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Phytochemical studies on *Cladophora* species from the Nil River Edges, Egypt

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Abstract
Cladophora algal species was subjected to phytochemical and antioxidant studies. The phytochemical studies revealed that it contains flavonoids, tannins, carbohydrates/ glycosides, sterols, terpenoids, sulphates, chlorides and free from saponins and alkaloids. Also the free and combined sugars, amino acids, phenolic acids, fatty acids, hydrocarbons, sterols and minerals were analysed quantitatively. Determination of phenolic acids was carried out and the flavonoid compounds were isolated from this alga. From this study *Cladophora* algal species can be considered as a promising antioxidant agent.

Keywords: phytochemical, *cladophora* algal species, nil river edges

Introduction

Algae are used for many purposes: in food industry, animal feeding, medicine, cosmetic industry and for soil enrichment. There are also many secondary ways for using algae: producing alginates and derivate of algae used in industry. Nowadays researchers use to analyze algae for medical purposes, because they have a strong potential against many diseases, in alimentation because they act like protective and functional additives (New Man *et al.,* 2003; Horincar *et al.,*2011) [73, 44]. Algae were reported to produce a wide variety of bioactive secondary metabolites as antimicrobial, antiviral and antioxidant (Schaeffer and Krylov, 2000; Del Val *et al.,* 2001 Zbakh *et al.,* 2014) [85, 33, 106] antifeedant, antihelmintic, antihypertinsive (Mudassir *et al.,* 2005) [69] and cytotoxic agents and the bioactive substances included alkaloids, polyketides, cyclic peptide, polysaccharide, phlorotannins, diterpenoids, sterols, quinones, lipids and glycerols (Cabrita *et al*., 2010; Kumar *et al*., 2012, Tang *et al*., 2012 and Kumbhar *et al*., 2014) [16, 56, 93, 57].

Filamentous green algae constitute an important component of algal communities in freshwater habitats. Nevertheless, their enormous morphological plasticity and the difficulty of observing some features have complicated their taxonomy. These and other facts explain the scarcity of data on their ecology. The filament is an excellent adaptative form: the plant grows rapidly in length and can use new volumes of water maintaining the area/volume ratio constant. Branches represent a variation which permits the algae a better use of water in confined spaces and a better control of absorption. Secession from simple filaments with fast growth to branched filaments with a slower growth can be observed very frequently in freshwater ecosystems (Margalef, 1983) [64].

The filamentous alga *Cladophora* is a common inhabits of freshwater locations. It is called blanket weed in some places, not an inappropriate name when in late summer dense floating rafts of *Cladophora* can be found both at the pond's edge and in the open water, buoyed up with the oxygen generated by its own photosynthesis. *Cladophora* is capable of branching, and seems to produce little or no mucilaginous secretion. This, and the fact that salts tend to crystallize on the filaments of older specimens, gives it a rougher, grittier feel than other filamentous algae. It is also more readily colonized by epiphytic diatoms and other algae, and provides a protected foraging environment for the smaller pond creatures such as protozoa, worms, small crustaceans and insect larvae (Fabrowska *et al.,* 2015 and Pochon. *et al.,* 2015) [35, 77].

There are various reports on the chemical constituents of the green algae *Cladophora* species in different parts of the world (Feng *et al.,* 2007; Nirmal Kumar *et al.,* 2009; Krish & Das, 2014 and Zbakh *et al.,* 2014) [36, 74, 54, 106].Also Several of chemical investigations on *Cladophora* species yielding antioxidant, antimicrobial and anti-cancer substances were reported (Kuniyoshi *et al.,* 1985; Abdel-Raouf *et al.,* 2008 and Al-Saif *et al.,*2014) [58, 2, 12].

Materials and Methods

A *Cladophora* samples was collected from Nil River pond edges, Al-Mansoura, Egypt during summer season, (2014). The temperature of water ranged between 23 ^{0}C and 24^{0}C and estimated PH 6.8 to 7. The samples were identified according (Leliaert and Coppejans, 2003) [61]. Fresh specimens of studied species was prepared and kept in the algal laboratory at Botany and Microbiology Department, Faculty of Science (Girls branch) Al-Azhar University. The samples were air dried at room temperature and ground to powder and stored in plastic bags in a dry place until use.

Algal extraction

One hundred grams of the dry plant material were extracted with successive selective solvents according to polarity pet. ether (B.p.40 -60^{0}C), ether, acetone, chloroform, ethyl acetate, ethyl alcohol 95 %, ethyl alcohol 70% and water. The residue obtained from each solvent was dried and weighted.

Preliminary phytochemical screening

The ethyl alcohol 70 % extract was used for detecting the presence of flavonoids, tannins, carbohydrates, glycosides, saponins, steroids, sulphates, chlorides and alkaloids. Also used for the detection of free and combined sugars, amino acids, phenolic acids, fatty acids, hydrocarbons, sterols and minerals

Composition analysis

The total ash content was estimated according to Askar and Treptow (1993) [16], and the minerals were determined according to AOAC (1991) [1] by using the atomic absorption spectrophotometer (Unicam M.A.929).

Determination of free and combined sugars

Free and combined sugars were determined by using paper chromatography, ascending method spray reagent (Partridge, 1949) [76]. The quantity of sugars was measured by using High Performance Liquid Chromatography (HPLC) apparatus (The Regional Center for Mycology and Biotechnology, Al-Azhar University).

Determination of fatty acids, hydrocarbons and sterols

Fatty acids, hydrocarbons and sterols were separated and identified by (HPLC) apparatus, as their methyl ester derivatives using ethereal solution of diazomethane method Vogel (2000) [102].

Identification and determination of free and protein amino acids

The determination of the free and combined amino acids were carried out by using LKB & plus high performance amino acid analyzer (LKB. Biochrom, LTD England) according to Sadasivam and Manickam (1996) [84].

Extraction and identification of free phenolic acids

The free phenolic acids were isolated from algal species according to Danny *et al.* (2003) [30] and separated by High Performance Liquid Chromatography (HPLC) instrument (Knauer, Germany) equipped with a Model 7125 injection valve (Rheodine, Cotati, CA, USA) with a 50 µl sample loop, under computer control (Knauer, HPLC version 211a). The flow rate was 1.0 ml / min and detection was carried out by UV at 280 mm (The Regional Center for Mycology and Biotechnology, Al-Azhar University).

Spectroscopic Determinations

Total Carbohydrates

Anthrone reagent was prepared as described by Trevelyan & Harrison (1952) [95]. The reagent was freshly prepared each day and used within 12 hr. Anthrone reagent (5 ml.) was pipette into thick walled Pyrex tubes (150 x 25 mm.) and chilled in ice water. The solution under test (1 ml.) was layered on the acid, cooled for a further 5 min. and then thoroughly mixed while still immersed in ice water. The tubes were loosely fitted with corks, heated as required in a vigorously boiling, constant level water bath and then cooled in water for 5 min. Absorption spectra were determined in spectrophotometer (Unicam SP. 500). The measurements of test solutions and of reagent blanks were made against water as a reference. The relation between scale readings and amounts of sugars was not strictly linear, and it was necessary to use calibration curves for the different sugars.

Total phenolics and flavonoids

Determination of total phenolics

The total phenolic contents of *Cladophora* sp. extract were determined according to the method described by Malik and Singh (1980) [63]. The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as mg catechol equivalent of phenol/g of sample.

Determination of total flavonoids

The aluminum chloride method was used for the determination of the total flavonoid content of the algal extract according to the method described by Bag *et al*., (2015) [19]. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample.

Total soluble protein

Total soluble proteins were determined quantitatively according to the Bradford method (Bradford, 1976) [22] and expressed as mg protein/g fresh weight. Bovine serum albumin was used as the standard.

Antioxidant activity (Free Radical DPPH Scavenging Activity)

Antioxidant activity was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) is a well-known radical and a trap (scavenger) for other radicals. Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction. The free radical scavenging activity was measured according to established methods (Yen and Duh, 1994) [105] with some modifications. A methanol sol. of the test compound was prepared, 40 µg/mL of each extract in methanol was added to 3 mL of a solution of 0.004% DPPH in methanol. The mixture was shaken vigorously and allowed to stand for 30 min. at room temperature in the dark. The absorbance of the resulting solution was measured at 515 nm with UV-visible spectrophotometer (Milton Roy, Spectronic 1201). Ascorbic acid was used as positive control. Percentage inhibition of the sample was calculated by the following equation:

$$\% \text{ Activity} = [(Ac-At) / Ac] \times 100$$

Where, Ac = Absorbance of the control (zero min.).

At = Absorbance of the test sample + DPPH at 16 min.

Isolation of flavonoid compounds

Two hundred grams of the algal powder were defatted then extracted in soxhlet with 70% ethanol. Ten g alcoholic algal extract dissolved in hot distilled water (100ml) then extracted with ether (5X100) followed by chloroform (5x100) followed

by ethyl acetate (5x100) and finally with butanol (using separatory funnel). Each solvent was dried to give residues,2g, 2g and 1g respectively. The separated residues were subjected to Thin Layer chromatography, TLC (System1: butanol: acetic acid: water (4:1:5), System 2: acetic acid: water (15%) and System 3: ethyl acetate –methanol –water (30:5:4). Bands of each flavonoid were Exposure to ammonia vapour. Then observed under UV light and /or spraying with $FeCl_3$, about 30 chromatograms were scratched then dissolved in methanol and submitted to a column of sephadex LH-20. Three compounds were isolated and identified by GC/Mass.

GC/Mass. Chromatography

Pure compounds were subjected to GC/Mass investigation using (SHIMADZU GC/Mass QP 5050 A) instrument employing the following conditions: column: DB5, (0.53mm IDX-1. 5μm.film) carrier gas: He (1ml/min); injector temp. (280C^0) detector temp. (280 C^0) column temp. 60 C^0, (0.5 min.) -150C^0 (1min.) at 10C^0/min.-250C^0 (2min) at 10C^0/min. Mass spectra: Electron empact70ev.

Results and Discussion

Phytochemical studies

The results obtained from the Preliminary phytochemical Screening carried on *Cladophora* sp. illustrated in Table 1, showed that *Cladophora* sp. contains flavonoids, tannins, carbohydrates/ glycosides,, sterols, terpenoids, sulphates, chlorides but free from alkaloids and saponins, our results corroborated with the previous studies on *Cladophora glomerata* by Mohamed *et al.,* 2013 [67].

Table 1: Preliminary phytochemical Screening

Experiment	Result
Alkaloids	-
Flavonoids	+
Terpenoids	+
Saponins	-
Tannins	+
Carbohydrates	+
steroids	+
Sulphates and Chlorides	+

Also from the data illustrated in Table 2 it was observed that *Cladophora* sp. contain a high content of Mg followed by Ca and Na respectively (0.05,0.04 and 0.02 gm/100gm dry wt.).Where Chloride record the lowest value (0.006 gm/100gm dry wt.) this results are disagree with the results obtained by Machado *et al*. (2013) [62]. Our results are uncorroborated with the previous studies on *Cladophora glomerata* by Zbikowsk *et al*. (2007) [107] as they reported that Ca, Mg, Na and K concentrations were ranged between 1.2&10.0,4.1&29.5 3.9&47.5μg/g. The ash content record 10% this result was disagree with Akkoz *et al*. (2009) [6] as they reported that *Cladophora glomerata* ash content was 2.4%. Khuantrirong and Traichaiyaporn (2011) [50] found that the ash content in their study on *Cladophora sp.* were ranged from 14.7 %to 16.86 % at different levels of phosphorus concentrations, and the level of Ca, Mg, Na and K were ranged between 6585-6863,2512-2658 and 241.6-255.3mg/100g^{-1}but agree with Ghazala and Shameel (2005) [38] for their study on some fresh green algae.

Table 2: The percentage of different components in the studied *Cladophora* species

Mineral	gm/100gm dry wt.	Fatty acids	mg%
Cl	0.006	Docosanoic(bhenic) acid	3.1
Mg	0.05	7,10,13-Elcosatrienoic acid	0.2
Ca	0.04	13-Docosenoic acid (erucic)	0.7
Na	0,02	5,8,11,14-Elcosatetraenoic(archidonic) acid	9.5
K	0.01	Tricosanoic acid	0.5
Ash	10	5,8.11,14,17-Elcosapentanoic acid	10
Free sugars	mg%	Tetracosanoic acid	0.03
Arabinose	0.6	Free amino acids	gm/16 gm N
Galactose	0.3	Alanine	0.16
Glucose	0.8	Glycine	0.13
Maltose	0.2	Beta alanine	0.12
Xylose	0.1	Phenyl alanine	0.13
Combined sugars	mg %	Valine	0.13
Arabinose	0.1	Leucine	0.11
Galactose	0.2	Iso leucine	0.07
Maltose	0.3	Serine	0.09
Phenolic acids	mg %	Threonine	0.08
P-hydroxy benzoic	o.4	Methionine	0.06
O-hydroxy Benzoic	0.7	Glutamic	0.03
Gallic	0.2	Histidine	0.05
Caffeic	0.5	Arginine	0.17
Isochlorogenic	0.8	Lysine	0.12
P-coumaric	0.3	Tyrosine	0.06
Gentasic	0.6	Phenylalanine	0.13
Proto-catechuic	0.2	Combined amino acids	gm/16 gm N
Chlorogenic	0.9	Cysteine	0.15
Sinapic	0.1	Glycine	0.14
Ellagic	0.7	Alanine	0.31

Ursolic	0.3	Beta alanine	0.17
Betulinic	0.6	Phenyl alanine	0.4
Total	7.2	Valine	0.17
Fatty acids	mg%	Leucine	0.19
Caproic acid	0.8	Iso leucine	0.24
Capric acid	0.99	Serine	0.14
Lauric acid	0.39	Threonine	0.22
Myristic acid	4.00	Methionine	0.32
Pentadecanoic acid	1.00	Aspartic	0. 16
Palmitic acid	5.35	Glutamic	0.18
Palmitoleic acid	3.3	Arginine	0.21
Margaric acid	0.18	Lysine	0.01
Stearic acid	4.1	Histidine	0.02
Oleic acid	7.5	Tyrosine	0.03
Linoleic acid	6.6	Sterols	mg%
Hexacosanoic acid	0.3	Cholesterol	0.07
Eicosanoic acid	0.19	Campasterol	0.04
Linolenic acid	19.2	Stigmasterol	0.03
11-Elcosenoic acid	0.9		

Five free sugars were recorded; glucose and arabinose were present as large amounts (0.80 mg % and 0. 60 mg % respectively) while maltose and xylose were the minute free sugars (0.20 mg % and 0.10 mg %) respectively. On the other hand maltose had the greatest percentage of the combined sugars (0.30mg %). This data disagreed with data obtained by Ramin, 2013 [80] from his study on *Cladophora Glomerata* alga and Ramana and Rao, 1991 [79] as they reported that green seaweed *Cladophora socialis* contained galactose (58.3%), arabinose (31.8%), xylose (10.6%) this different may attributed to the nature of growth between the two species our was fresh water where the other one was marine. On the other hand this results agree with Athbi *et al.,* 2012 [17] to their results obtained from their study on the algal *Cladophora crispata*. *Cl. Crispate* contain rhaminose, galactose, xylose and ribose in concentrations (11.94,12.3,10.5 and 13.3%) respectively, thirty one phenolic acids were recorded for *Cladophora sp.* Chlorogenic acid and iso- Chlorogenic Chlorogenic were present as large amounts (0.90 mg % and 0.80 mg % respectively) while gallic and sinapic were present as lowest amounts (0.20 mg % and 0.01 mg % respectively), this results agree with the results obtained by Cha *et al.,* 2014 [27] from *Cladophora wrightiana* and Dash *et al.,* 2014 [31] they reported that the phenolic content can be the responsible for the antioxidant activity of the algae. on the other hand this results disagree with Sheikh *et al.,* 2009 [90] to their results obtained from their study on the algal *Cladophora patentiramea.*

The free amino acids are recorded sixteen acids, Arginine is recorded the highest level followed by alanine (0.17 and 0.16 gm/16 gm N respectively),where Glutamic and Histidine are recorded the lowest level (0.03 and 0.05 gm/16 gm N respectively).While Cysteine, Aspartic, Glutathione, Aspragine, Aurine, Glutamine, Proline, Hydro proline and Gama amino putiric are absent the present results disagree with the results obtained by Rani, 2007 [81] on *Cladophora vagabunda* as he reported that The major constituents of the amino acid were aspartate, glutamate, glycine, valine, lysine, histidine, arginine, and proline. There were concomitant increases in the acidic amino acids: aspartate and the glutamate and the basic amino acids: lysine, histidine and arginine in response to salinity stress. The appearance of proline at hypersalinity alone showed that it acts as an osmoticant.on the other hand the results agree with Krisna Dewi1 *et al.,* 2014 [55] for their study on *Cladophora* sp. and agree with Tkachenko, 2003 [94] results on *Cladophora vagabunda & Cladophora vagabunda,* as he reported that the contamination of the aquatic environment by detergents was responsible for the increase in the content of amino acids involved in the processes of osmosis regulation and detoxication of pollutants. Combined amino acids are recorded nineteen acids, Phenyl- alanine, cystien and alanine showed the highest level (0.4, 0.33 and 0.31 gm/16 gm N respectively).Where Lysine, Histidine and Tyrosine are recorded the lowest level (0.01, 0.02 and 0.03 gm/16 gm N respectively).

Fatty acids composition revealed that Linolenic acid,5,8.11,14,17-Elcosapentanoic acid, 5,8,11,14-Elcosatetraenoic(archidonic) acid, Oleic acid, Linoleic acid and Palmitic acid are the most constituents (19.2,10.00, 9.5, 7.5, 6.6 and 5.35 mg% respectively) this results agree with Carballeira *et al.,* 1997 [25] for their study on *Cladophora coelothrix* and agree with Ghazala *et al.,* 2013 [39] for their results obtained from the study of *Cladophora glomerata* also agree with Ivanova *et al.,* 2012 [45] in the presence of the same kind of Fatty acids in *Cladophora vagabunda*, also agree with Sharmila and Jeyanthi Rebecca, 2012 [89] and Haq *et al.,* 2013 [41] from GC-MS Analysis of esters of fatty acid present in biodiesel produced from *Cladophora vagabunda* and *Cladophora* sp.

Table 3: Total flavanoids, total protein and total fat content in the studied *Cladophora* species.

Rutin (mg/kg)	**Quercetin (mg/kg)**	**Kaempherol (mg/kg)**	**Total Flavonoids**	**Total protein (%)**	**Total fat (%)**
1.5	1.2	1.7	1.2	3.1	0.3

From the data illustrated in Table 3 it was observed that three flavanoids namely rutein, quercetin and kaempherol were present in the extract in the algal sample studied. Kaempherol content was found to show a remarkable highest level among the three flavonoids (1.7 mg/kg) and a considerable low level

(1.2 mg/kg) was recorded for Quercetin. This results were disagree with the results obtained by Al-Saif *et al.,* 2014 [12] for the algal species *Cladophora socialis*, These results are in agreement with several reports (Ruperez *et al.,* 2002; Nagai and Yukimoto, 2003; Matanjun *et al*., 2008; Jaganathan and Mandal, 2009) [83, 71, 63, 46]. Flavonoids comprise a large group of naturally occurring compounds widely distributed in the plant kingdom and some of these compounds have been reported to contain various and potent biological activities including antioxidative tissue protective and tumor static effects as well as the inhibition of hepatic cholesterol biosynthesis (Kim *et al.,* 2007; Krant *et al*., 2005; Volk, 2009; Matanjun *et al*., 2008) [51, 53, 103, 65]. Moreover, the results of this study suggested that flavonoids can be used clinically to treat patients with hypercholesterolemia and hypertension (Abdel-Raouf *et al*., 2011) [3]. Present results were in accordance with an earlier report (Jaganathan and Mandal 2009) [46] which stated that Quercetin and Kaempherol have evolved as promising pharmacological agents in the treatment of cancer. It is well known that the increase in the levels of flavonoids in the daily diet may reduce the incidence of certain human diseases. The health benefits of flavonoids may be due to their interaction with various biological systems and show antioxidant capacity, free-radical scavenging activity, anticancer activity, and cardiovascular disease prevention, while some flavonoids exhibit potential for anti- HIV functions.

Table 4: DPPH Radical Scavenging activity

Plant extract Concentration in (µg/ml)	DPPH Radical Scavenging activity %
25	10 ± 3.4
50	27 ± 0.5
75	32 ± 0.9
100	43± 0.7
125	55± 1.9
150	66± 5.6
175	73± 0.6
200	89± 1.3

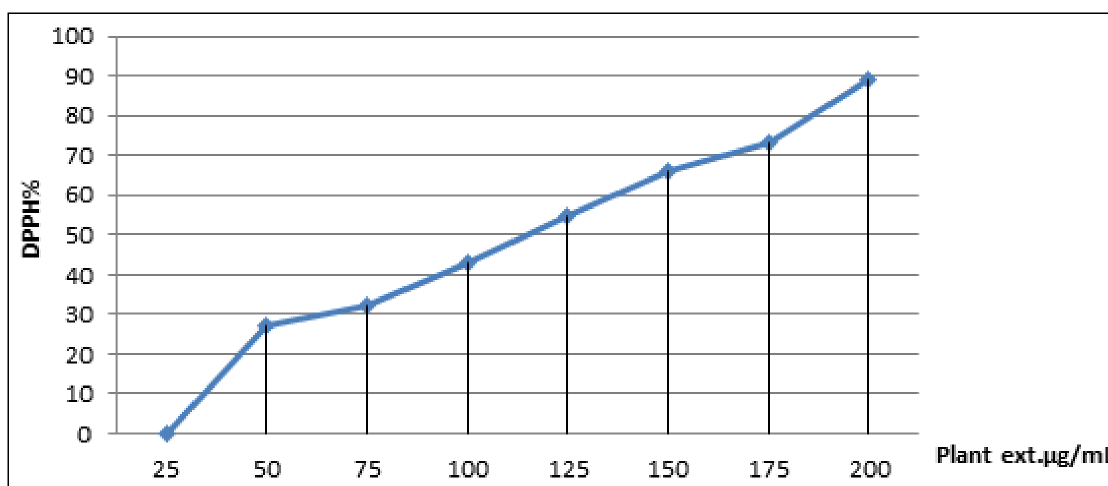

Fig 1: Radical-scavenging activity of algal extract.

Free radical DPPH scavenging potential of the plant extract has been evaluated spectro-photometrically (Table 4 & Figure 1). Results indicate that algal extract has a considerable scavenging potential at the concentration of 200 µg/mL (89.13%). The results agree with Keser *et al*., 2012 [49]. Results revealed the role of free radicals in the emergence of such lifestyle diseases such as atherosclerosis, heart attack, stroke, cancer, diabetes, senile cataracts and accelerated aging. Structurally polyphenols contain number of Hydroxyl groups (HO-), which are responsible for antioxidant characteristics and potential of polyphenols (flavonoids). Because of these OH groups, polyphenols are able to chelate transition metal ions, such as iron, and copper. Generally, these metal ions are involves to initiate various free radical chain reactions. Additionally they are also capable to inhibit enzymes such as xanthine oxidase and NADPH oxidase. These enzymes catalyze the production of large amounts of reactive oxygen (Gawron-Gzella *et al*., 2012) [37].

The extracts of *Cladophora* sp. (Figure 1) showed good antioxidant activity and effective scavenging of the DPPH radical. The percentage of inhibition was 98.13% for the *Cladophora* sp. extract, at the concentration of 200 µg/mL. (IC50=20.00 mg/mL). Antioxidant activity of the genus *Cladophora* agree with results obtained by (Sheikh *et al.* 2009; Soltani *et al.* 2011 and Laungsuwon& Chulalaksananukul 2013) [90, 92, 60]. Zubia *et al.* 2007 [108] reported that the extracts of *C. prolifera* exhibited also relatively high DPPH radical scavenging activities (IC50=16.66 mg/mL). Phenols and polyphenols such as flavonoids which are a large group of compounds, widely found in macroalgae. These compounds exert potent antioxidant activity not only because of their capacity to donate electrons or hydrogen atoms, but also because of their stable radical intermediates. In the study done by Soltani *et al.* 2011 [92] high phenolic content has been measured for *Cladophora* species showing a maximum of 3077 ± 105 mg gallic acid equivalent per gram of dry weight of extract.

Table 5: Three flavonoidal compounds were isolated and identified with chemical methods and Mass spectrum.

Compound	m. p.	Wt.(mg)	R_F value			Detection methods				Compound Name
			S1	S2	S3	Visible	UV	UV/NH_4OH	$AlCl_3$	
Compound 1	314-315	0.2	0.5	0.3	0.7	Yellow	Yellow	Bright-Yellow	Orange	Quercetin
Compound 2	277-281	0.7	0.86	0.14	0.19	-	Yellow	Yellow	Yellowish-green	Kaempherol
Compound 3	179-185	0.5	0.65	0.59	0.75	Yellow	Yellow	Dark Yellow	Yellow	Rutin
Compound	EI-MS									
Quercetin $C_{15}H_{10}O_7$	303(M^+) (100%), 257 (5%), 229(60%), 165(25%) and 137 (10%).									
Kaempherol $C_{15}H_{10}O_6$	287(M^+) (100%), 258(15%), 229(15%), 153(40%), 129(20%) and 69(10%).									
Rutin $C_{27}H_{30}O_{16}$	611(M^+) (100%), 465(20%), 449(5%), 303(35%), 129(15%), 85(15%) and 70(10%).									

System1: butanol: acetic acid: water (4:1:5), System 2: acetic acid: water (15%) and System 3: ethyl acetate –methanol –water (30:5:4).

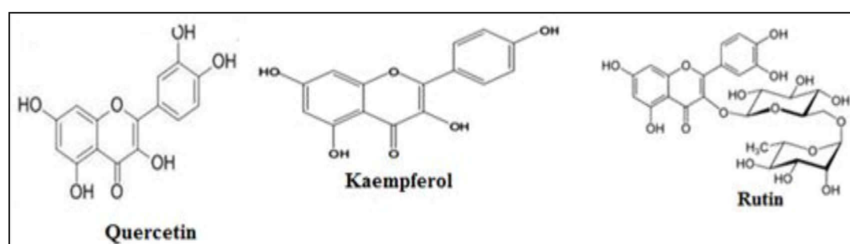

Fig 2: Structure of the three flavonoidal compounds.

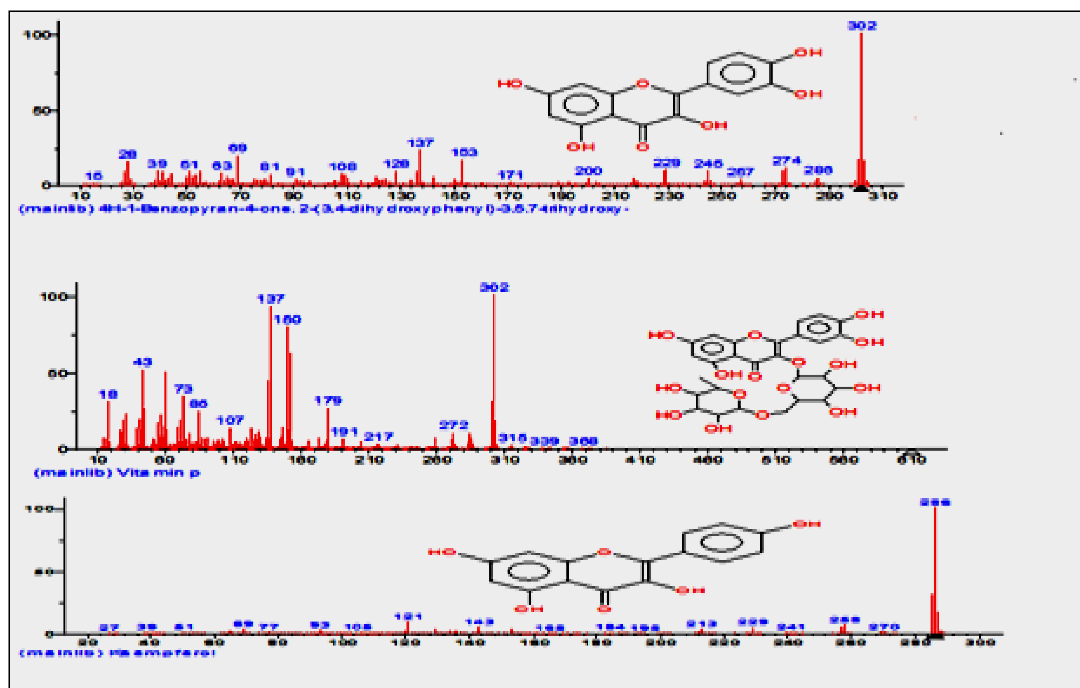

Fig 3: GC/Mass for the three flavonoid compounds.

From the data illustrated in Table 5 and Figure 2&3 it was observed that three flavanoids namely rutein, quercetin and kaempherol were isolated and identified using TLC and GC Mass methods. Many literatures proved that flavonoids have antioxidant effects (DeGroot, 1994; Halliwell, 1995; Korkina and Afanas, 1997; Shoskes 1998 and Mozaffari *et al.*2011) [32, 40, 52, 91, 68]. Also they have antiatherosclerotic effects (Arai *et al.* 2000 and Vinson *et al.* 2006) [15, 101]; anti-inflammmatory effects (Damas *et al.*1985 and Xing *et al.*2013) [29, 104], on the other hand they have antitumor effects (Caltagirone *et al.* 2000 and He *et al.* 2012) [24, 43], anti-thrombogenic effects (Osman *et al.*1998 and Bojié *et al.*2011) [75, 21], anti–osteoporotic effects (Uchida *et al.* 2010 and Lagari *et al.* 2012) [96, 59] and finally they have anti- viral effects (Harris *et al.* 2003 and Andres *et al.* 2009) [42, 14].

Conclusion

In the light of previous findings, the filamentous alga *Cladophora* is a common in habitatant of freshwater locations. It is called blanket weed in some places, it can be concluded that *Cladophora* sp. contains flavonoids and high molecular weight phenolic compounds which may be exhibited high antioxidant potential activities and has radical scavenging activity. The isolated compound may be exhibited pharmaceutical activity. Further research linked to the isolation of the bioactive compounds is in progress. Further

green algae have potential to return pharmaceutically useful, which can be harnessed for the development of drugs for use in management of human pathogens, cancer, tumor, AIDS and many human degenerative diseases. There is great scope for further investigations toward drug development.

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