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Phytochemical and Biological Studies on Some Egyptian Seaweeds

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Extracts of four species of seaweeds, *Ulva lactuca* L. (green), *Liagora farinosa* Lamouroux (red), *Padina pavonia* L. and *Turbinaria ornata* Turn (brown), were screened for their antimicrobial, and antimalarial activities, and binding affinity for human opioid receptors. Phytochemical analysis led to the isolation and identification of 10 constituents: fucosterol, stearic acid, palmitic acid, palmitoleic acid, oleic acid, myristic acid, *p*-hydroxy benzoic acid, β-sitosterol, glycerol-1-olyl-3-palmotyl-2-galactoside, and glycerol-1,3-diolyl, The last two compounds displayed strong binding affinity to delta opioid receptors.

Keywords: Seaweeds, Macroalgae, Sterols, Antimicrobial, Antimalarial, Opioid receptors.

As a consequence of increasing demand for biodiversity in the screening programs seeking therapeutic drugs from natural products, there is now greater interest in marine organisms, especially algae. The ability of seaweeds to produce secondary metabolites of potential interest has been reported [1-3]. There are many reports on biologically active compounds derived from macroalgae [4-6]. In this study we report the chemical and biological screening of four algal extracts.

The methanolic extracts of *Ulva lactuca* L., *Liagora farinosa* Lamouroux, and *Padina pavonia* L. displayed more than 40% inhibition in the primary antimicrobial assay, but in the secondary assay, the brown alga *P. pavonia* extract compared with the other tested species showed moderate antimicrobial activity against *Candida krusie, Cryptococcus neoformans, Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* (MRSA), with IC_{50} values of 22.3, 59.9, 18.9 and 16.6 µg/mL, respectively, while *U. lactuca* extract showed weak antimicrobial activities against *C. neoformans*, *S. aureus* and MRSA, with IC_{50} values of 128.5, 95.6 and 111.3 µg/mL, respectively. Among the tested species for antimalarial assay against *Plasmodium falciparum, U. lactuca* and *P. pavonia* displayed mild activities, with IC_{50} values of 60.0 and 54.0 µg/mL for the D6 clone, and 66.0 and 56.0 µg/mL for the W2 clones, respectively.

The affinities of the acetone extract of the four species to the human opioid receptors were evaluated. The result showed that *U. lactuca* and *T. ornata* exhibited 64.7% and 52.9% binding affinity to δ opioid receptors, respectively, while *P. pavonia* and *L. farinosa* extracts displayed 69.3% and 52.5% binding affinity to μ opioid receptors. *T. ornata* extract showed 50.1% binding affinity to k-opioid receptors .

Ten compounds were isolated from the four algal extracts and their structures established as fucosterol [7], palmitic acid [8], glycerol-1-olyl-3-palmotyl-2-glactoside [8], oleic acid [8-9], palmitoleic acid [10], β-sitosterol [7], glycerol-1,3-diolyl [8], *p*-hydroxy benzoic acid [9], myristic acid [9] and stearic acid [11] by a combination of spectroscopic and chemical methods of analysis, as well as by comparing with literature values. The identification of fatty acids was confirmed by methylation followed by GCMS. The structures of the two glycerides were confirmed by GCMS after alkaline hydrolysis with 5% alcoholic KOH. The isolated compounds displayed no antimicrobial or antimalarial activities. The two glycerides, glycerol-1-olyl-3-palmotyl-2-glactoside and glycerol-1,3-diolyl showed 91.9% and 80.7% binding affinity to human δ opioid receptors respectively, while the latter displayed 60.9% and 72.7% binding affinity to *k* and μ receptors, respectively.

Experimental

General: ^{1}H and ^{13}C NMR spectra were recorded on a Varian AS 400 NMR spectrometer in $CDCl_3$. MS were obtained on an Agilent Series 1100 SL mass spectrometer equipped with an ESI source. GC/MS analysis was carried out on a HP 6890 series GC, equipped with a split/splitless capillary injector, a HP 6890 Series injector, auto sampler, and a DB-5 ms column (30 m x 0.25 mm x 0.25 µm, Agilent). The GC was interfaced to a HP 5973 quadrupole mass selective detector through a transfer line set at 240°C. The injector temperature was 250°C, and 1 µL injections were used in the split (1:10) mode with helium as carrier gas. The oven temperature was raised from 70°C to 200°C at a rate of 2°C/min, then maintained at 200°C for 15 mins. The total run was 80 mins. CC was performed on silica gel (EM Science, 60 Å, 230-400 mesh ASTM, Bakerbond Polar PlusC18 bonded phase) and SepahexLH-20 (25–100 lm, lipophilic, Sigma–Aldrich). TLC was carried out on aluminum-backed plates pre-coated with silica gel F254 (20 x 20 cm, 200 lm, 60 Å, Merck).

Plant material: Four marine algal samples were collected from the Red Sea shores in Marsa Alam, Egypt, In July 2010. The algae were identified by Dr Ibraheem B. Ibraheem, Dept. of Botany, Faculty of Science, and voucher specimens are kept at the Faculty of Science, Beni-Suef University, Egypt.

Extraction and isolation: Algal samples were cleaned, washed with water, air dried, and ground. Two hundred g of each dried sample was separately extracted by maceration in MeOH at room temperature for 6 days and filtered. The filtrates were dried to give residue yields of 6.0 g for *T. ornata*, 7.45 g for *U. lactuca*, 3.3 g for *L. farinosa* and 2.15 gm for *P. pavonia*.

T. ornata extract (6g) was subjected to Si gel VLC eluted with hexanes, EtOAc and finally MeOH. Fractions (13) were collected (500 mL each). Fractions 3-4 (502.9 mg) were chromatographed using a Si- SPE column with hexanes: EtOAc (9:1) as eluting solvent to yield 9 subfractions. Subfraction 3 afforded fucosterol (140 mg) and subfraction 5 gave palmitic acid (25.7 mg). Fraction 10 (184.5 mg) was subjected to Sephadex LH-20 eluting with MeOH to yield 5 subfractions. Subfractions 2-4 (50 mg) were chromatographed on a Si column eluting with mixtures of DCM: MeOH to yield glycerol-1-olyl-3-palmotyl-2-galactoside (10 mg) and oleic acid (10 mg).

L. farinosa (3.3 g) was subjected to Si gel VLC eluting with hexanes, and EtOAc, followed by MeOH, to yields 12 fractions. Fractions 1-4 (135 mg) were chromatographed on a C-18 SPE column using MeOH: H_2O (65:35) to yield 8 subfractions. Subfraction 3 afforded palmitic acid (25.5 mg); subfraction 4 palmitoleic acid (15.3mg); and subfaction 7 β-sitosterol (19.1 mg). Fractions 5-6 (76.7 mg) were chromatographed on a C-18 SPE column (MeOH/H_2O 60:40) to yield glycerol-1,3-diolyl (8.9 mg).

U. lactuca (7.45 g) and *P. pavonia* (2.15 g) extracts were separately subjected to Si gel VLC, as above, followed by C-18 SPE CC to give *p*-hydroxy benzoic acid (10 mg), myristic acid (115 mg), stearic acid (46.8 mg) and palmitic acid (26.8 mg) from *U. lactuca*, and fucosterol (71 mg) from *P. pavonia*.

Esterification of fatty acids: The isolated fatty acids were separately dissolved in 0.5 mL dry diethylether and diazomethane solution was added (6 drops) twice. The ethereal mixture was evaporated. The residue was dissolved in dry diethylether and subjected to GC/MS.

Alkaline hydrolysis of glycerol-1-olyl-3-palmotyl-2-glactoside and glycerol-1,3-diolyl: Two mg of the two compounds in 5% KOH-MeOH (2 mL) was refluxed for 4 h. The reaction mixtures were neutralized with 2 N HCl and extracted with $CHCl_3$, and the $CHCl_3$ layer subjected to GC/MS analysis. GC/MS after methylation with diazomethane gave palmitoyl and olyl methyl esters for the first compound and olyl methyl ester for the second compound. The sugar in the first compound was identified (TLC) as D-galactose by comparison with an authentic sample.

Antimicrobial bioassay: Crude extracts were tested for antimicrobial activity against *Candida albicans* ATCC 90028, *C. glabrata* ATCC90030, *C. krusei* ATCC 6258, *Asperigillus fumigates* ATCC 90906, Methicillin-resistant *Staphylococcus aureus* ATCC 33591, *S. aureus* ATCC 29213, *Cryptococcus neoformans* ATCC 90113, *Escherichia coli* ATCC 35218, *Pseudomonus aeruginosa* ATCC 27853, and *Mycobacterium intracellulare* ATCC 23068 [12-13].

Antimalarial bioassay: Antimalarial activity of the crude extracts was determined *in vitro* on chloroquine sensitive (D6, Sierraleon) and resistant (W2, Indo-China) strains of *Plasmodium falciparum* using a previously reported method [12].

Human opioid binding assay: The human opioid receptors binding affinity of the acetone extracts of the 4 algae, as well as the isolated compounds, was evaluated using a reported method [14].

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