Indian J. Fish., 60(1) : 83-86, 2013

Bioactive sterols from the brown alga *Anthophycus longifolius* (Turner) Kützing, 1849 (= *Sargassum longifolium*)

I. RAJENDRAN, KAJAL CHAKRABORTY*, K. K. VIJAYAN* AND P. VIJAYAGOPAL*

Mandapam Regional Centre of Central Marine Fisheries Research Institute, Marine Fisheries P. O., Mandapam Camp, Ramanathapuram – 623 520, Tamil Nadu, India

**Division of Marine Biotechnology, Central Marine Fisheries Research Institute, Kochi – 682 018, Kerala, India*

e-mail: cmfrirajendran@gmail.com

ABSTRACT

Anthophycus longifolius (= Sargassum longifolium), a brown seaweed commonly found in the Gulf of Mannar region, south India was investigated for bioactive compounds against human as well as marine fish/shellfish pathogens. The fractionation of ethanolic extract by bioassay guided mode yielded two compounds by column chromatography. The compounds were spectrally characterised using proton nuclear magnetic resonance spectroscopy (^{1}H NMR) and mass spectrometry as fucosterol and hexadec-4-enoic acid. These compounds were characterised for the first time from the alcoholic extract. The antibacterial activity of the compounds against human and aquaculture pathogens by disc diffusion method showed that hexadec-4-enoic acid was more antagonistic to *Vibrio parahaemolyticus*, *Pseudomonas fluorescens*, *Vibrio vulnificus*, *Vibrio harveyii*, *Aeromonas hydrophilla* and *Pseudomonas fluorescens* when compared to fucosterol.

Keywords: *Anthophycus longifolius* (=*Sargassum longifolium*), Brown algae, Fuctosterol, Hexadec-4-enoic acid, Seaweed extracts, Steroids

Introduction

Marine organisms are treasure houses of novel organic compounds as has been evidenced by the proliferative reports available in this field (Blunt *et al*., 2008). Marine macroalgae belonging to Chlorophyceae, Rhodophyceae and Phaeophyceae are rich sources of important industrial products like agar, carrageenan and alginic acid apart from wide range of pharmacologically important bioactive compounds. Compounds with anti HIV (Witvrouw *et al*., 1994), haemostatic and bacteriostatic activities have been reported from macroalgae, and brown algae in particular (Fritton, 2003; Jung *et al*., 2006). *Anthophycus longifolius* (Turner) Kützing *(= Sargassum longifolium)* is a common species in the Gulf of Mannar (GOM). Padmini Sreenivasa Rao *et al*. (1991) carried out a comparative study on the total sterol content of brown algae of Indian coast and *A. longifolius (=S. longifolium)* was one of the species investigated. As part of our investigations on isolation and characterisation of potential bioactive compounds from seaweeds, the crude extract prepared from *A. longifolius (=S. longifolium),* was used for isolation of compounds with antagonistic activity aganst human as well as marine fish/shellfish bacterial pathogens.

Materials and methods

Seaweed collection and preparation of extracts

A. longifolius (=S. longifolium) was collected during April 2008 from the Mandapam coast of GOM and identified according to Kaliaperumal and Kalimuthu (1997). The alga was shade dried and transported to the laboratory,washed in tap water, to remove epibionts, sand and other debris. It was then shade dried again and coarsely powdered using a domestic mixer grinder. The algal powder (850 g) was repeatedly extracted with 3 l ethanol at 75 °C with occasional shaking to get green extract. The pooled extract was filtered and concentrated under reduced pressure in rotary evaporator (Superfit) to get a dark green gum (4 g). The crude gum was chromatographed on neutral alumina column using petroleum ether - ethyl acetate (PE 60-80 °C/EtOAc) gradient and then gradient of EtOAc and methanol with thin layer chromatography (TLC) monitoring. The like fractions were pooled to get fractions I and II. The fractions of 200 ml were collected and pooled based on their TLC behavior. TLC was performed on glass strips and spots visualised by blowing iodine vapour. From the pooled eluates, compounds 1 and 2 were recovered and rechromatographed over neutral alumina to finally get fractions I and II. Fraction I (waxy solid) was purified repeatedly over neutral alumina column with PE-EA gradient to get the pure compound 1 (TLC, PE/EA 8.5:1.5), 120 mg, m.p.126-127 °C (Blunt and Munro, 2008).

^{1}H and 2D NMR were done using Bruker 300 MHz in $CDCl_3$ and ^{13}C NMR at 75 MHz at IRPHA-DST facility of School of Chemistry, Madurai Kamaraj University (MKU). Chemical shifts were recorded in δppm with TMS as internal standard. IR spectra were recorded by FT-IR

spectroscopy (Shimadzu) in KBr pellets at USIC-MKU. Electron impact (EI) mass spectra were recorded on mass spectrometer (JEOL GCmate).

The compounds isolated were tested for antagonistic activity against human as well as aquaculture pathogens by disc diffusion method at a concentration of 20 µg 20 μl^{-1} $disc^{-1}$ (*i. e.*, 1 mg ml^{-1}). Human pathogens tested were *Vibrio parahaemolyticus* (ATCC 17809) and *Pseudomonas fluorescens,* while fish pathogens tested were *Vibrio parahaemolyticus* (MTCC 451), *Vibrio vulnificus*, *Vibrio harveyii*, *Aeromonas hydrophila* and *Pseudomonas fluorescens*. The compounds were tested with 20 µg loading on 6 mm disc.

Results and discussion

Fucosterol

The column purified fraction I (waxy solid) on further purification by column chromatography furnished compound 1 (TLC, PE/EtOAc 8.5:1.5), 120 mg, m.p.126- 127 °C (Blunt and Munro, 2008). The spectral data (Table 1) were compared with available literature and the compound was identified as fucosterol (Fig. 1), [(3*â*,20R,24E) -stigmasta-5,24(28)-dien-3-ol]. The overlapped multiplets of portons between δppm 1.6 to 2.5 were not listed. The connectivity between the protons and respective hydrogens were further confirmed by their 2D NMR experiments apart from their δ ppm values. In IR spectrum the wave number values for hydroxyl and unsaturation were seen by their strong band at 3425 and 1666. Marine algae are the store house of bioactive sterols (Al Easa *et al.,* 1995). Fucosterol is a characteristic sterol prevalent among brown algae (Scheuer, 1978). It is reported that this sterol has good bioactivity potential for reducing the blood cholesterol level and has wide spectrum anti-diabetic (Lee *et al.*, 2004), butyrylcholinesterase inhibitory (Yoon *et al*., 2008), antihypertensive (Jung *et al*., 2006), and antioxidant (Lee *et al.*, 2003) activities. This is the first report of the isolation of this C_{29} sterol from the brown alga *A. longifolius (=S. longifolium).*

(E)-Hexadec-4-enoic acid

Fraction II was repeatedly purified over alumina column to finally get compound 2 as white solid, 186 mg

Table 1. Spectral data of compound 1

^{13}C NMR chemical shift assignment			
Position of carbon	δ ppm	Position of carbon	δ ppm
1	36.4	15	24.3
2	31.6	16	37.2
3	71.8	17	56.7
4	42.3	18	13.2
5	140.7	19	19.4
6	121.7	20	35.2
7	29.7	21	18.7
8	36.5	22	34.7
9	50.0	23	31.8
10	39.7	24	146.95
11	21.0	25	28.2
12	25.6	26	22.1
13	42.2	27	22.2
14	55.7	28	115.5
		29	11.8

^{1}H NMR chemical shift assignment		
δ ppm	Position	IR cm^{-1}
0.69 (3H, s)	CH_3-18	3425 (O-H *str*), 2933, 2856 (C-H *str*)
0.97 (3H, d)	CH_3-21	1666 (C=C *str*), 1463 (CH_3 *def*)
0.99 (3H, d)	CH_3-26/CH_3-27	1332, 1377 (2° alcohol C-O *str*, O-H *def*)
1.01 (3H, s)	CH_3-19	
1.57 (3H, d)	CH_3-29	
3.52 (1H, m)	CH -3	
5.18 (1H, m)	CH -28	

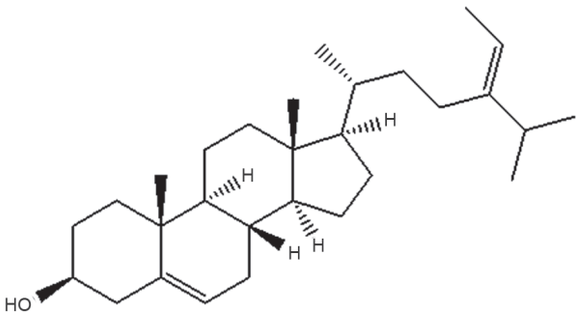

Fig. 1. Fucosterol 1

(TLC, PE/EtOAc 6.5:3.5), m.p.54-55 °C and the spectra were recorded as for compound 1. The spectral data (Table 2) were analysed to find it to be (E)-hexadec-4-enoic acid (Fig. 2). It is intriguing to isolate this compound in good quantity, as it is isolated from this alga for the first time. It gave the characteristic ^{1}H and ^{13}C NMR values typical of a straight chain carboxylic acid as shown in Table 2. The carboxylic proton peak appeared at 9.1 with very light broad intensity. The methylene carbons are clustered around 29 – 29.6 δppm. IR spectrum showed carboxyl carbon at 1706 cm^{-1}.

Antibacterial activity

The antibacterial activity of compounds 1 and 2 against human as well asfish pathogens tested by disc diffusion method showed an overall zone of inhibition ranging from

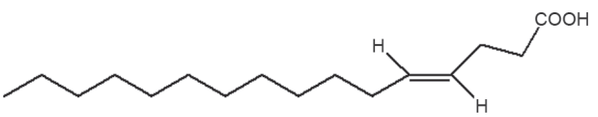

Fig. 2. (E) hexadec - 4 - enoic acid 2

6.3 to 8.3 mm in case of both the compounds (Table 3). Compound 2 showed better inhibitory effect than that of compound 1 which had no activity against *P. fluorescens* (of both human and fish strains) , when tested in triplicate.

The results of the present investigations have clearly indicated that the brown alga *A. longifolius (=S. longifolium)* is a potential source of valuable compounds with antibacterial activities and there is ample scope for developing products from this algal species for bacterial disease management iin human as well as in aquaculture systems.

Acknowledgements

The authors wish to express their sincere gratitude to Mr. J. R. Ramalingam, Technical Officer (Retd.), Mandapam Regional Centre of CMFRI for the identification of the algal specimen and Dr. N. Kaliaperumal Principal Scientist (Retd.), Mandapam Regional Centre of CMFRI for the clarification regarding the reclassification of the species taken for the present study. The authors also thank USIC, M K University and Sophisticated Analytical Instrument Facility, IIT Madras, Chennai for recording IR

Table 2. Spectral data of compound 2

^{13}C NMR chemical shift assignment		^{1}H NMR chemical shift assignment	
Position of carbon	δ ppm	δ ppm	Position
1	180.32	0.88 (3H, *t*)	CH_3-14
2	34.07	1.26 (20H, *br s*)	-$(CH_2)_n$-
3	24.65	1.63 (2H, *m*)	-CH_2-CH_2-C=O
4 to 11 clustered from 27.2 to 29.6		2.35 (2H, *t*	-CH_2-C=O
12	31.9	9.1 (1H, *br*)	-COOH
13	22.7		
14	14.12		
IR cm^{-1}			
2921, 2850 (C-H *str*), 1706 (C=O *str*, alip. acid), 1299 (C-O *str*)			

Table 3. Antibacterial activity of compounds 1 and 2 against human and aquaculture pathogens (20 µg loading on 6 mm disc)

Compound	Human pathogens		Aquaculture pathogens				
	*V. para.**	*P. fluores.*	*V. para.#*	*V. vulnif.*	*V. harv.*	*A. hydro.*	*P. fluores.*
1	7.5, 6.0, 7.6	6.3, 7.1, 6.9	7.1, 6.8, 7.0	7.1, 6.0, 7.5	7.8, 7.5, 7.5	6.0	6.0, 6.5, 6.8
2	8.1, 8.3, 8.0	6.0	7.9, 7.6, 8.0	7.6, 8.0, 7.8	8.3, 8.0, 7.8	8.4, 7.9, 8.0	6.0

Table represents triplicate results of experimental data.
*V. para.**-- *Vibrio parahaemolyticus* ATCC 17809; *V. para.# - Vibrio parahaemolyticus* MTCC 451; *P. fluores.- Pseudomonas fluorescenes*
V. vulnif.- Vibrio vulnificus: *V. harv.- Vibrio harveyii*; *A. hydro.- Aeromonas hydrophila*

spectra and Mass spectra respectively and also acknowledge the help rendered by Prof. S. Muthusubramanian, Department of Organic Chemistry, MKU , in interpretation of the spectra . Thanks are due to the Director, CMFRI, Kochi for the encouragement.

References

Al Easa, H. S., Kornprobst, J. M. and Rizk, A. M. 1995. Major sterol composition of some algae from Quatar. *Phytochem*., 39(2): 373-374.

Blunt, J. W., Copp, B. R., Hu, W. P., Munro, M. H. G., Nothcote, P. T. and, Prinsep, M. R. 2008. Marine natural products. *Nat. Prod. Rep*., 25: 35-94.

Blunt, J. W. and Munro, M. H. G. 2008. *Dictionary of marine natural products*. Chapman & Hall/CRC Taylor & Francis, 1766 pp.

Fritton, J. H. 2003. Brown marine algae: A survey of therapeutic potentials. *Alt. Comp. Therapy*, 9(1): 29-33.

Jung, H. A., Hyun, S. K., Kim, H. R. and Choi, J. S. 2006. Angiotensin-converting enzyme I inhibitory activity of phlorotannins from *Ecklonia stolonifera*. *Fish. Sci*., 72: 1292-1299.

Kaliaperumal, N. and Kalimuthu, S. 1997. Seaweed potential and its exploitation in India. *Seaweed Res. Utilization* 19 (1 & 2): 33-40.

Lee, Y. S., Lee, Y. S., Jung, S. H., Kang, S. S. and Shin, K. H. 2003. Anti-oxidant activities of fucosterol from the marine algae *Pelvetia siliquosa*. *Arch. Pharm. Res*., 26: 719-722.

Lee, Y. S., Shin, K. H., Kim, B. K. and Lee, S. H. 2004. Anti-diabetic activities of fucosterol from *Pelvetia siliquosa*. *Arch. Phar. Res*., 27: 1120-1122.

Padmini Sreenivasa Rao, P., Sreenivasa Rao, P. and Karmarkar, S. M. 1991. Chemical investigations of Indian Phaeophyceae 3. Total sterol content from different species of Indian *Sargassum*. *Seaweed Res. Utilization,* 14: 15-19.

Scheuer, P. J. 1978. *Marine natural products - Chemical and biological perspective*. Vol II,. Academic Press, NY, 80 pp.

Witvrouw, M., Desmyter, J. and De Clercq, E. 1994. Antiviral portrait series: 4. Polysulfates as inhibitors of HIV and other enveloped viruses. *Antivir. Chem. Chemother.,* 5 (6): 345-359.

Yoon, N. Y., Chung, H. Y., Kim, H. R. and Choi, J. S. 2008. Acetyl- and butyrylcholinesterase inhibitory activities of sterols and phlorotannins from *Ecklonia stolonifera*. *Fish. Sci*., 74: 200-207.

Date of Receipt : 05.01.2012
Date of Acceptance : 21.09.2012