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PRELIMINARY SCREENING OF PHYTOCHEMICAL AND BIOLOGICAL ACTIVITY OF Sargassum lapazeanum

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ABSTRACT. Algae are exposed to substantial stress, in response, these organisms have developed efficient defense systems, such as protective secondary metabolite synthesis, making algae a primary source of bioactive compounds with a wide spectrum of biological activities. Thus, algae show potential for use in treatments of thrombotic, infectious, and chronic degenerative diseases. Therefore, the objective of this study was to evaluate the phytochemical compounds and pharmacological activity of an extract obtained from *Sargassum lapazeanum*, an alga endemic to the Gulf of California. Algae were collected in the intertidal zone of Tarabillas beach (Bahia de La Paz, BCS). The biological activities of the ethanolic extract and its fractions were evaluated using chromatographic techniques. In addition, a bioautographic assay of hemolytic activity was conducted, and phytochemical profiles and acute toxicity in *Artemia franciscana* were evaluated. The relationships among the main extract components were also determined. The ethanolic extract exhibited significant antioxidant and hemolytic activity, which was mainly attributed to its content of anthrones, anthraquinones, and unsaturated triterpenes. Its toxicological activity reached an LC_{s0} value of 225.1 µg mL⁻¹, which was mainly attributed to alkaloids, flavonoids, anthrones, and saponins. The results suggest that *Sargassum lapazeanum* has great pharmacological potential with biomedical applications.

Key Words: antioxidant, flavonoids, hemolytic, toxicity, triterpenes.

Estudio preliminar de la actividad fitoquímica y biológica de Sargassum lapazeanum

RESUMEN. Las algas están expuestas a un gran estrés, en respuesta, estos organismos han desarrollado eficientes sistemas de defensa, como la síntesis de metabolitos secundarios protectores, lo que las convierte en una de las principales fuentes de compuestos bioactivos con un amplio espectro de actividades biológicas. Así, las algas tienen potencial para su uso en tratamientos de enfermedades trombóticas, infecciosas y crónico degenerativas. Por lo tanto, el objetivo de este estudio, fue evaluar los compuestos fitoquímicos y la actividad farmacológica de un extracto obtenido de *Sargassum lapazeanum*, un alga endémica del Golfo de California. Las algas fueron recolectadas en la zona intermareal de la playa Tarabillas (Bahía de La Paz, BCS). Se evaluó la actividad biológica del extracto etanólico y sus fracciones mediante técnicas cromatográficas, se realizó un ensayo bioautográfico de la actividad hemolítica y se evaluaron los perfiles fitoquímicos y la toxicidad aguda en *Artemia franciscana*. Así también se determinó la relación entre los componentes fitoquímicos y la actividad biológica. El extracto etanólico mostró una importante actividad antioxidante y hemolítica, atribuida principalmente a su contenido de antronas, antraquinonas y triterpenos insaturados. Su actividad toxicológica alcanzó un valor LC₅₀ de 225.1 μ g mL⁻¹, atribuida principalmente a compuestos alcaloides, flavonoides, anonas y saponinas. Los resultados sugieren que *Sargassum lapazeanum*, tiene un gran potencial farmacológico con aplicación biomédica.

Palabras clave: antioxidante, flavonoides, hemolítico, toxicidad, triterpenos

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INTRODUCTION

Some marine organisms produce secondary metabolites, which increase the biological efficiency of species and generate interspecific and intraspecific changes that affect community dynamics (Lobban & Harrison, 1994; Pelletreau & Targett, 2008). In particular, photosynthetically active macroalgae serve as food sources for other organisms and possess various carbon acquisition mechanisms while playing important ecological roles that help maintain environmental quality (Raven & Hurd, 2012). Currently, the number of studies aimed at isolating novel biologically active compounds from macroalgae has increased worldwide (Frikha et al., 2011; Hakim & Patel, 2020; Yang et al., 2023). By in large, these studies have searched for bioactive compounds via bioassayguided fractionation (Murillo-Alvarez, 2001; Akbari et al., 2020).

Multiple biotic and abiotic factors affect the production of secondary metabolites and the structural chemistry of the seaweed, including herbivory, substrate availability, temperature, irradiance, nutrient concentrations, physiological conditions, and the reproductive stage of the alga (Lobban & Harrison, 1994; Pelletreau & Targett, 2008). Among secondary metabolites, terpenoids, phlorotannins, polyphenols, volatile hydrocarbons, and products of mixed biogenetic origin are of particular interest. These secondary metabolites exhibit chemical compositions, which are responsible for the potential use of algae as fertilizers, fodder, and food supplements (Rindi *et al.*, 2012; Kim & Chojnacka, 2015).

Sargassum lapazeanum is a subtropical species that is only distributed in the Gulf of California (Dawson, 1944; Rocha-Ramírez & Siqueiros-Beltrones, 1990; Rivera & Scrosati, 2006), and like other macroalgae, creates microhabitats that provide refuge for

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other organisms, however, this alga has not been studied very much, so it is necessary carry out research focused on evaluation the potential of *S. lapazeanum* as a source of secondary metabolites, which is essential for better understanding of its biology and ecological interactions with the environment and other *Sargassum* species (Pelletreau & Targett, 2008). This study intends to expands the current knowledge of the phytochemistry and the biological activity (antioxidant, hemolytic, and cytotoxic) of *S. lapazeanum*.

MATERIALS AND METHODS

Biological material

Sargassum lapazeanum was manually collected in the intertidal zone of Tarabillas beach (24° 27' 55.1" N, 110° 41' 20.1" W) in Bahía de La Paz, BCS, Mexico. Algae were collected at a maximum depth of 1 m in the intertidal zone, washed with tap water to remove epiphytes or sand, and dried in the sun. Once dry, the samples were ground to a particle size of 1 mm with a manual mill and stored in plastic bags.

Ethanolic extract (EE)

Dried algae (300 g) were macerated with ethanol (96%) at room temperature for three days. After which, the extract was filtered with filter paper (Whatman No. 4), and the algal residue was extracted five times under the same conditions. All obtained filtrates were pooled and concentrated to dryness under reduced pressure at 40 °C in a rotary evaporator (Yamato, Orangeburg, NY, USA). The obtained ethanolic extract (EE) was stored at -18 °C.

Ethanolic extract fractionation

Three grams of EE were adsorbed in 60 g of normal-phase silica gel and subjected to solid-liquid separation by sequential washing with n-hexane, dichloromethane, acetone, methanol, and water. Thinlayer chromatography (TLC) was used to combined the eluates, and six primary fractions were obtained (F0-F5). Each fraction was analyzed by TLC using normal-phase silica gel plates developed in dichloromethane-methanol eluting solution (9:1). The F4 fraction was selected for chromatographic column fractionation (CCF) with normal-phase silica gel and partitioned in a polarity gradient with n-hexane, dichloromethane, methanol, and water. Nine secondary fractions were obtained (FF0-FF8). The antioxidant and hemolytic activity, cytotoxicity, and phytochemical profiles of the nine fractions were evaluated.

Free radical scavenging activity bioautographic assay

The bioautographic assay was conducted based on the methodology described by Kannan *et al.* (2010), which uses the CCF technique. For this, glass plates were covered with normal-phase silica gel 60. A total of 20 μ g mL⁻¹ of the secondary fractions (FF0-FF8) and EE previously diluted in methanol were applied to the plates. The chromatographic plate was further developed in the solvent system (dichloromethane:methanol, 9:1) and dried at room temperature. The TLC plate was sprayed with 0.4% 2,2-diphenyl-1-picrylhydrazil (DPPH) in methanol and left in the dark for 30 min at room temperature. The presence of discolored (white) areas that contrast with the violet background of the developer solution indicated the presence of scavenging activity.

Hemolytic activity

The EE and FF0, FF2, FF3, FF4, FF6, FF7, and FF8 fractions were evaluated in TLC plates that were developed in the same way as those mentioned in the previous section. Hemolysis was evaluated by bioautographic TLC according to the method of Singh et al. (2022). Blood was obtained from an apparently healthy individual and suspended (9:1) in anticoagulant solution 3.5% sodium citrate. The blood was centrifuged at 3000 rpm for 10 min, and the supernatant was discarded. The erythrocytic pellet was washed with a phosphate-saline solution and centrifuged at 3000 rpm for 10 min, and the supernatant was discarded. This process was repeated three times. The obtained sediment was suspended in phosphate-buffered saline (PBS) at pH 7 for a final concentration of 3% (v/v). The TLC plate was covered by this saline solution and allowed to stand vertically for 30 min at room temperature. At the end of the reaction, the discolored areas (white) were evaluated in contrast to the pink background of the developing solution, as these indicate erythrocyte hemolysis.

Acute toxicity in Artemia franciscana

The cytotoxicity of the EE and secondary fractions (FF0-FF8, except FF5) were evaluated in A. franciscana Linnaeus, following the methodology of McLaughlin et al. (1998). For each extract, three concentrations were analyzed (by triplicate) to determine the dose-response relationship. The assay was performed in flat-bottom 96-well polystyrene microplates. The extracts were prepared in concentrations of 10, 100, and 1000 µg mL⁻¹ dissolved in 5% dimethylsulfoxide (DSF) and filtered seawater. Then, 15 larvae of A. franciscana were added to each well. Sodium dodecylsulphate (DSD) and DSF were used as positive and negative controls, respectively. The plates were incubated for 24 h at 35 °C. After which, live larvae were counted, and the LC₅₀ value was estimated using the statistical method of Probits.

Phytochemical screening

A preliminary phytochemical screening of EE and secondary fractions (FF0-FF8) was conducted following the methods of Harborne (1990). Chromatographic plates were prepared as previously described. After which, they were exposed to the following developing solutions: Dragendorff reagent (Sigma-Aldrich, St. Louis, MO, USA) for alkaloids; Lieberman-Burchard reagent for unsaturated sterols and unsaturated and saturated triterpenes; 5% FeCl₃ in n-butanol and 5% HCl for phenols, pyrogallic tannins, and catecholic tannins; 1% AlCl₃ in ethanol for flavonoids; 10% KOH in ethanol for coumarins, anthraquinones, and anthrones; and 10% H₂SO₄ in ethanol for saponins. Subsequently, the plates were analyzed with ultraviolet light at 250 and 360 nm. The colorimetric reactions were interpreted as the presence or absence of the chemical groups mentioned above.

Phytochemistry and biological activity

The retention factor (Rf) was used to assess the relationship between phytochemistry and pharmacological activity. This value expresses the position of a substance on the chromatographic layer. Rf values were calculated by dividing the distance traveled by the compound of interest by the distance traveled by the eluent (Kenndler, 2004). The compounds present in the fractions were observed in a TLC plate developed with a 10% H_2SO_4 . Finally, pharmacological activities were related based on the similarity of the Rf values and those of the chemical compounds of the phytochemical analysis.

RESULTS

The fractionation process of *S. lapazeanum* is shown in Figure 1. The F2 and F3 fractions obtained from solid-liquid extraction were pooled due to their chromatographic similarity (Fig. 1a). Fraction F4 was chosen for further fractionation on a chromatographic column (Fig. 1b) because it had the highest number of compounds with high polarity.

DDPH scavenging activity

The autographic assay revealed relevant activity in the EE and its fractions. The FF4 and FF8 fractions exhibited the highest activity within the solvent system used for CCF (dichloromethane:methanol, 9:1; Fig. 2), which was reflected in the high intensity of decolorization of the DPPH solution.

Hemolytic activity

Notably, fractions FF3, FF4 and FF6 showed the highest hemolytic activity according to the bioautographic assay. Most of these compounds were weakly polar within the solvent system (dichloromethane:methanol, 9:1). However, compounds of high polarity were also found in fraction FF6. Also of note, fraction FF7 contained an isolated compound of medium polarity, although compounds with higher hemolytic activity were present in the FF3 fraction, which was reflected in the discoloration intensity (Fig. 3).

Toxicity test in Artemia franciscana

Figure 4 shows the results of the mean lethal concentration (LC₅₀). These values were inversely proportional to the toxicities shown by the fractions. With the exception of FF6 from the EE of *S. lapazeanum*, all fractions were found to have high acute toxicity against *A. franciscana*. The EE presented a mortality of 85% with a CL_{50} of 225.1 µg mL⁻¹. After fractionation, maximum mortality (81%) was presented by the FF3 fraction (CL₅₀ of 443.7 µg mL⁻¹).

Phytochemical screening

The main compounds in the *S. lapazeanum* fractions were flavonoids and alkaloids, which were present in almost all fractions. Phenols, tannins, and saturated sterols or triterpenes were only present in FF2, FF3, and FF4. The FF3 fraction was shown to have the highest amount of phytochemical compounds tested. The fractions that showed the best fractionation, which was reflected in superior isolation between compounds, were FF1 with alkaloids and flavonoids,

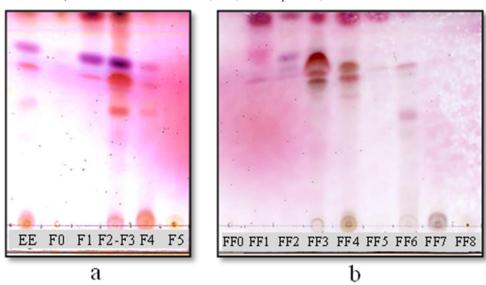


Figure 1. Chromatographies of: a) crude extract (EE) and primary fractions; b)secondary fractions. The analysis was conducted on normal phase silica gel chromatographic plates, developed in the dichloromethane:methanol (9:1) eluent system and sprayed with a 0.25% vanillin solution in 10% H_2SO_4 .

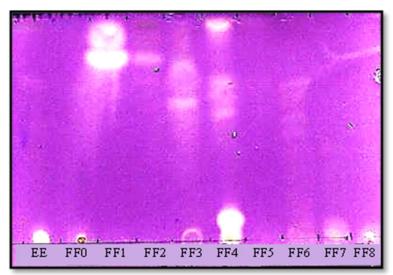


Figure 2. Autographic assay showing the antioxidant activity of the secondary fractions obtained from column fractionation (FF0-FF8) and the crude extract (EE) of *Sargassum lapazeanum*. The analysis was performed on a normal-phase silica gel thin layer chromatographic plate, developed in the dichloromethane:methanol (9:1) eluent system, and sprayed with the 0.04% DPPH solution.

FF5 with alkaloids and a minimal presence of unsaturated sterols, and FF7 with unsaturated triterpenes and a minimal presence of alkaloids (Table 1).

Evaluation of biological activity

The relationship between antioxidant and hemolytic activity and the results of the phytochemical assay were analyzed by means of the Rf value. It was observed that this relationship is inversely proportional to the polarity of the compound. Thus, it can be inferred that the antioxidant activity of *S. lapazeanum* is attributable to alkaloid compounds, triterpenes, unsaturated sterols, catechol tannins, anthrones, and anthraquinones, while its hemolytic activity is attributable to alkaloids, triterpenes, unsaturated sterols, phenols, pyrogallic tannins, and flavonoids. Among the compounds with antioxidant activity, the alkaloids with an Rf value of 0.82 in the FF1 fraction, anthrones with an Rf value of 0.95 in the FF0 fraction, and anthraquinones with an Rf value of 0.96 in the FF2 fraction were noteworthy. Of the compounds with hemolytic activity, flavonoids with an Rf value of 0.76 in the FF2 fraction and the unsaturated triterpenes with an Rf value of 0.87 in the FF3 fraction stand out. Unsaturated sterols with Rf values of 0.70 and 0.72 in the FF5 and FF2 fractions, respectively, are also noteworthy (Table 2).

DISCUSSION

Seaweeds are known to be major sources of antioxidant compounds, including sulfated polysaccharides, terpenes, phlorotannins, carotenoid pigments

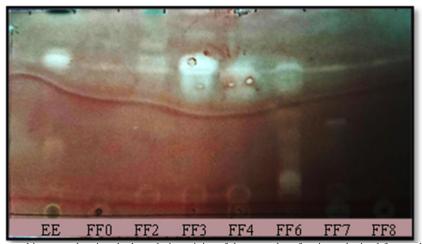
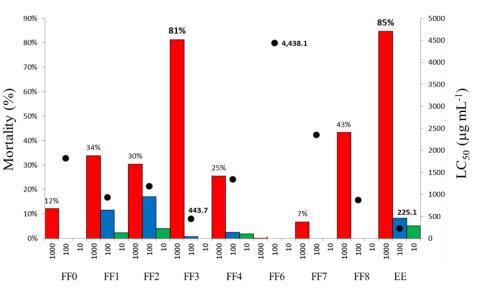
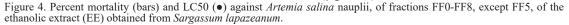


Figure 3. Bioautographic assay showing the hemolytic activity of the secondary fractions obtained from column fractionation (FF0-FF8) with the exception of FF1 and FF5 and the crude extract (EE) OF *Sargassum lapazeanum*. The analysis was performed on a normal-phase silica gel thin layer chromatographic plate, developed in the dichloromethane:methanol (9:1) eluent system, and sprayed with the 3% red blood cell suspension in PBS.



Extract concentration (µg mL⁻¹)



(e.g., astaxanthin and fucoxanthin), sterols, catechins, and proteins (Indu & Seenivasan, 2013) . In *Sargassum* species, compounds, such as meroterpenoids, phlorotannins, fucoidans, sterols, and glycolipids, have been isolated and found to demonstrate antibacterial, antifungal, antiviral, anti-inflammatory, anticoagulant, antioxidant, hypoglycemic, lipid-lowering, protective of the nervous system, and antimelanogenic activity, suggesting that these species are rich sources of agents with the potential to support and improve human health (Liu *et al.*, 2012; Indu & Seenivasan, 2013; Srivastava, 2013; Subramanian *et al.*, 2014; Yende *et al.*, 2014; Park *et al.*, 2015; Mehdinezhad *et al.*, 2016).

In the present study, the DPPH free radical scavenging activity of the polar compounds present in the fractions of *S. lapazeanum* was demonstrated. This algal species exhibits high potential as a source of antioxidant compounds. Based on the phytochemical screening results, these compounds could include alkaloids, triterpenes, unsaturated sterols, catecholic tannins, and/or coumarins such as anthraquinones and anthrones. When observing the relevant scavenging activity of DPPH by coumarins (anthrones and anthraquinones), it may be asserted that this group of polar compounds contributes to the important antioxidant activity observed in *S. lapazeanum*.

In recent years, progress has been made regarding the pharmaceutical applications of coumarins, especially those based on their antioxidant properties (Kostova *et al.*, 2006). Studies that have sought to develop innovative agents by evaluating the interactions among coumarins and ROS have concluded that various coumarins are able to capture free radi-

Table 1. Phytochemical screening of the ethanolic extract fractions from Sargassum lapazeanum. Fraction

Chemical compounds	FF0	FF1	FF2	FF3	FF4	FF5	FF6	FF7	FF8
Alkaloids	-	Х	Х	Х	Х	Х	Х	X*	-
Unsaturated sterols	-	-	Х	Х	Х	X *	Х	-	-
Unsaturated triterpenes	-	-	Х	Х	Х	-	Х	Х	-
Sterols or Saturated triterpenes	-	-	X *	X *	Х	-	-	-	-
Phenols	-	-	Х	-	-	-	-	-	-
Pyrogallic tannins	-	-	-	Х	Х	-	-	-	-
Catecholic tannins	-	-	X *	X *	-	-	-	-	-
Flavonoids	Х	Х	Х	Х	Х	-	Х	-	Х
Coumarins	-	-	-	-	Х	-	-	-	-
Anthraquinones	-	-	Х	Х	-	-	Х	-	-
Anthrones	Х	-	-	Х	Х	-	Х	-	Х
Saponins	Х	-	-	Х	Х	-	-	-	Х

* Minimal presence

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Biological activity Rf			Chemical compound	Fraction	
		0.48	Anthrones	FF6	
		0.49	Flavonoids	FF2	
		0.52	Flavonoids and Anthraquinones	FF4, FF6	
		0.53	Coumarins and Anthraquinones	FF3, FF4	
		0.54	Flavonoids	FF3	
		0.55	Alkaloids, Flavonoids and Anthraquinones	FF1, FF2, FF4	
0.56 A			Alkaloids, Unsaturated triterpenes, Flavonoids and Anthraquinones	FF6, FF7	
		0.57	Unsaturated triterpenes	FF2	
		0.59	Alkaloids	FF6	
Hemolytic		0.60	Alkaloids, Pyrogallic tannins and Flavonoids	FF1, FF2, FF3	
		0.61	Anthraquinones	FF2	
Antioxidant 0.62		0.62	Unsaturated triterpenes, Catecholic tannins and Anthraquinones	FF2, FF3, FF6	
		0.63	Alkaloids, Unsaturated triterpenes, Unsaturated sterols, Pyrogallic tannins and Flavonoids	FF1, FF2, FF3 FF4, FF6	
Hemolytic	Hemolytic 0.6		Pyrogallic tannins and Flavonoids	FF3, FF4	
		0.65	Sterols	FF4	
		0.66	Alkaloids, Sterols and Flavonoids	FF1, FF2, FF3 FF4	
Hemolytic		0.67	Unsaturated triterpenes and Phenols	FF2, FF4	
		0.68	Sterols	FF2	
Hemolytic		0.69	Anthraquinones and Saponins	FF2, FF3	
Antioxidant and molytic	He-	0.70	Unsaturated sterols	FF5	
Antioxidant and molytic	He-	0.71	Unsaturated sterols and Unsaturated triterpenes	FF2, FF3, FF ⁴ FF5, FF6, FF7	
Antioxidant and He- 0 molytic		0.72	Unsaturated sterols	FF2	
		0.74	Saponins	FF3, FF4	
Hemolytic		0.76	Flavonoids	FF2	
Antioxidant		0.78	Alkaloids and Anthraquinones	FF1, FF2	
Antioxidant 0.82		0.82	Alkaloids	FF1	
		0.85	Alkaloids	FF1	
Hemolytic		0.87	Unsaturated triterpene	FF3	
Hemolytic 0.88		0.88	Alkaloids and Unsaturated triterpene	FF1, FF2	
		0.93	Unsaturated triterpene	FF2	
Antioxidant		0.95	Anthrones	FF0	
Antioxidant		0.96	Anthraquinones	FF2	
		0.97	Alkaloids and Flavonoids	FF0, FF1	
		0.98	Alkaloids and Unsaturated triterpene	FF0, FF2	
		0.99	Unsaturated triterpene and Flavonoids	FF2, FF3	

Table 2. Rf values of the chemical compound and the biological activity of the FF0-FF8 fractions obtained from Sargassum lapazeanum.

cals and may protect against tissue damage caused by the degeneration of oxidants, including oxidative stress associated with aging (Martín-Aragón, 1994). Hydroxycoumarins are typical phenolic compounds that act as potent metal chelators and free radical scavengers. For example, coumarin 7-hydroxyl-3-(1,1-dimethylprop-2-enyl), which was isolated from *Sargassum wightii* and *Sargassum polycystum*, may be involved in the antioxidant and antitumor activities shown by this species (Yuvaraj & Arul, 2014). It is highly likely that *S. lapazeanum* produces similar coumarins to those reported in other *Sargassum* species. Erythrocytes provide a simple way to study the protective or toxic effects of a wide variety of substances and processes associated with oxidative stress (Mole & Sabale, 2014). In the present study, the hemolytic activity of polar *S. lapazeanum* compounds could be related to alkaloid compounds, triterpenes, unsaturated sterols, phenols, pyrogallic tannins, and flavonoids. Triterpenes exhibited the most evident hemolytic activity; however, there is no evidence of hemolytic terpenes in *Sargassum* species. Given that antibacterial activity is the main biological activity attributed to *Sargassum* species, it is probable that these types of compounds have both antibacterial and hemolytic potential (Devi *et al.*, 2013; Kannan *et al.*, 2013; Asha-Kanimozhi *et al.*, 2015). Triterpenes glycosides or terpenoid saponins are also included in this group. These compounds are mainly known for their ability to interact with cell membranes by modifying structural and functional properties, although they have also been shown to possess a large pharmacological spectrum of compounds with remarkable hemolytic and cytotoxic activities (Popov, 2002). It is probable that the compounds with hemolytic activity present in *S. lapazeanum* are glycosidic (i.e., triterpenoid saponins) due to the great hemolytic potential that has been reported for this phytochemical group (Devi *et al.*, 2013; Asha-Kanimozhi *et al.*, 2015).

The highest toxicity of S. lapazeanum against A. franciscana was observed in samples that mainly shared alkaloid compounds, flavonoids, anthrones, and saponins. Some authors mention that notable lethality was evident in *A. franciscana* based on LC_{50} values <1000 µg mL⁻¹, indicating the presence of a potent cytotoxic agent or probable insecticide, although additional studies are needed (Ara et al., 1999). Within the Sargassum genus, LC_{50} values have been reported for different compounds and species, including the hexanic extract of S. myriocystum (LC₅₀ of 273.28 μ g mL⁻¹) and the methanolic extracts of S⁰. of 275.28 µg mL⁻¹ and the memanine extracts of 5. wightii (LC₅₀ of 161 µg mL⁻¹), S. polycystum (LC₅₀ of 250 to 500 µg mL⁻¹ and 502 µg mL⁻¹ to 617 µg mL⁻¹), S. swartzii LC₅₀ of 928 µg mL⁻¹ to 61 µg mL⁻¹ ¹), and S. binderi (735 µg mL⁻¹ to 121 µg mL⁻¹). The different LC₅₀ values of the last three species are due to the type of extract examined (Ara *et al.*, 1999; Orbes et al. 2002; hyperparai et al. 2012; Daud et al. Orhan et al., 2003; Iyapparaj et al., 2012; Daud et al., 2015; Kurniatanty et al., 2015; Asha-Kanimozhi et al., 2015). Sargassum lapazeanum exhibited an LC₅₀ value of 225.1 μ g mL⁻¹ in the case of the crude ex-tract, followed by an LC₅₀ value of 443.7 μ g mL⁻¹ in the FF3 fraction. This reflects moderate toxicological activity, which may be the result of the synergistic effect of several compounds present in the EE. Thus, fractionation could enhance toxicity to match or exceed the toxicity of the EE.

The flavonoids present in S. lapazeanum showed relevant hemolytic activity. Sulfated flavonoids are a group of conjugated metabolites in which the sulfate component represents an adaptation to the marine environment (Jensen et al., 1998). These compounds have important effects on plant biochemistry and physiology because they act as antioxidants, enzyme inhibitors, and precursors of pigments that protect against light damage and toxic substances. In addition, these compounds are involved in defense mechanisms against infection. For example, flavonoids isolated from an aqueous extract of S. polycystum were found to be responsible for important toxicological activities (Arsianti et al., 2020). Toxicity results with A. franciscana were similar to those obtained from assays that have evaluated anticancer, antitumor, antiproliferative, and larvicidal activities against mosquito species such as Aedes aegypti and

Culex quinquefasciatus (Ara *et al.*, 1999; Ali *et al.*, 2013; Kurniatanty *et al.* 2015). It is very probable that the flavonoids present in *S. lapazeanum* that exhibited relevant toxicological and hemolytic activity are of a sulfated nature, which suggests that they may perform well as anticancer agents.

Preliminary analysis of bioactive compounds present in *Sargassum lapazeanum* showed that this alga has a high potential as a source of antioxidant compounds, hemolytic and cytotoxic activity, although further research is needed to isolate bioactive compounds and their possible application in pharmaceuticals.

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REFERENCES

- Akbari, V., Safaiee, F. & Yegdaneh, A. (2020). Bioassay-guided fractionation and antimicrobial activities of *Padina australis* extracts. *Jundishapur Journal of Natural Pharmaceutical Products*, 15(4), e68304. https://doi.org/10.5812/jjnpp.68304.
- Ali, M. Y., Ravikumar, S. & Beula, J. M. (2013). Mosquito larvicidal activity of seaweeds extracts against Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus. Asian Pacific Journal of Tropical Disease, 3(3), 196-201. https://doi. org/10.1016/S2222-1808(13)60040-7
- Ara, J., Sultana, V., Ehteshamul-Haque, S., Qasim, R. & Ahmad, V. U. (1999). Cytotoxic activity of marine macro-algae on Artemia salina (Brine shrimp). Phytotherapy Research, 13(4), 304-307. https://doi.org/10.1002/ (SICI)1099-1573(199906)13:4<304:AID-PTR439>3.0.CO;2-9
- Arsianti, A., Bahtiar, A., Wangsaputra, V. K., Azizah, N. N., Fachri, W, Nadapdap, L. D., Fajrin, A. M., Tanimoto, H. & Kakiuchi, K. (2020). Phytochemical composition and evaluation of marine algal *Sargassum polycystum* for antioxidant activity and *in vitro* cytotoxicity on HeLa cells. *Pharmacognosy Journal*, 12(1), 88-94. https:// doi.org/10.5530/pj.2020.12.14
- Asha-Kanimozhi, S., Johnson M. & Renisheya-Joy-Jeba-Malar, T. (2015). Phytochemical composition of Sargassum polycystum C. Agardh and Sargassum duplicatum J. Agardh. International Journal of Pharmacy and Pharmaceutical Sciences, 7(8), 393-397.
- Daud, N., Mohd-Noor, N. N., Alimon, H. & Rashid, N. A. (2015). Preliminary toxicity test and phy-

tochemical screening of *Sargassum polycystum* crude extracts from marine macroalgae. *ES*-*TEEM Academic Journal*, 11(1), 109-116.

- Dawson, E. Y. (1994). The marine algae of the Gulf of California. Allan Hancook. *Pacific Expeditions*, 3(10), 389-454.
- Devi, J. A. I., Balan, G. S. & Periyanayagam, K. (2013). Pharmacognostical study and phyotochemical evaluation of brown seaweed Sargassum wightii. Journal of Coastal Life Medicine, 1(3), 199-204. https://doi.org/10.12980/ JCLM.1.2013C959
- Frikha, F., Kammoun, M., Hammami, N., Mchirgui, R. A., Belbahri, L., Gargouri, Y., Miled, N. & Ben-Rebah, F. (2011). Chemical composition and some biological activities of marine algae collected in Tunisia. *Ciencias Marinas*, 37(2), 113-124. https://doi.org/10.7773/cm.v37i2.1712
- Hakim, M. M. & Patel, I. C. (2020). A review on phytoconstituents of marine brown algae. Future *Journal of Pharmaceutical Sciences*, 6(129), 1-11. https://doi.org/10.1186/s43094-020-00147-6
- Harborne, J. B. (1990). Methods in plant biochemistry: 1. In: Dey, P. M. & Harborne, J. B. (Eds.), *Plant phenolics* (pp. 283-323). London Academic Press. https://doi.org/10.1002/pca.2800020110
- Indu, H. & Seenivasan, R. (2013). In vitro antioxidant activity of selected seaweeds from Southeast Coast of India. *International Journal of Pharmacy and Pharmacautical Sciences*, 5(2), 474-484.
- Iyapparaj, P., Ramasubburayan, R., Raman, T., Das, N., Kumar, P., Palavesam. A. & Immanuel, G. (2012). Evidence for the antifouling potentials of marine macroalgae Sargassum wightii. Advances in Natural and Applied Sciences, 6(2), 153-162.
- Jensen, P. R., Jenkins, K. M., Porter, D. & Fenical, W. (1998). Evidence that a new antibiotic flavone glycoside chemically defends the sea grass *Thalassia testudinum* against zoosporic fungi. *Applied Environmental Microbiol*ogy, 64(4), 1490-1496. https://doi.org/ 10.1128/ aem.64.4.1490-1496.1998
- Kannan, R. R. R., Arumugarm, R., Meenakshi, S. & Anantharaman, P. (2010). Thin layer chromatography analysis of antioxidant constituents from seagrasses of Gulf of Mannar Biosphere Reserve, South India. *International Journal of ChemTech Research*, 2(3), 1526-1530.
- Kannan, R. R. R., Arumugam, R. & Iyapparaj, P. (2013). In vitro antibacterial, cytotoxicity and haemolytic activities and phytochemical analy-

sis of seagrasses from the Gulf of Mannar, South India. *Food Chemistry*, *136*(3-4), 1484-1489. https://doi.org/10.1016/j.foodchem.2012.09.006

- Kenndler, E. (2004). *Introduction to chromatography*. Institute for Analytical Chemistry. University of Viena.
- Kim, S. E. & Chojnacka, K. (2015). Marine Algae Extracts: Process, Products, and Applications. Vol 2. John Wiley & Sons.
- Kostova, I. (2006). Synthetic and natural coumarins as antioxidants. Mini Reviews in Medicinal Chemistry 6(4): 365-374. https://doi. org/10.2174/138955706776361457
- Kurniatanty, I., Tan, M. I., Ruml, T. & Sumarsono, S. H. (2015). Potencial cell proliferation inhibitor isolated from Indonesian brown algae (Pheophyceae). *International Journal of Pharmacy and Pharmaceutical Sciences*, 7(11), 140-143.
- Liu, L., Heinrich, M., Myers, S. & Dworjanyn, S. A. (2012). Towards a better understanding of medicinal uses of the brown seaweed Sargassum in traditional Chinese medicine: A phytochemical and pharmacological review. Journal of Ethnopharmacology, 142(3), 591-619. https://doi. org/10.1016/j.jep.2012.05.046
- Lobban, C. S. & Harrison, P. J. (1994). Seaweed Ecology and Physiology. Cambridge University Press.
- Martín-Aragón, S. (1994). Cumarinas en la prevención del estrés oxidativo dependiente de la edad. Tesis de Doctorado. Universidad Complutense de Madrid. Madrid.
- McLaughlin, J. L., Rogers, L. L. & Anderson, J. E. (1998). The use of biological assays to evaluate botanicals. *Drug Information Journal*, 32, 513-524. https://doi.org/10.1177/009286159803200223
- Mehdinezhad, N., Ghannadi, A. & Yegdaneh, A. (2016). Phytochemical and biological evaluation of some *Sargassum* species from Persian Gulf. *Research in Pharmaceutical Sciences*, 11(3), 243-249.
- Mole, M. N. & Sabale, A. B. (2014). Antimicrobial, antioxidant and hemolytic potential of brown macroalga Sargassum. World Journal of Pharmacy and Pharmaceutical Sciences, 3(8), 2091-2104.
- Murillo-Álvarez J. I. (2001). Compuestos con actividad antimicrobiana y citotóxica aislados de recursos naturales de Baja California Sur, México. Tesis de Doctorado. Centro de Investigaciones Biológicas del Noroeste, S.C. La Paz, B. C. S. 447 pp.

- Orhan, I., Wisespongpand, P., Atici, T. & Sener, B. (2003). Toxicity propensities of some marine and fresh-water algae as their chemical defense. *Journal of Faculty of Pharmacy of Ankara Uni*versity, 32(1), 19-29. https://doi.org/10.1501/Eczfak_0000000384
- Park, S. Y., Seo, I. S., Lee, S. J. & Lee, S. P. (2015). Study on the health benefits of brown algae (Sargassum muticum) in volunteers. Journal of Food and Nutrition Research, 3(2), 126-130. https:// doi.org/10.12691/jfnr-3-2-9
- Pelletreau, K. N. & Targett, N. M. (2008). Chap. 6. New perspectives for addressing patterns of secondary metabolites in marine macroalgae. In: Amsler, C. D. (Ed.). Algal Chemical Ecology (pp 121-146). Germany. Springer-Verlag Berlin Heidelberg.. doi:10.1007/978-3-540-74181-7
- Popov, A. M. A. (2002). A comparative study of the hemolytic and cytotoxic activities of triterpenoids isolated from ginseng and sea cucumbers. *Biology Bulletin, 29*, 120-128. https://doi. org/10.1023/A:1014398714718
- Raven, J. A. & Hurd, C. L. (2012). Ecophysiology of photosynthesis in macroalgae. *Photosynthesis Research*, 113, 105-125. https://doi.org/10.1007/ s11120-012-9768-z
- Rindi, F., Soler-Vila A. & Guiry, M. D. (2012). Taxonomy of marine macroalgae used as sources of bioactive compounds. In: Hayes, M. (Ed.) *Marine Bioactive Compounds*. pp. 1-53. Boston, M. A. Springer. https://doi.org/10.1007/978-1-4614-1247-2_1
- Rivera, M. & Scrosati, R. (2006). Population dynamics of Sargassum lapazeanum (Fucales, Phaeophyta) from the Gulf of California. México. Phycologia, 45(2), 178-189. https://doi.org/10.2216/05-47.1
- Rocha-Ramírez, V. & Siqueiros-Beltrones, D. (1990). Review of the species of the genus Sargassum C. Agardh recorded for Bahia de La Paz, B.C.S., Mexico. Ciencias Marinas, 16(3), 15-26. https:// doi.org/10.7773/cm.v16i3.702
- Singh, R., Sharma, R., Mal, G. & Varshney, R. (2022). A comparative analysis of saponin-enriched fraction from *Silene vulgaris* (Moench) Garcke, *Sapindus mukorossi* (Gaertn) and *Chlorophytum borivilianum* (Santapau and Fernandes): an in vitro hemolytic and cytotoxicity evaluation. *Animal Biotechnology*, 33(1), 193-199. https://doi. org/10.1080/10495398.2020.1775627
- Srivastava, Y. (2013) (Ed). Advances in food science and nutrition. Science and Education Development Institute, Nigeria.

- Subramanian, G., Stephen, J., Poornaselvi, M. & Anusha, M. B. (2014). Phytochemical screening, free radical scavenging and antioxidant activities of Sargassum vulgare. International Journal of Advances in Interdisciplinary Research, 1(5), 1-4.
- Yang, Y., Hassan, S. H. A., Awasthi, M. K., Gajendran, B., Sharma, M., Ji, M. K. & Salama, E. S. (2023). The recent progress on the bioactive compounds from algal biomass for human health applications. *Food Bioscience*, 51, 102267. https:// doi.org/10.1016/j.fbio.2022.102267
- Yende, S. R., Harle, U. N. & Chaugule, B. B. (2014). Therapeutic potential and health benefits of Sargassum species. Pharmacognosy Reviews, 8(15), 1-7. https://doi.org/10.4103/0973-7847.125514
- Yuvaraj, N. & Arul, V. (2014). In vitro antitumor, anti-inflammatory, antioxidant and antibacterial activities of marine brown alga Sargassum wightii collected from Gulf of Mannar. Global Journal of Pharmacognosy, 8(4), 566-577. https://doi. org/10.5829/idosi.gjp.2014.8.4.84281

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