medicinal plants: A systematic review. *Frontiers in microbiology*, 7(906): 1-14.

11. Kusari, S., Pandey, S.P., Spiteller, M. (2013). Untapped mutualistic paradigms linking host plant and endophytic fungal production of similar bioactive secondary metabolites. *Phytochemistry*, 91: 81-87.
12. Costelloe, C., Metcalfe, C., Lovering, A., Mant, D. and Hary, A.D. (2010). Effect of antibiotic prescribing in primary care and antimicrobial resistance in individual patients. *Systemic Review and Meta-analysis*, 340: 2096.
13. Mayank, G., Vijay, C.V., Gautam M.K., Gopal, N. (2015). Isolation and evaluation of antimicrobial activities of endophytic fungal extract from *Mallotus phillippinensis* Muell. *Applied Microbiology Open Access*, 1(1): 1-5.
14. Sharma, D., Pramanik, A., Agarwal, K.P. (2016). Evaluation of bioactive secondary metabolites from endophytic fungus *Pestalotiopsis neglecta* BAB-5510 isolated from leaves of *Cupressus torulosa* D.Don. *3 Biotech*, 6(210): 1-14.
15. Mishra, Y., Singh, A., Batra, A., Sharma, M.M. (2016). Understanding the biodiversity and biological applications of endophytic fungi: A review. *Journal of Microbial and Biochemical Technology*, S8(004): 1-11.
16. Verma, V.C., Kharwar, R.N., Strobel, G.A. (2009). Chemical and functional diversity of natural products from plant associated endophytic fungi. *Natural Products Communications*, 4 (11): 1511-1532.
17. Suryanarayanan, T.S., Kumaresan, V., Johnson, J.A. (1998). Foliar fungal endophytes from two species of the mangrove *Rhizophora*. *Microbiology*, 44: 1003–1006.
18. Bills, G.F. (1996). Isolation and analysis of endophytic fungal communities from woody plants. In: Endophytic fungi in grasses and woody plants (eds. S.C. Redlin and L.M. Carris). APS Press, St. Paul, Minnesota, USA, 31-65.
19. Barnett, H.L. and Hunter, B.B. (1998). Illustrated genera of Imperfect Fungi. APS Press, St. Paul, Minnesota, USA.
20. Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Heled, J., Kearse, M., Moir, R., Stones-Havas, S., Sturrock, S., Thierer, T. and Wilson, A. (2010). Geneious v5.1, Available from http://www.geneious.com.
21. Radji, M., Sumiati, A., Rachmayani, R., Elya, B. (2011). Isolation of fungal endophytes from *Garcinia mangostana* and their antibacterial activity. *African Journal of Biotechnology*, 1: 103-107.
22. Sardessai, Y., Pai Angle, G., Joshi, A., Carvalho, S., Bhobe, M. (2014). Antimicrobial activity of metabolic extract of the rhizomes of *Costus igneus*. *Journal of Pharmaceutical Chemical and Biological Sciences*, 2(3): 176-185.

23. Khan, R., Islam, B., Akram, M., Shakil, S., Ahmad, A., Ali, S.M., Siddiqui, M., Khan, A.U. (2009). Antimicrobial activity of five herbal extracts against Multi Drug Resistant (MDR) strains of bacteria and fungus of clinical origin. *Molecules*, 14: 586-597.
24. Bhardwaj, A., Sharma, D., Jadon, N., Agarwal, P.K. (2015). Antimicrobial and phytochemical screening of endophytic fungi isolated from spikes of *Pinus roxburgii*. *Archieves of Clinical Microbiology*, 6(3): 1-9.
25. Devi, N.N. and Singh, M.S. (2013). GC-MS Analysis of metabolites from endophytic fungus *Colletotrichum gloeosporioides* isolated from *Phlogacanthus thyrsiflorus* Nees. *International Journal of Pharmaceutical Sciences Review and Research*, 23(2): 392–395.
26. Shah, T., Joshi, K., Mishra, S., Otiv, S., Vijay K. (2016). Molecular and cellular effects of vitamin B12 on human trophoblast cells in presence of excessive folate. *Biomedicine and Pharmacotherapy*, 84: 526-524.
27. Singh, D.K., Sharma, V.K., Kumar, J., Mishra, A., Verma, S.K., Sieber, T.N., Kharwar, R.N. (2017). Diversity of endophytic mycobiota of tropical tree *Tectona grandis* Linn.f.: Spatiotemporal and tissue type effects. *Scientific Reports*, 7(1): 3745.
28. Fovo, J.D., Dostaler, D. and Bernier, L. (2017). Influence of culture media and temperature on growth and sporulation of *Lasiodiplodia theobromae*, *Pestalotiopsis microspora* and *Fusarium oxysporum* Isolated from *Ricinodendron heudelotii* in Cameroon. *International Journal of Current Microbiology and Applied Sciences*, 6(6): 3098-3112.
29. Kharwar, R.N., Mishra, A., Gond, S.K., Stierle, A., Stierle, D. (2011). Anticancer compounds derived from fungal endophytes: their importance and future challenges. *Natural Product Reports*, 28: 1208-1228.
30. Firakova, S., Sturdikova, M. and Muckova, M. (2007). Bioactive secondary metabolites produced by microorganisms associated with plants. *Biologia*, 62: 251-257.
31. Sharma, V.K., Kumar, J., Singh, D.K., Mishra, A., Gond, S.K., Verma, S.K., Kumar, A., Singh, G., Kharwar, R.N. (2017). Induction of cryptic metabolite production through epigenetic tailoring in *Colletotrichum gloeosporioides* isolated from *Syzygium cumini*. In Diversity and Benefits of Microorganisms from the Tropics (eds. JL Azevedo and MC Quecine), 169-184.
32. Beau, J., Mahid, N., Burda, W.N., Harrington, L., Shaw, L.N., Mutka, T. (2012). Epigenetic tailoring for the production of anti-infective cytosporones from the marine fungus *Leucostoma persoonii*. *Marine Drug*, 10: 762-774.