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Phytochemical Profile and Heavy Metals Contents of Codium Tomentosum And Sargassum Hornschuchi

Zaid Najah[1], Khaled M. Elsherif[*2], Mona Alshtewi[3] and Hajer Attorshi[3]

1. Faculty of Science, Chemistry Department, Al-Mergheb University, **LIBYA**
2. Faculty of Science, Chemistry Department, Benghazi University, **LIBYA**
3. Faculty of Education, Chemistry Department, Tripoli University, **LIBYA**

Email: zaidly@yahoo.co.uk,elsherif27@yahoo.com,hajerm29@gmail.com,Timorlank300@gmail.com

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ABSTRACT

Phytochemicals and heavy metals analysis of Codium tomentosum (green algae) and Sargassum hornschuchi (brown algae) were carried out with a view to assess their therapeutic values. The results showed that the bioactive compounds like alkaloids, flavonoids, tannins, glycosides and saponins were present. The tests were performed on different extracts with different polarity; the aqueous extract was the richest extract with secondary metabolites in both algae. Heavy elements concentrations in both algae were also investigated. The zinc concentration was found to be high; 131.3 and 194.2 ppm, in both algae. The micronutrients levels in both studied algae was in same order: Zn > Mn > Fe > Cu. The toxic metals levels were also similar in both cases in following order: Pb > Cd > Hg.

Keywords: Codium tomentosum and Sargassum hornschuchi, Phytochemical Screening, Heavy metals.

INTRODUCTION

Marine natural products have attracted the attention of biologists and chemists for the last five decades [1-3]. About 30,000 species of algae are found at all places where there is light and moisture and are found in abundance in sea. To date approximately 16,000 marine natural products have been isolated from marine organisms [4]. The four types of algae (brown, red and green algae) are rich in molecules with antiviral, antioxidant, antifungal and antimicrobial activities [5], in addition to their biological importance as a potential source of new drugs, algae are a source of food for fishes, cattle and man. Algae are also used as fertilizers and became important components of some creams and other cosmetic materials, few algae that excrete toxic substances pollute marine water [4].

The bioactive metabolites of seaweeds comprise a very wide range of chemical classes including brominated phenols, oxygen heterocyclics, nitrogen heterocyclics, sulphur nitrogen heterocyclics, sterols, terpenoids, polysaccharides, peptides, proteins, halogenated ketone, alkanes and cyclic polysulphides [6]. Codium tomentosum Stackhouse (Figure 1.) is a small green seaweed in the family Codiaceae in the Phylum Chlorophyta, which grows to 30 cm in length. The fronds are solid and spongy with a felt-like or velvety touch, and are covered with colourless hairs which are visible when it is submerged [7].

Figure 1. Codium Tomentosum Stackhouse

Sargassum hornschuchi C. Agardh (Figure 2.) is a genus of brown (class Phaeophyceae) macroalgae (seaweed) in the order Fucales. Numerous species are distributed throughout the temperate and tropical oceans of the world, where they generally inhabit shallow water and coral reefs [7].

Figure2. Sargassum hornschuchi C. Agardh

Recently, Algae were deemed as a bio indicator for evolution of heavy metals pollution in marine environments because of their accumulation capacity of these elements, but algae usually attach only with free metals ions. In literature [8-10] it was found that, on international level scientists have conducted studies on the heavy metal analysis in different types of samples. Some algae exhibit high ability to remove heavy metals from both food and body by expelling them out of the body [11].

The quantity of these metals effected with several factors such as season, sampling position in the shoreline, coexistence of several metals, salinity and water temperature [12]. Heavy metals pollution in the marine environment and the following use in food chain by aquatic organisms and humans put public health at risks. Existence of minor concentrations of toxic heavy metals like Cd, Hg, Cr and Pb could lead to dangerous effects on marine life. Thus, regular monitoring of these metals level is very crucial, especially in Libyan coasts where a few data is available about marine pollution.

This study will focus in measuring some elements concentrations in selected algae in specified area in addition to qualitative phytochemical assessment of different algal extracts.

MATERIALS AND METHODS

Collection and preparation of extracts: Algal material was collected by hand from Tajoura coast during April 2014. To remove epiphytes, debris and other marine organisms, collected samples were washed thoroughly firstly with tap water then with distilled water systematically, The collected samples were dried in electrical oven at 70°C for 72 h then grinded into fine powder using electrical blender (Hommer, 350 Watt). The extraction started by soaking 1.50 g of powder in 1.0 L of ethanol for 72 h at room temperature, then reduced on hot plate, extracted with gradual increasing solvents using hexane, ethyl acetate and chloroform was performed. Extracts were evaporated to dryness on hotplate then cooled to room temperature before conserved in fridge for further phytochemical analysis. Aqueous extract was prepared by heating 1.00 g of the powder in 100 mL distilled water at 60°C for 20 min, the mixture cooled down to room temperature, filtered and stored in fridge for next step.

Wet Digestion Procedure for Heavy Metals Analysis: 5.0 g of dry weight of powder algal material was digested using 65 % Nitric acid in microwave (Start D brand) for 20 min at 200°C, after cooling to room temperature, the digested solutions were diluted to 25 mL with distilled water then used for heavy metals analysis in Shimadzu 7000 atomic absorption spectrometer. Mercury was analyzed separately by digesting 0.50 g of sample powder in Conc. H_2SO_4 + Conc. HNO_3 in closed digesting flask on water bath for 2 h at 60°C. The mixture was left to cool to room temperature, filtered, diluted to 250 mL with distilled water then introduced to Mercury Analyzer 254 instrument for Hg concentration measurement.

Apparatus: Atomic absorption measurements were made using a Shimadzu AA-7000 with Deuterium lamp (D2-lamp) background correction and hollow cathode lamps (Figure 3.). Air–acetylene flame was used for determination of all the elements. The working range, wavelength, slit, and detection limits are given in table 1.

Figure 3. Atomic Absorption Spectrophotometer AA-7000 (Flame Model)

Table 1. The analytical characteristics used with AAS determination

Metal	Working range, ppm	Detection Limits, nm	Wavelength, nm	Slit, mm
Iron (Fe)	0.0-10.0	0.015	248.3	0.2
Copper (Cu)	0.0-1.0	0.007	213.9	1.0
Zinc (Zn)	0.0-5.0	0.021	324.8	0.5
Lead (Pb)	0.0-1.0	0.002	217.0	1.0
Cadmium (Cd)	0.0-1.0	0.003	228.8	0.5
Manganese (Mn)	0.0-2.0	0.005	279.5	0.2

The AMA254 Advanced Mercury Analyzer (Figure 4.) is used to determine total mercury content without sample pre-treatment or sample preconcentration. The detector, wavelength, interference filter, and instrument range are given in Table 2.

Figure 4. The AMA254 Advanced Mercury Analyzer

Table 2. The analytical characteristics used with AMA254 Advanced Mercury Analyzer

Detector	Wavelength	Interference Filter	Instrument Range
Silicon UV Diode	253.65 nm	254 nm, half-width 9 nm	5 ppb to 5 ppm

Reagents: All the reagents used were of the analytical purity (Merck, Germany). The working solutions were prepared immediately before the analysis from the basic solution with 1000 mg L^{-1} concentration for all metals. For the preparation of standard solutions high purity Milli-Q water was used. The glassware and polyethylene containers used for analysis were washed with tap water, then soaked over the night in 6 M HNO_3 solution and rinsed several times with ultra-pure water to eliminate absorbance due to detergent.

RESULTS AND DISCUSSION

Phytochemical evaluation revealed the presence of different secondary metabolites between the studied algae (Table 3). Tests showed the presence of alkaloids in both algae in aqueous and chloroform extracts in addition to Codium hexane extract, the rest of extracts was negative for alkaloids assessment. Alkaloids are well known for their biological activities such as antimalarial, antiasthma, anticancer, cholinomimetic, vasodilatory, antiarrhythmic, analgesic, antibacterial and antihyperglycemic activities [13-17].

The basic nutrient, carbohydrate was found in water, ethanol and chloroform Codium extracts, in contrast to the brown algae Surgassum, the carbohydrates was noticed only in aqueous extract. These findings indicate the richness of Codium with carbohydrates and explain the common use of Codium as salad in some countries. Glycosides were found in most extracts for both seaweeds except ethanol Codium extract and ethyl acetate Surgassum extract. Glycosidic aglycones could be one of the important chemical classes such as vitamins, polyphenolics, alkaloids and terpenoids, these motifs exhibit many biological activities such as antibiotic drugs, schizophrenia treatment, immunomodulatory and hypolipemic activity [18].

The investigation illustrates the presence of saponins and tannins and terpenes only in aqueous extracts for both algae, while phlobatannins were only found in water extract of codium. Phytosterols are very important natural products because of their relation to other well known steroidal compounds especially sex hormone [19]. These secondary metabolites were found in both aqueous extracts in addition to ethanolic extract of brown algae surgassum. Tests for proteins and quinines gave negative results. The important type of natural products, flavonoids were noticed in Surgassum aqueous extract in addition to ethyl acetate, chloroform and hexane extracts of green algae codium, flavonoids are well known for their biological activity particularly as antioxidants [20].

Table 3. Phytochemical screening of C. tomentosum and S. hornschuchi

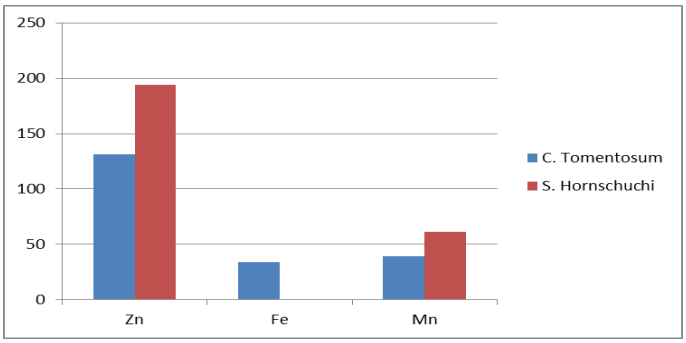
Extract	Water		EtOH		EtOAc		$CHCl_3$		Hexane	
Test	A	B	A	B	A	B	A	B	A	B
Alkaloids	+	+	-	-	-	-	+	+	-	+
Carbohydrates	+	+	+	-	-	-	+	-	+	-
Glycosides	+	+	-	+	+	-	+	+	+	+
Saponins	+	+	-	-	-	-	-	-	-	-
Phytosterols	+	+	-	+	-	-	-	-	-	-
Tannnins	+	+	-	-	-	-	-	+	-	-
Phlobatinnins	+	-	-	-	-	-	-	-	-	-
Flavonoid	-	+	-	-	+	-	+	-	+	-
Protiens	-	-	-	-	-	-	-	-	-	-
Terpenes	+	+	-	-	-	-	-	-	-	-
Quinones	-	-	-	-	-	-	-	-	-	-

A = C. tomentosum B = S. hornschuchi

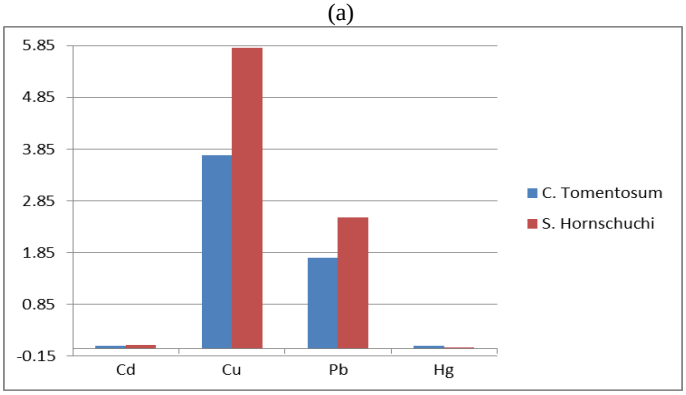
Table 4 shows the heavy metals composition of the two algae and that C. tomentosum contained the highest concentration of Fe which is 33.85 ppm. Fe is crucial for the building up of red corpuscles, which in turn are essential for formation of hemoglobin, the oxygen-carrying pigment in red blood cells. It is also used against anemia, tuberculosis and disorder of growth. Zinc is found in both algae and is high in both algae at 131.3 and 194.2 ppm. It is very important for nerve function, male fertility, stimulation of vitamins and formation of red and white corpuscles, healthy function of the heart and normal growth. Copper was found in both algae with nearly at same concentration (5.725 and 5.800 ppm). The function of Cu is to help in the absorption of iron. It is also important for the cellular defense, protection of the mucous membranes, anti-anemic and for the formation of iron hemoglobin. Pb, Cd, and Hg were present in low amounts in both algae studied. Lead ranged from 1.750 ppm to 2.525 ppm. In high concentration, Pb can cause abnormal brain and nerve function and it tends to displace vital minerals like calcium in the body. Concentrations of Cd were comparable in both algae (0.0500 and 0.0625 ppm). High blood levels of Cd can cause acute renal failure, hardening of the arteries (atherosclerosis), and high blood pressure. Mercury is found in both algae in low concentration at 0.0413 and 0.0182 ppm. Intoxication by elemental mercury or by methylmercury is revealed primarily by changes in behavior and by neurological signs. Specific sensory symptoms are also prominent in human methylmercury poisoning. Methylmercury may induce alterations in the normal development of the brain of infants and may, at higher levels, induce neurological changes in adults. Children exposed to methyl mercury prior to birth may experience negative effects on their mental development. Manganese is found in both algae and is high in both algae at 39.43 and 61.43 ppm. Manganese effects occur mainly in the respiratory tract and in the brains. Symptoms of manganese poisoning are hallucinations, forgetfulness and nerve damage. Manganese can also cause Parkinson, lung embolism and bronchitis. When men are exposed to manganese for a longer period of time they may become impotent.A syndrome that is caused by manganese has symptoms such as schizophrenia, dullness, weak muscles, headaches and insomnia. The concentration of all heavy metals and their concentrations in both algae were compared in figure 5.

Table 4. The content of heavy metals in Codium tomentosum and Sargassum Hornschuchi

Element	Codium tomentosum, ppm	Sargassum hornschuchi, ppm
Cd	0.0500 ± 0.0004	0.0625 ± 0.0050
Cu	5.725 ± 0.0458	5.800 ± 0.0464
Pb	1.750 ± 0.0140	2.525 ± 0.0202
Zn	131.3 ± 1.0504	194.2 ± 1.5539
Fe	33.85 ± 0.2708	0.5630 ± 0.0045
Mn	39.43 ± 0.3154	61.43 ± 0.4914
Hg	0.0413 ± 0.0010	0.0182 ± 0.0006



(a)



(b)

Figure 5. Concentrations of heavy metals in both algae (a) high level metals (b) low level metals

APPLICATIONS

Due to these algae contain many health beneficial substances such as flavonoids; these are anti-oxidant materials, and they have low content of heavy metals, these algae are appropriate for preparation of medicinal extracts.

CONCLUSIONS

It has been concluded that the codium tomentosum and sargassum hornschuchi algae showed the presence of alkaloids, flavonoids, tannins, glycosides and saponins. However, the analysis of chosen algae and their extracts showed the presence of Fe, Cu, Zn, Pb, Cd, Hg, and Mn. The zinc and Mn concentrations in both algae were rather high and Pb, Hg, and Cd concentrations were significantly lower.

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AUTHORS' ADDRESSES

1. **Zaid Najah**
Head of Chemistry Department – Faculty of Science,
Al-Mergheb University, Al Khoms – Libya
E-mail: zaidly@yahoo.co.uk
Tel: +218911051327
2. **Khaled M. Elsherif**
Chemistry Department – Faculty of Science,
Benghazi University,Benghazi – Libya
E-mail: elsherif27@yahoo.com
Tel: +218923920803
3. **Hajer Attorshi**
Chemistry Department- Faculty of Education.
Tripoli University, Tripoli – Libya
E-mail: hajerm29@gmail.com
4. **Mona Alshtewi**
Chemistry Department – Faculty of Education,
Tripoli University, Tripoli – Libya
E-mail:Timorlank300@gmail.com