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SHORT COMMUNICATION

Antioxidant activity of a halogenated monoterpene isolated from a Namibian marine algal *Plocamium* species

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Abstract The antioxidant potential of various marine natural products is well documented. The aim of this study was to evaluate the antioxidant potential of a rare halogenated monoterpene, namely; 1*E*,3*R*,4*S*,5*E*,7*Z*-1-bromo-3,4,8-trichloro-7-(dichloromethyl)-3-methylocta-1,5,7-triene (**1**) for the first time. This compound was isolated from a Namibian red algal *Plocamium* species. The antioxidant activity of the compound was evaluated using a series of antioxidant assays, namely; 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH), reducing power, nitric oxide (NO) and hydrogen peroxide (H_2O_2). The compound demonstrated remarkable DPPH, NO and H_2O_2 scavenging activities with IC_{50} values of 0.05 ± 0.01, 4.18 ± 0.22 and 5.58 ± 1.11 mM, respectively. The reducing power of the compound increased with an increase in concentration. These results were compared to the absorbance of ascorbic acid, which was used as a standard control in all the antioxidant assays. The results strongly suggest that compound **1** is a promising antioxidant agent with potential commercial applications.

Keywords Red algae · *Plocamium* species · Halogenated monoterpene · Antioxidant activity

Introduction

Marine algae, popularly known as seaweeds, contain high levels of minerals, vitamins, essential amino acids, carbohydrates and dietary fiber (Suresh Kumar et al. 2015). Seaweeds are marine plants and as a result are photosynthetic, meaning they are exposed to both light and oxygen which is known to lead to the formation of free radicals and other strong oxidizing agents, but there is no evidence of structural oxidative damage on seaweed (Heo et al. 2003). This had led scientists to suggest that cells of seaweeds have protective antioxidative defense systems (Matanjun et al. 2008), which could be in the form of secondary metabolites neutralizing the formation of free radicals. The antioxidant potential of a variety of edible seaweeds with potential application in the food and medical industry was reported by Bhattacharjee and Islam (2014). Specific applications of seaweed include treating vitamin deficiencies, alleviating various intestinal disorders (Collins et al. 2016), wound dressing (Pin et al. 2013), cosmetic preparations (Martins et al. 2014) and as anti-diabetic agents (Chin et al. 2015). This study investigated the antioxidant potential of a halogenated monoterpene isolated from a *Plocamium* species collected in Namibia. The compound was evaluated for free radical scavenging activity using a battery of assays.

Materials and methods

Sample collection

The *Plocamium* sample used in this study was collected in May and December 2014 from Swakopmund and Henties Bay, Namibia, at low tide. A voucher specimen (LK320),

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identified by Lineekela Kandjengo, is deposited in the herbarium of the Sam Nujoma Campus, University of Namibia, Namibia. The collected samples were transported on ice to Windhoek and then stored at −20 °C until further processing for analysis.

Compound isolation and identification

The isolation and characterization of 1*E*,3*R*,4*S*,5*E*,7*Z*-1-bromo-3,4,8-trichloro-7-(dichloromethyl)-3-methylocta-1,5,7-triene (Fig. 1) from a Namibian *Plocamium* specimen, was previously reported (Knott et al. 2016; Louw et al. 2017). Due to an overlap in characteristics of *Plocamium* species, the identity of the *Plocamium* specimen from which compound 1 was isolated can only be ascertained by DNA analysis (Louw et al. 2017).

In vitro antioxidant assays

DPPH radical scavenging activity

The DPPH free radical scavenging activity of compound **1** was determined using a method previously described (Kapewangolo et al. 2013) with slight modifications. The DPPH solution (90 µM) was mixed with various concentrations of the compound (0.02, 0.04, 0.08, 0.16, 0.32, 0.65, 1.29 and 2.58 mM). The mixture was incubated in the dark at room temperature for 30 min and the absorbance was measured at 520 nm using a SpectraMax M2 plate reader (Molecular Devices, USA). Ascorbic acid was used as a positive control.

Reducing power assay

The reducing power of compound **1** was investigated using a method described by Soni and Sosa (2013), with slight modifications. Four concentrations (1.29, 2.58, 10.33 and 20.65 mM) of the compound in 0.2 M phosphate buffer (pH 6.6) were mixed with 200 µL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. Once the mixture was cooled, 200 µL of 10% trichloroacetic acid was added and the mixture was centrifuged at 3000 rpm for 10 min. The upper layer of the solution (200 µL) was mixed with 200 µL of distilled water and 200 µL of 0.1% ferric chloride. The absorbance was measured at 700 nm using a SpectraMax M2 plate reader (Molecular Devices, USA). Ascorbic acid was used as a positive control.

Nitric oxide radical scavenging activity

The nitric oxide assay was carried out according to a method previously described (Govindarajan et al. 2003). A volume of 300 µL of sodium nitroprusside (10 mM) in phosphate buffer was mixed with different concentrations (2.58, 5.16, 7.74, 10.33 and 12.91 mM) of compound **1** in ethanol. The mixture was incubated at 37 °C for 2 h. After incubation, the solution was mixed with 300 µL of Griess reagent and the resultant mixture was further incubated in the dark for 30 min. The absorbance of a pink chromophore that was formed during the diazotization of the nitrite with sulphanilamide and the subsequent coupling with naphthylene diamine was measured at 546 nm using a SpectraMax M2 plate reader (Molecular Devices, USA). Ascorbic acid was used as a control.

Scavenging of hydrogen peroxide

The hydrogen peroxide scavenging assay was carried out as previously described (Saeed et al. 2012). Briefly, different concentrations (2.58, 5.16, 7.74, 10.33, and 12.91 mM) of compound **1** were mixed with a 3% hydrogen peroxide solution in phosphate buffer (0.04 M, pH 7.36). This solution was then incubated for 10 min at room temperature. The absorbance of the resultant mixture was measured at 230 nm using a SpectraMax M2 plate reader (Molecular Devices, USA). Ascorbic acid was used as a control.

Results and discussion

A concentration-dependent response was obtained for compound **1** for the DPPH radical scavenging activity (Fig. 2a). The compound demonstrated excellent DPPH radical scavenging activity with an IC_{50} value of 0.05 ± 0.01 mM. The IC_{50} value of ascorbic acid with DPPH was 0.02 ± 0.004 mM.

The reducing power of compound **1** as a function of its concentration is illustrated in Fig. 2b. Reducing power is associated with antioxidant activity which insinuates that these molecules should be electron donors in order to reduce Fe^{3+} to Fe^{2+} (Chanda and Dave 2009). Compound **1** demonstrated ferric reducing activity in a dose-dependent manner (Fig. 2b); the reducing power of the compound as well as that of ascorbic acid (standard antioxidant) increased with an increase in concentration. The absorbance of the compound was higher than that of ascorbic

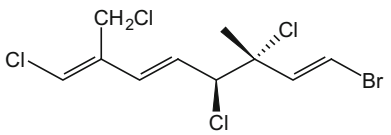

Fig. 1 1*E*,3*R*,4*S*,5*E*,7*Z*-1-bromo-3,4,8-trichloro-7-(dichloromethyl)-3-methylocta-1,5,7-triene (**1**) (Knott et al. 2016)

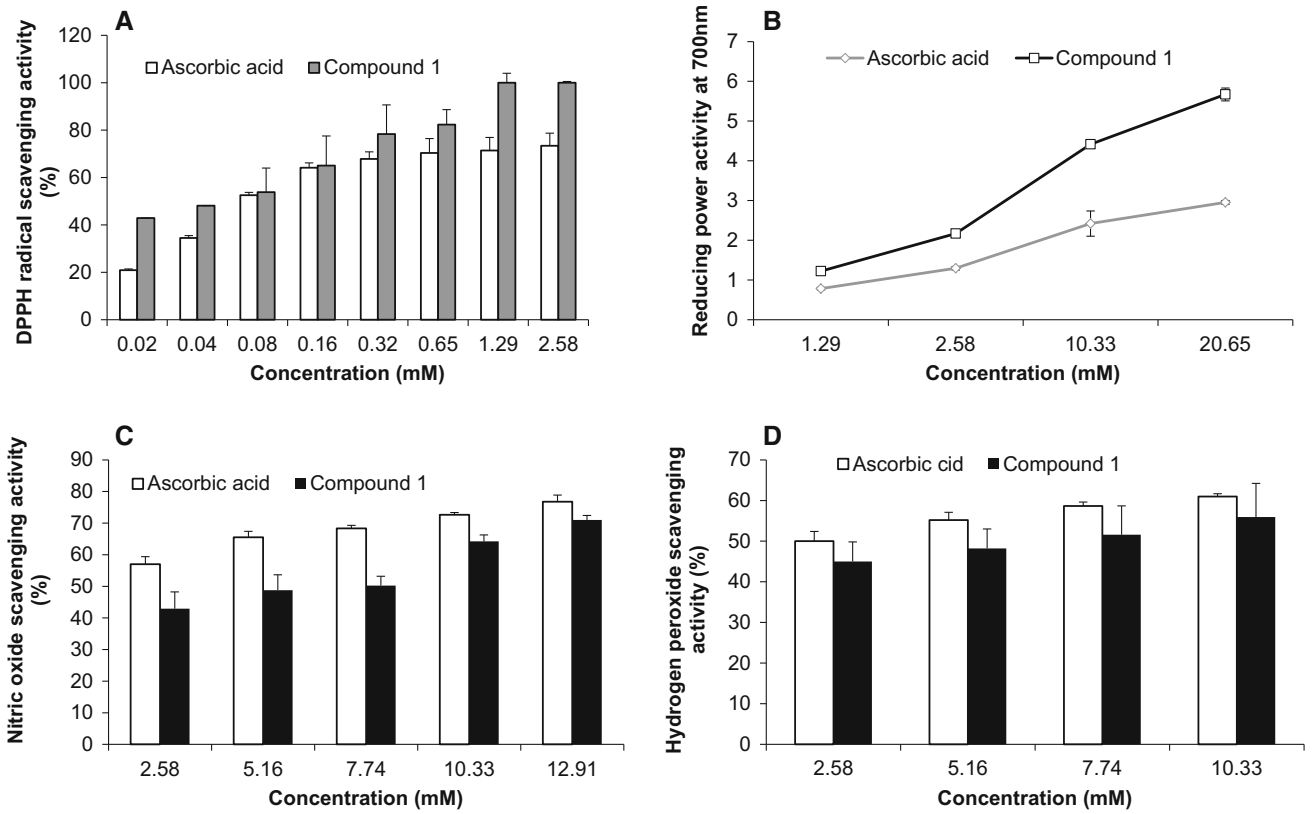

Fig. 2 Antioxidant activities of the halogenated monoterpene (compound **1**) at various concentrations. Each value represents a mean ± SD (n = 6): **a** DPPH radical scavenging activity, **b** reducing power, **c** nitric oxide scavenging activity, **d** hydrogen peroxide radical scavenging activity. Ascorbic acid was used as the control

acid (Fig. 2b), indicating good ferric reducing activity compared to the standard antioxidant.

The effect of compound **1** on scavenging NO radical is illustrated in Fig. 2c. Compound **1** demonstrated NO radical scavenging activity with the highest concentration tested (12.91 mM) inhibiting 64% of the NO radicals, while the lowest concentration tested (2.58 mM) only inhibited 45%. The NO scavenging activity of the compound was compared to that of the standard control, ascorbic acid (Fig. 2c). The IC_{50} value of the compound in scavenging NO radicals was 4.18 ± 0.22 mM while that of ascorbic acid was 3.12 ± 0.06 mM. NO is involved in immune system response to foreign pathogens in plants (Bellin et al. 2013) and humans (Schairer et al. 2012), however, serum levels of NO has been linked to various disorders leading to this molecule being identified as a therapeutic target (DeRojas-Walker et al. 1995; Kouti et al. 2013).

The ability of compound **1** to scavenge H_2O_2 radicals was also tested. Similarly to the other antioxidant experiments in this study, compound **1** was capable of scavenging H_2O_2 in a concentration dependent manner (Fig. 2d). The IC_{50} values obtained for compound **1** and ascorbic acid in the H_2O_2 scavenging assay were 5.58 ± 1.11 and 7.84 ± 1.76 mM respectively. Under normal physiologic conditions, H_2O_2 serves as a signal molecule (Sies 2014) but overproduction of this molecule could lead to cellular stress (Wen et al. 2013). H_2O_2 inhibitors could therefore be of therapeutic importance.

This is the first study of its kind to investigate the antioxidant potential of the halogenated monoterpene, 1*E*,3*R*,4*S*,5*E*,7*Z*-1-bromo-3,4,8-trichloro-7-(dichloromethyl)-3-methylocta-1,5,7-triene, which was isolated from a Namibian red algal *Plocamium* species. As reported by Cristiane et al. (2007) and Fleita et al. (2015), other marine compounds from red algal species which have demonstrated antioxidant activity include sulfated polysaccharides. Bromophenols isolated from another red algae, *Vertebrata lanosa*, reportedly demonstrated cellular antioxidant effect (Olsen et al. 2013). While extracts and semi-purified fractions from the marine red algae *Rhodomela confervoides* reportedly exhibited in vitro antioxidant activity (Wang et al. 2009). The reported evidence of the antioxidant potential of various seaweeds all point towards the potential of this marine resource as a rich source of natural antioxidants.

Various red seaweeds are consumed in many countries as a functional food (Mouritsen et al. 2013) and also used

in herbal medicine preparation (Collins et al. 2016). In addition, the potential application of marine algae in the cosmetic industry is also well described (Martins et al. 2014). The various application of seaweeds could be attributed to many health benefits of these marine natural products, which includes its ability to scavenge free radicals (Collins et al. 2016). Oxidative stress, caused by the accumulation of free radicals in a biological system, is linked to biological damage (Li et al. 2016). This study demonstrates the potential free radical protective effect of a halogenated monoterpene.

Conclusion

This study provided new evidence of the in vitro antioxidant potential of a halogenated monoterpene isolated from a Namibian *Plocamium* species. The compound demonstrated in vitro radical scavenging activity in all assays. The highest scavenging activity was obtained with DPPH where the lowest IC_{50} value was obtained. These results suggested that *Plocamium* species could be a promising source of antioxidants which require further investigation to develop them into potentially useful commercial products.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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