Available online on 15.09.2020 at http://jddtonline.info



Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

© 2011-18, publisher and licensee JDDT, This is an Open Access article which permits unrestricted non-commercial use, provided the original work is properly cited



Open Access

**Research Article** 

# Evaluation of Phytocompounds and Chemical Elements Present In Selected Species of Seaweeds, to Sustain Future Quantitative Analysis for Bioactive Compounds

Valera Dias<sup>1\*</sup>, Salomão Bandeira<sup>1</sup>, Eutilério Chaúque<sup>2</sup>, Maurício Lipassula<sup>1</sup>, Aidate Mussagy<sup>1</sup>

<sup>1</sup> Department of Biological Sciences, Eduardo Mondlane University, Maputo, Mozambique

<sup>2</sup> Department of Chemistry, Eduardo Mondlane University, Maputo, Mozambique

### ABSTRACT

**Introduction:** The specific physiological capacity of marine organisms, including seaweeds, to survive in extreme environmental conditions is associated to the production of secondary metabolites. Seaweeds are known as powerful source of a broad range of bioactive compounds. **Objective:** The aim of this study was to investigate the phytocompounds and chemical elements present in eight species of seaweeds which occur around Inhaca Island, Mozambique. Specifically, *Halimeda cuneata, Pseudorhizoclonium africanum, Pseudocodium devriesii, Dictyota suhrii, Gracilaria salicornia, Hypnea rosea, Laurencia natalensis* and *Jania adhaerens.* **Methods:** For phytocompounds and chemical elements screening, seaweeds samples were dried, ground to powder and extracted using methanol as solvent. The analyses were performed using GC-MS analysis, Energy Dispersive X-ray Fluorescence, and colorimetric protocols for phytocompound analysis. **Results:** A total of 82 phytocompounds were identified. Phytol and Z-8-Methyl-9-tetradecenoic were present in all samples analysed, while Cetene, 9-Octadecenoic acid (Z)-methyl ester, Desmoterol, Octadecanoic acid, and Oleic acid were the less common phytocompound identified. Campesterol, gama-Sitoesterol, Cholest-5-enol, 24-propylidene-(3.beta) are phytosterols only identified in green seaweeds. The concentration of chemical elements among the seaweeds species was different. However, Ca, Cl and K were presented in high concentration in some of the seaweeds analysed. **Conclusion**: Overall, the seaweeds analysed in this study, seems to be good candidate for further biotechnological application and deserve further investigation.

Keywords: seaweeds, methanolic extracts, phytocompounds, minerals, Inhaca Island.

Article Info: Received 21 July 2020; Review Completed 26 August 2020; Accepted 05 Sep 2020; Available online 15 Sep 2020

# Cite this article as:



Dias V, Bandeira S, Chaúque E, Lipassula M, Mussagy A, Evaluation of Phytocompounds and Chemical Elements Present In Selected Species of Seaweeds, to Sustain Future Quantitative Analysis for Bioactive Compounds, Journal of Drug Delivery and Therapeutics. 2020; 10(5):232-239 http://dx.doi.org/10.22270/jddt.v10i5.4422

### \*Address for Correspondence:

Valera Dias, Eduardo Mondlane University, Department of Biological Sciences, PO Box 257, Maputo, Mozambique

### **INTRODUCTION**

In recent years, marine resources have been tested to find potential compounds for the development of products with different applications, as they are a rich source of novel biocompounds<sup>1,2</sup>. In fact, the specific physiological capabilities of marine organisms, allow them to survive in extreme conditions and, provide a great potential for production of secondary metabolites compared to common organisms in terrestrial environments<sup>3</sup>. Among the marine resources investigated so far, seaweeds or marine macroalgae are known as rich in a broad range of compounds<sup>4</sup>, and researchers suggest them as a promising resource to provide novel biochemically active substances<sup>5,6</sup>. Biocompounds from seaweeds can be used as natural products in different industry such as pharmaceuticals, cosmetic, human nutrition, animal feed, fertilizer and bioplastics production<sup>7</sup>. These industries mainly search for antioxidants,

antimicrobial, anti-parasitic, anti-inflammatory, antiallergic, antifeedant, anti-trombic, anticarcenogenic and anti-ulcer<sup>8,9</sup>. The seaweeds metabolites with biological interest include alkaloids, phenols, flavonoids, saponins, steroids, and terpenoids<sup>9,10</sup>, and the techniques used to their extraction have been intensively reviewed<sup>4</sup>. Among the different procedures to analyse seaweeds metabolites, methanolic extracts was found to have the highest reducing power in comparison with other solvents such as chloroform, acetone and ethanol<sup>2</sup>. However, results are controversy among different studies and seem to be species specific<sup>4</sup>.

Despite the broad application of seaweed extracts worldwide<sup>11-13</sup>, in Mozambique where nearly 300 species of seaweeds have been registered these resources are under exploited<sup>14</sup>. To the best of our knowledge there is no scientific information in Mozambique reporting the phytochemical characterization and application of seaweeds

metabolites. Therefore, the purpose of the present study was to carry out a preliminary screening to investigate the phytocompounds and chemical elements present in eight species of seaweeds which occur around Inhaca Island, southern Mozambique. Specifically, the study evaluated the presence of phytocompounds and chemical elements in three species of Chlorophyta, Halimeda cuneata Hering, devriesii Weber Pseudocodium Bosse and Pseudorhizoclonium africanum (Kützing) Boedeker; one species of Phaeophyta, Dictyota suhrii G. Murray and four species of Rhodophyta Gracilaria salicornia (C. Agardh) E.Y. Dawson, Hypnea rosea Papenfuss, Laurencia natalensis Kylin and Jania adhaerens J.V. Lamouroux. All these species were previously described for Mozambique with exception to D. suhrii until recently known only to KwaZulu/Natal in South Africa<sup>15</sup>. These species were chosen due to the availability and group diversity along Mozambican coast. Mozambique seaweed flora is still poorly studied<sup>16</sup>, the country having quite a handful of papers highlighting to Inhaca<sup>16,17</sup>, Xai-Xai<sup>18</sup>, Mozambique Island<sup>19</sup>, Mecúfi<sup>20</sup> and Quirimbas archipelago<sup>21</sup>. The results of the present study might open to new opportunities and venues of research and development on seaweeds exploitation and brings solution to the gaps of our understanding of valuable seaweeds in Mozambigue and the Western Indian Ocean region.

### **MATERIALS AND METHODS**

### **Collection of seaweeds**

This study consisted of sampling of target species of seaweeds in Inhaca Island, and further laboratorial analysis of chemical elements and phytochemical compounds composition. The seaweed sampling was carried in October of 2018 at Ponta Mazónduè (near Farol) in Inhaca Island, in intertidal zone, during low spring tide. The north-eastern region of the island presents dense seaweed vegetation which extensive tracts are exposed during the low tidal range<sup>22</sup>. Eight species of seaweeds were sampled, specifically: three species of Chlorophyta, H. cuneata Hering, P. africanum and P. devriesii Weber Bosse; one species of Phaeophyta, D. suhrii and four species of Rhodophyta, G. salicornia (C.Agardh) E.Y.Dawson, H. rosea Papenfuss, L. natalensis Kylin and J. adhaerens J.V. Lamouroux. For Identification, books and field guide for seaweeds were used <sup>15-23</sup>, and their voucher specimens kept at the LMU herbarium at the Department of Biological Science, Eduardo Mondlane University.

Approximately 200 g of the seaweed were hand-picked, and a knife was used to remove de seaweed when necessary. The samples were transported in a basket with seawater, to avoid drying, to the Eduardo Mondlane University's laboratory. In the laboratory, the samples were cleaned to remove epiphytes, necrotic parts, and rinsed with distilled water to remove salts, sand particles and any associated detritus. Next, the samples were oven dried at 50 °C for 72 hours, and ground in an electric mixer, the powdered samples were weighed, and stored in a cool dry place until further analyses.

# Identification of phytocompounds using Gas chromatography-mass spectrometry (GC-MS) analysis

The analyses of phytochemical compounds followed an adapted<sup>24</sup> procedure. Briefly, each powered seaweeds sample was weighted, transferred to flask, treated with the Methanol until the powder was fully immersed, incubated overnight and filtered through a Whatmann filterpaper along with Sodium sulphate was wetted with absolute alcohol. The filtrates were then concentrated to 1 ml by bubbling nitrogen gas in to the solution. The extract contains

both polar and non-polar components of the material and 2  $\mu$ l sample of the solution was employed in GC-MS for analysis of different compounds. The GC - MS analysis was carried out using a Agilent 7820A GC System Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold, column - 5 MS 30 m (length) 250 µm (inner diameter) 0.25 µm (film). The instrument was set to an initial temperature of 110 °C, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280 °C, at the rate of an increase of 5 °C/min, and maintained for 9 min. Injection port temperature was ensured as 250 °C and Helium flow rate as one ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z). Interpretation on Mass-Spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) 2014, having more 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST 2014 library. The name, molecular weight and structure of the components of the test materials were ascertained. The compounds identified in this study are limited to the volatile and volatilizable compounds, which must be capable to retain in the column used; additionally, the components responsible for the observed peaks must be included in the library database.

### **Concentration of chemical elements in seaweed samples**

An amount of 5 g of each seaweeds powder were used to assess the semi-quantitative concentrations of the most frequent chemical elements by the methods of X-ray fluorescence scanning (Energy Dispersive X-rav Fluorescence, EDXRF, Shimadzu 7000, Japan). This is a relatively effective qualitative and semi-quantitative technique to analyse major, minor and trace elements<sup>25,26</sup>. Basically, the semi-quantitative analysis of elements was done at an accelerating voltage of 50 kV, 30 A, and at a detection time of 280 s with a Shimadzu EDX-700. The elements' concentrations were expressed as a percentage of their relative atomic abundances.

### Qualitative analysis of secondary metabolic compounds

Standard colorimetric method was used for qualitative analysis of phenolic compounds. Methanolic extracts were prepared for each seaweed sample. An amount of 2 g of sample were extracted with 40 ml of methanol for 90 minutes and concentrated on a rotavapor up to 10 ml at 60 <sup>o</sup>C. The phytochemical screening of different algal extracts was assessed using standard method as described<sup>27</sup>. Phytochemical screening was carried out to identify the major natural chemical groups such as alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins, quinones and glycosides.

*Test of coumarins (NaOH test)*: 1mL of each methanolic extract was added to a test tube containing 1 mL of 10% NaOH and the appearance of a yellow colour indicated the presence of coumarins <sup>27</sup>.

**Test of tannins (FeCl<sub>3</sub> test):** 1mL of each methanolic extract was added to a test tube containing 1 mL of 5% FeCl<sub>3</sub> and the appearance of a blue colour indicated the presence of hydrolyzable tannins and the appearance of a green colour indicated the presence of condensed tannins<sup>27</sup>.

**Test of Flavonoids:** 1 mL of each methanolic extract was added to a test tube containing 1mL of chloroform and 1mL of 1% NH<sub>4</sub>OH was added, and the appearance of a yellow color in the ammonia layer indicated the presence of flavonoids. The subclasses of flavonoids were identified as follows:

*Flavonols (Shinoda test)*: 1mL of each methanolic extract was added to a test tube containing a piece of magnesium tape (about 1 cm) and 2-3 drops of 32% HCl were placed. The appearance of an orange colour indicated the presence of flavones and the appearance of a red colour indicated the presence of flavonols<sup>27</sup>.

*Flavanones* (*NaOH test*): 1mL of each methanolic extract was added to a test tube containing 1 mL of 10% NaOH and the appearance of a yellow to orange colour indicated the presence of flavanones<sup>27</sup>.

*Test of saponin (foam test):* 1mL of each extract methanolic extract was added to a test tube containing 1 mL of distilled water and stirred permanently for 3 minutes. Persistent foaming for more than 5 minutes indicated the presence of saponins<sup>27</sup>.

**Test of alkaloids (Mayer test):** 1 ml of each methanolic extract was added to a test tube containing 6-12 drops of 2% HCl and filtered. To the filtrate, 2-3 drops of Mayer's reagent were added and the appearance of a yellow precipitate colour indicated the presence of alkaloids<sup>27</sup>.

**Test of steroid** *(Liebermann-Burchard test):* 1mL of each methanolic extract was added to a test tube containing about 10 drops of anhydrous acetic acid and then 2-3 drops of concentrated sulfuric acid were added. The colour change from blue to green indicated the presence of steroids<sup>27</sup>.

# **RESULTS AND DISCUSSION**

Seaweeds are potential sources of novel bioactive compounds which might bring new insights to industries such as pharmacology, animal feed, cosmetics, and others9. This study allowed to identify 82 phytocompounds from methanolic extract of eight seaweeds species (Table 1), by applying GC-MS, a high sensitive equipment which allow to identify volatile molecules from the samples<sup>28</sup>; being also the most precise methods to identify various phytocompounds in extracts from different species<sup>29</sup>. From the extracts analysed in this study, J. adhaerens and P. devriesii are the species with more phytocompounds identified, 36 and 33, respectively, these findings might indicate that these species are rich in the phytocompounds. According to Domettila et al. <sup>30</sup>, the seaweeds with a range of secondary metabolites might be strong candidates to be applied in pharmacy technology.

Among the species of seaweeds, the more frequent phytocompounds identified were n-Hexadecanoic acid, Cholesterol, Phytol, Phytol acetate, cis-13-Eicosenoic acid, Tetradecanoic acid, and Z-8-Methyl-9-tetradecenoic acid. Particularly, Phytol and Z-8-Methyl-9-tetradecenoic were found to be commonly present in all of the extracts analysed. However, n-Hexadecanoic acid was detected in the methanol blank sample. Therefore, it was not a surprised that it appeared in all samples. Phytol is a common compound found in plants and seaweeds. It is a key acyclic diterpene alcohol that is a precursor for vitamins E and K<sup>31</sup>. In other studies, it was reported to have antibacterial activities against *Staphylococcous aureus* and antifungical activities against *Ganoderma boninense*<sup>32</sup>.

On the other hand, some compounds were detected in only one species of seaweed in this study, which include Cetene (*J. adhaerens*), Desmosterol (*D. suhrii*) and Oleic acid (*P. africanum*). From these three compounds, Cetene (also known as 1-Hexadecene) was intensively reported in extract of plants such as *Citrus limon*<sup>33</sup>, *Gleichenia pectinata*<sup>34</sup> and

*Hyptis verticillata*<sup>35</sup>. Kebelmann et al.<sup>36</sup> reported that Cetene where detected only in three species of polar seaweeds (*Monostroma arcticum, Sphacelaria plumose and Kallymenia antarctica*), out of thirteen species evaluated. In a study performed in fungus *Berkleasmium* sp. Dzf12, Mou *et al.*<sup>37</sup> reported that Cetene showed antimicrobial and antioxidant effects.

Campesterol, Linoleic acid ethyl ester, gama-Sitoesterol, Cholest-5-en-ol -24-propylidene-(3.beta) are all phytosterols only found in H. cuneata, P. devriesii (both Chlorophyta). Phytosterols are metabolites synthesized by plant and algae, with great relevance to the development of new drugs and functional foods. According to Ogbe et al.<sup>38</sup> phytosterols may promote the health of humans and animals when they are included in their diet as natural foods or in enriched food supplements, for a regular period. These compounds are benefic, probably, due to their ability to lower cholesterol levels<sup>39</sup>. Campesterol, in particular, showed to have chemo-preventive effects against different types of cancer<sup>40</sup>, and this phytocompound was reported in Dictyota dichotoma and Sargassum granuliferum<sup>41</sup> and several other species of Phaeophyta from Antarctica<sup>42</sup>.

The qualitative colorimetric methods, used to evaluate some groups of phytocompounds (Table 2), revealed that condensed tannins and alkaloids were present in most of methanolic extracts, analysed in the present study. Tannins were similarly obtained in methanolic extracts in different studies, such as the ones with the brown seaweeds D. dichotoma<sup>27</sup> and Sargassum wightii<sup>10</sup>; red seaweeds Jania rubens, Corallina mediterranea and Pterocladia capillacea<sup>2</sup>; and in green seaweed Cladophora glomerata, Ulva lactuca and *Ulva reticulata*<sup>10</sup>; the obtained results suggest that tannins are common phytocompounds in seaweeds. In terms of application, Tannin-containing drugs are used as astringent, as healing agents in inflammation, gonorrhea, and seems to have antiviral, antibacterial and antiulcer activity9. Looking at alkaloids, some studies reported that these compounds were present in extracts of *D. dichotoma*<sup>27</sup>, in J. rubens, C. mediterranea and P. capillacea<sup>2</sup>, but it was absent in all species analysed by Abirami and Kowsalya<sup>24</sup> and by Mansuya et al.<sup>10</sup>. This phytocompound was mentioned to inhibit the growth of both Gram-positive and Gram-negative bacteria<sup>43</sup>.

In this study, the test for saponins and steroids were positive only in the methanolic extracts of two species of Chlorophyta (*H. cuneata* and *P. devriesii*). However, in other studies these two phytocompounds were detected in *S. wrightii*<sup>9</sup> and *D. dichotoma*<sup>27</sup>, which are both Phaeophyta. Mansuya et al.<sup>10</sup> detected these two phytocompounds in all six species analysed. Saponins present diverse biological characteristics such as antimicrobial, anti-inflammatory, anti-feedent and hemolytic effects<sup>44</sup>, while steroids are reported to have an important role in nutrition, cosmetics<sup>27</sup> and especially in medicine<sup>45</sup>.

The test for coumarins was positive only in two Rhodophyta species (*G. salicornia* and *H. rosea*). Although tests for coumarins revealed positive in species of Rhodophyta – by El-Din and Al-Ahwany<sup>2</sup> – in methanolic extract of *J. rubens, C. mediterranea and P. capillacea*, in the present study it revealed negative in methanolic extract of *J. adhaerens* and *L. natalensis*, which are also Rhodophyta. These results might indicate that these phytocompounds are specific to some species of Rhodophyta. In terms of application, coumarins are known as anti-coagulant to treat lymphedema<sup>46</sup>.

2 n 2 m	
A cuneata A cuneata A cosea A	L. natalensis
1 [1,1'-Bicyclopropyl]-2-octanoic acid, 2'-he1yl-,	
methyl ester	x
2 11-Octadecenoic acid, methyl ester x x x x	
3 12,15-Octadecadiynoic acid, methyl ester x	
4 12-Methyl-E,E-2,13-octadecadien-1-ol x	
5 15-Tetracosenoic acid, methyl ester, (Z)- x	
6 17-Octadecynoic acid x	
7 1-Heptatriacotanol x x x x	
8 1-He1adecanol, 2-methyl x	
9 2-Pentadecanone, 6,10,14-trimethyl- x x x	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	
11 5,8,11,14-Elcosatetraenoic acid, methyl ester, (allZ)- x x   12 C. Hadra 1a, 44,7a, trimestad	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	x
5,6,7,7 atetranydrobenzoruran-2(4H)-one	
13 6-Octadecendic acid, intelligitester, (Z) x	X
14 7,10-Herauetaulenoic aciu, methyl ester x x x x x x x x x	Х
(2methylo1iranyl)-	
16 8 11 14-Ficosatrienoic acid $(7.7.7)$ -	
17 91215-Octadecatrienoic acid $(7,7,7)$ -	
18 9 12-Octadecadienoic acid (7.7)- methyl ester	x
19 9.12-Octadecadienovi chloride. (Z.Z)- x x	*
20 9-He1adecenoic acid, methyl ester, (Z)-	
21 9-Nonadecene x	
22 9-Octadecenoic acid (Z)-, methylester x x	
23 alphaTerpineol x	
24 Arginine <b>x x</b>	х
25 Benzenepropanoic acid, 3,5-bis(1,1dimethylethyl)-4-	
hydro1y-, methyl ester	X
26 betaD-Glucopyranose, 4-ObetaDgalactopyranosyl x	
27 Campesterol x x	
28 Cetene x	
29 Cholest-5-en-3-ol, 24-propylidene-, (3.beta.) x x	
30 Cholesterol x x x x x x x	
31 cis-13-Eicosenoic acid x	
32 cis-5,8,11,14,17-Eicosapentaenoic acid <b>x</b>	
33 cis-Vaccenic acid x x x	
34 Cyclododecane x	
35 Desmosterol X	
36 Dodecanoic acid, 3-hydroly- X X   27 E 15 Hanta daesaal T	
37 E-15-Heptadecenal X X	
So   E-2-1etilduetell-1-01     20   Ficessatetraenois osid mothul octor (all7)	v
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Х
41 Fthyl Oleate <b>v v</b>	
42 3-Eicosene (E)	
43 exo-2.7.7-trimethylbicyclo[2.2.1]beptan-2-ol x	
44 gamma-Linolenic acid, methyl ester x	
45 gamma-Sitosterol <b>x x</b>	

Table 1: Chemical composition of methanolic extracts of eight species of seaweeds analysed using GC–MS. Chloro-Chlorophyta, Phaeo-Phaeophyta, Rhodo-Rhodophyta.

(Continued on next page)

# Table 1. (continued)

			Chloro. Phaeo.				Rhodo.			
	Name of the compound	H. cuneata	P. devriescii	P. africanum	D. suhrii	G. salcornia	H. rosea	J. adhaerens	L.natalensis	
46	Geranyl vinyl ether								х	
47	Glycerol 1-palmitate						x			
48	Heptadecane	х		х	х			x	х	
49	Heptadecanoic acid, 10-methyl-, methyl ester					х		х	х	
50	He1adecanoic acid, 2-hydro1y-					v				
	1(hydro1ymethyl)ethyl ester					А				
51	He1adecanoic acid, methyl ester		Х	х	х		х	X	х	
52	Imidazole, 2-amino-5-[(2-carbo1y)vinyl]-							x	х	
53	Isopropyl palmitate								х	
54	l-Gala-l-ido-octose					х				
55	Linoleic acid ethyl ester	х	Х							
56	Methyl stearate		Х							
57	Methyl tetradecanoate	х	X					x		
58	Neophytadiene		Х	х				X		
59	n-He1adecanoic acid	х	Х	х	х	х	х		х	
60	Octadecanoic acid				х					
61	Oleic Acid			х						
62	Palmitoleic acid	х				х	x			
63	Pentadecanoic acid	N. H			x	х		x		
64	Phthalic acid, butyl undecyl ester				$I_{Dec}$			x		
65	Phthalic acid, di(2-propylpentyl) ester	х	X		1975					
66	Phytol	х	х	х	x ()	x	x	x	х	
67	Phytol, acetate		X	х	x	x	х	х	x	
68	Pterin-6-carbo1ylic acid		X			19		х	х	
69	R-Limonene	-an				x			х	
70	Stigmastan-3,5-diene	х		х						
71	Tetracosanoic acid, methyl ester	x		х					х	
72	Tetradecane, 2,6,10-trimethyl-		х	х						
73	Tetradecanoic acid	x	x	х	х	х	х	х	х	
74	Tetradecanoic acid, 12-methyl-, methyl ester	2						X		
75	trans-13-Octadecenoic acid	2				х		х		
76	Trichloroacetic acid, heladecyl ester							х		
77	Undec-10-ynoic acid, dodecyl ester	x	X					X		
78	2-(13,14-Epo1y)tetradec-11-en-1-ol acetate	-	x	x	<b>*</b>	x				
79	<i>L</i> , <i>L</i> - <i>L</i> ,5-Pentadecadien-1-ol							X		
80	2-10-1etradecen-1-ol acetate				х	X		X	х	
81	Z-5-Nonadecene	x	x	x						
82	Z-8-Methyl-9-tetradecenoic acid	X	X	X	Х	X	X	X	X	

Table 2: Qualitative description of phytochemical compounds in methanolic extracts of eight species of seaweeds, collected in Inhaca Island. Chloro-Chlorophyta, Phaeo-Phaeophyta, Rhodo-Rhodophyta.

			Chloro.		Phaeo.	Rhodo.				
	Phytocompounds	H. cuneata	P. devriescii	P. africanum	D. suhrii	G. salicornia	H. rosea	J. adherensis	L. natalensis	
1	Condensed tannins	+	+	+	+	-	+	+	+	
2	Hydrolyzable tannins	-	-	-	-	-	-	-	-	
3	Coumarinas	-	-	-	-	+	+	-	-	
4	Flavanones	-	-	+	+	+	+	-	-	
5	Flavonols	-	-	-	-	-	-	-	-	
6	Saponins	+	+	-	-	-	-	-	-	
7	Alkaloids	+	+	-	+	+	+	+	+	
8	Steroids	+	+	-	-	-	-	-	-	

(+) positive; (-) negative.

Seaweeds possess a range of chemical elements required by humans, such as calcium, sodium, magnesium, potassium, phosphorus, iodine, iron, and zinc. However, the quality and quantity may differ among individuals, species, habitats, maturity, and environmental conditions<sup>47</sup>. In fact, the semiquantitative analysis in present study revealed a different percentage of elements in the samples evaluated, even within the same group of seaweeds (Table 3). For instance, within the group of Rhodophyta J. adhaerens presented 85.27% (Ca), 1.62% (Cl), 7.54% (Si) while G. salicornia presented 6.26% (Ca), 51.60% (Cl), 0.84% (Si), respectively. In a study by El-Din and Al-Ahwany<sup>2</sup>, the sequence of the most common elements was Ca, Mg, Na, followed by K, in all three species analysed. Ito and Hori47 analysed different species of seaweeds, and also revealed that Ca, Mg and K were the most common elements; where K was present in high proportion in Phaeophyta species: Padina arborescens, Hizikia fusiforme, and Sargassum thunbergii), Ca was in high proportion also in Phaeophyta (Scytosiphon lomentaria and Sargassum tortile), while Mg was in high proportion in Chlorophyta conglobata, (Ulva Ulva pertusa and Enteromorpha compressa). Results from the present study also show high proportion of Ca, in some seaweed such as H. cuneata and J. adherensis (72.04% and 85.27%), and in K in seaweeds such as D. suhrii, G. salicornia and H. rosea (28.50%, 35.84% and 38.36%). Low atomic weight elements including H, C, N, Na and Mg were not evaluated in our analysis, due to instrumental limitations (EDX-7000). The notable results in our analysis were referent to Cl which was in high proportion in three species analysed namely P. devriesii, G. salicornia and H. rosea (51.05%, 51.60% and 40.51%, respectively). The level of Cl may indicate that these species may be used as potential antibiotics. According to Kim<sup>48</sup>, Cl is the most common disinfectant. Therefore, the seaweed identified with high proportion of chlorine may be potential agent to be incorporated in antimicrobial products. The less represented elements such as Copper, Manganese, and Zirconium, were below 0.05%, in most dried samples of seaweeds; Zinc and Chromium did not exceed 0.02% in all dried samples analysed in this study.

Table 3: Concentration of chemical elements in dried samples of eight species of seaweeds from Inhaca Island. Chloro-Chlorophyta, Phaeo-Phaeophyta, Rhodo-Rhodophyta.

			Chloro.		Phaeo.	Rhodo.					
Cher	mical nents	H. cuneata	P. devriescii	P. africanum	D. suhrii	G. salcornia	H. rosea	. adherensis	L. natalensis		
1	Са	72.038	13.63	4.474	27.077	6.263	8.045	85.272	26.528		
2	Cl	10.758	51.053	3.644	25.015	51.595	40.51	1.623	28.114		
3	Si	5.904	3.881	5.561	7.331	0.838	3.063	7.539	9.066		
4	S	3.959	12.739	5.645	9.941	n.d	7.503	1.395	10.742		
5	Fe	2.413	1.879	1.821	1.037	0.37	1.164	1.467	1.641		
6	Sr	1.513	0.207	0.033	0.202	0.044	0.051	0.593	0.212		
7	Br	1.353	n.d	n.d	n.d	0.29	n.d	n.d	n.d		
8	К	0.937	3.327	1.568	28.498	35.837	38.362	1.598	19.109		
9	Ti	0.423	n.d	0.119	0.139	0.048	0.309	0.203	0.283		
10	Cu	0.062	0.107	0.032	0.045	0 <mark>.0</mark> 28	0.037	0.053	0.053		
11	Mn	0.035	n.d	0.025	0.084	0.013	0.043	0.046	0.034		
12	Zn	0.01	0.016	0.01	0.006	0.003	0.006	0.005	0.009		
13	Ва	n.d	2.774	0.274	0.397	n.d	0.385	0.207	3.437		
14	Cr	n.d	n.d	0.012	n.d	n.d	n.d	n.d	n.d		
15	Ι	n.d	1.652	n.d	n.d	0.391	n.d	n.d	0.625		
16	Р	n.d	0.175	0.057	0.153	0.329	0.395	n.d	0.007		
17	Rb	n.d	n.d	n.d	0.014	0.02	0.01	n.d	n.d		
18	Ru	n.d	0.683	n.d	n.d	n.d	n.d	n.d	n.d		
19	Zr	n.d	0.032	0.014	0.049	0.004	0.086	n.d	0.131		

n.d - not detected.

To the best of our knowledge, this is one of the first studies carried out on wider methanolic extracts of seaweed species from Mozambique. A total of 82 phytocompounds were identified, where *P. devriesii* and *L. natalensis* showed more phytocompounds. Phytol and Z-8-Methyl-9-tetradecenoic were commonly present in all of the extracts, while sterols Campesterol, Cholest-5-en-3-ol 24-propylidene-(3.beta), Ethyl Oleate and gamma-Sitosterol were only found in *H.* 

*cuneata* and *Padyna devriesii*. Further colorimetric analysis revealed existence of phytocompound such as condensed tannins, alkaloids, coumarins, saponins in selected seaweed species. However, hydrolyzable tannin and flavonols tests were negatives in all eight species investigated. The semiquantitative analysis of chemical elements showed to be variable among the seaweeds species. The elements such as Ca, Cl and K were present in high concentration in some of the seaweeds analysed. From this observation, seaweeds with high concentration of chlorine will be investigated for their potential use as antibiotic, as this element is the most common in disinfectants. Indeed, while this study highlights wider qualitative or semi-quantitative analysis it could support additional studies to emphasize the role of seaweeds in potential new drugs or healthy food.

### Acknowledgements

This work was funded by WIO-RISE (Western Indian Ocean Region Initiative in Science and Education), SIDA (Swedish International Development Cooperation Agency) and UEM (University of Eduardo Mondlane). The authors would like to thank the Department of Biological Science and the Department of Chemistry at UEM for technical support. Our grateful goes to Mr Amós Nhaca for the field and laboratorial assistance.

### REFERENCES

- 1. Agatonovic-Kustrin S, Morton DW. Cosmeceuticals Derived from Bioactive Substances Found in Marine Algae. Oceanography, 2013; 1:106. doi:10.4172/2332-2632.1000106.
- 2. El-Din SM, El-Ahwany AMD. Bioactivity and phytochemical constituents of marine red seaweeds (*Jania rubens, Corallina mediterranea* and *Pterocladia capillacea*). Journal of Taibah University for Science, 2016; 10:471-484.
- Blunt JW, Copp BR, Hu WP, Munro MH, Northcote PT, Prinsep MR. Marine natural products. Natural Product Research, 2007; 24:31-86. doi:10.1039/b603047.
- Pérez MJ, Falqué E, Domínguez H. Antimicrobial Action of Compounds from Marine Seaweed. Marine Drugs, 2016; 14, 52; 10.3390/md14030052.
- 5. Taskin E, Ozturk M, Taskin E, Kurt O. Antibacterial activities of some Marine algae from the Aegean sea (Turkey). African Journal of Biotechnology, 2007; 6:2746-2751.
- Melpha Y, Manchu N, James JE. Phytochemical evaluation of two brown seaweeds from Muttom and Rasthacaud coasts of Tamil Nadu, India. Journal of Chemical and Pharmaceutical Research, 2014; 6:566-569.
- Gullón B, Gagaoua M, Barba FJ, Gullón P, Zhang W, Lorenzo JM. Seaweeds as promising resource of bioactive compounds: Overview of novel extraction strategies and design of tailored meat products. Trends in Food Science & Technology, 2020; 100:1–18.
- 8. Cox S, Abu-Ghannam N, Gupta S. Effect of Processing Conditions On Phytochemical Constituents Of Edible Irish Seaweed *Himanthalia Elongata*. Journal Of Food Processing And Preservation, 2011; 36:348–363.
- 9. Marimuthu J, Antonisamy, Essakimuthu P, Narayanan J, Anantham B, Tharmaraj RJJM, Arumugam. Phytochemical characterization of brown seaweed *Sargassum wightii*. Asian Pacific Journal of Tropical Disease, 2012; 2:S109–S113.
- 10. Mansuya P, Aruna P, Sridhar S, Kumar J, Babu S. Antibacterial Activity and Qualitative Phytochemical Analysis of Selected Seaweeds from Gulf of Mannar Region. Journal of Experimental Sciences, 2010; 1:23-26.
- 11. Osman MEH, Aboshady AM, Elshobary ME. Production and characterization of antimicrobial active substance from some macroalgae collected from Abu- Qir bay (Alexandria) Egypt. African Journal of Biotechnology, 2013; 12:6847-6858.
- Shanmugam J, Devi RK, Viswanathan S, Nallamuthu T. Antibacterial and antioxidant activity of red seaweeds from Kilakarai, Rameswaram, Tamilnadu, India. Journal of Pharmaceutical and Biomedical Sciences, 2013; 32:1386– 1395.
- 13. Tanniou A, Vandanjon L, Incera M, Serrano-Leon E, Husa V. Assessment of the spatial variability of phenolic contents and associated bioactivities in the invasive alga *Sargassum muticum* sampled along its European range from Norway to Portugal. Journal of Applied Phycology, 2014; 2:1215–1230.
- Bandeira SO. Seaweed resources of Mozambique. In: The Seaweed Resources of the World (Eds) Critchley AT; Ohno M, Japan International Cooperation Agency (JICA). 1998, 403– 408.

- De Clercket O, Bolton JJ, Anderson RJ, Coppejans E. Guide to the seaweeds of KwaZulu-Natal, Scripta Botanica Belgica. 2005, 33. i-283.
- Coppejans E; Richmond MD; Clerck O; Rabesandratona R. Marine Macroalgae. Seaweeds. In: MD Richmond (ed) A Guide to the seashores of eastern Africa and the western Indian Ocean Islands. Sida. 1997, 70-97.
- 17. Critchley AT, Aken ME, Bandeira SO, Kalk M. A revised list of marine algae from Inhaca Island, Mozambique. South African Journal of Botany, 1994; 37:261–265.
- Isaac WE. Some Marine Algae from Xai-Xai, South African Journal of Botany, 1957; 23:75-102.
- Massingue AO, Bandeira SO. Distribution of Seagrasses and Common Seaweeds around Nampula Province (Northern Mozambique) with emphasis to Mozambique Island. Western Indian Ocean Journal of Marine Science, 2005; 4:175–183.
- Bandeira SO, António CM, Critchley AT. A taxonomic listing, including new distribution records, for benthic, intertidal seaweeds from Mecúfi, northern Mozambique. South African Journal of Botany, 2001; 67:492-496.
- Carvalho AM, Bandeira SO. Seaweed flora of Quirimbas Archipelago, northern Mozambique. In: Chapman ARO, Anderson RJ, Vreeland VJ and Davison IR (eds). Proceedings of the XVIIth International Seaweed Symposium, Cape Town, South Africa. 2003. Oxford University Press. ISBN 019 850742 9.
- 22. Critchley AT, Aken ME, Bandeira SO, Kalk M. A revised list of marine algae from Inhaca Island, Mozambique. South African Journal of Botany, 1997; 63:426-435.
- 23. Richmond MD. A guide to the Seashore of Eastern Africa and the Western Indian Ocean Islands. Wiomsa. 2010. p 464.
- 24. Abirami RG, Kowsalya S. Cosmeceuticals Derived from Bioactive Substances Found in Marine Algae. International Research Journal of Microbiology, 2012; 3:328-332.
- 25. Sitko R, Zawisza B. Quantification in X-Ray Fluorescence Spectrometry, X-Ray Spectroscopy. IntechOpen, 2012; doi: 10.5772/29367.
- 26. Noda T, Tsuda S, Mori M, Takigawa S, Matsuura-Endo C, Kim S. Determination of the phosphorus content in potato starch using an energy-dispersive X-ray fluorescence method. Food Chemistry, 2006; 95:632–637.
- 27. Deyab M, Elkatony T, Ward F. Qualitative and Quantitative Analysis of Phytochemical Studies on Brown Seaweed, *Dictyota dichotoma*. International Journal of Engineering Development and Research, 2016; 4:674-678.
- 28. Yogeswari S, Ramalakshmi S, Neelavathy R, Johnpaul M. Identification and Comparative Studies of Different Volatile Fractions from *Monochaetia kansensis* by GCMS. The Global Journal of Pharmacology, 2012; 6:65-71.
- 29. Garg K, Shrivastava B, Bhargava A. GC-MS Analysis of Methanol and Ethyl Acetate Extract of fruits of *Sphaeranthus indicus*. Journal of Drug Delivery and Therapeutics, 2019; 9:28-30.
- 30. Domettila C, Joselin J, Jeeva S. Phytochemical analysis on some south Indian seaweeds. Journal of Chemical and Pharmaceutical Research, 2013; 5: 275-278.
- Nandagopalan V, Gritto MJ, Doss A. GC-MS analysis of bioactive components of the methanol extract of *Hibiscus tiliaceus* Linn. Asian Journal of Plant Science and Research, 2015; 5:6-10.
- 32. Aziz S, Jafarah N, Yusof B, Zetty Z. Phytol-containing seaweed extracts as control for *Ganoderma boninense*. Journal of oil palm research, 2019; 31: 238-247.
- Kalsoom R, Haider MS, Chohan C. Phytochemical analysis and antifungal activity of some medicinal plants against *Alternaria* specie isolated from onion. Journal of Animal and Plant Sciences, 2020; 30:1–7.
- Femi-Adepoju A, Fatoba PO, Adepoju A, Oluyori AP. Phytochemical analysis, antimicrobial activity and identification of phytoconstituents in *Gleichenia pectinata* (Willd.) C. Presl. International Journal of Biomedical and Advance Research, 2018; 9:400-406.
- Egbung E, Anosike C, Utu-Baku A, Ogar I, Nna V. Phytochemical evaluation and GC-MS analysis of *Hyptis verticillata* cultivated in Calabar Cross River State, Nigeria. International Journal of Biological and Chemical Sciences, 2018; 11:2548-2559.

- 36. Kebelmann K, Hornung A, Karsten U, Griffiths G. Thermochemical behaviour and chemical product formation from Polar seaweeds during intermediate pyrolysis. Journal of Analytical and Applied Pyrolysis, 2013; 104:131-138.
- 37. Mou Y, Meng J, Fu X. Antimicrobial and antioxidant activities and effect of 1-hexadecene addition on palmarumycin C2 and C3 yields in liquid culture of endophytic fungus *Berkleasmium sp.* Dzf12. Molecules, 2013; 18:15587-15599.
- Ogbe R. A Review of Dietary Phytosterols: Their occurences, metabolism and health benefits. Asian Journal of Plant Science and Research, 2015; 5:10 - 21.
- Lopes G, Sousa C, Valentão P, Andrade PB. Sterols in Algae and Health. In Bioactive Compounds from Marine Foods (eds Hernández-Ledesma B, Herrero M). 2013, DOI:10.1002/9781118412893.ch9.
- Choi JM, Lee EO, Lee HJ. Identification of campesterol from *Chrysanthemum coronarium* L. and its antiangiogenic activities. Phytotherapy Research, 2007; 21:954-959. doi:10.1002/ptr.2189.
- 41. Bakar K, Mohamad H, Latip J, Seng T; Herng M. Fatty acids compositions of *Sargassum granuliferum* and *Dictyota dichotoma* and their anti-fouling activities. Journal of Sustainability Science and Management, 2017; 12:8 16.

- Pereira H, Barreira L, Figueiredo F. Polyunsaturated Fatty acids of marine macroalgae: potential for nutritional and pharmaceutical applications. Marine Drugs, 2012; 10:1920-1935.
- 43. Cowan MM. Plant Products as Antimicrobial Agents. Clinical Microbiology, 1999; 12:564-582.
- 44. Desai S, Desai DG, Kaur H. Saponins and their biological activities. Pharma Times, 2009; 41:13-16.
- 45. Okwu DE. Improving the Nutritive Value of Cassava Tapioca Meal with Local Spices. Journal of Nutraceuticals, Functional & Medical Foods, 2001; 3:43-50.
- 46. Jeeva S, Domettila C, Anantham B, Mahesh M. Preliminary phytochemical studies on some selected seaweeds from Gulf of Mannar, India. Asian Pacific Journal of Tropical Biomedicine, 2012; 2:S30–S33.
- 47. Ito K, Hori K. Seaweed: Chemical composition and potential food uses. Food Reviews International, 1989; 5:101-144.
- Kim J, Pitts B, Stewart P, Camper A, Yoon J. Comparison of the Antimicrobial Effects of Chlorine, Silver Ion, and Tobramycin on Biofilm. Antimicrobial agents and chemotherapy, 2008; 52:1446-1453.

JDDT