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Marine-derived fungus, *Penicillium aurantiogriseum* AUMC 9757: a producer of bioactive secondary metabolites

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Abstract: Thirty-six isolates comprising 23 species of fungi belonging to 8 genera isolated from five regions in Mediterranean Sea (Alexandria) were screened for production of indole alkaloids. Twenty-two isolates gave positive reactions (blue spots on TLC) with Van Urk's reagent and were regarded as indole alkaloids producers. *Penicillium aurantiogriseum* AUMC 9757 was isolated from sea sediment, was selected as the most active producer of indole alkaloids for biological evaluation (antimicrobial and antitumor activities). The crude extract of the strain exhibited high activities against four bacterial strains (*Staphylococcus aureus*, *Bacillus cereus*, *B. subtillus* and *Salmonella* sp.), four fungal strains (*Fusarium solani*, *Alternaria alternata*, *Aspergillus flavus* and *A. ochraceus*) and liver carcinoma cell line (HEPG2). The maximum concentration (100 µg/ml) killed 82.76% of the viable cells, while 50 µg/ml killed 80.52% of the viable cells. The cytotoxicity bioassay using brine shrimp eggs revealed that, there was no mortality in the tested samples at different concentrations. The present study identified *P. aurantiogriseum* from marine sediment as a potential producer of safe bioactive compounds which can be used as antimicrobial and anticancer compounds.

Key words: Marine fungi, *P. aurantiogriseum*, alkaloids, antimicrobial and antitumor.

Introduction

Many fungi are adapted to the presence of salt even up to quite high concentrations. Marine fungi are recognized as a prolific source of biologically active secondary metabolites. A number of reviews, describing the importance and attractiveness of marine fungal metabolites, have been published (Proksch *et al*. 2002, Bugni and Ireland 2004, Somei and Yamada 2005, Blunt *et al*. 2006, Pivkin *et al*. 2006 and Smetanina *et al*. 2007). Potential of marine fungi in providing biologically-active metabolites and new compounds is greater than that of terrestrial counter parts (Cuomo *et al*. 1995).

Alkaloids are a large group of secondary metabolites produced by many organisms, which contain almost 40% metabolites of microorganisms (Blunt *et al*. 2011). These N-containing metabolites usually biosynthesized by a convergence of multiple biosynthetic pathways from many kinds of amino acids (Khadem and Marles 2012) and they have the novelty and diversity of structures.

Screening members of the genus *Penicillium* for the alkaloids production revealed that most of them produce alkaloids belonging to various structural groups, mainly clavines, diketopiperazines, benzodiazepines and quinolines (Kozlovsky *et al*. 2000, Rundberget and Wilkins 2002 and Xin *et al*. 2007). In particular, they have a variety of biological activities such as antimicrobial, antitumor, antipredator, antiinflammatory, and antiviral (Zhuravleva *et al*. 2012). From this point and due to biological activity of alkaloids, we conduct this work to explore new strains which have the ability for indole alkaloid production and determining the biological activity of these compounds.

Materials and Methods

Samples collection

A total of 10 samples of the sediments and sea water were collected from five regions from the Mediterranean Sea (Alexandria). These samples were collected in sterile containers and kept in refrigerator at 4°C for further processing.

Fungal survey

The collected samples were screened for isolation fungal strains on Czapek-Dox agar. The medium composition was (per liter): Sucrose 30.0g, Agar 15.0g, $NaNO_3$ 3.0g, K_2HPO_4 1.0g, KCl 0.5g, $MgSO_4 \cdot 7H_2O$ 0.5g, $FeSO_4 \cdot 7H_2O$ 0.01g and Chloramphenicol 50 mg/l as bacteriostatic agent. All medium ingredients were dissolved in sea water then autoclaved at 121°C for 20 minutes. Afterwards, the collected samples were diluted at 10 and 100-fold with autoclaved filtered sea water, aliquots of 0.1 ml of diluted samples (Awad and Kraume 2011) put into Petri-dish followed by 20 ml of Czapek-Dox agar medium (3 replicates). Plates were incubated at 30 °C for 1-2 weeks to allow for development of pigment on colonies to facilitate complete differentiation of fungal types. Repeated sub-culturing on Czapek-Dox agar was done to obtain pure cultures. Isolates were identified according to morphological features, cultural characteristics such as pigmentation of the mycelium. Identification was accomplished using

appropriate taxonomic techniques (Moubasher 1993 and Pitt and Hocking 2009).

Screening of fungal isolates for indole alkaloids production

All the fungal strains isolated from marine water and sediment were maintained on Czapek-Dox agar medium (Table 1). Spores from a five to seven days old culture were transferred into 250 ml Erlenmeyer flask containing Abe 's medium (per liter) (Manitol 50g, Succinic acid 5.4g, $MgSO_4.7H_2O$ 0.3g, KH_2PO_4 1g). The pH of medium was adjusted to 5.4 with concentrated ammonia. After inoculation, the flasks were agitated on rotary shaker (160 rpm) at 25^{o}C for 10 days. Thereafter the content of each flask was filtered (El-Shanawany *et al*. 2005).

Detection of indole alkaloid production

Indole alkaloids were detected by using Van Urk's reagent (P. dimethylaminobenzaldhyde 0.125 g, 5% $FeCl_3$ 1 ml, 65% v/v sulphuric acid 100 ml). One ml of the filtrate was heated with 2 ml of reagent which gives blue color with positive result (Van Urk 1929).

Extraction of bioactive compounds

The filtrate was extracted two times using organic solvent (chloroform) in a separation funnel. The extract was evaporated to near drying by using rotary evaporator.

Antimicrobial activity

Screening for antibacterial activity

The antibacterial activity of the marine-derived fungus, *P. aurantiogriseum* crude extract was tested against four different pathogenic bacterial strains. Three of them are Gram-positive (*Staphylococcus aureus*, *Bacillus cereus*, *B. subtillus*) and one Gram-negative (*Salmonella* sp.). Aliquots of the tested bacterial cultures were introduced into sterile Petri-dishes then poured with sterilized nutrient agar medium then allowed to solidify; filter paper discs were saturated with crude extract and placed on the surface of agar medium. All plates were incubated at 37^{o}C for 24 h, and were observed for the formation of inhibition zones around the discs.

Screening for antifungal activity

The antifungal activity of the marine-derived fungus (crude extract) was tested against four different fungal strains which belong to *Aspergillus ochraceus*, *A. flavus*, *Fusarium solani*, and *Alternaria alternata*. Aliquots of the tested fungal cultures were introduced into sterilized Petri-dishes then poured with sterilized Czapek's medium and then allowed to solidify. Wells of 0.5 cm were made in the medium by sterilized cork borer, and 150 µl of crude extract were transferred into each well. All plates were incubated at 28^{o}C for 3-5 days, and were observed for the formation of inhibition zones around the wells.

Antitumer activity

The crude extract of marine-derived fungus, *Penicillium aurantiogriseum* was tested against hepatic cellular carcinoma (HEPG2).

A) Cell seeding for sulforhodamine B (SRB) assay (original monolayer 96-well plate seeding)

Potential cytotoxicity of the compound (s) was tested using the method of Skehan *et al.* (1990). Cells under investigation were trypsinized and proper dilution in the compatible media was made. Aliquots of 100 µl cell suspension containing 1000 cells were seeded into flat bottom 96-well plate (according to the cell line doubling time, and operator handling usually ranges from 500-2000 cell per well). Negative control: a lane that only contains media, while positive control is a lane that contains cells but not treated. Each plate must accommodate at least 24 peripheral wells filled with PBS; to guard against media drying. Plates were incubated in a humidified 37ºC, 5% CO_2 chamber for 24 hr. Another aliquots of 100µl media containing the crude extract concentration ranging from 1 ng/ml to 100 µg/ml were added to treated lanes, and blank media to the +ve, and –ve control lanes (this is considered the zero time for treatment). Plates were incubated in a humidified 37ºC, 5% CO2 chamber for another 72 hrs (for the long incubation period the media might need to be changed, and some time PBS washing is recommended according to the treatment under investigation.

B) SRB (sulforhodamine B) assay procedure (for simple 96-well plate format)

On the day of analysis, the 96 well-plates were centrifuged at 1000 rpm, and 4 ºC for 5 min. The media containing the crude extract solution were removed. Fixation was performed by 150 µl of 10% TCA, added and incubated at 4 ºC for 1 hr. The fixative solution was removed and washed 5 times with double distilled water. Aliquots of 70 µl of 0.4 % SRB solution were added and incubated for 10 min at room temperature in a dark place. Plates were washed 3 times with 1% acetic acid, and left to air-dry overnight. To dissolve SRB-bound protein, aliquots of 150 µl of 10 mM Tris- HCl were added and shaken for 2 min. The absorbance was measured at 540 nm (Skehan *et al.* 1990).

Cytotoxicity bioassay

Brine shrimp lethality bioassay (McLaughlin 1991, Krishnaraju *et al*. 2005 and Al-Bari *et al*. 2007) was carried out to investigate the cytotoxicity

of the crude extract of *P. aurantiogriseum*. Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a conical shaped vessel (1L), filled with sterile artificial seawater (prepared using sea salt 38 g L^{-1}) under constant aeration at 30°C for 48 hrs. After hatching, active nauplii (larvae, matured shrimp) free from egg shells were collected from the brighter portion of the hatching chamber and used for the assay. The extract was dissolved in Dimethyl Sulfoxide (DMSO) (not more than 50 µl in 5 ml solution) plus sea water (3.8% NaCl in water) to attain concentrations of 1, 5, 10, 20, 40 µg ml^{-1}. A tube containing 50 µl DMSO diluted in 5 ml was used as a control. Ten nauplii were drawn through a glass capillary and placed in each tube. The number of the nauplii that died after 24 hrs at room temperature was counted. Experiments were conducted together with a control and different concentrations of the test substances in a set of three tubes per dose. The findings are presented graphically by plotting log concentration versus percentage mortality of nauplii from which lethal dose, 50% (LD50) was determined by extrapolation (Goldstein *et al*. 1974).

Results and Discussion

Biodiversity of marine derived fungi

Diverse filamentous fungi were recovered from the water and sediment samples of five coastal environs along Mediterranean Sea (Alexandria). Thirty-six isolates comprising 23 species of fungi belonging to 8 genera were isolated and identified in this study (Table 1). Of all these, *Aspergillus* was the most dominant genus with 8 species (36.11 % of total isolates), followed by *Penicillium* with 4 species (19.4%), *Fusarium* with 3 species (19.4 %), *Scopulariopsis* with 3 species (8.33 %) and others with 5 species (13.9 %). In this respect, Mabrouk *et al.* (2010) isolated 88 fungal isolates from different algae, sea grasses and decaying wood samples collected from Abou-keer, Alexanderia, Egypt. They found that the marine fungal genera were *Acremonium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Dendryphiopsis*, *Fusarium*, *Moniliella*, *Penicillium*, *Scopulariopsis*, and *Verticillium.* Saravanan and Sivakumar (2013) isolated a total of 41 fungal species from marine systems of East Coast of Tamil Nadu, India by plating techniques. They recovered 24 species of fungi from sediment samples whereas water samples yielded 30 species and natural substrates with 24 species. *Aspergillus* was the most common genus represented by 14 species followed by *Penicillium* and *Cladosporium*.

Screening of marine derived isolates for indole alkaloids

Thirty-six isolates belonging to 23 marine fungal species were screened for their potentiality to synthesize indole alkaloids. Twenty-two isolates gave positive reactions (blue spots on TLC) with Van Urk's reagent and were regarded as indole-alkaloid producers, namely *Alternaria chlamydospora* (1 isolate), *Aspergillus aegyptiacus* (1), *A. flavus* (1), *A. tamarii* (2), *A. ustus* (1), *A. versicolor* (4), *Fusarium solani* (1), *Penicillium aurantiogriseum* (2); *P. citrinum* (3), *P. corylophilum* (1), *Scopulariopsis brevicaulis* (1), *S. candida* (1), *S. halophilica* (1), *Trichoderma viride* (1) and *Verticillium* sp. (1) (Table 1 and Fig 1). Vinokurova *et al*. (2003) studied the ability of 13 strains belonging to 10 species of the genus *Penicillium* to produce alkaloids. Most of these strains produced identical ranges of alkaloids when grown on wheat and synthetic Abe's medium. A family of prenylated indole alkaloids derived from tryptophan, proline and two isoprene units are produced by various fungi of the genera *Aspergillus* and *Penicillium*. These alkaloids are characterized by a diketopiperazine or a bicyclo[2,2,2]diazaoctane ring and a 1,7-dihydropyrano-[2,3-g]indole ring system. Among them notoamides were produced from a marine-derived *Aspergillus* sp. (Kato *et al*. 2007, Tsukamoto *et al*. 2008, 2010), stephacidins A & B, from *Aspergillus ochraceus* (Qian-Cutrone *et al*. 2002), versicolamide B from *Aspergillus versicolor* (Greshock *et al*. 2008) and aurantiomides A–C from *Penicillium aurantiogriseum* (Xin *et al*. 2007).

Screening for antimicrobial efficacy

The screening of fungal strains for indole alkaloids production revealed that *P. aurantiogriseum* was regarded as a producer of mixture of indole alkaloids and exhibited many blue spots on TLC. A strain of *P. aurantiogriseum* isolated from sediment was selected as the most active producer of indole alkaloids to test its antimicrobial and antitumor activity (Figs 2 & 3). The culture filtrate of this strain was subjected to extraction using chloroform. The crude extract exhibited activities against four bacterial strains (*Staphylococcus aureus, Bacillus cereus, Bacillus subtillus, Salmonella* sp.) and against four fungal strains (*Fusarium solani*, *Alternaria alternata*, *A. flavus*, *A. ochraceus*). It is interesting to observe that the crude extract of *P. aurantiogriseum* achieved the best inhibition when tested against fungi. Fungi of the genus *Penicillium* are promising objects in the search for new biologically active compounds (Dreyfuss and Chapela 1994). Tsuda *et al*. (2005) attained three pyrrolidine alkaloids, scalusamides A-C (1-3), from the cultured broth of *Penicillium citrinum*, each of 1-3 was found to be a mixture of epimers at C-7. Scalusamide A (1) exhibited antifungal and antibacterial activities. Song *et al*. (2012) isolated three new alkaloids, including auranomides A and B, a new scaffold containing quinazolin-4-one substituted with a pyrrolidin-2-iminium moiety, and auranomide C,

Table 1: Screening of active producer isolates for indole alkaloids

Genera and species	No. of isolates	Source	Indole alkaloids production
Alternaria alternata (Fries) Keissler	1	Sediment	-ve
Alternaria chlamydospora Mouchacca	1	Sediment	+ve
Aspergillus aegyptiacus Moubasher & Moustafa	1	Sea water	+ve
Aspergillus flavus Link	1	Sediment	+ve
Aspergillus niger van Tieghem	2	Sea water (1), Sediment (1)	-ve -ve
Aspergillus parasiticus Speare	1	Sediment	-ve
Aspergillus tamarii Kita	2	Sediment	+ve
Aspergillus terreus Thom	1	Sediment	-ve
Aspergillus ustus (Bainier) Thom & Church	1	Sediment	+ve
Aspergillus versicolor (Vuill.) Tirab.	5	Sea water (3), Sediment (2)	+ve (4) -ve (1)
Fusarium dimerum Penzig	1	Sediment	-ve
Fusarium moniliforme Sheld.	1	Sediment	-ve
Fusarium solani (Martius) Saccardo	5	Sediment	+ve (1) -ve (4)
Penicillium aurantiogriseum Dierckx	2	Sea water (1), Sediment (1)	+ve +ve
Penicillium citrinum Thom	3	Sediment	+ve
Penicillium corylophilum Dierckx	1	Sediment	+ve
Penicillium purpurogenum Stoll	1	Sea water	-ve
Scopulariopsis brevicaulis (Saccardo) Bainier	1	Sediment	+ve
Scopulariopsis candida Vuill.	1	Sediment	+ve
Scopulariopsis halophilica Tubaki	1	Sediment	+ve
Talaromyces sp.	1	Sediment	-ve

as well as two known metabolites auranthine and aurantiomides C from the marine-derived fungus *P. aurantiogriseum*. The quinazolin-4-one ring system has been consistently recognized as a promising pharmacophore because of its broad spectrum pharmacological activities as antibacterial and antifungal agents (Pandey *et al*. 2009 and Mohamed *et al*. 2010).

Screening for antitumor activity

The obtained crude extract of *P. aurantiogriseum* was evaluated for potential cytotoxicity, against hepatic cellular carcinoma (HEPG2). The cell line was treated with serial concentrations of 6.25, 12.5, 25, 50 and 100 µg/ml. Results in Table (2) showed that the maximum concentration 100 µg/ml killed 82.76 % of the viable cells, while the minimum concentration 6.25 % killed 52.54% of the viable cells. Similarly, Song *et al*. (2012) isolated Auranomide B from marine-derived fungus *Penicillium aurantiogriseum* which exhibited the most potent inhibitory effect against human myelogenous leukemia HEPG2 cells, with an inhibitory effect 73.28. Mabrouk *et al*. (2011) extracted 11 compounds from *Penicillium brevicompactum* which was isolated from the associated marine alga *Pterocladia* sp. They reported that the maximum concentration of compound 9 (100 µg/ml) killed approximately 40% of the viable infected liver cells and also killed approximately 50% of the viable infected lung cells at concentration equal to 91.6 µg/ml. They concluded that compound 9 can be recommended as an anticancer compound.

In conclusion: The results of this study revealed that *P. aurantiogriseum* is a good producer of safe bioactive compounds which can be used as antimicrobial and an anticancer compound. Further studies are required to separate and identify these compounds in a pure state and determine which compound has the biological activity to use in application.

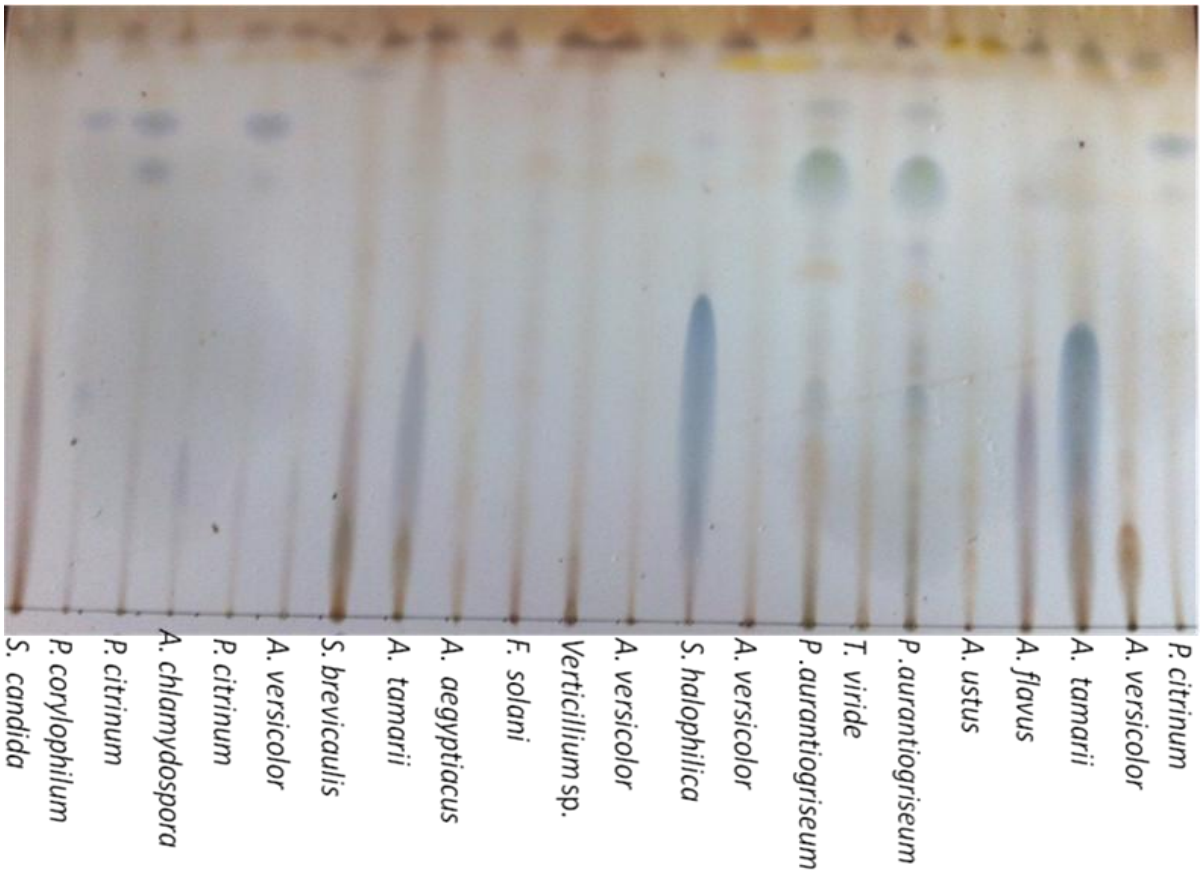

Fig 1: Running extract of tested isolates on TLC Kieselgure using system (Chloroform: Methanol: 25% ammonia; 90:10:0.1)

Table 2: Sensitivity of cancer liver cells to different concentrations of crude extract of *P. aurantiogriseum* AUMC 9757 (AUMC = Assiut University Mycological Center).

Concentration µg/ml	Viability %
Control (0)	100
6.25	47.46396
12.5	41.27069
25	36.99947
50	19.48745
100	17.24506

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References

Al-Bari MA, Sayeed, MA, Khan A, Islam MR, Khondokar MP, Rahman MS and Islam MAU (2007): In-*vitro* antimicrobial activities and cytotoxicity of ethyl acetate extract from *Streptomyces maritimus*. Biotechnology 6: 81-85.

Awad, MF and Kraume M (2011): Mycoflora of Activated Sludge with MBRs in Berlin, Germany. World Academy of Science, Engineering and Technology 54: 566-570.

Blunt JW, Copp BR, Munro MHG, Northcote PT and Prinse MR (2006): Marine natural products. Natural Product Reports 23: 26-78.

Blunt JW, Copp BR, Munro MHG, Northcote PT and Prinsep MR (2011): Marine natural products. Natural Product Reports 28(2): 196-268.

Bugni TS and Ireland CM (2004): Marine-derived fungi: a chemically and biologically diverse group of microorganisms. Natural Product Reports 21: 143-163.

Cuomo V, Palomba I, Perretti A, Guerriero A, D'Ambrosio M, and Pietra F (1995): Antimicrobial activities from marine fungi. Journal of Marine Biotechnology 2: 199-204.

Dreyfuss MM and Chapela IH (1994): Potential of fungi in the discovery of novel, low-molecular weight pharmaceuticals. In: The Discovery of Natural Products with Therapeutic Potential, Gullo VP (ed.), Boston, London, Butterworth-Heinemann, pp. 49-79.

El-Shanawany A, Barakat AA and Abdel-Hadi AM (2005): Ergot alkaloids agroclavine-1 and epoxyagroclavine-1 from *Penicillium citrinum* MU-534. The African Journal of Mycology and Biotechnology 13(1): 71-82.

Goldstein A, Arnow L and Kalkan SM (1974): Principles of Drug Action. 2nd edn., Wiley Biomedical Health Publication, pp. 376-381.

Greshock TJ, Grubbs AW, Jiao P, Wicklow DT, Gloer JB and Williams RM (2008): Isolation, structure elucidation, and biomimetic total synthesis of versicolamide B, and the isolation of antipodal (-)-stephacidin A and (+)-notoamide B from *Aspergillus versicolor* NRRL 35600. Angewandte Chemie International Edition 47: 3573–3577.

Kato H, Yoshida T, Tokue T, Nojiri Y, Hirota H, Ohta T, Williams RM and Tsukamoto S (2007): Notoamides A–D: prenylated indole alkaloids isolated from a marine-derived fungus, *Aspergillus* sp. Angewandte Chemie International Edition 46: 2254–2256.

Khadem S and Marles RJ (2012): Chromone and flavonoid alkaloids: occurrence and bioactivity. Molecules 17: 191-206

Kozlovsky A, Vinokurova NG, Adanin VM and Gräfe U (2000): Piscarinines, new polycyclic diketopiperazine alkaloids from *Penicillium piscarium* NKM F-691. Natural Product Letters 14: 333-340.

Krishnaraju AV, Rao TV, Sundararaju D, Vanisree M, Tsay H, and Subbaraju GV (2005): Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. International Journal of Applied Science and Engineering 3(2): 125-134.

Mabrouk AM, Kheiralla ZH, Hamed ER, Youssry AA and Abd El Aty AA (2011): Physiological studies on some biologically active secondary metabolites from marine-derived fungus *Penicillium brevicompactum*. www.Gate2Biotech.com. 1(1): 1-15.

Mabrouk AM, Kheiralla ZH, Hamed ER, Youssry AA and Abd El Aty AA (2010): Screening of some marine-derived fungal isolates for lignin degrading enzymes (LDEs) production. Agriculture and Biology Journal of North America 1(4): 591-599.

McLaughlin JL (1991): Bench-top bioassays for the discovery of bioactive compounds in higher plants. Brenesia 34: 1-14.

Mohamed MS, Kamel MM, Kassem EM, Abotaleb N, Abd El-moez SI and Ahmed MF (2010): Novel 6,8-dibromo-4(3H) quinazolinone derivatives of anti-bacterial and anti-fungal activities. European Journal of Medicinal Chemistry 45: 3311-3319.

Moubasher AH (1993): Soil fungi of Qatar and other Arab Countries. The Scientific and Applied Research Centre, University of Qatar, Doha, Qatar, 566 pp.

Pandey SK, Singh A and Singh A (2009): Nizamuddin antimicrobial studies of some novel quinazolinones fused with [1,2,4]-triazole, [1,2,4]-triazine and [1,2,4,5]-tetrazine rings. European Journal of Medicinal Chemistry 44: 1188-1197.

Pitt JI and Hocking AD (2009): Fungi and Food Spoilage. 3rd ed, Springer, 519 pp.

Pivkin MV, Kuznetsova TA and Sova VV (2006): Marine Fungi and their Metabolites; Dalnauka: Vladivostok, 133 pp.

Proksch P, Edrada RA and Ebel R (2002): Drugs from the seas: current status and microbiological implications. Applied Microbiology and Biotechnology 59:125-134.

Qian-Cutrone J, Huang S, Shu YZ, Vyas D, Fairchild C, Menendez A, Krampitz K, Dalterio R, Klohr SE and Gao Q (2002): Stephacidin A and B: two structurally novel, selective inhibitors of the testosterone-dependent prostate LNCaP cells. Journal of the American Chemical Society 124: 14556-14557.

Rundberget T and Wilkins AL (2002): Thomitrems A and E, two indole alkaloid isoprenoids from *Penicillium crustosum* Thom. Phytochemistry 61: 979-985.

Saravanan R and Sivakumar T (2013): Biodiversity and biodegradation potentials of fungi isolated from marine systems of East Coast of Tamil Nadu, India. International Journal of Current Microbiology and Applied Science 2: 192-201

Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S and Boyd MR (1990): New colorimetric cytotoxicity assay for anti-cancer drug screening. Journal of National Cancer Institute 82: 1107-1117.

Smetanina OF, Kalinovsky AI, Khudyakova YV, Pivkin MV, Dmitrenok PS, Fedorov SN, Ji H, Kwak JY and Kuznetsova TA (2007): Indole alkaloids produced by a marine fungus isolate of *Penicillium janthinellum* Biourge. Journal of Natural Products 70: 906-909.

Somei M and Yamada F (2005): Simple indole alkaloids and those with a non-rearranged

monoterpenoid unit. Natural Product Reports 22: 73-103.

Song F, Ren B, Yu K, Chen C, Guo H, Yang N, Gao H, Liu X, Liu M, Tong Y, Dai H, Bai H, Wang J and Zhang L (2012): Quinazolin-4-one coupled with pyrrolidin-2-iminium alkaloids from marine-derived fungus *Penicillium aurantiogriseum*. Marine Drugs 10: 1297-1306.

Tsuda M, Sasaki M, Mugishima T, Komatsu K, Sone T, Tanaka M, Mikami Y and Kobayashi J (2005): Scalusamides A-C, new pyrrolidine alkaloids from the marine-derived fungus *Penicillium citrinum*. Journal of Natural Products 68: 273-276.

Tsukamoto S, Kato H, Samizo M, Nojiri Y, Onuki H, Hirota H and Ohta T (2008): Notoamides F–K, prenylated indole alkaloids isolated from a marine-derived *Aspergillus* sp. Journal of Natural Products 71: 2064-2067.

Tsukamoto S, Umaoka H, Yoshikawa K, Ikeda T, and Hirota H (2010): Notoamide O, a structurally unprecedented prenylated indole alkaloid, and notoamides P–R from a marine-derived fungus, *Aspergillus* sp. Journal of Natural Products 73: 1438-1440.

Van Urk HW (1929): A new sensitive reaction for the ergot alkaloids, ergotamine, ergotoxine and ergotinine and its adaptation to examination and colorimetric determination of ergot preparations. Pharmaceutisch Weekblad 66: 473-481.

Vinokurova NG, Khmel nitskaya II, Baskunov BP and Arinbasarov MU (2003): Occurrence of indole alkaloids among secondary metabolites of soil *Aspergillus* spp. Applied Biochemistry and Microbiology 39: 192-196.

Xin ZH, Fang Y, Du L, Zhu T, Duan L, Chen J, Gu QQ, and Zhu WM (2007): Aurantiomides A–C, quinazoline alkaloids from the sponge-derived fungus *Penicillium aurantiogriseum* SP0-19. Journal of Natural Products 70: 853-855.

Zhuravleva OI, Afiyatullov SS, Denisenko VA, Ermakova SP, Slinkina NS, Dmitrenok PS and Kim NY (2012): Secondary metabolites from a marine-derived fungus *Aspergillus carneus* Blochwitz. Phytochemistry 80: 123-131.