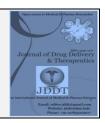
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Research Article

Qualitative Analysis of Phytocompounds of *Liagora divaricata* and *Trematocarpus flabellatus*

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ABSTRACT

Introduction: Phytocompounds are a powerful chemical group obtained from natural resources that exhibit a range of biological activities. **Objective:** This study explored the phytocompounds constituents of two species of Rhodophyta, *Liagora divaricata* and *Trematocarpus flabellatus* in order to give a preliminary view of qualitative diversity of potentially bioactive compounds. **Methods:** Approximately 200g of each species were hand-picked at in Chongoene, Mozambique, during a low spring tide. Voucher specimens were identified and stored at the LMU *herbarium* in the Department of Biological Science, University of Eduardo Mondlane. Samples were cleaned and dried at 50°C for 72 hours before grounding using an electric mixer. Powdered samples were extracted with methanol solvent. Phytocompounds samples were analysed using the GC-MS and identified based in NIST mass spectral library. **Results:** A total of 42 phytocompounds were identified. The common identities from both seaweeds species include Cholesterol, Desmosterol, Heptadecane, Hexadecanoic acid methyl ester, n-Hexadecanoic acid, Neophytadiene and Phytol. **Conclusion:** Due to the relevance of these phytocompounds in different industries such as pharmacy, nutrition, agriculture and cosmetic, the identified seaweeds might be good candidates for further research in terms of isolating and validating their activity. Particular attention should be given to Neophytadiene as it is a strong bioactive compound, and can be used for several applications.

Keywords: Phytocompounds, Liagora divaricata, Trematocarpus flabellatus, Neophytadiene

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INTRODUCTION

Phytocompounds are a chemical group obtained from natural sources (plants, seaweeds and microalgae) that exhibit a range of biological activities ^{1,2,3}. There has been growing interest in the application of these bioactive compounds in recent years ^{4,5}, attracting to the investigation of different species. Among the organisms evaluated for novel phytocompounds, a huge effort has been given to marine habitants^{6,7}. Marine organisms habit in complex environments, usually exposed to extreme conditions of temperature, salinity and pressure. They, therefore, produce diverse secondary metabolites that cannot be found elsewhere ⁶.

Seaweeds or macroalgae are one of the richest marine sources of several types of biologically active metabolites ^{8,9}, including alkaloids (e.g. Galanthanine), terpenoids (e.g. Phytol), steroids (e.g. Desmosterol), tannins (e.g.

Octoplorethol), PUFAs (e.g. α -linolenic acid), etc ^{10,11,12}. Seaweeds present a wide spectrum of useful biological properties, which include antibacterial, antiviral, antifungal, antitumor, anti-inflammatory, anti-proliferative, anti-cancer, antioxidant, analgesic, algicidal, larvicidal and insecticidal activities 13,14 These properties are tools for biotechnological application in different fields such as medicine, cosmetics, food industry, fertilizers and animal feed 15,16,17. A comprehensive review of phytocompound in seaweeds can be found in Tyśkiewicz et at ¹⁸ and Rengasamy et al ¹⁹. However, there are still diverse species of seaweeds that have not been characterized¹⁰.

Despite the broad application of seaweed in different industries worldwide ^{20,21,22}, this resource in underexploited in Mozambique where nearly 300 species of seaweeds have been documented ²³. To the best of our knowledge, there is no scientific information in Mozambique reporting the phytochemical characterization and application of seaweeds

metabolites. Therefore, the present study aimed to give a preliminary view of phytocompounds from seaweeds in Mozambique. We analysed two species of Rhodophyta (red seaweeds), *Liagora divaricata* and *Trematocarpus flabellatus* that occur in Chongoene. Finding of this study may contribute to the development of a new focus on phytocompounds exploitation and bring a solution to the scientific knowledge gaps in Mozambique and the region.

MATERIALS AND METHODS

Seaweed collection

Seaweed sampling was carried out in September 2018 at Chongoene, Mozambique, in the intertidal zone, during a low spring tide. Two species of Rhodophyta, L. divaricata and T. flabellatus were sampled. The specimens were identified using field guides for seaweeds ^{24,25}. Approximately 200g of seaweed was hand-picked. A knife was used to remove the seaweeds when necessary. The samples were transported to the Eduardo Mondlane University's laboratory in a basket with seawater to prevent drying. In the laboratory, the samples were cleaned to remove epiphytes and necrotic parts. Samples were rinsed with distilled water to remove salts, sand particles and any associated detritus (miscellaneous) before voucher identifications and storage at the LMU herbarium at the Department of Biological Science, Eduardo Mondlane University. Thereafter, the samples were dried at 50°C for 72 hours and were ground in an electric mixer. The powdered samples were weighed and stored in a cool place until further analyses.

Preparation of seaweed extracts

An amount of 10g of each powered sample of seaweeds was transferred into test tubes, treated with Methanol until the powder was fully immersed before overnight incubation. Samples were filtered through a Whatman paper along with Sodium sulphate, which was wet with absolute alcohol. Filtrates were concentrated to 1ml by bubbling nitrogen gas into the solution.

Identification of phytocompounds using GC-MS

The analyses of phytochemical compounds were performed according the method described by Abirami and Rajendran ²⁶, with minor modifications. The extract contains both polar and non-polar components of the material, and 2µl sample of the solution was employed in GC-MS for analysis of different compounds. The GC-MS analysis was carried out using an Agilent 7820A GC System Gas Chromatography equipped and coupled with a mass detector Turbo mass gold, column - 5MS, 30m (length) 250µm (inner diameter) 0.25µm (film). The instrument was set to an initial temperature of 110°C and was maintained at this temperature for 2 minutes. At the end of this period, the oven temperature was raised to 280°C, at the rate of 5°C/min for a constant of 9 minutes. Injection port temperature was ensured as 250°C and Helium flow rate as 1ml/min.

The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z). Interpretation of Mass-Spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) 2016 with more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. The compounds in GC-MS analysis were identified based on the comparison of the retention time and mass spectra with the references present in the NIST mass spectral library. The compounds identified in this study were 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 were included in the library database.

RESULTS AND DISCUSSION

GC-MS is highly sensitive equipment and one of the most precise in identifying various compounds in extracts from different solvents²⁷. In this study, GC-MS enabled the identification of 42 phytocompounds from the methanolic extracts of the red seaweed *L. divaricata*, 32 phytocompounds (Table 1) and *T. flabellatus*, 17 phytocompounds (Table 2). From the phytocompounds identified, seven were common to both extracts, namely: Cholesterol, Desmosterol (both sterols), Heptadecane (Alkane), Hexadecanoic acid methyl ester, n-Hexadecanoic acid (both fatty acid), Neophytadiene and Phytol (both terpenes). The relevance of these phytocompounds is discussed in this study.

Sterols obtained from seaweeds can be used in fields such as pharmacy, nutrition, and cosmetics ²⁷. Indeed, diet containing sterol may reduce the risk of heart disease ²⁹. These compounds are also associated with anti-inflammatory, antibacterial, anti-fungicidal, anti-ulcerative and anti-tumoral effects ³⁰. Similar to the results of this study, Cholesterol has been identified in several other seaweeds studies ^{31,32,33}. Desmosterol is another sterol found in different species of seaweeds ³⁴, such as *Porphyra sp.* and *Laminaria sp.*³⁴.

A range of fatty acid can be found in seaweeds ^{35,29}. Some of them, such as polyunsaturated fatty acid, are considered essential for humans and animals, and help to prevent the growth of atherosclerotic plaque, reduce blood clotting, blood pressure and improve immune functions ^{36,29}. In their study, Manilal et al ³⁷ reported that fatty acids from seaweeds possessed proprieties that can be used as ecofriendly anti-fouling. Additionally, fatty acids from seaweeds are a rich substitute for PUFAs that can be used in food formulations ³⁸. The fatty acids that were identified in both seaweeds analysed in this study - Hexadecanoic acid methyl ester and Hexadecane - were also registered in green seaweeds, Ulva lactucaand Ulva fasciata ³⁹. Both compounds showed anti-cancer properties in a study conducted in Dictyota bartayresiana (brown seaweed), and they were suggested to be an alternative to synthetic drugs available in the market ⁴⁰.

The last group of seaweeds that occur in both species analysed in this study, belong to terpenes. Terpenes are the major class of secondary metabolites with a range of roles in mediating antagonistic and beneficial interaction ⁴¹. However, most of them demonstrate qualities of toxins and/or repellents. Indeed, among seaweed phytocompounds, terpenes have merged as the principal chemical defence against grazing by herbivores ⁴². Some terpenes from plants show that they are important in resistance to diseases caused by fungi and bacteria. Nevertheless, the functionality and application of many terpenes have not yet been explored 43.

In this study, the terpenes, Neophytadiene and Phytol, were present in methanolic extracts of the two species analysed. Both phytocompounds were detected in several plants and some microalgae⁴⁴. According to Wei et al ⁴¹, red seaweeds are rich in terpenes. Phytol is a common terpene found in plants and seaweeds and is a precursor for vitamins E and K ⁴⁵. Additionally, Phytol has antibacterial activities against *Staphylococcous aureus* and antifungal activities against *Ganoderma boninense* ⁴⁶. Similar to Phytol, Neophytadiene is

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an acyclic diterpene. Bhardwaj et al ⁴⁷ studied the seaweed *Turbinaria ornata* and found that Neophytadiene has potential use in inflammatory disorder. Additionally, several studies reported that phytocompounds have strong antibacterial, antifungal, antipyretic, antioxidant, analgesic and vermifugic qualities ^{48.49}.

Among the phytocompounds identified in this study, Neophytadiene has been reported as a very strong bioactive phytocompound. Therefore, its total ion chromatogram and GC-MS spectrum from both species analysed (*L. divaricata* and *T. flabellatus*) are presented as supplementary information, in this study. Further quantitative analysis and additional assays, might elucidate the biological activities of Neophytadiene, in *L. divaricata* and *T. flabellatus*. Nevertheless, the present results are useful bases for the posterior investigation to evaluate these species of seaweeds as potential sources of bioactive compounds.

Table 1: Phytocompounds identified from the methanolic extract of the seaweed *L. divaricata*, by GC-MS. The phytocompounds highlighted are the ones that were found in methanolic extracts of both seaweeds species analysed in this study.

	Name	DB Formula	RT	Hits (DB)
1	.alphaTerpineol	C10H18O	4.779	10
2	1,2-15,16-Diepoxyhexadecane	$C_{16}H_{30}O_2$	23.266	9
3	1-Heptadecene	C17H34	25.009	10
4	1-Octanol, 2-butyl-	C12H26O	13.236	10
5	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	18.479	1
6	2-Piperidinone, N-[4-bromo-n-butyl]-	C ₉ H ₁₆ BrNO	28.37	1
7	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	19.22	10
8	3-Eicosene, (E)-	C20H40	21.368	10
9	4-tert-Butylcyclohexyl acetate	$C_{12}H_{22}O_2$	8.229	1
10	7-Hexadecenoic acid, methyl ester, (Z)-	C17H32O2	19.672	8
11	7-Tetradecene	C14H28	8.762	10
12	9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	23.482	10
13	Benzenepropanoic acid, 3,5-bis(1,1-	C ₁₈ H ₂₈ O ₃	20.458	2
	dimethylethyl)-4-hydroxy-, methyl ester			
14	Cholesterol	5 C ₂₇ H ₄₆ O	39.276	9
15	Cyclododecane	C12H24	8.593	10
16	Desmosterol	C27H44O	40.062	2
17	Dodecanal	C12H24O	19.152	10
18	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	11.663	1
19	E-15-Heptadecenal	C17H32O	17.393	10
20	Heptadecane	C17H36	15.431	10
21	Hexadecanoic acid, methyl ester	C17H34O2	20.082	10
22	Hexadecen-1-ol, trans-9-	$C_{16}H_{32}O$	13.084	10
23	Methyl stearate	$C_{19}H_{38}O_2$	23.841	6
24	Methyl tetradecanoate	$C_{15}H_{30}O_2$	15.997	8
25	Neophytadiene	C ₂₀ H ₃₈	18.361	10
26	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	20.738	10
27	Octan-2-one, 3,6-dimethyl-	C10H20O	4.588	3
28	Phthalic acid, butyl undecyl ester	C23H36O4	18.978	1
29	Phytol	C ₂₀ H ₄₀ O	23.609	10
30	Tricyclo[4.2.1.1(2,5)]dec-3-en-9-ol, acetate,	$C_{12}H_{16}O_2$	9.426	1
	stereoisomer			
31	Undec-10-ynoic acid, dodecyl ester	$C_{23}H_{42}O_2$	23.03	10
32	Z,Z-2,5-Pentadecadien-1-ol	C15H28O	6.669	1

	Name	DB Formula	RT	Hits (DB)
1	17-Octadecynoic acid	$C_{18}H_{32}O_2$	18.86	10
2	1-Heptatriacotanol	C37H76O	32.21	1
3	Cholest-5-en-3-ol, 24-propylidene-, (3.beta.)-	C ₃₀ H ₅₀ O	44.156	4
4	Cholesterol	C ₂₇ H ₄₆ O	39.277	10
5	cis-13-Eicosenoic acid	C20H38O2	20.458	6
6	Desmosterol	C27H44O	40.126	7
7	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	36.316	1
8	Heptadecane	C17H36	15.426	10
9	Hexadecanoic acid, methyl ester	C17H34O2	20.086	10
10	Neophytadiene	C20H38	18.365	10
11	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	21.372	10
12	Oleic Acid	$C_{18}H_{34}O_2$	24.209	10
13	Phytol	C ₂₀ H ₄₀ O	23.621	10
14	Phytol, acetate	C22H42O2	19.219	10
15	Tetradecanoic acid	C14H28O2	16.729	10
16	Z-10-Tetradecen-1-ol acetate	C16H30O2	13.079	10
17	Z-8-Methyl-9-tetradecenoic acid	C15H28O2	17.393	10
			111	

Table 2: Phytocompounds identified from the methanolic extract of the seaweed *T. flabellatus*, GC-MS. The phytocompounds highlighted are the ones that were found in methanolic extracts of both seaweeds species analysed in this study.

CONCLUSION

GC-MS analysis allowed the identification of 42 phytocompounds from methanolic extracts of the red seaweed L. divaricate and T. flabellatus. Diverse groups of secondary metabolites were found within the phytocompounds, such as sterols (Cholesterol and Desmosterol), fatty acids (Hexadecanoic acid methyl ester and n-Hexadecanoic acid), and terpenes (Neophytadiene and Phytol). Due to their relevance in different industries such as pharmacy, nutrition, agriculture and cosmetic, these types of seaweed are good candidates for further research in terms of isolating and validating the phytocompounds identified in this study. Particular attention should be given to Neophytadiene as this is a strong bioactive compound with several applications. To the best of our knowledge, this is the first time that secondary metabolites from red seaweed types L. divaricata and T. flabellatus have been evaluated in the region. The results provide new insights regarding the importance of these marine resources.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Supplementary Information

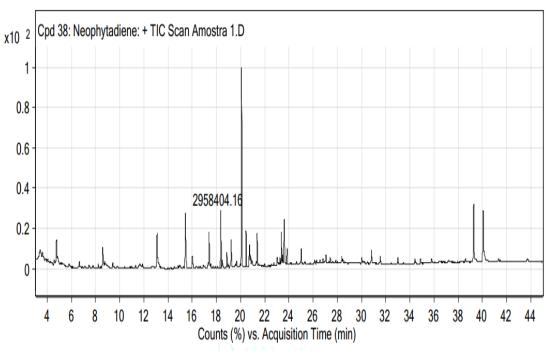


Figure 1: Total ion chromatogram (TIC) of *Liagora divaricata* methanolic extract highlighting the presence of Neophytadiene phytocompound, analysed in GC-MS.

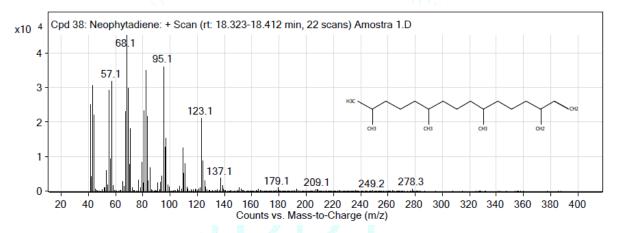
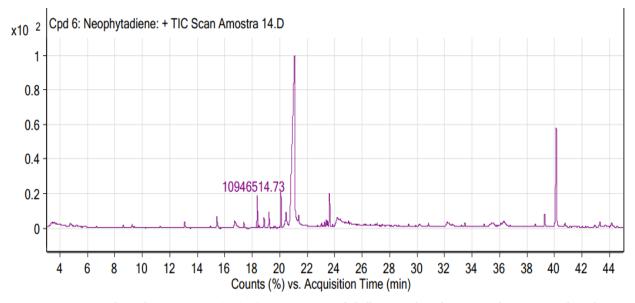
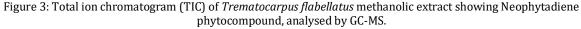


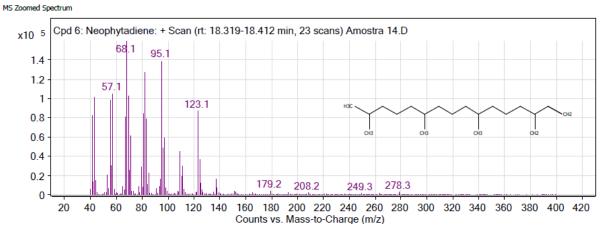
Figure 2: GC-MS spectrum of the phytocompound Neophytadiene from Liagora divaricata methanolic extract.







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Figure 4: GC-MS spectrum of the phytocompound Neophytadiene from Trematocarpus flabellatus methanolic extract.
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