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 Chongone, Mozambique, during a low spring tide. Voucher specimens were identified and stored at the LM Un herbarium in the Department of Biological Science, University of Eduardo Mondlane. Samples were cleaned and dried at 50°C for 72 hours before grinding 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 identifications 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

INTRODUCTION

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

Seaweeds or macroalgae are one of the richest marine sources of several types of biologically active metabolites, including alkaloids (e.g. Galanthamine), terpenoids (e.g. Phytol), steroids (e.g. Desmosterol), tannins (e.g. Octoplorethol), PUFAs (e.g. α-linolenic acid), etc. Seaweeds present a wide spectrum of useful biological properties, which include antibacterial, antiviral, antifungal, antitumor, anti-inflammatory, anti-proliferative, anti-cancer, antioxidant, analgesic, algidial, larvicidal and insecticidal activities. These properties are tools for biotechnological application in different fields such as medicine, cosmetics, food industry, fertilizers and animal feed. A comprehensive review of phytocompound in seaweeds can be found in Tyśkiewicz et al and Rengasamy et al. However, there are still diverse species of seaweeds that have not been characterized.

Despite the broad application of seaweed in different industries worldwide, this resource is underexploited in Mozambique where nearly 300 species of seaweeds have been documented. 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), Liatraga divaricata and Trematocarpus flabellatus that occur in Chongono. 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 Chongono, 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. 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 powdered 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 to the method described by Abirami and Rajendran, with minor modifications. The extract contains both polar and non-polar components of the material, and 2ml 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 set at 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 massespectral 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 solvents. 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. Indeed, diet containing sterol may reduce the risk of heart disease. These compounds are also associated with anti-inflammatory, antibacterial, anti-fungical, anti-ulcerative and anti-tumoral effects. Similar to the results of this study, Cholesterol has been identified in several other seaweeds studies. Desmosterol is another sterol found in different species of seaweeds, such as Porphyra sp. and Laminaria sp.

A range of fatty acid can be found in seaweeds. 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. Desmosterol is another sterol found in seaweeds analysed in this study – Hexadecanoic acid methyl ester and Hexadecane – were also registered in green seaweeds, Ulva lactuca and Ulva fasciata. 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.

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. 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. 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.

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 microalgae. 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. Additionally, Phytol has antibacterial activities against Staphylococcus aureus and antifungal activities against Ganoderma boninense. Similar to Phytol, Neophytadiene is
an acyclic diterpene. Bhardwaj et al.\(^1\) 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.\(^2,3\)

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. alpha-Terpineol                                                   | C\(_{10}\)H\(_{18}\)O | 4.779  | 10        |
| 2. 1,2,15,16-Diepoxyhexadecane                                        | C\(_{16}\)H\(_{30}\)O\(_2\) | 23.266 | 9         |
| 3. 1-Heptadecene                                                     | C\(_{12}\)H\(_{26}\)O | 13.236 | 10        |
| 4. 1-Octanol, 2-butyl-                                                | C\(_{12}\)H\(_{26}\)O | 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\(_{6}\)H\(_{12}\)BrNO | 28.37  | 1         |
| 7. 3,7,11,15-Tetramethyl-2-hexadecan-1-ol                             | C\(_{20}\)H\(_{40}\)O | 19.22  | 10        |
| 8. 3-Eicosene, (E)-                                                | C\(_{20}\)H\(_{40}\)O | 21.368 | 10        |
| 9. 4-tert-Butylcyclohexyl acetate                                     | C\(_{12}\)H\(_{22}\)O\(_2\) | 8.229  | 1         |
| 10. 7-Hexadecenoic acid, methyl ester, (Z)-                          | C\(_{17}\)H\(_{32}\)O | 19.672 | 8         |
| 11. 7-Tetradecane                                                   | C\(_{14}\)H\(_{28}\) | 8.762  | 10        |
| 12. 9-Octadecenoic acid (Z)-, methyl ester                           | C\(_{19}\)H\(_{38}\)O | 23.482 | 10        |
| 13. Benzenepropanoic acid, 3,5-bis(1,1-dimethylthyl)-4-hydroxy-, methyl ester | C\(_{18}\)H\(_{28}\)O\(_3\) | 20.458 | 2         |
| 14. Cholesterol                                                      | C\(_{27}\)H\(_{46}\)O | 39.276 | 9         |
| 15. Cyclododecane                                                   | C\(_{22}\)H\(_{34}\) | 8.593  | 10        |
| 16. Desmosterol                                                      | C\(_{27}\)H\(_{46}\)O | 40.062 | 2         |
| 17. Dodecanol                                                       | C\(_{12}\)H\(_{24}\)O | 19.152 | 10        |
| 18. Dodecanoic acid, methyl ester                                    | C\(_{13}\)H\(_{26}\)O\(_2\) | 11.663 | 1         |
| 19. E-15-Heptadecenal                                               | C\(_{17}\)H\(_{32}\)O | 17.393 | 10        |
| 20. Heptadecane                                                    | C\(_{17}\)H\(_{36}\) | 15.431 | 10        |
| 21. Hexadecanoic acid, methyl ester                                  | C\(_{17}\)H\(_{36}\)O\(_2\) | 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 | 23.841 | 6         |
| 24. Methyl tetradecanoate                                            | C\(_{15}\)H\(_{30}\)O | 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-                                       | C\(_{10}\)H\(_{20}\)O | 4.588  | 3         |
| 28. Phthalic acid, butyl undecyl ester                               | C\(_{22}\)H\(_{38}\)O\(_4\) | 18.978 | 1         |
| 29. Phytol                                                          | C\(_{20}\)H\(_{40}\)O | 23.482 | 10        |
| 30. Tricyclo[4.2.1.1(2,5)]dec-3-en-9-ol, acetate, stereoisomer       | C\(_{12}\)H\(_{16}\)O | 9.426  | 1         |
| 31. Undec-10-ynoic acid, dodecyl ester                               | C\(_{23}\)H\(_{42}\)O\(_2\) | 23.03  | 10        |
| 32. ZZ-2,5-Pentadecadien-1-ol                                       | C\(_{13}\)H\(_{20}\)O | 6.669  | 1         |
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.

| Name                           | DB Formula | RT  | Hits (DB) |
|--------------------------------|------------|-----|-----------|
| 1 17-Octadecynoic acid         | C_{18}H_{32}O_{2} | 18.86 | 10        |
| 2 1-Heptatriacetal            | C_{7}H_{16}O    | 32.21 | 1         |
| 3 Cholest-5-en-3-ol, 24-propylidene-,(3,beta)- | C_{25}H_{50}O | 44.156 | 4         |
| 4 Cholesterol                   | C_{27}H_{44}O   | 39.277 | 10       |
| 5 cis-13-Eicosenoic acid       | C_{20}H_{38}O_{2} | 20.458 | 6         |
| 6 Desmosterol                  | C_{27}H_{44}O   | 40.126 | 7         |
| 7 E,E,Z-1,3,12-Nonadecatriene-5,14-diol | C_{19}H_{36}O_{2} | 36.116 | 1         |
| 8 Heptadecane                  | C_{17}H_{36}    | 15.426 | 10        |
| 9 Hexadecanoic acid, methyl ester | C_{17}H_{34}O_{2} | 20.086 | 10        |
| 10 Neophytadiene                | C_{19}H_{38}    | 18.365 | 10        |
| 11 n-Hexadecanoic acid         | C_{16}H_{32}O_{2} | 21.372 | 10        |
| 12 Oleic Acid                  | C_{18}H_{36}O_{2} | 24.209 | 10        |
| 13 Phytol                      | C_{14}H_{22}O_{4} | 23.621 | 10        |
| 14 Phytol, acetate             | C_{22}H_{42}O_{2} | 19.219 | 10        |
| 15 Tetradecanoic acid          | C_{16}H_{32}O_{2} | 16.729 | 10        |
| 16 Z-10-Tetradecen-1-ol acetate | C_{16}H_{30}O_{2} | 13.079 | 10        |
| 17 Z-8-Methyl-9-tetradecenoic acid | C_{15}H_{28}O_{2} | 17.393 | 10        |

CONCLUSION

GC-MS analysis allowed the identification of 42 phytocompounds from methanolic extracts of the red seaweed L. divaricata 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.

Figure 3: Total ion chromatogram (TIC) of *Trematocarpus flabellatus* methanolic extract showing Neophytadiene phytocompound, analysed by GC-MS.
Figure 4: GC-MS spectrum of the phytocompound Neophytadiene from *Trematocarpus flabellatus* methanolic extract.