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BIOACTIVITIES OF EXTRACTS, DEBROMOLAURINTEROL AND FUCOSTEROL FROM MACROALGAE SPECIES

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ABSTRACT

Parasitic diseases including malaria, and other numerous microbial infections and physiological diseases are threatening the global population. Tanzanian coast shores are endowed with a variety of macroalgae (seaweeds), hitherto unsystematically explored to establish their biomedical potentials. Thus, antiplasmodial activity using malarial imaging assay, antimicrobial activity using microplate dilution technique, antioxidant activity using DPPH radical scavenging method and cytotoxicity using brine shrimp test were carried out on crude extracts from the selected species of algae (Acanthophora spicifera, Cystoseira myrica, Cystoseira trinodis, Laurencia filiformis, Padina boryana, Sargassum oligocystum, Turbinaria crateriformis, Ulva fasciata and *Ulva reticulata) occurring along the coast of Tanzania. The extracts showed antimicrobial activities with MIC ranging from 0.3- 5.0 µg/mL against Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli, Candida albicans and Cryptococcus neoformans; DPPH radical scavenging activity at EC_{50} 1.0- 100 µg/mL and cytotoxicity on brine shrimp larvae with LC_{50} value ranging from20 - 1000 µg/mL. The extracts from C. myrica and P. boryana inhibited growth of Plasmodium falciparum (3D7 strain) by 80 and 71%, respectively at 40 µg/mL while a sesquiterpene debromolaurinterol (**1**) which was chromatographically isolated from C. myrica exhibited antiplasmodial activity with IC_{50} 20 µM whereas a sterol fucosterol (**2**) from P. boryana showed weak activity at 40 µM. Bioactivities portrayed by the investigated extracts indicate their ingredients as potential sources of bioactive agents that warrant further explorations.*

Keywords: Macrolgae, antiplasmodial, antimicrobial, antioxidant, cytotoxicity, DPPH radical scavenging, debromolaurinterol and fucosterol.

INTRODUCTION

Parasitic diseases such as malaria, numerous microbial infections and physiological diseases arising from cellular oxidation causing cancer, aging, heart diseases and several other chronic diseases are

threatening the global population. Drug-resistant pathogens and other resistance mechanisms are hindering the effectiveness of the existing drugs, thus prompting the need to find novel curative agents with possible mechanism of action different from the conventional drugs currently in use. Among the potential sources of such therapeutic ingredients are marine algae that have provided a wealthy alternative resource for the discovery of new bioactive compounds. Algae are such important organisms constituting the branch of phycology that are ubiquitous throughout the world due to their adaptability in diverse environmental conditions. A variety of secondary metabolites that possess novel chemical structures and interesting pharmacological activities have been isolated from these sources (Patra et al. 2008, Kim and Himaya 2011, Liang et al. 2012, Gouveia et al. 2013, Brkljaca et al. 2015).

Macroalgae (seaweeds) included in the present investigations, some of which are being used in ethnomedicine, have attracted significant investigation for bioactive constituents with a range of biological and physiological activities such as antimicrobial including antituberculosis (Daniel et al. 2009, Nurul et al. 2010, Tajbakhsh et al. 2011, Vijayabaskar and Shiyamala 2011, Pridawati et al. 2014, Poonam 2014, Ravikumar et al. 2016, Manal et al. 2016), cytotoxicity including antitumour (Ayyad et al. 2003, Ali 2010, Permeh et al. 2012, Meenakshi et al. 2014), antiviral including anti-HIV (Ayyad et al. 2003, Gabriella et al. 2010), antioxidant (Patra et al. 2008), hypoglycaemic activity (Manal et al. 2016), antidiabetic (Kim and Himaya 2011), anticoagulant, anticonvulsant and anti-inflammatory (Ayyad et al. 2003). Such bioactive metabolites could be used as templates for developing future drugs against resistant pathogens and for both chronic and non-chronic physiological diseases. Tanzanian coast is endowed with a variety of seaweeds from which systematic studies are needed to establish their biomedical potentials. Thus, in the present work, crude extracts from nine species of macroalgae (*Acanthophora spicifera, Cystoseira myrica, Cystoseira trinodis, Laurencia filiformis, Padina boryana, Sargassum oligocystum, Turbinaria crateriformis, Ulva fasciata and Ulva reticulata*) occurring along the coast of Tanzania were investigated for antiplasmodial, antimicrobial, antioxidant properties. Their preliminary cytotoxicity status was also evaluated using brine shrimp larvae lethality test.

MATERIALS AND METHODS

Collection and identification of algae

Nine marine algae *Acanthophora spicifera* (SB 001), *Cystoseira myrica* (SB 002), *Cystoseira trinodis* (SB 003), *Laurencia filiformis* (SB 004), *Padina boryana* (SB 005), *Sargassum oligocystum* (SB 006), *Turbinaria crateriformis* (SB 007), *Ulva fasciata* (SB 008) and *Ulva reticulata* (SB 009) were collected during spring low tide in August 2012 by hand picking from the shores of Oyster Bay and Mji Mwema, Dar es Salaam, Tanzania. All the algal species reported in this study were identified by Dr. Amelia S. Buriyo and their specimens were preserved in the Herbarium, Botany Department, University of Dar es Salaam (UDSM).

Extraction

The under-shade air dried, pulverized samples were consecutively soaked twice for 48 hours in petroleum ether, dichloromethane and methanol. The filtered crude extracts were concentrated *in vacuo* using a rotary evaporator while maintaining the water bath temperature below 40 °C to avoid thermal decomposition of labile compounds. The weights of the crude extracts were determined and about 50 mg were taken from each extract to carry out

antiplasmodial, antimicrobial, antioxidant and cytotoxicity assays.

Bioassays

Antimicrobial assay of crude extracts

The antimicrobial activity of selected algal extracts was done by broth microdilution technique using sterile flat bottomed 96 well polystyrene microtiter plates (Ellof 1998). The assay was carried out at the Institute of Traditional Medicine (ITM), Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam. The gram positive bacteria, *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* (clinical isolates) and gram negative bacteria, *Pseudomonas aeruginosa* (ATCC 29953) and *Escherichia coli* (ATCC 25922) were used as representative bacteria for determination of antibacterial activity while *Candida albicans* (ATCC 90028) and *Cryptococcus neoformans* (clinical isolates) were used as representative fungi for determination of antifungal activity of the crude extracts. Test solutions were prepared by dissolving 20 mg of extracts in 0.1 mL of dimethyl sulfoxide (DMSO) and diluted with 0.9 mL of broth to make a concentration of 20 mg/mL. The 50 µL of test solution was pipette and added into the first well of each row of plates preloaded with 50 µL of broth. Then serial dilution of concentrations 5, 2.5, 1.25, 0.625 and 0.312 µg/mL were performed by transferring the test sample from first row wells to wells of the next rows, down to the last rows. The 50 µL from the last row wells were discarded. This was followed by addition of 50 µL of solution containing the test organisms (0.5 McFarland dilutions) to each of the wells. Wells in two columns were used as growth controls, while other two were used for gentamycin as positive control for antibacterial activity and fluconazole for antifungal activity. DMSO, a solvent to which extracts were dissolved was used as a negative control. The microtitre plates were incubated at 37 °C for 24 h. After the incubation period, 30µL of a 0.2% *p*-iodonitrotetrazolium chloride (INT) was added to the wells followed by the incubation at 37 °C for 30 min. Presence of microbial growth was indicated by change of INT colour to pink (the colourless tetrazolium salt acted as an electron acceptor and reduced to a pink coloured formazan product by a biologically active organism) while absence of growth was indicated by observing no colour change. The lowest concentration at which microbial growth was inhibited was recorded as the minimum inhibitory concentration (MIC). All determinations were performed in triplicate.

DPPH scavenging activity

The free radical scavenging activity of the algal crude extracts were evaluated using the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Maisuthisakul et al. 2007, Aronsson et al. 2016). The test was done at Botany Department, UDSM. Test solution of 5mM DPPH was prepared by dissolving 0.005 gm of DPPH in 1000 µL of methanol. 100 µL of 5mM DPPH was mixed with 4.9 mL of each two-fold serially diluted extract containing 1g/mL concentration of the test sample stock solution. Then serial dilution in the range of 1:10, 1: 10^2, 1:10^3 and 1:10^4 were prepared and placed in the dark at 37 ºC for 30 min. The absorbance of DPPH solution without algal extract was recorded and labeled as A_o and considered as control. The absorbance was read at 517 nm using a spectrophotometer (UV-Vis spectrophotometer, model 6305, JENWAY). The absorbance of each sample which contained DPPH was then recorded as A_1 and the absorbance of each sample without DPPH as A_s, which was used for error correction arising from unequal colour of the sample solutions was also recorded. The percentage of DPPH radical scavenging activity of each plant extract was determined by using following calculation:

DPPH radical scavenging activity (%) = $\frac{[Ao-(A1-As)]}{Ao}$ x 100

The percentage of DPPH radical scavenging activity was plotted against the plant extract concentration (µl/ml) to determine the amount of extract necessary to decrease DPPH radical concentration by 50% called EC_{50}. The EC_{50} value of each extract was estimated by sigmoid non-linear regression using GNUPLOT software.

Brine Shrimp Cytotoxicity Test

The Brine shrimp test was carried out at the Institute of Traditional Medicine (ITM), Muhimbili University of Health and Allied Sciences (MUHAS) following standard procedures (Meyer et al. 1982, Nondo et al. 2011). Brine shrimp (*Artemia salina*) larvae were used as indicator organism for preliminary determination of cytotoxicity of the crude extracts. Artificial sea water was prepared by dissolving 3.8 g of sea salt in 1 L of distilled water and then filtered. The solution prepared was then filled into two divided compartments of a tank. Shrimp eggs were spread into the covered part of a tank, and a lamp illuminated the uncovered part to attract the hatched shrimps. After 48 hours of hatching, the matured nauplii were collected. Each algal extract under study was tested at concentration of 240, 120, 80, 40 and 24 µg/mL dissolved in a DMSO in triplicate vials having 10 brine shrimp larvae. The fourth vial which was used as negative control had only DMSO and brine shrimp larvae whereas cyclophosphamide was used as a standard positive control. The number of survivors from 10 brine shrimp larvae after 24 h exposure was noted and the concentration where 50% lethality rate of the nauplii observed was calculated as LC_{50} using Log probit analysis. Log probit analysis was used to determine log dose regression lines for mortality in relation to concentration used and thereafter LC_{50} values were determined from the best-fit line.

Antiplasmodial Assay

Antiplasmodial activity was determined using malaria imaging assay method (Duffy and Avery 2012, Nyandoro et al. 2017). This assay was done at Discovery Biology, Eskitis Institute for Drug Discovery, Griffith University, Australia. The crude extracts were diluted in 100% DMSO while the stock solutions of the reference compounds were prepared at 10 mM in 100% DMSO (artesunate, puromycin, dihydroartemisinin and pyrimethamine); and at 10 mM in 100% H_2O (pyronaridine and chloroquine). Stock solutions of the extracts were diluted to a final assay concentration of 20-400 µg/mL, depending on the stock solution concentration, while the reference antiplasmodials were diluted to 40 µM. The final DMSO concentration for all extracts was 0.4% in the assay. The extracts were tested in 11-points dose-response using three concentrations per log dose.

Plasmodium falciparum parasites (3D7, chloroquine sensitive strains) were grown in Roswell Park Memorial Institute (RPMI) 1640 supplemented with 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 5% AB human male serum, 2.5 mg/mL Albumax II and 0.37 mM hypoxanthine. Parasites were subjected to two rounds of sorbitol synchronization before undergoing treatment. Ring stage parasites were exposed to the experimental extracts and reference compounds in 384-wells imaging microplates (Perkin Elmer Cell Carrier) as previously described (Duffy and Avery 2012, Nyandoro et al. 2017). Plates with the samples were incubated for 72 hours at 37 °C, 90% N_2, 5% CO_2, 5% O_2, then the parasites were stained with 2-(4-amidinophenyl)-1H-indole-6-carboxamidine (DAPI) and imaged using an Opera QEHS micro-plate confocal imaging system (Perkin Elmer). The digital images obtained

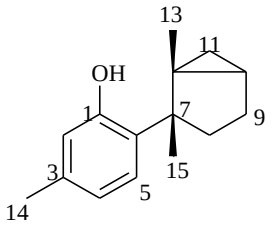
were then analyzed using the Perkin Elmer Acapella spot detection software where spots which accomplish the criteria established for a stained parasite count. The % inhibition of parasite replication was then calculated using DMSO and dihydroartemisinin (DHA) control data. The experiments were carried out in two independent biological replicates, each consisting of two technical replicates. Raw data was normalized using the in-plate positive and negative controls to obtain normalized % inhibition data, which was then used to calculate IC_{50} values, through a four parameter logistic curve fitting in GraphPad Prism v.6.

General chromatographic and spectroscopic methods

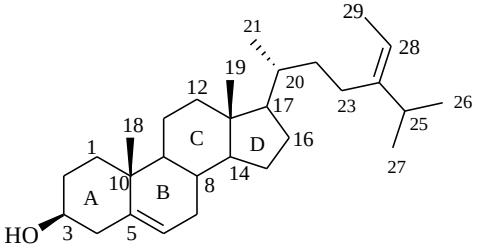
Isolation of secondary metabolites were achieved using silica gel 60 (Merck 230-400 mesh) and Sephadex® LH-20 (Pharmacia) column chromatography using solvents of analytical grade, namely petroleum ether (b.p 40–60 ^{o}C), dichloromethane, ethyl acetate and methanol throughout the investigation. Isolation process was monitored using the thin layer chromatography (TLC) where pre-coated chromatography plates of analytical grade (Merck, Kieselgel 60 F_{254}, 0.20 mm) were used for TLC analysis. The UV positive spots on TLC plates were detected using UV light at wavelengths, 254 and 365 nm. The plates were then sprayed using anisaldehyde reagent (prepared by mixing 90 mL of methanol, 3.5 mL of *p*-methoxybenzaldehyde, 4 mL of glacial acetic acid, 2.5 mL of concentrated sulphuric acid and then shaken thoroughly), followed by heating at about 110 ˚C for identification of UV negative spots and color changes of UV positive spots. 1D (^{1}H and ^{13}C) and 2D (COSY, TOCSY, NOESY, HSQC, and HMBC) NMR experiments were recorded on Bruker Avanace III HD 800 MHz spectrometer utilizing deuterated methanol or chloroform. LC-MS chromatograms were recorded on a Perkin-Elmer PE SCIEX 150 EX instrument utilizing H_2O/MeCN 80:20-20:80 gradient solvent systems and 0.2% HCO_2H. All spectroscopic measurements were done at Department of Chemistry and Molecular Biology, University of Gothenburg in Sweden.

Isolation of compounds 1 and 2

Dark greenish adhesive methanolic crude extract (24 g) of *Cystoseira myrica* yielded debromolaurinterol (**1**, 15.2 mg, yellowish oil) from repeated silica gel column chromatography using 2.5-20% petroleum ether-ethyl acetate gradient solvent system. Fucosterol (**2**, 15.3 mg, white solid) was isolated from the dark greenish gelatinous methanolic crude extract (15 g) of *Padina boryana* when subjected to silica gel column chromatography using 20-50% petroleum ether-ethyl acetate gradient solvent system followed by 1:1 methanol:dichloromethane charcoal column chromatography (to remove chlorophyll and other colouring matters), and thereafter further purified by sephadex filtration (100% methanol).



1



2

Debromolaurinterol (**1**). Yellowish oil, yield 15.2 mg, UV positive; anisaldehyde–pink; IR (film), ν_{max}3316.3, 2943.5, 2832.0, 2170.7, 2045.1, 1745.2, 1732.8, 1656.0, 1449.3, 1415.8, 1113.8, 1020.8 and 596.1 cm^{-1}; MS *m/z* 217 [M^++H]; ^{1}H NMR data ($CDCl_3$, 799.88 MHz; *J* in Hz): δ 6.55 (H-2, *s*, 1H), 6.70 (H-4 *d*, 7.8, 1H), 7.40 (H-5, *d*, 7.8, 1H), 2.11 (H-8, *dd*, 13.3, 8.1, 1H), 1.31 (H-8, *m*, 1H), 1.95 (H-9, *dddd*, 12.2, 12.0, 8.1, 4.4, 1H), 1.65 (H-9, *dd*, 12.2, 8.0, 1H), 1.12 (H-10, *ddd*, 8.1, 4.2, 4.0,1H), 0.58 (H-11, *dd*, 4.4, 4.4, 1H), 0.51 (H-11, *dd*, 7.9, 4.7, 1H), 1.32 (H-13, *s*, 3H), 2.27 (H-14, *s*, 3H), 1.42 (H-15, *s*, 3H); ^{13}C NMR data ($CDCl_3$, 201.15 MHz): δ 154.1 (C-1), 117.5 (C-2), 136.9 (C-3), 121.0 (C-4), 129.0 (C-5), 131.6 (C-6), 48.1 (C-7), 36.2 (C-8), 25.5 (C-9), 24.5 (C-10), 16.5 (C-11), 29.9 (C-12), 19.0 (C-13), 20.8 (C-14), 23.8 (C-15).

Fucosterol (**2**). White solid, yield 15.3 mg, UV negative; anisaldehyde–green; IR (film), ν_{max}3410.8, 1667.7, 1464.0, 1377.5, 1332.7, 1109.3, 1087.2, 985.8, 881.3, 800.1, 740.2, 590.2and 503.4 cm^{-1}; MS *m/z* 412; ^{1}H NMR data ($CDCl_3$, 799.88 MHz; *J* in Hz) : δ 1.87 (H-1, *m*, 1H), 1.08 (H-1, *m*, 1H), 1.85 (H-2, *m*, 1H) and 1.52 (H-2, *m*, 1H), 3.51 (H-3, *m*, 1H), 2.28 (H-4, *dd*, 10.3, 3.0, 1H), 2.23 (H-4, *dd*, 11.0, 2.19, 1H), 5.34 (H-6, *dd*, 2.9, 3.0, 1H), 1.90 (H-7, *m*, 1H), 1.28 (H-7, *m*, 1H), 1.40 (H-8, *m*, 1H), 0.96 (H-9, *m*, 1H), 1.52 (H-11, *m*, 1H), 1.48 (H-11, *m*, 1H), 2.09 (H-12, *m*, 1H), 1.88 (H-12, *m*, 1H), 1.15 (H-14, *m*,1H), 1.60 (H-15, *m*, 1H), 1.10 (H-15, *m*, 1H), 2.05 (H-16, *m*, 1H),1.14 (H-16, *m*, 1H), 1.01 (H-17, *m*), 0.67 (H-18, *s*, 3H), 1.03 (H-19, *s*, 3H), 2.22 (H-20, *m*), 1.0 (H-21, *d*, 6.2, 3H), 1.43 (H-22, *m*, 1H), 1.12 (H-22, *m*, 1H), 2.0 (H-23, *m*, 1H), 1.55 (H-23, *m*, 1H), 1.48 (H-25, *m*, 1H), 0.99 (H-26, *d*, 6.6, 3H), 0.99 (H-27, *d*, 6.6, 3H), 5.16 (H-28, *m*), 1.56 (H-29, *d*, 7.1, 3H); ^{13}C NMR data ($CDCl_3$, 201.15 MHz) : δ 37.4 (C-1), 31.8 (C-2), 71.9 (C-3), 42.4 (C-4), 140.8 (C-5), 121.8 (C-6), 28.4 (C-7), 36.5 (C-8), 50.2 (C-9), 36.6 (C-10), 21.2 (C-11), 25.8 (C-12), 42.4 (C-13), 55.9 (C-14), 24.4 (C-15), 39.8 (C-16), 56.8 (C-17),12.0 (C-18), 19.5 (C-19), 34.9 (C-20), 18.9 (C-21), 35.3 (C-22), 32.0 (C-23), 147.1 (C-24), 32.0 (C-25), 22.2 (C-26), 22.3 (C-27), 115.6 (C-28), 13.3 (C-29).

RESULTS AND DISCUSSION

Antimicrobial activity

Twenty five crude algal extracts were evaluated for antimicrobial assay against six microbial strains, the results of which are recorded at minimum inhibition concentrations (MIC) as presented in Table 1. Petroleum ether, dichloromethane and methanolic algal extracts showed varying antimicrobial activity with MIC values ranging from 0.312 to 5.0 µg/mL against the tested organisms (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans* and *Cryptococcus neoformans*). The most active extract was dichloromethane extract of *Laurencia filiformis* which showed potent antimicrobial activity to all the tested pathogens while

Padina boryana extracts remained inactive. Dichloromethane and methanol extracts of *L. filiformis* was the most active against *P. aeruginosa*, *S. aureus*, *S. pyogenes* and *E.coli*, the potency being higher or similar to positive control (standard drug gentamycin). However, *L. filiformis* extracts exhibited very weak activity against the fungal strains tested when compared to the positive control (fluconazole). The dichloromethane and methanol extracts of *L. filiformis* exhibited lowest MIC at 1.25 µg/mL each against *E. coli* whereas the same extracts exhibited MIC at 0.312 and 0.625 µg/mL, respectively against *P. aeruginosa*. Dichloromethane and methanol extracts of *Ulva reticulata* also exhibited MIC at 0.312 and 0.625 µg/mL, respectively against *P. aeruginosa*. Dichloromethane and methanol extracts of *L. filiformis* exhibited MIC at 0.625 µg/mL against *S. aureus* while only dichloromethane extract of *L. filiformis* showed MIC at 0.312 µg/mL followed by methanolic extracts of *L. filiformis* and dichloromethane extract of *Sargassum oligocystum* which exhibited MIC at 1.25 µg/mL against *S. pyogenes*. Thus, *L. filiformis* is observed to be the most non-selective extracts showing activity to all Gram positive and negative bacteria species tested. In the previous studies, antibacterial activity of members within the genus *Laurencia* has been reported to be attributed to the presence of sesquiterpenoids (Wratten and Faulkner 1977, Kladi et al. 2007, Daniel et al. 2009, Davis and Vasanthi 2011, Liang et al. 2012, Li et al. 2012b, Nai and Wang 2013).

Fungal species were found to be the most resistant to test extracts among the tested organisms. However, dichloromethane extracts of *L. filiformis*, *U. fasciata* and *U. reticulata* exhibited the lowest MIC at a range of 2.5 - 5.0 µg/mL against *Candida albicans* and *Cryptococcus neoformans* while other extracts remained inactive. Chemical constituents such as acrylic acid, phlorotannins, terpenoids and steroids are reported to be present in these algal species (Mtolera and Semesi 1996), thus could be responsible for the observed antifungal activity.

Table 1: Antimicrobial activity of algae species crude extracts (MIC values, µg/mL)

Extract	Extracting Solvent	Gram negative bacteria		Gram positive bacteria		Yeast	
		EC	**PA**	**SA**	**SP**	**CA**	**CN**
Acanthophora spicifera	P. ether	NA	NA	NA	5.0	NA	NA
Acanthophora spicifera	DCM	5.0	2.5	5.0	5.0	5.0	5.0
Acanthophora spicifera	MeOH	5.0	2.5	5.0	NA	5.0	5.0
Cystoseira myrica	MeOH	5.0	5.0	2.5	2.5	5.0	5.0
Cystoseira trinodis	P. ether	5.0	NA	5.0	5.0	NA	NA
Cystoseira trinodis	DCM	5.0	NA	5.0	5.0	NA	NA
Cystoseira trinodis	MeOH	5.0	NA	5.0	2.5	NA	NA
Laurencia filiformis	P. ether	2.5	2.5	1.25	2.5	2.5	5.0
Laurencia filiformis	DCM	1.25	0.312	0.625	0.312	2.5	2.5
Laurencia filiformis	MeOH	1.25	0.312	0.625	1.25	2.5	5.0
Padina boryana	P. ether	NA	NA	NA	NA	NA	NA

Padina boryana	DCM	NA	NA	5.0	NA	NA	NA
Padina boryana	MeOH	NA	NA	NA	NA	NA	NA
Sargassum oligocystum	P. ether	2.5	5.0	5.0	2.5	5.0	5.0
Sargassum oligocystum	DCM	5.0	5.0	1.25	1.25	NA	5.0
Sargassum oligocystum	MeOH	5.0	5.0	5.0	5.0	NA	5.0
Turbinaria crateriformis	P. ether	NA	NA	5.0	NA	NA	NA
Turbinaria crateriformis	DCM	2.5	5.0	1.25	2.5	NA	NA
Turbinaria crateriformis	MeOH	2.5	5.0	5.0	5.0	NA	NA
Ulva fasciata	P. ether	5.0	NA	NA	NA	NA	NA
Ulva fasciata	DCM	2.5	2.5	NA	5.0	5.0	NA
Ulva fasciata	MeOH	2.5	2.5	2.5	5.0	5.0	NA
Ulva reticulata	P. ether	2.5	0.625	2.5	2.5	5.0	5.0
Ulva reticulata	DCM	2.5	0.625	2.5	2.5	5.0	2.5
Ulva reticulata	MeOH	2.5	0.625	1.25	2.5	5.0	2.5
Gentamycin	-	1.25	1.25	1.25	1.25	-	-
Fluconazole	-	-	-	-	-	0.312	0.312

EC- *E. coli*; PA- *P. aeruginosa*; SA- *S. aureus*; SP- *S. pyogenes*; CA- *C. albicans* and CN- *C. neoformans;* NA – Not active

DPPH scavenging activity

Antioxidant activity of the selected crude extracts were evaluated using DPPH radical scavenging assay, the results which varied in the range of EC_{50} 1 to 100 µg/mL are reported in Table 2. The most active extracts that exhibited the highest DPPH radical scavenging activity at EC_{50} 1 µg/mL are the methanolic extracts of *C. myrica* and dichloromethane extracts of *T. crateriformis* and *U. reticulata*. These were ten times active than ascorbic acid (EC_{50} 10 µg/mL), a standard antioxidant used in the present study. Dichloromethane extracts of *P. boryana* exhibited EC_{50} 5.5 µg/mL, being also active than ascorbic acid. Dichloromethane extracts of *L. filiformis* demonstrated antioxidant activity similar to ascorbic acid. Other extracts exhibited mild activity ranging from EC_{50} 55 to 100 µg/mL. The radical scavenging activity of the investigated extracts (Table 3) may be due to the presence of flavonoids and/or other phenolic compounds, class of natural products well acknowledged for such activity (Herawati and Firdaus 2013, Imdadul et al. 2014). The most active antioxidant extracts may be considered ideal candidate for further exploration against physiological diseases arising from cellular oxidations.

Brine shrimp cytotoxicity

Crude extracts of selected species were subjected to cytotoxic assay against brine shrimp larvae. The results whose LC_{50} values ranged from 20 to 1000 µg/mL are presented in Table 2. The dichloromethane extract of *L. filiformis* showed the most potent cytotoxic activity with LC_{50} 20 µg/mL, similar to the positive control cyclophosphamide (LC_{50} 16.36 µg/mL), a well-known anticancer drug (Santosh et al. 2011). Moreover, the methanolic extract of *U. reticulata*, petroleum ether extract of *P. boryana*, petroleum ether and methanolic extracts of *C. myrica* and *U. fasciata* exhibited moderate activity with LC_{50} values ranging from 36.86 to 89.21 µg/mL. On the

other hand, mild activity was exhibited by the dichloromethane extracts of *P. boryana*, *U. fasciata*, *A. spicifera* and petroleum ether extract of *U. reticulata* whose LC_{50} ranged from 111.44 to 276.13 µg/mL. Extracts of other species exhibited activity with LC_{50} greater than 1000 µg/mL, hence considered inactive. Some of the investigated species extracts are used in folk medicine, thus those with low toxicity (Table 2) may be considered to be safe.

Table 2: Radical scavenging (EC_{50}, µg/mL) and cytotoxic (LC_{50}, µg/mL) activities of algal species extracts

Extract	Extracting solvent	DPPH RS activity	Cytotoxicity
Acanthophora spicifera	P. ether	100	NA
Acanthophora spicifera	DCM	100	294.95
Acanthophora spicifera	MeOH	100	NA
Cystoseira myrica	MeOH	1	69.56
Cystoseira trinodis	P. ether	NA	NA
Cystoseira trinodis	DCM	100	NA
Cystoseira trinodis s	MeOH	NA	NA
Laurencia filiformis	P. ether	NA	NA
Laurencia filiformis	DCM	10	<20.00
Laurencia filiformis	MeOH	10	>1000
Padina boryana	P. ether	NA	68.33
Padina boryana	DCM	5.5	124.62
Padina boryana	MeOH	50	NA
Sargassum oligocystum	P. ether	NA	NA
Sargassum oligocystum	DCM	100	>1000
Sargassum oligocystum	MeOH	1000	>1000
Turbinaria crateriformis	P. ether	100	NA
Turbinaria crateriformis	DCM	1	>1000
Turbinaria crateriformis	MeOH	100	NA
Ulva fasciata	P. ether	100	NA
Ulva fasciata	DCM	10	150.17
Ulva fasciata	MeOH	10	89.21
Ulva reticulata	P. ether	100	276.13
Ulva reticulata	DCM	1	>1000
Ulva reticulata	MeOH	100	36.86
Ascorbic acid	-	10	-
Cyclophosphamide	-	-	16.36

NA = not active

Antiplasmodial activity of *Cystoseira myrica* and *Padina boryana* extracts, debromolaurinterol (1) and fucosterol (2)

Phycochemical studies of *C. myrica* and *P. boryana* led to isolation of a sesquiterpene debromolaurinterol (**1**) and a sterol fucosterol (**2**), respectively. The compounds were identified by spectroscopic methods providing data similar to those previously reported for the corresponding compounds (Irie et al. 1970, Okamoto et al. 2001, Rajendran el. 2013). When evaluated for antiplasmodial activity, the methanolic crude extract of *C. myrica* and *P. boryana* inhibited growth of *Plasmodium falciparum* (3D7 strain) by 80% and 71%, respectively at concentration of 40 µg/mL whereas debromolaurinterol (**1**) from the extracts of *C. myrica* exhibited potent antiplasmodial activity with IC_{50} 20 µM while fucosterol (**2**) from *P. boryana* extract showed weak activity at 40 µg/mL (Table 3). The compounds were not tested for other bioactivities due to paucity of the isolated amounts. In addition to antiplasmodial activity hereby reported for compound **1**, previously isolated from red alga, *Laurencia intermedia* (Irie et al. 1970) and from sea hares namely *Aplysia kurodai* (Okamoto et al. 2001) and *A. californica* (Kokate et al. 2008), it is also known to possess antimicrobial (Kokate et al. 2008) and Na, K- ATPase inhibitory activities (Okamoto et al. 2001). Fucosterol (**2**) is a characteristic sterol prevalent among members of class Phaeophyceae and have been previously isolated from numerous brown algae and other sources (Bouzidi et al. 2014, Abdul et al. 2016). The compound is known to possess wide spectrum of bioactivity including cytotoxicity, antidiabetic, antioxidant, anti-inflammatory, hepatoprotective, antihyperlipidemic, antifungal, antihistaminic, anticholinergic, antiadipogenic, antiphotodamaging, anti-osteoporotic, blood cholesterol reducing, blood vessel thrombosis preventive and butyryl cholinesterase inhibitory, antidepressant and anticonvulsant activities (Zhen et al. 2015, Abdul et al. 2016). Thus, antiplasmodial activities of compounds **1** and **2** hereby reported adds to other already known bioactivities.

Table 3: Antiplasmodial activity of crude extracts and compounds from *Cystoseira myrica* and *Padina boryana*

Extract/Compounds/Standard	**Antiplasmodial activity**
Cystoseira myrica methanol extract	80%[a]
Padina boryana methanol extract	71%[a]
Debromolaurinterol (**1**)	92%[a]; 20[b]
Fucosterol (**2**)	9%[a]
Artesunate	0.00048[b]
Puromycin	0.023[b]
Dihydroartemisinin	0.00011[b]
Pyrimethamine	0.0025[b]
Pyronaridine	0.0036[b]
Chloroquine	0.0045[b]

[a]Percent growth inhibition at 40 µg/mL; [b] IC_{50} in μM.

CONCLUSION

Bioactivities of algal extracts from the reported investigations reveal biomedical potentials of these bio-resources that merit further explorations. The most antibacterially active extract was found to be dichloromethane extract of *L. filiformis* portraying activity comparable to standard drug used. However, the same extract was also undesirably the most toxic. On the other side, the methanolic extracts of *C. myrica* and dichloromethane extracts of *T. crateriformis* and *U. reticulata* manifested good antioxidant activity being ten times active than ascorbic acid (a standard antioxidant), hence ideal candidate for further exploration against physiological diseases arising from cellular oxidations. Some of the investigated species extracts are used in folk medicine. Therefore, while preliminary toxicity test as shown in the present investigations indicated most of them to be less toxic, further toxicity study are required to evaluate their safety for human use.

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