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SCREENING AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS FROM BROWN ALGAE (*TURBINARIA CONOIDES* J. AGARDH.) KUZING. THROUGH GC-MS ANALYSIS

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ABSTRACT

Turbinaria conoides (brown algae) was investigated for the richness of its bioactive compounds. The chloroform extracts of *T. conoides* was analyzed through GC/MS analysis which was indicated that the presence of more than 18 active compounds were identified *viz*., fatty acids (Hexadeconic acid (71.01%)), Arachidonic acid (6.90%), n-Tetracosanol-1(11.50)), steroids (Stearic acid (3-(octadecyloxy) propyl ester (CAS) (1.52%), diterpenoids (Geranylgeraniol (0.66%) Neophytadiene (0.23%), heterocyclic compounds (1H-Isoindole, 2,3-dihydro- (CAS) (9.68%)) and phenols (OO′-Biphenol 4,4',6,6'-tetra-T-butyl (0.34%)), remains are unknown compounds. The present investigation concluded that, brown algae, *T. conoides* proved to be the most potential seaweed for the development of drugs for Pharmaceutical industries and probiotic application to silkworm rearing in Sericulture Industries.

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INTRODUCTION

Seaweeds or marine algae grow in the intertidal as well as in the subtidal area up to a certain depth where 0.1% photosynthetic light is available; they are one of the ecologically and economically important living resources of the world ocean (Balamurugan *et al*., 2013). Seaweeds or marine macroalgae are potential renewable resources in the marine environment. About 6000 species of seaweeds have been identified and are grouped into different classes including green (Chlorophytes), brown (Pheophytes), and red (Rhodo phytes) algae (Abbott, 1995). They are well known as an excellent source of biologically active compounds. Marine macroalgae are primitive non-flowering plants without root, stem and leaves. They comprise one of the commercially important marine renewable resources (Selvaraj *et al*., 2010; Domettila *et al*., 2013). They have been a source for the production of a variety of major metabolites such as polysaccharides, lipids, proteins, carotenoids, vitamins, sterols, enzymes, antibiotics and many other fine chemicals (Raghukumar, 2011; Mišurcová, 2011; Ambrozova *et al*., 2014). Brown algae are not only the source of major metabolites but are an extensive source of secondary metabolites too. More than 600 secondary metabolites have been isolated from marine algae. Many of the secondary metabolites of brown algae are toxic substances which act as chemical defense systems for protecting them from grazers (Faulkner, 1986; Machu *et al*., 2015). Marine algae contain phenolic compounds, terpenoids, phlorotannins, steroids, amino acids, halogenated alkanes and ketones, cyclic polysulphides, fatty acids and acrylic acid and their various biological activities of seaweeds has been studied by Rajasulochana *et al*. (2009). The present study was undertaken to isolate and characterize the compounds from the brown algae of *Turbinaria conoides* and to analyze the potent bioactive compounds by GC-MS analysis.

MATERIALS AND METHODS

Collection area

Turbinaria conoides was collected randomly from the intertidal regions of the Mandapam (Lat. 09º 17.417′N; Long. 79º08.558′E) coast (Ramanathapuram District) of the Gulf of Mannar, Southeast coast of India.

Sample preparation and extraction

The collected seaweed were washed successively with tap water, distilled water and air dried under shade for two weeks. The dried plant material was ground to 2 mm or smaller particle size. The algae powder (25 g) was extracted through soxhlet apparatus with temperature of 40ºC of 6 hours with 150 ml of organic solvents of acetone and chloroform were used. After 6 hours extraction the solvents were

evaporated from crude extracted by rotary evaporator. The concentrated dried extracts were weighed dissolved in 2 mL of the respective solvents and stored at 4ºC till antifungal assay was conducted (Solomon *et al*., 2008; Manivannan *et al*., 2011).

GC-MS analysis

The chloroform extract of *T. conoides* was subjected to GC-MS (Hewlett Packard) analysis. Phyco-constituents were detected using a Hewlett Packard 5890 Series II gas chromatographic system (Hewlett Packard, Waldbronn, Germany) equipped with HP-5971 mass selective detector (MSD, Hewlett Packard, Palo Alto, CA, USA) and capillary column (30m×0.25mm×0.25mm) was used with helium at a 1 ml min^{-1} as a carrier gas, GC oven temperature was kept at 110ºC for two minutes and programmed to 280ºC at the rate of 5ºC min^{-1} and kept constant at 280ºC for10 minutes. The split ratio was adjusted to 1:20 and injection volume was 2 μL. The injection and detector temperature was 250ºC. The GC-MS electron ionization mode was 70 eV. Mass range was from m/z 45- 450 amu.

For the gas chromatographic-mass spectrometric analysis of the volatile compounds a 30 Mts, ID: 0.25 mm, FILM: 0.25ìm DB-5MS fused-silica capillary columns (Agilent, USA) were used at a helium flow rate of 1 ml/min. The oven temperature was kept at 80ºC for 1 min and raised to 300ºC for 5min. The identification of compounds was obtained by comparison with Wiley 275 Mass Spectra Library (Manilal *et al*., 2010).

RESULTS AND DISCUSSION

A high resolution mass spectrum equipped with a data system in combination with Gas Chromatography was used for the chemical analysis of active brown seaweed. The crude chloroform extract of *T. conoides* based on spectral data by GC-MS analysis was found to be a mixture of volatile compounds. The GC-MS analysis of *T. conoides* of extract revealed the presence of more than 21 active compounds including carbohydrates, amines (amino acids), fatty acid, steroids, phenols and heterocyclic compounds and other unidentified components at different quantities. GC-MS profiles of *T. conoides* chloroform extracts identified the major compounds *viz*., OO'-Biphenol, 4, 4', 6, 6'-tetra-T-butyl (phenols),

Hexadeconic acid, 3-Ethenylcholestan, Cholestan-26-oic acid and 3,7,12-trihydroxy-, (3à,5á,7à,12à)- (CAS) (fatty acids),

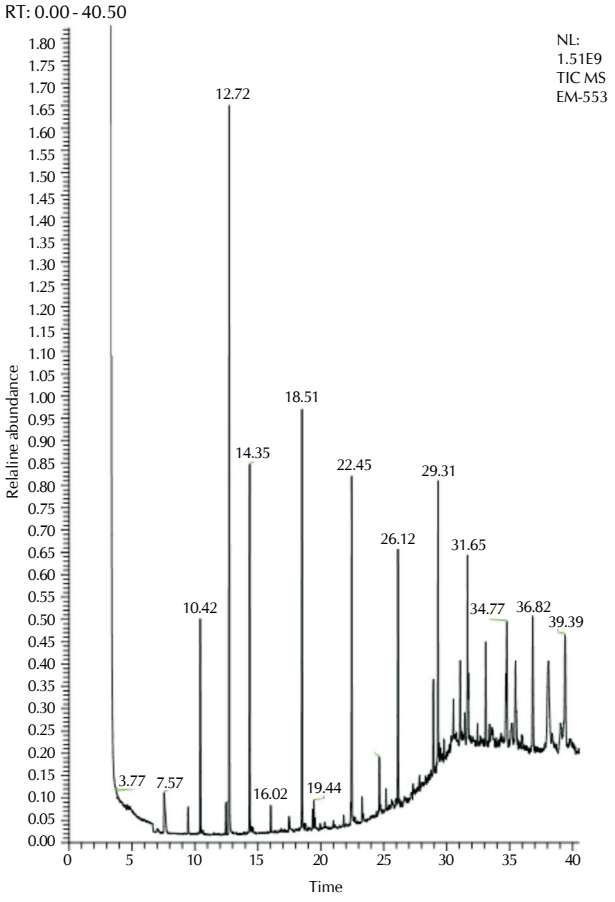

Figure 1: GC-MS chromatogram of chloroform extract of *T. conoides*

Table 1: GC-MS analysis of phytochemicals identified from chloroform extract of *T. conoides*

RT	Compound Name	Molecular formula	Molecular weight	Area (%)	Functional Groups
3.16	1H-Isoindole, 2,3-dihydro- (CAS)	C_8H_9N	119	9.68	Heterocyclic compounds
6.57	3,6-Di(pyrrolidinomethyl)-1,2-dithiine	$C_{14}H_{22}N_2S_2$	282	0.24	
12.48	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	518	0.12	
35.47	á-D-Mannofuranoside, farnesyl-	$C_{21}H_{36}O_6$	384	0.66	Carbohydrates
39.39	Stearic acid, 3-(octadecyloxy)propyl ester (CAS)	$C_{39}H_{78}O_3$	594	1.52	Steroids
38.05	Cholestan-26-oic acid, 3,7,12-trihydroxy-, (3à,5á,7à,12à)- (CAS)	$C_{27}H_{46}O_5$	450	1.42	Fatty acids
12.72	Hexadeconic acid	$C_{13}H_{18}O_2$	206	71.01	
38.05	3-Ethenylcholestan-3-ol	$C_{29}H_{50}O$	414	1.42	
28.94	O O'-Biphenol, 4,4',6,6'-tetra-T-butyl-	$C_{28}H_{42}O_2$	410	0.34	Phenols
31.42	Etioporphyrine	$C_{32}H_{15}D_{23}N_4$	478	0.18	Amines
34.75	2-amino-4-(1,3-benzodioxol-5-yl)-1-(dimethylamino) -5-oxo-1,4,5,6,7,8-hexahydro-3-quinolinecarbonitrile	$C_{19}H_{20}N_4O_3$	352	1.00	
33.62	Bis (3,6,9 trioxatetraethylene) crowno-N,N,N',N '-tetramethyl-p-phanediamine	$C_{32}H_{52}N_4O_6$	588	0.32	
19.44	Neophytadiene	$C_{20}H_{38}$	278	0.23	Diterpenoids
35.47	Geranylgeraniol	$C_{20}H_{34}O$	290	0.66	

steroids (Stearic acid, 3-(octadecyloxy) propyl ester (CAS), hetero cyclic compounds (1H-Isoindole,2,3-dihydro-(CAS); 3,6-Di (pyrroli dinomethyl)-1,2-dithiine and Cyclo hepta siloxane, tetra decamethyl-), carbohydrate (á-D-Manno furanoside, farnesyl-) and amines (Etioporphyrine; 2-amino-4-(1,3-benzo dioxol-5-yl)-1-(dimethyl amino)-5-oxo-1,4,5,6,7,8-hexahydro-3 quinolinecarbonitrile and Bis (3,6,9 trioxatetra ethylene) crowno-N,N,N′,N′-tetramethyl-p-phanediamine) representing in Table 1and their retention time indicated in Fig. 1. *Turbinaria ornata* showed the presence of n-hexa decanoic acid which is also called as palmitic acid (Neela mathi and Kannan, 2016). Similar GC-MS studies found in the species *viz*,. *Sargassam wightii* (Prameetha and Sree Kumari, 2016) and *Gracilaria edilus* (Hebsibah, 2010), *Sebastiania chamaelea* (Shanthi sree *et al*., 2010), *Costus specious, Gloriossa superba* and *Rauvolfia serpentine* (Rakesh dadsena *et al*., 2013) characterized the biochemical compounds. Battu *et al*. (2011) analyzed the preliminary phytochemical screening of ethanolic (70%) extract of three marine algae like, *Chaetomorpha antennina*, *Gracilaria corticata* and *Ulva fasciata* showed positive results for bioactive compounds like steroids, terpenoids, alkaloids, glycoside, amino acids, carbohydrates, saponins and oils. Hebsibah *et al*. (2010) analyzed the secondary metabolites like phenolic compounds, terpeniods, glycosides, proteins and glycoprotein's from three solvents of brown seaweed (*Sargassum wightii*). Phenolic compounds in particular are considered as one of the most important classes of natural antioxidants. Their molecules are formed by one or more aromatic rings with one or more hydroxyl groups (Hemat *et al*., 2007). Different phenols and fatty acids such as Phenol, 2, 4 – Bis (1, 1 - Dimethylet, Pyrrolo [1, 2 - A] Pyrazine - 1, 4 - Dione, 1 - Octadecanol, Hexadecanoic Acid, 2, 3 - Bis [(Tri methylsil)] with antimicrobial activity and pharma ceutical importance were identified through GC-MS by Kalaivani *et al*. (2016). El Shafay *et al*. (2016) identiûed the antibacterial fatty acids, cyclo pentane acetic acid, and 10,13-octadeadienoic acid as principal components of ethanol extracted *Sargassum vulgar* and diethyl ether extracted *Sargassum fusiforme*. The brown seaweed, *T. conoides*, was analyzed the chemical composition found to contain a higher concentration of fatty acids, protein, phenols, amines, steroids *etc.*

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