Free Radicals and Antioxidants xxx (2013) 1–10

Contents lists available at ScienceDirect

Free Radicals and Antioxidants

journal homepage: www.elsevier.com/locate/fra

Original article

Isolation, identification and radical scavenging activity of phlorotannin derivatives from brown algae, *Ecklonia maxima*: An experimental and theoretical study

Henry M. Mwangi [a], Jan Van Der Westhuizen [b], Jeanine Marnewick [c], Wilfred T. Mabusela [a,d,**], Mwadham M. Kabanda [e,*], Eno E. Ebenso [e]

[a] *Department of Chemistry, University of the Western Cape, Bellville 7535, South Africa*
[b] *Department of Chemistry, University of Free State, Bloemfontein 9301, South Africa*
[c] *Antioxidant and Oxidative Stress Unit, Cape Peninsula University, Bellville 7535, South Africa*
[d] *South African Herbal Science and Medicine Institute, University of the Western Cape, Bellville 7535, South Africa*
[e] *Department of Chemistry, North-West University (Mafikeng Campus), Mmabatho 2735, South Africa*

A R T I C L E I N F O

Article history:
Received 17 June 2013
Accepted 23 October 2013
Available online xxx

Keywords:
Brown algae
Ecklonia maxima
Phlorotannins
Radical scavenging activity
Hydrogen atom transfer mechanism

A B S T R A C T

Aim & background: Phlorotannins are a family of polyphloroglucinols with numerous biological activities including anticancer, antimalarial and antioxidant. They are highly sought for utilization in food ingredients, animal feeds, fertilizers and medicines. This work reports the isolation, from brown algae, of four phlorotannin derivatives namely phloroglucinol (**1**), eckol (**2**), 7-phloroeckol (**3**) and 2-phloroeckol (**4**). Their radical scavenging activity was assayed to elucidate their capacity to scavenge free radical species. Their structural and electronic features were then compared across structures to provide an explanation for the differences in their radical scavenging properties. Moreover, the two main radical scavenging mechanisms, namely hydrogen atom transfer (HAT) and electron transfer (ET), were checked to determine the preferred mode of radical scavenging.
Methods: Polyphenols were determined spectrophotometrically according to the Folin–Ciocalteu colorimetric methods and the antioxidant assays were determined by means of *ORAC assay* and the Trolox equivalent antioxidant capacity (TEAC) assay. Theoretical studies were performed by means of the Density Functional Theory (DFT) method.
Results: Theoretical predictions indicate that the radical scavenging activities follow the order **1** < **2** < **4** < **3**. Theoretical study also indicates that ET mechanism could be more significant than HAT mechanism because of the high BDE values.
Conclusion: Overall, the results suggest that the position of substitution of phloroglucinol unit on eckol (**2**) plays a significant role in determining the radical scavenging ability of the resulting eckol derivatives.

1. Introduction

Phloroglucinols are polyphenolic derivatives, many of which are found in nature in different environments including plants,[1] marine organisms,[2–6] and some bacteria species.[7] They exhibit various biological activities such as anticancer,[8–13] antiprotozoal,[1,14–16] antimicrobial[17–20] and antioxidant.[2–6,21] Because of their promising biological activities, they are increasingly investigated for their potential utilization in food, cosmetic and pharmaceutical applications for the design of dietary supplements and useful pharmacological drugs. Among phloroglucinol derivatives investigated for their dietary utilization are phlorotannins. Phlorotannins are found abundantly in marine brown algae species such as *Ecklonia cava*. Brown algae are found abundantly in East Asian countries where they are utilized as ingredients in food, animal feeds, fertilizers and medicines.[22] Recently, however, different marine brown algae species have been discovered worldwide and are increasingly investigated for their human beneficial bioactivity components, such as phlorotannins. One of the human beneficial effects of phlorotannins is that they are natural antioxidants – they

* Corresponding author. Tel.: +27 18 389 2352; fax: +27 18 389 2051.
** Corresponding author.
E-mail addresses: wmabusela@uwc.ac.za (W.T. Mabusela), mbyechura@gmail.com (M.M. Kabanda).

2231-2536/$ – see front matter Copyright © 2013, SciBiolMed.Org and Phcog.Net, Published by Reed Elsevier India Pvt. Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fra.2013.10.006

have the ability protect the human body from cellular or molecular damage by reactive oxygen species including superoxide anion radical, hydroxyl radical (HO•) and alkyl radical (ROO•) free radicals. Free radical species have the potential to cause oxidative damage to almost all major groups of biological macromolecules (e.g., DNA), and have shown to lead to a number of degenerative diseases such as cardiovascular diseases, gastrointestinal degeneration diseases and cancer.[23,24] Therefore, the search for natural antioxidant poses an interesting area of study because of the potential beneficial effects on human health.[25–28]

The current study investigates the antioxidant radical scavenging activity of phlorotannin derivatives isolated from brown algae species, *Ecklonia maxima*, found on the Western Cape region of South Africa. *E. maxima* is considered a member of *Ecklonia* species and contains a variety of compounds, including carotenoids, fucoidans, and phloroglucinol derivatives, that play diverse biological and ecological roles. *Ecklonia* species are increasing utilized for therapeutic applications as they exhibit antioxidant and anti-inflammatory activities,[29] radical scavenging activity,[30,31] anticancer,[32] antibacterial activity[33] and HIV-1 reverse transcriptase activity.[34,35]

The objectives of the study include the isolation and characterisation of the different phloroglucinol derivatives from the *E. maxima* EtOAc fraction, assaying the antioxidant activity of the isolated phlorotannins (using the Oxygen radical absorption capacity (ORAC) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS))) and utilizing theoretical methods to explain the differences in the antioxidant radical scavenging properties of the isolated phloroglucinol derivatives. Although there have been various experimental studies on the antioxidant activities of marine organisms, such as fungi and brown algae, there is scarce theoretical studies detailing the structural and electronic properties of antioxidants from marine organisms.[36] Moreover, although some of the phlorotannins isolated from *E. maxima* (see Fig. 1) have previously been isolated from other species of brown algae,[4,37] the theoretical study reported in this work forms the first attempt to explain the effects of structural and electronic features on their antioxidant activity and provides a tentative explanation for their possible mechanism of action as radical scavengers.

The antioxidant radical scavenging activity is mainly governed by the hydrogen atom transfer (HAT) and electron transfer mechanism.[38–41] In the HAT mechanism, the antiradical property of phenol derivatives (ArOH) is related to their ability to transfer their phenolic H-atom to a free radical. The H-atom abstraction is described by the reaction:

$$R^{\bullet} + ArOH \rightarrow RH + ArO^{\bullet} \quad (1)$$

The termination of further chain reactions depends strongly on the stability of the ArO• radical species formed. This means that factors enhancing the stability of the ArO• radical increase the antiradical activity. The ability of phenolic antioxidants to donate a hydrogen atom is mainly governed by the homolytic O–H bond dissociation enthalpy (BDE):

$$BDE = H_r(ArO^{\bullet}) + H_h(H^{\bullet}) - H_p(ArOH) \quad (2)$$

where H_r is the enthalpy of the radical generated by H abstraction, H_h is the enthalpy of the H atom, and H_p is the enthalpy of parent compound. A low O–H BDE value, usually associated with greater ability to donate the H atom, corresponds to high radical scavenging ability by the phenolic compound.[42–45]

The ET mechanism is governed by the capacity of the studied compound to transfer an electron and is better described in terms of the ionization potential (IP).

$$R^{\bullet} + ArOH \rightarrow ArOH^{\bullet +} + R^{-} \quad (3)$$

The stability of the radical cation is better described by the IP value; the lower the IP, the easier is the electron abstraction.

2. Materials and methods

2.1. Chemicals and reagents

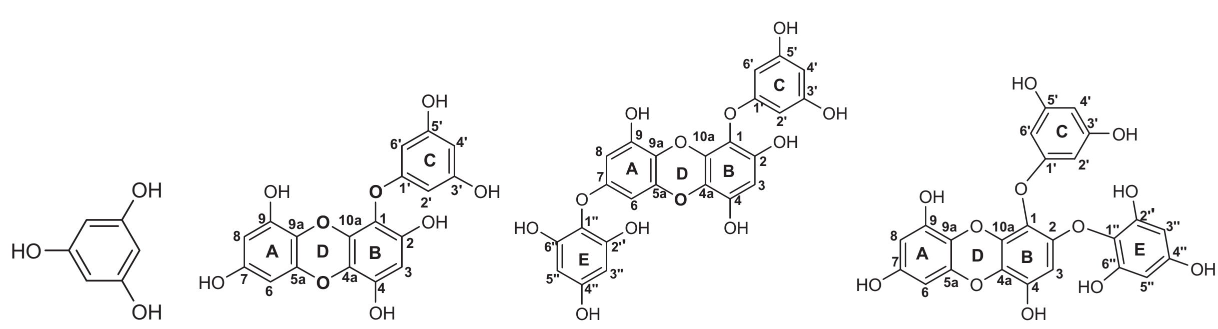
Methanol, Folin Reagent, Sodium Carbonate, Gallic acid standard were purchased from Sigma–Aldrich Chemical Company, South Africa. Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), sodium bisulphite and formic acid were purchased from Fluka Chemicals (South Africa). Phosphate buffer, Fluorescein sodium salt, AAPH (2,2′-Azobis (2-methylpropionamidine) dihydrochloride), Sodium acetate buffer, TPTZ (2,4,6-tri[2-pyridyl]-s-triazine), L-Ascorbic acid were purchased from Sigma–Aldrich, South Africa.

2.2. Plant materials

The brown seaweeds were collected from the rocky reefs off the coast of the western part of the Western Province, South Africa (between January and May 2011). The collected materials were then freeze-dried, pulverized and deposited in the laboratory.

2.3. Preparation of methanolic/ethanolic extracts and fractions

The freeze-dried pulverized material of *E. maxima* (300 g) was extracted three times with 80% MeOH and then filtered. The filtrate



phloroglucinol (**1**) eckol (**2**) 7-phloroeckol (**3**) 2-phloroeckol (**4**)

Fig. 1. Structures of the phloroglucinol derivatives isolated from the brown algae, *Ecklonia maxima*, and the atom numbering utilized throughout the study. The OH atoms are numbered with the same number as the C atom to which they are attached.

Please cite this article in press as: Mwangi HM, et al., Isolation, identification and radical scavenging activity of phlorotannin derivatives from brown algae, *Ecklonia maxima*: An experimental and theoretical study, Free Radicals and Antioxidants (2013), http://dx.doi.org/10.1016/j.fra.2013.10.006