Journal of Basic and Environmental Sciences, 6 (2019) 8-11

ISSN
Online: 2356-6388
Print: 2536-9202

Research paper Open Access

Phthalate Ester Derivative Isolated from The Coral Associated Fungus "*Aspergillus Sp*. 2c1-Egy"

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Abstract

The culture of the fungus *Aspergillus sp*. 2C1-EGY was isolated from the soft coral; *Lobophyton* sp. the fungus was extracted with organic solvents. The combined crude extracts were subjected to silica gel column, and final purification by Crystillization which successively led to the isolation, structural elucidation and biological activities of the compound 1. The structure of the compound 1 was determined on the basis of NMR and mass spectral data analysis, as well as comparison of their NMR data with those in the literature. antioxidant activity with DPPH radical scavenging activity and Acetylcholinesterase (AChE) inhibitory activity were evaluated.

Keywords: Coral Associated fungus; Aspergillus sp. 2C1-EGY, phthalate ester, Biological Activity.
Received; 15 Sept. 2018, Revised form; 10 Nov. 2018, Accepted; 10 Nov. 2018, Available online 1 Jan. 2019

1. Introduction

Biologists and chemists of the world have been attracted towards marine natural products have been isolated from marine organisms which have been reported in approximately 6,800 publications, proving marine microorganisms to be a invaluable source for the production of novel antibiotic, anti tumor, and anti inflammatory agents. The marine fungi particularly those associated with marine alga, sponge, Coral, invertebrates, and sediments appear to be a rich source for secondary metabolites, possessing Antibiotic, antiviral, antifungal, antiyeast activities [1-3], Cytotoxic Potential of the Coral-Associated Fungus Aspergillus sp. 2C1-EGY Against Human Colon Cancer Cells [14] and Antimicrobial activity [15]. Besides, recent investigations on marine filamentous fungi looking for biologically active secondary metabolites indicate the tremendous potential of them as a source of new medicines. the chemistry and biological activity of the major bioactive alkaloids, polyketides, terpenoids, isoprenoid and non-isoprenoid compounds, quinones, isolated from marine fungi [4-7]. The organic extract of the culture of a Coral-derived fungus Aspergillus sp. 2C1-EGY. yielded Compound 1. We present herein the isolation, structural elucidation and biological activities of these compounds.

2. Results and Discussion

2.1. Fungal Registration in GenBank

***Aspergillus sp*. 2C1-EGY**

Accession No. KP001511
PubMed:
https://www.ncbi.nlm.nih.gov/nuccore/KP001511
This fungus was isolated from the fresh soft coral; ***Lobophyton* sp.**

2.2. Purification of the Compound

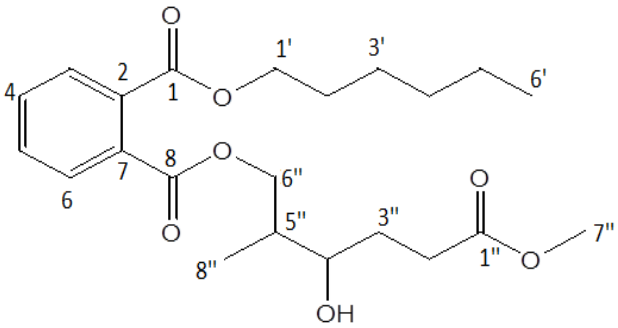
The culture of the fungus ***Aspergillus sp*. 2C1-EGY**. was extracted with organic solvents. The combined crude extracts were subjected to silica gel column, and final purification by Crystillization which successively led to the isolation of Compounds 1 (Figure 1).

2.3. Structure Elucidation of the Compound

The structure of the compound was elucidated using IR, NMR and MS data was obtained as:

EI-MS data showed molecular ion peak at m/z 408 and thus a molecular formula of $C_{22}H_{32}O_7$. IR spectrometric analysis of compound **1** showed characteristic absorbance at 3345 cm-1 representing OH and bands at 1728 cm^{-1}, representing carbonyl group and at 1072~1250 cm-1, signifying C-O band. indicated the presence of an aromatic system (1627, 1400, 829 and 702 cm−1) and an ester moiety (1729 and 1262 cm^{-1}).The ^{1}H NMR spectrum , indicated four aromatic protons resonating as two symmetrical 2H multiplets, at δH 7.26 (two protons at C-3/C-6) and δH 7.29 (two protons at C-4/C-5) which indicated symmetrical substitution pattern; which indicated a symmetrical substitution pattern. The presence of multiplit at δH 4.20 (2H,) and δH 4.19 ppm (2H,) indicated two oxymethylene groups adjacent to a methine group. A singlet (3H, -OCH3) at δH 3.64 along with a methylene triplet at δH 2.30 (m) were evident as an indication of a methyl ester group on two side chain, multiplet at δH 1.28 - 1.64 was evident and was assigned to 5 methylenes of the side chains. Also, a 2H multiplet was observed at δH 1.68 and assigned as two methine protons on the two side chains methyl signal at 0.91 (t, 9H). ^{13}C NMR- carbon resonances (δ 10.6, 13.7, 22.6,

23.4, 28.6, 30.1, 38.4, 67.6, 128.4, 130.5, 132.1, 167.3,172 .0) assigned as two quaternaries, three methane and five methylene carbons with two methyl groups. This data is analogous to ^{1}H NMR data and helped in providing the Carbon backbone of the molecule. By comparison of the recorded ^{1}H and ^{13}C-NMR data to those published in literature, compound 1 was identified as 2″-(Methoxycarbonyl)-4″-hydroxy-5″-methylpentyl-monohexyl phthalate (A13). The mass spectrum of the expected molecule was analyzed and found to be 408 (M^{+}) (Figure 1). which corresponds to $C_{22}H_{32}O_7$. Based on IR, NMR and MS data the compound was identified as 2″-(Methoxycarbonyl)-4″-hydroxy-5″-methylpentyl-monohexyl phthalate (A13). Comparison of ^{1}H and ^{13}C NMR data of isolated compound with published data indicated that the isolated compound was a phthalic acid ester derivative [8].



2″-(Methoxycarbonyl)-4″-hydroxy-5″-methylpentyl-monohexyl phthalate (A13)

Fig (1): Structure of Compound 1

2.4. Biological Activities

2.4.1DPPH radical scavenging activity:

The compound showed very very low activity (Figure 2A)

2.4.2Acetylcholinesterase (AChE) inhibitory activity:

The compound showed very very low activity (Figure 2B)

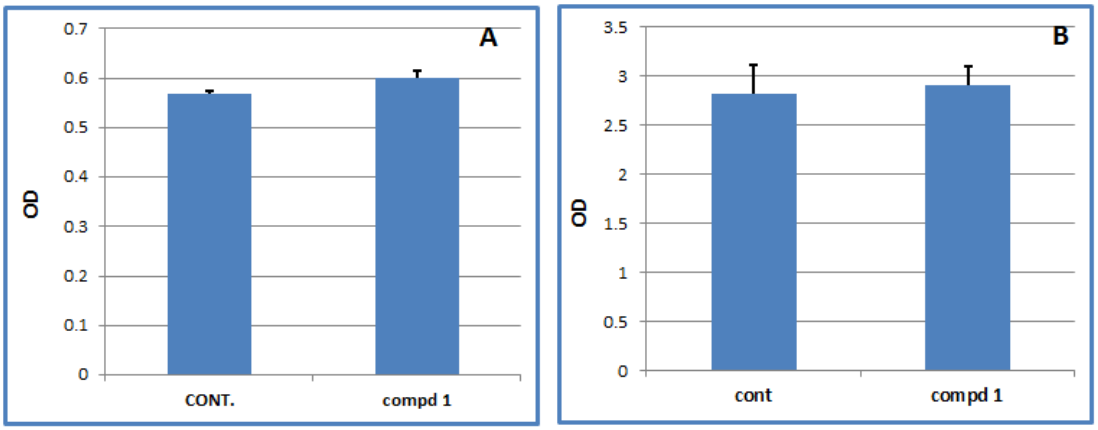


Fig (2): DPPH radical scavenging activity (A) and Acetylcholinesterase (AChE) inhibitory activity (B)

2.5. Phytochemical test

Chemical tests were carried out on the fungal extract using standard procedures to identify the constituents as described by [9,10]

3. MATERIALS AND METHODS

3.1. General

^{1}H and ^{13}C NMR spectra were recorded at 400.13 MHz on a Bruker DRX-600 spectrometer, equipped with a TCI Cryo Probe TM, fitted with a gradient along the Z axis and on Bruker instruments at 400 MHz. Samples for NMR analysis were dissolved in DMSO; a downfield shift of the signal of the solvent was used as the internal standard.

3.2. Soft coral materials

Soft coral samples; *Lobophytons*p. was collected from Hurghada coast, Red Sea, Egypt. The site is Shaa'b Al areq latitude, N 27° 25′ 08.9″, E 33° 51′ 0.5″ the samples were collected at depth of 5m - 8m in January 2013 and kept frozen until the work-up. The morphological taxonomy of the soft corals was identified by Mohamed A. Ghani – environmental researcher -Red Sea Marine parks, Hurghada, Red Sea, Egypt.

3.3. Preparation of animal material

Small pieces of inner tissue of fresh Soft coral materials were rinsed three times with sterile sea water (SW); then aseptically cut into small cubes, approx. (0.5 cm^3). A total of 50 - 75 cubes of each sample were placed on different

isolation media. During the initial investigations, cubes from Soft coral sample were placed in EtOH (70 %) for various times between 5 and 30s and subsequently squeezed three times in sterile sea water (SW) before inoculation.

3.4 Isolation of Fungi from Soft coral sample

A measured area of Soft coral tissue (about $1cm^3$) was excised from the middle internal mesohyl area of the Soft coral using a sterile scalpel. These Soft coral cubes were placed directly on the surface of the agar plates[7] or the excised tissue was then homogenized with sterile aged sea water, using a sterile mortar and pestle. The resultant homogenization was serially diluted until 10^{-6} and preincubated at room temperature for 1hr for the activation of dormant cells. From dilution 10^{-3} to 10^{-6} 0.1ml of each dilution was used to inoculate suitable solid medium containing antibacterial antibiotics. The plates were then incubated at 30ºC for 7-14 days [8]. The appeared single fungal colonies were picked up and inoculated on PDA (sea water) slants. The medium used in isolation exhibited the following composition (g/L): yeast extract (1), glucose (1), Ammonium nitrate (1), peptone (0.25), agar (20) and sea water (1000). The pH was adjusted to 7.4 [9]. The medium [10] was supplemented with Streptomycin sulphate (0.1g/L) and Penicillin G (0.1g/L).

3.5. Screening medium (Wickerham Medium for Liquid Culture)

For both shake and static cultures this broth medium with the following ingredients (g/L): Yeast extract (3), Malt extract (3), peptone (5), glucose (10) and sea water to make 1000 mL

The culture medium

A. The isolates ***Aspergillus sp*. 2C1-EGY was** grown on **Rice solid medium** of the following ingredients: rice (100g) and distilled water 100ml in 1l-Erlenmeyer flasks.

3.6. Extraction of secondary metabolites

The rice solid medium was directly extracted with ethyl acetate, followed by filtration and evaporation [11].

4. Extraction and Isolation

The fungal cultures were filtered through cheesecloth, and the filtrate was extracted with EtOAc (3 x 20 L, 24 h each). The organic extracts were concentrated in vacuo to yield an oily residue, which was subjected to silica gel column chromatography (CC) (petroleum ether, EtOAc v/v, gradient (100:0–0:100) to generate six fractions (Fr. 1–Fr. 5). Fr. 3 was isolated by CC on silica gel eluted with petroleum ether–EtOAc (v/v, gradient 100:10–10:100), and then subjected to Sephadex LH-20 CC eluting with mixtures of Petroleum ether; $CHCl_3$: MeOH (2:1:1), and further purified by using column silica gel eluted with 80% MeOH/H2O to obtain compound **1** (5.0 mg)

4. 1. Thin layer chromatography (TLC)

Thin layer chromatography was performed on silica-gel 60 TLC plate and developed using different solvent systems to obtain best separation. There were two methods to visualize the compound. Using UV-light and vanilin sulfuric acid that sprayed at plat TLC and heated on hot plate until the color changes on the TLC plate.

4.2. Open Column chromatography (OCC)

Purification by column chromatography conducted with modification methods. The solvents used were n-hexane (1), chloroform (2), chloroform: ethyl acetate (1: 1) (3), ethyl acetate

(4), and ethyl acetate: methanol (2:1) (5).

5. Biological activity

5. 1. DPPH radical scavenging activity

DPPH radical scavenging activity of all extracts was analyzed according to a modified procedure of Matsushige and his group [12]. 1 ml of methanol solution for each extract (100µg/ml) was added to 1 ml of methanol solution of DPPH (60µM). The prepared solutions were mixed and left for 30 min at room temperature. The optical density was measured at 520 nm using a spectrophotometer (UV-1650PC Shimadzu, Japan). Mean of three measurements for each compound was calculated.

5.2. Acetylcholinesterase (AChE) inhibitory activity

To investigate the AChE-inhibitory activity we followed the method previously described [13], with slight modified spectrophotometric procedure. Electric-eel AChE (Sigma) was utilized as source of cholinesterase. Acetylthiocholine iodide (Sigma) was used as substrate for AChE, to perform the reaction. 5,5-Dithiobis-(2-nitrobenzoic acid) (DTNB, Sigma) was utilized for the determination of cholinesterase assay. Investigated samples were solubilized in ethanol. Reaction mixture contained 150 µL of (100 mM) sodium phosphate buffer (pH 8.0), 10 µL of DTNB, 10 µL of test-extract solution and 20 µL of acetylcholinesterase solution were mixed and incubated for 15 min (25°C). 10 mL of acetylthiocholine was added to initiate the reaction. Hydrolysis of acetylthiocholine was monitored by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine, at a wavelength of 412 nm (15 min). All the reactions were performed in triplicate in 96-well micro-plate.

Acknowledgements: This work is financially supported by the bilateral projects within the Executive Program of Scientific and Technological Cooperation between Arab Republic of Egypt and Italian Republic for the years 2013 – 2016, Project no. [A2-12-15].

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