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The acute toxicity of *Sargassum fuiltans* (Børgesen) Børgesen and *Sargassum natans* (Børgesen) Børgesen on some rats of wistar stock

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1 ABSTRACT

Sargassum fluitans (Børgesen) Børgesen and *Sargassum natans* (Børgesen) Børgesen (Sargassaceae) are two macroscopic, pelagic and invasive brown marine algae found in waters and beaches of many countries, including Côte d'Ivoire. The objective of this study is to contribute to the fight against the pollution of *Sargassum* on Ivorian beaches by enhancing the value of these species (*Sargassum fluitans* and *Sargassum natans*). To reach that objective, the study was to evaluate the acute toxicity and characterize the active principles of the aqueous and ethanol extracts of these two algae. Phytochemical sorting by tube characterisation has revealed the presence of active principles with interesting pharmacological properties. These include polyterpen sterols, polyphenols, catechic tannins, saponins and polysaccharides. The acute toxicity tests were carried out for 14 days on female rats of Wistar strain according to the OECD guideline no.423 (OECD 423, 2001). The single oral administration of 2000, 3000 and 5000 mg/kg as body weight (BW) of the four extracts to the animals did not result in mortality. Moreover, no clinical sign of toxicity was recorded. These different doses of extracts had no significant effects ($p > 0.05$) on the mass weight of the animals compared to the control animals given distilled water. Thus, these different algae extracts had a LD_{50} higher than 5000 mg/kg of BW according to the OECD guideline. These algae extracts were almost non-toxic or relatively harmless. This study showed the innocuousness of *Sargassum fluitans* and *Sargassum natans* extracts as well as their phytochemical composition.

2 INTRODUCTION

Sargasso are macroscopic, brownish-coloured algae. There are nearly 1000 described taxa all over the world, making *Sargassum* the richest genus concerning the Fucales order (Guiry and Guiry, 2008; 2013). Several scientific studies conducted on this genus assign it various pharmacological benefits including antiviral, anticancerous, anticoagulant, antioxidant, antibacterial, antimicrobial properties, etc. (Smit, 2004; Stiger *et al.*, 2004; Plougerné *et al.*, 2006, 2008; Mattio, 2008;). In Cote d'Ivoire and all along the Gulf of Guinea, two invasive pelagic species, *Sargassum fluitans* and *Sargassum natans* have flooded marine waters and stranding massively on the beaches of these countries since 2011. From 2015 to the present, this scourge has become a socioeconomical issue (Guiry and Guiry, 2013; Sankaré *et al.*, 2016). All sectors of

activity in the flooded areas are affected. In fact, these algae disrupt many economic activities, especially the hotel industry, tourism, fishing, navigation and rendering the beaches inaccessible. Annual loses for hotel owners in many affected countries, mainly Guadeloupe, Martinique, are estimated at millions of euros. (Anonyme, 2017). At the ecological level, these algae constitute an obstacle to sea turtle nesting and hinder the sea journey of new-borns, resulting in the loss of hundreds, even thousands of them (Johnson *et al*., 2012; 2014). At present, there is no means to prevent or stop Sargasso arrivals at open sea and their stranding on beaches (Mattio, 2008; Sankaré *et al*., 2016; Anonyme, 2017). In response to this disaster, the Convention of Abidjan on Biodiversity organised two workshops in November 2015 in Freetown, Sierra Leone, and in August 2016 in Monrovia, Liberia, bringing together eight concerned countries in the sub-region. One of the major decisions taken at these meeting was to take initiatives to enhance the value of these algae. In addition, since the beginning of 2000s, throughout the world, algae are more and more reared and used as feed additives with phytobiotic powers, replacing the current chemical antibiotics. Most of these algae extracts contain numerous molecules capable of inhibiting the growth of digestive bacteria *in vitro* and have beneficial effects on the zootechnical parameters of some animals such as poultry (Guardia, 2011). In Côte d'Ivoire, apart from the systematic aspects addressed by Komoé *et al.* 2016; Sankaré *et al.* 2016, the literature on Sargasso is almost non-existent, especially the pharmacological data. It is within this perspective that this work is being carried out with the aim of evaluating the safety of *S. fluitans* and *S. natans* in order to enhance their value as food additives.

3 MATERIAL AND METHOD

3.1 Collection and Drying of Planting Material:

This material was made up of two algae species, *Sargassum fluitans* and *Sargassum natans*, collected in October 2018 in the area of South Comoe more precisely on the beaches of the cities of Assinie and Grand Bassam, located respectively at 80 and 20 Km from the district of Abidjan. These algae were identified by Dr KOMOE Koffi, Assistant Professor and Phycologist at the Laboratory of Natural Environment and Biodiversity Conservation of the Biosciences Faculty at the Félix Houphouet-Boigny University- Abidjan, and then confirmed by the National Center of Floristics (CNF) of that University. The algae collected on the beaches were sorted, washed and rinsed several times with tap water in order to remove any extraneous matter before being dried at Laboratory at a temperature of 18°C. Once dried, they were mash and finely powdered using a blender.

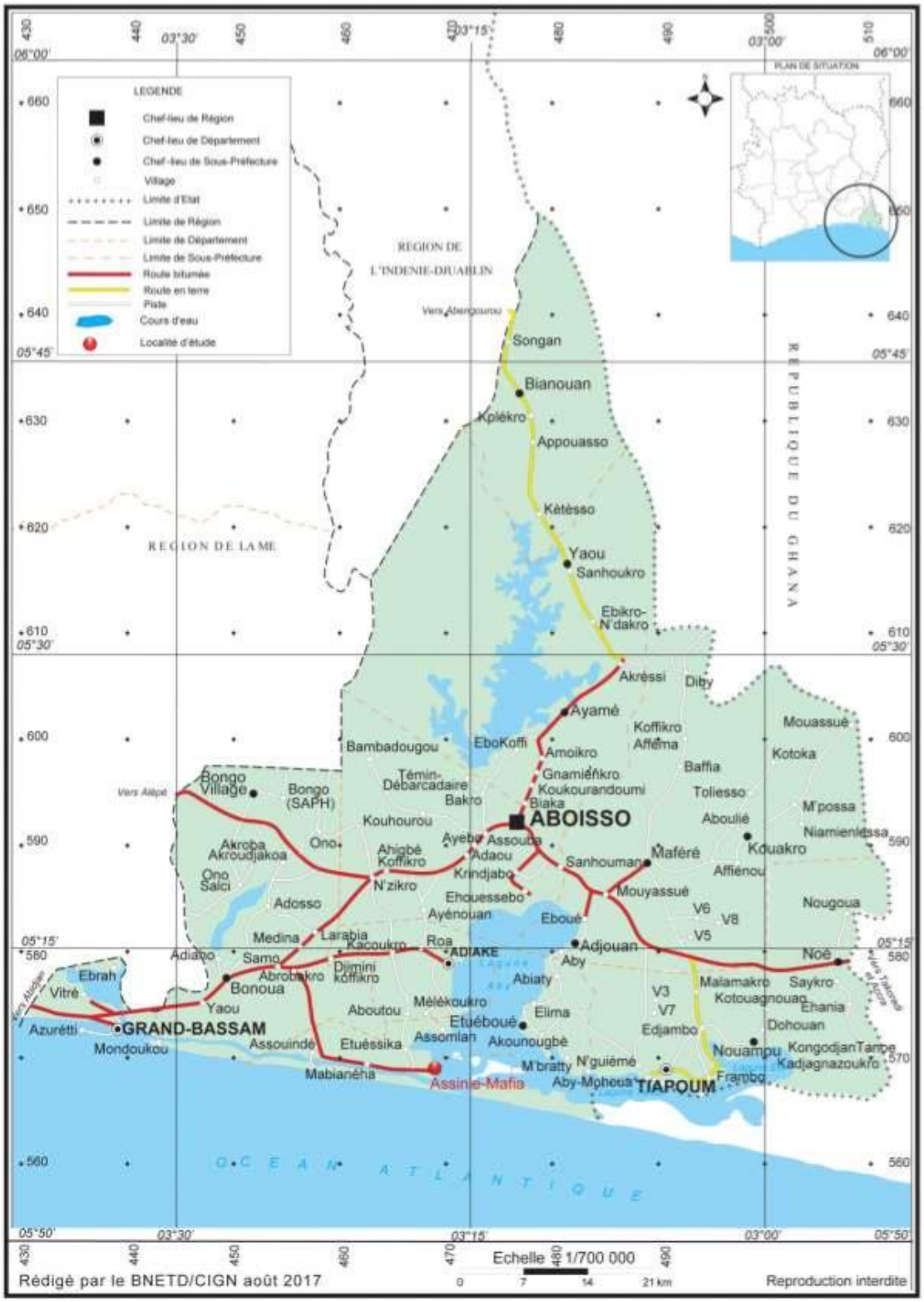

Figure 1: Map of the South Comoe area (Source: BNETD/CING August 2017)

3.2 Extracts Preparation: One hundred (100) g of algae powder were macerated in one litre of distilled water using a blender and then filtered through a square of white fabric. The operation is repeated with the pomace. The liquid obtained is filtered three consecutive times on cotton wool and then once on Wattman N°1 filter paper. The filtrates were dried in an oven at 50 °C (Zirihi, 2001). After drying, the extract is collected and finely powdered. The extract mass is determined using an electrical balance. The same operation was repeated with 70% of the ethanol for the ethanolic extract. The efficiency was calculated with the following formula:

$$\text{Efficiency} = \frac{\text{weight of obtained extract}}{\text{weight of initial powder}} \times 100$$

3.3 Phytochemical Study: Extracts underwent a phytochemical sorting using the tube reaction method to determine the major chemical groups present. They are qualitative analyses based on colouring and/or precipitation reactions (Karumi *et al.*, 2004; Benariba *et al.*, 2013).

3.3.1 Identification of Saponins: Introduce 10 ml of the extract solution into a test tube, shake strongly in a vertical position for 15 to 20 seconds then leave it to stand for 10 minutes. The presence of persistent foam > to 1 cm indicates the presence of Saponins.

3.3.2 Identification of Sterols and Polyterpene by the reaction of LIEBERMANN (Karumi *et al.*, 2004; Benariba *et al.*, 2013). Evaporate at dryness 05 ml extract solution on a sand bath. Then hot dissolve the residue in 1 ml of acetic anhydride. Subsequently, 0.5 ml of concentrated sulfuric acid was poured along the tube wall. The appearance of a purple or violet ring at the interphase, turning blue then green indicates a positive reaction.

3.3.3 Identification of Polyphenols by the reaction with ferric chloride (Karumi *et al.*, 2004; Benariba *et al.*, 2013). To 2 ml of extract solution, a drop of 2% ferric chloride alcoholic solution was added. The appearance of a blackish blue or more or less dark green colouring indicates the presence of polyphenolic derivatives.

3.3.4 Identification of Tannins (Karumi *et al.*, 2004; Benariba *et al.*, 2013). Search for catechic tannins using the Stiasny reagent (formol 30%, concentrated HCl 1/0,5) which enables us to distinguish catechic tannins (by precipitation) from gallotannins (by saturation). Evaporated to dryness 05 ml of the extract solution on a sand bath, and then add 15 ml of the Stiasny reagent to the solution. Then, bring to a water bath at 80 °c for 30 minutes and cool to room temperature. The appearance of precipitation in large flakes indicates the presence of catechic tannins.

3.3.4. 1 Search of Gallotannins by the $FeCl_3$ reagent (Karumi *et al.*, 2004; Benariba *et al.*, 2013). Saturate the filtrate obtained from the characterisation reaction of the catechic tannins with sodium acetate, and then add three drops of 2% FeCl3. The presence of an intense blue-blackish-black colouring indicates the presence of gallotannins.

3.3.5 Identification of Quinoid Substances (Karumi *et al.*, 2004 ; Benariba *et al.*, 2013). Evaporated to dryness on a sand bath 02 ml of extract solution, then triturated the residue in 05 ml of 1/5 chlorhydric acid. Bring the solution to the boiling water bath for half an hour. After cooling, extract the hydrolysate with 20 ml of chloroform in a test tube, then collect the chloroform phase in another test tube by adding 0.5 ml of ammonia diluted to 1/2. The presence of a red to purple colouring indicates the presence of quinoids.

3.3.6 Identification of Flavonoids by the reaction called cyanidin (Karumi *et al.*, 2004; Benariba *et al.*, 2013). Evaporate to dryness on a sand bath, 02 ml of extract solution and allow cooling. Then recover the residue by trituration with 05 ml of alcohol. Collect the mixture in a test tube and add 2-3 magnesium shavings. There is an absence of pinkish-orange or purplish colouring. The addition of 3 drops of isoamyl alcohol to intensify this colouring indicates the presence of flavonoids.

3.3.7 Identification of Alkaloids by the reagents of Dragendorff and Burchard (Karumi *et al.*, 2004; Benariba *et al.*, 2013). Evaporate to dryness 6 ml of the extract solution, and then recover it with 6 ml of alcohol at 60°. Then, divide the said solution into 2 test tubes.

- Add 2 drops of Dragendorff reagent to the first tube. The appearance of a precipitate or an orange colouring indicates the presence of alkaloids
- In the second tube, add two drops of Bouchard reagent. The presence of a reddish-brown precipitate indicates a positive reaction.

4. Study of the acute toxicity

4.1 Animals conditioning: A total of 39 female Wistar-type rats, from six to eight weeks old and weighing between 120 and 160 g were used. All of these rats were from the pet store of the Secondary school teachers training School (ENS) of Abidjan, and were acclimatised more than a week before the start of the experiment. The ambient temperature was 26 to 30 °C and under a photoperiod of twelve (12) hours of light and 12 hours of darkness. They had free access to food (a mixture of bakery bread flour, maize and dry fish) and had uninterrupted access to tap water in baby bottles.

4.2 Evaluation of toxicity: Acute toxicity was determined in accordance with OECD Guideline n° 423 of for the chemical substance tests, adopted in 2001. Five (5) batches of three female rats were formed, including one control group. The day before the treatment, all the rats were weighed and starved for, but had water at their disposal until 3 hours before the administration of the extract. They were weighed again the next day before the force-feeding. For the first stage, the batch 1(control) received 1 ml of distilled water per 100 g body weight and the rats of the four other batches received each the initial dose of 2000 mg/kg body weight in a volume of 1 ml / 100 g body weight orally using a gastric tube. The animals were observed individually for the first 30 minutes after administration **of** the extracts, then hourly for 4 hours and 24 hours later. The observations were based on the following clinical signs: Apathy, excitement, breathing difficulties, refusal of food, mouth and/or nose bleeding, abdominal pain, convulsion, trembling, diarrhoea, coma, etc. Mortality was assessed within 24 hours. The observation continued for 14 days and the rats were weighed every two days at the same time. As there were no deaths after the first day, the same protocol was repeated 24 hours later with the 3000 mg/kg dose and another 24 hours with the 5000 mg/kg body weight dose.

5 RESULTS

5.1 Outputs: The average Outputs are obtained from three extractions (3 x100 g) macerated powder.

Table 1: Result of the average output

Extracts	Mass of macerated powder (g)	Weight of 1 (g) extract	Weight of 2 (g) extract	Weight of 3 (g) extract	Output (%)
SF_{H2O}	100	4,5	5,27	4,68	4,75 ± 0,45
$SF_{ETH\ (70\%)}$	100	0,61	0,51	0,58	0,56 ± 0,05
SN_{H2O}	100	5,34	5,11	6,01	5,48 ± 0,46
$SN_{ETH\ (70\%)}$	100	0,52	0,82	0,63	0,65 ± 0,15

5.2 Detection of major chemical groups present in the studied algae: The results of the phytochemical sorting revealed the presence of some large chemical groups, which are Polysaccharides, Polyphenols, Catechic Tannins, Alkaloids and polyterpen Sterols and Saponins (Table 2 and 3).

Table 2: Result of the phytochemical sorting of *Sargassum* fluitans (SF)

Extracts	Sterols	Poly-phenols	Flavonoids	Tannins		Quinoid Substances	Alkaloids			Saponins	Polysac-charides
SF	Polyter-pens			Gal.	Cat.		D	B	VM		
H_2O	+	+	-	-	+	-	-	-	-	+	+
ETH 70 %	+	+	-	-	-	-	-	-	-	-	+

ETH = Ethanolic; H_2O = Distilled water; Cat =Catechic; Gal = Gallic; D = Dragendorff; B= Bouchardât; VM =Vasen-Mayer.

Table 3: Results of the phytochemical sorting of *Sargassum natans* (SN)

Extracts	Sterols	Poly-phenols	Flavonoids	Tannins		Quinoid Substances	Alkaloids			Saponins	Polysac-charides
SN	Polyter-pens			Gal.	Cat.		D	B	VM		
H_2O	+	+	-	-	+	-	-	-	-	+	+
ETH 70 %	+	+	-	-	-	-	-	-	-	-	+

ETH = Ethanolic; H_2O = Distilled water; Cat =Catechics; Gal = Gallic; D = Dragendorff; B= Bouchardât; VM =Vasen-Mayer.

5.3 Acute Toxicity: The single oral administration of 2000, 3000 and 5000 mg/kg as body weight (BW) of SF_{H2O}, $SF_{ETH\ (70\%)}$, SN_{H2O} and $SN_{ETH\ (70\%)}$ to the animals did not result in any mortality. No clinical sign of toxicity was also recorded. These different doses of extract would also have no significant effects ($p > 0.05$) on the mass weight of animals compared to the control animal that received only distilled water (Table 3). Thus, these different plant extracts would have an LD50 higher than 5000 mg/kg of BW according to the OECD guideline no.423 (OECD 423, 2001). Indeed, according to the Hodge and Sterner (1943) toxicity scale, these plant extracts would be almost non-toxic or relatively harmless depending on whether the LD50 is between 5000 and 15000 mg/kg of BW or higher than 15000 mg/kg of BW (Table 4).

Table 4: Rate of some ponderable benefits of animals on 14th day

Extracts	**Doses (mg/Kg of B.W.)**			
	Control	**2000**	**3000**	**5000**
SF_{H2O}	31,46 ± 0,48	24,67 ± 1,82	33,52 ± 4,74	31,27 ± 6,32
$SF_{ETH\ (70\%)}$	31,46 ± 0,48	26,29 ± 1,09	24,77 ± 4,63	32,89 ± 5,00
SN_{H2O}	31,46 ± 0,48	27,27 ± 7,25	28,69 ± 6,72	26,20 ± 8,28
$SN_{ETH\ (70\%)}$	31,46 ± 0,48	24,89 ± 5,00	39,48 ± 3,88	30,60 ± 8,95

Table 5: Toxicity class, according to the toxicity scale of **Hodge and Sterner (1943)**

Index or toxicity class	Terms currently used	Toxicological endpoint (LD_{50})
1	Extremely toxic	$DL_{50}) \leq 1\ mg/kg$
2	Highly toxic	1 mg/kg $\leq DL_{50} \leq 50$ mg/kg
3	Moderately toxic	50mg/kg$\leq DL50 \leq$500 mg/kg
4	Slightly toxic	500 mg/kg$\leq DL50 \leq 5\ g/kg$
5	Almost toxic	5 g/kg$\leq DL50 \leq 15\ g/kg$
6	Relatively inoffensive	DL50$\geq$15 g/kg

6. DISCUSSION

The phytochemical study carried out revealed the presence of major chemical groups in the aqueous and ethanol extracts of *Sargassum fluitans* and *Sargassum natans*. These are polysaccharides, polyphenols, catechic tannins, polyterpene sterols and saponosides. These results are in line with those of Maya (2017) and Stiger-Pouvreau *et al* (2014) who have demonstrated in their studies that the *Sargassum* genus was rich in polysaccharides and phenolic compounds, particularly phlorotannins. Peng *et al.* (2013) also revealed in their study that the *Sargassum* genus is rich in active principles such as secondary metabolites, polysaccharides, proteins, minerals, vitamins, fibres. Indeed, the bioactive properties of plants are mainly due to secondary metabolites (Sofowora, 1996) and primary metabolites such as polysaccharides (Ye *et al.*, 2008). According to Koné *et al.* (2007) and Kamanzi (2002), flavonoids, catechic tannins, saponosides and alkaloids are responsible for the antibacterial activity of certain plants. Similarly, Praven and Kumud, (2012) have demonstrated that plant extracts rich in tannins have a potential for anti-inflammatory activity. According to Gutman and Ryu (1996), polyphenols have anticancerous properties. Plouguerné *et al* (2013) demonstrated that total lipids from the brown alga *Sargassum vulgare* have a very strong antiviral activity against the herpes simplex 1 (HSV-1) and herpes simplex 2 (HSV-2) viruses. In addition, according to Pham *et al* (2013), polysaccharides from *Sargassum mcclurei* have a high anticancer activity. Pugh *et al* (2001) have also found that polysaccharides from spirulina (*Spirulina platensis*) are potent immune cell activators and increase the level of human tumour necrosis factor. All these results confirm the claims that Sargasso has interesting pharmacological properties and could justify its use in phytotherapy. In fact, Sargasso is used in several Asian countries for its medicinal properties (Masuda *et al.* 1993, Hong *et al.* 2007), as food (Wondimu *et al.* 2007) or for its alginate content (Saraswathi *et al.* 2003). Many species have anti-inflammatory activities (Dar *et al.* 2007). In his synthesis on the medical and pharmacological uses of natural products from algae, Smit (2004) listed several properties attributed to Sargasso, including antiviral, blood vessel genesis stimulating, anticancer, fibrinolytic cell proliferation reducing, antithrombic and anticoagulant properties. Sargasso also contains phenolic compounds (Stiger *et al.* 2004, Plougerné *et al.* 2006), with antioxidant and antimicrobial properties. A single-dose acute oral toxicity evaluation revealed that aqueous and ethanolic extracts of *Sargassum fluitans* and *Sargassum natans* at doses of 2000 mg/kg; 3000 mg/kg and 5000 mg/kg of body weight (bw) resulted in no mortality among rats. In addition, no signs of apparent toxicity were recorded with respect to general behaviour such as apathy, excitement, respiratory disturbances, refusal of food, mouth and/or nose bleeding, abdominal pain, convulsions, tremors, diarrhoea and coma among the rats throughout the study. The toxicity of the chemicals is based on their LD50 value, which is the dose that kills 50% of the test animals. The single oral administration of the aqueous and ethanol extracts revealed that the 50% lethal

dose (LD50) is higher than 5000 mg/kg of BW. According to the Globally Harmonised Classification System of OECD 423 (OECD 423, 2001), aqueous and ethanol extracts of *Sargassum fluitans* and *Sargassum natans* can be classified in category 5 and considered as a non-toxic substance by the oral route. The same method was used by Koné *et al.* (2009) to indicate that the LD50 of the aqueous extract of *Sacoglottis gabonensis* is higher than 5000 mg/kg of BW. However, these results are inconsistent with those of Alwashli *et al.* (2012) who have demonstrated that the methanolic extract of *Rumex nervosus Vahl* induced apparent undesirable effects (sedative action and paralysis of the forelegs) among mice at a dose of 500 mg/kg with an LD50 of 1028 mg/kg of BW between the 1st and 7th day after administration of the extract. Extracts of *S. fluitans* and *S. natans* could therefore be a good material for further pharmacological studies.

7 CONCLUSION

This study concluded that the aqueous and ethanol extracts of *Sargassum fluitans* and *Sargassum natans* contain primary and secondary metabolites known for their interesting therapeutic and pharmacological effects. The study of the acute toxicity of the aqueous and ethanolic extracts of these two algae, by the oral route, has revealed that they have no apparent toxic effect at the doses tested, with an LD50 higher than 5000 mg/kg of BW. These different test doses also had no significant effect ($p > 0.05$) on the weight mass of the animals compared to the control animals that received distilled water. This result seems to be encouraging in favour of the use of these algae as feed additives with therapeutic and/or zootechnical properties. However, it would be interesting to carry out further work, such as subacute and chronic toxicity, to confirm the safety of extracts of these algae by the oral route.

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