INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 15, No. 4, p. 531-538, 2019

RESEARCH PAPER OPEN ACCESS

Molecular identification and hemolytic activity of four jellyfish-associated marine fungi from Cagbatano Bay, Pio Duran, Philippines

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Key words: Jellyfish associated fungi, *Catostylus* sp., Cytotoxicity, Hemolysis, Philippines

http://dx.doi.org/10.12692/ijb/15.4.531-538 Article published on October 30, 2019

Abstract

This study aims to preliminarily investigate and explore the potential of jellyfish-associated marine fungi in the Philippines for cytotoxicity indicators. The fungi were isolated from the sub-umbrellar tissues of the jellyfish *Catostylus* sp. collected from the coasts of Pio Duran in the Philippines. Fungal strains were molecular identified using their internal transcribed spacer (ITS) regions. Crude extracts were obtained by ethyl acetate (EtOAc) extraction of rice-fermented culture supernatants. Qualitative preliminary metabolite screening on the crude extracts and *in vitro* hemolysis screening of crude EtOAc extracts were conducted. Four fungal strains were isolated which revealed 93% to 100% similarity to *Aspergillus nomius, Aspergillus fumigatus*, *Aspergillus tamarii* and *Penicillium citrinum*. Alkaloids, phenols, and terpenoids were present in all the crude extracts. Likewise, all crude fungal extracts demonstrated hemolytic activity in mouse erythrocytes with the highest hemolytic activity (EC_{50} of 3.62µg/ml) observed in *Aspergillus tamarii* crude extract. This result provided robust baseline information that cnidarian-associated fungi species are promising sources of marine bioactive compounds which could be later developed for novel drug candidates.

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Introduction

Nowadays, the marine environment plays an important role in drug discovery programs. In terms of biodiversity, marine environments are among the richest and most complex ecosystems. Harsh chemical and physical conditions in the environment have been important drivers for the production of a variety of molecules with unique structural features (Rocha *et al.,* 2011). Evolutionary development has equipped many marine organisms with the appropriate mechanisms to survive this hostile environment in terms of extreme temperatures, changes in salinity and pressure, as well as overcoming the effects of mutation, bacterial and viral pathogens (Jimeno *et al.,* 2004). These marine molecules can exhibit various types of biological activities with compounds having potential applications in the areas like cancer, inflammation, microbial infections, and various other deadly diseases (Jain *et al.,* 2008; Rawat *et al.,* 2006).

Fungi are occurring naturally in marine environment. Marine fungi play an important ecological role in the marine environment being the primary decomposers, as pathogens, and as obligate symbionts of marine invertebrates (Belofsky *et al.,* 2000). It is believed that most novel natural products found in extracts of marine invertebrates are synthesized, either in part or in their entirely, by the symbiotic microbes that are intimately associated with these marine organisms (Meenupriya and Thangaraj, 2010). Fungi isolated from these marine invertebrates are of considerable importance as they are promising sources of unique secondary metabolites with significant biomedical potential (Imhoff, 2016).

Marine fungi that have antimicrobial activities have been isolated in some jellyfish species (Yue *et al.,* 2015; Liu *et al.,* 2011). However, few studies had been done regarding fungi symbionts of Cnidarian species and their potential biological activities in the Philippines. Hence, the present study was conducted to preliminarily investigate and explore the potential of jellyfish-associated marine fungi in the Philippines particularly its hemolytic activity in view of its possible pharmaceutical exploitation and discovery of new compounds that have therapeutic properties.

Material and methods

Fungi Isolation

In this study, jellyfish samples were collected from the waters of Pio Duran along the west coast of Cagbatano situated in between the coasts of Oas, Albay and Donsol, Sorsogon in the Philippines. After capture, living specimens were collected in sterile polythene bags and transported to the laboratory within minimum possible time. Tissue samples from its inner umbrella were excised manually from the living individuals. Following the protocol done by Liu *et al.* (2011), under aseptic conditions, a five (5) successive rinse of sterile natural seawater was done to remove contaminants and microorganisms attached on the jellyfish surface, the tissue were homogenized, and inoculated for sporulation on agar plates containing malt extract agar (MEA), which was prepared with 75% seawater, containing glucose (20g/L), malt extract (30g/L), agar (15g/L) and peptone (5g/L). The plates were incubated at a temperature of 28°C for 7 up to ~14 days.

Molecular procedures and phylogenetic analysis

After obtaining pure isolates, each fungal species was submitted to Macrogen Inc. (South Korea) for molecular identification. Using the genomic DNA of the pure fungal cultures, taxonomic identification was achieved by DNA amplification and sequencing of the fungal ITS region using primer ITS1: Forward 5' TCCGTAGGTGAACCTGCGG 3' and Reverse ITS4 5' TCCTCCGCT TATTGATATGC 3'. DNA sequences were compared to the sequences within the NCBI database using the BLASTN algorithm. Neighbor-joining analyses were performed in MEGA X and computed using Kimura 2-parameter model method, followed by bootstrap analysis with 1,000 replicates for phylogenetic analyses. The fungus *Paecilomyces variotii* (JN8509969) served as the outgroup taxon.

Fungal Fermentation and Extraction

After identification, each isolate was then cultured into a solid rice medium (RM: 100g rice to 110ml

100% seawater) which was sterilized at 121°C for 15 min (Yue *et al.*, 2015). Afterwards, fungal mycelium samples from the different cultures were introduced into different flasks containing the sterile media and were incubated at 28°C for 45 days. After incubation, the moldy rice was macerated in 250ml ethyl acetate (EtOAc) and was left closed for another 72h. Extraction was done using a homogenizer with 2 × 90 cycles of running for 20 second with a 5 second interval. The homogenate was subject to filtration with medical gauze and the organic layer was collected. Afterwards, the EtOAc solutions was concentrated and evaporated to dryness at a reduced pressure using a rotary evaporator (BIOBASE RE100-Pro) to yield the crude extracts of each fungal species.

Qualitative Metabolite Screening

Chemical tests were carried out on the crude fungal EtOAc extracts using qualitative standard procedures to screen for its biochemical constituents.

Test for Alkaloids

Approximately, 0.2g of the extract was weighed in a test tube and warmed with 2% sulphuric acid (H_2SO_4) for 2 minutes. Then, it was filtered in separate test tube and few drops of Dragendorff reagent were added and observed for the presence of orange red precipitate which shall be the resulting basis for the presence of alkaloid (Elezabeth and Subramanian, 2013).

Test for Terpenoids

Fiveml of each extract was mixed in 2ml of chloroform, and concentrated H_2SO_4 (3ml) was carefully added in a test tube to form a layer. A reddish-brown coloration of the interface shall form to show positive results for the presence of terpenoid (Edeoga *et al.*, 2005).

Test for Saponins

About 1g of each extract was boiled in 10ml of distilled water in a water bath and filtered. 5ml of the filtrate was mixed with 2.5ml of distilled water and was shaken vigorously in a test tube for a stable persistent froth up to 2 cm. Honeycomb-like frothing which persists on warming is indicative of the presence of saponin. The frothing was mixed with 3 drops of olive oil and was shaken vigorously, then observed for the formation of emulsion (Edeoga *et al.*, 2005).

Test for Phenols

The crude extract was mixed with 2ml of 2% solution of Ferric chloride ($FeCl_3$). A blue-green or black coloration indicated the presence of phenols (Yadav and Agarwala, 2011).

Experimental Animals

To assess the erythrocyte lysis activity of each extract, C3H/HeJ strain mice purchased from St. Luke's Medical City- Quezon City, Philippines were utilized as the animal subject of the assay. All animals were provided with food and water *ad libitum* and were maintained under 12 hours dark and 12 hours light cycles. Furthermore, acclimation duration of the animal subjects was done for 14 days. The treatment and use of the animals in this experiment was approved as per norms set by the Animal Care and Use Committee of Bicol University (with assigned control number: PRF-2017-06-009) following in accordance with the ethical standards of the Institutional Animal Care and Use Committee (IACUC).

Hemolytic assay

The hemolysis test was performed in 96-well plates following the method described by Costa-Lotufo *et al.* (2005). Each well received 50µl of the test extracts with concentrations ranging from 2 to 1000µg/ml. The two-fold serial dilution method continued until the 10th well. In a separate well, the negative control contained only the vehicle (DMSO 0.5%), while, in the other, 50µl of the positive control (Triton X-100 0.1%) in 0.85% saline was also added, to obtain 100% hemolysis. Finally, each well received 100µl of 0.85% NaCl solution containing 10 mM $CaCl_2$ with a 2% suspension of mouse erythrocytes. After incubation at room temperature for 30 min and vibrated for 1 min inside the ELISA reader (BIOBASE-EL10B Elisa Microplate Reader), the liberated hemoglobin was measured spectroscopically as absorbance at 540 nm. To calculate for the percentage (%) hemolysis the formula below was employed.

$$\% \text{ Hemolysis} = \frac{\text{sample absorbance} - \text{negative control absorbance}}{\text{positive control absorbance} - \text{negative control absorbance}} x\ 100$$

Statistical Analysis

Results are presented as mean ± S.E.M. Eventual significant differences ($p < 0.05$) were evaluated by the nonparametric Kruskal-Wallis Test, followed by the post hoc Mann-Whitney U Test using SPSS (v. 20) software. The EC_{50} values and their 95% confidence intervals (CI 95%) were obtained by nonlinear regression by probit analysis by means of GraphPad Prism 6.0 software.

Results

Interest in the present study centers particularly on the exploration of bioactive secondary metabolites from jellyfish-derived marine fungi species which could elicit cytotoxic activity. It was assumed that Cnidarian species harbor potential fungi species which may contain novel compounds which could promote significant cytotoxic activity through hemolysis.

Isolation, identification and phylogenetic analysis of the Catostylus sp. associated marine fungi

From the sub-umbrella tissue of the white morph medusa, four different associated fungi were isolated (Fig. 1). Molecular analysis from their ITS regions identified the isolates as *Penicillium citrinum* KT844552, *Aspergillus fumigatus* KU687812, *Aspergillus tamarii* KP784375, and *Aspergillus nomius* JN709035 (Table 1). Based on phylogenetic analysis, WSUm02-2 clustered together within the genus *Penicillium* while WSUm01-1, WSUm02-1 and WSUm02-4 with the genus *Aspergillus* (Fig. 2).

Table 1. Identification of fungal strains isolated from the jellyfish sub-umbrella tissue samples based on DNA analysis of the internal transcribed spacer (ITS) region. The closest relatives in GenBank according to BLAST search were presented.

Isolates	Query Length (bp)	Accession Number	Closest Strains	Similarity (%)
WSUm01-1	591	KP784375	*Aspergillus tamarii*	100
WSUm02-1	589	KT972124	*Aspergillus fumigatus*	100
WSUm02-2	1,241	KT844552	*Penicillium citrinum*	99
WSUm02-4	1,555	JN709035	*Aspergillus nomius*	93

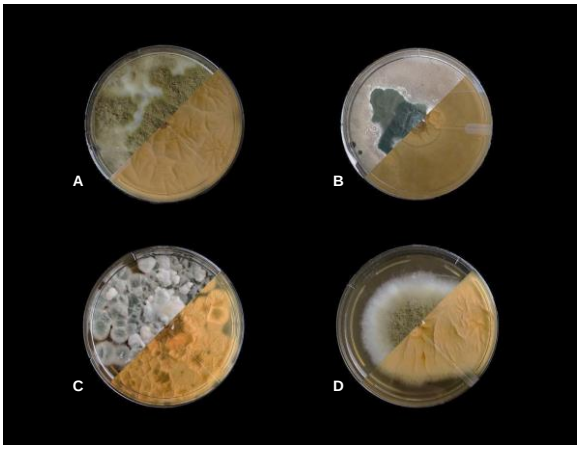

Fig. 1. Seven-day old purified fungal cultures obtained from the jellyfish *Catostylus* sp. grown in Malt Extract Agar (MEA) media with amendments. Photos are presented such that both the surface and reverse views of the plates are depicted. Fungal species obtained from the subumbrellar tissue of the white morph *Catostylus* sp. i.e. **(A)** *Aspergillus tamarii,* **(B)** *Aspergillus fumigatus,* **(C)** *Penicillium citrinum* and **(D)** *Aspergillus nomius.*

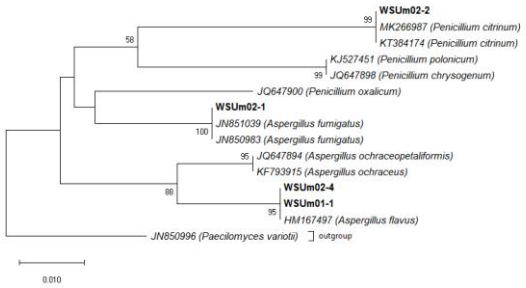

Fig. 2. Neighbor-joining phylogenetic tree from analysis of available ITS sequences of identified marine-derived fungi. The ITS sequences obtained in this work were indicated in bold. Bootstrap values were indicated at nodes of each branch based on a neighbor-joining analysis of 1000 replicates. Bootstrap scores > 50 are presented at the nodes. Scale bar was equal to 0.01 substitutions per nucleotide position. Evolutionary analyses were conducted in MEGA X.

Qualitative metabolite screening

The crude extracts of the different fungal species were subjected to a preliminary phytochemical screening following a standardized conventional protocol for the presence of selected secondary metabolites. Based on the intensity of the colorimetric reaction and froth

formation (in the case of saponin), results revealed that, alkaloids, phenols, and terpenoids were present in all the crude extracts. Observable changes were distinctly notable in *P. citrinum* for its high terpenoid content compared to the other fungal extracts however, a notable absence of saponin content in *P. citrinum* was also observed (Table 2).

Table 2. Qualitative metabolite screening of crude extracts from jellyfish-derived marine fungi using standardized phytochemical screening protocols: Dragendorff's test for alkaloids, Salkowski test for terpenoids, Ferric chloride test for phenols, and froth test for saponins.

Fungal species	Alkaloids	Terpenoids	Phenols	Saponins
Aspergillus tamarii	+	++	+	++
Penicillium citrinum	++	+++	++	-
Aspergillus nomius	+	++	+	++
Aspergillus fumigatus	+	++	+	+

(-) absence, (+) traces, (++) moderate abundance, (+++) high abundance

Hemolytic activity of the isolated fungi

In order to determine whether the cytotoxic activities of the fungal extracts were related to membrane disruption, their ability to induce lysis in mouse erythrocytes were tested. The hemolytic activity (expressed as the *percent hemolysis*) of the crude ethyl acetate extracts of *Aspergillus fumigatus* and *Penicillium citrinum* significantly ($p < 0.05$) increased in a dose-dependent manner whereas *Aspergillus tamarii* and *Aspergillus nomius* extracts exhibited significant ($p < 0.05$) maximal hemolytic activities in the concentrations 31µg/ml (88.2%) and 63µg/ml (96.9%), respectively (Fig. 3). Hemolytic potentials were investigated across all fungal extracts, with *Aspergillus tamarii* as having the most effective EC_{50} value of 3.62µg/ml, followed by *Aspergillus nomius, Aspergillus fumigatus* and *Penicillium citrinum*, which exhibited EC_{50} values of 4.55, 5.48 and 7.45µg/ml, respectively (Fig. 4).

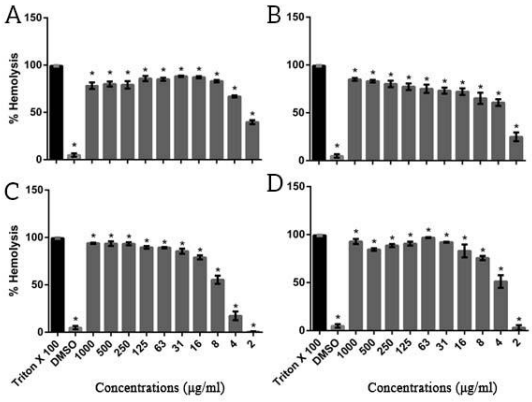

Fig. 3. Dose-response effect of fungal extracts (A-D) *Aspergillus tamarii*, *Aspergillus fumigatus*, *Penicillium citrinum* and *Aspergillus nomius*, respectively evaluated by hemolysis assay. Results are expressed as mean ± SEM, each made in 5 replicates. Significant differences (*$p < 0.05$) versus the positive control (Triton X-100) were tested by Kruskal-Wallis Test, followed by the post hoc Mann-Whitney U Test.

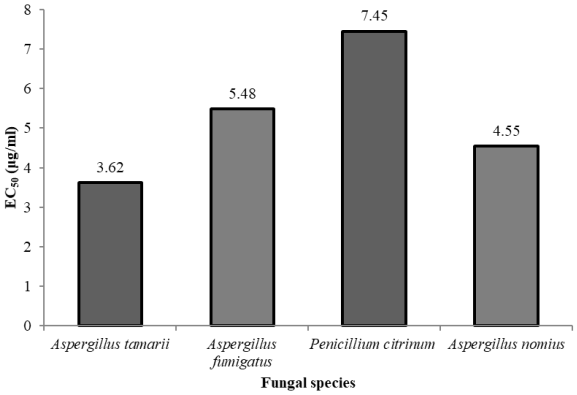

Fig. 4. Hemolytic activity of the fungal extracts on mouse erythrocytes utilizing 2% RBC suspension. The total hemolysis was obtained with 50µl of Triton X-100 (1%) and 30min incubation period. The EC_{50} and 95% confidence interval (CI 95%) were obtained by non-linear regression. Data are presented as the EC_{50} with 95% confidence interval of 2.40-5.45, 3.95-7.61, 5.90-9.40 and 3.43-6.05 for *A. tamarii, A. fumigatus*, *P. citrinum,* and *A. nomius*, respectively.

Discussion

Fungal secondary metabolites play a major role within the large group of natural products originating from marine microorganisms as they are a source of plentiful structurally unique and biologically active secondary metabolites. In recent decades, much effort has been directed toward using natural products as a

source of novel anticancer drugs. Marine-derived fungi have shown in recent years to produce a plethora of new bioactive secondary metabolites with biological activities mainly focused in the areas of antibiotic and anticancer properties. There lies immense scope of exploring marine fungus to obtain oncologic leads and drug (Virupakshaiah, 2014).

Upon morphological and molecular analyses, the isolates are identified as *Penicillium citrinum, Aspergillus tamarii*, *Aspergillus fumigatus* and *Aspergillus nomius*. Based on the reconstruction of phylogenetic tree the isolates were closely related with the genera *Penicillium* and *Aspergillus*. The first group formed a clade within the genus *Penicillium* with WSUm02-2 isolate showing a 99% homology with *Penicillium citrinum*. The second group consisted of WSUm01-1, WSUm02-1 and WSUm02-4, which all belonged to the genus *Aspergillus*. These sequences showed 93-100% similarity with *Aspergillus tamarii*, *Aspergillus fumigatus* and *Aspergillus nomius*, respectively. The *Aspergillus* and *Penicillium* genera are among the most chemically inventive of all fungi, producing a wide array of secondary metabolites (Frisvad, 2015), which demonstrated promising biological activities in terms of antibiotic activities (Artasasta *et al.,* 2017; Zheng *et al.,* 2015; Meenupriya and Thangaraj, 2012; Zhang *et al.,* 2012), cytotoxic, antiproliferative, and anti-cancer potentials (Salendra *et al.,* 2019; Artasasta *et al.*, 2017; Ma *et al.,* 2016; Li *et al.,* 2013; Swathi, 2013; Lee *et al.,* 2013) and free radical scavenging activity (Ruma *et al.,* 2013; Chen *et al.*, 2002) among others. It is also notable that both *A. tamarii* (WSUm01-1) and *A. nomius* (WSUm02-4) joined at a common branch with *A. flavus* since these three species belong to the same subgenus classification under section *Flavi* (Varga and Samson, 2008) which interestingly, the former are *Aspergillus* species that phenotypically resemble *A. flavus* (Tam *et al.,* 2014) with their characteristic yellow to golden conidia.

All the fungal extracts proved positive for hemolytic activity, more notably with the *Aspergillus tamarii* extract exhibiting the most effective EC_{50} against the other extracts. While *Penicillium citrinum* was found out to exhibit the least half-maximal effective concentration which is probably due to the absence of saponins in the extract. It is our understanding that the combined structural diversity and synergistic complementarity of all these mentioned bioactive families could explain the pronounced hemolytic activity demonstrated by every fungal extract. Hence, this study has shown that fungi isolates from cnidarian species are showing high hemolytic activity which could be sources of bioactive compounds with pharmacologic applications.

Acknowledgements

The authors wish to acknowledge Sheldon Rey Boco for his technical assistance on jellyfishes.

References

Artasasta MA, Yanwirast AD, Handayani S. 2017. Cytotoxic activity screening of ethyl acetate fungal extracts derived from the marine sponge *Neopetrosia chaliniformis* AR-01. Journal of Applied Pharmaceutical Science **7,** 174- 178.

Belofsky G, Anguera M, Jensen P, Fenical W, Köck M. 2000. Oxepinamides A ± C and Fumiquinazolines H ± I: Bioactive Metabolites from a Marine Isolate of a Fungus of the Genus *Acremonium*. Chemistry-A European Journal **6,** 1355- 1360.

Chen CH, Shaw CY, Chen CC, Tsai YC. 2002. 2, 3, 4-Trimethyl-5, 7-dihydroxy-2, 3-dihydrobenzofuran, a novel antioxidant, from *Penicillium citrinum* F5. Journal of Natural Products **65,** 740- 741.

Costa-Lotufo L, Khan M, Ather A, Wilke D, Jimenez P, Pessoa C, *et al.* 2005. Studies of the anticancer potential of plants used in Bangladeshi folk medicine. Journal of Ethnopharmacology **99,** 21-30.

Edeoga HO, Okwu DE, Mbaebie BO. 2005. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology **4,** 685- 688.

Elezabeth DV, Subramanian A. 2013. Identification of phytochemical constituents and antimicrobial activity of *Indigofera suffruticosa* leaves. International Journal of Current Biotechnology **1,** 6- 10.

Fang W, Lin X, Zhou X, Wan J, Lu X, Yang B, Liu Y. 2014. Cytotoxic and antiviral nitrobenzoyl sesquiterpenoids from the marine-derived fungus *Aspergillus ochraceus* Jcma1F17. MedChemComm **5,** 701- 705.

Frisvad JC. 2015. Taxonomy, chemodiversity, and chemoconsistency of *Aspergillus, Penicillium,* and *Talaromyces* species. Frontiers in Microbiology **5,** 773.

Imhoff JF. 2016. Natural products from marine fungi—Still an underrepresented resource. Marine Drugs **14,** 1- 19.

Jain R, Sonawane S, Mandrekar N. 2008. Marine organisms: Potential source for drug discovery. Current Science **94,** 292.

Jimeno J, Faircloth G, Fernández Sousa-Faro J, Scheuer P, Rinehart K. 2004. New marine derived anticancer therapeutics — a journey from the sea to clinical trials. Marine Drugs **2,** 14- 29.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution **35,** 1547- 1549.

Lee YM, Kim MJ, Li H, Zhang P, Bao B, Lee KJ, Jung JH. 2013. Marine-derived *Aspergillus* species as a source of bioactive secondary metabolites. Marine Biotechnology **15,** 499- 519.

Li YX, Himaya SWA, Dewapriya P, Zhang C, Kim SK. 2013. Fumigaclavine C from a marine-derived fungus *Aspergillus fumigatus* induces apoptosis in MCF-7 breast cancer cells. Marine Drugs **11,** 5063-5086.

Liu J, Li F, Kim E, Li J, Hong J, Bae K, *et al.* 2011. Antibacterial Polyketides from the Jellyfish-Derived Fungus *Pacilomyces variotii*. Journal of Natural Products **74,** 1826- 1829.

Ma YM, Liang XA, Zhang HC, Liu R. 2016. Cytotoxic and antibiotic cyclic pentapeptide from an endophytic *Aspergillus tamarii* of *Ficus carica*. Journal of Agricultural and Food Chemistry **64,** 3789-3793.

Meenupriya J, Thangaraj M. 2010. Isolation and molecular characterization of bioactive secondary metabolites from *Callyspongia* spp. associated fungi. Asian Pacific Journal of Tropical Medicine **3,** 738-40.

Meenupriya J, Thangaraj M. 2012. Bioprospecting of Potent Fungal Strains from Marine Sponge Hyatella cribriformis from Gulf of Mannar Coast. International Conference on Bioscience, Biotechnology and Healthcare Sciences. Singapore 72-75.

Park MS, Fong JJ, Oh SY, Kwon KK, Sohn JH, Lim YW. 2014. Marine-derived *Penicillium* in Korea: diversity, enzyme activity, and antifungal properties. Antonie Van Leeuwenhoek **106,** 331- 345.

Rawat D, Joshi M, Joshi P, Atheaya H. 2006. Marine peptides and related compounds in clinical trials+. Anti-Cancer Agents in Medicinal Chemistry **6**, 33-40.

Rocha J, Peixe L, Gomes N, Calado R. 2011. Cnidarians as a source of new marine bioactive compounds—an overview of the last decade and future steps for bioprospecting. Marine Drugs **9,** 1860- 1886.

Ruma K, Sunil K, Prakash HS. 2013. Antioxidant, anti-inflammatory, antimicrobial and cytotoxic properties of fungal endophytes from *Garcinia* species. International Journal of Pharmacy and Pharmaceutical Sciences **5,** 889-897.

Salendra L, Lin X, Chen W, Pang X, Luo X, Long J, Yang B. 2019. Cytotoxicity of polyketides and steroids isolated from the sponge-associated fungus *Penicillium citrinum* SCSIO 41017. Natural Product Research 1- 9.

Swathi J, Narendra K, Sowjanya KM, Satya AK. 2013. Marine fungal metabolites as a rich source of bioactive compounds. African Journal of Biochemistry Research **7,** 184- 196.

Tam EW, Chen JH, Lau EC, Ngan AH, Fung KS, Lee KC, Woo, PC. 2014. Misidentification of *Aspergillus nomius* and *Aspergillus tamarii* as *Aspergillus flavus*: characterization by internal transcribed spacer, β-tubulin, and calmodulin gene sequencing, metabolic fingerprinting, and matrix-assisted laser desorption ionization–time of flight mass spectrometry. Journal of Clinical Microbiology **52,** 1153- 1160.

Varga J, Samson R. 2008. Aspergillus in the genomic era. In: Phylogenetics and taxonomy of *Aspergilli*. The Netherlands: Wageningen Academic Publishers 17- 53.

Virupakshaiah DBM. 2014. *In silico* screening of secondary metabolites derived from marine fungi for anticancer study. Journal of Advanced Bioinformatics Applications and Research **5,** 78- 82.

Wang W, Liao Y, Zhang B, Gao M, Ke W, Li F, Shao Z. 2019. Citrinin Monomer and Dimer Derivatives with Antibacterial and Cytotoxic Activities Isolated from the Deep Sea-Derived Fungus *Penicillium citrinum* NLG-S01-P1. Marine Drugs **17,** 46.

Yadav RNS, Agarwala M. 2011. Phytochemical analysis of some medicinal plants. Journal of Phytology **3,** 10- 14.

Yue Y, Yu H, Li R, Xing R, Liu S, Li P. 2015. Exploring the Antibacterial and Antifungal Potential of Jellyfish-Associated Marine Fungi by Cultivation-Dependent Approaches. PLoS ONE **10,** 1- 16.

Zhang X, Sun Y, Bao J, He F, Xu X, Qi S. 2012. Phylogenetic survey and antimicrobial activity of culturable microorganisms associated with the South China Sea black coral *Antipathes dichotoma*. FEMS Microbiology Letters **336,** 122- 130.

Zhang XY, Bao J, Wang GH, He F, Xu XY, Qi SH. 2012. Diversity and antimicrobial activity of culturable fungi isolated from six species of the South China Sea gorgonians. Microbial Ecology **64,** 617- 627.

Zheng CJ, Huang GL, Xu Y, Song XM, Yao J, Liu H, Sun XP. 2016. A new benzopyrans derivatives from a mangrove-derived fungus *Penicillium citrinum* from the South China Sea. Natural Product Research **30,** 821- 825.

Zuluaga-Montero A, Ramírez-Camejo L, Rauscher J, Bayman P. 2010. Marine isolates of *Aspergillus flavus*: denizens of the deep or lost at sea?. Fungal Ecology **3,** 386-391.