Indian Journal of Natural Products and Resources
Vol. 8(4), December 2017, pp. 322-328

Bioactive compounds from marine *Stachybotrys* sp. QL23

Ahmed S. Abdel-Razek[1,2*], Mohamed El-Awady[3], Amal Z. Hassan[4], Faten Y. Yassin[4], Mohsen Asker[3] and Mohamed Shaaban[1,4]

[1]Organic and Bioorganic Chemistry Department, Faculty of Chemistry, Bielefeld University, D-33501 Bielefeld, Germany

[2]Microbial Chemistry Department, [3]Microbial Biotechnology Department., Genetic Engineering and Biotechnology Division,

[4]Chemistry of Natural Compounds Department, Division of Pharmaceutical Industries Research, National Research Centre, Giza, Egypt

Received 21 March 2017; Revised 30 January 2018

During our ongoing search for bioactive compounds from microorganisms, the marine fungus *Stachybotrys* sp. QL23 was isolated from a sediment sample collected from Qarun Lake, El Fayoum governorate, Egypt and biologically screened to produce bioactive compounds. The fungal extract showed moderate to high activity against Gram-positive and Gram-negative bacteria assayed. The genotypic characterisation of *Stachybotrys* sp QL23 has been deduced basically on its 18S rRNA gene sequencing. Pre-chemical studies of the strain extract using different chromatographic techniques afforded the major bioactive metabolites, ergosterol (1) and linoleic acid (2), and their chemical structures were assigned using 1D and 2D NMR and Spectroscopy and HR-EI mass spectrometry. In this article, we present the isolation and taxonomical characterisation of the producing fungal strain, along with the study of antimicrobial activity of the strain extract and compounds 1-2 using a set of microorganisms. The study of the QL23 using cervix carcinoma cell line (KB-3-1) extract has been reported as well.

Keywords: Bioactive compounds, Marine fungi, *Stachybotrys* sp., Structure elucidation, Taxonomy.

IPC code; Int. cl. (2015.01)- A61k 36/00

Introduction

Marine microorganisms provide an enormous number of potentially useful natural therapeutic agents; some of them are in clinical use for a long time. However, the rapid development of resistance is forcing researchers to explore new sources of potent bioactive natural products. Moreover, current cancer therapies suffer from a low therapeutic index and many cancers are not curable with the current therapy. Therefore, new drugs need to be more specific with lower side effects and less toxicity than the ones currently in use[1]. Marine microorganisms often produce biologically active and chemically intriguing secondary metabolites[2]. Chemists on a worldwide basis have paid attention during the past four decades towards the potential of marine sources for isolation of novel metabolites with interesting biological and pharmacological properties[3,4].

Fungi are distributed in nature; eukaryotic, heterotrophic microorganisms, and often live symbiotically through interacting with other species or hosts (such as plants, algae, insects or other animals) for survival forming a relationship called "symbiosis".

Symbiosis can be beneficial to one or both organisms. They have been used for a long time by mankind for many purposes including food production (beer, wine, leavened bread, soy foods), treatments and in everyday life. Thousands of years ago, fungi grown on roasted green corn have been used to treat intestinal diseases by Mayans[5–7]. Alternatively, fungi are a rich source of bioactive secondary metabolites[8,9]. Many therapeutic agents such as cyclosporine and mycophenolic acid are representing immunosuppressive agents[10], along with their antimicrobial activities[11]. Recently, the non-immunosuppressive cyclosporine DEBIO-025 is a potent inhibitor of hepatitis C virus replication in vitro[12]. Fusidic acid[13] and griseofulvin[14], which possess antimicrobial activity and other novel semisynthetic antifungal drugs (e.g. anidulafungin and caspofungin) have been derived from fungal metabolites[11,15].

For decades, many antibiotics have been reported to be produced by fungi[16,17]. Many other anti-microbially-active metabolites have been isolated

*Correspondent author:
E-mail: ahmedshukri_sci@yahoo.com

from marine fungi including *Stachybotrys chlorohalonata*[18]. Two new cytochalasins having antimicrobial activity have been isolated from the fungus *Stachybotrys charatum*[19].

Materials and Methods

General experimental procedures

All NMR (Nuclear Magnetic Resonance) spectra [^{1}H NMR, ^{13}C NMR, Distortionless Enhancement by Polarization Transfer (DEPT), Correlation Spectroscopy (COSY), Heteronuclear Multiple-Quantum Correlation Spectroscopy (HMQC) and Heteronuclear Multiple-Bond Correlation Spectroscopy (HMBC)] were measured on Bruker Avance DRX 500 and DRX 600 MHz spectrometers using standard pulse sequences and referenced to residual solvent signals. HR-EI-MS (High Resolution Electro ionization Mass Spectroscopy) was determined using GCT Premier Spectrometer. Column chromatography was carried out on silica gel 60 (0.040–0.063 mm, Merck) and Sephadex LH-20 as the stationary phases. Preparative TLC (0.5 mm thick) and analytical TLC were performed with pre-coated Merck silica gel 60 PF254+366, Germany. *Rf* values of the strain extract and pure compounds, and visualisation of their chromatograms was carried out under UV light (254 and 366 nm) and further by spraying anisaldehyde/sulphuric acid followed by heating.

Isolation of the producing strain

The marine fungal strains were obtained from marine water and sediment samples collected from El Natron Valley Lake, Beheira governorate, Egypt and Qarun Lake, Fayium governorate, Egypt. It was cultivated on potato dextrose agar (PDA) medium (gL^{-1}) (potato infusion, 200 g; dextrose, 20 g and 20.0 g agar and 1000 mL of 50 % sea water). The pH was adjusted to 7.2 as following: The sediment samples were suspended in sterile water and incubated into reciprocal water bath at 30 °C for 30 min, and then the samples have been serially diluted under aseptic conditions, then aseptic pipetting of 100 µl from each dilution on potato dextrose agar plates have been done. The plates were then incubated at 35 °C for 6-8 weeks. The colonies with distinct morphological characteristics were selected and transferred onto freshly prepared solid media and stored in a refrigerator at 4 °C. The strain is deposited in the collection of the Microbial Chemistry Department, National Research Centre (NRC) Egypt.

DNA isolation and 18S rRNA gene sequencing

Genomic DNA of the strain was isolated and sequenced using 3730xl DNA analyzer. The DNA concentration was 100 ng/µL, volume 20 µL. The PCR product was detected by agarose gel and was visualised by (UV) fluorescence after ethidium bromide staining[20]. The results of 18S rRNA gene sequence were compared to the available database at GenBank by using BLAST software (blastn) on National Centre Biotechnology Information (NCBI). The phylogenetic tree was constructed using neighbour-joining tree method.

Fermentation on rice medium, working-up and isolation

The spore suspension of the selected strain QL23 was inoculated into 100 mL of ISP2 medium composition: malt extract, 10 g/L; yeast extract, 4 g/L and glucose, 4 g/L at 30 ^{o}C for 3 days as the seed culture. About 5 mL of seed culture was used to inoculate 1 L Erlenmeyer flasks (5 flasks) containing rice medium composition: 100 g commercial rice; 150 mL of 50 % sea water. The flasks were incubated for 14 days at 22 ^{o}C. The methanol was separated from rice by filtration under vacuum. After filtration, the water/methanol fraction was evaporated to remove methanol using rotary evaporator (Heidolph)[21]. After complete evaporation of methanol, the water phase was re-extracted by ethyl acetate. The obtained ethyl acetate extracts were finally in vacuo concentrated to dryness and then applied to working up stages. The crude extract (11 g) was separated by column chromatography on silica gel column chromatography (column 3×100 cm^{2}), using gradient elution with n-hexane- DCM and DCM-MeOH. Based on TLC monitoring, visualised by UV and anisaldehyde/ sulfuric acid spray, two fractions were obtained: FI (0.95 g) by which after purification using silica gel column afforded linoleic acid (Fig.1b). Fraction II was dissolved in MeOH to get white precipitate which by crystallisation from Dichloromethane (DCM)/MeOH afford fraction AFII. The latter was applied for purification using Sephadex LH-20 affording ergosterol (Fig. 1a).

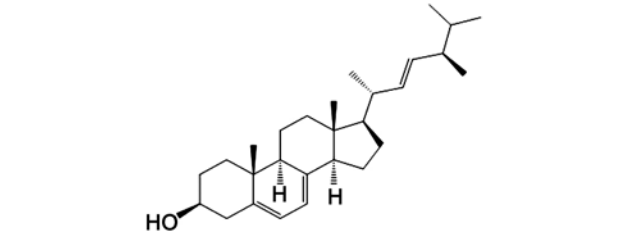
Antimicrobial assay using Agar diffusion test

Antimicrobial activities testing of the crude extract and pure compounds were carried out against a set of microorganisms using the agar diffusion technique. Paper-disk diffusion assay[22] with some modifications have been followed to measure the antimicrobial activity. 20 mL of medium seeded with test organism

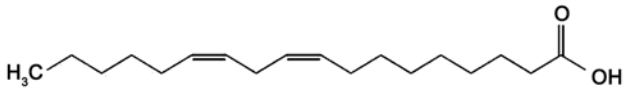
were poured into 9 cm sterile Petri dishes. After solidification, the paper disks were placed on inoculated agar plates and allowed to diffuse the loaded substances into the refrigerator at 4 °C for 2 h. The plates were incubated for 24 h at 30 °C. Both bacteria and yeasts were grown on nutrient agar medium (g/L): beef extract, 3 g; peptone, 10 g and agar 20 g. The pH was adjusted to 7.2. Fungal strain was grown on potato dextrose agar (g/L): potato extract, 4 g; dextrose, 20 g; agar, 15 g (pH 6). After incubation, the diameters of inhibition zones were measured with a wide panel of test microorganisms comprising Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Micrococcus luteus* DSMZ 1605, *Staphylococcus warneri* DSMZ 20036, and *Staphylococcus aureus* ATCC 6538), Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* DSMZ 1058 and *Pseudomonas agarici* DSMZ 11810), yeasts (*Candida albicans* ATCC 10231, *Saccharomyces cerevisiae* ATCC 9080), and the fungus *Aspergillus niger* NRRL A-326.

Cytotoxicity assay

The human cervix carcinoma cells KB-3-1 were cultivated as a monolayer in DMEM (Dulbecco's modified Eagle medium) with 4.5 g/L glucose, l-glutamine, sodium pyruvate and phenol red, supplemented with 10 % foetal bovine serum (FBS). The cells were maintained at 37 °C and 5.3 % CO_2-humidified air. On the day before the test, the cells (70 % confluence) were detached with trypsin-ethylene diamine tetra acetic acid solution (0.05 %; 0.02 % in DPBS) and placed in sterile 96-well plates in a density of 10000 cells in 100 µL medium per well. The dilution series of the compounds were prepared from stock solutions in DMSO of concentrations of 100 mM, 50 mM or 25 mM. The stock solutions were diluted with culture medium (10 % FBS) down to the picomolar range. The dilution prepared from the stock solution was added to the wells. Each concentration was tested in six replicates. Dilution series were prepared by pipetting liquid from well to well. The control contained the same concentration of DMSO as the first dilution. After incubation for 72 h at 37 °C and 5.3 % CO_2-humidified air, 30 µL of an aqueous resazurin solution (175 µM) was added to each well. The cells were incubated under the same conditions for 5 h. Subsequently, the fluorescence was measured. The excitation was affected at a wavelength of 530 nm, whereas the emission was recorded at a wavelength of 588 nm. The IC_{50} values were calculated as a sigmoidal dose-response curve using GraphPad Prism 4.03. The IC_{50} values equal the drug concentrations, at which vitality is 50 %[23,24].



1(a)



1(b)

Fig. 1—a) Ergosterol and b) Linoleic acid

Results and Discussion

During our ongoing search for bioactive compounds from microorganisms, four marine fungal strains were isolated and biologically screened to produce bioactive compounds. Antimicrobial activity testing of the crude extracts obtained from isolated strains (Table 1) was carried out against a set of microorganisms using the agar diffusion technique.

The strain QL23 showed high activity against Gram-positive *S. aureus* (12 mm) and Gram-negative *P. aeruginosa*, (12 mm) and *P. agaraci* (13 mm) and the fungus *Aspergillus niger* (14 mm) but showed moderate activity against Gram-positive *B. subtilis*

Table 1—Antimicrobial activities of QL23 crude extract and isolated compounds using agar diffusion test (mm diameter)

	EC[a]	BS[b]	Psa[c]	Ml[d]	Stw[e]	Sta[f]	Psae[g]	Ca[h]	Sac[i]	An[j]
QL23 crude extract	-	10	13	9	8	12	12	-	10	14
Ergosterol (1)	-	-	-	-	-	-	-	9	-	11
Linoleic acid (2)	-	12	-	10	8	-	-	-	-	-

[a]*E. coli* DSMZ 1058, [b]*Bacillus subtilis* DSMZ 704, [c]*Pseudomonas agarici* DSMZ 11810, [d]*Micrococcus luteus* DSMZ 1605, [e]*Staphylococcus warneri* DSMZ 20036; [f]*Staphylococcus aureus*; [g]*Pseudomonas aeruginosa*; [h]*Candida albicans*; [i]*Saccharomyces cerevisiae*; [j]*Aspergillus niger*(-) = no activity detected

and *S. cerevisiae*. Low activities were detected against two Gram-positive *M. luteus* and *S. warneri* (9 & 8 mm, respectively). The Strain QL23 has been selected for large scale fermentation. The classification of the strain QL23 has been deduced basically on its 18S rDNA gene sequencing. The strain QL23 was isolated from a sediment sample collected from Qarun Lake, El-Fayoum governorate, Egypt. Chemical studies on extract obtained from the strain using different chromatographic techniques afforded ergosterol. The chemical structures of these compounds were assigned according to 1D and 2D NMR and HR-EI mass spectrometry.

Genotypic identification of selected strain

A 999 bp DNA sequence for a single colony of strain QL23 has been amplified, since the phylogenetic tree indicates the existence of an inter-relationship between several related sequences to strain QL23. Consequently, phylogenetic analysis of 18S rDNA gene sequence confirmed the highly closed relation of the strain QL23 to *S. cholohalonata* (99 %), *S. chartarum* (99 %), *S. subreniforms* (99 %) *and S. xanthohalonata* (99 %). It has been confirmed that the strain QL23 belongs to the genus *Stachybotrys* spp. The results were supported by neighbour-joining based phylogenetic tree (Fig. 2). We have assigned its name as *Stachybotrys* sp. QL23. The strain QL23 has been recorded in GenBank database with accession no. KY348337.

Fermentation, working-up and structure elucidation

The strain QL23 was cultivated on solid rice medium. The methanol extract of the strain exhibited high activity against Gram-positive *S. aureus* and Gram-negative *P. aeruginosa* and moderate activity against Gram-positive *B. subtilis* and the yeast *S. cerevisiae* (Table I)**.** *In vitro* cytotoxicity assay of QL23 extract against the human cervix carcinoma cell line (KB-3-1), revealed that it has no activity. In the chemical screening monitored by TLC, the fungal extract exhibited numerous bands in a wide polarity range: some of them contained a group of non-UV-absorbing compounds detected as intensive violet to blue bands with anisaldehyde/sulfuric acid. Separation of metabolites produced by the strain using a series of chromatographic techniques afforded ergosterol and linoleic acid.

Ergosterol

Compound 1 was obtained as middle polar colorless solid, displaying an UV absorbance (254 nm) on Thin Layer Chromatography (TLC). On spraying with anisaldehyde/sulfuric acid, compound 1 exhibited a violet color turned into grey. An intensive study of compound 1 using different spectroscopic means including ^{1}H, ^{13}C NMR spectroscopy and EI-MS and comparison with related literatures, confirmed its structure as ergosterol[25]. Ergosterol was assayed against set of microorganisms revealing that it has low to moderate antifungal activity against

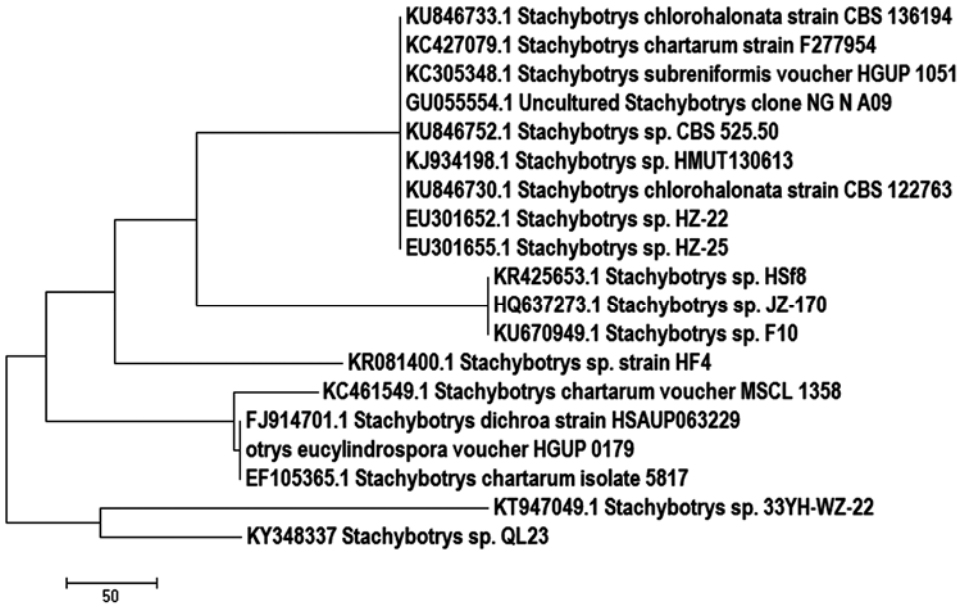


Fig. 2—Neighbour-joining phylogenetic tree of strain QL23 based on 18S rDNA gene sequence, showing its close relationship to Stachybotrys species

C. albicans and *A. niger*, respectively (Table 1); deducing that although the presence of ergosterol in the cell membrane of fungi, it may cause inhibition to some fungal species at certain concentrations.

Fungal sterols are widely reported from various fungal species such as *Aspergillus fumigates*[26], *Pleurotus ostreatus*[27] and from numerous *Emericella* strains[28-30]. Ergosterol, a principle and common constituent of the fungal species *Polyporus*, *Poria*, and mushroom, can act as therapeutic agent for mucosal mast cell-related diseases, at where it has high capability to suppress the degranulation of mBMMCS (Mucosal-type murine bone marrow-derived mast cells)[31]. Moreover, ergosterol was deduced to have *in vitro* anti-liver fibrosis[32].

Linoleic acid

Compound 2 was obtained as low polar colorless solid, exhibiting no UV absorbance or fluorescence. However, the compound was detected by spraying with anisaldehyde/sulfuric acid, exhibiting a violet staining turned later into blue. A Study of the spectroscopic properties of compound 2 using Nuclear Magnetic Resonance (^{1}H NMR, ^{13}C NMR) spectroscopy and Electrospray Ionization Mass Spectrometry (ESI-MS), and comparison with corresponding literature[33] confirmed its structure as linoleic acid. Linoleic acid from strain QL23 exhibited strong activity against Bacillus subtilis, moderate activity against *M. luteus* and low activity against *S. warneri*.

Linoleic acid is one of the essential fatty acids (EFA). EFA are group of unsaturated fatty acids which are necessary to human body functions and must be obtained from other sources[34]. These fatty acids have various medicinal properties and useful in some diseases such as cardiovascular diseases, skin permeability, insulin resistance, cancer and depression[35]. Recently, it has been reported that linoleic acid has antiplasmodial activity[36]. Moreover, Linoleic acid belongs to Omega 6 fatty acid[37,38]. The latter reduces symptoms of nerve pain in people with diabetic neuropathy, breast pains, blood pressure, rheumatoid arthritis and help in osteoporosis[39–42].

Stachybotrys sp. is one of biotechnologically important autochthonous filamentous fungi. The species of *Stachybotrys* were reported to produce β-farnesene, α-curcumene, β-bisabolene and cuparene compounds which are of great biological and pharmacological properties[43]. Major class of secondary metabolites produced by *Stachybotrys* species is represented by spirocyclic drimanes[44]. Many other new metabolites like stachyin A and stachyin B[45]. Moreover, many *Stachybotrys* species have been applied in various biotechnological applications. It has been reported to have a significant role in decreasing soil toxicity[46]. *Stachybotrys chartarum* was used to produce silver nanoparticles with antimicrobial activity[47]. Accordingly, this highlights the importance of these fungal species as a source of many bioactive compounds. Many approaches, such as using different media, variation in fermentation parameters, co-cultivation should be studied for the discovery of new drug leads from such interesting fungal species.

Acknowledgement

The authors are thankful to Prof (Dr) N. Sewald, Bielefeld University, for his lab facilities and support during the purification, structural elucidation of the obtained compounds. We thank NMR and MS Departments in Bielefeld University for the spectral measurements. This research work has been financed by the National Research Centre (NRC) foundation project (P101115).

References

1 Habbu P, Warad V, Shastri R and Madagundi S, Antimicrobial metabolites from marine microorganisms, *Chin J Nat Med*, 2016, **14**(2), 101-116.

2 Shaaban M, El-Metwally M M and Laatsch H, New bioactive metabolites from *Penicillium purpurogenum* M M, *Z Naturforsch B: Chem Sci*, 2016, **71**(4), 287-296.

3 Faulkner D J, Marine natural products, *Nat Prod Rep*, 1987, **4**(5), 539–576.

4 Faulkner D J, Marine natural products, *Nat Prod Rep*, 1988, **5**(6), 613-663.

5 Buss T and Hayes M A, in *Biodiversity: New leads for pharmaceutical and agrochemical industries*, edited by Wrigley S K, Hayes M A, Thomas R, Chrystal E J T and Nicholson N, (Royal Society of Chemistry, Cambridge), 2000, 75-85.

6 Strobel G, Daisy B, Castillo U and Harper J, Natural products from endophytic microorganisms, *J Nat Prod*, 2004, **67**(2), 257-268.

7 Wu Z, Liu D, Proksch P, Guo P and Lin W H, Punctaporonins H-M: caryophyllene-type sesquiterpenoids from the sponge-associated fungus *Hansfordia sinuosae*, *Mar Drugs*, 2014, **12**, 3904-3916.

8 Pang K L, Ovary D P, Jones E B G, Calado M L, Burgaud G, Walker A K, *et al.*, "Marine fungi' and 'marine-derived fungi' in natural product chemistry research: Toward a new consensual definition, *Fungal Biol Rev*, 2016, **30**(4), 163-175.

9 Zhou Y, Debbab A, Wray V, Lin W H, Schulz B, Trepos R, *et al.*, Marine bacterial inhibitors from the sponge-derived

fungus *Aspergillus* sp., *Tetrahedron Lett*, 2014, **55**, 2789-2792.

10 Butler M S, The role of natural product chemistry in drug discovery, *J Nat Prod*, 2004, **67**(12), 2141-2153.

11 Aw M M, Brown N W, Itsuka T, Gonde C E, Adams J E, Heaton N D, *et al*., Mycophenolic acid pharmacokinetics in pediatric liver transplant recipients, *Liver Transpl*, 2003, **9**(4), 383-388.

12 Paeshuyse J, Leyssen P, Mabery E, Boddeker N, Vrancken R, Froeyen M, *et al*., A novel, highly selective inhibitor of pestivirus replication that targets the viral RNA-dependent RNA polymerase, *J Virol*, 2006, **80**(1), 149-160.

13 Godtfredsen W, Roholt K and Tybring L, Fucidin: a new orally active antibiotic, *Lancet I*, 1962, **1**(7236), 928–931.

14 Grove J F, Macmillan J, Mulhol-Land T P C and Rogers M A T, Griseofulvin, 1952, *Int J Chem Soc*, 3949-3958.

15 Flisiak R, Horban A, Gallay P, Bobardt M, Selvarajah S, Wiercinska-Drapalo A, *et al*., The cyclophilin inhibitor Debio-025 shows potent anti-hepatitis C effect in patients coinfected with hepatitis C and human immunodeficiency virus, *Hepatology*, 2008, **47**(3), 817–826.

16 Brian P W and Hemming H G, Production of antifungal and antibacterial substances by fungi;preliminary examination of 166 strains of Fungi Imperfecti, *J gen Microbiol*, 1947, 158-167.

17 Synytsya A, Monkai J, Bleha R, Macurkova A, Ruml T, Ahn J, *et al.*, Antimicrobial activity of crude extracts prepared from fungal mycelia, *Asian Pac J Trop Biomed*, 2017, **7**(3), 257-261.

18 Butti R, Guntagani S and Muvva V, Antimicrobial profile of *Stachybotrys chlorohalonata* isolated from the marine habitats, *J Pharm Res*, 2014, **8**(8), 1059-1066.

19 Zhang Y, Tian R, Liu S, Chen X, Li X and Che Y, Alachalasins A–G, new cytochalasins from the fungus *Stachybotrys charatum*, *Bioorg Med Chem,* 2008, **16**, 2627–2634.

20 Kumar V, Bharti A and Gusain O, An improved method for isolation of genomic DNA from filamentous actinomycete, *Bisht J Sci Engg Tech Mgt*, 2010, **2**(2), 10-13.

21 Shaaban M, Abdel-Razik A S, Abdel-Aziz M S, Abou Zied A A and Fadel M, Bioactive secondary metabolites from marine *Streptomyces albogriseolus* isolated from red sea coast, *J Appl Sci Res*, 2013, **9**(3), 996-1003.

22 Baur A W, Kirby W M, Sherris J C and Truck M, Antibiotic susceptibility testing by a standardized single disk method, *Am J Clin Pathol*, 1966, **45**(4), 493-496.

23 Awantu A F, Lenta B N, Bognerb T, Fonganga Y F, Ngouelaa S, Wansib J D, *et al*, Dialiumoside, an Olean-18-ene Triterpenoid from *Dialium excelsum, Zeitschrift für Naturforschung B*, 2011, **66**(6), 624-628.

24 Sammet B, Bogner T, Nahrwold M, Weiss C and Sewald N, Approaches for the synthesis of functionalized cryptophycins, *J Org Chem*, **75**(20), 2010, 6953-6960.

25 Nagia M M S, El-Metwally M M, Shaaban M, El-Zalabani S M and Hanna A G, Four butyrolactones and diverse bioactive secondary metabolites from terrestrial *Aspergillus flavipes* MM2: Isolation and structure determination, *Org Med Chem Lett*, 2012, **2**(1), 1-8.

26 Alcazar-Fuoli L, Mellado E, Garcia-Effron G, Lopez J F, Grimalt J O, Cuenca-Estrella J M, *et al*., Ergosterol biosynthesis pathway in *Aspergillus fumigates*, *Steroids*, 2008, **73**(3), 339-347.

27 Ramos-Ligonio A, Lopez-Monteon A and Trigos A, Trypanocidal activity of ergosterol peroxide from *Pleurotus ostreatus*, *Phytother Res*, 2012, **26**(6), 938-943.

28 Kawahara N, Nakajima S, Satoh Y, Yamazaki M and Kawai K, Studies on fungal products. XVIII.: Isolation and structures of a new fungal depsidone related to nidulin and a new phthalide from *Emericella unguis*, *Chem Pharm Bull*, 1988, **36**(6), 1970-1975.

29 Liangsakul J, Pornpakakul S, Sangvichien E, Muangsin N and Sihanonth P, Emervaridione and varioxiranediol, two new metabolites from the endophytic fungus, *Emericella variecolor*, *Tetrahedron Lett*, 2011, **52**, 6427-6430.

30 Malmstom J, Christophersen C, Barrero A F, Oltra J E, Justicia J and Rosales A, Bioactive metabolites from a marine-derived strain of the fungus *Emericella variecolor*, *J Nat Prod*, 2002, **65**(3), 364-367.

31 Kageyama-Yahara N, Wang P, Wang X, Yamamoto T and Kadowaki M, The inhibitory effect of ergosterol, a bioactive constituent of a traditional Japanese herbal medicine saireito on the activity of mucosal-type mast cells, *Biol Pharm Bull*, 2010, **33**(1), 142-145.

32 Peng Y, Tao Y, Wang Q, Shen L, Yang T, Liu Z, *et al*., Ergosterol is the active compound of cultured mycelium *Cordyceps sinensis* on antiliver fibrosis, *Evid Based Complement Alternat. Med*, 2014, **4**(1), 1-12.

33 Shaaban M, *Bioactive secondary metabolites from marine and terrestrial bacteria: Isoquinoline quinones, bacterial compounds with a novel pharmacophor*, Ph D Thesis, Georg-August University, Göttingen, 2004.

34 Das U N, Essential fatty acids: Biochemistry, physiology, and pathology, *Biotechnol J*, 2006, **1**, 420-439.

35 Undurti N D, Can essential fatty acids reduce the burden of disease(s)?, *Lipids Health Dis*, 2008, **7**(9), 1-5.

36 Melariri P, Campbell W, Etusim P and Smith P, *In vitro* and *in vivo* antimalarial activity of linolenic and linoleic acids and their methyl esters, *Adv Stud Biol*, 2012, **4**(7), 333-349.

37 Gaullier J M, Halse J, Hoye K, Kristiansen K, Fagertun H, Vik H, *et al*., Supplementation with conjugated linoleic acid for 24 months is well tolerated by and reduces body fat mass in healthy overweight humans, *J Nutr*, 2005, **135**(4), 778-784.

38 Larsen T M, Toubro S and Astrup A, Efficacy and safety of dietary supplements containing CLA for the treatment of obesity: evidence from animal and humans studies, *J Lipid Res*, 2003, **44**(12), 2234-2241.

39 Harris W, Omega-6 and omega-3 fatty acids: partners in prevention, *Curr Opin Clin Nutr Metab Care*, 2010, **13**(2), 125-129.

40 Kast R E, Borage oil reduction of rheumatoid arthritis activity may be mediated by increased cAMP that suppresses tumor necrosis factor-alpha, *Int Immunopharmacol*, 2001, **1**(12), 2197-2199.

41 Kenny F S, Pinder S E, Ellis I O, Gee J M, Nicholson R I, Bryce R P, *et al*., Gamma linolenic acid with tamoxifen as primary therapy in breast cancer, *Int J Cancer*, 2000, **85**(5), 643-648.

42 Vanhala M, Saltevo J, Soininen P, Kautiainen H, Kangas A J, Ala-Korpela *et al*., Serum omega-6

polyunsaturated fatty acids and the metabolic syndrome: a longitudinal population-based cohort study, *Am J Epidemiol*, 2012, **176**(3), 253-260.

43 Wilkins K, Nielsen K F, and Din S U, Patterns of volatile metabolites and non-volatile trichothecenes produced by isolates of *Stachybotrys*, *Fusarium*, *Trichoderma*, *Trichothecium* and *Memnoniella*, *Environ Sci Pollut Res*, **10**, 162-166.

44 Jarvis B B, Salemme J and Morais A, *Stachybotrys* toxins. 1, *Nat Toxins*, 1995, **3**(1), 10-6.

45 Wu B, Oesker V, Wiese J, Malien S, Schmaljohann R and Imhoff J F, *Spirocyclic drimanes* from the marine fungus *Stachybotrys* sp. strain MF347, *Mar Drugs*, 2014, **12**(4), 1924-1938.

46 Annibale A D, Rosetto F, Leonardi V, Federici F, and Petruccioli M, Role of autochthonous filamentous fungi in bioremediation of a soil historically contaminated with aromatic hydrocarbons, 2006, *Appl Environ Microbiol*, **72**(1), 28-36.

47 Abdelghany T, *Stachybotrys chartarum*: A novel biological agent for the extracellular synthesis of silver nanoparticles and their antimicrobial activity, 2013, *Indones J Biotechnol*, **18**(2), 75-82.