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GC-MS analysis and preliminary test of phytochemical screening of crude ethanolic extract of green algae, *Cladophora Glomerata (L.) Kütz* from Caspian Sea

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Abstract: Alga, with the scientific name of *Cladophora Glomerata (L.) Kütz* is a plant in the family of Cladophoracea was collected in summer from the Caspian Sea in Babolsar, Mazandaran, Iran and dried in shade for one week. The extraction of the dried plant was obtained by different Extraction techniques of plants like Maceration (in ethanol, ethyl acetate and n-hexane) and Hot Continuous Extraction (Soxhlet in methanol). The screening tests of plant photochemistry, showed the presence of Flavonoid, Terpenoid, Saponin, Tanin, Glycoside from Cladophora ethanolic extraction. The obtained plant extraction was analyzed by the Gas Chromatography-Mass (GC-Mass) spectroscopy. n-hexadecanoic acid-ethyl ester (11.46%), marmesin or furanocoumarin (17.39%) and oleic acid (17.74%) contributed more percentage than the other compounds.

Keywords: Alga, *Cladophora Glomerata (L.) Kütz* ., extraction, phytochemical screening test, GC-MS analysis.

Introduction

In recent years, natural products have been playing a major role in the search for novel drugs or drug candidates against infectious diseases, inflammation, cancer and many other illnesses. They are an ongoing and inspiring source for researchers due to their enormous structural diversity and complexity. The marine algae represents a largely unexplored source for the isolation of novel bioactive compounds and may become even more so as knowledge on marine natural products. Alga and their extracts are one of the most commonly used natural biological active raw materials [1]. Green algae are widely used in the life science as the source of compounds with diverse structural forms and biological activities. Marine and fresh water algae have been historically and exceptionally rich source of pharmacologically active metabolites [2].

Macroalgae has been recognized as a source of bioactive secondary metabolites with antitumor [1,2], antibacterial [3,4], antioxidant [5,6], anti-inflammatory [5-7] and antifouling activities. This is due to the presence of biologically active compounds such as carbohydrates, fatty acids, brominated phenols, sterols, polysaccharides, peptides, proteins, acrylic acid, chlorophyllides, terpenes and heterocyclic carbons [8-12].

Result and Discussion

The present paper reports the results of a research aimed to verify the GC-MS analysis and preliminary test of phytochemical screening of crude ethanolic extract of green algae Cladophora from the floating benthic populations of south coast of the Caspian Sea in Babolsar, Mazandaran, Iran, in order to understand the efficacy of this algae as a foodstuff as well as in medicine and recommends its use in human diets and commercial purpose. Gas chromatography and mass

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spectroscopy analyses were carried out on the alcoholic extract of *Cladophora Glomerata (L.) Kütz* and various bioactive compounds were identified. Active principles with their retention time (RT), molecular formula (MF), molecular weight (MW), concentration (%) and nature of the compounds are presented in Table 1.

Figure 1: Green Algae, *Cladophora Glomerata (L.) Kütz* from Caspian Sea

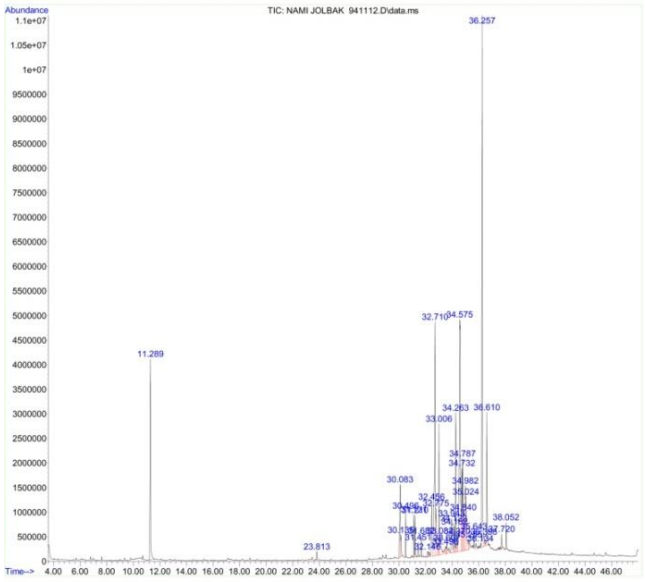


Figure 2: GC-MS Chromatogram of methanolic extract of *Cladophora Glomerata (L.) Kütz*

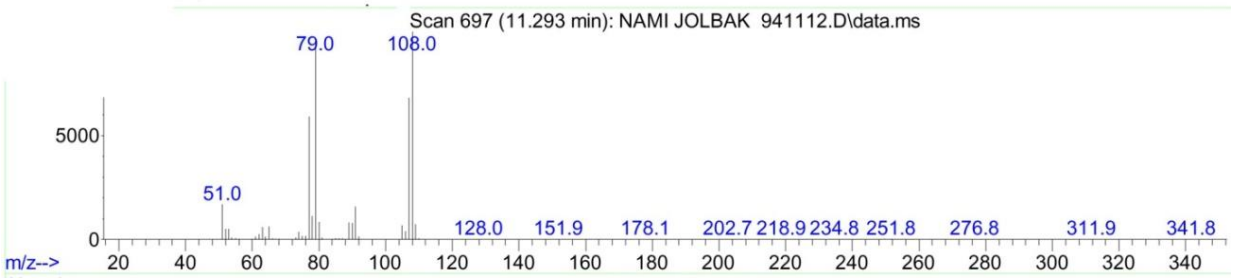


Figure 3a: Benzyl Alcohol

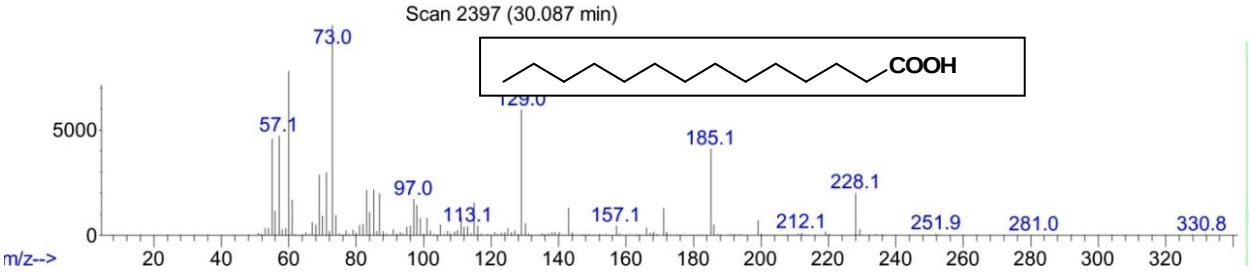


Figure 3b: Tetradecanoic acid

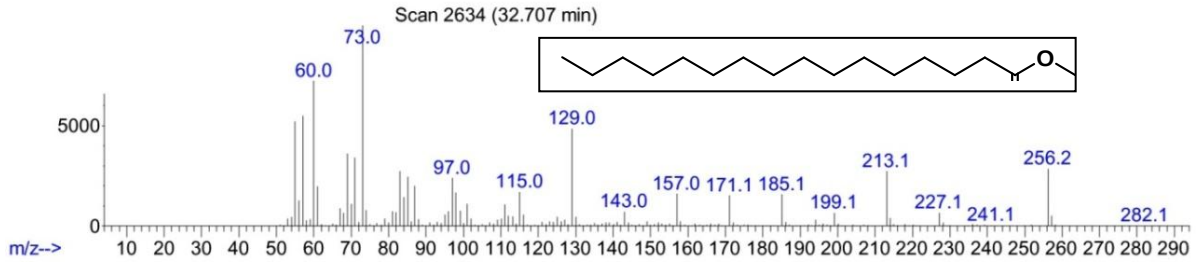


Figure 3c: n-Hexadecanoic acid-ethyl ester

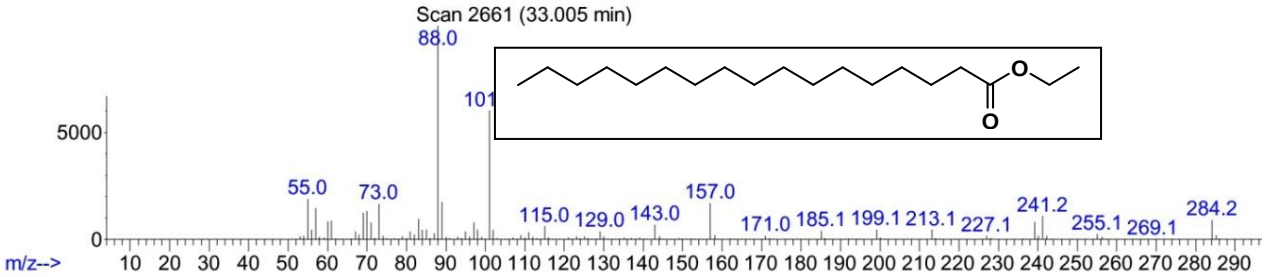


Figure 3d: Hexadecanoic acid ethylester

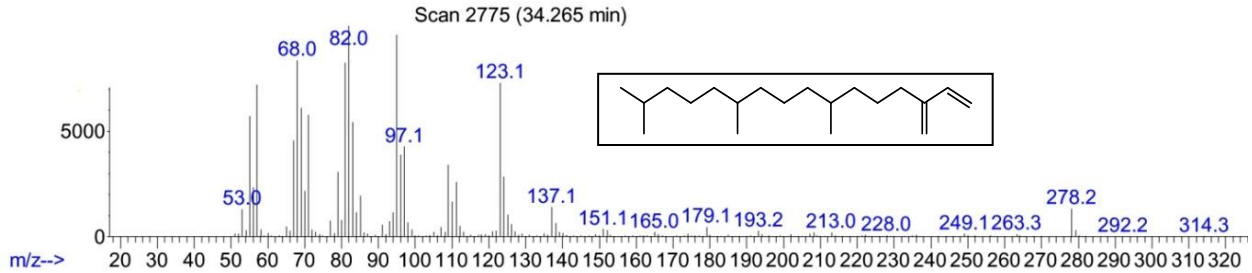


Figure 3e: Neophytadiene

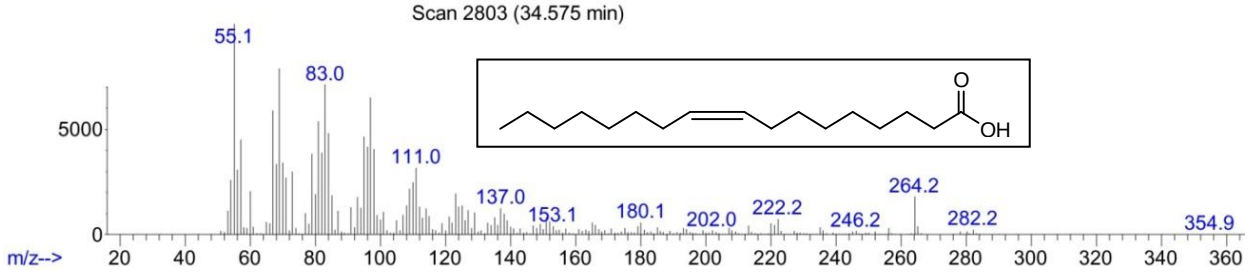


Figure 3f: Oleic acid

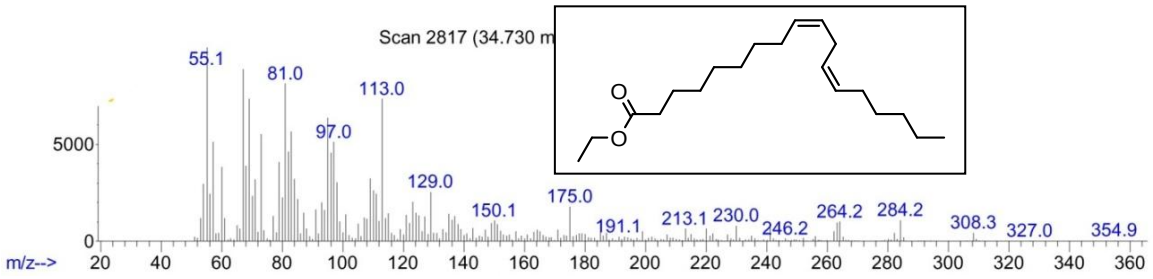


Figure 3g: Linoleic acid ethyl ester

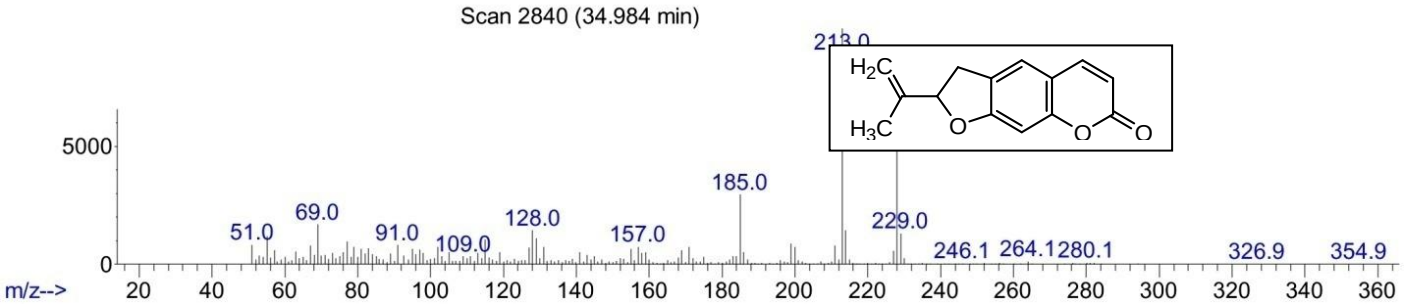


Figure 3h: 2-Isopropenyl-2,3-dihydrofuro[3,2-g]chromen-7-one

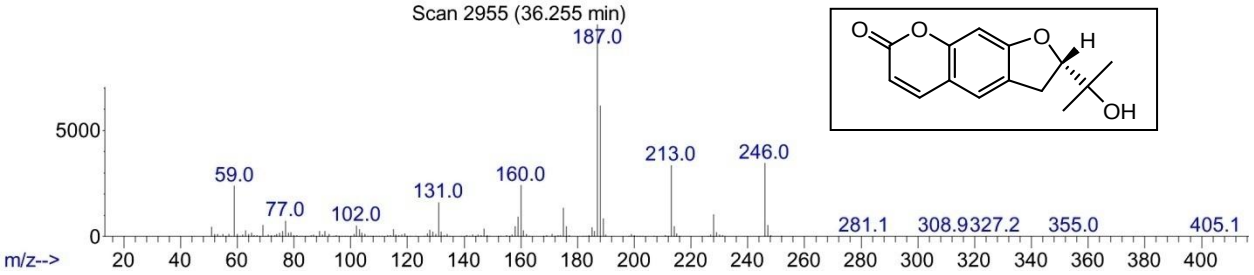


Figure 3i: Marmesin

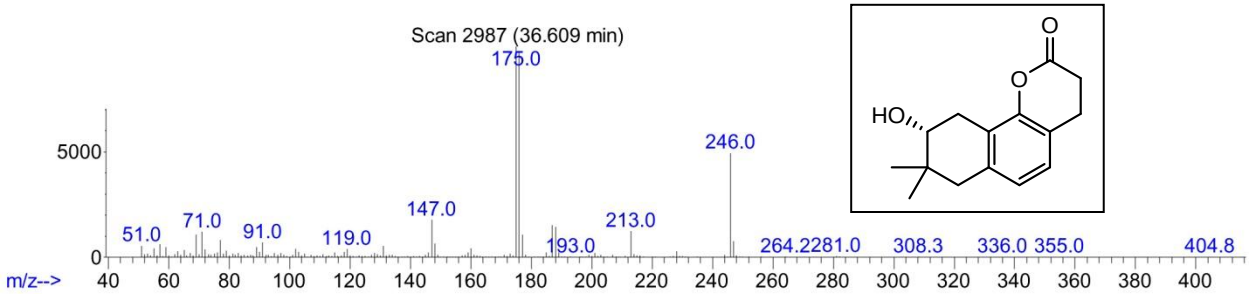
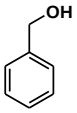
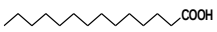
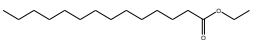
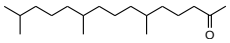
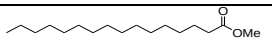
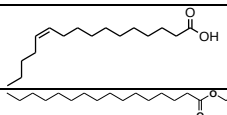
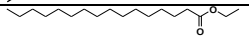
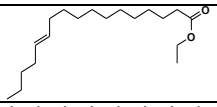
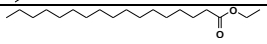
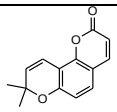


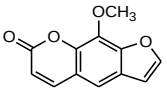
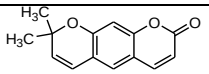
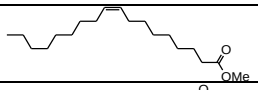
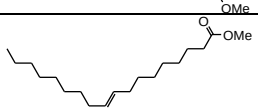
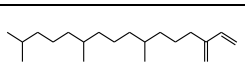
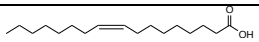
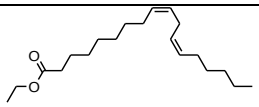
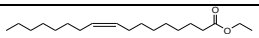
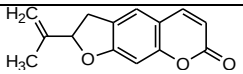
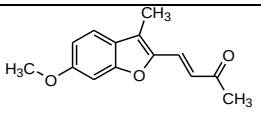
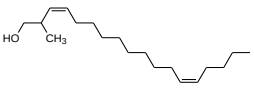
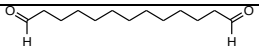
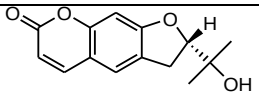
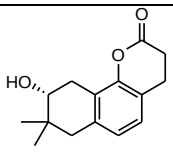
Figure 3j: 2H,8H-Benzo[1,2-b:3,4-b']dipyran-2-one, 9,10-dihydro-9-hydroxy-8,8-dimethyl- (R), (Jatamansinol)

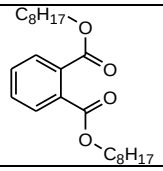
Knowledge of chemical constituents of plants is desirable because such information will be important for synthesis of chemical substances [13]. It could be qualified for application in pharmaceutical industry [17-20].

Therefore, present study revealed that in the aerial part extract of *Cladophora Glomerata (L.)* Kütz were identified 25 different compounds by GC-MS (Table**1**). Especially, n-Hexadecanoic acid-ethyl ester, Oleine, Z-11-Hexadecenoic acid, Marmesin, 2H,8H-Benzo[1,2-b:3,4-b']dipyran-2-one, 9,10-dihydro-9-hydroxy-8,8-dimethyl-(R) neophtadiene, benzyl alcohol and tetradecanoic acid contributed more percentage than the other compounds. Some other compounds were identified. n- hexadecanoic acid-ethyl ester (11.46%), marmesin or furanocoumarin (17.39%) and oleic acid (17.74%) were extracted from the *Cladophora Glomerata (L.) Kütz* which is higher than that of the other extracted compounds (Figure **2**, Figure 3a-j). These compounds act as anemiagenic, insectifuge, antiandrogenic, dermatitigenic antioxidant, anticancer and use for treatment of asthma and lymphedema, for increasing the plasma anti-thrombin levels [22]. The identified compounds of the *Cladophora Glomerata (L.) Kütz*., their retention indices, percentage composition, chemical structure and activities are given in Table **1**.

Table1: Phytocomponents identified in the ethanolic extract of *Cladophora Glomerata (L.) Kütz* by GC-MS

retention time (RT)	Name of the compound	Molecular Formula (MF)	Molecular weight (MW) ($g \cdot mol^{-1}$)	Peak area%	Structure	Nature of compound	Activity
11.29	Benzyl Alcohol	C_7H_8O	108.14	7.58		Alcohol	Bacteriostatic, cosmetics and topical drugs.
30.09	*Tetradecanoic acid*	$C_{14}H_{28}O_2$	228.37	3.17		fatty acid	cancer preventive, nematicide, Lubricant, hypercholesterolemic
30.49	*Tetradecanoic acid, ethyl ester*	$C_{16}H_{32}O_2$	256.42	1.05		fatty acid ethyl esters	cosmetics, soaps, perfumes, and flavoring
31.21	6,10,14-Trimethyl-2-pentadecanone	$C_{18}H_{36}O$	268.47	1.14		Diterpenoid	
32.14	Hexadecanoic acid, methyl ester	$C_{17}H_{37}O_2$	270.45	0.32		fatty acid methyl esters	Antibacterial, antifungal, antimicrobial
32.45	*Hexadecenoic -acid, Z-11*	$C_{16}H_{30}O_2$	254.41	2.37		Unsaturated Fatty acids	can prevent initial bacterial adhesion
32.70	*n- Hexadecanoic acid-ethyl ester*	$C_{19}H_{36}O_2$	282.42	11.46		Palmitic acid ester	Antioxidant
32.77	*Hexadecenoic -acid-ethylester (Z)11*	$C_{19}H_{36}O_2$	282.56	2.12		fatty acid ethyl esters	antimicrobial
33.00	*Hexadecanoic acid ethylester*	$C_{18}H_{36}O_2$	284.48	3.04		fatty acid ethyl esters	antimicrobial
33.08	*Seselin*	$C_{14}H_{12}O_3$	228.24	1.53		Coumarins	anticoagulant, antimicrobial, Anti-inflammatory, Antioxidant

33.49	*9-Methoxy-7H-furo[3,2-g][1] benzopyran-7-one (Xanthotoxin)*	$C_{12}H_8O_4$	216.18	0.47		Coumarins	anticoagulant, antimicrobial, Anti-inflammatory, Antioxidant
33.94	*2H,8H-Benzo[1,2-b:5,4-b']dipyran-2-one, -8,8-dimethyl*	$C_{14}H_{14}O_4$	280.45	2.48		Coumarins	anticoagulant, antimicrobial, Anti-inflammatory, Antioxidant
34.12	Octadecenoic -۹ acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.48	0.88		fatty acid methyl esters	Antibacterial, antifungal, antimicrobial
34.17	*Octadecenoic -۹ acid, methyl -(ester, E)*	$C_{19}H_{36}O_2$	296.48	0.74		fatty acid methyl esters	Antibacterial, antifungal, antimicrobial
34.26	*Neophytadiene*	$C_{20}H_{38}$	278.57	3.44		Olefine	Antiproliferative
34.57	*Oleic acid*	$C_{18}H_{34}O_2$	282.46	17.74		fatty acid	Cancer preventive, Anemiagenic, Insectifuge, Antiandrogenic, .Dermatitigenic
34.73	*Linoleic acid ethyl ester*	$C_{20}H_{36}O_2$	308.49	3.11		fatty acid ethyl esters	antimicrobial
34.78	*Ethyl Oleate*	$C_{20}H_{38}O_2$	310.52	2.73		fatty acid ethyl esters	a primer pheromone in honeybees
34.98	*2-Isopropeny-2,3-dihydrofuro[3,2-g]chromen-7-one*	$C_{14}H_{12}O_3$	228.24	3.15		furanocoumarin	anticoagulant, antimicrobial, Anti-inflammatory, Antioxidant
35.02	4-(6-Methoxy-3-methyl-2-benzofuranyl)-3-buten-2-one	$C_{14}H_{14}O_3$	230.25	2.84		Benzofuran	antiplasmodial activity
35.26	*2-Methyl-Z,Z-3,13-octadecadienol*	$C_{19}H_{36}O$	280.49	0.40		Fatty Alcohols	Not found
35.64	*Tridecanedial*	$C_{13}H_{24}O_2$	212.33	0.47		Aldehydes	Not found
36.25	*Marmesin*	$C_{14}H_{14}O_4$	264.26	17.39		furanocoumarin	For treatment of asthma and lymphedema, for increasing the plasma anti-thrombin levels
36.60	*2H,8H-Benzo[1,2-b:3,4-b']dipyran-2-one, 9,10-dihydro-9-hydroxy-8,8-dimethyl- (R), (Jatamansinol)*	$C_{14}H_{14}O_4$	246.25	3.66		Coumarins	anticoagulant, antimicrobial, Anti-inflammatory, Antioxidant

38.05	*1,2-Benzenedicarboxylic acid, diisooctyl ester*	$C_{24}H_{38}O_4$	390.55	0/57		diisooctyl ester Phthalic acid,	antibacterial

Generally, alkaloids, saponins, tannins, flavonoids and phenolic compounds are important antimicrobials [23, 24]. This lends support to the present study which revealed that those phytochemicals were identified in *Cladophora Glomerata (L.) Kütz* Similarly, previous studies have revealed that alga could act as antimicrobial, antioxidant and antibacterial drugs [25, 26].

Table **2** indicated the different phytochemical components of *Cladophora Glomerata (L.) Kütz* The ethanol extracts from *Cladophora Glomerata (L.) Kütz* contained a number of phytochemical such as alkaloids, flavonoids, glycoside, phenols, saponins and tannins. This data corroborated the findings of other authors where these compounds exhibited antimicrobials activities [27]. The presence of flavonoids indicates the natural occurring phenolic compound, with beneficial effects in the human diet as antioxidants and neutralizing free radicals [28]. Tannins are group of polymeric phenolic compound and cause local tumors. Terpenoids and steroids were reported to be active against antibacterial activity [29]. Saponins have the properties of precipitating and coagulating red blood cells, anti- inflammatory. Alkaloids are used in medicines for reducing headache and fever [30].

Table 2: Phytochemical analysis of ethanol of *Cladophora Glomerata (L.) Kütz* .

Phytochemical components Test	Resalts
Tannins	+
Saponins	+
Flavonoids	+
Terpenoids	+
Glycoside	+
Alkaloids	-

Conclusions

In conclusion, ethanol extract of aerial parts of *Cladophora Glomerata (L.) Kütz* possess significant antibacterial activity and this potential may be due to the presence of bioactive compounds like alkaloids, tannins, saponins, triterpenoids and phenolic compounds. Hence, the present study was justified on its use in the traditional folk medicine. GC-MS analysis also identified a variety of natural bioactive compounds n-Hexadecanoic acid-ethyl ester, Oleic acid, Z-11-Hexadecenoic acid, Marmesin, ۴H,8H-Benzo[1,2-b:3,4-b']dipyran-2-one, 9,10-dihydro-9-hydroxy-8,8-dimethyl-(R) neophtadiene, benzyl alcohol and tetradecanoic acid. However, a further detailed study on *Cladophora Glomerata (L.) Kütz* is necessary for the development of novel drugs in the area of anticancer, anti-inflammatory, antiandrogenic, antiarthritic and anticoronary activity.

Experimental

Phytochemical Screening

The preliminary Phytochemical analysis of the extracts carried out using ethanol extract and on the powdered specimens using standard procedures to identify the various constituents described previously [13-15].

Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Collection and preparation of algal extracts

Alga, with the scientific name of *Cladophora Glomerata (L.) Kütz* was carried out in the southern coast of the Caspian Sea in the city of , Mazandaran, Iran, in summer 2015. Samples of *Cladophora Glomerata (L.) Kütz* were collected manually from the rock. Biomass was rinsed with fresh water to eliminate other materials such as sand, shells, etc. The macroalgae were stored in the laboratories and dried at room temperature for one week, ground in a blender.

Dried materials were coarsely ground before extraction.

Preparation of plant extracts

50 gram of the powdered alga was weight in a beaker and percolated with 150 ml ethanol this beaker was proper sealed with aluminum foil and left for 72 hours. The solution was then filtered using a funnel filled in a filter paper and the extract obtained. The extract obtained were concentrated using rotary evaporator. The extract were stored in a universal bottle and refrigerated of 4^{o} C prior to use [16].

Determination of tannins

About 2 ml of the extract was stirred with 2ml of distilled water and few drops of ferric chloride ($FeCl_3$) solution were added. Formation of green precipitate was indication of presence of tannins.

Determination of Alkaloids

3 ml of extract was stirred with 3 ml of 1% HCl on steam bath. 1 ml of mixture was taken separately in two test tubes. Few drops of Dragendorff's reagent were added in one tube and occurrence of orange red precipitated was taken as positive. Two the second tube Mayer's reagent was added and appearance of buff colored precipitate was taken as positive test for presence of alkaloids.

Determination of Saponins

5 ml of extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

Determination of Terpenoids

2 ml of the organic extract was dissolved in 2ml of $CHCl_3$ and evaporated to dryness. 2ml of conc. H_2SO_4 was then added and heated for about 2 minutes. Development of a grayish color indicates the presence of terpenoids Test for glycosides To 2 ml of extract with dilute HCl and 2 ml Sodium nitropruside in pyridine and sodium hydroxide solution were added. Formation of pink to blood red color indicates the presence of Cardiac glycosides.

Determination of flavonoids

To 3 ml of extract, 3ml of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for presence of flavonoids.

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