ORIGINAL ARTICLE

Gas Chromatography-mass Evaluation of Terpenoids from Persian Gulf *Padina tetrastromatica* sp.

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Abstract

In this research work, brown algae, *Padina tetrastromatica*, collected from Bushehr coasts in Persian Gulf, was extracted by non-polar and polar solvents to the isolation of terpenoid compounds. For isolation of various terpenoids, n-hexane and chloroform/ethyl acetate (1:1) were used as extraction solvents, after removal of the solvents, the gas chromatography/mass spectrum spectra of the fraction were obtained. Identification of organic constituents was made by comparison of their mass spectra and retention indices with those given in the literature and authentic samples. n-hexane fraction is consisted from alpha-humulene, decane, 2-methyl-phenol, tetradecanoic acid, and hexadecanoic acid, and chloroform-ethyl acetate fraction is consisted from pentadecane, hexadecane, 2,6,10,14-tetramethyl-hexadecane, 2,6,11-trimethyl-dodecane, 1,2-benzenedicarboxylic acid, and diisooctyl ester.

Key words: Brown algae, extract, gas chromatography–mass spectrum, *Padina tetrastromatica,* Persian Gulf, terpenoid

INTRODUCTION

Marine resources are promising organisms for the preparation of biologically active compounds. Many marine organisms are able to produce substances derived from secondary metabolism. These metabolites, also called natural products, are present from bacteria and algae to echinoderms, mollusks, tunicates, and vertebrates through the sponges and corals.[1] The brown algae (*Phaeophyceae*) are a class of almost exclusively marine organisms that have been explored for the bioactivity potential of its metabolic products. In all living organisms, terpenoids play a role in respiration chain electron transport (ubiquinone and menaquinone) as well as in cell wall and membrane biosynthesis and stability.[2] To date, a large number of marine terpenoid structures are known.[3]

Terpenes comprise primary and secondary metabolites, all derived from the five-carbon isoprene entity.[4] Terpenoids, or isoprenoids, are a large family of compounds including carotenoids, tocopherol, phytol, sterols, and hormones. There are tens of thousands of known terpenoid compounds and likely many more that have not yet been described.

The knowledge of metabolites has helped to characterize the algal contents at different chemical studies. With such wide range of biological functions, terpenoids have extensive applications in the fields of pharmaceuticals, cosmetics, colorants, disinfectants, fragrances, flavorings, and agrichemicals. Several terpenoids have also been used as drugs to benefit human health, such as artemisinin used as an antimalarial drug.[5]

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Figure 1: Persian Gulf brown algae, *Padina tetrastromatica* species

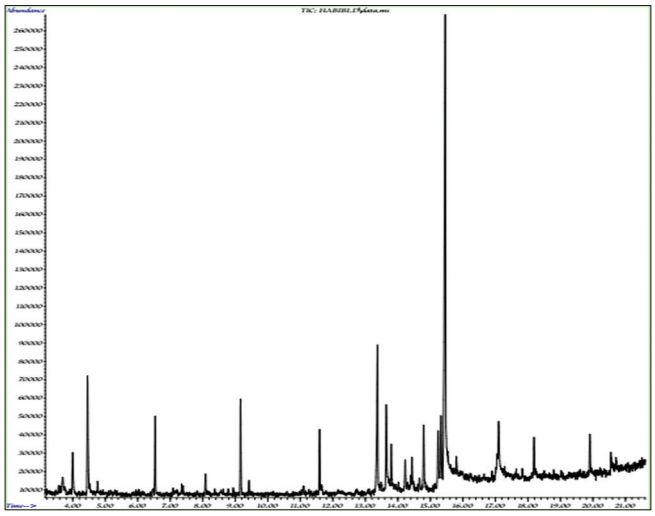

Figure 2: Gas chromatography spectrum of the n-hexane extract of *Padina tetrastromatica*

In this research work, we report the gas chromatography (GC)-mass analyzing results of several compounds corresponded to organic extracts from Persian Gulf marine Algae, *Padina tetrastromatica* species for possible pharmacological activity of them.

MATERIALS AND METHODS

Collection of sample

The brown algae were randomly collected from the Bushehr tide coasts Persian Gulf, July 2014. All samples were transported to the laboratory for identification and characterization. Taxonomic was done and certified by Marine Science and Technology University of Khoramshahr. The sample was identified as *P. tetrastromatica* as shown in Figure 1. The brown algae samples were washed with tap water to remove dirt, sand, and then dried in room temperature. The dried algae were ground in a mixer.

Preparation of the extract

About 100 g of algal powder were extracted with 1:3 volumes (v/w) of ethyl acetate, chloroform, and n-hexane, respectively, by maceration method for 72 h at room temperature. The resulting solution was filtered through cotton sterile filter. The extract was concentrated using rotary evaporator at 40°C. The crude extracts were obtained by freeze-drying and stored at −20°C for thin-layer chromatography (TLC) and GC-mass analysis.

TLC accomplished the presence of chemical compounds on silica gel (Merck, Germany) Polygram SIGL/UV254 plates.

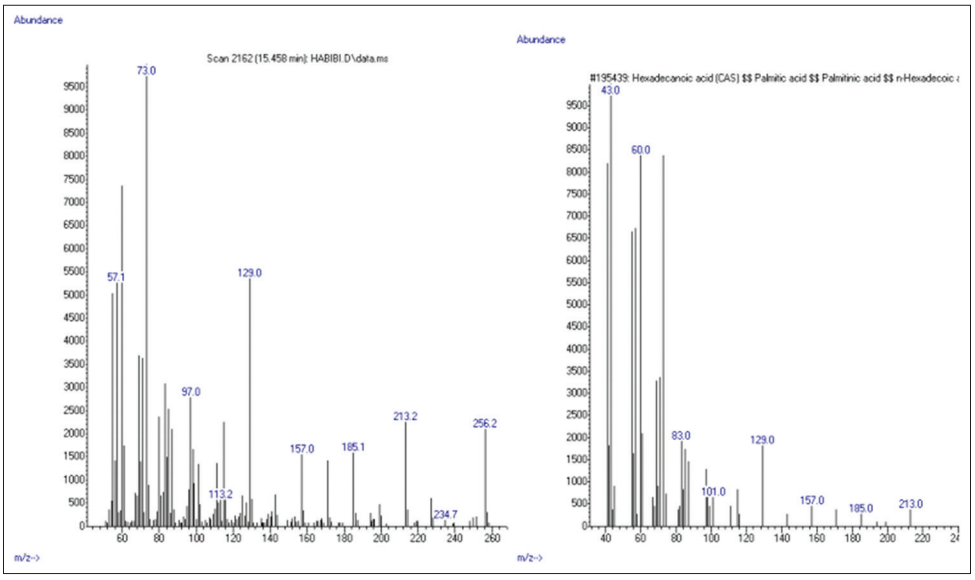

Figure 3: Mass spectrum of the n-hexane extract of *Padina tetrastromatica* with RT=15.458 (left) and n-hexadecanoic acid reference (right)

GC–mass spectrum (MS)

GC–MS Agilent GC 7890, Mass 5975 fitted with a fused HP-5 ms (5% phenyl methylpolysiloxane) capillary column (0.25 mm × 30 m, 0.25 µm film thickness) with helium gas at 1 ml/min are used. The sample was injected (sampling time, 5 min). The respective temperatures and ionization chamber were 290°C and 280°C. Temperature programs for the column oven were as follows: Program, 60°C for 1 min, elevated to 130°C at 20°C/min, then to 210°C at 10°C/min, then to 260°C at 10°C/min, and then to 300°C at 10°C/min; it was finally maintained at 300°C.

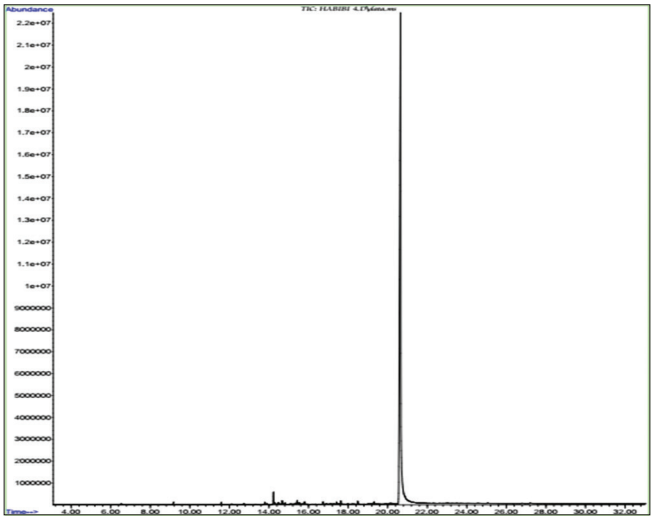

Figure 4: Gas chromatography spectrum of ethyl acetate-chloroform (1:1) extract of *Padina tetrastromatica*

Identification of compounds

Interpretation of mass spectra was done according to the National Institute of Standards and Technology (NIST 08s), WILEY 8 and FAME.

RESULTS

The ethyl acetate-chloroform (1:1) and n-hexane extracts of *P. tetrastromatica* were tested to TLC for the preliminary compound separation and then to GC–MS analysis to characterize the compound. GC–MS of *P. tetrastromatica* sp. [Figures 2-5 and Tables 1 and 2] revealed compounds, namely alpha-humulene, decane, 2-methyl-phenol, tetradecanoic acid, and hexadecanoic acid in n-hexane extract and pentadecane, hexadecane, 2,6,10,14-tetramethyl-hexadecane,

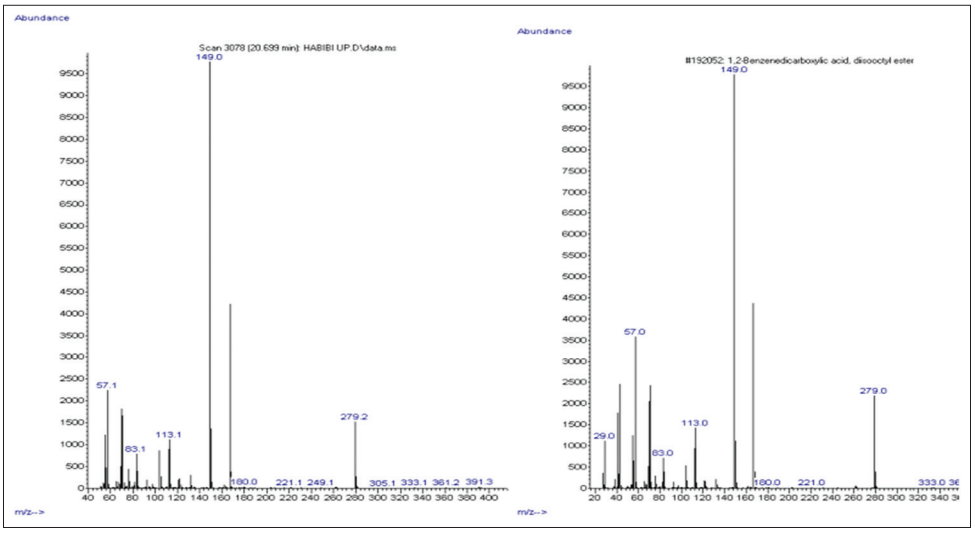

Figure 5: Mass spectrum of ethyl acetate-chloroform (1:1) extract of *Padina tetrastromatica* with RT=20.699 (left) and 1,2-benzenedicarboxylic acid, diisooctyl ester reference (right)

Table 1: Bioactive of some components identified in the n-hexane extract of *P. tetrastromatica*

Entry	RT	Name of compounds	Peak area %	Nature of compound
1	3.688	Alpha-humulene	1.12	Sesquiterpene
2	4.002	Decane	2.35	Monoterpene
3	4.454	2-methyl-phenol	6.19	Aromatic
4	13.375	Tetradecanoic acid	9.08	Sesquiterpene
5	15.458	Hexadecanoic acid	32.59	Sesquiterpene

P. tetrastromatica: Padina tetrastromatica, RT: 3.688, 4.002, 4.454, 13.375, 15.458

Table 2: Bioactive of some components identified in the ethyl acetate-chloroform (1:1) extract of *P. tetrastromatica*

Entry	RT	Name of compounds	Peak area %	Nature of compound
1	10.405	Pentadecane	0.50	Sesquiterpene
2	11.590	Hexadecane	0.74	Sesquiterpene
3	13.901	2,6,10,14-tetramethyl-hexadecane	0.34	Diterpene
4	14.823	2,6,11-trimethyl-dodecane	0.44	Sesterpene
5	20.699	1,2-benzenedicarboxylic acid, diisooctyl ester	94.50	Aromatic ester

P. tetrastromatica: Padina tetrastromatica, RT: 10.405, 11.590, 13.901, 14.823, 20.699

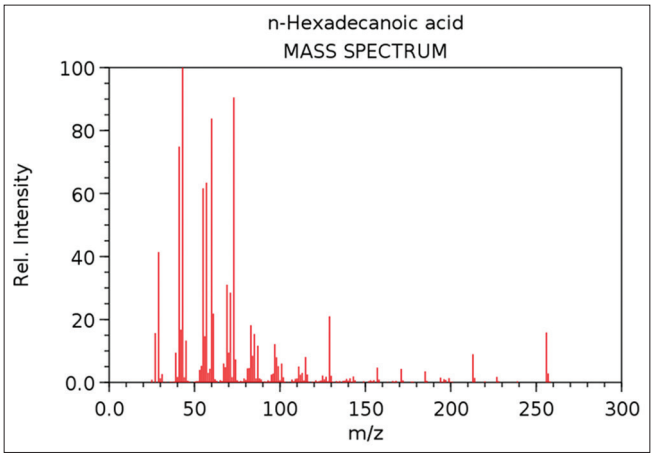

Figure 6: Mass spectrum the reference of n-hexadecanoic acid

2,6,11-trimethyl-Dodecane, 1,2-benzenedicarboxylic acid, and diisooctyl ester in ethyl acetate-chloroform (1:1) extract, were identified terpenoid compounds with high abundant (32.59 and 94.50%). MS spectra of five mentioned compounds are shown in Figures 2 and 3.

DISCUSSION

Marine supplies are a potent source for unexplored chemical moieties that may have vital biological and economic properties. Marine seaweeds have proven its importance as several of compounds of the economic significance are isolated and mass produced across the globe. Very little of these living organisms in Persian Gulf was studied, and therefore, it needs to evaluate all contents of algae.

In this study, GC–MS as a good method can be analyzed the unknown organic materials from Persian Gulf brown algae *P. tetrastromatica* sp. We obtained mixtures of terpenoids compounds with other compounds such as aromatic esters and phenols. GC investigation of isolated mixture showed at least five different compounds, namely alpha-humulene, decane, 2-methyl-phenol, tetradecanoic acid, and hexadecanoic acid in n-hexane extract and pentadecane, hexadecane, 2,6,10,14-tetramethyl-hexadecane, 2,6,11-trimethyl-dodecane, 1,2-benzenedicarboxylic acid, and diisooctyl ester in ethyl acetate-chloroform (1:1) extract. Major of these compounds

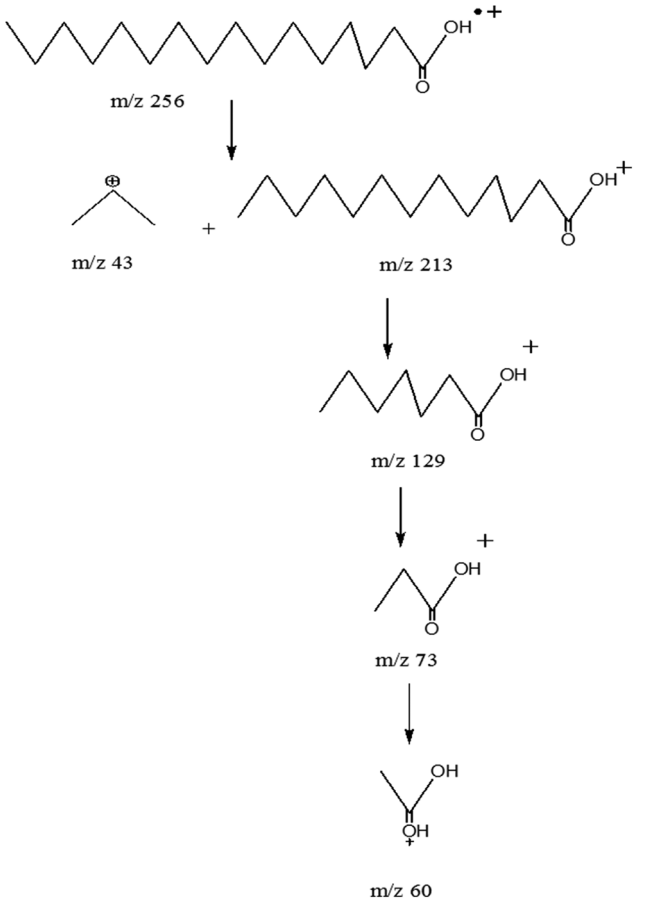

Figure 7: Fragmentation pattern of n-hexadecanoic acid

belong to terpenoid family such as sesquiterpene, monoterpene, and diterpene, as they are shown in Tables 1 and 2. For example, the reference MS and fragmentation pattern corresponded to n-hexadecanoic acid were displayed in Figures 6 and 7.

CONCLUSION

Persian Gulf brown algae *P. tetrastromatica* sp. produces numerous unique biomaterial of potential medicinal value. This species has several components. From it, to groups of five different compounds were identified by GC–MS for n-hexane and ethylacetate-chloroform (1:1) extracts. Most of these compounds which are belonged to terpenoid family,

are widely can used in pharmaceutical, medicinal, cosmetic and other fields.

REFERENCES

1. Blunt JW, Copp BR, Munro MH, Northcotec PT, Prinsep MR. Marine natural products. Nat Prod Rep 2010;27:165-237.
2. Mazid M, Khan T, Mohammad F. Role of secondary metabolites in defense mechanisms of plants. Biol Med 2011;3:232-49.
3. De Paula JC, Vallim MA, Teixeira VL. What are and where are the bioactive terpenoids metabolites from *Dictyotaceae* (*Phaeophyceae*). Brazil J Pharm 2011;21:216-28.
4. Ruzicka L. The isoprene rule and the biogenesis of terpenic compounds. Experientia 1953;9:357-96.
5. Van Agtmael MA, Eggelte TA, van Boxtel CJ. Artemisinin drugs in the treatment of malaria: From medicinal herb to registered medication. Trends Pharm Sci 1999;20:199-205.

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