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Screening for antiinflamatory agents from *Sargassum wightii* and *hypnea musciformis* from Gulf of Mannar, India

V.Kathiravan[1], L.Palanikumar[1,2], M.Rajesh Kannan[1], N.D.Kannan[3], N.Panneerselvam[1,*]

[1]Department of Botany and Research Center, The Madura College (Autonomous), Madurai, Tamil Nadu, 625011 (INDIA)

[2]School of Nanobioscience and Chemical Engineering, Ulsan National Institute of Science and Technology, (REPUBLIC OF KOREA)

[3]School of Biotechnology, Madurai Kamaraj University, Madurai, Tamil Nadu, 625020, (INDIA)

E-mail: npanneer1958@gmail.com

ABSTRACT

In the present work, the extracted compounds were identified from *Sargassum wightii* and *Hypnea musciformis* using gas chromatography and mass spectrometer (GC-MS). The potential phytochemicals such as triterpenes, carotenoids, steroids, glycoside, flavonoids and phenols were identified from ethanolic extracts of *S. wightii* and *H. musciformis.* One among the important antioxidant eicosianoids – prostaglandin and leukotriene were identified from *H. musciformis*. Both the seaweeds had higher triterpenes compounds. This is the first report on the chemical composition of *H. musciformis* from the Southeast coast of India. The result of this study suggests that these seaweeds can be considered as a potential source for the extraction of bioactive compounds, which might be used as anti-inflammatory compounds or dietary supplements or in production in the food industry.

KEYWORDS

Seaweeds;
Anti-inflammatory;
Sargassum wightii;
Hypnea musciformis.

INTRODUCTION

Inflammation has been connected with several diseases such as atherosclerosis, cancer, neurodegenerative diseases, diabetes, parkinson's disease and other deadly diseases, further, inflammation can also result due to genetic defects and immunoregulation and mechanism defects[1]. Several commercially available anti-inflammatory agents such as Vioxx, Celebrex, NSAID's can damage Cox-2 Inhibitor, cause heart problems and may lead to death[1]. Pathogenic organisms can easily develop resistance to those synthesic drug molecules. Therefore, a natural resource of anti-inflammatory production is required in this era for obtaining those bioactive agents which are rich in marine resources. Macro algae is one of the well known resources for screening several bioactive compounds[2].

Seaweeds are primitive on-flowering plants with pseudo stem and roots. These seaweeds have been used by humans for medicine and food for several decades. Seaweeds are rich source of antioxidants and several other biomolecules which may play a vital role in biomedical and food industries. The enormous amount of studies has revealed the significance of bioactive compounds from seaweeds. Some of them are major pharmaceuticals, biomedical and nutraceutical importance to reduce risk fac-

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tors of diet-related chronic diseases[2]. Traditionally, many seaweeds have been used as ingredients in both medicinal and food preparations around the world[3]. The edible seaweeds from the Phaeophyceae (brown), Chlorophyceae (green) and Rhodophyceae (red) are common in Pacific and Asian diets[4].

Macroalage can grow well in the aquatic environment in a huge scale and they can easily withstand several environmental fluctuation such as salinity and deviating tidal currents. Several studies have reported the extraction of anti-inflammatory agents from macro algae. Indian coastal environment ranges around 7000 kms with several diversities[5]. It has a rich source of several macro algal species such as brown, green, red and Gulf of Mannar is one among the hot spot of biodiversity which covers more than 3600 plant and animal species.

The algae synthesize a different compounds such as carotenoids, terpenoids, xanthophylls, chlorophyll etc. and many of these compounds are used to treat diseases like cancer, acquired immune-deficiency syndrome (AIDS), inflammation, pain, arthritis, as well as viral, bacterial, and fungal infections[6]. Seaweeds have lower lipid content[7] and C18, C20 polyunsaturated fatty acid (PUFA) contents were higher than terrestrial vegetables[3]. Omega-3 PUFAs also presents in seaweeds, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that were beneficial in clinical and nutraceutical applications[8,9].

Sargassum wightii and *Hypnea musciformis* are well known diversely distributed seaweeds of Gulf of Mannar, Southeast coast of India. *S. wightii* is belonging to the class Phaeophyceae, family Sargassaceae and order Fucales is widely distributed in tropical and temperate oceans. A wide range of bioactive properties has been reported from this species[10]. Sulfated polysaccharides of *S. wightii* can act therapeutically against Cyclosporine A (CsA)-induced hepatotoxicity[11]. Methanolic extract of *S. wightii* possess antimicrobial activity against human pathogenic bacteria[12].

Hypnea musciformis is typically found in shallow water attached to sandy hard substrate or as an epiphyte on other algae. They belong to the class Rhodophyceae, family Hypneaceae and order Gigartinales. A protein fraction rich in aggulutinins, obtained from *H. musciformis* showed anti fungal activity[13]. The methanolic extract of the *H. musciformis* exhibited strong antibacterial activity against the gram positive and seven gram negative bacteria[14]. The medical importance of these seaweeds is still unexplored. Hence, in this study the ethanolic extract of *S. wightii* and *H. musciformis* were analyzed by GC-MS.

Many literatures have reported the presence of bioactive compounds from crude extracts before fractionization, isolation and purification[15]. Some of them have reported the anti-inflammatory activities. However, it can understood that solvent and extraction conditions may vary[1] and sometimes, it may lead to erroneous results. Ethanol extracts are considered to be one among the most advantageous solvent in biomedicine and pharmacology, due to ease of isolation, purification, evaporation and concentrating the samples at required mass.

MATERIALS AND METHODS

Seaweed collection

The seaweeds used for this study were collected from the coastal regions of Mandapam (09° 17'N, 79° 07'E), the Gulf of Mannar, Southeast coast of India. The collected samples were immediately transported to the laboratory with gentle aeration and were rinsed thoroughly with sterile water to remove the epiphytes, sand particles and other debris. The species were identified at the Department of Botany, The Madura College, Madurai where the voucher specimens were deposited.

Ethanol extraction

The modified method of Sajiki and Kakimi[16] was used to extract the active compounds from the seaweeds. Briefly, the fresh seaweeds was cut finely (2–3 mm) and to 15 g of the sample 2 ml of water was added. An 8 ml volume of ethanol (EtOH) was added to the sample, and homogenized for 20 min. The homogenate was placed in a cold room, shaken for 3 h and centrifuged at 1000 g for 15 min. The resulting extracting solution was evaporated to dryness in a rotary flash evaporator (Supervac, China)

and the residue was dissolved in 0.1 ml methanol. The final residue thus obtained was then subjected to GC-MS analysis.

GC-MS analysis

The composition of the ethanolic extracts was analyzed on a Thermo GC - trace ultra ver: 5.0 and gas chromatograph interfaced to a Thermo MS DSQ II (GC-MS) equipped with a DB 35 - MS capillary standard non - polar column (30 MTS x 0.25 mm ID x 0.25 μM Film). Helium gas was used as the carrier gas at a constant flow rate 1 ml/min and an injection volume of 1 μl was employed. The oven temperature was programmed from 70° C to 250° C at 10 min hold. The constituents of the ethanolic extract were identified by comparing retention times of GC peaks with software adapted to handle mass spectra and chromatograms was a National Institute Standard and Technology (NIST).

RESULT AND DISCUSSION

In the present investigation, the chemical composition of the ethanolic extracts of *S. wightii* and *H. musciformis* were analyzed by GC–MS. The identified components and their molecular formula, molecular weight, structure and activity were summarized in TABLE 1. Totally, twenty six compounds were identified from combined data, which consisted of seven triterpenes, three steriods, three eicosanoids, two carotenoids, two drug like compounds, one glycoside, one flavanoid, one phenol, one flavonol and five other compounds.

Triterpenes were the major components in both seaweeds, 6 compounds from *S. wightii* and 5 compounds from *H. musciformis* were identified. The majority of these exhibit anti-inflammatory activity. The identified triterpens are glycyrrhetinic acid, eburicoic acid, hopane-29-acetate, absinthin and acetylglycyrrhetinic acid. Lanosta-7,9(11),20(22)-triene-3β,18-diol, diacetate were reported to have anti-protozoal activity[17]. Triterpenoids are the metabolites of isopentenyl phosphate oligomers and constitute the largest class of secondary metabolites and have been shown to suppress the growth of a variety of cancer cells without exerting any toxicity in normal cells[18-21].

Seaweeds have shown to be a good source of unsaponofiable, non toxic sterols that have medicinal importance[22,23]. One steroid 4,4-Dimethyl-5alpha-cholesta-8,14,24-trien-3beta-ol found in *S. wightii* and two steroids such as cholic acid and holothurinogenin 2,4 were identified from *H.musciformis*.

Eicosanoids molecules 9,11-Dideoxy-11 alpha, 9-alpha-epoxymethano-PGF2, 17-phenyl-trinor-PGF2α and ablukast were identified from *H. musciformis*. PGF2 reported to have the activity of thromboxane mimetic and mimicking the hydro osmotic effect of vasopressin activation of type c phospholipase[24,25]. 17-phenyl-trinor-PGF2α acting as the analogs of PGF2α[26]. Ablukast is a leukotriene molecule can be used to treat inflammatory skin conditions[27].

H. musciformis found to have carotenoid compounds 3,4,5-trimethoxybenzoic acid and 3,4,3',4'-tetrahydrospirilloxanthin. Several reports have demonstrated the function of different carotenoids in the prevention of degenerative diseases, and this has been attributed to their antioxidant properties[28].

Compounds structurally related to the drugs such as iloprost and viprostol were identified from *S. wightii*. These drugs possess biological activities of analogous to prostaglandin I_2 (PGI_2)[29] and PGE_2[30]. Stable PGI_2 analog iloprost extensively used as the treatment of pulmonary hypertension, showed potent anti-inflammatory and endothelium-dependent anti-edemagenic effects in several models of acute lung injury[31,32]. PGE_2 has been also shown to regulate *in vivo* and *in vitro* endothelial permeability[33].

Glycoside molecule martynoside reported to have the property of anticancer, cytotoxic, antimetastatic[34] and antioxidative[35] was found in *H. musciformis*. Furthermore, lucenin-2, carnosol and pachypodol were present in this seaweed.

In our study we investigated the two seaweeds, both had a totally different chemical composition. This is the first report of chemical composition of *H. musciformis* by GCMS analysis. Some of the previous works have explained about the fatty acid profile of *H. musciformis*[14,36]. This revealed that *H. musciformis* has higher bioactive compounds as

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TABLE 1 : Chemical composition of methanolic extracts from two selected species of seaweeds by gc-ms

Constituent	*S. wightii*	*H. musciformis*	Molecular Formula	MW	Activity
Triterpenes					
Glycyrrhetinic acid	+	-	$C_{30}H_{46}O_4$	470	Anti inflammatory[37,38]
Eburicoic acid	+	+	$C_{31}H_{50}O_3$	470	Anti inflammatory[39]
Hopane-29-acetate	+	+	$C_{32}H_{54}O_2$	470	Anti inflammatory[40]
Absinthin	+	+	$C_{30}H_{40}O_6$	496	Anti inflammatory[41]
Acetylglycyrrhetinic acid	-	-	$C_{32}H_{48}O_5$	512	Anti inflammatory[37,38]
Lanosta-7,9(11),20(22) triene-3β,18-diol, diacetate	+	+	$C_{34}H_{52}O_4$	524	Antiprotozoal[17]
Fomlactone A	-	+	$C_{33}H_{50}O_5$	526	---
Carotenoid					
3,4,5-trimethoxybenzoic acid	-	+	$C_{10}H_{12}O_5$	212	Antimicrobial[42]
3,4,3',4'-Tetrahydrospirilloxanthin	-	+	$C_{42}H_{64}O_2$	600	---
Steroid					
4,4-Dimethyl-5alpha-cholesta-8 14,24-trien-3beta-ol,	+	-	$C_{29}H_{46}O$	410	---
Cholic acid	-	+	$C_{24}H_{40}O_5$	408	---
Holothurinogenin 2,4	-	+	$C_{30}H_{48}O_5$	488	---
Glycoside					
Martynoside	-	+	$C_{31}H_{40}O_{15}$	652	Anticancer, cytotoxic, antimetastatic[34] and antioxidative[35]
Flavanoid					
Lucenin-2	-	+	$C_{27}H_{30}O_{16}$	610	Antimicrobial[43]
Phenol					
Carnosol	-	+	$C_{20}H_{26}O_4$	330	Antioxident, antiinflammatory chemopreventive[44]
Flavonol					
Pachypodol	-	+	$C_{18}H_{16}O_7$	344	Antimutagenic[45]
Eicosanoids					
Prostoglandin					
9,11-Dideoxy-11 alpha, 9-alpha-epoxymethano- PGF2	-	+	$C_{21}H_{34}O_4$	344	Thromboxane mimetic. Mimicking the hydroosmotic effect of vasopressin activation of type c phospholipase[24,25]
17-phenyl-trinor-$PGF_{2\alpha}$	-	+	$C_{23}H_{32}O_5$	388	Analogs of PGF2α[26]
Leukotriene					
Ablukast	-	+	$C_{28}H_{34}O_8$	498	Treatment of inflammatory skin conditions[27]
Drug like compounds					
Iloprost	+	-	$C_{22}H_{32}O_4$	360	Analogs of PGI_2[29]
Viprostol	+	-	$C_{23}H_{36}O_5$	392	Antihypertensive PGE_2 analog[30]
Others					
6-methoxy2phenyl-hexahydro 2Hpyrano[3,2-d][1,3]dioxine-7,8-diol Dasycarpidan-1-methanol, acetate (ester)	-	+	$C_{14}H_{18}O_6$	282	---
	-	+	$C_{20}H_{26}N_2O_2$	326	---
Effusanin E	-	+	$C_{20}H_{28}O_6$	364	Antibacterial[44]
Strongyloster	+	-	$C_{30}H_{50}O$	426	---
Renillafoulin A	-	+	$C_{24}H_{32}O_9$	464	---

+ Present; - Absent; MW: Molecular Weight

compared to *S. wightii*. Our study substantiate that an appropriate choice of seaweeds may form a promising strategy to isolate bioactive compounds that can be used for health benefit such as to prevent inflammation, cardiovascular diseases and anti-microbial.

In conclusion, GC-MS analysis revealed *S. wightii* and *H. musciformis* are rich sources of a great variety of bioactive compounds. Many of these compounds acts as antioxidants, anti-inflammatory, chemopreventive and antimicrobial. These biologically active compounds that may serve as candidates for the discovery of new drugs. However, further studies will need to be undertaken to ascertain fully its pharmacological activity.

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SUPPORTING INFORMATION

The structure of the identified compounds from GC-MS analysis was given as supplementary.

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