International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 4 | OCT-DEC | 2018 | 291-310
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

ASSESSMENT OF ANTIOXIDANT, ANTI-LIPID PEROXIDATION, DNA DAMAGE PROTECTION AND ANTICANCER ACTIVITIES OF MARINE MANGROVE FUNGI

J.S. Kushveer, Rashmi Mishra, B. Devadatha, Mohd Imran K Khan and V.V. Sarma*

Fungal Biotechnology Lab, Department of Biotechnology, School of Life Sciences, Pondicherry University, Kalapet, Pondicherry-605014, India

***Corresponding Author Email: veerkush1992@gmail.com**

ABSTRACT

Marine fungi are still a less explored group of microorganisms for novel medicinal properties that may have medicinal and pharmacological applications. In the present study, 55 marine fungi were screened for in-vitro antioxidant potential. Three marine fungi were shortlisted based on their higher antioxidant activity and were subjected to further studies including estimation of total phenolic & flavonoid content, qualitative screening of secondary metabolite groups and anticancer activity. Potential marine fungal isolates were identified and characterized based on their morphology and molecular phylogenetic analyses. The antioxidative properties of crude extracts derived from marine fungi were evaluated employing various in-vitro methods viz., DPPH, ABTS, nitric oxide radical scavenging assay, reducing power assay, anti-lipid peroxidation assay, oxidative DNA damage protection and cell viability assay. Among the three fungi, Marasmiellus sp. (PUK-64) showed highest antioxidant activity followed by Hypoxylon sp. (PUK-4) and Scedosporium aurantiacum (PUK-60). Marasmiellus sp. not only showed highest radical scavenging activity but also had highest phenolic and flavonoid contents. This study has demonstrated a notable antioxidant potential in the selected marine fungi in addition to appreciable anti-cancerous activity.

KEY WORDS

Cytotoxicity; free radicals; phylogenetic analysis; polyphenol content; secondary metabolites

1. INTRODUCTION

Marine fungi are an affluent source of chemically unique as well as structurally diverse skeletons of bioactive secondary metabolites. Several natural products derived from marine sources have been reported (Blunt et al. 2013). In the past one decade several publications on marine organisms as a source of natural products have appeared reporting different biological activities including antioxidant, antifungal, anticancer and anti-inflammatory (Kijjoa & Sawangwong 2004). According to Deshmukh et al. (2018), in the past few decades, more than half potentially approved drugs came from marine organisms, reflecting the therapeutic importance of marine based secondary metabolites. The diverse and unique chemical molecules found in marine fungi reflect on their different facets including competitive ability, nutritional choices, communications, defense mechanisms and survival instincts to harsh environments. Due to the harsh habitats in which they thrive, they have developed distinct secondary metabolic pathways that are different from terrestrial fungi (Hasan et al. 2015). But the relevance of secondary metabolites from marine fungi is not well documented and remain underrated in drug discovery pipeline even though they express interesting levels of metabolite profiles (Imhoff 2016).

During metabolic processes, reactive oxygen species (ROS) are generated. These chemically reactive molecules increase tolerance to and profitably affect the metabolic and cellular processes, and immunity. However, beyond threshold concentrations, they may create adverse pathological conditions inside the living organisms (Sugiharto et al. 2016). Often termed as molecular sharks, these unstable free radicals are known to damage cell membrane molecules, mitochondria and DNA which cause diseases such as Alzheimer's disease, Parkinsons' disease, heart dysfunction, cancer, aging, etc. (Finkel & Holbrook 2000). Antioxidants act by scavenging these harmful radical species, thereby, protecting the human body from various clinical conditions associated with radical damage and are proclaimed to be involved in alleviating vascular cardiac diseases, asthma, inflammation, arthritis, neurodegeneration, diabetes and Parkinson's disease (Arora & Chandra 2011).

Antioxidants play a vital role in food preservation by preventing oxidation processes and are used in many dietary supplements, nutraceuticals and functional food ingredients (Shahidi & Zhong 2015). Commercially available antioxidants which are exploited in food industries such as butylated hydroxytoluene (BHT), tert-Butylhydroquinone (TBHQ) and butylated hydroxyanisole (BHA) have been found to be cancer causing; consequently, ban has been imposed on their use (Abdullah et al. 2012). Despite the advent of effective chemotherapeutic drugs, cancer still causes high mortality and the drugs used often have high side effects. Antioxidants play a major role in inhibiting the free radical damage produced as a result of chemical reactions. Due to the complex nature of secondary metabolites, a single method to evaluate the antioxidant activity is not sufficient.

Various studies proved that plants and microbes are a rich source of antioxidants. However, very few studies have been conducted on antioxidant activities of marine fungi. These few reports suggest that marine fungi could be a good source of natural antioxidants that could aid in the blockage of distinct oxidative stresses (Hong et al. 2015). In this paper we report the results of a qualitative screening of different groups of secondary metabolites, quantification of phenolic and flavonoid content in the crude extracts and tests on antioxidant potential shown by marine fungi. Further, the ability of crude extracts in the prevention of DNA damage and cytotoxicity against human cancer cell lines are also reported.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Ascorbic acid, 2, 2' - azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), potassium persulphate, disodium hydrogen phosphate, sodium dihydrogen phosphate, trichloroacetic acid, potassium ferricyanide, sodium carbonate, gallic acid, Folin-Ciocalteu, sodium nitrite, aluminium chloride, ferric chloride, 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) and Dulbecco's Modified Eagle's medium (DMEM) were supplied by Himedia, Mumbai, India. Quercetin; 2, 2'diphenyl-1-picrylhydrazyl (DPPH); butylated hydroxytoluene (BHT) were procured from Sigma Aldrich, India. All the chemicals and reagents used in this study were of analytical grade.

Cell lines: Cancer cell lines; HeLa and MCF7 were procured form NCCS, Pune (INDIA)

2.2. Sample collection, isolation and morphological studies

Decaying wood samples of different mangrove plants and salt marsh plants such as *Avicennia marina, Aegiceras corniculatum, Excoecaria agallocha, Rhziophora apiculata* and *Suaeda monoica* were collected from mangroves of Muthupet (10.4 °N, 79.5 °E), Kaveri River Delta, Tiruvarur district, Tamil Nadu, India. The samples were processed and micro-morphological studies were carried out as described in Devadatha et al. (Devadatha et al. 2017). For Basidiomycetes, a small tissue of basidioma was inoculated on sea water agar and malt extract seawater agar (MEA). Single spore isolates were obtained and axenic cultures were preserved as described in Devadatha et al. (Devadatha et al. 2017).

2.3. Molecular Identification

Total genomic DNA from the fresh mycelium of each culture was extracted using Thermo Scientific Gene JET Plant Genomic DNA Purification kit. The ITS (Internal Transcribed Spacer) regions were amplified using the primer pairs ITS1 and ITS 4 and amplification reactions were carried out according to White et al. (White et al. 1990). The amplification reactions were performed, and sequencing was carried out at Macrogen Inc. (Seoul, Korea).

2.4. Phylogenetic analyses

The obtained ITS sequences were subjected to BLAST search engine of NCBI to retrieve closer taxa for phylogenetic analyses. Three separate data sets were prepared for the phylogenetic analyses. Reference taxa datasets of ITS sequences were aligned using an online MAFFT server (http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley 2013) and the portions which were unaligned were further aligned manually with the help of BioEdit sequence alignment editor. Phylogenetic trees were generated using maximum-likelihood and maximum parsimony. Maximum-likelihood (ML) analysis was performed at CIPRES Science Gateway platform (http://www.phylo.org/portal2/, (Miller et al. 2010) using the RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al. 2008; Stamatakis 2014). Parsimony analysis was carried out in PAUP (Phylogenetic analysis Using Parsimony).4.0b10 with the heuristic search option 1000 random- addition sequences with a tree bisection and reconnection (TBR) branch swapping algorithm (Swofford 2003). The Phylograms are visualized in FigTree 1.4.0 program (Rambaut & Drummond 2017) and reorganized in Microsoft power point (2007) and Adobe Illustrator® CS5 (15.0.0, Adobe®, San Jose, CA).

2.5. Fermentation and extraction

Fresh axenic cultures of marine fungi were grown on MEA and incubated for 7-14 days. Actively growing fungal mycelial agar plugs were then transferred to Erlenmeyer flasks (2L) containing malt extract broth base medium prepared by using an equal amount of sterilized sea and distilled water (dH_2O) followed by an incubation at room temperature under shaking condition at 120 rpm. Fungal secondary metabolites were harvested after 25 days of fermentation. The secondary metabolites from the broth were extracted by mixing the broth with twice the volume of ethyl acetate and concentrated using Buchi rotary evaporator at 37 °C. The concentrated crude extracts of individual fungi were used to perform different experiments.

2.6. Determination of polyphenol content & secondary metabolites screening

2.6.1. Total phenolic content (TPC)

Total phenolic content (TPC) of marine fungal crude (MFC) extract was quantified by Folin-Ciocalteu colorimetric assay as reported by Kim et al. (Kim et al. 2003). Briefly, 0.1 mL of MFC (1mg/mL) was mixed with 0.1 mL Folin-reagent followed by incubation for 5 min. Then added 1mL of Na_2CO_3 (7.5%) and diluted with dH_2O. Reaction mixture was incubated for 1.5 hrs in dark and absorbance was recorded at 750 nm. Gallic acid was used as a standard phenolic. The TPC was calculated and expressed in terms of µg of Gallic acid equivalents (GAE) per mL of crude sample quantified on the basis of standard curve of gallic acid.

2.6.2. Total flavonoid content (TFC)

Total flavonoid content (TFC) of the MFC extracts was performed according to Saeed et al. (Saeed et al. 2012) with few modifications. Reaction mixture contained 0.3 mL of MFC, 3.4 mL of 30% methanol, 150 µL of $NaNO_2$ (0.5M) and 150 µL of $AlCl_3.6H_2O$ (300 mM), added sequentially. Later, 1 mL of NaOH (1M) was added after 5 min of incubation and absorbance was recorded at 415 nm. Quercetin (QE) was used as a standard and the results were expressed as µg of quercetin equivalents (QE) per mL of crude sample quantified on the basis of standard curve of quercetin.

2.6.3. Screening of secondary metabolites in fungal crude extracts

The marine fungal crude (MFC) extracts were screened for the presence or absence of tannins, terpenoids, steroids, flavonoids, cardiac glycosides, alkaloids, anthraquinones and phlobatannins. The concentration of MFC extracts used for the analyses was 1mg/mL (Gul et al. 2017).

2.7. Antioxidant activities

2.7.1. DPPH radical scavenging activity

Antioxidant potential of the MFC extracts was determined by DPPH radical scavenging assay as described by Shen et al. (Shen et al. 2010) with few modifications. An aliquot of 1.9 mL of freshly prepared DPPH solution (100µM) was mixed with 0.1 mL of test sample over a concentration range (1-50 µg/mL). Reaction mixture was incubated for 20 min in dark and absorbance was recorded at 517 nm with Butylated Hydroxytoluene (BHT) and ascorbic acid (AA) as standards. The percentage (%) inhibition of DPPH radicals was calculated using the following equation:

$$\% \text{ DPPH inhibition} = [(A_c - A_t)/ (A_c)] * 100$$

Where,

A_c = Absorbance of control

A_t = Absorbance of test sample

2.7.2. ABTS radical scavenging activity

The ABTS radical scavenging activity was performed according to the method outlined by Zheleva-Dimitrova et al. (Zheleva-Dimitrova et al. 2010) with few

modifications. A mixture of ABTS solution (7mM) and potassium persulphate (2.45mM) solution was kept for 16-19 h in dark to produce a dark coloured solution comprising ABTS radical cations. Test samples (0.1mL) were introduced at various concentration range (0.5-25 µg/mL) with 1.9 mL of ABTS solution. Then absorbance was read at 745 nm after 15 min. AA and BHT were used as standards. Percentage scavenging inhibition was calculated as mentioned above.

2.7.3. Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity of MFC was estimated following Boora et al. (Boora et al. 2014) with few modifications. An aliquot of 250 µL of 10 mM sodium nitroprusside (SNP) in phosphate buffered saline was added to 500 µL of different concentration range of test sample (5-200 µg/mL) and incubated at ambient temperature for 180 min. Finally, an equal volume of freshly prepared Griess reagent (1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid) was mixed. Absorbance was read at 546 nm. AA was used as a standard and % inhibition was calculated as mentioned above.

2.7.4. Reducing power activity

The reducing power of test samples was tested by following the method outlined by Rahman et al. (Rahman et al. 2015) with some modifications. Reaction mixture contained 0.500 mL of MFC extracts at various concentration range (1.25-25 µg/mL), mixed with 0.500 mL of phosphate buffer (200mM) and 0.500 mL of 1 % potassium ferricyanide solution. The reaction mixtures were incubated for 20 min at 50 °C for reaction completion. Then 0.500 mL of 10 % trichloroacetic acid (TCA) solution was mixed into the test tubes. Later on 0.500 mL from above reaction mixture was added to 0.500 mL of dH_2O and 0.100 mL of 0.1 % ferric chloride ($FeCl_3$) solution. The absorbance of the solution was read at 700 nm after 5 min. AA was used as a positive control.

2.7.5. Lipid peroxidation (LPO) inhibition activity

Lipid peroxidation or thiobarbituric acid reactive substances (TBARS) activity of MFC extracts was performed with slight modifications (Ohkawa et al. 1979). Briefly, 1 mL of 10% fresh chick liver, homogenised in 10% KCl, was prepared. The reaction was initiated by adding 0.1 mL of $FeSO_4$ (25 µM) in 1 mL of chick liver homogenate, 0.1 mL of MCF extract (10-200 µg/mL) & 0.1 mL of KH_2PO_4 (10mM) and volume was made up to 2 mL with dH_2O, followed by incubation at ambient temperature for 1 h. The reaction was stopped by adding 1 mL of 5% TCA. Finally, 1mL TBA (0.375 % in 0.5 N HCL) was added to reaction mixture & tubes were boiled for 20 min in water bath. After cooling, reaction mixture was centrifuged at 3500 rpm for 10 min. The supernatant was collected, and absorbance was read at 532 nm. QE was used as a positive control. Lipid peroxidation inhibition was calculated as mentioned above.

IC_{50} values for DPPH, ABTS, NO and LPO radical scavenging were calculated using linear regression plot of range of concentration of MFCs against mean percentage scavenging of respective antioxidant activity.

2.7.6. Oxidative DNA damage protection activity

Oxidative DNA damage protection activity was measured on pUC19 plasmid DNA as per the protocol of Akhter et al. (Akhter et al. 2013) with minor changes. An amount of 250 ng of the total plasmid DNA was incubated with Fenton's reagent (30 mM H_2O_2, 50 mM ascorbic acid and 80 mM $FeCl_3$) in the presence and absence of MFC extracts with a concentration range (5 - 50 µg/mL) and the reaction mixture was made up to 20 µL with de-ionised dH_2O and 0.25% bromophenol loading dye (prepared in 50% of glycerol). The reaction mixture was incubated at 37°C for 30 min. After gel electrophoresis and ethidium bromide staining the gel was visualized for DNA bands under UV in Gel Doc system (Biorad).

2.8. Anticancer activity

2.8.1. Culturing of cell lines

Human Cancer cell lines HeLa and MCF7 were grown in DMEM containing 10% FBS (Fetal bovine serum) and L-Glutamine-Penicillin-Streptomycin solution at 37 °C in 5% CO_2 condition.

2.8.2. MTT cell viability assay

To perform MTT assay, 96 well tissue culture plate was seeded with 10^3 number of cells. After 16 hours of seeding, when cells were attached to the culture plate successfully, the cells were treated with the filter sterile MFC dissolved in 50% DMSO at different concentrations (5-500 µg/mL). DMSO (50%) was used as a control. About 72 hours of post MFC treatment, the MTT assay was performed as described in Singh et al (Singh et al. 2016). All the experiments were performed in triplicates.

2.9. Statistical analyses

All experiments were performed thrice, and values were represented as mean with their standard derivations. Data were analyzed using the Microsoft excel and graph pad prism version 5.04. Statistical analyses were performed with one-way ANOVA, and *p* values < 0.05 were considered as significant. The Pearson's correlation coefficient (r) and coefficient of determination (R^2) between TPC-TFC and antioxidant activities were estimated to determine the relationships among them.

3. RESULTS

3.1. Morphological description of marine fungi

***Marasmiellus* sp. (PUK-64)**

Basidiomatal Caps 0.5-1cm, across, convex to flattened, white; Stem 2–6 x 1–2 mm; white, membranous. Flesh white, thin. Gills and Basidiospores were not observed and did not develop after incubation (Figure 1A).

***Culture characteristics*:** Colonies on malt extract agar slow growing, reaching 30-45 mm diameter after 25 days of incubation at room temperature, hyaline, reverse light yellow, flexuous, cottony and circular.

Notes: Morphological data and the phylogenetic analysis results show that this taxon belong to the genus *Marasmiellus* (Figure 1A, Figure 3).

***Hypoxylon* sp. (PUK-4)**

Stromata 310–770×400–655 µm diam, glomerate to hemisphaerical. *Asci* 65–160 ×7–13 µm, 8-spored, unitunicate, cylindrical, pedicellate, with discoidal apical ring bluing in Lugol's reagent, 1–2.5 × 2–3.5 µm. *Ascospores* 9–15 × 4–7 µm, uniseriate, one-celled, ellipsoid-in equilateral, with narrowly rounded ends, brown to dark-brown, with a straight germ slit (Figure 1B).

Notes: Hypoxylon is characterized by stromata that are superficial, pulvinate or effuse to erumpent, hemispherical to spherical, with or without KOH-extractable pigments, Asci 8-spored, unitunicate, cylindrical, pedicellate, persistent, with a J+ apical ring bluing or not bluing in Melzer's reagent, discoid. Ascospores ellipsoid or short fusoid, inequilateral, slightly inequilateral or nearly equilateral, with acute, narrowly to broadly rounded ends, pale brown to dark brown, or blackish-brown, with straight, sigmoid, or spiral germ slit (Wendt et al. 2018). Based on the above morphological resemblances, the present taxon was found to belong to *Hypoxlon*. Further, molecular analysis also confirms the placement under *Hypoxlon.*

***Scedosporium aurantiacum* (PUK-60)**

Synnemata solitary to gregarious, erect, dark brown, 170–1110 µm tall with a cylindrical stipe, 10–25 µm wide dark gray, smooth-walled, terminate into a slimy head of conidia. *Conidiophores* synnematous, solitary, branched, often reduced to conidiogenous cells, growing laterally bearing single verticil of conidiogenous cell. Three types of conidia: (i) smooth-walled, obovoid, or sub cylindrical (ii) cylindrical or claviform with a wide truncate base; (iii) sessile, solitary, lateral, brown, smooth, and thick-walled, mostly obovoid (Figure 1C).

Notes: *Scedosporium aurantiacum* is characterized by *Graphium*-like synnemata with large, erect bundles of hyphae terminating in a dense aggregate of conidiogenous cells. Conidia develop from a short extension of the conidiogenous cells with annellidic development (Gueho 1991; Lackner et al. 2014; Ramirez-Garcia et al. 2018). The present taxon shares similar morphological characteristics with *Scedosporium aurantiacum* and this is the first report of *Scedosporium* sp. from marine habitat. *Scedosporium* species are reported from various substrates such as soil, water, sewage , clinical samples and found as an opportunistic pathogen causing Scedosporosis in humans (De Hoog et al. 2000; Ramirez-Garcia et al. 2018). The above three species did not fit into any existing species of their respective genera. Further taxonomic work is in progress and they would be communicated separately as new species.

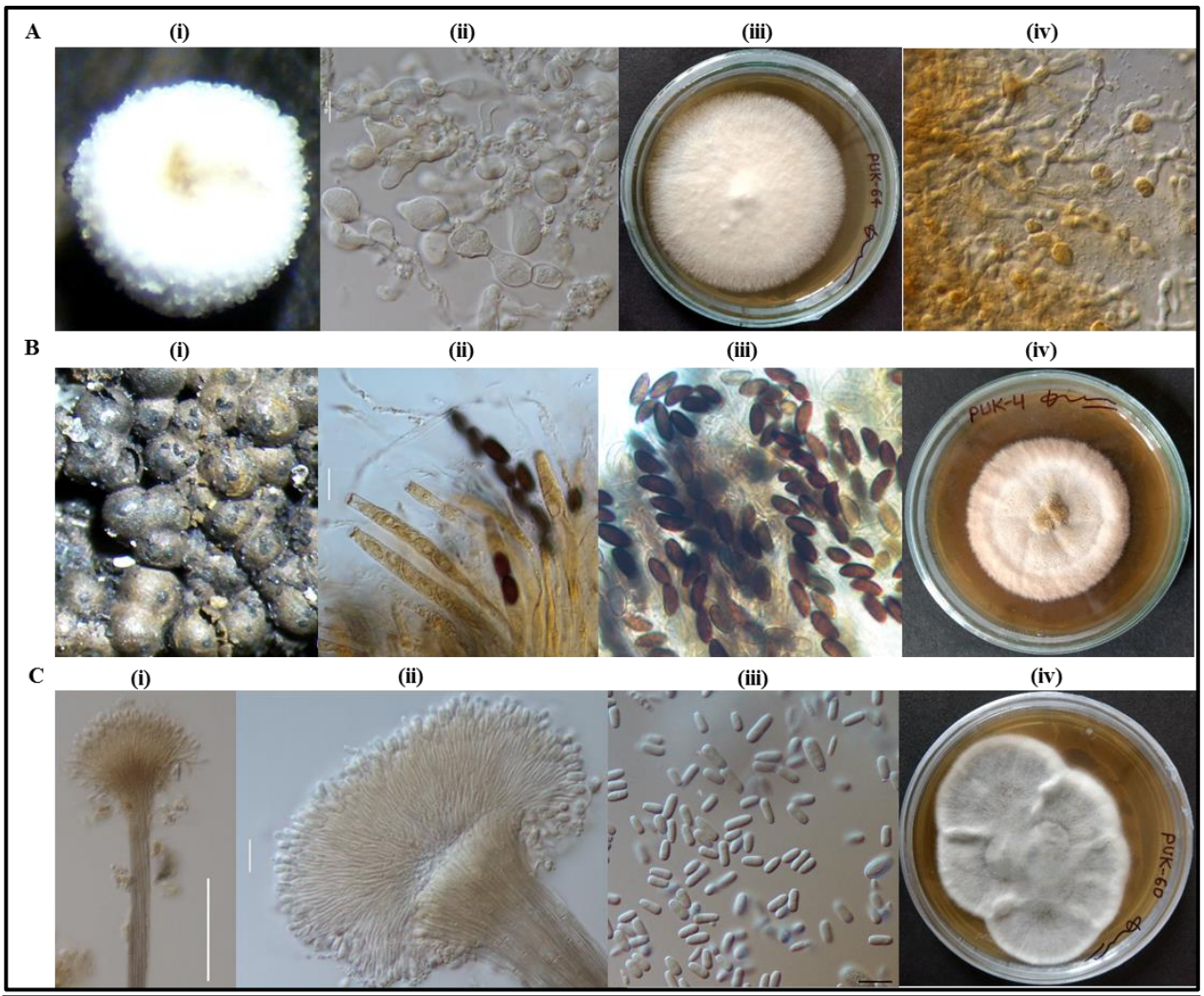


Figure 1. Morphological description of marine fungal isolates: **A-** *Marasmiellus* sp. (i) Basidioma, (ii) Basidia, (iii) Culture, (iv) Conidiophore bearing golden yellow ellipsoidal conidia. **B-** *Hypoxylon* sp. (i) Ascomata, (ii) Asci, (iii) Ascospores, (iv) Culture. **C-** *Scedosporium aurantiacum* (i) Synnema, (ii) Slimy head, (iii) Cylindrical to obovoid conidia, (iv) Culture.

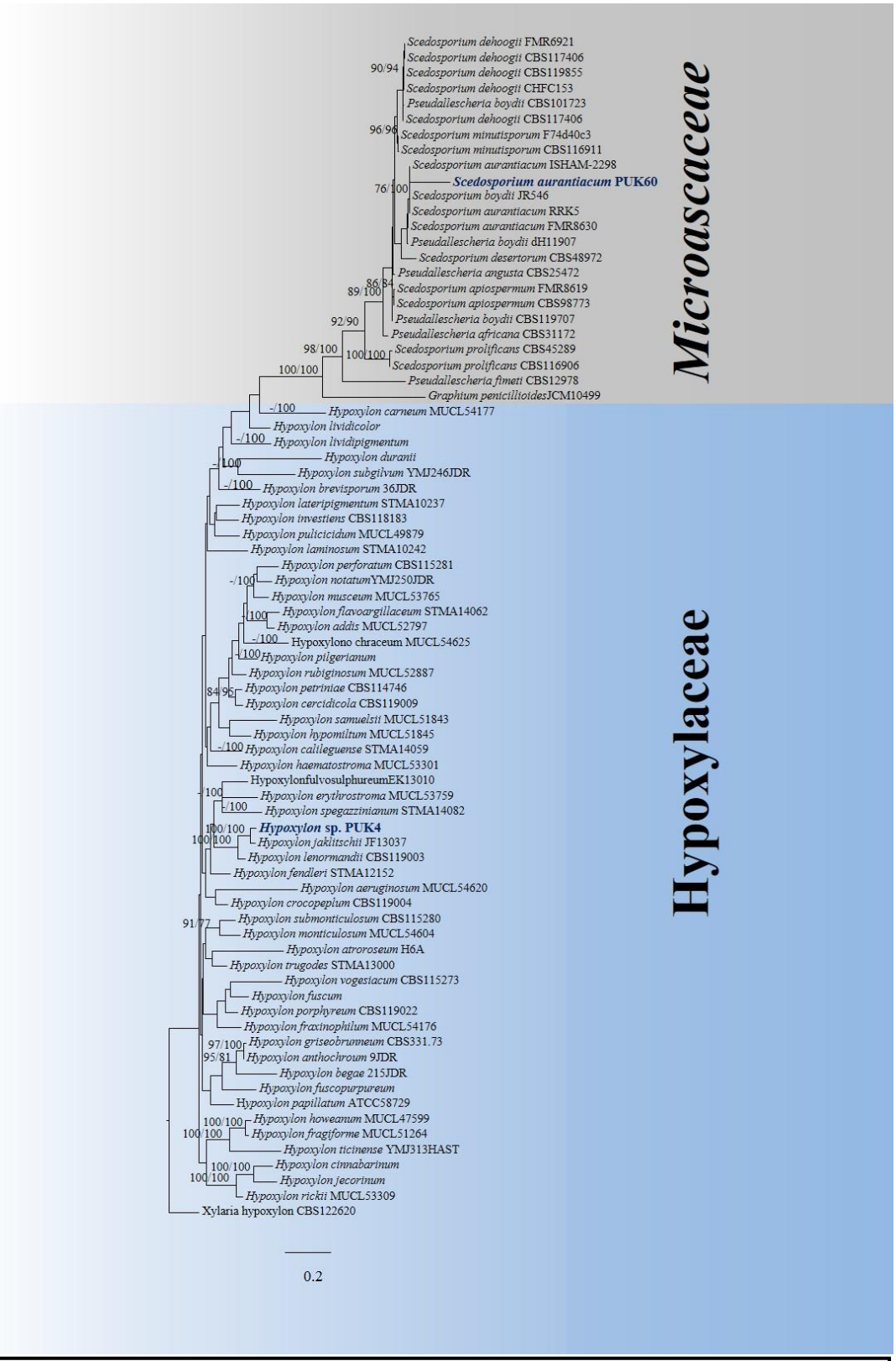


Figure 2. Phylogram of *Scedosporium aurantiacum* and *Hypoxylon* sp., generated through maximum parsimony and maximum likelihood analyses based on ITS sequence data. Maximum likelihood and Maximum parsimony bootstrap values ≥75% are given at the nodes. The isolate used in the present study is in blue colour.

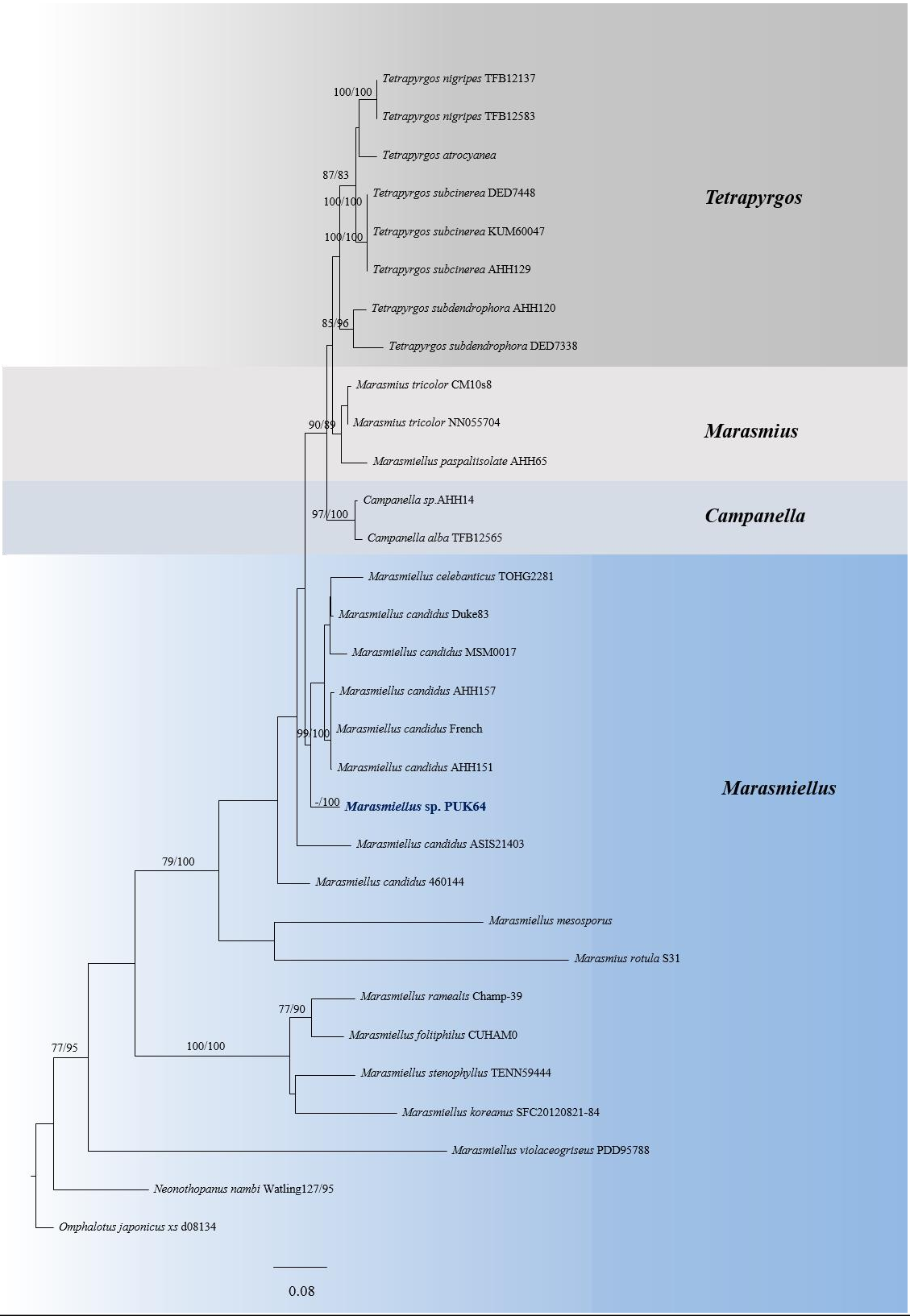


Figure 3. Phylogram of *Marasmiellus* sp., generated through maximum parsimony and maximum likelihood analyses based on ITS sequence data. Maximum likelihood and Maximum parsimony bootstrap values ≥75% are given at the nodes. The isolate used in the present study is in blue colour.

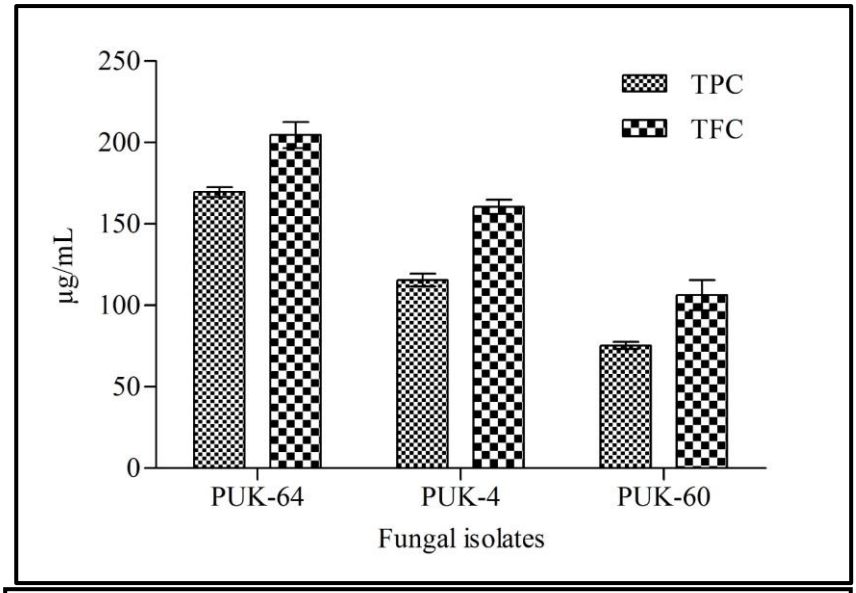


Figure 4. Total phenolic content (TPC) and total flavonoid content (TFC) of different marine fungal crude extracts expressed as µg GAE/mL of crude and µg QE/mL of crude, respectively. Each value represents a mean ± SD (n = 3). GAE – Gallic acid equivalent, QE – Quercetin equivalent.

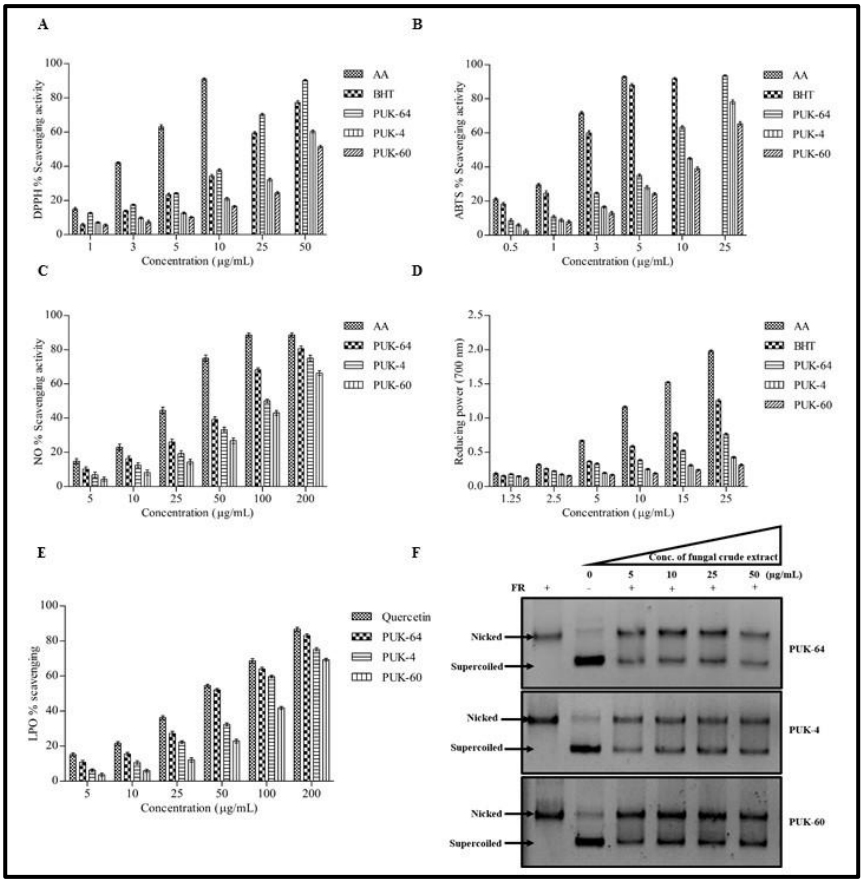


Figure 5. Antioxidant activities of the selected marine fungal crude extracts at various concentrations. Each value represents a mean ± SD (n = 3), $p<0.05$ was considered significant. **A-** DPPH radical scavenging activity, **B-** ABTS radical scavenging activity, **C-** Nitric oxide (NO) radical scavenging activity, **D-** Reducing power activity, **E-** Lipid peroxidation (LPO) inhibition activity, **F-**

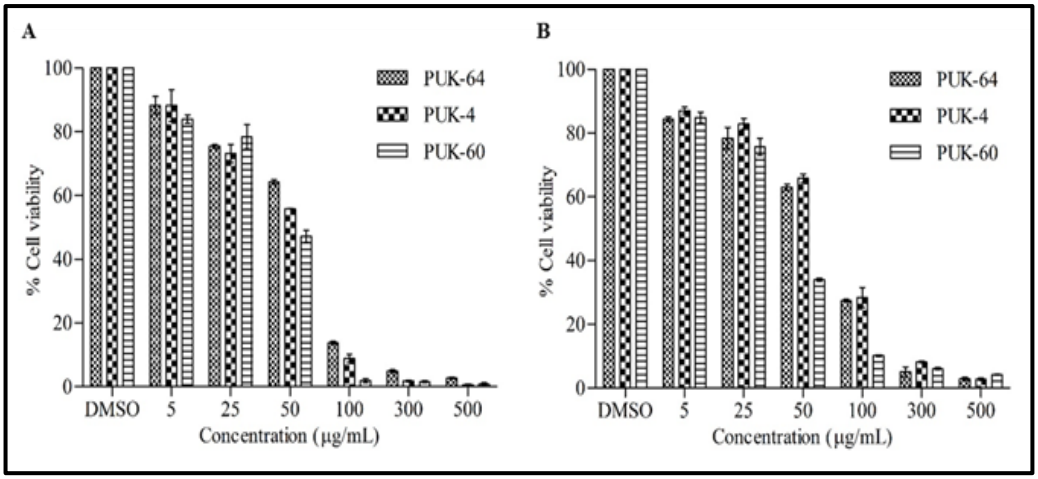


Figure 6. Anticancer activity of selected marine fungal crude extracts on different cancer cell lines. Each value represents a mean ± SD (n = 3): Percentage cell viability of **A-** HeLa cells and **B-** MCF7 breast cancer cells, when treated with marine fungal crude at different concentrations. DMSO (dimethyl sulfoxide) was used as control.

3.2. Molecular phylogeny and identification

Out of 55 different taxa, we have shortlisted 3 cultures that have shown hyper antioxidant activity and only these selected three taxa were taken up for molecular identification in the present study. Phylogenetic trees (Figure 2 & 3) resulted from both Maximum Likelihood (ML) and Maximum parsimony (MP) analyses were similar in overall topologies. Phylogenetic analysis resulted from Marasmiaceae (Figure 3) showed that *Marasmiellus* sp. (PUK-64) clustered within the genus *Marasmiellus* sharing sister relation with *Marasmiellus candidus* with significant bootstrap support from 100% MP. Phylogenetic analysis inferred from Hypoxylaceae and Microascaceae (Figure 2) showed that *Hypoxylon* sp. (PUK-4) belongs to *Hypoxylon* sharing a sister relation with *H. jaklitschii* and *H. lenormandii* with significant bootstrap support from 100% ML and 100% MP. The third taxon belongs to *Scedosporium aurantiacum* (PUK-60) nested together within *Scedosporium* genus with significant bootstrap support from ML 76% and MP 100% (Figure 2). The sequence data has been submitted to NCBI and the Gene Bank accession numbers of ITS region of the 3 fungi are as follows: *Marasmiellus* sp. (MG946760), *Hypoxylon* sp. (KY863509) and *Scedosporium aurantiacum* (MF182397).

3.3. Screening of secondary metabolites in MFCs

Qualitative estimation of the chemical constituents of the three MFC extracts revealed the presence of bioactive constituents such as tannins, terpenoids, steroids, flavonoids, cardiac glycosides, alkaloids, anthraquinone and phlobatannins as the main chemical constituents. (Supp. Table 2).

Supplementary Table 1: List of fungal isolates screened for antioxidant activity and their IC_{50} values (µg/mL). Each value in the table is represented as mean ± SD (n = 3). *The code numbers denote in-house codes given by us to different marine fungal isolates and maintained in our laboratory.

S. No.	Fungal Isolates*	Mean ± SD	S. No.	Fungal Isolates	Mean ± SD
1	PUFD-18	442.82±3.32	29	FD-6	164.04±0.37
2	DM-28	431.42±4.81	30	FD-37	158.03±0.23
3	FD-98	412.12±8.03	31	FD-62	135.67±1.19
4	FD-551	405.78±4.56	32	FD-505	131.89±0.50
5	DM-30	380.89±2.56	33	FDSP-3	124.28±0.55
6	FD-5	378.40±5.36	34	EA-1	116.82±0.90
7	FD-103	343.51±2.64	35	DM-31	114.24±0.67
8	FD-530	325.90±0.51	36	FD-69	109.68±0.29
9	FD-11	292.14±0.70	37	FD-89	109.12±1.59
10	FD-48	278.96±1.62	38	FD-65	105.73±0.74

11	FD-519	278.28±1.70	39	FD-45	105.10±1.02
12	FD-57	277.99±0.87	40	FD-52	102.67±1.10
13	FD-8	269.10±2.62	41	FD-504	93.67±0.49
14	DM-350	263.83±1.14	42	FD-9	89.01±0.30
15	DE-18	263.34±1.19	43	FD-87	83.65±0.41
16	FD-76	261.35±1.52	44	DT-1	83.92±0.83
17	FD-526	239.91±0.31	45	DE-2	82.78±0.66
18	FD-72	234.23±0.80	46	FD-16	78.97±0.65
19	FD-53	224.37±1.26	47	FD-3	78.47±0.70
20	FD-56	202.26±0.67	48	FD-79	76.93±0.33
21	FD-538	200.62±2.99	49	FD-501	77.35±0.26
22	FD-83	194.56±1.07	50	FD-71	69.89±0.48
23	FD-10	174.82±0.81	51	PUFD-9	65.35±0.77
24	FD-41	170.69 ± 1.06	52	FD-34	53.94±0.33
25	FD-61	170.02±0.50	**53**	**PUK-60**	**49.59±0.43**
26	FD-49	169.15±2.91	**54**	**PUK-4**	**40.37±0.49**
27	DM-6	168.38±1.20	**55**	**PUK-64**	**16.13±0.24**
28	FD-503	164.40±1.40			

Supplementary Table 2: Qualitative analysis on different secondary metabolite groups of compounds produced by selected marine fungi. Scores denote: + = weakly positive, ++ = moderately positive, +++ = strongly positive extracts.

Secondary metabolite groups	Fungal isolates		
	PUK-64	PUK-4	PUK-60
Tannins	+	+	+
Terpenoids	+	+	+
Steroids	+	++	+++
Flavonoids	++	+	+
Cardic glycosides	+	++	+++
Alkaloids	+++	+	+
Anthraquinones	-	-	-
Phlobatannins	-	-	-

3.3.1. Estimation of total phenolic content

The total phenolic content of the marine fungal crude extracts, was calculated from the gallic acid standard calibration curve (R^2=0.999), was found to be 169.59±3.01, 115.52±3.98 and 75.40±2.14 µg GAE/mL in *Marasmiellus* sp. (PUK-64), *Hypoxylon* sp. (PUK-4) and *Scedosporium aurantiacum* (PUK-60), respectively (Table 1, Figure 4). Phenolic content was found to be the highest in PUK-64, while PUK-60 showed least content as evident from Figure 4.

3.3.2. Estimation of total flavonoid content

The total flavonoid content of the marine fungal crude extracts, was calculated from the quercetin standard calibration curve (R^2=0.996), was found to be 204.61±5.01, 160.53±4.45 and 106.27±9.34 µg QE/mL in PUK-64, PUK-4 and PUK-60, respectively (TABLE 1, Figure 5).

3.4. Antioxidant activities

3.4.1. DPPH free radical scavenging activity

The scavenging activity of the 55 MFC extracts towards DPPH free radicals was carried out and the results were expressed in terms of IC_{50} values wherein a lower IC_{50} value indicates stronger ability of the extracts (Supp. Figure 1 & Table 1). PUK-64 showed highest percentage (%) scavenging activity of 90.32±0.48, followed by PUK-4 with 60.37±0.84 and PUK- 60 with 51.49±0.76 %, whereas AA and BHT that served as standards have shown 90.94±0.76 and 77.46±0.99 % scavenging, respectively (Figure 5A). Standard AA and BHT were found to have an IC_{50} value of 3.82±0.05 and 19.48±0.17 µg/mL respectively. In comparison to standards i.e., AA and BHT, isolates PUK-64, PUK-4, PUK-60 extracts had IC_{50} values of 16.13±0.24, 40.37±0.49, and 49.59±0.43 µg/mL respectively (Table 2). PUK-64 was found to be

more potent than BHT standard. PUK-60 showed least free radical scavenging activity.

3.4.2. ABTS radical scavenging activity

All the MFC extracts have shown ABTS radical scavenging activity. Percentage scavenging of the isolates PUK-64, PUK- 4 and PUK- 64 were found to be 93.58±0.47, 78.13±1.12 and 65.39±1.19 % respectively. Furthermore, for AA and BHT that served as standards have shown 92.93±0.53 and 91.82±0.65 % scavenging, respectively (Figure 5B). Results of the present study show that ABTS radical scavenging ability of the three MFC extracts could be ranked as PUK-64> PUK-4 >PUK-60. The IC_{50} values of ABTS radical cation scavenging activity for AA and BHT was 1.95±0.03 and 2.42±0.07 µg/mL respectively. Similarly, IC_{50} values of PUK-64, PUK-4 and PUK-60 were found to be 7.63±0.07, 14.15±0.16 and 17.36±0.40 µg/mL, respectively (Table 2).

3.4.3. Nitric oxide radical scavenging activity

In the present study nitric oxide radical scavenging activity was assayed and was found to be highest in PUK-64 followed by PUK-4 and PUK- 60 with 80.66±1.57, 75.07±1.72 and 66.35±1.37 % radical scavenging activity respectively while AA showed 88.63±1.26 % radical scavenging activity (Figure 5C). The IC_{50} values of the three MFC extracts were found to be 68.4±1.3, 92.0±3.4 and 137.4±2.8 µg/mL in PUK-64, PUK-4 and PUK-60 respectively, whereas it was 30.52±1.09 µg/mL in the case of standard AA (Table 2).

3.4.4. Reducing power activity

The reducing power of MFC extracts was found to be increasing with an increase in the concentration of MFC which is comparable to the standard AA. PUK-64 has shown a higher reductive power than PUK-4 and PUK-60. PUK-60 possessed least reductive capacity (Figure 5D).

3.4.5. Lipid peroxidation inhibition activity

In this assay, TBARS level was assessed in decreasing order by increasing the concentration of the test samples. The three selected MFC extracts showed a significant decrease in lipid peroxide radical scavenging potency. PUK-64 showed highest % radical scavenging ability with 83.11±1.04, followed by PUK-4 and PUK-60 (Figure 5E) with the magnitude of 75.24±0.96 and 69.37±0.77. On the basis of IC_{50} values, the pattern of lipid peroxidation of the three MFC extracts could be ranked as PUK-64>PUK-4>PUK-60 (Table 2).

Table 1. Values of total phenolic content (TPC) and total flavonoid content (TFC) of selected marine fungal crude extracts. Each value in the table is represented as mean ± SD (n = 3). GAE - gallic acid equivalent, QE - quercetin equivalent.

Fungal isolates	Total phenolic content (µg GAE/mL of crude)	Total flavonoid content (µg QE/mL of crude)
Marasmiellus sp. (PUK-64)	169.59±3.01	204.61±5.01
Hypoxylon sp. (PUK-4)	115.52±3.98	160.53±4.45
Scedosporium aurantiacum (PUK-60)	75.40±2.14	106.27±9.34

Table 2. IC_{50} values (µg/mL) of radical scavenging activities of selected marine fungal crude extracts. Each value represents mean ± SD (n = 3). AA = Ascorbic acid, BHT = Butylated hydroxytoluene, QE = Quercetin, TBARS = Thiobarbituric acid reactive substance.

Test samples	DPPH activity IC_{50} Value (µg/mL)	ABTS activity IC_{50} Value (µg/mL)	Nitric oxide activity IC_{50} Value (µg/mL)	TBARS activity IC_{50} Value (µg/mL)
Marasmiellus sp. (PUK-64)	16.13±0.24	7.63±0.07	68.4±1.26	48.12±0.61
Hypoxylon sp. (PUK-4)	40.37±0.49	14.15±0.16	92.00±3.39	81.47±1.31
Scedosporium aurantiacum (PUK-60)	49.59±0.43	17.36±0.40	137.44±2.82	135±1.11
AA	3.82±0.05	1.95±0.03	30.52±1.09	-
BHT	19.48±0.17	2.42±0.07	-	-

QE	-	-	-	43.67±0.97

Table 3. Correlations between the IC_{50} values of antioxidant activities and phenolic and flavonoid contents of selected three marine fungal crude extracts. r = Pearson's correlation coefficient & R^2 = coefficient of determination. Each value is represented as mean ± SD (n = 3). TPC -Total phenolic content and TFC - total flavonoid content, NO – nitric oxide, LPO – lipid peroxidation.

Antioxidant activities	TPC	TFC
IC_{50} of DPPH radical scavenging activity	r = 0.9435 R^2= 0.8902	r = 0.8857 R^2= 0.7845
IC_{50} of ABTS radical scavenging activity	r = 0.9635 R^2= 0.9284	r = 0.9147 R^2= 0.8368
IC_{50} of NO radical scavenging activity	r = 0.9973 R^2= 0.9947	r = 0.9974 R^2= 0.9948
IC_{50} of LPO inhibition activity	r = 0.9979 R^2= 0.9959	r = 0.9782 R^2= 0.9569

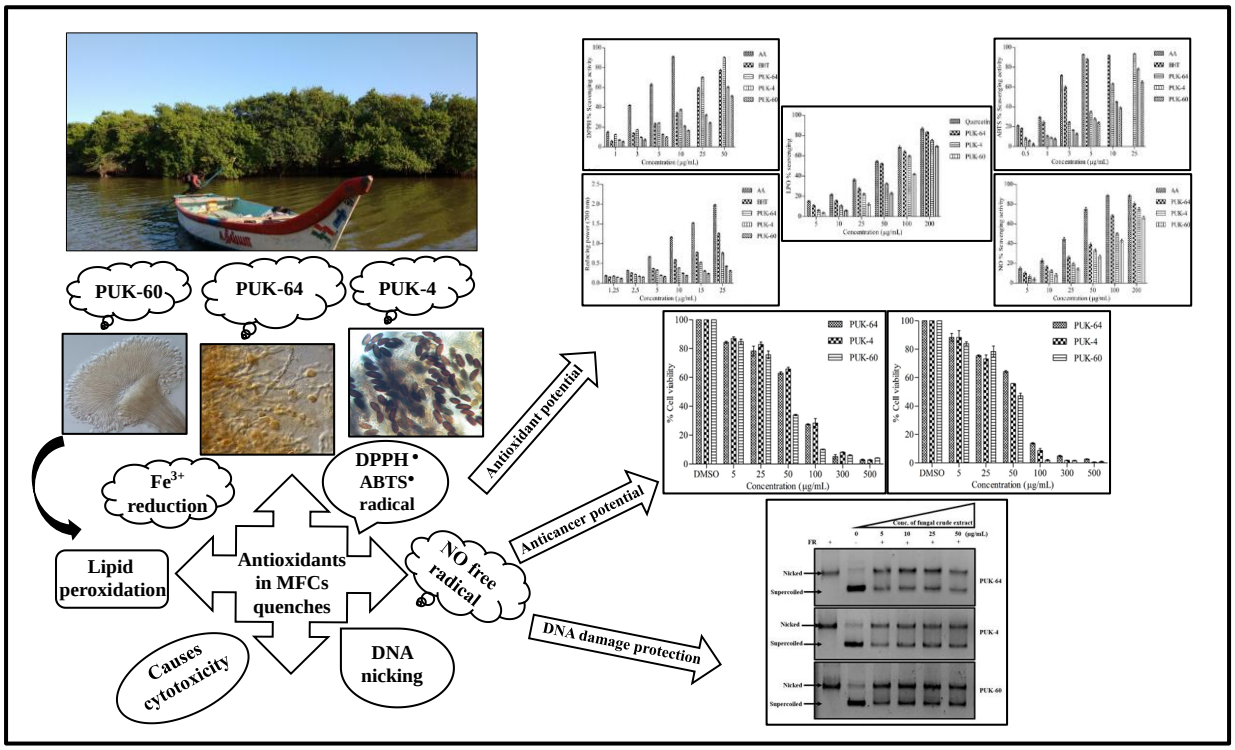
3.4.6. Oxidative DNA damage protection assay

Oxidative DNA damage assay was performed to investigate the protecting ability of the three selected MFC extracts and shielding of free radical attack on pUC19 plasmid DNA. We observed that with increase in the concentration of MFC extracts, the nicking of supercoiled DNA into circular and open form was mitigated as evident from the Figure 5F. The hydroxyl radical generated from iron mediated decomposition of H_2O_2 produces nicks in DNA strand as shown in lane 1. The lane 2 shows the native pUC19 plasmid DNA. The results showed that introduction of MFC extract at various concentrations range (5-50 µg/mL), caused a visible reduction in the formation of open circular form of DNA (lane 3-6) and hence, an increase in the supercoiled form of pUC19 plasmid DNA compared to the FR induced plasmid DNA damage.

3.5. Anticancer activity

MTT assay was performed to observe the cytotoxic potential of MFC extracts on HeLa and MCF7 cancer cells. Cell viability data reflected a high cytotoxicity in MFC extract treated cells when compared to the DMSO control. The effect of MFC extracts was found to be dose dependent as an increase in concentration of MFC decreased the viability of cancer cells (Figure 6A & B).

Graphical abstract:



Concept Figure:

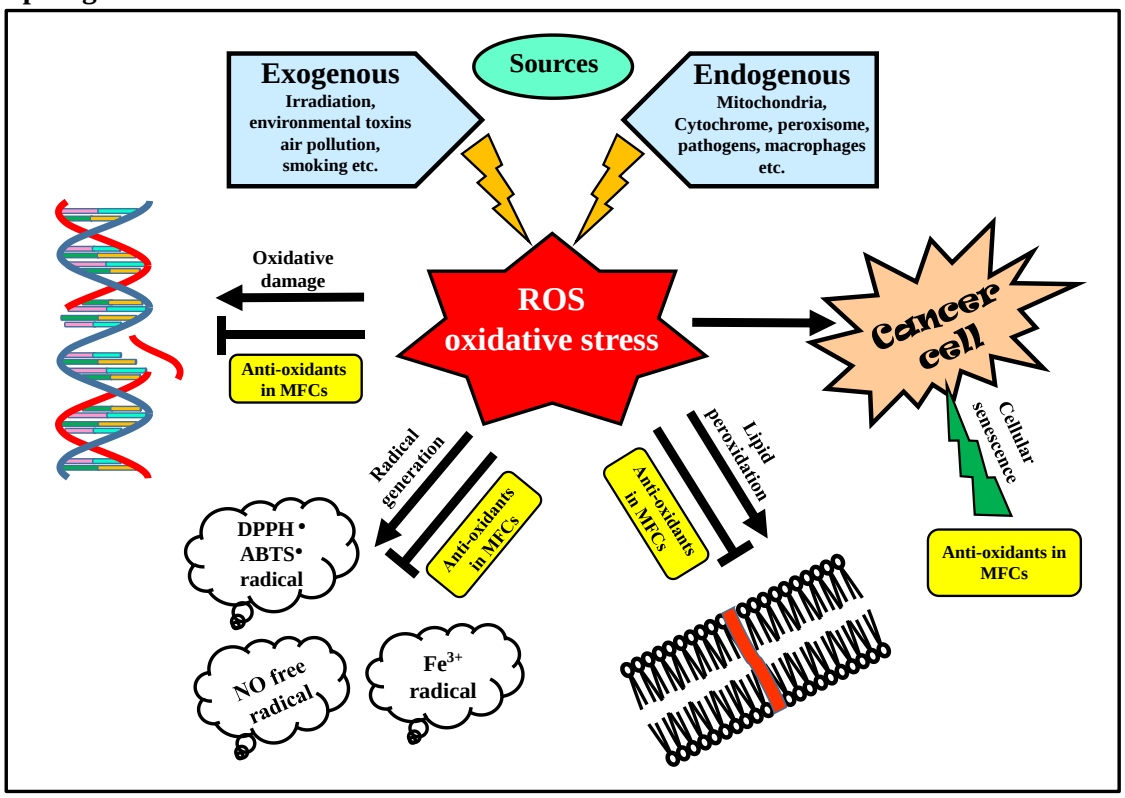
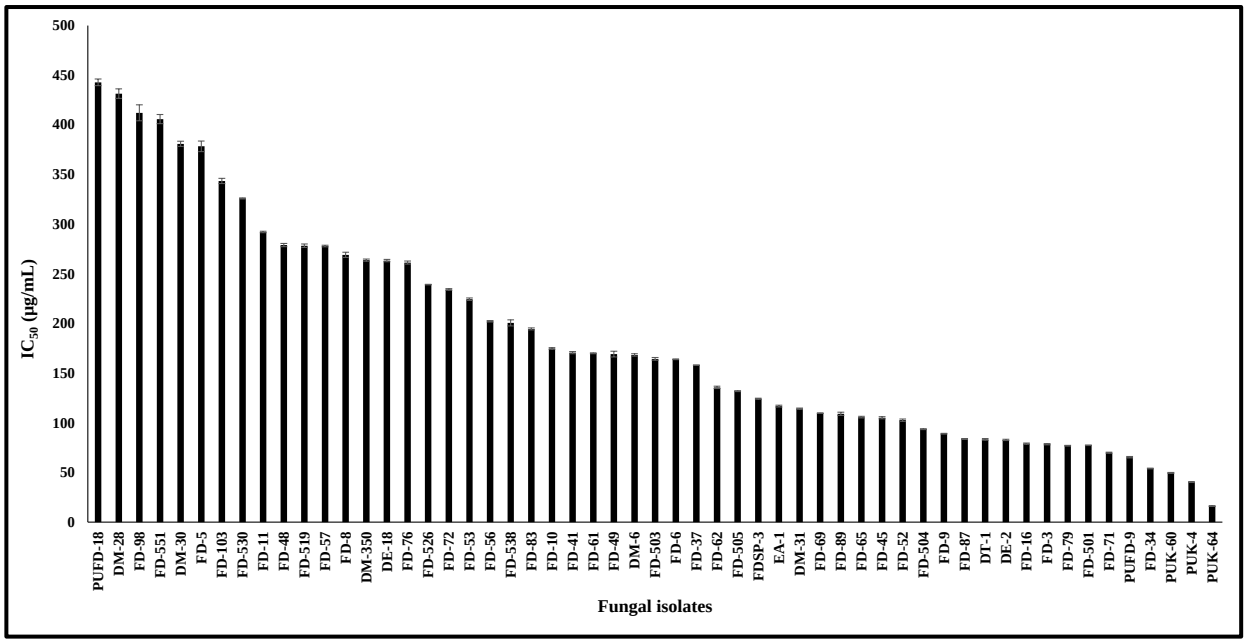


Figure 7. Proposed plausible action of marine fungal crude extract depicting antioxidant potential, oxidative DNA damage protection and anticancer activity. MFC – marine fungal crude, ROS – reactive oxygen species, NO – nitric oxide.



Supplementary Figure 1. Results of preliminary screening of marine fungi for potential antioxidant activity (DPPH Activity). Each value in the figure is represented as mean ± SD (n = 3)

4. DISCUSSION

The marine environment is an abundant source for natural product discovery and novel compounds of medicinal value. Being the second most productive ecosystem, mangroves harbour fungi as the second largest host substrata after driftwood (Khot et al. 2012). Developing in intertidal zone, mangroves are coastal biotopes of ecological, social and economic significance (Latha & Mitra 2004). Marine fungi from mangroves have constantly intrigued researchers as unique chemical scaffolds and novel bioactive metabolites have come into picture (Thatoi et al. 2013). This study presents the results of screening of antioxidant potential in 55 marine mangrove fungal isolates from Muthupet mangroves, East coast of India. Based on the initial screening, three potent isolates, i.e., *Marasmiellus* sp. (PUK- 64), *Hypoxylon* sp. (PUK- 4) and *Scedosporium aurantiacum* (PUK- 60) were shortlisted and these alone were taken up for further studies including morpho-molecular identification (Figures. 1-3). These isolates were subjected to a thorough investigation of their antioxidant potential assessed via *in-vitro* antioxidative activities based on various scavenging abilities such as DPPH, ABTS, NO, LPO, reducing power assays and DNA damage protection. Fungal secondary metabolites provide basic information about bioactive importance of a fungal extract. In this study we have estimated the chemical constituents present in the three marine fungal extracts. The nature of phenols as effective antioxidants is very well acknowledged (Dimitrios 2006). Phenolic compounds have redox properties, which allow them to act as scavengers. Hydroxyl groups of the phenolic compounds play an important key role in facilitating the free radical scavenging ability and used as a basis for rapid screening of antioxidant potential (Baba & Malik 2015). Similarly, flavonoids such as flavones, condensed tannins and flavanols have free OH groups, especially 3-OH. These OH groups allow flavonoids for free radical scavenging potential in vitro and also show antioxidant potential in vivo (Shimoi et al. 1996). Among the three fungi, PUK-64 showed the highest amount of flavonoid content, while PUK-60 possessed the least (Figure 4). Same trend was observed for TPC values with PUK- 64 possessing highest value and PUK- 60 having the least. The values of TPC and TFC was found to be significant higher with a value of 169.59±3.01, 115.52±3.98 and 75.40±2.14 µg GAE/mL as TPC and 204.61±5.01, 160.53±4.45 and 106.27±9.34 µg QE/mL as TFC in PUK-64, PUK-4 and PUK-60, respectively when compared to earlier reports of various endophytes isolated from *Justicia wynaadensis* ranging from 4.8±0.08 to 20.74±0.96 mg GAE/g dry extract of TPC and 2.1±0.08 to 8.75±0.6 mg CE/g dry extract of TFC (Das et al. 2017).

DPPH free radical scavenging assay is the most common procedure for the determination of antioxidant capacity of the extract. DPPH• radical is a stable, coloured radical with nitrogen in the centre (Ani & Naidu 2011), relies on the reduction of methanol DPPH solution in the presence of a hydrogen donating compound (Abdullah et al. 2012). *Marasmiellus* sp. (PUK-64) is a Basidiomycete and it displayed a significantly higher potential with IC_{50} value of 16.13±0.24 µg/mL and 90.32±0.48 % scavenging (Figure 5A) which is quite more than other Basidiomycetes such as *Gandomerma dankaliensis* reported in earlier studies with % scavenging of only 59.28% (Abdel-Monem et al. 2013). Results of the present study suggest that PUK-64 crude extracts comprise secondary metabolites that could donate hydrogen to a free radical to scavenge the potential damage and interestingly, have more IC_{50} value than a very well-known standard BHT.

ABTS* radical, a blue green coloured chromophore and serves the basis of measuring the scavenging activity of water as well as lipid soluble antioxidant compounds (Rice-Evans et al. 1996). The chromophore colour reduces in proportion to the increasing antioxidant concentration. All the potential marine fungal isolates in this study possessed strong scavenging activity with PUK- 64 having the IC_{50} value of 7.6 µg/mL (Figure 5B). When compared to other studies, isolates of this study showed significantly values of % scavenging of 93.58±0.47, 78.13±1.12 and 65.39±1.19 % for all three isolates viz., PUK- 64, PUK- 4 and PUK- 60 respectively, when compared with other fungal strain such as *Rhizopus oryzae* with scavenging % of 14.2.(Sugiharto et al. 2016).

Nitric oxide (NO) is generated from amino acid by vascular endothelial cells, phagocytes, and neurons etc. NO is widespread intracellular and intercellular signalling molecule involved in the regulation of certain physiological process (Kelm et al. 1997; Parul et al. 2013). Excess concentration of NO becomes adverse when it reacts with other free radicals such as superoxide radical, forming a highly reactive peroxynitrite anion (ONOO−). Nitrite ions (NO^{2}–) form

when the nitric oxide formed from SNP reacts with oxygen. Diazotization of NO^{2-} ions with sulphanilamide acid followed by a coupling with naphthyl ethylenediamine, results in pinkish red colour. A decrease in absorbance is used to measure the amplitude of NO^{2-} radical scavenging (Boora et al. 2014). PUK-64 depicted the highest capability of scavenging NO radical followed by PUK-4 and PUK-60 (Figure 5C). Our finding shows that marine fungi possess higher NO radical scavenging activity viz., 80.66±1.57, 75.07±1.72 and 66.35±1.37 % shown by PUK-64, PUK-4 and PUK-60, in contrast to earlier studies on endophytic fungi viz. *Trichoderma* sp., *Colletotrichum* sp. which have shown only 62.39 and 59.20 % NO scavenging (Kumaresan et al. 2015).

In reducing power assay, formation of green colour in the test solution from yellow represents the reducing capability of a sample. The reducing power assay is based on the transformation of Fe^{3+} to Fe^{2+} in the presence of a sample, resulting in the formation of Perl's Prussian blue, could be monitored by measuring it at 700 nm (Saeed et al. 2012). Increase in absorbance at 700 nm indicated an increase in the reducing capability of MFCs. In the present study all the three selected fungi have shown a reduction of Fe^{3+}/ferricyanide complex to the ferrous form, thus demonstrating their reducing power. All the potential marine fungal isolates in this study possessed strong reducing power activity i.e., showing significant values (Figure 5D) when compared with other soil fungal strain such as *Aspergillus* sp. (Arora & Chandra 2011).

The protective effect of MFC extracts against $FeSO_4$ induced lipid peroxidation was estimated in chick liver sample. One of the important naturally occurring lipid peroxidation end product is known as malondialdehyde (MDA), which is considered as the main marker in lipid peroxidation. One molecule of MDA reacts with two molecules of thiobarbituric acid via a Knoevenagel type condensation to yield a chromophore with absorbance maximum at 532 nm. Due to the reactivity of TBA with various reactive substances in the biological sample, a more widely accepted terminology called thiobarbituric acid reactive substances (TBARS) is now commonly used (Grotto et al. 2009; Zeb and Ullah 2016). PUK-60 showed least radical scavenging activity. Our finding shows that marine fungi possess higher lipid peroxidation inhibition activity of 83.11±1.04% in PUK- 64, 75.24±0.96 in PUK-4 and PUK- 60 in 69.37±0.77 (Figure 5E), while with earlier studies on filamentous fungus *Curvularia lunata* and white-rot fungus, *Ceriporiopsis subvermispora* with (Enoki et al. 1999; Paraszkiewicz et al. 2010).

Hydroxyl radicals are well known to induce nicks in DNA strands resulting in its open or relaxed or circular forms. When plasmid DNA gets exposed to Fenton's reagent, it results in strand breaks, fragmentation of deoxy sugars and modification of bases (Golla & Bhimathati 2014). Every molecule of biological system damage in the process of lipid peroxidation and possess the binding capacity to bind with the DNA causing strand breaks, mutation and carcinogenesis (Shabbir et al. 2013). All the three MFC extracts tested showed a strong DNA damage protection (Figure 5F)when compared with mushroom *Ganoderma lucidum* (Sa-ard et al. 2015).

MTT assay is very sensitive and colorimetric assay for assessing cell metabolic activity. Cellular oxidoreductase enzymes are capable of reducing the tetrazolium dye MTT to its insoluble formazan, which has a purple colour (van Meerloo et al. 2011). We found that all the three MFC extracts exhibited more than 85% and 70% cytotoxicity in HeLa and MCF7 cells respectively at 100 µg/mL concentration (Figure 6A & B). When cells were treated at higher concentrations of MFC extracts, we observed cytotoxicity close to 100% suggesting that these fungal extracts exhibit strong anti-cancer properties, which need to be explored further. All MFC extracts were showing significant anticancer activity when compared to earlier reports on anticancer studies from marine fungi e.g. *Eurotium cristatum* (Almeida et al. 2010).

The correlation between TPC-TFC and IC_{50} values for DPPH, ABTS, NO, and LPO scavenging activities are presented in Table 3. Previous studies have demonstrated that there is a linear correlation between total phenolic and flavonoid content and antioxidant potential of certain endophytic fungi & mushrooms such as *Pleurotus ostreatus* and *Pleurotus eryngii* (Gąsecka et al. 2016; Pan et al. 2017). In the present study we have also found a strong correlation between TPC-TFC and IC_{50} values of DPPH, ABTS, nitric oxide and TBARS scavenging activity, which indicates that there is an important role for phenolic compounds and flavonoids in antioxidant potential of the three MFC extracts. Both the classes of compounds have an important role in antioxidant potential, but phenolic content displayed better correlation when compared to flavonoid content.

An interesting observation of the present study is that all the three MFC extracts showed that they produce higher amount of TFCs and TPCs especially flavonoids, have higher antioxidant activity and also significant anticancer activity thus showing a strong correlation. We surmise, based on the results of the present study, that, those extracts that have higher TFCs and TPCs also have higher antioxidant and anticancerous activities. Future research on these lines should throw more light on such a correlation. We have a provided a concept figure depicting the functioning of the bioactive compounds present in the marine fungal crude extracts through probable pathways to bring out antioxidant activity and anticancerous activity. As depicted in Figure 7, some of the probable pathways could be that the secondary metabolites of the marine fungal crude extracts prevent DNA damage and/or, act as radical scavengers, prevent lipid peroxidation and in turn also have cytotoxic activity against human cancer cells. Future research on similar lines may throw more light on the hypothesis proposed by us.

5. CONCLUSIONS

Several challenges and obstacles related to marine fungal studies have barred researchers to explore their full potential. Difficulties faced in collection, preservation and cultivation of marine fungal isolates are some of the major limiting factors. Therefore, more efforts are required to explore the so-called "oceans' dark matter." The present study represents one such effort and showed that some marine fungi possess higher antioxidant activity, almost on par with the standard compounds. In addition to the antioxidant nature of marine fungi we also studied applications such as DNA damage protection and anticancer property. Hence, in the series of experiments performed, the results indicated that TFC was found to be more in MFC extracts as compared to TPC. There is a direct correlation of phenolics and flavonoids with antioxidant activities in the three MFCs tested, which clearly indicate that phenolics and flavonoids might be responsible for antioxidant activities of the MFC. Among the 55 isolates screened, a strong anti-oxidant activity was observed in *Marasmiellus* sp. (PUK-64) followed by *Hypoxylon* sp. (PUK-4) and *Scedosporium aurantiacum* (PUK-60) isolates. More queries will be addressed in future studies targeting these marine fungi including isolation of particular bioactive compounds responsible for anti-oxidant activity and exploring the possibilities of using them as chemo-preventive and therapeutic agents. MFC extracts also showed a strong anticancer activity against human cancer cells indicating that they may serve as a possible source to combat the cancer and various other diseases. To our knowledge, this is the first report on *in vitro* antioxidant and anticancer potential of marine fungal crude extracts from species belonging to the genera *Marasmiellus*, *Hypoxylon* and *Scedosporium aurantiacum* from mangrove environment.

ACKNOWLEDGEMENTS

We gratefully acknowledge The Ministry of Earth Sciences (MOES), Govt. of India for a funding of the project (MOES/36/OO1S/Extra/40/2014/PC-IV dt.14.1.2015) through which the marine fungi were isolated and used in the present study. The district forest office of Tiruvarur under which Muthupet mangroves come and Tamil Nadu forest department are thanked for providing permission to collect the mangrove samples from which the marine fungi were isolated. First author would like to thank the Pondicherry University for providing a university fellowship. The department of Biotechnology, Pondicherry University is thanked for providing the facilities. We are also thankful to Dr. Arunkumar Dhayalan for his support and encouragement.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Abdel-Monem N, Abdel-Azeem AM, El Ashry ESH, Ghareeb DA, Nabil-Adam A. 2013.Assessment of secondary metabolites from marine-derived fungi as antioxidant. Open J Med Chem. 3:60.

Abdullah N, Ismail SM, Aminudin N, Shuib AS, Lau BF. 2012. Evaluation of selected culinary-medicinal mushrooms for antioxidant and ACE inhibitory activities. Evidence-Based Complement Altern Med. 2012.

Akhter F, Hashim A, Khan MS, Ahmad S, Iqbal D, Srivastava AK, Siddiqui MH. 2013. Antioxidant, α-amylase inhibitory and oxidative DNA damage protective property of Boerhaavia diffusa (Linn.) root. South African J Bot. 88:265–272.

Almeida AP, Dethoup T, Singburaudom N, Lima R, Vasconcelos MH, Pinto M, Kijjoa A. 2010. The in vitro anticancer activity of the crude extract of the sponge-associated

fungus Eurotium cristatum and its secondary metabolites. J Nat Pharm. 1:25–29.

Ani V, Naidu KA. 2011. Antioxidant potential of bitter cumin (Centratherum anthelminticum (L.) Kuntze) seeds in in vitro models. BMC Complement Altern Med. 11:40.

Arora DS, Chandra P. 2011. Antioxidant activity of Aspergillus fumigatus. ISRN Pharmacol. 2011.

Baba SA, Malik SA. 2015. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii Blume. J Taibah Univ Sci. 9:449–454.

Blunt JW, Copp BR, Keyzers RA, Munro MHG, Prinsep MR. 2013. Marine natural products. Nat Prod Rep. 30:237–323.

Boora F, Chirisa E, Mukanganyama S. 2014. Evaluation of nitrite radical scavenging properties of selected Zimbabwean plant extracts and their phytoconstituents. J Food Process. 2014.

Das M, Prakash HS, Nalini MS. 2017. Antioxidative and antibacterial potentials of fungal endophytes from justicia wynaadensis heyne: an ethnomedicinal rain forest species of western ghats. Asian J Pharm Clin Res. 10:203–209.

Deshmukh SK, Prakash V, Ranjan N. 2018. Marine fungi: A source of potential anticancer compounds. Front Microbiol. 8:2536.

Devadatha B, Sarma VV, Wanasinghe DN, Hyde KD, Jones EBG. 2017. Introducing the new Indian mangrove species, Vaginatispora microarmatispora (Lophiostomataceae) based on morphology and multigene phylogenetic analysis. Phytotaxa. 329:139–149.

Dimitrios B. 2006. Sources of natural phenolic antioxidants. Trends Food Sci Technol. 17:505–512.

Enoki M, Watanabe T, Nakagame S, Koller K, Messner K, Honda Y, Kuwahara M. 1999. Extracellular lipid peroxidation of selective white-rot fungus, Ceriporiopsis subvermispora. FEMS Microbiol Lett. 180:205–211.

Finkel T, Holbrook NJ. 2000. Oxidants, oxidative stress and the biology of ageing. Nature. 408:239.

Gąsecka M, Mleczek M, Siwulski M, Niedzielski P. 2016. Phenolic composition and antioxidant properties of Pleurotus ostreatus and Pleurotus eryngii enriched with selenium and zinc. Eur Food Res Technol. 242:723–732.

Golla U, Bhimathati SSR. 2014. Evaluation of antioxidant and DNA damage protection activity of the hydroalcoholic extract of Desmostachya bipinnata L. Stapf. Sci World J. 2014.

Grotto D, Maria LS, Valentini J, Paniz C, Schmitt G, Garcia SC, Pomblum VJ, Rocha JBT, Farina M. 2009. Importance of the lipid peroxidation biomarkers and methodological aspects for malondialdehyde quantification. Quim Nova. 32:169–174.

Gueho E. 1991. Taxonomy of the medical species of Pseudallescheria and Scedosporium. J Mycol Med. 118:3–9.

Gul R, Jan SU, Faridullah S, Sherani S, Jahan N. 2017. Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from Ephedra intermedia Indigenous to Balochistan. Sci World J. 2017.

Hasan S, Ansari MI, Ahmad A, Mishra M. 2015. Major bioactive metabolites from marine fungi: A Review. Bioinformation. 11:176.

Hong J-H, Jang S, Heo YM, Min M, Lee H, Lee YM, Lee H, Kim J-J. 2015. Investigation of marine-derived fungal diversity and their exploitable biological activities. Mar Drugs. 13:4137–4155.

De Hoog GS, Guarro J, Gene J, Figueras MJ. 2000. Atlas of clinical fungi, Centraalbureau voor Schimmelcultures. Utrecht, The Netherlands.:276–282.

Imhoff JF. 2016. Natural products from marine fungi—Still an underrepresented resource. Mar Drugs. 14:19.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 30:772–780.

Kelm M, Dahmann R, Wink D, Feelisch M. 1997. The nitric oxide/superoxide assay insights into the biological chemistry of the NO/O2 interaction. J Biol Chem. 272:9922–9932.

Khot M, Kamat S, Zinjarde S, Pant A, Chopade B, RaviKumar A. 2012. Single cell oil of oleaginous fungi from the tropical mangrove wetlands as a potential feedstock for biodiesel. Microb Cell Fact. 11:71.

Kijjoa A, Sawangwong P. 2004. Drugs and cosmetics from the sea. Mar Drugs. 2:73–82.

Kim D-O, Jeong SW, Lee CY. 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem. 81:321–326.

Kumaresan S, Karthi V, Senthilkumar V, Balakumar BS, Stephen A. 2015. Biochemical constituents and antioxidant potential of endophytic fungi isolated from the leaves of Azadirachta indica A. Juss (Neem) from Chennai. J Acad Ind Res. 3:355.

Lackner M, de Hoog GS, Yang L, Ferreira Moreno L, Ahmed SA, Andreas F, Kaltseis J, Nagl M, Lass-Flörl C, Risslegger B, et al. 2014. Proposed nomenclature for Pseudallescheria, Scedosporium and related genera. Fungal Divers. 67:1–10.

Latha R, Mitra S. 2004. Mangrove fungi in India. Curr Sci. 86:1586.

van Meerloo J, Kaspers GJL, Cloos J. 2011. Cell sensitivity assays: the MTT assay. In: Cancer cell Cult. [place unknown]: Springer; p. 237–245.

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic

trees. In: Gatew Comput Environ Work (GCE), 2010. [place unknown]: Ieee; p. 1–8.

Ohkawa H, Ohishi N, Yagi K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 95:351–358.

Pan F, Su T-J, Cai S-M, Wu W. 2017. Fungal endophyte-derived Fritillaria unibracteata var. wabuensis: diversity, antioxidant capacities in vitro and relations to phenolic, flavonoid or saponin compounds. Sci Rep. 7:42008.

Paraszkiewicz K, Bernat P, Naliwajski M, Długoński J. 2010. Lipid peroxidation in the fungus Curvularia lunata exposed to nickel. Arch Microbiol. 192:135–141.

Parul R, Saha P, Kundu SK. 2013. In vitro nitric oxide scavenging activity of methanol extracts of three Bangladeshi medicinal plants. pharma Innov. 1.

Rahman MM, Islam MB, Biswas M, Alam AHMK. 2015. In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. BMC Res Notes. 8:621.

Rambaut A, Drummond A. 2017. FigTree version 1.4. 0. 2012.

Ramirez-Garcia A, Pellon A, Rementeria A, Buldain I, Barreto-Bergter E, Rollin-Pinheiro R, de Meirelles JV, Xisto MIDS, Ranque S, Havlicek V. 2018. Scedosporium and Lomentospora: an updated overview of underrated opportunists. Med Mycol. 56:S102–S125.

Rice-Evans CA, Miller NJ, Paganga G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. 20:933–956.

Sa-ard P, Sarnthima R, Khammuang S, Kanchanarach W. 2015. Antioxidant, antibacterial and DNA protective activities of protein extracts from Ganoderma lucidum. J Food Sci Technol. 52:2966–2973.

Saeed N, Khan MR, Shabbir M. 2012. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC Complement Altern Med. 12:221.

Shabbir M, Khan MR, Saeed N. 2013. Assessment of phytochemicals, antioxidant, anti-lipid peroxidation and anti-hemolytic activity of extract and various fractions of Maytenus royleanus leaves. BMC Complement Altern Med. 13:143.

Shahidi F, Zhong Y. 2015. Measurement of antioxidant activity. J Funct Foods. 18:757–781.

Shen Q, Zhang B, Xu R, Wang Y, Ding X, Li P. 2010. Antioxidant activity in vitro of the selenium-contained protein from the Se-enriched Bifidobacterium animalis 01. Anaerobe. 16:380–386.

Shimoi K, Masuda S, Shen B, Furugori M, Kinae N. 1996. Radioprotective effects of antioxidative plant flavonoids in mice. Mutat Res Mol Mech Mutagen. 350:153–161.

Singh RK, Awasthi S, Dhayalan A, Ferreira JMF, Kannan S. 2016. Deposition, structure, physical and invitro characteristics of Ag-doped β-Ca 3 (PO 4) 2/chitosan hybrid composite coatings on Titanium metal. Mater Sci Eng C. 62:692–701.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 30:1312–1313.

Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web servers. Syst Biol. 57:758–771.

Sugiharto S, Yudiarti T, Isroli I. 2016. Assay of antioxidant potential of two filamentous fungi isolated from the Indonesian fermented dried cassava. Antioxidants. 5:6.

Swofford DL. 2003. PAUP*: phylogenetic analysis using parsimony, version 4.0 b10.

Thatoi H, Behera BC, Mishra RR. 2013. Ecological role and biotechnological potential of mangrove fungi: a review. Mycology. 4:54–71.

Wendt L, Sir EB, Kuhnert E, Heitkämper S, Lambert C, Hladki AI, Romero AI, Luangsa-ard JJ, Srikitikulchai P, Peršoh D. 2018. Resurrection and emendation of the Hypoxylaceae, recognised from a multigene phylogeny of the Xylariales. Mycol Prog. 17:115–154.

White TJ, Bruns T, Lee S, Taylor JL. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protoc a Guid to methods Appl. 18:315–322.

Zeb A, Ullah F. 2016. A simple spectrophotometric method for the determination of thiobarbituric acid reactive substances in fried fast foods. J Anal Methods Chem. 2016.

Zheleva-Dimitrova D, Nedialkov P, Kitanov G. 2010. Radical scavenging and antioxidant activities of methanolic extracts from Hypericum species growing in Bulgaria. Pharmacogn Mag. 6:74.

Received:06.08.18, Accepted: 08.09.18, Published:01.10.2018

***Corresponding Author:**
V.V. Sarma*
Email: **veerkush1992@gmail.com**