International Journal of Applied and Natural Sciences (IJANS)
ISSN (P): 2319–4014; ISSN (E): 2319–4022
Vol. 9, Issue 4, Jun–Jul 2020; 37–50
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International Academy of Science, Engineering and Technology
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EVALUATION OF THE MASS COLLECTION OF SARGASSUM IN RIVERA MAYA FOR PHARMACEUTICAL AND INDUSTRIAL HIGH SUPPLIES

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ABSTRACT

Since 2015, an excess of sargassum has been observed on the beaches of the Mexican Caribbean that has generated a negative impact due to its contamination of the environment and its effects on the tourism sector. Two species are commonly found in the Mexican Caribbean, namely Sargassum natans and Sargassum fluitans. Currently, much of the biomass of sargassum collected is that which from what is stranded on the beach. It is collected by locals and the tourism sector with the objective of minimizing the negative effects caused by sargassum stranded on the seashore. The present investigation characterizes sargassum biomass collected on the beaches, which may have differences in composition from sargassum collected offshore, to determine its potency for use as a raw industrial material. Sargassum extracts were characterized in different solvents by High-Performance Liquid Chromatography (HPLC), nuclear magnetic resonance spectroscopy, atomic absorption spectroscopy for trace minerals, mass spectrometry for lipid analysis, and Fourier Transform Infrared Spectroscopy (FTIR). The average composition of the sargassum extract was 11 % polyphenols, 12 % polysaccharides of alginic acid, 0.2 % trace minerals, 0.85 % fatty acid esters containing hydrocarbon chains from C8 to C20, a calorific value of 2260 KJ / Kg, ash percentage of 16 %, and energy content of 540 kcal / Kg.

KEYWORDS: *Fluitans, Natans, Sargassum, Polysaccharides, Polyphenols, Fatty Acids*

Article History

Received: *30 Apr 2020* | ***Revised:*** *09 Jun 2020* | ***Accepted:*** *16 Jun 2020*

INTRODUCTION

The excess of sargassum washing up on beaches in the Caribbean originates from the Sargasso Sea, located in the open North Atlantic Ocean near Bermuda [1, 2]. This sea stretches 1000 km wide and 3200 km long and is estimated to hold up to 10 million metric tons of sargassum. It is known as “the golden floating rainforest.” Sargassum is also found in the Northern Gulf of Mexico [3, 4]. Previous studies have suggested that the influx of sargassum in the Caribbean is due to a rise in water temperatures and low winds, which both affect ocean currents [5, 6]. Large amounts of sargassum are becoming entrained in currents that head toward the Eastern Caribbean Islands. The spread of sargassum has also been linked to increased nitrogen loading due to pollution of the oceans through human activities of increased sewerage, oils, fertilizers, and global climate change.

The species that predominate in the Mexican Caribbean are Sargassum natans and Sargassum fluitans [7]. These are free-floating seaweeds that do not attach to the ocean floor; their movements depend solely on ocean currents [8,9]. While sargassum seems to be a nuisance, it is integral to marine life, serving as an essential habitat for over 250 species of fish and invertebrates. These organisms use sargassum as nurseries, feeding grounds, and shelter. Sargassum can also be extremely important to endangered and migratory species such as sea turtles and whales [10, 11, and 12]. Seaweeds are of economic importance. Sargassum has usefulness for humans: from the biomass of these macroalgae, we can obtain alginates; phycocolloids with thickening, gelling, and stabilizing properties; fertilizers; and feed for animals. Sargassum is also widely used in the cosmetics industry [12, 13, and 14].

The present impact of sargassum throughout the entire Caribbean region is worrying. With the massive arrival of sargassum, the beaches have lost their scenic component, and with it their value for tourist use. Therefore, tourism service providers and local populations have reacted in various ways to mitigate its impacts. In Mexico, efforts have focused mainly on collecting biomass on beaches, mainly due to problems of tourist importance, but the foundations have not been laid to face the problem in the long term1, [15, and 16]. For this reason, it is particularly important to determine the components of interest in the stayed sargassum and collect it from the beaches of the Mexican Caribbean to value its potential and its components of economic interest. The analyses were focused mainly on evaluating valuables compounds such as polysaccharides, Poly phenols and fatty acid esters, trace minerals and heavy metals. Polysaccharides in seaweed are an important source for alginate and fucoidan extraction [17, 18]. Phenolic compounds are an important group of secondary metabolites that exhibit antioxidant activity, among other biological functions [19]. The extraction of Poly phenols is demanding due to their chemical structure and their interaction with other food components [20].

MATERIALS AND METHODS

Collection and Identification

Sargassum natans and fluitans were collected by handpicking on the beaches of Puerto Carrillo along the Riviera Maya coast of Mexico. The samples were placed in plastic containers with seawater and transported to our laboratory. We carried out their classification according to differential morphological characteristics and other characteristics collected from the literature [12, 13, and 21]. The presence of both Sargassum fluitans and Sargassum natans was confirmed, with a clear predominance of the former species. The sargassum collected was washed with tap water followed by distilled water to remove any mineral particles and organisms attached to the seaweed. Washed sargassum were dried on blotting paper and spread out at room temperature in shade. The dried sargassum was ground to a fine powder using a tissue blender. The powdered samples were then refrigerated for further use.

Preparation of the Extract

Many different extraction processes were applied for seaweed extraction-among them ethanol, is opropanol, acetone, ether, benzene and others-yielding results that depend of the type of sargassum and the proportion of the solvent [22, 23, 24, 25, 26, 27]. The sargassum powder sample was used to perform three different extractions in different solvents and solvents diluted in water at 20 %. We used 20 g of dried sargassum powder in 180 ml each of the following solvents: ethanol 96 %, methanol-acetone 80:20 % and formaldehyde 45 %. Subsequently, the 3 mixtures were repeated, adding 20 % of water to the liquid phase. The samples were kept in dark conditions for 72 hours with intermittent shaking. After incubation, the solutions were filtered through paper filters, the liquid phase was passed through a vacuum pump and the filtrate (crude extract) was collected.

Analysis Methods

Removal of traces of solvents and water was carried out by distillation under reduced pressure in a rotary evaporator assisted by a vacuum pump (Edwards RV3) at 100 torr with a hot water bath. Dry samples (absence of water and solvents) were obtained at 50 °C. Thin-layer chromatography was performed with a hexane-ethyl acetate phase in a ratio of 7:3 to visualize the components of low to medium polarity, and then the polarity of the phase was increased using an ethyl acetate mixture (methanol 1:1). A series of spots with rf of 0.5 were observed; however, other spots of higher polarity still remained at the retention point. For these, a phase of methylene chloride and methanol 1:1 and 20 µL of acetic acid were used, and the displacement of the more polar compounds was observed. To reveal plate chromatography, we applied ultraviolet light, which is essential to observe the aromatic rings characteristic of Poly phenols [28]. Bromocresol green was also used to reveal organic acids and bases, ninhydrin was used to reveal amino acids in general, and ammoniac serum sulfate was used to reveal carbohydrates linked to aromatic systems (alginates) [29]. A complete fraction was worked with HPLC by using a reverse phase column from which retention times were obtained. Once the information from the chromatography plates was obtained, the compounds were separated by column chromatography and evaluated by hydrogen nuclear magnetic resonance at 200-MHz.

Trace minerals were determined by means of atomic absorption spectroscopy using flame dispersion. The metals present were calcium, magnesium, sodium and potassium. Low-resolution mass spectrometry was performed with the least polar fraction of the plates and samples to determine fatty acids. The percentage contained in the sample was 0.2 % in 5 ml, which can be an interesting quantity at higher volumes / Long-chain fatty acids obtained featured a majority of 18 carbons. Thus, the final sample was derived by adding 2 drops of methanol and one drop of BF_3Et_2O for a fraction of 100 mg.

Finally, heavy metals determination was carried out in four solid samples of sargassum following the DFA Food Elemental Analysis Manual Section 4.4 standard, which uses inductively coupled plasma-atomic emission spectrometry equipment (ICP-OES) for the determination of elements. The calorific value test was performed in a triple run using dehydrated cellulose (Merck) as an internal reference, with a calorimetric pump (IKA model C-200).

RESULTS AND DISCUSSIONS

Below are the details of the results for the extraction performed with acetone-methanol mixture at 80:20. The following spectrograms show the functional group of the peaks presented by the liquid chromatography technique (HPLC). As shown in Figure 1, a peak retention time of 45.03 min was observed as a majority component, followed by a secondary peak 56.54 min and a tertiary peak at 73.00 min. In the table of Figure 1, these peaks correspond to 21.045 %, 16.54 % and 12.99 %, respectively. Once these data were obtained, separation was achieved. Next, 200MHz NMR spectra were defined, which eluted a carbohydrate structure for the majority peak, Poly phenols for the second, and a complex mixture of lipids for the third.

In Figure 2, a large number of low-frequency signals are visible in this spectrum. Double low-frequency peaks with a chemical shift of 5.55 ppm are characteristic of a diastereotopic carbohydrate system (Aldrich spectra collection/Reich reference). The multiplicity of signals is due to multiple carbohydrate cycles. On the other hand, in the aromatic zone with chemical displacement between 7.10 to 7.70 ppm, a depressed signal with varied multiplicity was observed. This corresponds to the aromatic system of the phenolic ring of the alginic component [30]. Alginate is a group of natural anionic polysaccharides derived from seaweed cell walls. It is of great value as a raw material for textiles, food, paper, and the pharmaceutical and cosmetic industries [31].

In Figure 3, the spectrum shows an abundant number of aromatic compounds, including between a chemical shift from 7.38 to 7.43 ppm. An inverted cone-shaped signal of unsystematic multiplicity was observed. This is a typical characteristic of polyphenols, as the hydrogen of the aromatic hydroxyl is exchanged with the deuterated methanol used to run the sample and translates into the existing signal at 4.71 ppm. In addition, the presence of a quartet with a chemical shift of 3.96 to 4.16 ppm, which is a characteristic signal of a methylene bounded to an aromatic system, indicates a spectrum that corresponds to the polyphenol group [32].

In Figure 4, the signal of aromatic compounds between 7-8 ppm is not observed. Only a cluster of signals between 1-4 ppm was observed. The complexity of the systems does not allow us to assign the multiplicity of signals correctly. However, they are signals of homologated amplitude in height and width. A clear triplet at 3.1 ppm was observed in Figure 4. This signal is characteristic of methine hydrogen in a carbohydrate. Thereby, the presence of free carbohydrates is indicated, probably from the release or breakdown of alginates with temperature. As an interesting fraction of low polarity compounds was found, they were isolated and taken to the NMR laboratory. Their spectrum was also obtained and is shown in Figure 5. A terminal methyl of one fatty acid chain was observed with a chemical shift of 0.88-0.89 ppm. A signal between 1.25 and 1.3 ppm corresponding to an aliphatic chain of various methylenes was observed. The signal between 2.26 and 2.34 ppm refers to an intermediate methylene toward a terminal methyl chain. However, the most interesting signal is a chemical shift of 3.66 ppm, which corresponds to a methoxyl derived from fatty acids: the sample was treated with a MeOH-BF3Et2O mixture. If a reaction took place, fatty acid methyl esters would be formed, and this spectrum shows a positive reaction.

The low-polarity fraction content was analyzed using mass spectrometry. The results are shown in Table 1 and the spectrums are shown in figures 6-11. The spectrums confirm the presence of triglycerides.

Figure 12 shows the FTIR spectrum for derivatives of alginic acids. The wide, low signal present at 3405 cm-1 indicates carboxyl groups from carboxylic acids. The width is given by the formation of weak hydrogen bonds intertwined. A tense Signal at 1720 cm-1 shows axial methyl characteristic of the carbohydrate ring.

In Figure 13, an overlapped spectrum is shown since it is a comparison between Sample 1 and Sample 2. The comparison was done to confirm the presence of poly phenols, where the broad band at 3344.62 cm^{-1} corresponds to the hydroxyl of an aromatic system. The width of the peak is due to the formation of low-energy hydrogen bonds, colloquially known as hydrogen bridges. Transmittance is not very pronounced due to the chemical environment surrounding the phenolic hydroxyl, so this is a mixture of various substituted phenols of different patterns. The untreated spectrum, called Sample 1, is shown in blue (Figure 13), while the spectrum with NaBH4-MeOH treatment is shown in red (Figure 13). The purpose of this test is based on reduction: if quino lines or some other aromatic derivative susceptible to reduction were present, the spectrum would change before and after treatment. This was not the case, because phenol cannot be reduced, and both spectrums present practically the same signals. Figure 14 shows the IR spectrum for the absent fraction of aromatic rings. Given the low solubility coupled with the characteristics of the sample, an elongated signal was observed at 2820 cm^{-1}. This signal is due to the elongation of the anomeric carbons of carbohydrates as complemented by the NMR spectrum. This corresponds to various rings of carbohydrates cyclized with each other. There is no open chain because this spectrum does not show any aldehyde carbonyl from hexose.

The results of the atomic absorption spectroscopy analysis shown in Table 2 were obtained using a standard method with the following characteristics: A hollow cathode (LCH) lamp flame fueled by an air-acetylene mixture was used. Our results correspond to the average of a triplicate test for each sample of the isopropanol-acetone extraction. Water with a coefficient of variation of less than 2 % was used as solvent. The sample had to be calcined due to the presence of organic matter.

The same methodology was applied to the remaining sargassum extracts with ethanol and formaldehyde, with and without water. Table 3 shows the results for all extracts. As can be observed in Table 3, the extract with formaldehyde presents major concentrations of Poly phenols and polysaccharides. It is well known that formaldehyde is used in the alginate extraction process to soften tissues and prevent pigmentation of alginate [33]. In addition, formaldehyde reacts with phenolic compounds found within the algae to produce insoluble products [34]. On the other hand, it is important to observe that despite the fact that the algae had already experienced previous decomposition because it was sampled from the beach (assuming several days of exposure to humidity and sunlight), it presented an average of 10 % of polysaccharides of alginic acid. It has been observed that while the polysaccharide percentages of alginic acid from dry sargassum powder range from 10 to 24 %, the extraction of sodium alginate is profitable [33-36]. For this case, the recollected sargassum can be industrially exploited to obtain sodium alginate. Poly phenols are antioxidant agents and can be extracted for use in the animal feed or cosmetic industries. The results of the Table 3 show a content of 11 % of poly phenols with methanol-acetone and 12 % with formaldehyde. These percentages of content are important to consider for industrial extraction applications. Methanol and acetone solvents are the best solution to extract fatty acid esters, yielding the greatest percentages of them. In order to analyze fatty acid ester types of every percentage from Table 3 in every extraction, we determined the content of hydrocarbon chains C8 to C20. In addition, trace minerals are important because they provide the essential nutrients that animals need to perform metabolic functions such as growth and development, immuno response, and reproduction. Deficiencies, even if moderate, can adversely affect the performance of the animal. The trace minerals detected were Ca, Na, Mg and K, which play an important role in animal feeding.

Table 4 presents the results for fatty acids in hydrocarbon chains C8 to C20 in the 3 extracts of methanol-acetone, ethanol and formaldehyde. From the results of Table 4, fatty acid esters of C11 to C17 are predominated. These may include lauric acid (C12), tridecyclic acid (C13), myristic acid (C14), palmitic acid (C16), margaric acid (C17) and others [37-38].

Table 5 shows calorific values, energy values and ash content. Algae biomasses are considered a viable option to produce biofuel because of their high yields of oil produced per dry weight [39]. The total lipid content of sargassum spp. ranges between 1.0 and 2.5 % 39. As shown in our results, sargassum presented an average of 2260 Kj / Kg and 540 Kca / kg. These values are indicative that sargassum, even when remained sargassum on beach, can be explored it to be applied for biofuel or as a nutritional complement in feeding animals.

Finally, Table 6 displays the results of heavy metals in the four different samples of sargassum collected. In our study, no worrisome levels of any of the elements studied in algal biomass (notwithstanding their possible use as agricultural fertilizer or animal feed) could increase the concentrations of toxic metals and salts in soils and / or incorporate them into the food chain. In all cases, values less than 0.1 mg / Kg were reported. It is possible that this is because sargassum remains on the shores of the beaches where it was sampled: the reduction processes of these metals may be due to photo catalytic acceleration [40, 41].

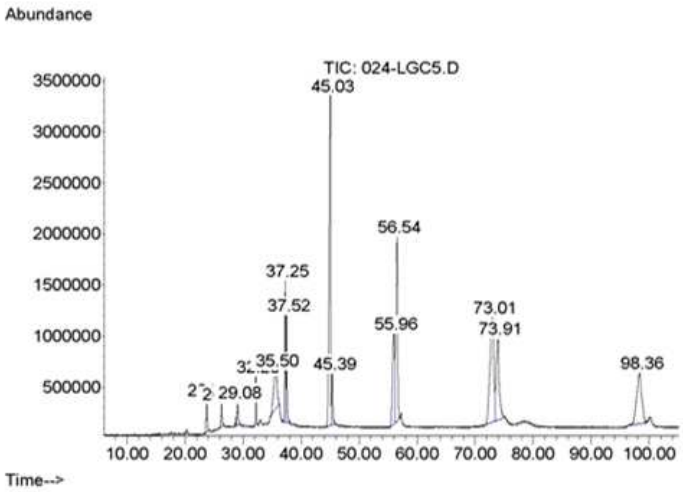

Figure 1: HPLC Chromatogram for the Entire Fraction.

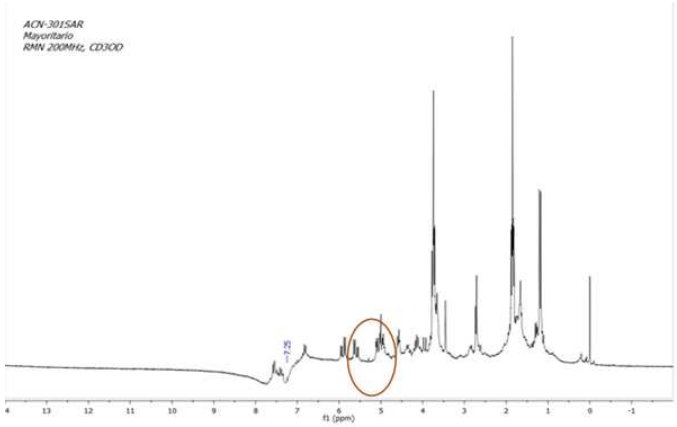

Figure 2: NMR Spectrum for the Majority Peak of HPLC with Remaining Time of 45.03 Min.

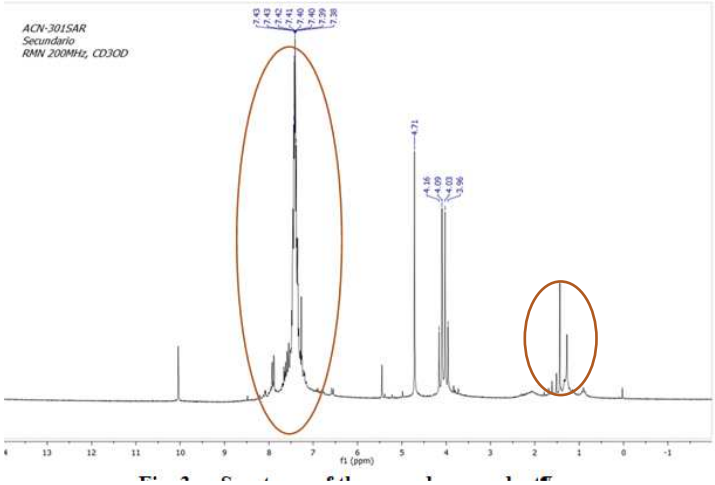

Figure 3: Spectrum of the Secondary Product.

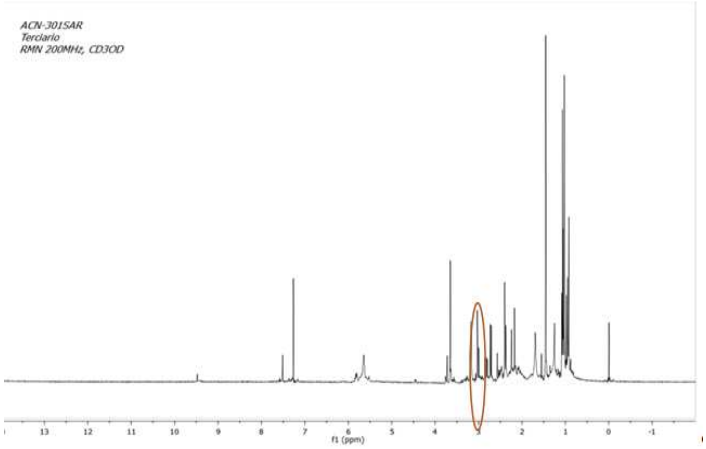

Figure 4: Carbohydrate Range Without Aromatic Ring.

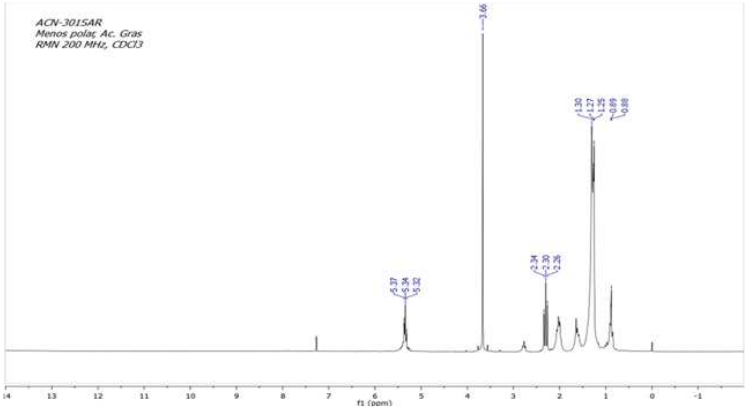

Figure 5: Methyl Ester Mixture From Fatty Acids from the Sargassum Extraction.

Table 1: Mass Spectrometry Results of Lipid Analysis (See Figures 6-11)

Entry	Molecular Ion(M / Z)	Percentage %	Molar Mass
1	183	4.03	457
2	127	2.01	397
3	183	16.2	638
4	453	21.4	593
5	155	11.6	554
6	184, 439	3.1	638

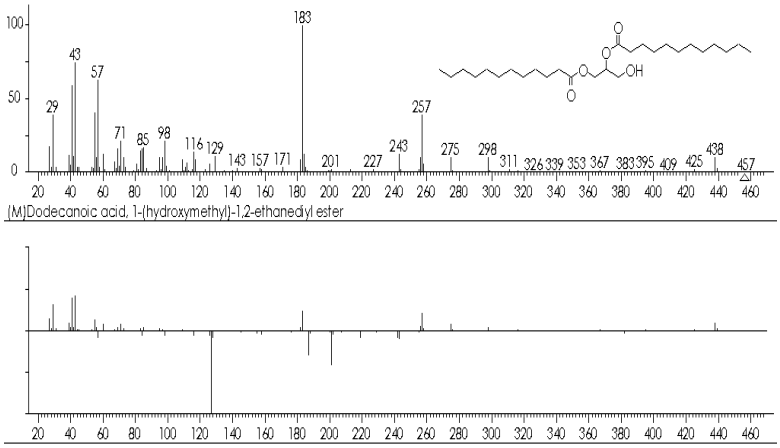

Figure 6: Triglyceride Content, 4 %.

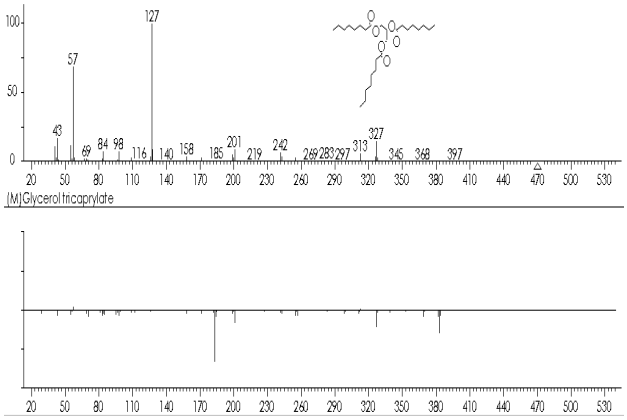

Figure 7: Triglyceride Content, 2.01 %.

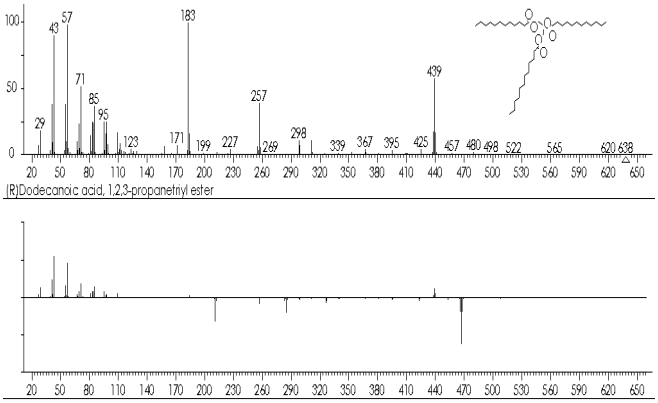

Figure 8: Triglyceride Content, 16 %.

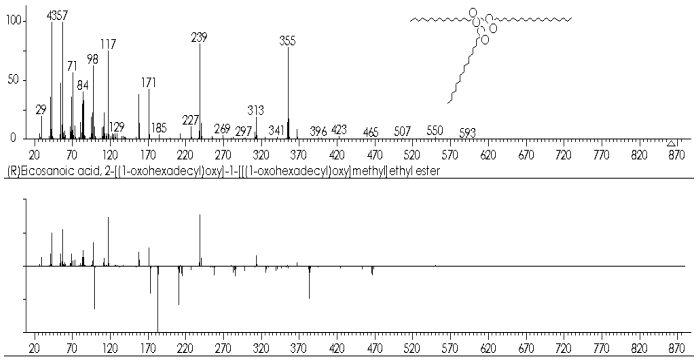

Figure 9: Triglyceride Content, 21 %.

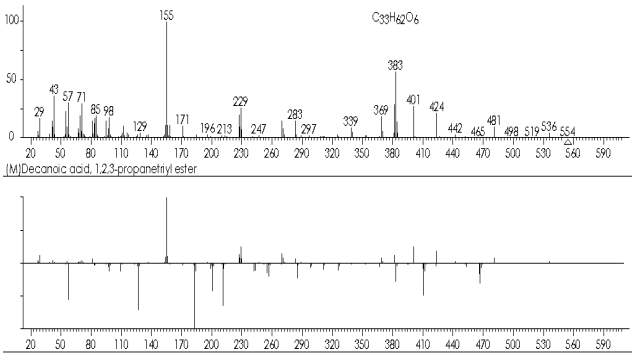

Figure 10: Fatty Acid Ester Content, 1.6 %.

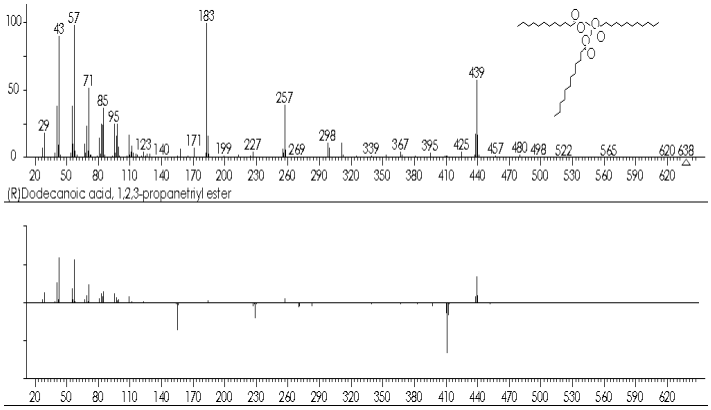

Figure 11: Triglyceride Content, 3 %.

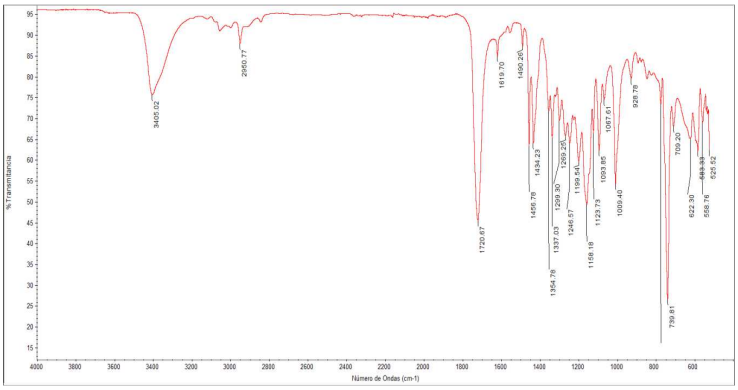

Figure 12: FTIR Spectrum for Derivatives of Alginic Acids.

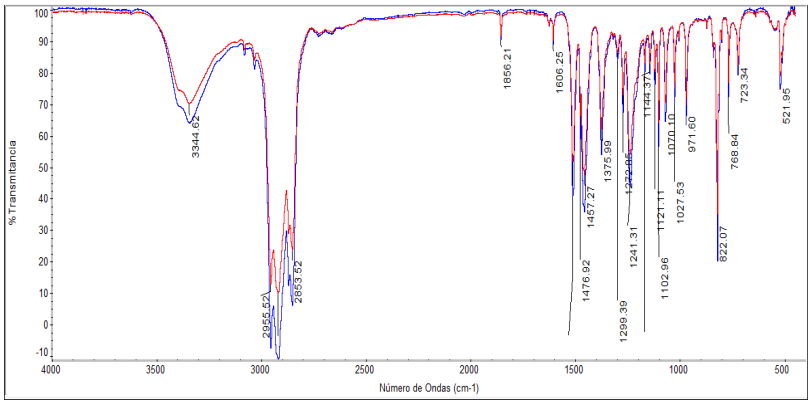

Figure 13: FTIR Fraction of Poly Phenols, Overlapping Spectrums.

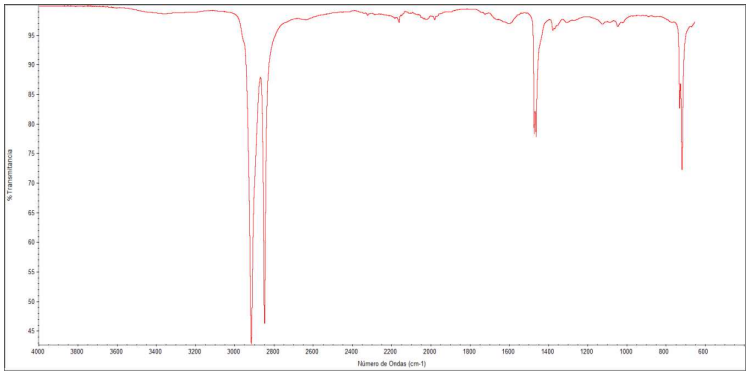

Figure 14: FTIR Spectrum, Fraction for Free Carbohydrates.

Table 2: Atomic Flame-Absorption Spectroscopy Results

Element	Wavelength	Overlap	Relative noise	Concentration	Response	Content %
Ca	243	0.5	1.7	50 ppm	1.0623	0.43
Mg	250	0.5	1.2	50 ppm	1.0089	0.25
Na	244	0.5	1.4	50 ppm	6.2425	1.87
K	260	0.5	1.5	50 ppm	5.3187	1.01

Table 3: Characterization of Powered Sargassum Extracts

Solvents	Poly Phenols	Alginic Acid Polysaccharides	Fatty Acid Esters	Trace Minerals	Insoluble Matter
CH□OH - C_3H_6O	11 %	12 %	0.85	Ca, 0.43 %, Mg, 0.25 %, Na, 1.87 %, K, 1.01 %	2 %
C_2H_5OH	6 %	6 %	0.43	Ca, 0.28 %, Mg, 0.19 %, Na, 1.02 %, K, 0.59 %	1%
CH_2O	12 %	13 %	0.22	Ca, 0.19 %, Mg, 0.14 %, Na, 0.83 %, K, 0.42 %	0.6 %
C_2H_5OH – H_2O	6 %	4 %	0.1	Ca, 0.1 %, Mg, 0.1 %, Na, 0.21 %, K, 0.13 %	1.7 %
CH□OH – C_3H_6O – H_2O	7 %	5 %	0.1	Ca, 0.12 %, Mg, 0.11 %, Na, 0.15 %, K, 0.10 %	1.5 %

Table 4: Hydrocarbon Chains of Esters, Percentage of Total Fatty Acid Esters

Solvents	Esters C6-C10	Esters C11-C17	Esters C18-C22
CH□OH - C_3H_6O	4 %	49 %	3 %
C_2H_5OH	4 %	17 %	1 %
CH_2O	<1 %	39 %	<1 %

Table 5: Sargassum Calorific Test Results

Sample	Calorific Value, Kj / Kg	Energetic Value, Kcal / Kg	Ash %
CH□OH-C_3H_6O	2512	600	16
C_2H_5OH	2050	490	16
CH_2O	2219	530	17

Table 6: Heavy Metals Results

Sample Number	As 1890, Mg / Kg	Cd 2288, Mg / Kg	Hg 1849, Mg / Kg	Pb 2203 Mg / Kg
1	0.016	0.0006	0.0	0.015
2	0.001	0.0	0.0	0.005
3	0.011	0.0	0.0	0.012

CONCLUSIONS

In conclusion, the stayed sargassum sampled from the beaches of Puerto Morelos in the Mexican Caribbean has high component values of polyphenols, polysaccharides, fatty acid esters and trace minerals. These components have great value for the animal feed and cosmetic industries, as mentioned before. No worrisome levels of any of the heavy metal elements studied were present in the biomass of the analyzed sargassum. The calorific and energetic values of the sargassum represent an interesting result that indicates sargassum's application for biofuel or as a nutritional complement in feeding animals. Sargassum represents a nuisance to both fishermen and beach lovers. Since biological, chemical and mechanical removal approaches may not be effective, attention should be focused on how the pelagic masses can be economically useful.

ACKNOWLEDGMENTS

Acknowledgments to FORDECYT-Mexico for funding support under project No. 297614 “Technological innovation to valorize the mass collection of sargassum in Rivera Maya for pharmaceutical and industrial high supplies”.

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