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Enrichment and purification of marine polyphenol phlorotannins using macroporous adsorption resins

Jiyoung Kim [a,1], Minseok Yoon [a,1], Hyejin Yang [a], Jinho Jo [a], Daeseok Han [a], You-Jin Jeon [b,**], Suengmok Cho [a,*]

[a] Korea Food Research Institute, Sungnam 463-746, Republic of Korea
[b] Department of Marine Life Science, Jeju National University, Jeju 690-756, Republic of Korea

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ABSTRACT

Phlorotannins are one of the most important bioactive polyphenols; however, their purification using chromatographic methods has not been explored. Here, we studied purification of phlorotannins from the crude phlorotannin extract (CPhE) of the brown seaweed *Ecklonia cava* using macroporous adsorption resins. For purification of phlorotannins, four resins (HP-20, SP-850, XAD-7HP, and XAD-2) were screened. Among them, HP-20 resin showed the highest adsorption and desorption capacities. In static adsorption tests, the adsorption capacity of HP-20 increased with increasing temperature (25–45 °C). Optimal conditions for the dynamic experiments can be summarized as follows: total phlorotannin content (TPhC) in loading solution: 1.5 mg PGE/mL, processing volume: 4 BV, flow rate: 1 mL/min, temperature: 45 °C, desorption solvent: 40% ethanol solution. After purification, TPhC (452 mg PGE/g) and arsenic (180 μg/g) of CPhE increased and decreased to 905 mg PGE/g and 48 μg/g, respectively. Recovery rate of phlorotannins from CPhE was 92%.

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1. Introduction

Phlorotannins, brown seaweed polyphenols, are oligomers and polymers of the monomeric unit phloroglucinol (1,3,5-trihydroxybenzene) (Shibata et al., 2004). They are structurally different from terrestrial plant polyphenols based on gallic acids or flavones (Shibata, Fujimoto, Nagayama, Yamaguchi, & Nakayama, 2002). Phlorotannins have a high structural diversity like ginsenosides in ginseng (Ramarajan, Somasundaram, Subramanian, & Pandian, 2012; Shibata et al., 2004; Sugiura, Tanaka, Katsuzaki, Imai, & Matsushita, 2013; Xiang, Shang, Gao, & Zhang, 2008), and individual phlorotannin constituents are structurally similar (Fig. 1) (Koivikko, Loponen, Pihlaja, & Jormalainen, 2007). Approximately 150 phlorotannins including trifucol, triphlorethol A, bieckol, dieckol, and eckol have been identified from various brown seaweeds thus far (Isaza Martínez & Torres Castañeda, 2013; Kang, Heo, Kim, Lee, & Jeon, 2012; Kang et al., 2012). They are specifically connected by aryl-aryl bonds (e.g. fucols), ether bonds (e.g. phlorethols, hydroxyphlorethols), or dibenzodioxin linkage (e.g. eckol and carmalols) (Balboa, Conde, Moure, Falqué, & Domínguezm, 2013; Sugiura et al., 2009). The molecular weight of phlorotannins ranges from 250 to 1738 Da (Isaza Martínez & Torres Castañeda, 2013). Among phlorotannin constituents, dieckol is the most abundant, and has been considered to be an indicator compound in phlorotannin extracts from brown seaweeds (Cho et al., 2012a; Shibata et al., 2002; Shibata et al., 2004).

Phlorotannins exhibit a variety of different biological properties, including antioxidative (Zou et al., 2008), anti-inflammatory (Sugiura et al., 2013), antiallergenic (Shim, To, Lee, & Kim, 2009), neuroprotective (Yoon, Chung, Kim, & Choi, 2008), and memory-enhancing effects (Myung et al., 2005). Recently, Cho et al. (2012a) and Cho et al. (2012b) reported that the phlorotannin extract could induce sleep by modulating the benzodiazepine binding site of the gamma-aminobutyric acid type A receptor.

Although many studies on phlorotannins have been reported in the past two decades, this area is still growing exponentially (Isaza Martínez & Torres Castañeda, 2013). In particular, most studies on phlorotannins describe structural identification and biological activities (Okuda & Ito, 2011; Vo, Ngo, & Kim, 2012). However, information on the enrichment and purification of phlorotannins is limited. Phlorotannins have been used as a crude extracts from brown seaweeds, due to high structural diversity and difficulty in mass purification. For human consumption, ethanol extraction

* Corresponding author. Tel.: +82 31 780 9314; fax: +82 31 709 9876.
** Co-Corresponding author. Tel.: +82 64 754 3475; fax: +82 64 756 3493.
E-mail addresses: youjinj@jejunu.ac.kr (Y.-J. Jeon), smcho@kfri.re.kr (S. Cho).
[1] Jiyoung Kim and Minseok Yoon contributed equally to this work.

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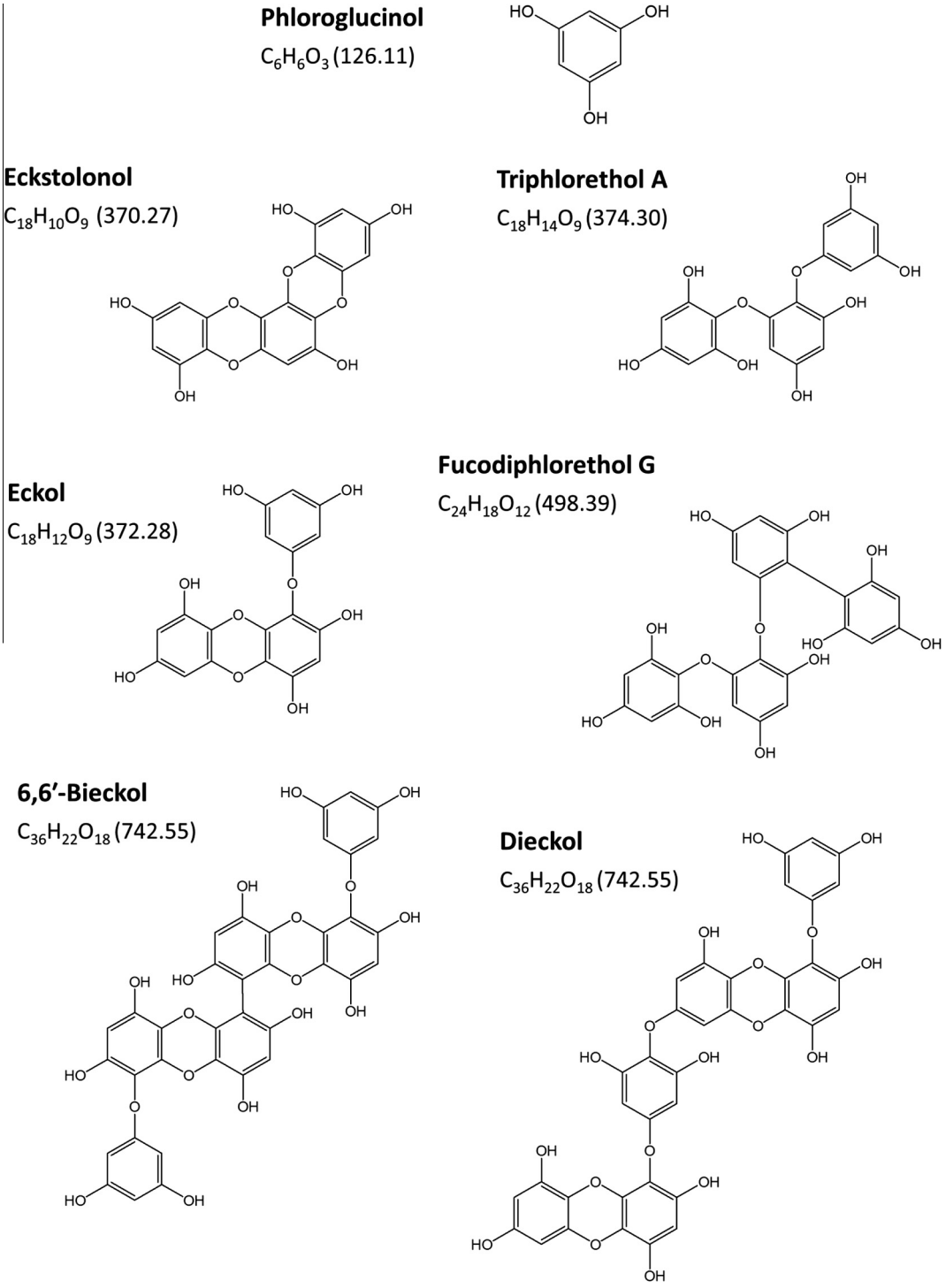

Fig. 1. Molecular structure, chemical formula (molecular weight) of phlorotannins from *Ecklonia cava*.

(Kim et al., 2006) and enzyme hydrolysis (Cho et al., 2012b) have been utilised for the preparation of phlorotannins. However, total phlorotannin content of the extracts generated using these protocols does not exceed 50% (Cho et al., 2012a; Cho et al., 2012b; Kamiya et al., 2010). Therefore, for a high-purity phlorotannin preparation, additional processes are needed. In previous reports, liquid–liquid fractionation on the laboratory-scale was applied to purify phlorotannins from crude brown seaweed extracts (Cho et al., 2012a; Kang et al., 2012). In particular, the ethyl acetate fraction was rich in phlorotannins (Cho et al., 2012a; Shibata et al., 2004). However, this purification process is not suitable for production production of high-purity phlorotannins for human consumption.

The objective of this study was to develop an efficient process for enrichment and purification of phlorotannins from crude ethanol extracts of a brown seaweed, *Ecklonia cava,* using macroporous adsorption resins. Adsorption kinetics and thermodynamics of the selected resin (HP-20) were systemically investigated through static adsorption and desorption experiments. Dynamic adsorption and desorption tests were also performed for the purification of

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