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Volatile Constituents of the Brown Algae *Padina pavonia* (L.) Gaill. and *Hydroclathrus clathratus* (C. Agardh) Howe and their Antimicrobial Activity

Nagwa E. Awad[1*] • Hemaia M. Motawe[1] • Mohamed A. Selim[2] • Azza A. Matloub[1]

[1] Pharmacognosy Department, National Research Centre, Dokki, Cairo, Egypt
[2] Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt

Corresponding author: * NagwaEA@hotmail.com

ABSTRACT

GC/MS analysis of the volatile oils of *Padina pavonia* (L.) Gaill. and *Hydroclathrus clathratus* (C. Agardh) Howe led to identify 54 volatile constituents in the former, or 75.64% of total constituents and 39 in the latter, or 92.67% of total constituents. The volatile constituents of both brown algae consisted mainly of esters (25.06 and 42.25%), hydrocarbons (20.38 and 12.63%) and fatty acids (6.59 and 11.68%). The antimicrobial activity of the volatile fractions of these algae was tested on 12 microorganisms (6 bacteria, 2 yeasts and 3 fungi). The volatile fraction of *P. pavonia* exhibited obvious antimicrobial activity against *Bacillus cereus* compared with amoxycillin as the reference drug while the volatile fraction of *H. clathratus* showed pronounced antimicrobial activity against *Saccharomyces cerevisiae* compared with canestin.

Keywords: hydrodistillation, marine algae, micro-organism, odoriferous hydrocarbons

INTRODUCTION

Brown algae release volatile compounds which belong to different groups (aliphatic and aromatic hydrocarbons, acids, esters, phenols, alcohols, aldehydes, ketones, terpenes, etc). These biomarkers, which are responsible for the behavior of organisms, act as allelochemicals, defensive compounds, attractants and alarming pheromones, etc. (Kamenarska *et al.* 2002). Odoriferous C_{11} hydrocarbons are emitted by all species of brown algae (El Hattab *et al.* 2007) which are affected by algal growing conditions, e.g. nutrients, mineral composition, and temperature of sea water (Kajiwara *et al.* 1989).

GC/MS analysis of the volatile fraction of *Padina pavonia*, collected from the Adriatic Sea, revealed the presence of free fatty acids, aromatic esters, phenols, benzyl alcohol, terpenes, sulfur containing compounds, aromatic hydrocarbons and benzaldehyde (Kamenarska *et al.* 2002). On the other hand, GC/MS analysis of the volatile constituents of *Hydroclathrus clathratus* showed the presence of phytol, free fatty acids and esters of fatty acids (Sagami *et al.* 1990).

P. pavonia possesses moderate antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* (Kamenarska *et al.* 2002), antifungal against *Macrophomina phaseolina*, *Rhizocotonia solani* and *Fusarium solani* (Sultana *et al.* 2005), cytotoxicity against KB cells (Ktari and Guyot 1999) and antitumour activity against lung (H460) and liver (HepG2) human carcinoma cell lines (Awad *et al.* 2008). The methanol extract of *P. pavonia* has high antibacterial activity against *Staphylococcus aureus* (Chiheb *et al.* 2009). On the other hand, the hot water extract of *H. clathratus* showed potential anti-viral activity against *Herpes simplex virus* types 1 and 2 and moderate antirespiratory syncytial virus activity (Wang *et al*. 2008).

The aim of this study was to identify the volatile constituents and their antimicrobial activity of *Padina pavonia* (L.) Gaill. and *Hydroclathrus clathratus* (C. Agardh) Howe collected from Egyptian Sea shore, as nothing has been published about this subject.

MATERIALS AND METHODS

Algal material

The two brown algae *P. pavonia* (L.) Gaill. (Family Dictyotaceae) and *H. clathratus* (C. Agardh) Howe (Family Scytosiphonaceae) were collected from the Red Sea coasts at Hurghada, Egypt during May, 2002 and authenticated by Prof. Dr. S. Shaalan, Professor of Phycology, Faculty of Science, Alexandria University.

Analysis of the volatile constituents

Pure, fresh homogenized algae (1 kg) were hydrodistilled in a modified Likens-Nickerson apparatus (Macleod and Cave 1975) using *n*-pentane (AR grade). The *n*-pentane layer was evaporated under pressure to yield faint-yellow oil. GC/MS analysis was done using a Finnigan SSQ 7000 (USA) GC/MS spectrophotometer equipped with library software Wiley 138 and NBS 75 under the following conditions: DB-5 fused silica capillary column, 30 m in length, 0.32 mm i.d. and 0.25 µm film thickness, carrier gas, helium at a flow rate of 10 ml/min, temperature programmed 50-260°C at a rate of 5°C/min, ion source temperature 180°C, ionization voltage 70 eV, detector, flame ionization detector (FID).

The identification of the constituents was performed depending on the fragmentation of the obtained spectra and comparing with those of available authentic material or published data (Mass Spectrometry Data Center 1974; Jennings and Shibamoto 1980; Adams 1989, 1995) and a library database [Wiley (Wiley Institute, USA) and NIST (National Institute of Technology, USA)]. Quantitative determination was carried out based on peak area measurements of the gas chromatogram.

Microbiological activity

The antimicrobial activity of the volatile constituents was tested against several microbes. Pure strains of bacteria, yeasts and fungi were kindly provided by the Microbial Genetics Department, National Research Center, Egypt. The bacterial strains used were *Bacillus cereus* (Gram positive, G^+), *Bacillus subtillis* (G^+), *Staphylo-*

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coccus aureus (G^+), *Escherichia coli* (Gram negative, G^-), *Pseudomonas fluorescens* (G^-) and *Pseudomonas aeruginosa* (G^-). The yeast strains were *Saccharomyces cerevisiae* and *S. carles*, while the fungi were *Aspergillus niger*, *A. flavus* and *Diplodia oryzea*.

The antimicrobial activity of the volatile fraction was determined by the antibiotic assay method (Gnanamanickam and Mansfield 1981). The bacteria were cultured on Lauria-Bertani Medium (LB medium) (Moniatis *et al.* 1980), while the yeasts were cultivated on Yeast Extract Peptone Medium (YEPD medium) (Dillon *et al.* 1985). The fungi were cultured on Potato-Dextrose Agar (PDA) growth medium (Subba 1977). The oils were sterilized by filtration through bacterial membrane filter (0.45 µm, 2.5 mm diameter, Millipore, USA). The concentration of the volatile fraction was used at 100 µg/disc. The discs, after being air dried, were firmly applied to the surface of inoculated agar plates. The diameters of inhibition zones were measured per applied disc after incubation at 37°C for 24 h with the bacteria strains, while those containing yeast and fungi were incubated at 30°C for 48-72 h. Amoxycillin (Medical Union Pharmaceuticals Co., Egypt) as antibacterial (100 µg/disc) and canestin (Alexandria Co., Egypt) as antifungal (100 µg/disc) were used as reference drugs.

Statistical analysis

All values were expressed as the mean of inhibition zone (mm) with three replicates for each treatment. Data were subjected to paired-samples *t*-test using SPSS (ver. 9.0). $P < 0.05$ was regarded as significant.

Table 1 Result of GC/MS analysis of the volatile constituents of the brown alga *Padina pavonia* (L.) Gaill.

Compounds	Molecular formula	RR_t*	Relative (%)	$[M^+]$	B.P.
2-Hexanone,3-methyl	$C_7H_{14}O$	0.068	1.56	114	43
Nonane	C_9H_{20}	0.134	0.51	128	43
2,3-Octanedione	$C_8H_{14}O_2$	0.18	0.14	142	43
Decane	$C_{10}H_{22}$	0.217	0.68	142	43
2-Methyldecane	$C_{11}H_{24}$	0.227	0.12	156	43
Allyl hexanoate	$C_9H_{16}O_2$	0.28	0.66	156	41
Undecane	$C_{11}H_{24}$	0.29	0.89	156	57
Dictyopterene D	$C_{11}H_{16}$	0.33	3.22	148	91
Dictyopterene A	$C_{11}H_{18}$	0.345	0.60	150	79
Estragole	$C_{10}H_{12}O$	0.35	0.32	148	148
Dodecane	$C_{12}H_{26}$	0.36	0.52	170	57
Decanal	$C_{10}H_{20}O$	0.376	0.12	156	57
Anethole	$C_{10}H_{12}O$	0.42	1.25	148	148
Tetradecane	$C_{14}H_{30}$	0.52	0.34	198	43
β-Cubebene	$C_{15}H_{24}$	0.54	2.14	204	161
Germacrene D	$C_{15}H_{24}$	0.543	0.33	204	91
Pentadecane	$C_{15}H_{32}$	0.546	4.43	212	57
Tridecanol	$C_{13}H_{28}O$	0.55	0.17	200	43
8-Isopropylidene bicyclo[3.2.1] octan-2-one	$C_{12}H_{19}ON_3$	0.57	0.13	221	43
2,6-Di-*t*-butyl-4-hydroxy-benzaldehyde	$C_{15}H_{22}O_2$	0.57	0.38	234	219
Santalol	$C_{15}H_{24}O$	0.59	2.30	220	121
1-Hexadecene	$C_{16}H_{32}$	0.593	0.28	224	43
Hexadecane	$C_{16}H_{34}$	0.60	0.94	226	57
8-Heptadecene	$C_{17}H_{34}$	0.64	1.41	238	55
Heptadecane	$C_{17}H_{36}$	0.65	1.86	240	57
1-Octadecene	$C_{18}H_{36}$	0.66	1.49	252	41
Nor-Decyl bromide	$C_{10}H_{21}Br$	0.67	0.26	220	135
4,9-Di-*nor*-propyl dodecane	$C_{18}H_{38}$	0.69	0.32	254	57
1-Formyl heptadecane	$C_{18}H_{36}O$	0.695	1.20	268	43
4-(1-Methyl,1-phenyl ethyl)phenol	$C_{15}H_{16}O$	0.70	0.30	212	197
6,10,14-Trimethyl pentadecan-2-one	$C_{18}H_{36}O$	0.719	1.53	268	43
Isobutyl phthalate	$C_{16}H_{22}O_4$	0.735	1.26	278	149
Hexadecanol-1	$C_{16}H_{32}O$	0.74	0.40	242	43
Nor-octadecanol	$C_{18}H_{38}O$	0.74	7.60	270	55
Dibutyl phthalate	$C_{16}H_{22}O_4$	0.77	0.92	278	149
Palmitic acid	$C_{16}H_{32}O_2$	0.785	1.40	256	43
4-*Nor*-propyl heptadecane	$C_{20}H_{42}$	0.787	0.36	282	57
Totarene	$C_{20}H_{32}$	0.81	0.28	272	41
2,6,10,14-Tetramethyl heptadecane	$C_{21}H_{44}$	0.822	0.24	296	57
Vinyl stearate	$C_{20}H_{38}O_2$	0.829	0.22	310	57
Geranyl geraniol	$C_{20}H_{32}O$	0.84	1.09	288	41
Phytol	$C_{20}H_{40}O$	0.848	2.39	296	71
Methyl eicosa-5,8,11,14,17-pentaenoate	$C_{21}H_{32}O_2$	0.85	0.46	316	79
Methyl eicosa-5,8,11,14-tetraenoate	$C_{21}H_{34}O_2$	0.87	0.92	318	41
Oleic acid	$C_{18}H_{36}O_2$	0.89	5.19	284	43
Nor-tricosane	$C_{23}H_{48}$	0.92	0.14	324	57
Dioctyl adipate	$C_{22}H_{42}O_4$	0.94	0.49	370	129
n-tetracosane	$C_{24}H_{50}$	0.95	0.26	338	57
9-*Nor*-octyl heptadecane	$C_{25}H_{52}$	0.98	0.40	352	57
Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	1:00	19.75	390	149
Nor-hexacosane	$C_{26}H_{54}$	1.02	0.35	366	57
10-*Nor*-propyl-10-*nor*-butyl eicosane	$C_{27}H_{56}$	1.06	0.45	380	57
Di-*nor*-octyl phthalate	$C_{24}H_{38}O_4$	1.067	0.38	390	149
7-*Nor*-hexyl docosane	$C_{28}H_{58}$	1.11	0.29	394	57

* Relative retention time, calculated relative to Bis(2-ethylhexyl)phthalate (Rt= 46:25)

RESULTS AND DISCUSSION

The yields of volatile oils of fresh algae *P. pavonia* and *H. clathratus* were 0.023 and 0.037% (w/w), respectively.

Fifty four and 39 compounds were identified which represent 75.64 and 85.20% of the total volatile compounds released from *P. pavonia* and *H. clathratus*, respectively. **Tables 1** and **2** show that the volatile constituents of both algae are composed of hydrocarbons (20.38 and 12.63%), sesquiterpenes (4.77 and 2.41%), alcohols (11.65 and 2.13%), aldehydes (1.70 and 10.07%), ketones (3.23 and 1.15%), methoxy (1.57 and 0.32%), acids (6.59 and 11.68%), esters (25.06 and 42.25%), phenol derivatives (0.30 and 0.70%) and miscellaneous (0.39 and 1.86%), respectively.

Bis-2-ethylhexyl phthalate was identified as the princeple constituent in both algae (19.75 and 40.22% for *P. pavonia* and *H. clathratus*, respectively). This result was also found in the red alga *Corallina officinalis* L. (Awad *et al.* 2001, 2003). Other authors recorded that phthalate constituted a major component in *Bangia atropurpurea* (Chen 2004) and *Sargassum wightii* (Sastry and Rao 1995). Dibutyl phthalate was detected in some edible brown algae *Undaria pinnatifida* and *Laminaria japonica* as a natural product (Namikoshi *et al.* 2006).

Many fatty acid esters such as, methyl eicosa-5,8,11,14-tetraenoate, methyl eicosa-5,8,11,14,17-pentaenoate which known to be useful for the treatment of atherosclerosis (Awad *et al.* 2003), allyl hexanoate and vinyl stearate have been detected in *P. pavonia*, while dioctyl adiptate has been identified in both algae. Furthermore, the fatty acid propionic acid was detected as a major free acid in *H. clathratus*, while oleic acid was found in *P. pavonia* in a moderate concentration. Meanwhile, palmitic acid was present in both algae as a minor fatty acid.

The presence of free fatty acids was cited in the oils of both algae (Sakagami *et al.* 1990; Kamenarska *et al.* 2002).

Dictyopterenes A and D, which are odoriferous C_{11} hydrocarbons and identified in the essential oils of *Dictyopteris* spp. (Moore 1976; El Hattab *et al.* 2007), have been detected here for the first time in *P. pavonia*. Characteristic aroma dictyopterenes have been identified as constituents of brown algae with male gamete-attracting activity (Kajiwara 2005).

Furthermore, sesquiterpenoid compounds: *β*-cubebene, germacrene D, and santalol have been detected for the first time in *P. pavonia* and *H. clathratus*. These compounds were detected in *Dictyopteris* spp. (Yamamoto *et al.* 2000; El Hattab *et al.* 2007). Also, anethole and its isomer estragole have been detected in both algae for the first time.

Kajiwara *et al.* (2006) identified cubenol (as the major component), (*E,Z*)-2,6-nonadienal, (*E*)-2-no-nenal, (*Z,Z*)-3,6-nonadienal, (*E,Z*)-2,6-nonadienol, (*E*)-2-nonenol, myristic acid, and *ω*-hexadecenoic acid in volatile oils isolated from edible kelps (*Laminaria angustata*, *L. japonica*, *Kjellmaniella crassifolia*, *Costaria costata*, *Ecklonia cava*, *Alaria crassifolia*, *Undaria pinnatifida*).

Table 2 Result of GC/MS analysis of the volatile constituents of the brown alga *Hydroclathrus clathratus* (C. Agardh) Howe.

Compounds	Molecular formula	RR_t*	Relative (%)	$[M^+]$	B.P.
Propionic acid	$C_3H_6O_2$	0.07	11.13	74	59
Nor-decane	$C_{10}H_{22}$	0.22	0.07	142	43
Undecane	$C_{11}H_{24}$	0.29	0.16	156	57
Estragole	$C_{10}H_{12}O$	0.419	0.10	148	148
Anethole	$C_{10}H_{12}O$	0.42	0.22	148	148
Bicyclo[4.1.0] heptane, 7-pentyl	$C_{12}H_{22}$	0.48	0.45	166	82
6-Methyl tridecane	$C_{14}H_{30}$	0.485	0.11	198	57
2-Phenyl-3-methyl butanol-2	$C_{11}H_{16}O$	0.50	0.17	164	121
β-Ionone	$C_{13}H_{20}O$	0.53	0.39	192	177
Pentadecane	$C_{15}H_{32}$	0.546	1.87	212	57
Butylated hydroxytoluene	$C_{15}H_{24}O$	0.548	0.70	220	205
Tridecanal	$C_{13}H_{26}O$	0.55	3.63	198	57
3,5-Dibutyl-4-hydroxy benzaldehyde	$C_{15}H_{22}O_2$	0.57	0.40	234	219
Santalol	$C_{15}H_{24}O$	0.585	2.41	220	121
Hexadecene	$C_{16}H_{32}$	0.59	0.41	224	43
Hexadecane	$C_{16}H_{34}$	0.60	0.71	226	57
1-Heptadecene	$C_{17}H_{34}$	0.64	0.23	238	43
Heptadecane	$C_{17}H_{36}$	0.65	3.44	240	57
1-Formyl heptadecane	$C_{18}H_{36}O$	0.66	3.97	268	82
5-Octadecene	$C_{18}H_{36}$	0.70	0.44	252	55
Octadecane	$C_{18}H_{38}$	0.70	1.45	254	57
1-Nonadecene	$C_{19}H_{38}$	0.71	0.80	266	97
6,10,14-Trimethyl 2- pentadecanone	$C_{18}H_{36}O$	0.72	0.76	268	43
Octadecanal	$C_{18}H_{34}O$	0.74	9.54	266	55
Dibutyl phthalate	$C_{16}H_{22}O_4$	0.77	0.34	278	149
Palmitic acid	$C_{16}H_{32}O_2$	0.784	0.55	256	43
Eicosane	$C_{20}H_{42}$	0.787	0.52	282	57
1,2,3,4,4a,5,8,9,12,12a Decahydro-1,4-methanobenzo- cyclodecene	$C_{15}H_{22}$	0.81	1.86	202	79
2,6,10,15-Tetra methyl heptadecane	$C_{21}H_{44}$	0.83	0.44	296	57
Geranylgeraniol	$C_{20}H_{32}O$	0.84	0.77	288	41
Phytol	$C_{20}H_{40}O$	0.85	1.19	296	71
Docosane	$C_{22}H_{46}$	0.87	0.40	310	57
Tricosane	$C_{23}H_{48}$	0.91	0.52	324	57
Dioctyl adipate	$C_{22}H_{42}O_4$	0.94	0.53	370	129
Nor-tetracosane	$C_{24}H_{50}$	0.943	0.24	338	57
Nor-pentacosane	$C_{25}H_{52}$	0.98	0.25	352	57
Bis(2-ethyl hexyl) phthalate	$C_{24}H_{38}O_4$	1.00	40.22	390	149
Nor-hexacosane	$C_{26}H_{54}$	1.02	0.12	366	57
Diisononyl phthalate	$C_{26}H_{42}O_4$	1.06	1.16	418	149

* Relative retention time, calculated relative to Bis(2-ethylhexyl)phthalate (Rt= 46:18)

Table 3 Inhibitory response of the volatile constituents of the brown algae *Padina pavonia* (L.) Gaill. and *Hydroclathrus clathratus* (C. Agardh) Howe on the tested microbes in comparison with standard antibacterial and antifungal substances.

Microorganisms	Mean of inhibition zones (mm) ± SEM			
	Volatile fraction		Reference drug	
	P. pavonia	*H. clathratus*	Amoxycillin (100 µg/ disc)	Canestin (100 µg/ disc)
Bacillus cereus	14 ± 0.58*	9 ± 0	10 ± 0	
Bacillus subtilis	10 ± 0.58[a]	8 ± 0*	24 ± 0.58[a]	
Staphylococcus aureus	9 ± 0 [a]	-	22 ± 0 [a]	
Escherichia coli	8 ± 0*	-	16 ± 0.58	
Pseudomonas fluorescens	14 ± 0.58*	9 ± 0.58*	26 ± 0.58	
Pseudomonas aeruginosa	9 ± 0.58	8 ± 0 [a]	9 ± 0 [a]	
Saccharomyces cerevisiae	-	12 ± 0.58[a]		11 ± 0.58 [a]
Saccharomyces carles	-	-		12
Aspergillus niger	8 ± 0.58	-		9 ± 0.58
Aspergillus flavus	12 ± 1.16*	-		20± 0.58
Diplodia oryzea	-	9 ± 0.58 [a]		14 ± 0.58 [a]

Each value represents the mean of inhibition zones (mm) of three replicates ± SEM (Standard Error of Mean)
*Significantly different from the reference drug at p< 0.05 according to paired-sample *t*-test
[a] The correlation and *t* cannot computed because the standard error of the difference is zero

Antimicrobial activity

The results of antimicrobial activity are summarized in **Table 3**. The volatile fraction of *P. pavonia* exhibited significant antimicrobial activity against *B. cereus* comparing with amoxycillin as reference drug. On the other hand, the volatile of *H. clathratus* showed pronounced antimicrobial activity against *S. cerevisiae* compared with canestin as reference material. In contrast, Ozdemir e*t al.* (2006) proved that the volatile oils of *Dictyopteris membranaceae* and *Cystoseira barbata* did not remarkably inhibit the growth of microorganisms.

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