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Secondary Metabolites and Certain Bioactivities of *Pterocladia capillacea* (S. Gmelin) Bornet and *Dictyopteris membranacea* (Stackhouse) Batters

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ABSTRACT

Twelve secondary metabolites of the red alga *Pterocladia capillacea* (S.Gmelin) Bornet and the brown alga *Dictyopteris membranacea* (Stackhouse) Batters, collected from the Mediterranean Coast of Egypt during February – March, and May – June, respectively, in two successive years, were isolated and their chemical structures were elucidated on the basis of their UV, IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data. Diisooctyl phthalate and cholesterol were isolated from both algae. In addition, 24 norcholest-5-en-3,7 dione, cholesterol, stigmasterol, linoleic acid and isodomoic acid were isolated from *P. capillacea*. On the other hand, three diterpenes (18,19-epoxyxenic-4-one-6, 9,13-triene, dictyolactone and 4-acetoxycrenulide) as well as fucosterol were isolated from *D. membranacea.* The anti-inflammatory, antioxidant and antimicrobial activities of different extracts of both algae were evaluated.

Keywords: anti-inflammatory, antimicrobial, antioxidant activities, diterpenes, sterols

INTRODUCTION

Many researchers had directed their attention towards the field of marine natural products chemistry, which encompasses the study of the chemical structures and biological activities of secondary metabolites produced by marine algae (Faulkner 1991). Due to an unusual environment compared with terrestrial organisms, marine algae produce a variety of substances having various unprecedented chemical structures and exhibiting significant biological activities. These substances was found to have nontoxic and effective medicinal influences for use in the treatment of human diseases (Zheng *et al*. 2001; Huleihel *et al*. 2001; Liao *et al*. 2003; Matsui *et al* 2003; Albuquerque *et al*. 2004; Arad (Malis) *et al*. 2006; Lane *et al*. 2006; Lim *et al*. 2006; Athukorala *et al*. 2006; Masato *et al.* 2008; Kamenarska *et al*. 2009; Kamenarska 2009). Marine algae were also reported to be used in traditional medicine, recommended for treating cancer (Ji *et al*. 1998). Algal preparations have been used as detoxifying agents (Morita *et al*. 2002). Red marine algae are thought to enhance the immune system's regulatory response, indicating imunomodulatory/antiviral activities (Ikegawa and Kobayashi 2003). Brown algae of the family Dictyotaceae produce a significant number of secondary metabolites, cyclic diterpenes of unique structures being produced by many members of the family (Paul *et al*. 2001).

This study aimed to isolate and elucidate the structure of secondary metabolites from the red alga *Pterocladia capillacea* (S.Gmelin) Bornet and the brown alga *Dictyopteris membranacea* (Stackhouse) Batters collected from the Mediterranean Coast of Egypt as well as to evaluate their antioxidant, anti-inflammatory and antimicrobial activities.

MATERIALS AND METHODS

Algal materials

The red and brown algal species used throughout the present work were collected from the beach of Abo-Kir, Alexandria, Egypt. The algae were authenticated by Prof. Samy Ahmed Shaalan, Professor of Botany, Faculty of Science, Alexandria University. These were found attached to the rocky belt, about 15 m from the sea-shore and at about 2 m depth. Freshly collected samples were repeatedly washed with sea-water followed by tap-water to remove sand, salt and any extraneous matters. Small samples of the algae were preserved in a solution containing 250 ml sea-water, 2.5 ml glycerol, 15 ml formalin and a few crystals of copper sulphate. For phytochemical and bioactivity studies, parts of the algae were kept frozen and the remaining were air-dried in the shade, ground and stored in dark coloured containers at room temperature (25-30°C) and humidity.

Experimental animals

Adult Sprauge Dawely albino rats weighing 130-150 g and albino mice weighing 25-30 g were obtained from the Animal House Colony of the National Research Centre, Egypt. They were kept under the same hygienic conditions and well-balanced diet and water. All animal procedures were performed after approval from the ethics committee of the National Research Centre and in accordance with the recommendations for the proper care and use of laboratory animals (NIH Publication No. 85-23, revised, 1985).

Material for antioxidant activity

Diphenyl picryl hydrazyl (DPPH) was purchased from Sigma Chemical Co. and ascorbic acid from Cid Co. Egypt (used as a reference antioxidant agent).

Received: 28 March, 2010. Accepted: 1 November, 2010.

Original Research Paper

Microorganisms

All test microorganisms were obtained from the Microbial Chemistry Department, National Research Centre, Egypt:
a. Gram-positive bacteria: *Bacillus cereus* and *Staphylococcus aureus*.
b. Gram-negative bacteria: *Escherechia coli* and *Pseudomonas fluoresens*.
c. Yeast strain: *Saccharomyces cerevisiae*.
d. Fungal strains: *Fusarium oxysporium* and *Aspergillus niger*.

Reference compounds for bioactivity evaluation

Ampicillin, Galaxo-Wellcome, Egypt, 100 µg/disc was used as a standard antibacterial. Clotrimazol, Bayer, Egypt, 100 µg/disc was used as a standard antifungal substance. Indomethacin (Kahira Pharm. Ind. Co. Egypt) was used as a standard anti-inflammatory agent. Carrageenan (Sigma Co.) was used for induction of acute inflammation in rats.

Apparatus

1. Nuclear magnetic resonance (NMR) spectrophotometer JEOL EX-300, 400, 500 MHz (for determination of ^{1}HNMR).
2. Infrared spectrophotometer (Pye Unicam, Cambridge, England).
3. Shimadzu PR-1 UV-visible recording spectrophotometer (UV 240 Graphicord).
4. Mass spectrometer (Varian MAT 711 Finnigan SSQ 7000OMM 7070E. PU 9712).
5. Elexsys Bruker 500, operated at X-band frequency (Germany) for evaluation of the antioxidant activity (ESR).

A. Phytochemical studies

1. Extraction and separation of compounds (1-6) from P. capillacea

Air-dried powdered thalli of *P. capillacea* (3 Kg) were exhaustively extracted by maceration with 70% ethyl alcohol. The filtrate was concentrated at a temperature not exceeding 40°C under reduced pressure, and successively partitioned with petroleum ether, chloroform and ethyl acetate until complete exhaustion. The remaining aqueous extract was evaporated to dryness and dissolved in methanol. Each extract was dried over anhydrous sodium sulphate, filtered, evaporated and weighed.

a. Petroleum ether extract (8 g) was chromatographed on a silica gel 60 (300 g) column (120 cm in height and 5 cm in diameter). Elution was successively carried out by hexane, ether, chloroform and methanol mixtures of increasing polarities. Fractions (100 ml each) were collected, evaporated, separately, to dryness under reduced pressure at a temperature not exceeding 40°C. Thin layer chromatographic examination [TLC] of fractions obtained was carried out using the solvent system benzene: ethyl acetate (6: 2, v/v). TLC plates were examined under UV and visualized by spraying with 60% H_2SO_4 and heated at 110°C for 5 min, similar fractions being pooled and further purification was carried out by preparative TLC. Four compounds were isolated: **1** eluted with hexane 100%, **2** eluted with hexane: ether (90: 10), **3** eluted with hexane: ether (80: 20) and **4** eluted with hexane: ether (40: 60). Compounds **2**, **3** and **4** tested positive for sterols and/or triterpenes with the Liberman-Burchard and Salkowski tests (Stahl 1969).

b. Chloroform extract (5 g) was chromatographed on a vacuum liquid column (VLC) [20 cm in length and 5 cm in diameter] packed with silica gel G (Type 60) Merck for TLC, and eluted with a hexane and ethyl acetate gradient. Compound **5** was separated from fractions eluted with hexane-ethyl acetate (80: 20, v/v) as the major spot.

c. Compound **6** was isolated from the successive methanol extract by preparative thin layer chromatography (PTLC) using the solvent system chloroform-methanol (90: 10, v/v).

2. Extraction and separation of compounds (7-12) from D. membranacea

Air-dried powdered thalli of *D. membranacae* (3 Kg) were extracted and fractionated as performed for *P. capillacea.*

a. Petroleum ether extract (8 g) yielded three compounds **7**, **8** and **9** from fractions eluted with hexane (100%), hexane: ether (80: 20, v/v) and hexane: ether (40: 60, v/v), respectively. They were purified by preparative thin layer chromatography PTLC using benzene: ethyl acetate (86: 14) solvent system.

b. Air-dried powdered thalli (2 Kg) were exhaustively extracted with dichloromethane by maceration and filtered. After stripping off the solvent, a dark-green residue (7.6 g) was obtained. VLC of 5 g of this extract yielded two compounds **10**, eluted by hexane: ether (90: 10, v/v) and **11** eluted by hexane: ether (80: 20, v/v).

c. Air-dried powdered thalli (1 Kg) were exhaustively extracted with chloroform: methanol (80: 20) by maceration and filtered. Stripping off the solvent yielded a dark residue (3.8 g). VLC of this extract yielded compound **12** which was eluted with hexane: ether (90: 10, v/v).

B. Bioactivity studies

1. Preparation of extracts for bioactivity evaluation

a. Total ethanol extract: The dried powder of each of the algae was exhaustively extracted by maceration with 95% ethanol. The extract in each case was evaporated under reduced pressure to give 22 and 31 g/100 g powdered algae, respectively.

b. Successive extracts: Successive extraction of the dried powder of each of the algae was carried out using solvents of increasing polarities, to give petroleum ether, chloroform, ethyl acetate and methanol successive extracts.

2. Antioxidant activity test

The antioxidant activity of the total ethanol extracts of both *P. capillacea* and *D. membranacea* was assessed by measuring the ability of each extract to scavenge the free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH) using electron spin resonance (ESR) spectroscopy (Calliste *et al.* 2001).

10^{-3} M solution of DPPH in methanol was prepared. Five mg of each extract, as well as, ascorbic acid were separately dissolved in 5 ml methanol. 1 ml DPPH solution + 1 ml MeOH were mixed and measured, considered as the reference solution. 1 ml DPPH solution + 1 ml of the MeOH solution of each extract or ascorbic acid standard were mixed and measured immediately.

The ESR signal of DPPH in MeOH appears at g = 2.006 characterizing the free radical. The decrease of this signal after mixing with the solution of each extract is taken as an indication of the antioxidant activity, measured as a double integration area (DIA).

$$\% \text{ Activity} = \frac{[\text{DIA (DPPH)} - \text{DIA (DPPH + extract)}]}{\text{DIA (DPPH)}} \times 100$$

3. Anti-inflammatory activity test

Paw swelling, or footpad oedema, is a convenient method for assessing inflammatory responses to antigenic challenges and irritants (Winter *et al*. 1962). This model uses carrageenan as an irritant to induce paw oedema. Typically, tested materials are assessed for acute anti-inflammatory activity by examining their ability to reduce or prevent the development of carrageenan-induced paw swelling. Nonsteroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, reduce paw swelling in a dose-dependent manner to a maximum of 60%.

Seventy-two adult male albino rats, divided into 12 groups (each of six animals) were orally treated with 50 and 100 mg/kg b.wt. of each extract, indomethacin (positive control), and saline (negative control). One hour after oral administration, all animals were given a sub-plantar injection of 0.1 ml of 1% carrageenan solution in saline in the right hind paw and 0.1 ml saline in the left hind paw. Four hours after oral administration, the rats were sac-

rificed. Both hind paws were separately, excised and weighed. Oedema % and oedema inhibition % were calculated according to the following equations, (Winter *et al*. 1962).

$$\% \text{ Oedema} = \frac{\text{(Wt. of right paw - wt. of left paw)}}{\text{Wt. of left paw}} \times 100$$

$$\% \text{ Oedema inhibition} = \frac{\text{(Mc-Mt)}}{\text{Mc}} \times 100$$

where Mc = the mean oedema in control group, and Mt = the mean oedema in the drug-treated group.

Data were statistically analyzed using the student's *t*-test (Snedecor and Cochran 1971).

Results with $P < 0.01$ were considered statistically significant.

4. Antimicrobial activity test

The antimicrobial activity test was carried out according to the antibiotic assay disc method (Gnanamanickam and Manafield 1981).

RESULTS AND DISCUSSION

Compounds 1-6 isolated from *P. capillacea* and 7-12 isolated from *D. membranacea* and their structure elucidation

1, 2-Benzenedicarboxylic acid diisooctyl ester [diisooctylphthalate] (1 and 7)

White crystals (11mg), with $R_f = 0.63$ in benzene: ethyl acetate (86: 14, v/v). UV λ_{max}nm($CHCl_3$): 204.8, 225.8, 273.8. EIMS, *m/z* (rel. int. %) 391 (8) [M^++H], 279 (88) [M^+-C_8H_{17}], 167 (90) [279-C_8H_{17}], 149 (100) [167-H_2O], 113 (44), 83 (28), 71 (70), 57 (87) and 43 (68). API$^+$ mass spectrum 392.2 (22), 391.2 (80), 281.1 (2), 279.1 (60), 261.1 (25), 257.2 (4), 231.1 (3), 216.0 (5), 215.1 (13), 214.1 (100). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) 7.5, 7.7 (4H, m, four aromatic protons), 4.23 (4H, m, two -OCH_2- groups), 2.35 (2H, m, two methyne groups), 1.27-1.56 (16H, m, eight methylene groups), 0.89-0.96 (12H, m, four methyl groups). Comparison with published data (Sastry and Rao 1995) indicated that compounds **1** and **7** are diisooctyl phthalate ($C_{24}H_{38}O_4$).

24-Norcholest-5-en-3, 7-dione (2)

Amorphous substance, (15mg), with $R_f = 0.73$ in benzene: ethyl acetate (6:2, v/v). IR γ_{max} (KBr) cm^{-1} 2917.77 (-CH_2), 2849.89 (-CH), 1706.21 (conjugated ketone), 1597 (-C=C-), 1464.95 (-CH_3) and 1408.81, 1296.11, 939.71, 722.79, 684.18. EIMS, *m/z* (rel. int. %) 384 (100) [M^+], 369 (7) [M^+-CH_3], 234 (4) [$C_{17}H_{30}$]$^+$, 204 (7) [$C_{13}H_{16}O_2$]$^+$, 180 (4) [$C_{13}H_{24}$]$^+$, 118 (16) [C_9H_{10}]$^+$ and 95.1 (29), 71.2 (30), 57.2 (38). ^{1}H NMR ($CDCl_3$, 300 MH_Z, δ ppm) 6.7 (1H, s, H-6), 1.26 (3H, *s*, H-19), 0.87 (3H, d, $J = 2.7$ Hz, H-21), 0.85 (3H, d, $J = 2.7$ Hz, H-26), 0.83 (3H, d, $J = 2.4$ Hz, H-25) and 0.76 (3H, s, H-18). When compared with published data, it was concluded that compound **2** is identical to 24-norcholest-5-en-3, 7-dione ($C_{26}H_{40}O_2$), which was previously isolated from the red alga *Melanothamnus somalensis* (Ahmed *et al*. 1996). This is the first report on the isolation of this compound from the red alga *P. capillacea*.

3β-Cholest-5-en-3-ol [cholesterol] (3 and 8)

White crystals (30 mg), with $R_f = 0.69$ in benzene: ethyl acetate (86: 14, v/v). IR γ_{max} (KBr) cm^{-1} 3423.8 (OH-) which is broad due to hydrogen bonding, 2923 (-CH_2), 2853.6 (-CH), 1672.4 (olefinic group -C=C-), 1460.1 (-CH_3) and 1377.3, 1276.8, 1123,742.5. EIMS *m/z* (rel. int. %) 386 [M^+] (100), 371 (45) [M-CH_3]$^+$, 368 (65) [M^+-H_2O], 353 (70) [M^+-H_2O-Me], 325 (20) [M^+-H_2O-isopropyl], 311 (16) [M^+-H_2O-C_4H_9], 301 (97) [M^+-C_6H_{13}], 273 (40) [M^+-side chain C_8H_{17}]$^+$, 255 (60) [M^+-side chain-H_2O], 227 (15) [255-C_2H_4], 213 (75), 145 (83), 105 (95), 81 (80), 57 (90). ^{1}H NMR ($CDCl_3$, 300 MH_Z, δ ppm) 5.35 (1H, d, H-6), 3.50 (1H, m, H-3), 2.27 (2H, d, $J = 1.8$ Hz, H-4), 1.99 (2H, m, H-12), 1.86 (2H, m, H-16), 1.83 (2H, m, H-1), 1.56 (2H, d, $J = 2.1$ Hz, H-2), 1.55 (2H, d, $J = 2.1$ Hz, H-7), 1.51 (1H, s, H-25), 1.49 & 1.48(2H, s, H-11), 1.44 (1H, d, $J = 4.2$ Hz, 1H-8), 1.259 (1H, s, H-20), 1.086 (1H, s, H-17), 1.06 (2H, s, H-15), 1.012 (3H, s, H-19), 0.97 (1H, s, H-9), 0.92 (3H, d, $J = 6.6$ Hz, H-21), 0.88 (3H, d, $J = 1.5$ Hz, H-27), 0.85 (3H, d, $J = 1.5$ Hz, H26) and 0.68 (3H, s, H-18). When compared with published data (Ahmad *et al*. 1996; Plouguerne *et al.* 2006) **3** was identified as 3β-cholest-5-en-3-ol (cholesterol) ($C_{27}H_{46}O$).

Stigmasta-5, 22-ien-3β-ol [stigmasterol] (4)

White crystals (14 mg), with $R_f = 0.64$ in benzene-ethyl acetate (86: 14 v/v). IR γ_{max} (KBr) cm^{-1} 3423.87 (broad due to hydrogen bonding), 2935.64 (-CH_2), 2857.74 (-CH), 1665.52 (olefinic group -C=C-), 1464.15 (-CH_3), 1376.28, 882.43 and 820.35 (characteristic for Δ^5 algal sterol). EI MS *m/z* (rel. int.%) 412 (3) [M^+], 383 (3) [M^+-C_2H_5], 272 (5) [383-C_8H_{15}], 229 (10) [272-C_3H_7], 212 (8) [229-H_2O], 145 (25), 105 (30), 95 (34), 83 (60), 71 (69), 55 (100). ^{1}H NMR ($CDCl_3$, 300MH_Z δ ppm) 5.35 (1H, d, H-6), 5.14 (1H, dd, $J = 15.0$, 6.5 Hz, H-22), 5.05 (1H, dd, $J = 15.0$, 9.0 Hz, H-23), 3.51 (1H, m, H-3), 2.37(1H, m, H-7a), 2.26 (1H, m, H-20), 1.95(1H, m, H-8), 1.25 (3H, s, H-19), 0.97 (3H, t, $J = 6.5$ Hz, H-29), 0.89 (3H, d, $J = 6.0$ Hz, H-27), 0.87 (3H, d, $J = 6.0$ Hz, H-26), 0.68 (3H, s, H-18). Comparison with published data (Bano *et al.* 1987; El-Askary 2005; Mohamed and Khaled 2005) compound **4** was identified as Stigmasta-5, 22-ien-3β-ol (stigmasterol) ($C_{29}H_{48}O$).

9, 12-Octadecadienoic acid [linoleic acid] (5)

Colourless oily substance (14mg), with $R_f = 0.58$. IR γ_{max} (KBr) cm^{-1} 3383.48 (OH), 2924.05 (-CH_2), 2853.81 (-CH), 1724.54 (-C=O), 1595.91 (olefinic group -C=C-), 1460.09 (-CH_3), 1310.6, 746.28 and 689.8. EI MS *m/z* (rel. int. %) M^+ 280, 123 (17), 109 (30), 95 (67), 67 (100). ^{1}H NMR (400 MHz, CDCl3, δ ppm) 5.35 (4H, m, H-9, 10, 12, 13), 2.7 (2H, m, H-11), 2.32 (2H, m, H-2), 2.01 (2H, m, H-8), 1.6 (2H, m, H-8), 1.26 (2H, m, H-6), 0.85 (3H, m, H-18). Comparison with published data (Lee and Chang 2000) compound **5** was identified as 9, 12 octadecadienoic acid [linoleic acid] ($C_{18}H_{34}O_2$)

2-Carboxy-4-(5-carboxy-1-methyl-2-hexenylidene)-3-pyrrolidine acetic acid [isodomoic acid](6)

White crystals (10mg), with $R_f = 0.57$ in chloroform-methanol (90:10 v/v). ESI/MS spectrum 312 $(M+H)^+$, 334.4 $(M+Na)^+$, 350.4 $(M+K)^+$. ^{1}HNMR (400 MHz, CD_3COOD, δ ppm) 4.8 was D_2O, 6.35, (1H d, $J = 16$ Hz, H-2'), 5.86 (1H, m, H-3'), 4.31 (1H, d, $J = 7$ Hz, H-2), 4.13 & 4.21 (2H, d, $J = 16$ Hz, H-5a & 5b), 3.81 (1H, d, H-3), 2.72 (2H, d, $J = 7$ Hz, H-6), 2.65 (1H, m, H-5'), 2.37 & 2.44 (2H, m, H-4'a & 4'b), 1.75 (3H, s, H-1'-Me), 1.61 (3H, d, $J = 7$ Hz, H-5'-Me). ^{13}CNMR (D20 - 100 MHz, δ ppm) 185.95 (C-8), 179.54 (C-7), 173.72 (COOH), 130.45 (C-2), 130.10 (C-4), 129.40 (C-3), 128.99 (C-5), 66.45 (C-4'), 47.66 (C-5'), 42.90 (C-3'), 41.82 (C-2'), 41.56 (C-1'), 37.59 (C-6), 17.22 (CH_3), 15.08 (CH_3). On comparison with the published data (Zaman *et al*. 1997), compound **6** was identified as, 2-carboxy-4-(5-carboxy-1-methyl-2-hexenylidene)-3-pyrrolidine acetic acid [isodomoic acid] ($C_{15}H_{21}NO_6$) previously isolated from the red alga *Chondria aromata* (Zaman *et al*. 1997). This is the first report on its isolation from red alga *P. capillacea*.

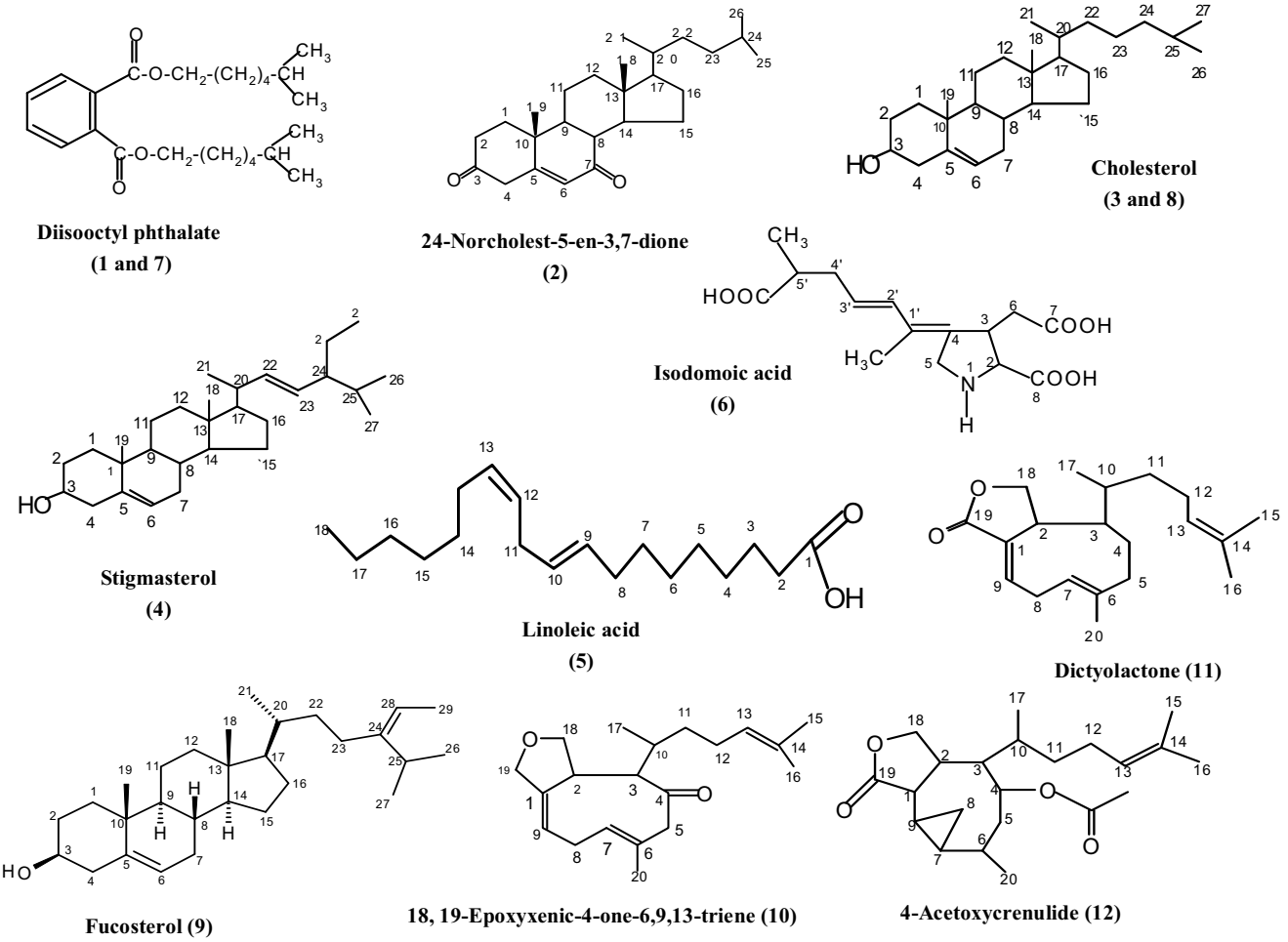

Fig. 1 Chemical structures of the compounds isolated from *P. capilacea* (1-6) and *D. membranacea* (7-12).

Stigmasta-5, 24(28-ien-3β-ol [Fucosterol] (9)

White crystals (20mg), with R_f = 0.66 in benzene-ethyl acetate (86:14 v/v). IR γ_{max} (KBr) cm^{-1} 3406 (OH), broad due to hydrogen bonding, 2928.1 (-CH_2), 2854.4 (-CH), 1666.3 (olefinic group -C=C-), 1463.2 (-CH_3) and 1376.5 (isopropyl group). CI/MS *m/z* 413 $[M^+ + H]^+$ and *m/z* 430 $[M^+ + NH_4]^+$. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) 5.38 (1H, d, *J* = 4.8 Hz, H-6), 5.18 (1H, q, H-28), 3.5 (1H, m, H-3), 1.58 (3H, d, *J* = 6.6 Hz, H-29), 1.07 (3H, s, H-19)), 0.99 (3H, d, *J* = 2.4 Hz, H-21), 0.97 (6H, d, *J* = 3.3 Hz, H-26, H-27) and 0.69 (3H, s, H-18). On comparison with published data, compound 9 was found identical with Stigmasta-5, 24(28)-en-3β-ol [fucosterol] ($C_{29}H_{48}O$) previously isolated from *Dictyopteris divaricata* (Song *et al.* 2005) and *Dictyota dichotoma* (Gedara *et al*. 2003). This is the first report on the isolation of this compound from the brown alga *D. membranacea*.

18, 19-epoxyxenic-4-one-6,9,13-triene (10)

Oily substance (8 mg), with R_f = 0.58 in benzene-ethyl acetate (86: 14 v/v). IR γ_{max} (KBr) cm^{-1} 2918.49 (for the methylene group), 2849.17 (for methine group), 1732.33 (for the carbonyl group), 1470.63 (for methyl group) 1378.15 (for the isopropyl group), and 1181.54 (for the olefinic group). EI-MS, *m/z* (rel. int. %) 302 (3) $[M]^+$, 256 (13) $[M^+ - CH_2, CH_3, OH]$, 213 (4) [256 - C_3H_7], 97 (20), 83 (33), 71 (38), 69 (57), 57 (83), 43 (100). ^{1}H NMR (300 MHz, CDCl3, δ ppm) 5.35 (1H, m, H-7), 5.33 (1H, s, H-9), 5.18 (1H, m, H-13), 4.15 (4H, d, *J* = 6.9 Hz, H-18 & H-19), 2.8 (1H, m, H-10), 2.8 (1H, m, H-8a), and 3.o8 (1H, d, *J* = 5.1 Hz, H-8b), 2.34 (1H, t, *J* = 7.8 Hz, H-2), 2.28 (2H, m, H-5), 2.02 (1H, s, H-3), 1.63 (3H, s, H-15), 1.58 (3H, s, H-16), 1.3 (3H, s, H-20) and 1.19 (2H, s, H-11), 0.88 (3H, m, H-17). In comparison with published data, compound D4 was identified as 18,19-epoxyxenic-4-one-6,9,13-triene ($C_{20}H_{30}O_2$) isolated previously from *Dictyota dichotoma* (Jacobi *et al.* 1979; Nobuyasu *et al*. 1982; Ishitsuka *et al*. 1988; Kim *et al*. 2006).

18, 19-epoxyxenic-19-one-6,9,13-triene [Dictyolactone] (11)

Crystalline (6.5mg), with R_f = 0.61 in the benzene-ethyl acetate (86:14 v/v). IR γ_{max} (KBr) cm^{-1} 2923.54 (-CH2), 2852.56 (methine), 1731.75 (carbonyl), 1462.95 (methyl) and 1377.34 (isopropyl), there is a band 1620 (olefinic). EIMS, *m/z* (rel. int. %) 302 (3) $[M]^+$, 247 (4) $[M^+-C_4H_7]$, 192 (2) $[247-C_4H_7]$, 179 (4) [192-CH], 127(7) $[179-C_4H_4]$, 111 (13) $[127-CH_4]$. Other fragments are 97 (26), 83 (34), 69 (47), 57 (73), 55 (94). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 6.07 (1H, d, *J* = 5.4 Hz, H-9), 5.8 (1H, m, H-10), 5.40 (2H, m, H-7 and H-3), 4.98 (1H, br. t, *J* = 3.3 Hz, H-13), 4.52 (1H, t, H-18a), 4.2 (1H, m, H-18b), 2.7 (2H, m, H-8), 2.68 (1H, m, H-2), 1.76 (3H, s, H-20), 1.65 (2H, m, H-4), 1.57 (6H, s, H-15, H-16), 1.52 (2H, m, H-5), 1.25 (2H, m, H-11), 1.23 (2H, m, H-12),), 0.89 (3H, d, *J* = 6.9 Hz, H-17). Comparison with published data (Panayiota *et al*. 2004; Kim *et al.* 2006), compound 11 was identified as the diterpene 18, 19-epoxyxenic-19-one-6,9,13-triene [dictyolactone] ($C_{20}H_{30}O_2$).

4-Acetoxycrenulide (12)

Colourless oily substance (8 mg) with R_f = 0.58 in hexane: ether (90: 10). High-resolution mass, *m/z* (rel. int.%) 361 (36), 360 (100), 35 (20), 324 (20), 239 (16), 231(63), 214 (40). ^{1}HNMR (400 MHz, CDCl3) δ ppm 5.45 (1H, s, H-4), 5.04 (1H, t, *J* = 7.5 Hz, H-13), 4.72 (2H, dd, H-18), 3.21

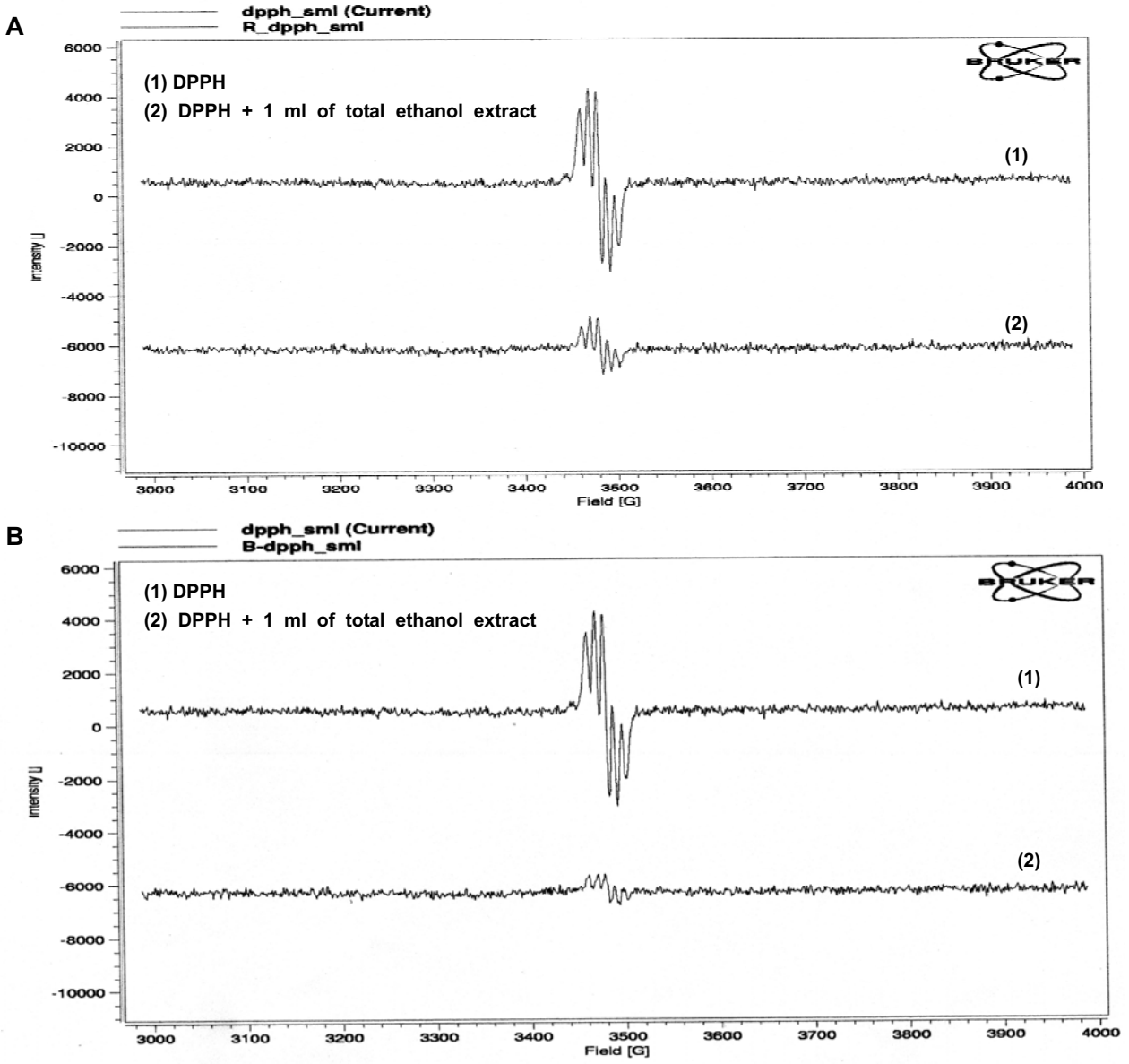

Fig. 2 Anti-oxidant activity of the total ethanol extracts of *Pterocladia capilacea* (A) and *Dictyopteris membranacea* (B) as compared with ascorbic acid (standard anti-oxidant agent) applying ESR method using DPPH as a source of free radical. (1) DPPH; (2) DPPH + 1 ml of total ethanol extract. Results are measured as the decrease in the double integration area. X-axis = Field; Y-axis = Intensity.

(1H,d, *J* = 8 Hz, H-3), 2.06 (1H, m, H-10), 2.03 (3H, s, H-acetate), 1.93 (2H, m, H-12a,b), 1.8 (1H, dd, dd, *J* = 5.1-3.1 Hz, H-5b), 1.78 (1H, dd, *J* = 5.1-3.1 Hz, H-5a), 1.69 (3H. s, H-15), 1.58 (3H, s, H-16), 1.49 (1H, m, H-9), 1.28 (1H, m, H-11b), 1.12 (1H, m, H-11a), 1.03 (3H, d, *J* = 5.5 Hz, H-8b), 1.01(3H, d, *J* = 6.8 Hz, H-17), 0.98 (3H, d, *J* = 6.8 Hz, H-20), 0.96 (1H, m, H-7), 0.39 (1H, m, H-8a). On comparison of the obtained data with those reported for acetoxycrenulide, a bicyclic cyclopropane-containing diterpenoid isolated from *Dictyota crenulata* (Sharon *et al*. 1982; Hao *et al*. 1983), compound–D6 could be identified as 4-acetoxycrenulide ($C_{22}H_{32}O_4$). This compound is a diterpene containing a bicyclic cyclopentane ring which is isolated for the first time from the brown alga *D. membranacea* (Stackhouse) Batters in the present work.

Bioactivities

1. LD_{50}

The LD_{50} of the total ethanol extract was carried out for *P. capillacea and D. membranacea* in a previous work (El-Rafie 2009) and it was found to be 7.4 and 7.8 g/kg b. wt. respectively and these high values of LD_{50} mean that these algae are safe to be used.

2. Antioxidant activity

As illustrated in **Fig. 2** it was found that the total ethanol extract of *D. membranacea* [brown alga] exhibited more free radical scavenging activity (85%) than that of *P. capillacea* [red alga] (76.2%). These results were in agreement with those reported by Zubia *et al.* (2007), and A-Reum *et al.* (2009) and Sheikh *et al.* (2009) who found that marine macroalgae have a great antioxidant potential which could be considered for future applications in medicine, food production and cosmetic industry.

3. Anti-inflammatory activity

A significant inhibition of the rat paw oedema weight induced by carrageenan was exhibited by some extracts of both studied algae. For *P. capillacea* the highest activity was exhibited by 100 mg/kg b.wt of petroleum ether successive extract (92.4% potency), followed by methanol successive extract (91.6% potency) and ethyl acetate successive extract (90.8% potency) in comparison with indomethacin (100% potency) as indicated in **Table 1** and **Fig. 3**, whereas, for *D. membranacae* the highest activity was exhibited by 100 mg/kg b.wt of chloroform successive extract (84.8% potency), followed by 100 mg/kg b.wt total ethanol

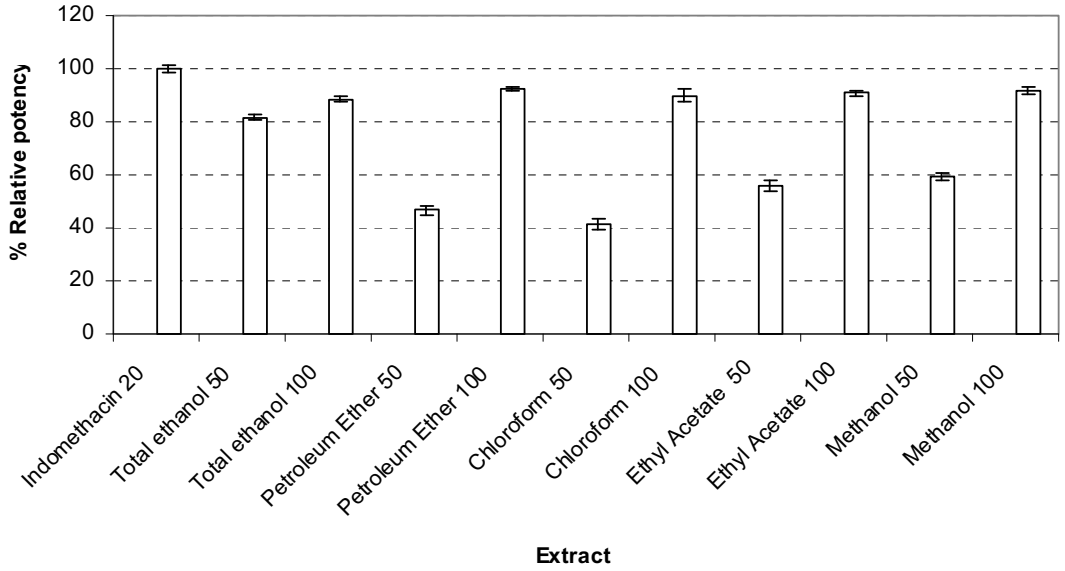

Fig. 3 Anti-inflammatory activity of total ethanol and successive extracts (petroleum ether, chloroform, ethyl acetate and methanol) of *P. capillacea*. Two dose levels (50 and 100 mg/kg b. wt.) were tested using indomethacin as a standard anti-inflammatory agent. Oedema % was calculated after four hours of injection of 1 % carrageenan solution. Values represent mean ± standard error.

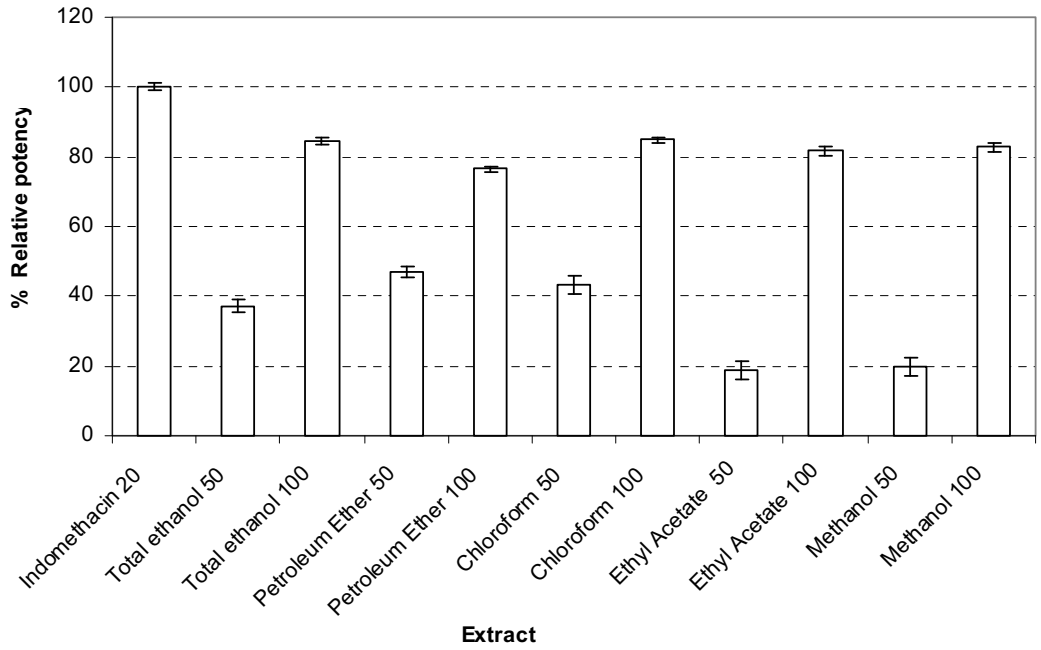

Fig. 4 Anti-inflammatory activity of total ethanol and successive extracts (petroleum ether, chloroform, ethyl acetate and methanol) of *D. membranacea*. Two dose levels (50 and 100 mg/kg b. wt.) were tested using indomethacin as a standard anti-inflammatory agent. Oedema % was calculated after four hours of injection of 1% carrageenan solution. Values represent mean ± standard error.

Table 1 Anti-inflammatory activity of total ethanol and successive extracts of *P. capillacea* (I) and *D. membranacea* (II) on male albino rats (n=6).

Group	Dose	Oedema (%) Mean ± S.E.		Inhibition (%)		Relative Potency %	
	(mg/kg b. wt)	I	II	I	II	I	II
Control	1 ml saline	59.3 ± 2.9	60.7 ± 2.6	-	-	-	-
Indomethacin	20	21.3 ± 1.2	22.3 ± 1.1	64.1	63.3	100	100
Total ethanol	50	28.3* ± 1.2	46.4* ± 1.8	52.3	23.6	81.6	37.3
	100	25.7 * ± 1.1	28.2* ± 0.9	56.7	53.5	88.5	84.5
Petroleum ether	50	41.5* ± 1.7	42.7* ± 1.7	30.0	29.7	46.8	46.9
	100	25.2 *± 0.9	31.3* ± 0.6	59.2	48.4	92.4	76.5
Chloroform	50	43.6* ± 2.3	44.1* ± 2.6	26.5	27.4	41.3	43.3
	100	25.1* ± 2.3	28.1* ± 0.7	57.7	53.7	90.0	84.8
Ethyl acetate	50	38.1* ± 1.8	53.5* ± 2.8	35.8	11.9	55.9	18.8
	100	24.8* ± 1.1	29.3* ± 1.2	58.2	51.7	90.8	81.7
Methanol	50	36.9 * ± 1.4	53.2* ± 2.4	37.8	12.5	59.0	19.8
	100	24.5* ± 1.3	28.9* ± 1.2	58.7	52.4	91.6	82.8

* Significantly different from the control at $p<0.01$

(84.5% potency) and methanol successive extract (82.8% potency) as illustrated in **Table 1** and **Fig. 4**. This activity may be attributed to their contents of chlorophylls, fucoidans (brown algae), eicosa-pentaenoic acids and other poly-unsaturated fatty acids (red algae). Cumashi *et al.* (2007) extensively studied the anti-inflammatory activity of nine

Table 2 Antimicrobial activity of total ethanol and successive extracts of *P. capillacea* (I) and *D. membranacea* (II).

Microorganism	Diameter of inhibition zone in mm (% activity)							
	Total ethanol		Petroleum ether		Chloroform		Ethyl acetate	
	I	II	I	II	I	II	I	II
Bacillus cereus (G+)	-	-	8 (44.4)	8 (61.5)	8.5 (47.2)	-	-	-
Staphylococcus aureus (G+)	9 (40.9)	8 (40)	10 (45.5)	-	-	-	-	-
Streptococcus pyogen (G+)	-	-	8 (21.1)	-	-	-	9.5 (25)	-
Escherichia coli (G -)	-	7 (29)	-	-	-	-	-	-
Pseudomonas flouresens (G -)	-	7 (25)	-	-	-	7.2 (25.7)	-	-
Candida albicans	-	-	-	-	-	9 (128.6)	-	7 (100)
Saccharomyces cerevisea	-	-	-	7 (41.2)	-	7.2 (42.2)	-	6.8 (40)
Asperagillus niger	-	-	-	2 (10)	-	-	-	7 (35)
Macrophomina phaseli	-	-	-	-	-	-	-	-
Fusarium oxysporium	-	-	-	8 (61.5)	-	-	-	-

different fucoidans from brown seaweeds and found that all fucoidans inhibited leucocyte recruitment in an inflammation model in rats.

Ben Aoun*et al.* (2010) also showed that the aqueous and organic extracts from *D. membranacea* collected from the Tunisian Mediterranean coast had high anti-inflammatory activity in carrageenan-induced rat paw edema assays in Wistar rats.

4. Antimicrobial activity

The total ethanol, petroleum ether, chloroform and ethyl acetate extracts of *D. membranacea*showed a wide range of antimicrobial activity against the tested microorganisms (G-, G+ and fungi) than those of *P. capillacae* as indicated in **Table 2**. These results were in agreement with those reported by Afzal and Shameel (2005) who found that the tested brown marine macroalgae exhibited potent antimicrobial activity against certain bacterial strains (G - and G +) and against fungi. Ibtissam *et al.* (2009) also found that brown marine macroalgae collected from the Atlantic and Mediterranean coast of Morocco present a significant capacity of antibacterial activities.

ACKNOWLEDGEMENTS

The authors are grateful to Prof. Dr. Amany Ameen Sleem, Pharmacology Dept., NRC, for carrying out the anti-inflammatory activity tests in her laboratory.

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