Dimeric naphtho-γ-pyrones and further diverse bioactive metabolites from the marine-derived *Aspergillus flavus* Af/MMA 2018

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Background and objective
Sponge-associated fungi are known for their production of structurally diverse secondary metabolites, many of which exhibit different pharmacological activities.
Materials and methods
Isolation and identification of fungal isolate from the marine sponge *Echinodictyum flabelliforme* collected from the Red Sea coast of Hurghada, Egypt, was done. Working up and purification with the ethyl acetate extract produced by the marine-derived *Aspergillus flavus* Af/MMA 2018 afforded nine bioactive compounds. Structure of the isolated compounds was determined on the basis of NMR (1D and 2D) and mass (EI, ESI, HRESI MS) spectra and by comparison with the corresponding literature studies. Biologically, the antimicrobial, antioxidant, and antitumor activities (using Ehrlich cells) of compounds were studied in comparison with the original extract.
Results and conclusion
Working up and purification of the ethyl acetate-extracted residue produced by the marine-derived *A. flavus* Af/MMA 2018 afforded nine diverse bioactive compounds: five dimeric naphtho-γ-pyrones, that is, aurasperone A (1), aurasperone B (2), aurasperone D (3), aurasperone F (4), and aurasperone E (5), along with β-sitosterol glucoside (6), cerebroside C (7), glyceryl linoleate (8), and linoleic acid (9). Cerebroside C showed strong antimicrobial activity against different test organisms, whereas aurasperone E showed maximum DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) scavenging activity (67.41%) after 1 h, and by using different concentrations, giving 98.99% at 1000 μg/ml. The maximum antitumor activity against Ehrlich ascites carcinoma cells (70.9%) was attributed to the dimeric naphtho-γ-pyrone aurasperone E.

Keywords:
Aspergillus flavus, biological activities, dimeric naphtho-γ-pyrones, marine-derived fungus

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Introduction

Marine microbes, especially fungi, have long been recognized as a potential source of new and biologically effective metabolites [1,2]. A large number of structurally diverse and bioactive fungal metabolites were isolated and characterized. Some of these were used for the development of valuable pharmaceuticals and pesticides [3–5]. Endophytic fungi have been occasionally investigated as a source of diverse potent bioactive compounds [6,7]. The most common endophytes are Ascomycota anamorphic members, and some are closely related to fungi that cause diseases in plants and animals [8]. *Aspergillus* spp. represent a valuable source of wide range of secondary metabolites used in diverse fields. *A. flavus* is one of the most important fungal species located in tropical environments owing to its industrial use and toxigenic potential [9]. Several diverse structural categories of bioactive secondary metabolites were reported from *A. flavus*, namely, pyranone, anthraquinone, anthrone, isocoumarin, pyrazinone-hydroxamic acid, and epithiodiketopiperazine [10].

In this study, *A. flavus* strain, isolated from the marine sponge *Echinodictyum flabelliforme*, was selected owing to its interest on the basis of our carried out chemical and biological screening studies. This encouraged us to identify its taxonomy and apply it to a large-scale fermentation for working up and purifying its desired secondary metabolites on the basis of diverse chromatographic techniques. In accordance, five dimeric naphtho-γ-pyrones, aurasperones A–B (1–2) and aurasperones D-F (3–5), along with β-sitosterol glucoside (6), cerebroside C (7), glyceryl linoleate (8), and linoleic acid (9) were obtained, and their

antimicrobial, antioxidant, and antitumor efficiency based on Ehrlich's cells were studied as well.

Materials and methods

The NMR spectra were measured on a Bruker AMX 300 (Gottingen, Germany) (300.135 MHz), a Varian Unity 300 (300.145 MHz), and a Varian Inova 500 (125.820 MHz) spectrometer. ESI MS was recorded on a Finnigan LCQ with quaternary pump Rheos 4000 (Flux Instrument). EI mass spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorkerosine as reference substance for EI HRMS. Flash chromatography was carried out on silica gel (230–400 mesh). R_f values were measured on Polygram SIL G/UV_{254} (Macherey-Nagel & Co., Gottingen, Germany). Size-exclusion chromatography was done on Sephadex LH-20 (Pharmacia).

Sampling and isolation of the fungal strain

The marine sponge *E. flabelliforme* was collected from the Red Sea coast of Hurghada, east Egypt (geographical coordinates: Latitude 27° 15' 26N, Longitude 33° 48' 46 E) at a depth of ~30 m. Small pieces of the dark violet marine sponge *E. flabelliforme* were rinsed three times with sterilized sea water to remove all loosely attached bacteria, and then aseptically cut into smaller pieces with sterile scalpel to reach the inner tissue surface. Next, 5 ml of sterilized sea water was added to each sample and incubated for 30 min into a reciprocal water bath at 30°C [11]. A serial of tenfold dilution was made with sterile sea water and plated (100 µl) on prepared potato dextrose agar medium (potato infusion, 200 g; dextrose, 20 g; and agar, 20.0 g and 1000 ml of 50% sea water, pH 6.0). The obtained agar plates were then incubated at 28°C for 6–8 weeks. The grown colonies with distinct morphological characteristics were picked up and transferred to other plates, followed by incubation for further 10 days, and periodically checked for culture purity. Then, they were stored in a refrigerator at 4°C [12]. A pure culture of the fungal isolate was deposited in the Microbial Biotechnology Department, NRC, Egypt, until further investigation.

Prescreening

The two fungal isolates FA and FB, obtained from the marine sponge *E. flabelliforme*, were fermented on a small scale on rice-solid media at 30°C for 7 days. After incubation, the culture media of both strains were individually extracted with ethyl acetate, followed by decantation and filtration. The organic extracts were concentrated *in vacuo* and then subjected to biological (antimicrobial, antioxidant, and Ehrlich's antitumor activities) and chemical screening (during thin layer chromatography (TLC), visualized by UV and spraying reagents).

Taxonomic identification of the producing fungus

For identification of the fungus, it was grown on Czapek-Dox medium (10 g/l glucose, 1 g/l $NaNO_3$, 1 g/l KCl, 0.5 g/l KH_2PO_4, 0.5 g/l $MgSO_4.7H_2O$, 1 ml, 20 g/l agar and 1000 ml of 50% sea water, pH 6.0) at 28°C for 7 days. Identification of the selected fungus isolate (FA) was carried out using the morphological characteristics, microscopic examination and DNA characterization. According to the morphological properties, the colony diameter, color of conidia, extracellular exudates, pigmentation, and the color of reverse mycelium of the fungus were characterized [13]. The microscopic features of the fungus were examined as well, showing conidial heads, fruiting bodies, degree of sporulation, and the homogeneity characteristics of conidiogenous cells by optical light microscope (10×90) Olympus CH40 [14]. However, taxonomic identification of the fungal strains was achieved by DNA amplification and sequencing of the fungal ITS region [15]. Pure isolated PCR product was sequencing together with the primer ITS1 (5/-TCCGTAGGTGAACCTGCGG-3/)/ITS4 (5/-TCCTCCGCTTATTGATATGC-3/). The 18S rRNA gene sequence was aligned using BLAST available at NCBI database (GenBank C, www.ncbi.nlm.nih.gov/Genbank/National Institute of Biotechnology Information, Bethesda, Maryland, USA). The phylogenetic tree was constructed using neighbor-joining tree method using the software MEGA7.

Large-scale fermentation and working up

The well-grown single colonies of *A. flavus* Af/MMA 2018 (FA) were inoculated into 100 ml of International System Project (ISP2) medium (g/l) [malt extract (10); yeast extract (4); glucose (4); 50% natural sea water; pH 6.0] and subjected to cultivation on shaker at 30°C for 3 days. The grown seed culture was served to inoculate 5×1 l Erlenmeyer flasks, each containing 100 g commercial rice and 150 ml 50% natural sea water. The seeded culture medium was then applied to static incubation at 28°C for 14 days [16]. After harvesting, the obtained yellowish-brown culture was soaked in ethyl acetate, followed by decantation and filtration. The remaining solid residue was resoaked in methanol followed by filtration. The aqueous methanol extract was concentrated *in vacuo*, and the remaining water residue was re-extracted with ethyl acetate. The

obtained unique yellowish-orange organic extracts were concentrated to dryness to yielding 6.5 g of a reddish-brown crude extract.

Isolation of the active constituents

The obtained extract was applied to column chromatography on silica gel eluted by cyclohexane-CH_2Cl_2-MeOH gradient and monitored by TLC to afford five fractions: I (0.62 g), II (0.51 g), III (1.59 g), IV (1.98 g), and FV (0.80 g). Fraction I was re-purified on silica gel column (DCM) followed by Sephadex LH-20 (DCM/40% MeOH) to afford colorless, semisolid linoleic acid (9, 16 mg) and colorless oil of glyceryl linoleate (8, 525 mg). Application of fraction II to PTLC (DCM/3% MeOH) followed by purification on Sephadex LH-20 (DCM/40% MeOH) led to isolation of two yellow solids of aurosperones: A (1, 13.5 mg) and B (2, 2 mg). Fraction III was purified using a silica gel column (DCM-MeOH) followed by PTLC (DCM/5% MeOH) and then Sephadex LH-20 (DCM/40% MeOH) to give further two yellow solids of aurasperones: D (3, 1 mg) and E (5, 5 mg). Purification of fraction IV via PTLC (DCM/6% MeOH) followed by Sephadex (MeOH) yielded aurasperone F (4, 24 mg) as fifth yellow solid. Purification of the polar fraction on silica gel column eluted with DCM-MeOH, followed by purification on Sephadex LH-20 (MeOH) resulted in two colorless solids of β-sitosterol glucoside (6, 4.8 mg) and cerebroside C (7, 15.9 mg). Spectroscopic data of the isolated compounds (1–9) are present in attached file 'Supplementary Data.'

Biological activity

Antimicrobial activity

The compounds 1–9 were dissolved in CH_2Cl_2/10% MeOH at a concentration of 1 mg/ml. Aliquots of 40 µl were soaked on filter paper discs (6 mm ?) and dried for 1 h at room temperature under sterilized conditions. The paper discs were placed on inoculated agar plats and incubated for 24 h at 37 °C for bacterial and 48–72 h (30°C) for the fungal isolates. For the fungal extract examination and the desired compounds, representative test microbes, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger*, were served. Isolates were obtained from the Microbial Biotechnology Department, NRC, Egypt. Both bacterial and yeast strains, they were grown on nutrient agar medium (g/l): beef extract 3; peptone 10; and agar 20. The pH was adjusted to 7.2. The fungal strain was grown on Czapek-Dox medium. The disc diffusion test has been done according to Bauer *et al.* [17].

Assessment of antioxidant activity

The free radical scavenging activity was assessed by the decoloration of DPPH radical solution evaluated spectrophotometrically at λ_{max}517 nm [18].

Assessment of the antitumor activity against Ehrlich cells

This test was performed using in-vitro assay. Viability of tumor percentages of tumor cells which was measured by modified cytotoxic trypan blue exclusion technique [19]. The in-vitro results were expressed as the inhibition ratio of tumor cell proliferation calculated as follows: the inhibition ratio of tumor cell proliferation (%)= [(A–B)/A]×100, where A and B are the average numbers of viable tumor cells of the control and the samples, respectively.

Results and discussion

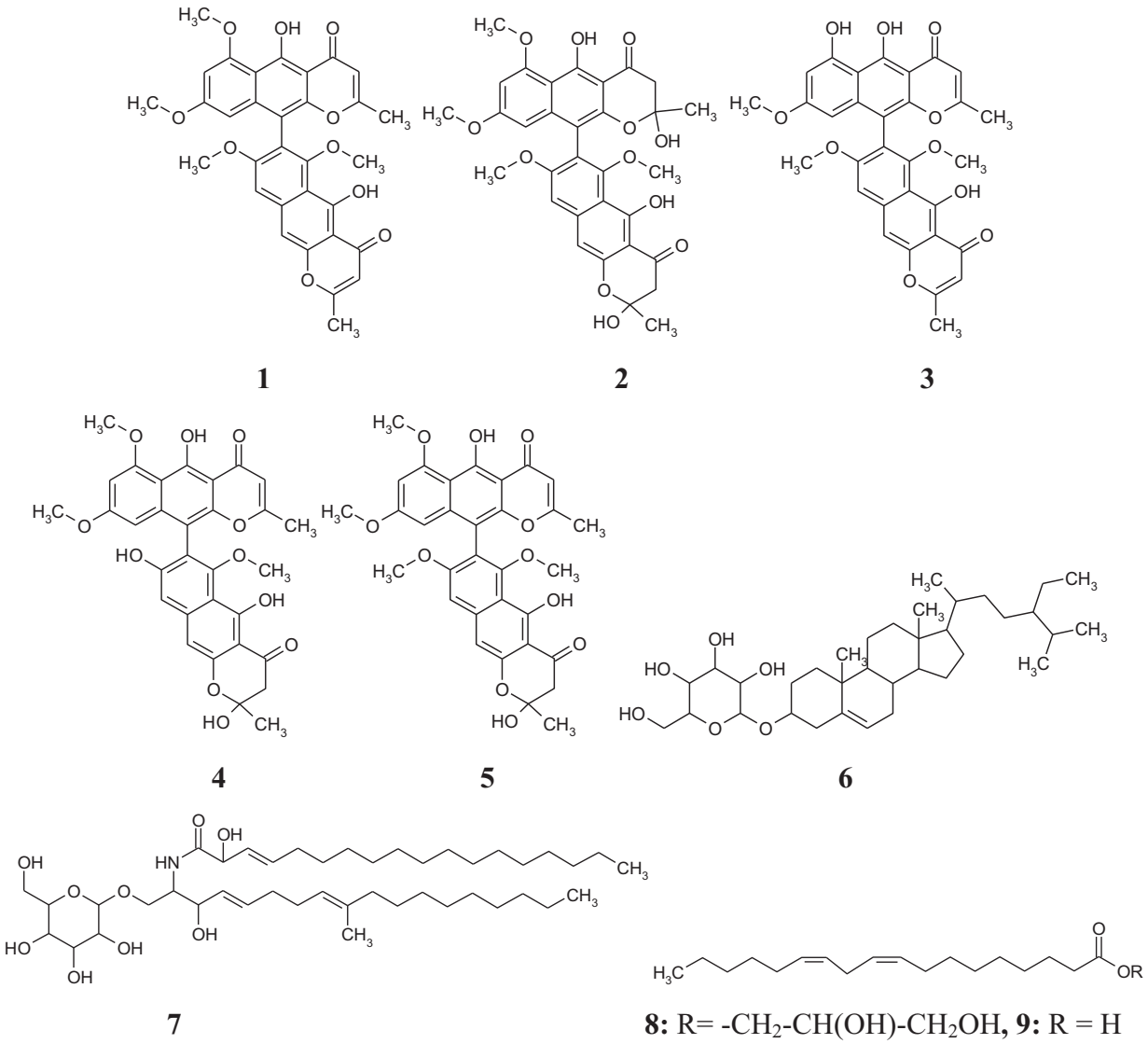
Isolation and prescreening study

During our searching for bioactive compounds from rare marine-derived fungal strains, two isolates FA and FB were isolated from marine sponge *E. flabelliforme* collected from the Red Sea coast at Hurghada, Egypt. Based on the biological prescreening, the crude extracts of both fungal isolates FA and FB (Table 1) showed high similarity in their moderate activity against *S. aureus*, *B. subtilis*, *P. areuginosa*, and *C. albicans*, meanwhile both extracts exhibited no activity against *E. coli* and *A. niger*. Alternatively, on studying the antioxidant activity of both extracts, it was shown that the fungal strain FA (conc. 1 mg/ml) exhibited higher antioxidant (93.6%) activity than those revealed by fungal extract FB (53.3%). Likewise, an antitumor testing of both extracts based on Ehrlich cells and antitumor activity, the fungal strain FA showed higher potency (71.8%) than those displayed by FB (32.8%). Therefore, the fungal strain FA was selected for a broad investigation and large-scale study.

Table 1 Antimicrobial activity of the crude extracts of fungal isolates (clear zone in mm)

Fungal extract	Gram positive		Gram negative		Yeast	Fungi
	Staphylococcus aureus	*Bacillus subtilis*	*Pseudomonas areuginosa*	*Escherichia coli*	*Candida albicans*	*Aspergillus niger*
FA	16	20	16	00	21	00
FB	18	21	18	00	21	00

Figure 1



Chemical structures of the fungal metabolites 1–9.

Taxonomical characterization of the producing fungus

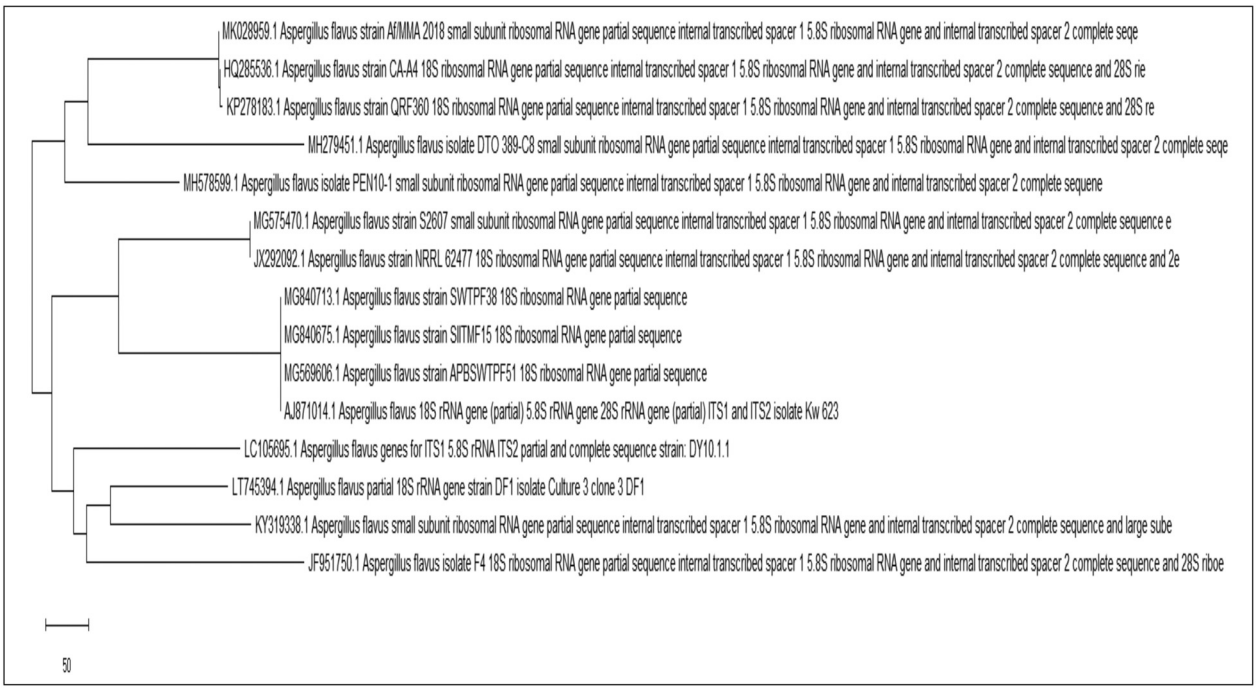
The selected fungal isolate (FA) was morphologically identified on Czapek-Dox agar medium. Based on its evaluation with the aid of light microscopic examination (Fig. S69, supplementary data), the colonies are flat, granular, with radial grooves, at first yellow but with age they are becoming bright to dark yellowish green. Typically, conidial heads radiate and then split into loose columns (mostly 300–400 μm in diameter). They are biseriate but with some heads directly on the vesicle with phialides (uniseriate). Conidiophore stipes are hyaline and rough. Conidia are globose to subglobose (diameter 3–6 μm), and this referred that isolate FA belonged to the *A. flavus* [13]. A subsequent phylogenetic analysis based on the 18S rRNA gene sequence of isolate FA was compared with reference. 18S rRNA quality arrangement was accessible in the GenBank and EMBL database acquired from the National Centre of Biotechnology Data database utilizing BLAST search (http://ncbi.nlm.nih.gov/BLAST/), establishing it as *A. flavus* Af/MMA 2018, having the accession number MK028959 (Figs 1 and 2).

Fermentation and structure elucidation

The fungus *A. flavus* Af/MMA 2018 was upscale cultivated on solid rice medium. After harvesting and scale up, the afforded crude extract was purified using a series of chromatographic techniques to deliver the mentioned nine secondary metabolites (1–9).

Structures of the compounds (1–9) were confirmed on the basis of different spectroscopic means (NMR and MS) (see supporting information) and comparison with the corresponding literatures. They were classified into five dimeric naphtho-γ-pyrones of

Figure 2



Phylogenetic tree of *Aspergillus flavus* Af/MMA 2018.

middle polarities and yellow solid appearance, namely, aurasperone A (1) [20,21], aurasperone B (2) [20,21], aurasperone D (3) [22,23], aurasperone F (4) [21], aurasperone E (5) [20]; one steroidal glycoside, β-sitosterol glucoside (6) [24]; a sphingolipid, cerebroside C (7) [25], bearing two extra methylene groups of 28 Daltons higher than cerebroside A (*m/z*: 725) [26]; and the fatty acid analogs, glyceryl linoleate (8) [26], and linoleic acid (9) [26].

Naphtho-γ-pyrones

Monomeric and dimeric naphtho-γ-pyrones are widespread in nature, especially in higher plants [23] (e.g. those belonging to the genera *Senna*, *Cassia*, and *Paepalanthus bromelioides*), and filamentous fungi (e.g., *Fusarium* and *Aspergillus*) [23] and recently from *Alternaria* [21]. The presence of monomeric flavosperones has been shown in a wide variety of earlier investigational studies on *A. niger, which* collectively revealed rubrofusarins [22,27], fonsecins [22,27], dimeric fonsecinones [28], aurasperones [22], nigerones [29], and asperpyrones [28], naphtho-γ-pyrone pigments. It is worthy herein to refer that this is the first time to report the dimeric naphtho-γ-pyrones (1–5) from *A. falvus*.

This class of compounds has drawn the attention of several research groups owing to their wide range of biological activities (antimicrobial, antimycobacterial, hepatoprotective, antimutagenic, antioxidant, cytotoxic, antitumor, antiallergic, reversal multidrug resistance of human epidermal KB carcinoma cells, strong hypotensive activity in cats, acute toxicity to mice and rats which act as a central nervous system depressant, interleukin-4 signal transduction inhibitor, inhibitor of Taq DNA polymerase, and HIV-1 integration inhibitor) [23].

β-sitosterol glucoside

Sterols represent the first choice of potential natural preventive dietary products. β-sitosterol is a phytosterol, structurally similar to cholesterol and is well spread in plants, fungi (e.g. endophytic *Trichoderma* spp. [30] and *Talaromyces purpureogenus* [31]), and animals [24]. As a secondary metabolite, it is used as a health-promoting constituent of natural foods. European food safety authority recommends that around 1.5 to 2–4 g/day be consumed of phytosterol in order to reduce blood pressure. In addition, for reducing the risk of heart attack, the US Food and Drug Administration has approved the role of foods containing phytosterol esters, and a low saturated fat and cholesterol diet. β-sitosterol-β-D-glucoside (6) has been proposed as a useful candidate for the development of new drugs to treat endotoxemia and inflammation in conjunction with nitric oxide. This compound reduces production of nitric oxide from RAW 264.7 cells induced by lipopolysaccharide [24]. In addition, it strongly inhibits the interleukin-6 activities of stimulated macrophages [32,33].

Cerebrosides

Cerebrosides (sphingolipids) consist of a hydrophobic part called ceramide, which is linked to one sugar moiety. Sphingolipids are widespread. They are the components of all eukaryotic cell membranes and abundant in plasma membranes. They play an important role in major cellular processes such as growth, cell differentiation, and morphogenesis [34]. In animals, they play important roles in general function of membrane, cell recognition, cell-to-cell contact, cell growth regulation, differentiation, and apoptosis. Sphingolipids justice transmembrane signal transduction via their effects on protein kinases linked with growth factor receptors and protein kinase C [25]. Recently, it has been discovered that sphingolipid metabolic products, ceramides, act as second messengers in pathway of the signal transduction involved in apoptosis. According to the literature studies, cerebrosides appear to be present almost in the most common and recently studied fungal producers, so far, they are known to function as cell differentiation inducers [35].

Linoleic acid derivatives

Linoleic acid and its derivatives are the main essential unsaturated fatty acids (EFA) that belong to Omega 6 fatty acids [36,37]. The latter are necessary to human body physiological processes and must be provided from other sources. These fatty acids have several medicinal applications and are useful in the treatment of some diseases such as cardiovascular diseases, skin permeability, insulin resistance, cancer, and depression [38]. In addition, linoleic acid has been recently reported to have antiplasmodial activity [39]; reduces symptoms of nerve pain in people with diabetic neuropathy, breast pains, blood pressure, and rheumatoid arthritis; and aids in osteoporosis [36].

Biological activity studies

Compounds (1–9) obtained from the fungus *A. flavus* Af/MMA 2018 were antimicrobially tested using paper-disk method (Table 2). According to this study, only cerebroside C (7) showed antimicrobial activity. The latter (7) has strong activity against gram-negative *P. aeruginosa*, gram-positive *S. aureus* and *B. subtilis*, and low activity against the yeast *C. albicans*, and no activity against *E. coli* and *A. niger*. Cerebroside derivatives reported from the marine fungus *A. flavus* and *Spathodea campanulata* have antibacterial activity against *S. aureus*, methicillin-resistant *S. aureus*, and multidrug-resistant *S. aureus* [40,41]. Alternatively, cerebrosides reported from *Fusarium* spp. were inactive against *Trichophyton rubrum* and *C. albicans*, whereas they showed strong antibacterial activities against *B. subtilis*, *E. coli*, and *Pseudomonas fluorescens* [42].

By assessing compounds (1–9) for antioxidant activity at a concentration of 200 μg/ml (Table 3), aurasperone E (5) showed maximum DPPH scavenging activity (67.41%), whereas the latter displayed the highest DPPH scavenging activity (98.99%) at concentration of 1000 μg/ml (Fig. 3). On the contrary, the other studied compounds (1–4, 6–9) exhibited no antioxidant activity.

A study of the antitumor activity of the obtained compounds (1–9) against Ehrlich ascites carcinoma cells [43] was carried out at concentration of 200 μg/ml (Table 4). Based on this study, aurasperone E (5) displayed the highest antitumor activity (70.9%) as well, whereas the other compounds displayed low activity. Aurasperone E (5), as one of the dimeric naphtho-γ-pyrones, is a secondary metabolite of industrial interest, mainly produced by filamentous fungi as *Aspergillus* spp., reporting various biological

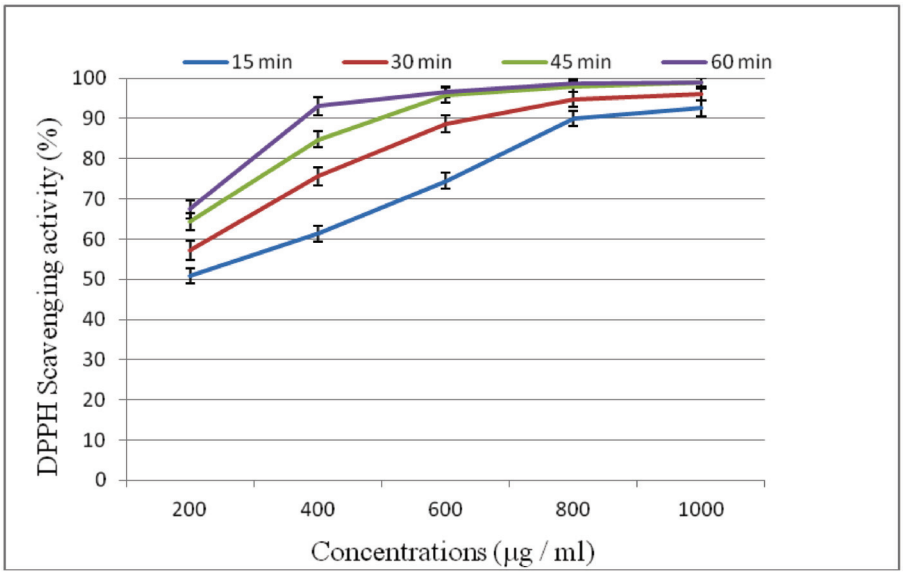
Table 2 Antimicrobial activity of compounds (1–9) (clear zone in mm)

Pure compounds	Gram positive		Gram negative		Yeast	Fungi
	Staphylococcus aureus	*Bacillus subtilis*	*Pseudomonas areuginosa*	*Escherichia coli*	*Candida albicans*	*Aspergillus niger*
Aurasperone A (1)	00	00	00	00	00	00
Aurasperone B (2)	00	00	00	00	00	00
Aurasperone D (3)	00	00	00	00	00	00
Aurasperone F (4)	00	00	00	00	00	00
Aurasperone E (5)	00	00	00	00	00	00
β-Sitosterol glucoside (6)	00	00	00	00	00	00
Cerebroside C (7)	18	18	16	00	10	00
Glycerol linoleate (8)	00	00	00	00	00	00
Linoleic acid (9)	00	00	00	00	00	00

Table 3 DPPH scavenging activity (%) of the pure compounds (1–9)

Compounds	Time (min.)			
	15	30	45	60
Aurasperone A (1)	17.05±1.31	20.71±1.22	25.70±2.10	29.66±1.99
Aurasperone B (2)	13.09±1.52	13.62±1.53	17.16±2.22	18.59±2.15
Aurasperone D (3)	15.77±1.97	17.78±1.74	22.01±1.84	22.80±1.97
Aurasperone F (4)	9.24±1.76	11.67±2.38	15.91±1.51	13.71±1.68
Aurasperone E (5)	50.77±2.14	57.21±1.87	64.34±1.25	67.41±1.54
β-Sitosterol glucoside (6)	14.97±2.48	22.82±1.59	25.57±1.33	26.55±1.98
Cerebroside C (7)	12.09±1.89	14.81±1.64	14.99±1.54	17.74±1.55
Glycerol linoleate (8)	9.38±2.12	15.33±1.15	15.44±2.05	17.32±2.45
Linoleic acid (9)	11.49±1.45	16.61±1.96	16.70±2.55	17.50±2.13

Figure 3



DPPH scavenging activity at different concentrations of aurasperone E (5).

Table 4 Antitumor activity (%) of the pure compounds (1–9)

Compounds	Concentrations (µg/ml)				
	200	400	600	800	1000
Aurasperone A (1)	10.2±1.4	14.5±1.6	16.7±1.9	17.4±1.7	19.1±1.8
Aurasperone B (2)	8.3±1.9	12.7±0.8	15.1±1.2	18.3±1.4	20.5±1.3
Aurasperone D (3)	13.7±1.5	19.1±1.7	26.7±2.1	30.1±1.9	31.9±1.7
Aurasperone F (4)	11.4±1.6	17.4±1.3	23.3±1.0	29.5±1.6	34.6±1.5
Aurasperone E (5)	36.5±1.8	45.2±1.1	56.4±1.2	63.7±1.4	70.9±1.6
β-sitosterol glucoside (6)	17.9±2.1	24.8±1.4	30.7±2.0	34.6±2.1	38.5±1.2
Cerebroside C (7)	21.1±2.6	28.9±2.1	36.9±1.8	40.4±1.8	42.3±1.1
Glycerol linoleate (8)	16.7±1.2	21.7±1.9	26.3±1.3	30.2±1.6	33.8±1.3
Linoleic acid (9)	25.3±2.3	32.5±2.1	38.1±1.7	45.3±1.5	49.7±1.0

activities including antioxidant and anti-cancer potentialities [44].

Conclusion

In this paper, we report isolation and identification of *A. flavus* Af/MMA 2018 from marine sponge *E. flabelliforme* collected from the Red Sea. Then, purification with the ethyl acetate extract afforded nine diverse bioactive compounds: five dimeric naphtho-γ-pyrones, i.e., aurasperone A (1), aurasperone B (2), aurasperone D (3), aurasperone F (4), and aurasperone E (5), along with β-sitosterol glucoside (6), cerebroside C (7), glycerol linoleate

(8), and linoleic acid (9). Biologically, cerebroside C (7) showed strong antimicrobial activity against different test organisms. Aurasperone E (5) showed maximum DPPH scavenging activity and maximum antitumor activity by Ehrlich ascites carcinoma cells.

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Conflicts of interest

There are no conflicts of interest.

References

1 Saleem M, Ali MS, Hussain S, Jabbar A, Ashraf M, Lee YS. Marine natural products of fungal origin. Nat Prod Rep 2007; 24:1142–1152.

2 Kossuga MH, Romminger S, Xavier C, Milanetto MC, do Valle MZ, Pimenta EF, *et al.* Evaluating methods for the isolation of marine-derived fungal strains and production of bioactive secondary metabolites. Rev Bras Farmacogn 2012; 22:257–267.

3 Rodriguez KF, Hesse M, Werner C. Antimicrobial activities of secondary metabolites produced by endophytic fungus of *Spondia mombin*. J Basic Microbiol 2000; 40:261–267.

4 Taechowisan T, Shen LY, Lumyong S. Secondary metabolites from endophytic Streptomyces aureofaciens CMUAc130 and their antifungal activity. Microbiology 2005; 151:1691–1695.

5 Onifade AK. Research trends: bioactive metabolites of fungal origin. Res J Biol Sci 2005; 2:81–84.

6 Carroll G. Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. Ecology 1988; 69:2–9.

7 Tanseer S, Anjum T. Modification of c and n sources for enhanced production of cyclosporin'a'by *Aspergillus terreus*. Braz J Microbiol 2011; 42:1374–1383.

8 Wilson D. Endophyte the evolution of a term, and clarification of its use and definition. Oikos 1995; 73:274–276.

9 Rodrigues P, Soares C, Kozakiewicz Z, Paterson RRM, Lima N. Identification and characterization of Aspergillus flavus and aflatoxins. In Communicating Current Research and Educational Topics and Trends in Applied MIcrobiology. In: Méndez-Vilas A, ed. Badajoz, Spain: FORMATEX; 2014; pp. 527–534.

10 Cary JW, Gilbert MK, Lebar MD, Majumdar R, Calvo AM. Aspergillus flavus secondary metabolites: more than just aflatoxins. Food Safety 2018; 6:7–32.

11 Hamed A, Abdel-Razek AS, Frese M, Wibberg D, El-Haddad AF, Ibrahim TMA, *et al.* New oxaphenalene derivative from marine-derived *Streptomyces griseorubens* sp. ASMR4. Z Naturforsch B 2017; 72:53–62.

12 Debbab A, Aly AH, Edrada-Ebel R, Müller WEG, Mosaddak M, Hakiki A, *et al.* Bioactive secondary metabolites from the endophytic fungus *Chaetomium* sp. isolated from Salvia officinalis growing in Morocco. Biotechnol Agron Soc Environ 2009; 13:229–234.

13 Klich MA, Pitt JI. A laboratory guide to the common Asperigillus species and their teleomorphs. In: 1st (eds.). Commonwealth scientific and industrial research organization, division of food processing. North Ryde, Australia 1992; pp. 106–108.

14 Ainsworth GC. Ainsworth and Bisby's dictionary of the fungi. In: 6 (eds.). Commonwealth mycological institute. Kew, Surrey, England; 1971; pp. 663.

15 Blunt JW, Copp BR, Hu WP, Munro MH, Northcote PT, Prinsep MR. Marine natural products. Nat Prod Rep 2007; 24:31–86.

16 Bara R, Zerfass I, Aly AH, Goldbach-Gecke H, Vijay R, Sass P, *et al.* Atropisomeric dihydroanthracenones as inhibitors of multi-resistant *Staphylococcus aureus*. J Med Chem 2013; 56:3257–3272.

17 Bauer AW, Kirby WM, Sherris JC, Truck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966; 45:493–496.

18 Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Tech 1995; 28:25–30.

19 Bennett JM, Catovsky D, Danniel MT, Galton DAG, Graanlink HR, Sultan C. Proposal for the classification of the acute leukamias. Br J Haem 1976; 33:451–458.

20 Priestap HA. New naphthopyrones from *Aspergillus fonsecaeus*. Tetrahedron 1984; 40:3617–3624.

21 Shaaban M, Shaaban KA, Abdel-Aziz MS. Seven naphtho-γ-pyrones from the marine-derived fungus *Alternaria alternata*: Structure elucidation and biological properties. Org Med Chem Lett 2012; 2:2–6.

22 Ghosal S, Biswas K, Chakrabarti DK. Toxic naphtho-gamma-pyrones from *Aspergillus niger*. J Agri Food Chem 1979; 27:1347–1351.

23 Bouras N, Mathieu F, Coppel Y, Lebrihi A. Aurasperone F a new member of the naphtho-gamma-pyrone class isolated from a cultured microfungus, Aspergillus niger C-433. Nat Prod Res 2005; 19:653–659.

24 Peshin T, Kar HK. Isolation and characterization of β-sitosterol-3-o-βd-glucoside from the extract of the flowers of *Viola odorata*. Br J Pharma Res 2017; 16:1–8.

25 Koga J, Yamauchi T, Shimura M, Ogawa N, Oshimai K, Umemura K, *et al.* Cerebrosides A and C, sphingolipid elicitors of hypersensitive cell death and phytoalexin accumulation in rice plants. J Biol Chem 1998; 273:31985–31991.

26 Naureen H, Asker MMS, Shaaban M. Structural elucidation and bioactivity studies of secondary metabolites from endophytic *Aspergillus niger*. Indian J Appl Res 2015; 5:74–81.

27 Ehrlich KC, DeLucca AJ, Ciegler A. Naphtho-gamma-pyrone production by *Aspergillus niger* isolated from stored cottonseed. Appl Environ Microbiol 1984; 48:1–4.

28 Akiyama K, Teraguchi S, Hamasaki Y, Mori M, Tatsumi K, Ohnishi K, Hayashi H. New dimeric naphthopyrones from *Aspergillus niger*. J Nat Prod 2003; 66:136–139.

29 Gorst-Allman CP, Steyn PS, Rabie CJ. Structural elucidation of the nigerones, four new naphthopyrones from cultures of *Aspergillus niger*. J Chem Soc Perkin Trans 1980; 1:2474–2479.

30 Zhao JL, Zhang M, Liu JM, Tan Z, Chen RD, Xie KB, Dai JG. Bioactive steroids and sorbicillinoids isolated from the endophytic fungus *Trichoderma* sp.Xy24. J Asian Nat Prod Res 2017; 19:1028–1035.

31 Kumari M, Taritla S, Sharma A, Jayabaskaran C. Antiproliferative and antioxidative bioactive compounds in extracts of marine-derived endophytic fungus *Talaromyces purpureogenus*. Front Microbiol 2018; 9:1777.

32 Kontogiorgis CA, Bompou EM, Ntella M, Berghe WV. Natural products from mediterranean diet: from anti-inflammatory agents to dietary epigenetic modulators. Anti Inflamm Anti-Allergy Agents Med Chem 2010; 9:101–124.

33 Gautam SS, Navneet Kumar S. The antibacterial and phytochemical aspects of Viola odorata Linn. Extracts against respiratory tract pathogens. Proc Natl Acad Sci, India B Biol Sci 2012; 82:567–572.

34 Youssef DTA, Ibrahim SRM, Shaala LA, Mohamed GA, Banjar ZM. New cerebroside and nucleoside derivatives from a Red Sea strain of the marine *Cyanobacterium moorea* producens. Molecules 2016; 21:324.

35 Barreto-Bergter E, Sassaki GL, de Souza LM. Structural analysis of fungal cerebrosides. Front Microbiol 2011; 5:239.

36 Abdel-Razek AS, El-Awady M, Hassan AZ, Yassin FY, Asker M, Shaaban M. Bioactive compounds from marine *Stachybotrys* sp.QL23. Indian J Natl Prod Resour 2017; 8:322–328.

37 Gaullier JM, 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; 135:778–784.

38 Undurti ND. Can essential fatty acids reduce the burden of disease(s)?. Lipids Health Dis 2008; 18:7–9.

39 Melariri P, Campbell W, Etusim P, Smith P. In vitro and *in vivo*antimalarial activity of linolenic and linoleic acids and their methyl esters. Adv Stud Biol 2012; 4:333–349.

40 Yang G, Sandjo L, Yun K, Leutou AS, Kim G, Choi HD, *et al.* Flavusides A and B, antibacterial cerebrosides from the marine-derived fungus *Aspergillus flavus*. Chem Pharm Bull 2011; 59:1174–1177.

41 Mbosso EJT, Ngouela S, Nguedia JCA, Beng VP, Rohmer M, Tsamo E. Spathoside, a cerebroside and other antibacterial constituents of the stem bark of *Spathodea campanulata*. Natl Prod Res 2008; 22:296–304.

42 Shu RG, Wang FW, Yang YM, Liu YX, Tan RX. Antibacterial and xanthine oxidase inhibitory cerebrosides from *Fusarium* sp.IFB-121, an endophytic fungus in Quercus variabilis. Lipids 2004; 39:667–673.

43 Hidaka T, Yano Y, Yamashita T, Watanabe K. Biological activity of macromomycin. J Antibiot 1979; 32:340–346.

44 Choque E, El Rayess Y, Raynal J, Mathieu F. Fungal naphtho-γ-pyrones secondary metabolites of industrial interest. Appl Microbiol Biotechnol 2015; 99:1081–1096.