Phlorotannins: from Isolation and Structural Characterization, to the evaluation of their Antidiabetic and Anticancer Potential

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Abstract: Phlorotannins are phenolic characteristic compounds of brown seaweeds that are only constituted by phloroglucinol (1,3,5-trihydroxybenzene). They are chain- and net-like structures of diverse molecular weights, and have been widely identified in *Ecklonia*, *Eisenia* and *Ishige* species. Since the time they were discovered in the 70s, phlorotannins have been suggested as a main factor responsible for the antimicrobial activities attributed to algae extracts. Currently, cumulative *in vitro* and *in vivo* research evidence the diverse bioactivities of phlorotannin extracts -such as antidiabetic, anticancer and antibacterial- pointing out their potential pharmacological and food applications. However, metabolomic studies and clinical trials are scarce, and thus, many phlorotannins health-beneficial effects in humans are not yet confirmed. This article reviews recent studies assessing the antidiabetic and anticancer activities of phlorotannins. Particularly, their potential to prevent and control the progression of these non-communicable diseases are discussed, considering *in vitro* and animal studies, as well as clinical interventions~~trials~~. In contrast to other approaches, we only included investigations with isolated phlorotannins or phlorotannin-rich extracts. Thus, phlorotannin extraction, ~~isolation~~purification and characterization procedures are briefly addressed. Overall, although considerable research showing the antidiabetic and anticancer potential of phlorotannins is now available, further clinical trials are still necessary to conclusively demonstrate the efficacy of these compounds as adjuvants for diabetes and cancer prevention or treatment.

Keywords: seaweeds, phlorotannins, ~~isolation~~purification, non-communicable diseases, antidiabetic potential, anticancer potential, *in vivo* studies.

1. Introduction

The burden of non-communicable diseases (NCDs) is continuously growing worldwide. Diabetes, cancer and cardiovascular diseases are the most prevalent chronic conditions, accounting for $3.3 trillion of the annual health care cost in the United States (NCCDPHP, 2019). ~~Unbalanced diet, low physical activity and unhealthy lifestyle are the main modifiable risk factors contributing to NCDs. This situation has led governments and the World Health Organization (WHO) to develop key public policies that promote healthier diets (Kaczorowski, Campbell, Duhaney, Mang, & Gelfer, 2016).~~ In line with the government's public policies oriented to reduce NCDs prevalence, ~~T~~the food industry has been ~~also engaged with lowering NCDs risks~~continuously working~~, through~~ on food reformulation,~~ consumer information~~ nutrition labelling improvements~~, promotion of healthy lifestyle and development of~~ , functional foods development and the general promotion of a healthy lifestyle (Bigliardi & Galati, 2013). Additionally, the scientific community has been increasingly investigating natural compounds that could prevent or treat chronic diseases. The research has been mainly focused on phytochemicals ("Phyto" means plant in Greek), plant-derived non-nutritive compounds with health-promoting activities, such as antioxidant, anti-inflammatory and anticancer (Liu, 2004).

Glossary of Abbreviations

iNOS: inducible nitric oxide synthase
COX-2: cyclooxygenase 2
CAT: catalase
SOD: superoxide dismutase
GSH-px: glutathione peroxidase
Bcl-2: β-cell lymphoma 2 (anti-apoptotic protein of the Bcl-2 family)
GLUT-4: glucose transporter type 4
Fas: cell surface death receptor
XIAP: X-linked inhibitor of apoptosis protein
FLIP: FLICE (caspase 8) -inhibitory protein
AKT: serine/threonine-specific protein kinase, also known as PKB (protein kinase B)
Bid: BH3 interacting domain death agonist (pro-apoptotic protein of the Bcl-2 family)
Bim: BH3 only protein (pro-apoptotic member of the Bcl-2 family)
Bak: Bcl-2 homologous antagonist/killer (pro-apoptotic protein of the Bcl-2 family)
Bax: Bcl-2-associated X protein (pro-apoptotic member of the Bcl-2 family)
p53: tumor suppressor protein (p) 53
NF-κB: nuclear factor kappa B, a transcription factor involved in stress responses and regulation of cell proliferation and apoptosis
Bcl-xl: β-cell lymphoma-extra large (anti-apoptotic protein of the Bcl-2 family)
PI3K: phosphatidylinositol 3-kinase
RAF-1: rapidly accelerated fibrosarcoma, a serine/threonine-specific kinase
ERK: extracellular signal-regulated kinase
EGFR: epidermal growth factor receptor, a proliferation-stimulating protein

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According to Liu (2004), phytochemicals are categorized into six major groups: phenolic compounds, alkaloids, nitrogen-containing compounds, organosulfur compounds, phytosterols and carotenoids. ~~According to Liu (2004), phytochemicals are bioactive non-nutritive compounds present in fruit, vegetables and other plant foods that have been related to reductions in the risks of major NCDs. They have been widely studied in the last two decades through *in vitro* assays, *in vivo* models and clinical trials, which have shed light into the structure-function relation responsible for their health-promoting effects. In plants, phytochemicals accomplish defence and reproductive functions (Huang, Xiao, Burton-Freeman, & Edirisinghe, 2016). They are categorized into six major groups: phenolic compounds, alkaloids, nitrogen-containing compounds, organosulfur compounds, phytosterols and carotenoids.~~ Phenolic compounds are the most studied phytochemicals since they are ubiquitous and abundant in all plant-based diets (Tsao, 2010). They are chemically defined as compounds having one or more aromatic rings with one or more hydroxyl groups (Liu, 2004). One high-value group of polyphenolic compounds –or polyphenols- only found in brown seaweeds are phlorotannins. These have attracted considerable interest because of their superior antioxidant capacity (Shibata, Ishimaru, Kawaguchi, Yoshikawa, & Hama, 2008; Wang et al., 2012) and valuable biological activities, such as anti-inflammatory, antihyperglycaemic and anti-tumour (Catarino, Silva, & Cardoso, 2017). Although phlorotannins have been widely studied through *in vitro* assays, *in vivo* models and some clinical trials, which have shed light into a possible structure-function relation, metabolomic studies addressing their biotransformation and conjugation in the body, and thus their effective bioavailability, are still scarce. This article reviews the available research assessing the diabetes- and cancer--preventive potential of phlorotannins, as well as their capacity to treat both NCDs. Biochemical and cell-based studies, animal assays and clinical interventions are presented and discussed; unlike other articles, we only focused on investigations with isolated phlorotannins or phlorotannin-rich extracts. Therefore, phlorotannins extraction, ~~isolation~~purification and characterization techniques are also introduced.

2. Phlorotannins and their Relevance

Phlorotannins are polyphenols unique to brown seaweeds (*Phaeophyta*). In contrast to terrestrial plant polyphenols, which are gallic acid and flavonoid polymers, phlorotannins are solely based on phloroglucinol (1,3,5-tri-hydroxybenzene) (Fig. 1, A). Phloroglucinol monomeric unit is synthesized via the acetate-malonate pathway and its condensation gives rise to chains- and net-like structures with diverse molecular weights: the phlorotannins (Shibata, Fujimoto, Nagayama, Yamaguchi, & Nakamura, 2002). Phlorotannins biogenesis is attributed to the Golgi apparatus and the

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endoplasmic reticulum, which has been seen to produce small phenolic-rich vesicles called physodes (Schoenwaelder & Clayton, 2000). Physodes are the reservoir of soluble phlorotannins, while a small fraction of insoluble phlorotannins is~~Phlorotannins are stored in soluble form into physodesand in an insoluble form.~~ associated with proteins and alginates of the cell wall. ~~In some cases, t~~ The content of phlorotannins can reach up to 30% of the algae dry weight, especially in species belonging to the Fucales order. However, this is extremely variable, depending on environmental conditions (e.g., temperature, UV radiation intensity, nutrient concentration, grazing pressure) and intrinsic factors (e.g., age, thallus morphology, growth rate) ~~can reach 25-30% of the alga dry weight (Singh & Sidana, 2013)~~ (Mannino & Micheli, 2020). They are classified into six major groups (Fig. 1, B-H), according to the type of linkages between phloroglucinol units and their content of hydroxyl groups: fucols, with aryl-aryl linkages; phlorethols, with aryl-ether linkages; fucophlorethols, with aryl-aryl and aryl-ether units; fuhalols, with aryl-ether linkages and additional OH groups in every third ring; carmalols, with a dibenzodioxin moiety and derived from phlorethols; and eckols, with at least one three-ring moiety with a dibenzodioxin element substituted by a phenoxyl group at C-4 (K.W. Glombitza & Pauli, 2003). ~~The main function of phlorotannins is to protect seaweeds from stress factors (e.g., UV radiation, herbivores, nutrients depletion).~~

Due to their polymeric structures, phlorotannins are ~~able to scavenge~~ potent free radicals scavengers; ~~interact with~~ they also modulate proteins and chelate metals (Ragan, Smidsrød, & Larsen, 1979; Stern, Hagerman, Steinberg, & Mason, 1996; Wijesinghe, Ko, & Jeon, 2011). These capacities explain the wide range of cellular and ecological roles of phlorotannins in seaweeds. They are involved in cell wall hardening, accomplishing structure and reproductive functions, such as protection of the zygote, adhesion of zygotes to substrate and wound healing. Moreover, phlorotannins constitute a defence against herbivory, desiccation, high UVB radiation and toxic heavy metals concentrations (Mannino & Micheli, 2020). For instance, it has been seen that under copper contamination, their concentration in the cell wall increases together with their exudation to the water, preventing copper from entering and damaging the photosynthetic system (Connan & Stengel, 2011).

Available evidence states that phlorotannins not only play important ecological roles in seaweeds but also would have beneficial health effects in humans. In fact, in the last fifteen years research has mainly focused on addressing the capacity of phlorotannins to regulate relevant physiological processes that affect body functions, such as digestion, metabolism, inflammation and cell

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proliferation. Antidiabetic, anticancer, antibacterial and anti-aging are just some of the potential health benefits that have been identified (Jang et al., 2015; H. J. Kim et al., 2018; Sharifuddin, Chin, Lim, & Phang, 2015). Although a lot of studies have involved crude brown seaweed extracts, many others were performed with purified phlorotannins or isolated compounds (Catarino et al., 2017). In this context, seaweeds from the Lessoniaceae family are the most studied, particularly the phlorotannins eckol (a phloroglucinol trimer) and dieckol (a phloroglucinol hexamer) (Fig. 1, G-H), found in high quantities in *Ecklonia*, *Eisenia* and *Ishige* species (Manandhar, Paudel, Seong, Jung, & Choi, 2019; Rosa et al., 2019). However, findings are still based on a large number of *in vitro* and animal studies, with only some clinical trials performed; thus, the health-promoting effects of phlorotannins in humans have not yet been confirmed. Otherwise, investigations into the development of extraction and purification processes oriented to the obtention of high yield phlorotannins extracts or isolated phlorotannins have also sharply increased, mainly those with food or pharmaceutical applications (Cikos, Jokic, Subaric, & Jerkovic, 2018). ~~Eckol (a phloroglucinol trimer) and dieckol (a phloroglucinol hexamer), found in high quantities in *Ecklonia*, *Eisenia* and *Ishige* species (Li et al., 2017), are two of the most studied phlorotannins.~~

3. Phlorotannins Extraction and Purification

Phlorotannins can be extracted from seaweeds by different methods. The most typical one is the traditional solid-liquid extraction (SLE) by maceration. This involves the contact of the matrix with high volumes of solvents during long periods, at room or high temperatures. In SLE methods the yield and the composition of the extracts depend on the solvent type, the solid-liquid ratio and the extraction time and temperature (Leyton et al., 2016; Li et al., 2017). As phlorotannins are moderately polar compounds, high yields have been achieved using methanol, ethanol, acetone or their aqueous mixtures (e.g., acetone 70%), using high temperatures and long extraction times (Catarino, Silva, Mateus, & Cardoso, 2019; Wang et al., 2012). However, similar extraction yields can be achieved using alternative environmentally friendly extraction techniques. For instance, Tanniou et al. (2013) demonstrated that pressurized hot liquid extraction (PHLE) with a 75:25 ethanol-water mixture yields *S. muticum* extracts with high polyphenol content and antioxidant activities; they found that PHLE has a performance similar to centrifugal partition extraction (CPE) with 50:50 ethyl acetate-water and classical SLE, but with higher productivity and eco-sustainability. The study also showed that supercritical CO_2 (SC-CO_2) extraction is not suitable for the recovery of phlorotannins from brown algae. However, the addition of water and ethanol as co-solvents in SC-CO_2 extraction

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has been shown to significantly increase the polyphenol yields obtained with pure CO_2 (Conde, Moure, & Domínguez, 2014; Saravana et al., 2017).

Other environmentally friendly processes applied to produce phlorotannin-rich extracts are microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE). An optimized aqueous MAE procedure (solid to liquid ratio 1:30, 160 °C, 3 min) was shown to increase by 70% the phlorotannin yield and purity of the extracts, and to reduce the extraction times compared to SLE with organic solvents, due to the MAE ability to decompose the cellular structure according to scanning electron microscopy images (Magnusson et al., 2017; R. Zhang et al., 2018). UAE with ethanol/water mixtures enhances the phenolic content and the antioxidant capacity, as well as shortens the maceration time in brown algae extractions (Agregán et al., 2019; Dang et al., 2017). In addition, Kadam, Tiwari, Smyth, and O'Donnell (2015) reported an optimized UAE method with aqueous-HCl as solvent (0.03 M HCl, 25 min, amplitude 114 µm), to obtain high yields of phenolic compounds, fucose and uronic acid from *Ascophyllum nodosum*.

Enzyme assisted extraction (EAE) is another green approach that takes advantage of the hydrolytic activity of proteases and carbohydrase to unbound cell wall phlorotannins. It has been mainly used as a pre-treatment to enhance the phlorotannin yields of alkaline SLE procedures (Leyton, Pezoa-Conte, Mäki-Arvela, Mikkola, & Lienqueo, 2017; Siriwardhana et al., 2008).

Overall, PHLE has been the most employed, environmentally friendly, technique to efficiently obtain phlorotannin-rich extracts with potential application in functional foods or nutraceutical products (Heavisides et al., 2018; Montero et al., 2016; Sánchez-Camargo et al., 2016; Sanz-Pintos et al., 2017; Tierney, Smyth, Hayes, et al., 2013). Nevertheless, PHLE is difficult to scale-up and in some cases may be economically unfeasible for industrial production due to the high-pressure that the equipment needs to withstand to keep the solvent in the liquid state (Cuevas-Valenzuela, Vergara-Salinas, & Pérez-Correa, 2017). Finally, it should be noted that after using even the most efficient extraction procedure, a significant fraction of non-extractable phlorotannins remains in the residue of the seaweed matrix, due to their strong associations with protein or dietary fibre, and additional hydrolysis steps are required to release them (Sanz-Pintos et al., 2017).

~~4.~~ ~~Phlorotannins Separation~~

Extraction methods recover not only phlorotannins but also pigments, alginates and other brown algae compounds. Therefore, additional separation steps are necessary to obtain purified

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phlorotannins for semi-preparative or analytical purposes. The main separation methods are liquid-liquid extraction and solid-phase-extraction (SPE) based on the polarity of the molecules, as well as dialysis based on the molecular size. Liquid-liquid extraction with ethyl acetate has been broadly employed to obtain enriched phlorotannin fractions from raw extracts (E. K. Kim et al., 2015; Li et al., 2017; S. R. Park, Kim, Jang, Yang, & Kim, 2018; R. Zhang et al., 2018). For instance, in E. K. Kim et al. (2015) ethyl acetate was used to obtain phlorotannins from an 80% methanol-water extract of *Ecklonia cava*, followed by the isolation of dieckol. Although ethyl acetate is a permitted flavouring and extraction solvent in the food and pharmaceutical industries, it is a toxic substance and can cause organ damage when repeatedly inhaled or ingested (TOXNET, 2015). Thus, SPE procedures, such as macroporous resins, silica gel and dispersants, are safer alternatives. Haider, Zhenxing, Hong, and Jamil (2009) demonstrated that macroporous resins are better than silica gel and polyvinylpolypyrrolidone in adsorbing/desorbing phlorotannins. Later, J. Kim et al. (2014) reported that HP-20 is a suitable macroporous resin to purify phlorotannins and to eliminate co-extracted arsenic from *E. cava* (recovery: 92%, purity: 90.5 %); in addition, Leyton, Vergara-Salinas, Pérez-Correa, and Lienqueo (2017) found that XAD-16N is a good option to purify phlorotannins from *M. pyrifera* (recovery: 42%). The capacity of phlorotannins to adsorb on cellulose and celite have also been exploited through dispersive purification steps (Ferreres et al., 2012; H. A. Lee, Lee, & Han, 2017; Sadeeshkumar et al., 2017).

Molecular weight cut-off (MWCO) dialysis is another food-grade approach to separate phlorotannins by size and to remove interferences. For example, Tierney, Smyth, Rai, et al. (2013) and Heffernan, Brunton, FitzGerald, and Smyth (2015) fractionated low and high molecular weight phlorotannins using a 3.5 kDa MWCO membrane; the low molecular fraction (<3.5 kDa) was then separated by reverse-phase flash-chromatography to get rid of small sugars.

Further separations by preparative chromatography combined with detectors (typically ultra-violet detectors) are needed to obtain isolated phlorotannins. Size exclusion chromatography with Sephadex LH-20 (Sadeeshkumar et al., 2016; C. Zhang et al., 2011; Zhou, Yi, Ding, He, & Yan, 2019), reverse-phase chromatography (Kirke, Smyth, Rai, Kenny, & Stengel, 2017; Yotsu-Yamashita et al., 2013) and thin-layer chromatography (Eom et al., 2012; Shibata, Yamaguchi, Nagayama, Kawaguchi, & Nakamura, 2002) are the standard methods. They are used alone or in tandem to reach a better fractionation. For instance, by sequentially using reverse-phase C18 chromatography, Sephadex LH-20 gel filtration and thin-layer chromatography on silica gel, Eom et al. (2012) were able to isolate

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two phlorotannins from *E. bicyclis*; the compounds were later identified as fucofuroeckol A and dioxinodehydroeckol by nuclear magnetic resonance (NMR).

Figure 2 presents a schematic representation of the general steps and the most used extraction and purification methods to obtain phlorotannin-rich extracts or isolated phlorotannins from brown seaweeds. It also shows the conventional techniques to characterize the phlorotannin content in the output product of each step, which is reviewed in the next section.

~~5.~~4. Phlorotannins Characterization and Identification

After the preparation of seaweed extracts (crude or purified), the next task is to characterize their phlorotannin content. Total phenolic content (TPC) in combination with *in vitro* antioxidant capacity are useful and economic approaches for preliminary screening (Sáyago-Ayerdi, Mercado-Mercado, Ramos-Romero, Torres, & Pérez-Jiménez, 2017). To estimate the TPC of seaweed extracts, the Folin-Ciocalteu (F-C) (Singleton & Rossi, 1965) and 2,4-dimethoxybenzaldehyde (DMBA) (Stern, Hagerman, Steinberg, Winter, & Estes, 1996) spectrophotometric assays are widely used. The results of both assays are commonly expressed as phloroglucinol equivalents (PE) per gram of dry extract or alga, and sometimes as gallic acid equivalents. Based on F-C method, typical phenolic contents in crude seaweed extracts range from 50 to 150 mg PE/g of dry extract (Eom et al., 2012; Li et al., 2017; Montero et al., 2016; Tierney, Smyth, Rai, et al., 2013).

To assess the *in vitro* antioxidant activity of phlorotannin extracts, DPPH (2,2′-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis(3-ethylben-zothiazoline-6-sulfonic acid)) are the most used analyses (Heffernan et al., 2015; Montero et al., 2016). DPPH and ABTS values are usually expressed as equivalents of reference antioxidants, such as Trolox and ascorbic acid, or as IC_{50} (half maximal inhibitory concentration). Through the same DPPH protocol, Heffernan et al. (2015) and Kirke et al. (2017) reported IC_{50} values between 4 and 19 μg of dry extract/mL. ORAC (oxygen radical absorbance capacity), one of the most widely used methods to assess the antioxidant capacity of plant extracts, has also been applied. In methanolic seaweed extracts and dichloromethane fractions, Pinteus et al. (2017) and Silva et al. (2019) reported ORAC values ranging from 213 to 4469 μmol Trolox equivalents/g dry weight; overall, although TPC and *in vitro* antioxidant assays are practical to screen for phlorotannin content, they also quantify other reducing agents. For this reason, more specific and sensitive methods are necessary to characterize the phlorotannin profiles of algae extracts with accuracy.

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Ultraviolet detection and diode array detection (DAD) are very useful techniques to determine the presence of phlorotannins in seaweed extracts, since their maximum absorption is at 280 nm (Agregán et al., 2017; Koivikko, Loponen, Pihlaja, & Jormalainen, 2007; Wang et al., 2012). However, few phlorotannins can be identified by these methods, because of the lack of commercial standards. Therefore, NMR and tandem mass spectrometry (MS^n) coupled with liquid chromatography are the main methods utilized to identify and determine the chemical structure of phlorotannins. Proton (^1H) and carbon-13 (^{13}C) NMR analyses of phlorotannins were first applied by Glombitza and his group in the 70s, when they identified phloroglucinol in different brown algae species (K. W. Glombitza, Rosener, Vilter, & Rauwald, 1973). In 1974, bifuhalol and diphlorethol were firstly isolated from an 80% ethanol extract from *C. tamariscifolia* and structurally elucidated by ^1H NMR (K.W. Glombitza, Rosener, & Müller, 1975). Up to now, the structure of more than one hundred phlorotannins have been identified using NMR (Jacobsen, Sorensen, Holdt, Akoh, & Hermund, 2019). ^1H quantitative NMR (qNMR) is an alternative method instead of the classic F-C to quantify phlorotannins, but it has been scarcely used (Jegou, Kervarec, Cerantola, Bihannic, & Stiger-Pouvreau, 2015; Parys et al., 2009). Investigating the phlorotannin content in *C. tamariscifolia*, Jegou et al. (2015) found that both F-C and qNMR methods showed the same trend in seasonal variation; however, qNMR demonstrated higher selectivity because it did not overestimate the results.

Another strategy for the advanced characterization of phlorotannins is MS^n, which enables the identification based on their mass-to-charge ratio (*m/z*) and fragmentation patterns (*m/z* of precursor and product ions, respectively) (Table I). Quadrupole time-of-flight (QqTOF) and triple quadrupole (QqQ) analysers have been broadly used to this aim (Heffernan et al., 2015; Hermund et al., 2018; Li et al., 2017; Tierney et al., 2014). MS^n spectrums allow knowing the polymerization degree (PD) and structures of the phlorotannins being analysed, although, in most cases, only a general elucidation is possible due to the high level of isomerization occurring in these compounds. Further isolation of individual phlorotannins and NMR analyses are mandatory to accurately determine the structure of the isomers. By a QqQ method, Li et al. (2017) detected 42 different phlorotannins with a PD of 2 to 12 in *S. fusiforme*; among them, they identified dieckol (*m/z*: 741; MS^2 *m/z*: 389, 600), eckol (*m/z*: 371; MS^2 *m/z*: 121, 140, 229, 246, 317, 335) and hexaphlorethol-A (*m/z*: 745; MS^2 *m/z*: 727, 621, 461, 339, 265, 247).

~~6.~~ Finally, some comments must be considered regarding the high-performance liquid chromatography (HPLC) process that should be done before MS^n identification, for the

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separation of the individual compounds. Classical reverse phase (RP) chromatography is the most used approach (Agregán et al., 2017; Ferreres et al., 2012; Li et al., 2017; Lopes et al., 2018; Wang et al., 2012). However, it has been seen that the separation of phlorotannins is not quite as effective through this method, since the hydrophobic stationary phases of RP columns weakly retain these compounds (Koivikko et al., 2007). HILIC (hydrophilic interaction liquid chromatography) is considered a better option in separating phlorotannins and has been widely applied in combination with MS^n (Heffernan et al., 2015; S. M. Kim et al., 2013; Melanson & MacKinnon, 2015; Montero et al., 2016).

~~7.~~5. Phlorotannins Bioactivities: Antidiabetic and Anticancer Potential ~~of Phlorotannins~~

Bioactivities can be defined as effects that some non-nutritive food compounds (e.g., phytochemicals, vitamins and fibre) exert in physiological or cellular activities, resulting in the promotion of health (Guaadaoui, Benaicha, Elmajdoub, Bellaoui, & Hamal, 2014). Since they were discovered, phlorotannins have been explored in terms of their bioactivities. Through simple *in vitro* assays, their protein-inhibition and antibacterial capacities were assessed first (Fukuyama et al., 1985; K.W. Glombitza, Wiedenfeld, & Eckhardt, 1978). Currently, phlorotannins are considered to be health-promoting compounds~~highly bioactive compounds~~, because extensive evidence has been gathered about their antioxidant (Wang et al., 2012), antihyperglycaemic~~diabetic~~ (S. H. Lee, Ko, Kang, Lee, & Jeon, 2016), anti-tumour ~~cancer~~ (Sadeeshkumar et al., 2017), anti-viral (Cho et al., 2019), antibacterial (H. J. Kim et al., 2018) and many other bioactivities ~~health-promoting effects~~ (Jang et al., 2015; Jung, Jin, Ahn, Lee, & Choi, 2013). However, these ~~most~~ findings are ~~research available is about~~mainly based on *in vitro* assays and animal testing on rodents; most comprehensive research involving metabolomic approaches and clinical interventions oriented to understand the bioavailability and the real effects of phlorotannins in humans are still limited. ~~crude algae extracts.~~

In this section, we present studies demonstrating the diabetes- and cancer-preventive potential of individual phlorotannins or purified phlorotannin extracts, as well as their capacity to reduce the progression of both NCDs.

~~7.1~~5.1 Diabetes Control and Prevention Potential

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Diabetes Mellitus (DM) is a group of chronic metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, action, or both. It is normally classified into insulin-dependent DM (type 1 diabetes) and non-insulin-dependent DM (type 2 diabetes). Type 2 diabetes is the most prevalent form of DM (90-95%); it is a global health epidemic, which has been triggered by the increasing obesity and aging of the world population. Chronic hyperglycaemia of DM causes high oxidative stress, inflammation and dysfunction of different organs, especially eyes, kidneys, nerves, heart and blood vessels (ADA, 2014). Therefore, the effective control of fasting and postprandial blood glucose is key to prevent diabetes or diabetic complications, and to improve the quality of life of diabetic patients (DeFronzo, 1999). Many synthetic drugs such as acarbose and rosiglitazone can reduce postprandial blood glucose, but they present adverse effects that could lead to secondary failures. For example, it has been reported that the thiazolidine class of drugs induces adipogenesis in cell culture models and increases weight gain in rodents and humans (K. R. Kim et al., 2006). They can also cause liver toxicity, headaches, hypoglycaemia, oedema and hypertension (Cantello et al., 1997). Phlorotannins, and particularly dieckol, also act as DM regulators, reducing hyperglycaemia and its negative effects by a combination of mechanisms of action ~~(Fig. 1)~~. These properties of phlorotannins have been verified in several *in vitro* and animal studies, and in a few clinical trials. Next, we summarize the evidence of the antihyperglycaemic and hyperglycaemia-protective effects of individual phlorotannins and phlorotannin-rich extracts, with a special focus on clinical trials.

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~~7.1.1~~5.1.1 *In vitro* assays

~~*7.1.1.1*~~*5.1.1.1* *Biochemical assays*

Carbohydrate-hydrolysing enzymes -α-glucosidase and α-amylase- are key factors responsible for elevating the blood glucose level after a meal (postprandial glycaemia); hence, many antidiabetic drugs, such as acarbose and miglitol, inhibit the activity of these enzymes. However, consuming synthetic drugs for long periods may have negative side effects, such as abdominal cramps, vomiting and ~~diarrhea~~diarrhoea (Hanefeld, 1998). Many researchers are looking for natural drugs able to inhibit α-glucosidase and α-amylase with no harmful effects. Phlorotannins are possible candidates to control such enzymes, since polyphenols of several plants have shown to inhibit them effectively. In fact, most investigated phlorotannins have exhibited lower IC_{50} values -or greater inhibitory activities- than acarbose. For instance, diphlorethohydroxycarmalol (DPHC) of *I. okamurae* presents an IC_{50} of 0.19 and 0.53 mM against α-glucosidase and pancreatic α-amylase, respectively, while the

values of acarbose are 1.05 and 1.10 mM (Heo et al., 2009). Additionally, it has been observed that phlorotannins with molecular weights over 500 Daltons exhibit higher inhibitory effects in both enzymes than compounds under 500 Daltons (S. H. Lee, Li, Karadeniz, Kim, & Kim, 2009; Moon et al., 2011; S. R. Park et al., 2018). S. R. Park et al. (2018) found that 6,8'-bieckol (MW: 742.55) and 2-O-(2,4,6-trihydroxyphenyl)-6,6'-bieckol (MW: 866.65) are more than fifteen-fold stronger α-glucosidase inhibitors than eckol (MW:372.29) and 2-phloroeckol (MW:496.38). Moon et al. (2011) hypothesized that t~~T~~his is probably explained by the higher number of hydroxyl groups present in large phlorotannins than in small ones ~~(Moon et al., 2011)~~. Another plausible reason is the poor absorption of high molecular weight phlorotannins in the upper gastrointestinal tract, described by Corona et al. (2016). As for the potency of studied phlorotannins for each enzyme, dieckol, fucodiphlorethol G, 6,6'-bieckol, 7-phloroeckol (S. H. Lee et al., 2009; S. H. Lee, Park, et al., 2010), DPHC (Heo et al., 2009), fucofuroeckol A and dioxinodehydroeckol (Eom et al., 2012) have shown higher inhibitory activity against α-glucosidase than against pancreatic α-amylase. Conversely, 2,7''-phloroglucinol-6,6'-bieckol (H. A. Lee et al., 2017) has shown better inhibitory capacity against pancreatic α-amylase. Additionally, kinetic and molecular docking analyses have been performed to confirm the type of inhibition and to develop guidelines to apply phlorotannins as antidiabetic drugs, showing that phlorotannins exhibit competitive and non-competitive inhibition against carbohydrate-hydrolysing enzymes (Eom et al., 2012; Kawamura-Konishi et al., 2012; Moon et al., 2011). For instance, after finding, through a kinetic assay, that 6,8'-bieckol is a competitive inhibitor of α-glucosidase, S. R. Park et al. (2018) further confirmed this result by an *in-silico* docking study which determined that 6,8'-bieckol anchors to amino acids located in the active site of the enzyme.

Besides carbohydrate-hydrolysing enzymes, the inhibitory capacity of phlorotannins against protein tyrosine phosphatase 1B (PTP1B), a negative regulator of intracellular insulin signalling, has also been addressed. While hydrolysing enzymes increase the influx of glucose from the intestine to the vessels, PTP1B suppresses glucose uptake by muscle, adipose tissue and liver cells. Hence, inhibitors of PTP1B, such as some phlorotannins, can enhance insulin action and reduce postprandial glycaemia and insulin resistance in DM patients (S. Lee & Wang, 2007). Moon et al. (2011) reported that most studied phlorotannins have similar or lower IC_{50} than ursolic acid, a natural compound present in many fruits and herbs that is a strong inhibitor of PTP1B. The study of Moon et al. (2011) also found a possible correlation between molecular mass and inhibitory activity, with dieckol and phlorofucofuroeckol A being stronger inhibitors than eckol and 7-phloroeckol. Moreover, almost all investigated phlorotannins exhibited non-competitive inhibition of PTP1B.

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~~7.1.1.2~~5.1.1.2 *Cell-based assays*

Several studies have demonstrated that hyperglycaemia can promote alternative glucose-metabolizing pathways in the mitochondria, thus generating intermediates that lead to the formation of reactive oxygen species (ROS), nitric oxide (NO), peroxynitrite ($ONOO^-$) and advanced glycation end products (AGE). Hyperglycaemia can also reduce antioxidant enzymes defence, thereby allowing ROS to accumulate. The combination of all these responses results in cellular and tissue damage, causing organs failure and dysfunction (Baynes & Thorpe, 1999). Dieckol and 6,6'-bieckol isolated from *E. cava* have exhibited a protective effect against glucose-induced oxidative stress in human umbilical vein endothelial cells (HUVEC) and in rat insulinoma cells (S. H. Lee, Han, Heo, Hwang, & Jeon, 2010; S. H. Lee, Park, et al., 2012; M. H. Park et al., 2015; M. H. Park et al., 2014). The studies showed that both compounds significantly inhibit cell death induced by high glucose treatments (30 mM), in a dose-dependent manner. The authors argued that dieckol and 6,6'-bieckol diminish intracellular ROS and NO levels, as well as the lipid peroxidation caused by high glucose concentrations. This antioxidant activity ~~is~~was related to a reduction of the overexpression of NO-producing enzymes (iNOS, COX-2), to an increased activity of antioxidant enzymes (CAT, SOD and GSH-px) and to a higher expression of the anti-apoptotic protein Bcl-2. Similarly, DPHC from *I. okamurae* has shown a protective effect against glucose-induce damage in RINm5F pancreatic β cells, as a result of an enhanced activity of antioxidant enzymes (S. H. Lee, Choi, et al., 2012).

~~7.1.2~~5.1.2 Animal assays

The following phlorotannins were shown to significantly alleviate postprandial hyperglycaemia in streptozotocin-induced diabetic and normal mice: dieckol, phlorofucofuroeckol A as well as 2,7''-phloroglucinol-6,6'-bieckol from *E. cava* (M. C. Kang et al., 2013; H. A. Lee et al., 2017; S. H. Lee, Choi, et al., 2012; S. H. Lee, Min, et al., 2012; You, Lee, Park, Lee, & Han, 2015), DPHC from *I. okamurae* (Heo et al., 2009) and octaphlorethol A from *I. foliacea* (S. H. Lee et al., 2014; S. H. Lee et al., 2016). Furthermore, the observed reductions were like those of control mice treated with acarbose at the same dose (10 or 100 mg/kg). The authors related this effect to the capacity of the compounds to strongly inhibit carbohydrate-hydrolysing enzymes, as observed in biochemical *in vitro* assays. In addition, dieckol (M. C. Kang et al., 2013), an AG-dieckol rich extract (100 mg/g) from *E. cava* (S. H. Lee, Min, et al., 2012) and octaphlorethol A (S. H. Lee et al., 2016), have shown the capacity to significantly lower blood glucose and serum insulin levels, as well as to significantly improve glucose tolerance in type 2 diabetic mice (C57bl/KsJ-*db/db*). Moreover, since diabetes is

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related to the oxidative stress induced by uncontrolled ROS production, *db/db* mice treated with dieckol, or the AG-dieckol rich extract, presented an increment in antioxidant enzymes activity (CAT, SOD and GSH-PX), showing less lipid peroxidation in the liver than the control group (M. C. Kang et al., 2013; S. H. Lee, Min, et al., 2012).

Besides the inhibition of carbohydrate-hydrolysing enzymes, other mechanisms of action have been explored to explain the antihyperglycaemic effects of phlorotannins in mice. Hepatic gluconeogenesis is a crucial target, as glucose output from the liver has an important contribution in fasting and postprandial hyperglycaemia. This metabolic pathway is controlled by insulin-regulated enzymes, such as glucose-6-phosphatase (G6Pase), phosphoenolpyruvate carboxykinase (PEPCK) and glucokinase (GK). It is known that G6Pase and PEPCK are up-regulated in DM, while the opposite occurs with GK (Davies, Khandelwal, Wu, Juurlink, & Roesler, 2001). An AG-dieckol rich extract and octaphlorethol A exhibited the capacity to down-regulate the activity and gene expression of G6Pase and PEPCK, and to up-regulate the activity of GK in C57bl/KsJ-*db/db* mice, thus diminishing blood glucose and increasing hepatic glycogen level (S. H. Lee et al., 2016; S. H. Lee, Min, et al., 2012).

Glucose metabolism in muscle is another target for elucidating antidiabetic mechanisms of phlorotannins, as muscle is the primary tissue for glucose homeostasis. A crucial regulator is AMPK (AMP-activated protein kinase), which promotes insulin-independent glucose uptake by GLUT-4 transporter, during exercise or low energy status (Musi et al., 2001). S. H. Lee et al. (2016) and M. C. Kang et al. (2013) found that octaphlorethol A and dieckol, respectively, activate AMPK phosphorylation and GLUT-4 expression in the muscle of C57bl/KsJ-*db/db* mice, thus increasing glucose uptake and utilization.

The effects of some phlorotannins in lipid metabolism of DM mice have also been addressed since hepatic lipid accumulation has been linked to the development of hepatic insulin resistance. S. H. Lee, Min, et al. (2012) reported that an AG-dieckol rich extract significantly lowers the plasma and hepatic free fatty acids, triglyceride and cholesterol levels in C57bl/KsJ-*db/db* mice. Moreover, it was demonstrated that octaphlorethol A markedly suppresses mRNA expression level of hepatic fatty acid synthase, a key enzyme that catalyses the synthesis of saturated long-chain fatty acids, as compared to control diabetic mice (S. H. Lee et al., 2016).

Additionally, as the gut microbiome plays an important role in the development of metabolic disease, type 2 DM and inflammatory responses (Patterson et al., 2016), the effect of

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phlorotannins at this level has also been studied. Yuan et al. (2019) fed diabetic C57BL/6J rats with a polyphenol-rich extract (21.13 mg GAE/g, high in phlorotannins) from *L. trabeculate*, and found positive effects not only on metabolic and antioxidant stress parameters but also on the dysbiosis (gut microbial imbalance) produced by diabetes. The four-week polyphenol-based treatment lowered the fasting blood glucose and the serum insulin levels as well as improved the serum lipid profile and the antioxidant response in DM rats. However, the most relevant finding was the recovery of some short-chain fatty acids (SCFAs) and the gut bacterial biodiversity, which were impaired in control (non-treated) DM rats. The polyphenol-based feeding produced an increase of acetic and butyric acid levels and a restoration of the abundance of some microbial communities involved in maintaining colon integrity and healthy insulin levels.

~~7.1.3~~ Clinical trials

Despite the strong evidence supporting the antihyperglycaemic effect of phlorotannins in mouse models, to date only four studies have assessed this response in humans. They were double-blind, randomized and placebo-controlled clinical trials exploring the capacity of phlorotannin-rich extracts on regulating glucose and insulin levels in blood. Paradis, Couture, and Lamarche (2011) and Murray, Dordevic, Ryan, and Bonham (2018) involved healthy adults in acute postprandial tests, analogous to those practiced in mice, while Shin, Kim, Park, Lee, and Hwang (2012) and S. H. Lee and Jeon (2015) included overweight or prediabetic individuals in long-term interventions.

Regarding acute interventions, Paradis et al. (2011) reported a significant 12.1 % reduction in the insulin incremental area under the curve (iAUC) in 23 non-diabetic adults that ingested a 500 mg single-dose of a commercially available blend from the brown seaweeds *Ascophyllum nodosum* and *Fucus vesiculosus* (InSea2, minimum 10% polyphenols), 30 min prior to the consumption of 50 g of available carbohydrate (sugar plus starch), in comparison with those who ingested the placebo. However, no significant difference was observed in postprandial blood glucose. Applying the same study design to 38 participants, Murray et al. (2018) investigated the effect of a low (500 mg) and a high dose (2000 mg) of a polyphenol-rich *Fucus vesiculosus* extract (28% polyphenols); they found no significant differences in the iAUC or postprandial peak concentrations in blood glucose and plasma insulin across the treatments (placebo, low dose, high dose). On the other hand, in the long-term trial of Shin et al. (2012), 97 overweight adults consumed a daily dose of 72 or 144 mg of a polyphenol extract from *Ecklonia cava* (rich in dieckol, 8,8'-bieckol, 6,6-bieckol and phlorofucofuroeckol A), or a placebo, for 12 weeks. After the treatment, significantly lower fasting blood glucose (4.9 % decrease) and body weight (2.0 % decrease) were observed in the high dose

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group. Likewise, in another 12-week intervention trial, 40 pre-diabetic individuals took 1500 mg per day of an AG-dieckol-rich extract (100 mg/g) from *E. cava*, showing a significant reduction in 2 h postprandial glycaemia in comparison with the 40 people placebo group (S. H. Lee & Jeon, 2015). However, no significant effect was seen in fasting plasma glucose, nor in the insulin level.

Overall, considering the little clinical evidence available today, it appears that many more long-term trials are mandatory to demonstrate the antidiabetic ability of phlorotannins in humans, contrary to the single dose studies reported in mice. Clinical trials also seem to suggest that phlorotannin treatments would only be useful in insulin resistant or prediabetic individuals and not in healthy people, although more assays are needed. Additionally, ~~,~~ Coe and Ryan (2016) have argued that these studies would be more relevant if they could identify those compounds and doses with~~that have~~ higher antihyperglyacemic or anti-insulinemic activities. ~~Moreover,~~ However, to assess any structure-function relationship, the effects of absorption, biotransformation and conjugation of phlorotannins in the gut, liver and cells should be further explored~~,~~ since these processes determine the active metabolites that finally exert the~~their ability to act as effective~~ bioactivity~~bioactive molecules~~ *in vivo*. ~~Nevertheless, only~~ ~~a~~ So far, the few *in vitro* and *in vivo* metabolomic studies that have dealt with these aspects have only detected some common dimers or trimers derivatives as common final metabolites resulting from substrates rich in high molecular weight phlorotannins (Baldrick et al., 2018; Corona et al., 2017; Corona et al., 2016). Therefore, this finding suggests that a structure-function approach of intact phlorotannin bioactivities would probably not be useful, at least in terms of their systemic effects since they do not correspond to the circulating molecules~~.~~

Hence, it has been well-demonstrated through *in vitro* and *in vivo* assays that phlorotannin-rich extracts and some isolated phlorotannins inhibit carbohydrate-hydrolysing enzymes from the gut, ~~. There is also strong evidence that they~~ inhibit gluconeogenesis enzymes, ~~and~~ promote ~~muscle~~ glucose metabolism as well as induce the growth and activity of healthy gut microbiota ~~*in vivo*~~ (Fig. 3). Moreover, the capacity of phlorotannins to modulate glucose homeostasis and improve insulin sensitivity in mice has been proven in a wide number of studies. However, clinical trials have been scarce, and they have not conclusively demonstrated yet the antidiabetic effect of phlorotannins in humans.

~~7.2~~5.2 Cancer Treatment and Prevention Potential

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Cancer is the generic term referring to the uncontrollable cell proliferation and tumour formation that can occur in any body part. This process is caused by the progressive transformation of a normal cell into a malignant cell, due to genetic and epigenetic alterations. Cumulative research has established that these alterations result from an interaction between genetic inheritance and external factors, such as an unhealthy diet, tobacco use, infections, UV radiation, environmental contamination and immune condition. Cancer cells proliferate without limit, avoid apoptosis and can also invade or metastasize other tissues or organs, resulting in death (ACS, 2019). According to GLOBOCAN (Global Cancer Observatory), cancer is the second major cause of death worldwide and was responsible for an estimated 9.6 million deaths in 2018, with a prediction of near 16.4 million deaths in 2040. Lung, breast and colorectum cancers show the highest incidence rates (Ferlay et al., 2018).

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The treatment of cancer requires one or more therapies, depending on the type of tumour and its progression, including surgery, radiotherapy, chemotherapy and immunotherapy. The common purpose of these therapies is to induce apoptosis of malignant cells and thereby reduce tumour size (ACS, 2019). However, treatments comprising radiotherapy and chemotherapy have detrimental effects since they also damage healthy cells. For instance, the drug cisplatin is often associated with nephrotoxicity and neurotoxicity (Karasawa & Steyger, 2015). Moreover, resistance to ionizing radiation and cancer drugs is common and is considered to be the origin of metastasis and relapse (Morrison, Schmidt, Lakhani, Reynolds, & Lopez, 2008). Despite decades of research, a treatment that effectively targets the main cancer hallmarks –uncontrollable cell proliferation, evasion of apoptosis signalling, invasion and metastasis- is still lacking. Between natural products, some phlorotannins, such as dieckol and phloroglucinol, have been described with the capacity to enhance the effects of anticancer drugs and as protectors from the cytotoxicity of chemotherapy and irradiation. Below we present a summary of the recent evidence about the mechanism of action of phlorotannins against those cancers with higher incidence and mortality rates. Since, as far as we know, there are no reports of clinical trials in the open literature, only cell-based and animal assays are considered.

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~~7.2.1~~5.2.1 ~~*In Vitro:* c~~Cell-based assays

~~*7.2.1.1*~~*5.2.1.1* *Induction of apoptosis:*

Apoptosis is the main mechanism of cell death regulating proliferation and tissue growth in multicellular organisms. Through apoptosis, redundant and potentially harmful cells are eliminated.

Thus, the induction of apoptosis is considered one of the primary defences against cancer (Cotter, 2009). Irradiation and the drug cisplatin have been used for years as inducers of apoptosis in several cancers, but with possible life-threatening effects (De Ruysscher et al., 2019; Karasawa & Steyger, 2015). Some plant-derived compounds, such as curcumin (Deguchi, 2015) and quercetin (Khan et al., 2016), can also induce apoptosis in cancer cells, with no detrimental effects. Dieckol, phloroglucinol, phlorofucofuroeckol A and dioxinodehydroeckol have shown the capacity to promote apoptosis by activating caspases, proteins that proteolytically dismantle most cellular structures ~~(Fig. 2)~~. Two major signalling pathways activate caspases: the extrinsic/death receptor pathway and the intrinsic/mitochondrial pathway. The extrinsic pathway is mediated by the binding of death ligands to specific cell surface receptors (e.g., Fas). The intrinsic pathway is activated by various stimuli, such as ROS and the pro-apoptotic members of Bcl-2 proteins. These stimuli increase mitochondria permeability, which provokes the release of cytochrome c into the cytosol, thus activating caspases (Jan & Chaudhry, 2019). Ahn, Yang, Lee, and Choi (2015) and Yoon et al. (2013) observed that dieckol induces caspase-dependent apoptosis of cancer cells by the activation of both extrinsic and intrinsic pathways. In SKOV3 ovarian cancer cells, dieckol from *E. cava* raised intracellular ROS, which increased the cytosolic cytochrome c and activated caspases, and also down-regulated the anti-apoptotic proteins XIAP, FLIP and AKT kinase (Ahn et al., 2015). In human hepatocellular carcinoma HepB3 cells, 100 µM dieckol from *E. stolonifera* up-regulated the pro-apoptotic Bcl-2 proteins (Bid, Bim and Bak), releasing cytochrome c into the cytosol and activating caspase-9. At the same concentration, ~~D~~dieckol also activated extrinsic caspases (caspase-3, -6, -7 and -8), and had no cytotoxic effect in non-cancerous kidney cells (Yoon et al., 2013). Phloroglucinol (50 µg/mL) induced apoptosis in HT-29 colon cancer cells via increasing the expression of caspases, Fas and Bax/Bak, Bcl-2 proteins involved in boosting the permeability of mitochondria, with no harmful effect in healthy intestine epithelial cells (M. H. Kang, Kim, & Nam, 2014). Phlorofucofuroeckol A (100 µM) isolated from *Eisenia bicyclis* ~~also~~ promoted apoptosis of HT-29 cells through the up-regulation of ATF3, an apoptosis mediator transcription factor (Eo et al., 2016). In breast cancer cells, dioxinodehydroeckol from *E. cava* increased the expression of caspases and pro-apoptotic proteins p53 and Bax, in a dose dependant manner (5-100 µM). It also reduced the expression of Bcl-2 anti-apoptotic proteins and NF-κB, a transcription factor that promotes cell proliferation, migration, tumour growth and inflammation (Kong, Kim, Yoon, & Kim, 2009). Moreover, Yang, Ahn, Choi, and Choi (2015) observed that 50 µg/mL of a phlorotannin-rich extract from *E. cava* (98% phloroglucinol equivalents) augmented the apoptotic capacity of cisplatin (5 µM)

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in ovarian cancer cells by down-regulating AKT kinase, NF-κB and its controlled anti-apoptotic proteins (Bcl-xl, XIAP, FLIP). Although the phlorotannin extract increased intracellular ROS in the cancer cells, it reduced cisplatin-induced ROS and cell death in normal kidney cells.

Regenerating gene protein (Reg) 3A accomplishes an essential role in pancreatic cancer (PaC) initiation and progression, as it promotes the survival, growth and proliferation of PaC cells. Even though eckol did not present apoptotic effects on PaC cells at assayed concentrations M. Zhang et al. (2019) described dieckol as a potential protector against PaC. M. Zhang et al. (2019) reported that eckol (10-20 µg/mL) inhibited Reg3A-promoted cell survival, proliferation and colony formation of SW1990 PaC cells, in a dose-dependent manner. Eckol also reverted the Reg3A-mediated upregulation of JAK2, STAT3, NF-κB and cyclin D1 proteins. Thus, M. Zhang et al. (2019) hypothesized that the effect of eckol against Reg3A-induced PaC progression would be related to its capacity to modulate the JAK2/STAT3 and NF-κB/cyclin D1 signaling pathways.

~~7.2.1.2~~ *Inhibition of angiogenesis and invasion:*

Tumour progression and metastasis require the formation of new blood vessels, a process known as angiogenesis. The vessels originated by angiogenesis are able to deliver nutrients and oxygen to proliferating tumour cells and to create vascular connections for tumour cells metastasis. Metastasis is the process by which cancer cells move from the original tumour and invade other tissues or organs, producing a secondary cancer. One way to inhibit tumour progression and metastasis is inhibiting angiogenesis and cancer cells invasion capacity (Steeg, 2016). Vascular endothelial growth factor (VEGF) and matrix metallopeptidases (MMPs) are key proteins in both processes. VEGF promotes cell motility and division of vascular endothelial cells, and MMPs cleave extracellular matrix (ECM) to provide space for the new vessels and the growing tumour (Y. Zhang, Dang, Wan, Yang, & Li, 2017). MMP-2 and MMP-9 are specifically incremented in tumours (Roomi, Monterrey, Kalinovsky, Rath, & Niedzwiecki, 2009). Dieckol from *E. cava* has been shown to inhibit cancer cells motility and to reduce the expression of MMP-2, MMP-9 and VEGF- (E. K. Kim et al., 2015; S. J. Park & Jeon, 2012; S. J. Park, Kim, & Jeon, 2012; C. Zhang et al., 2011). ~~In t~~The study of E. K. Kim et al. (2015)~~,~~ demonstrated the capacity of dieckol ~~also showed the capacity~~ (1-100 µM) to increase the expression of some MMPs inhibitors (TIMP-1 and TIMP-2) in MCF-7 human breast cancer cells~~(Fig. 2)~~. C. Zhang et al. (2011) related the dieckol-suppressive effect on~~f~~ cell invasion with its ability to down-regulate the transcription factor NF-κB in human fibrosarcoma cells. Using mouse melanoma and human fibrosarcoma cells, Park observed that cancer cell migration and invasion are mediated by intracellular ROS generation, and the dieckol-inhibitory effect on these processes depends on its

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ROS-scavenging activity (S. J. Park & Jeon, 2012; S. J. Park et al., 2012). According to the *ex vivo* assay of Kwon et al. (2012), ~~P~~phloroglucinol would also attenuate angiogenesis and invasion, as it inhibited the VEGF-induced migration~~reduced the capillary-like tube formation~~ of endothelial progenitor cells (EPCs) and their capillary-like tube formation capability~~their migration from the bone marrow~~, in a dose-dependent manner (2-100 µM) ~~(Fig. 2)~~.

~~7.2.1.3~~5.2.1.2 Sensitization of cancer stem-like cells to drugs:

A subpopulation of high-grade malignant cells with stem cell properties are responsible for cancer progression and metastasis. These cells easily self-renew, avoid apoptosis and migrate to other tissues. The inefficiency of radiotherapy and chemotherapy is explained by the resistance of cancer stem-like cells (CSCs); the treatments fail to eliminate all malignant cells, and survivor cells cause a relapse (Colak & Medema, 2014). Phlorotannins are recognized as potential adjuvants of traditional therapies because they sensitize CSCs ~~(Fig. 2)~~. Phloroglucinol (R. K. Kim, N. Uddin, et al., 2015) and eckol (Hyun et al., 2011) have exhibited this capacity in glioma and breast CSCs, respectively. Both compounds were shown to reduce self-renewal, sphere formation and anchorage-independent growth (tumorigenicity) abilities of CSCs. More interestingly, they also were reported to effectively enhance the cytotoxicity of anticancer drugs (e.g., cisplatin, temozolomide and etoposide) and ionizing radiation. As PI3K/AKT and RAF-1/ERK signalling regulate the maintenance of CSCs, the effects of phloroglucinol and eckol in these pathways were also assessed, finding that both phlorotannins inhibit AKT and ERK kinases activities.

~~7.2.2~~5.2.2 Animal assays

~~7.2.2.1~~5.2.2.1 Carcinogenesis inhibition (cancer prevention):

The chronic exposure to environmental carcinogens -such as radiation, tobacco smoke, alcohol, unhealthy food and some viral infections- is largely responsible for the increased cancer prevalence (Soffritti, Belpoggi, Esposti, Falcioni, & Bua, 2008). Carcinogens cause excessive ROS, which is sometimes unmanageable by cellular detoxifying and repairing systems, producing oxidative stress. Oxidative stress damages DNA, proteins and lipids, eventually resulting in cancer initiation (Mena, Ortega, & Estrela, 2009). Some phlorotannins have been described as protectors against radiation-induced skin carcinogenesis and tissue damage. Thus, in the study of Hwang, Chen, Nines, Shin, and Stoner (2006), dietary and topical administration of a phlorotannin-rich extract reduced the tumour multiplicity and volume in ultraviolet B-irradiated mice. Piao et al. (2015) revealed that the protective effect of DPHC against UVB-induced DNA damage is related to its capacity to increase the

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expression of proteins involved in the DNA repairing system. Phloroglucinol and eckol showed protective effects against ionizing radiation in mice, and thus they have been proposed as candidates to alleviate radiation-induced injuries in cancer patients (Ha et al., 2013; E. Park et al., 2008). Additionally, dieckol was reported to have protective effects against chemical-induced hepatocarcinogenesis. Sadeeshkumar et al. (2016) demonstrated that dieckol (40 mg/kg) lowered tumour incidence in N-nitrosodiethylamine (NDEA) treated rats, through reducing the oxidative damage and inducing the antioxidant cascade in the liver. Dieckol also supressed NDEA-initiated hepatocarcinogenesis by modulating xenobiotic metabolizing enzymes, inducing apoptosis cascade and inhibiting proliferation, invasion and angiogenesis signalling (Sadeeshkumar et al., 2017).

~~7.2.2.2~~5.2.2.2 *Inhibition of tumour progression and metastasis:*

Tumour progression is associated with more aggressive cancers that lead to local invasion and metastasis. To address the antitumorigenic effect of phlorotannins *in vivo*, human tumour xenografts (HTXs) are commonly used. HTXs involve the implantation of commercial or patient-derived cancer cell lines into immunodeficient mice that do not reject human cells (Richmond & Su, 2008). In agreement with the antiproliferative and antiangiogenic effects evidenced in cell-based assays, phlorotannins have also shown anticancer potential through the suppression of tumour progression in HTX models. In an ovarian cancer SKOV3 xenograft, dieckol (100 mg/kg) was observed to reduce the tumour volume and weight to similar levels of cisplatin (3 mg/kg); but without the kidney or liver toxicity produced by the drug (Ahn et al., 2015). In a similar ovarian cancer model, a combination of an *E. cava* phlorotannin-rich extract (150 mg/kg) with cisplatin (3 mg/kg) markedly enhanced the potency of cisplatin in reducing tumour volume, and also reversed the body weight loss and nephrotoxicity caused by the drug alone (Yang et al., 2015). Through breast cancer and glioma xenograft models, phloroglucinol (R. K. Kim, N. Uddin, et al., 2015) and eckol (Hyun et al., 2011), respectively, ~~have~~ exhibited the capacity to suppress the growth of ~~cancer~~ CSCs tumours ~~*in vivo*~~. Additionally, in S180 sarcoma tumour-bearing mice, eckol (1mg/kg) reduced tumour growth by inducing apoptosis and inhibiting proliferation of sarcoma cells; these effects were related to a boost of pro-apoptotic proteins (caspase-3 and -9) as well as to a reduction of anti-apoptotic proteins (Bcl-2 and Bax) and EGFR -a proliferation-stimulating protein- in the solid tumour. However, much more interesting was the finding that eckol stimulated the innate and adaptative immune responses -responsible for tumour surveillance- in sarcoma-bearing mice. Specifically, eckol activated the mononuclear phagocytic system, recruited and activated dendritic cells (DCs),

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increased the number of helper T cells ($CD4^+$) over suppressor T cells ($CD8^+$), induced type 1 helper T cells (Th1) anti-tumour response and activated cytotoxic T lymphocyte responses (M. Y. Zhang et al., 2019).

~~P~~Phloroglucinol ~~has also demonstrated *in vivo* a~~Angiogenesis and metastasis-inhibition effects have been assigned to phloroglucinol (R. K. Kim, Y. Suh, et al., 2015; Kwon et al., 2012). In the study of Kwon et al. (2012) phloroglucinol reduced the migration of EPCs from the bone narrow into peripheral blood as well as the number of capillary microvessels in the peritumoral region of a lung tumour-bearing mice. Moreover, R. K. Kim, Y. Suh, et al. (2015) showed that phloroglucinol is effective in attenuating metastasis of breast cancer cells to lung, and in extending the survival time of mice.

Therefore, phlorotannins have been demonstrated to induce apoptosis of cancer cells~~,~~ and inhibit angiogenesis, tumour progression and metastasis, ~~mainly~~ through diverse mechanisms of action ~~cell-based assays~~(Fig. 4). Also, animal studies have shown that some phlorotannins are able to prevent cancer initiation and progression. Thus, phlorotannins could be considered as potential adjuvants for existing cancer therapies and as cancer chemoprevention agents. A preliminary study on an overweight and obese population showed a modest improvement in DNA damage in the obese subset of the total population after consuming 100 mg brown seaweed (*Ascophyllum nodosum*) polyphenol-rich extract for 8 weeks (Baldrick et al., 2018); however, up to now there is no clinical evidence about the effects of phlorotannins on cancer patients. Clinical studies are still needed to prove the efficacy and safety of phlorotannins in cancer prevention and treatment.

~~8.~~6. Conclusions and Future Perspectives

The development of more comprehensive studies about phlorotannin bioactivities requires their isolation from seaweed extracts. This is especially relevant considering that other alga compounds, such as carbohydrates, pigments and even toxic heavy metals, are co-extracted with phlorotannins, limiting the extent of the findings. Different separation techniques have been applied to obtain individual compounds or phlorotannin-rich extracts, with classical solid-liquid extraction using 70% acetone or 80% methanol, followed by purification with ethyl acetate being the most popular method. However, this procedure is not in agreement with green-chemistry and is not optimum for food or pharmaceutical applications. PHLE with water or ethanol-water mixtures as solvents are better options, because of their low environmental impact, high productivity and selectivity. As

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PHLE is hard to scale up to industrial production, future research in this area should be focused on scaled-up operation and design of industrial-scale equipment to take benefits from improved extraction of potential bioactive phlorotannins.

So far, considerable research proving the antidiabetic and anticancer potential of purified phlorotannins has been accumulated, with biochemical and cell-based assays representing the vast majority. Nevertheless, these *in vitro* analyses do not consider the biotransformation and conjugation reactions occurring in the gut, liver and cells, which significantly affect the bioavailability and biological activities of phlorotannins. Therefore, more *in vivo* studies are necessary to determine the real effects of these compounds in tissues and organs. The performance of further animal assays and especially clinical interventions are necessary to definitively confirm the efficacy of phlorotannins as adjuvants for the prevention and/or treatment of diabetes and cancer.

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Código de campo cambiado

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Table 1. Mass spectrometric data and identification of phlorotannins determined through HPLC-MS^n in brown seaweeds extracts by different authors.

Polymerization Degree/ Identification[a]	Molecular formula	Molecular mass	Precursor ion MS^1 $[M-H]^-$, m/z	Product ions MS^2 $[M-H]^-$, m/z[b]	Product ions MS^3 [(M-H) →base peak]$^-$, m/z[c]	References
Monomers						
Phloroglucinol derivative		392	391	125		Agregán et al. (2017)
Phloroglucinol derivative		402	401	**205**[d], 125		Agregán et al. (2017)
Dimers						
Bifuhalol	$C_{12}H_{10}O_7$	266	265	247, 141, 139, 125, 123		Li et al. (2017)
Phlorethohydroxycarmalol		264	263	245, 219, 111		Li et al. (2017)
Phloroglucinol dimer derivative		518	517	**247**		Agregán et al. (2017)
Trimers						
Dioxinodehydroeckol	$C_{18}H_{10}O_9$	370	369.0249	**238**	195,167, 112	Lopes et al. (2018)
Dioxinodehydroeckol	$C_{18}H_{10}O_9$	370	369.0246	351, **325**, 307	307, 297, 281	Lopes et al. (2018)
Eckol	$C_{18}H_{12}O_9$	372	371	335, 317, 246, 229, 140, 121		Li et al. (2017)
Trifucol	$C_{18}H_{14}O_9$	374	373	**305**, 247, 229		Vissers, Caligiani, Sforza, Vincken, and Gruppen (2017)
Triphlorethol	$C_{18}H_{14}O_9$	374	373	**305**, 231		Vissers et al. (2017)
Fucophlorethol	$C_{18}H_{14}O_9$	374	373.0564	**355**, 329, 247, 231	229, 215	Lopes et al. (2018); Hermund et al. (2018)
Fucophlorethol	$C_{18}H_{14}O_9$	374	373.0560	**247**, 233	229	Lopes et al. (2018); Vissers et al. (2017)
Fucophlorethol	$C_{18}H_{14}O_9$	374	373.0564	355, 329, 229		Hermund et al. (2018)
Fucophlorethol	$C_{18}H_{14}O_9$	374	373.0590	329, 247, 229		Hermund et al. (2018)
Phlorethohydroxycarmalol		388	387	329, 262, 245, 123		Li et al. (2017)
Trifuhalol	$C_{18}H_{14}O_{10}$	390	389	375		Agregán et al. (2017)
Trifuhalol	$C_{18}H_{14}O_{10}$	390	389	353, 265, 263, 245		Li et al. (2017)
Trifuhalol	$C_{18}H_{14}O_{10}$	390	389.0	375, 265, 245		Montero et al. (2016)
Eckol derivative		402	401	371		Agregán et al. (2017)
Eckol derivative		542	541	**401**, 371		Agregán et al. (2017)
Eckol derivative		546	545	371		Agregán et al. (2017)
Dioxinodehydroeckol derivative		464	463	369		Agregán et al. (2017)

Polymerization Degree/ Identification[a]	Molecular formula	Molecular mass	Precursor ion MS^1 $[M-H]^-$, m/z	Product ions MS^2 $[M-H]^-$, m/z[b]	Product ions MS^3 [(M-H) →base peak]$^-$, m/z[c]	References
Tetramers						
Fucofuroeckol hydroxylated	$C_{24}H_{14}O_{12}$	494	493.0405	**475**, 367	431, **405**	Lopes et al. (2018)
Tetrafucol	$C_{24}H_{18}O_{12}$	498	497	**461**, 435, 371, 353, 231		Vissers et al. (2017)
Difucophlorethol	$C_{24}H_{18}O_{12}$	498	497.0715	**479**, 353, 331	**461**, 435, 353	Lopes et al. (2018)
Difucophlorethol	$C_{24}H_{18}O_{12}$	498	497.0719	**479**, 461, 355	420, 353, **337**	Lopes et al. (2018)
Fucodiphlorethol	$C_{24}H_{18}O_{12}$	498	497.0717	**479**, 371, 353, 339	**339**, 229	Lopes et al. (2018); Hermund et al. (2018)
Fucodiphlorethol	$C_{24}H_{18}O_{12}$	498	497.0713	479, **355**, 311	**311**, 229	Lopes et al. (2018)
Fucodiphlorethol	$C_{24}H_{18}O_{12}$	498	497.0715	**355**, 235	269, **229**	Lopes et al. (2018)
Fucodiphlorethol	$C_{24}H_{18}O_{12}$	498	497.0715	479, **373**, 265	233, **139**	Lopes et al. (2018)
Fucodiphlorethol	$C_{24}H_{18}O_{12}$	498	497.0729	479, 371, 353, 335, 247, 229		Hermund et al. (2018); Vissers et al. (2017)
Bisfucophlorethol	$C_{24}H_{18}O_{12}$	498	497.0716	**235**	207, **191**	Lopes et al. (2018)
Fuhalol tetramer		510	509	441, **384**, 373, 305, 261		Vissers et al. (2017)
Deshydroxetrafuhalol	$C_{24}H_{18}O_{13}$	514	513	**385**		Agregán et al. (2017)
Deshydroxetrafuhalol	$C_{24}H_{18}O_{13}$	514	513	499		Agregán et al. (2017)
Deshydroxetrafuhalol	$C_{24}H_{18}O_{13}$	514	513.0	391, 373, 264, 245, 219		Montero et al. (2016)
Deshydroxetrafuhalol	$C_{24}H_{18}O_{13}$	514	513	389, 373, 265, 246		Montero et al. (2016); Li et al. (2017)
Deshydroxetrafuhalol	$C_{24}H_{18}O_{13}$	514	513.0	475, 438, 391		Montero et al. (2016)
Deshydroxetrafuhalol	$C_{24}H_{18}O_{13}$	514	513.0	499, 437, 389, 263		Montero et al. (2016)
Fuhalolhydroxycarmalol		528	527	403, 263, 233, 139		Li et al. (2017)
Tetrafuhalol	$C_{24}H_{18}O_{14}$	530	529	**387**, 219		Agregán et al. (2017)
Tetrafuhalol	$C_{24}H_{18}O_{14}$	530	529	387		Agregán et al. (2017)
Tetrafuhalol	$C_{24}H_{18}O_{14}$	530	529	403, 389, 263, 245		Montero et al. (2016); Li et al. (2017)
Tetrafuhalol	$C_{24}H_{18}O_{14}$	530	529.4	513, 389, 262		Montero et al. (2016)
Tetrafuhalol	$C_{24}H_{18}O_{14}$	530	529.5	483, 465, 401, 389, 262, 245		Montero et al. (2016)
Hydroxytetrafuhalol	$C_{24}H_{18}O_{15}$	546	545	387		Agregán et al. (2017)
Hydroxytetrafuhalol	$C_{24}H_{18}O_{15}$	546	545	385		Agregán et al. (2017)
Hydroxytetrafuhalol	$C_{24}H_{18}O_{15}$	546	545.4	525, 513, 484, 403, 389, 375		Montero et al. (2016)

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Polymerization Degree/ Identification[a]	Molecular formula	Molecular mass	Precursor ion MS^1 $[M-H]^-$, m/z	Product ions MS^2 $[M-H]^-$, m/z[b]	Product ions MS^3 [(M-H) →base peak]$^-$, m/z[c]	References
Pentamers						
Trifucophlorethol	$C_{30}H_{22}O_{15}$	622	621.0899	**603**, 577, 559, 497, 477	585, 559, 477	Lopes et al. (2018)
Trifucophlorethol	$C_{30}H_{22}O_{15}$	622	621.0883	**603**, 495	585, 463, 477, 459	Lopes et al. (2018)
Fucotriphlorethol	$C_{30}H_{22}O_{15}$	622	621.0902	**603**, 495, 461, 355	461, 355	Lopes et al. (2018); Vissers et al. (2017)
Fucotriphlorethol	$C_{30}H_{22}O_{15}$	622	621.0887	**603**, 479, 461, 353	479, 335	Lopes et al. (2018)
Fucotriphlorethol	$C_{30}H_{22}O_{15}$	622	621.0885	**603**, 479, 461, 353	461, 353, 335	Lopes et al. (2018)
Fucotriphlorethol	$C_{30}H_{22}O_{15}$	622	621.0901	**603**, 463, 339	477, 463, 339	Lopes et al. (2018)
Fucotriphlorethol	$C_{30}H_{22}O_{15}$	622	621.0900	**603**, 479, 337, 229	479, 339, 229	Lopes et al. (2018)
Fucotriphlorethol	$C_{30}H_{22}O_{15}$	622	621.0880	603, 477, 373, 207		Hermund et al. (2018)
Fucotriphlorethol	$C_{30}H_{22}O_{15}$	622	621.0891	603, 585, 479, 371, 353, 335, 229, 205		Hermund et al. (2018)
Fucotriphlorethol	$C_{30}H_{22}O_{15}$	622	621.0879	603, 585, 477, 371, 245, 205		Hermund et al. (2018)
Fucotriphlorethol	$C_{30}H_{22}O_{15}$	622	621	495, 373, 355, 263, 247, 231		Li et al. (2017); Vissers et al. (2017)
Pentaphlorethol	$C_{30}H_{22}O_{15}$	622	621.5	603, 493, 357, 245		Montero et al. (2016)
Deshydroxypentafuhalol	$C_{30}H_{22}O_{16}$	638	637	633, 385, **247**		Agregán et al. (2017)
Deshydroxypentafuhalol	$C_{30}H_{22}O_{16}$	638	637.1	621, 513, 385, 262		Montero et al. (2016)
Deshydroxypentafuhalol	$C_{30}H_{22}O_{16}$	638	637.3	623, 513, 373		Montero et al. (2016)
Deshydroxypentafuhalol	$C_{30}H_{22}O_{16}$	638	637.5	633, 513, 273		Montero et al. (2016)
Deshydroxypentafuhalol	$C_{30}H_{22}O_{16}$	638	637.3	621, 513, 497, 389		Montero et al. (2016)
Deshydroxypentafuhalol	$C_{30}H_{22}O_{16}$	638	637	511, 388, 373, 265, 247		Li et al. (2017)
Trifuhalolhydroxycarmalol	$C_{30}H_{20}O_{17}$	652	651	632, 387, 265, 244		Li et al. (2017)
Fuhalol pentamer		652	651	607, 582, **509**, 465, 413, 339		Vissers et al. (2017)
Pentafuhalol	$C_{30}H_{22}O_{17}$	654	653	527, 513, 389, 263, 245		Li et al. (2017); Montero et al. (2016)
Pentafuhalol	$C_{30}H_{22}O_{17}$	654	653	637, 527, 513, 387, 263, 245		Montero et al. (2016)
Hydroxypentafuhalol	$C_{30}H_{22}O_{18}$	670	669.6	623, 527, 465, 403, 385, 341, 261		Montero et al. (2016)
Hydroxypentafuhalol	$C_{30}H_{22}O_{18}$	670	669.8	621, 541, 527, 463, 401, 337, 271		Montero et al. (2016)
Hydroxypentafuhalol	$C_{30}H_{22}O_{18}$	670	671.0	653, 637, 627, 544, 466, 247		Montero et al. (2016)
Hydroxypentafuhalol	$C_{30}H_{22}O_{18}$	670	669.0	651, 625, 607, 465, 403, 263		Montero et al. (2016)
Hydroxypentafuhalol	$C_{30}H_{22}O_{18}$	670	671.3	653, 637, 627, 467, 405, 349		Montero et al. (2016)

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Polymerization Degree/ Identification[a]	Molecular formula	Molecular mass	Precursor ion MS^1 $[M-H]^-$, m/z	Product ions MS^2 $[M-H]^-$, m/z[b]	Product ions MS^3 [(M-H) →base peak]$^-$, m/z[c]	References
Hexamers						
Dieckol	$C_{36}H_{22}O_{18}$	742	741	600, 389		Li et al. (2017)
Tetrafucophlorethol	$C_{36}H_{26}O_{18}$	746	745.1040	**727**, 601	709, 602, 585	Lopes et al. (2018)
Fucotetraphlorethol	$C_{36}H_{26}O_{18}$	746	745.1050	**727**, 601, 461, 335, 229	601, 583, 479, 353, 229	Lopes et al. (2018)
Fucotetraphlorethol	$C_{36}H_{26}O_{18}$	746	745.1048	**727**, 601, 479, 353, 229	601, 583, 461, 335, 229	Lopes et al. (2018)
Fucophlorethol hexamer	$C_{36}H_{26}O_{18}$	746	745.1058	727, 477, 311, 205		Hermund et al. (2018)
Fucophlorethol hexamer	$C_{36}H_{26}O_{18}$	746	745.1046	727, 619, 585, 477, 205		Hermund et al. (2018)
Fucophlorethol hexamer	$C_{36}H_{26}O_{18}$	746	745.1049	727, 709, 619, 583, 477, 203		Hermund et al. (2018)
Fucophlorethol hexamer	$C_{36}H_{26}O_{18}$	746	745	603, 497, 478, **371**, 355, 229		Vissers et al. (2017)
Fucophlorethol hexamer	$C_{36}H_{26}O_{18}$	746	745	619, **601**, 479, 461 355		Vissers et al. (2017)
Hexaphlorethol	$C_{36}H_{26}O_{18}$	746	745.3	727, 619, 603, 371, 355, 309		Montero et al. (2016)
Hexaphlorethol A	$C_{36}H_{26}O_{18}$	746	745	727, 621, 461, 339, 265, 247		Li et al. (2017)
Hexafucol	$C_{36}H_{26}O_{18}$	746	745	**709**, 601, 579, 455, 437, 289		Vissers et al. (2017)
Hexafucol	$C_{36}H_{26}O_{18}$	746	745	619, **601**, 497, 479, 353, 335, 229		Vissers et al. (2017)
Deshydroxyhexafuhalol	$C_{36}H_{26}O_{19}$	762	761	635, 621, 512, 387, 355, 263		Li et al. (2017)
Deshydroxyhexafuhalol	$C_{36}H_{26}O_{19}$	762	761.6	637		Montero et al. (2016)
Deshydroxyhexafuhalol	$C_{36}H_{26}O_{19}$	762	761.3	745, 637, 498, 389, 245		Montero et al. (2016)
Deshydroxyhexafuhalol	$C_{36}H_{26}O_{19}$	762	761.3	747, 637, 621, 513, 497, 245		Montero et al. (2016)
Deshydroxyhexafuhalol	$C_{36}H_{26}O_{20}$	778	777	529, **387**, 375		Agregán et al. (2017)
Deshydroxyhexafuhalol	$C_{36}H_{26}O_{20}$	778	777	529, **375**		Agregán et al. (2017)
Deshydroxyhexafuhalol	$C_{36}H_{26}O_{20}$	778	777	636, 513, 402, 387, 245		Li et al. (2017)
Deshydroxyhexafuhalol	$C_{36}H_{26}O_{20}$	778	777.7	651, 637, 529, 511, 387, 261, 245		Montero et al. (2016)
Deshydroxyhexafuhalol	$C_{36}H_{26}O_{20}$	778	777.3	763, 655, 529, 515, 388		Montero et al. (2016)
Fuhalolhydroxycarmalol		792	791	747, 385, 356, 261		Li et al. (2017)
Hexafuhalol B	$C_{36}H_{26}O_{21}$	794	793	667, 529, 403, 387, 263		Li et al. (2017)
Hexafuhalol	$C_{36}H_{26}O_{21}$	794	793. 1	775, 731, 651, 527, 511, 403, 387		Montero et al. (2016)
Hexafuhalol	$C_{36}H_{26}O_{21}$	794	793.7	777, 652, 589, 554, 511, 390, 311		Montero et al. (2016)
Hexafuhalol	$C_{36}H_{26}O_{21}$	794	793.3	667, 653, 529, 403, 387, 263		Montero et al. (2016)
Hexafuhalol	$C_{36}H_{26}O_{21}$	794	793.2	775, 749, 731, 527, 511, 483, 387, 245		Montero et al. (2016)
Hydroxyhexafuhalol	$C_{36}H_{26}O_{22}$	810	809.5	791, 775, 637, 511, 387		Montero et al. (2016)
Hydroxyhexafuhalol	$C_{36}H_{26}O_{22}$	810	809.7	791, 765, 747, 667, 543, 527, 405		Montero et al. (2016)

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Heptamers						
Fucophlorethol heptamer	$C_{42}H_{30}O_{21}$	870	869.1238	851, 833, 727, 601, 204		Hermund et al. (2018)
Fucophlorethol heptamer	$C_{42}H_{30}O_{21}$	870	869.1198	851, 744, 619, 583		Hermund et al. (2018)
Heptafucol	$C_{42}H_{30}O_{21}$	870	869	**833**, 708, 579, 455		Vissers et al. (2017)
Heptaphlorethol	$C_{42}H_{30}O_{21}$	870	869	**833**, 743, 725, 707, 619, 601, 495, 477, 371, 355, 335		Vissers et al. (2017)
Heptaphlorethol	$C_{42}H_{30}O_{21}$	870	869	**743**, 725, 477, 355		Vissers et al. (2017)
Heptaphlorethol	$C_{42}H_{30}O_{21}$	870	869.2	851, 745, 728, 306, 245		Montero et al. (2016)
Deshydroxyheptafuhalol	$C_{42}H_{30}O_{23}$	902	901	637, 635, 527, 513, 387, 262		Li et al. (2017)
Deshydroxyheptafuhalol	$C_{42}H_{30}O_{23}$	902	901.8	857, 775, 761, 637, 511, 387		Montero et al. (2016)
Fuhalolhydroxycarmalol		916	915	791, 681, 652, 387, 263		Li et al. (2017)
Heptafuhalol	$C_{42}H_{30}O_{24}$	918	917	785, 653, 527, 387, 373		Li et al. (2017)
Heptafuhalol	$C_{42}H_{30}O_{24}$	918	917.1	897, 873, 791, 777, 731, 653, 527, 389		Montero et al. (2016)
Heptafuhalol	$C_{42}H_{30}O_{24}$	918	917.3	900, 874, 856, 714, 634, 513		Montero et al. (2016)
Hydroxyheptafuhalol	$C_{42}H_{30}O_{25}$	934	933	914		Agregán et al. (2017)
Hydroxyheptafuhalol	$C_{42}H_{30}O_{25}$	934	933.8	889, 793, 747, 651, 525, 385		Montero et al. (2016)
Hydroxyheptafuhalol	$C_{42}H_{30}O_{25}$	934	933.4	914, 889, 792, 748, 650, 529		Montero et al. (2016)
Hydroxyheptafuhalol	$C_{42}H_{30}O_{25}$	934	933	914, 871, 773, 667, 651, 623, 511		Montero et al. (2016)
Octamers						
Phloroglucinol octamer	$C_{48}H_{34}O_{24}$	994	993	373		Agregán et al. (2017)
Fucophlorethol octamer	$C_{48}H_{34}O_{24}$	994	993	**957**, 849, 831, 709, 603, 353		Vissers et al. (2017)
Fucophlorethol octamer	$C_{48}H_{34}O_{24}$	994	993	**957**, 832, 371		Vissers et al. (2017)
Deshydroxyoctafuhalol	$C_{48}H_{34}O_{25}$	1010	1009.2	994, 968, 887, 872, 747, 621		Montero et al. (2016)
Deshydroxyoctafuhalol	$C_{48}H_{34}O_{27}$	1042	1041	901, 777, 653, 621, 527, 513, 387, 263		Li et al. (2017)
Deshydroxyoctafuhalol	$C_{48}H_{34}O_{27}$	1042	1041.3	979, 915, 901, 853, 777, 731, 651, 637, 528, 389		Montero et al. (2016)
Octafuhalol	$C_{48}H_{34}O_{28}$	1058	1057	917, 793, 543, 527, 262		Li et al. (2017)
Octafuhalol	$C_{48}H_{34}O_{28}$	1058	1057.2	1008, 915, 793, 652, 527, 387		Montero et al. (2016)

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Nonamers						
Fucophlorethol nonamer	$C_{54}H_{38}O_{27}$	1118	1117	**1081**, 973, 849, 833, 707, 353		Vissers et al. (2017)
Fucophlorethol nonamer	$C_{54}H_{38}O_{27}$	1118	1117	1081, 993, **973**, 745, 727, 709, 621, 603, 583, 495, 459, 353		Vissers et al. (2017)
Nonaphlorethol	$C_{54}H_{38}O_{27}$	1118	1117	**1081**, 956, 745, 727, 621, 603, 582, 497, 477, 371, 351		Vissers et al. (2017)
Deshydroxynonafuhalol	$C_{54}H_{38}O_{28}$	1134	1133.9	1115, 1007, 993, 885, 869, 760, 745, 620		Montero et al. (2016)
Deshydroxynonafuhalol	$C_{54}H_{38}O_{30}$	1166	1165.7	1146, 1040, 1025, 917, 899, 777, 653, 637, 389		Montero et al. (2016)
Decamers						
Fucophlorethol decamer	$C_{60}H_{42}O_{30}$	1242	1241	**1205**, 1097, 1079, 975, 745, 727, 601, 495		Vissers et al. (2017)
Decaphlorethol	$C_{60}H_{42}O_{30}$	1242	1241	1205, 1097, 1079, **745**, 727, 601, 477		Vissers et al. (2017)
Deshydroxydecafuhalol	$C_{60}H_{42}O_{31}$	1258	1257.7	1239, 1133, 1117, 1007, 885, 624, 573, 387		Montero et al. (2016)

a Fuhalols nomenclature was taken from Keusgen and Glombitza (1995)

b *Common losses*. Firstly, it is important to consider that aryl-ether bonds (C-O-C), characteristics of phlorethols, are more susceptible to rupture than aryl-aryl linkages (C-C), characteristic of fucols: 124, 125, 126 amu (phloroglucinol unit); 18 amu (water); 44 amu (ethylene and water); 62 (44+ 18); 140 (124+ 16); 142 (126+ 16/ 124+ 18); 144 (126+ 18); 158 (124+ 16+ 18); 160 (126+ 16+ 18/ 124+ 18+ 18); 170: 126 + 44; 248 (124+ 124); 250 (126+ 124); 262 (124+ 124+ 14); 264 (124+ 124+ 16); 266 (124+ 124+ 18); 268 (126+ 126+ 16/ 124+ 126+ 18); 282 (124+ 124+ 18 +16); 284 (124+ 124+ 18+ 18); 286 (124+ 126+ 18+ 18); 374 (124+ 124+ 126); 376 (126+ 126+ 124); 392 (124+ 124+ 126+ 18; 410 (126+ 126+ 126+ 16+ 16).

c Ions derived from the fragmentation of the most abundant ion in MS^1.

d Most abundant ions are shown in bold.

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Figures Captions

Figure 1. Chemical structure of phloroglucinol and examples of phlorotannins for each of the six major groups identified to date. A: Phloroglucinol monomeric unit; B: Trifucol; C: Tetraphlorethol B; D: Fucodiphlorethol A; E: Pentafuhalol B; F: Diphlorethohydroxycarmalol; G: Eckol; H: Dieckol.

Figure 2. Commonly used extraction and purification methods for obtaining phlorotannins from brown seaweeds. The flowchart presents the whole extraction/purification process from the dry alga to the isolated compound. Classical methods are shown on the left boxes and alternative methods on the right ones. In brackets are the most utilized resources (e.g., solvent, solid phase) in each technique. Circles indicate phlorotannins characterization and identification methods. Aox: antioxidant. See the text for the meaning of acronyms.

Figure 3~~1~~. Suggested mechanisms of action of phlorotannins in controlling hyperglycaemia and diabetes-related oxidative stress in the human body, according to *in vitro* and animal assays. The scheme represents the main stages and organs involved in high-carbohydrate meal processing. I: Digestion of polysaccharides in the mouth; II: Digestion of oligosaccharides in the gut; III: Absorption of glucose to the bloodstream; IV: Delivery of glucose to tissues and organs and assimilation of glucose enabled by insulin. ~~Essential e~~Enzymes~~involved in both processes~~ modulated by phlorotannins are enclosed in circles. ~~(Kawamura-Konishi et al., 2012; S. H. Lee et al., 2014; Moon et al., 2011).~~ CH-CH: polysaccharides; CH: oligosaccharides; ◎:up-regulated by phlorotannins; ⊗:down-regulated by phlorotannins. See the text and the glossary for the meaning of acronyms. Kawamura-Konishi et al., 2012; S. H. Lee et al., 2014; Moon et al., 2011.

Figure 4~~2~~. General ~~Main~~ cancer-associated targets of phlorotannins, according to cell-based and animal assays. The figure schematizes the ~~R~~reported effects of phlorotannins against cancer hallmarks –uncontrollable cell proliferation, angiogenesis and invasion~~ are represented~~. I: Induction of apoptosis (dieckol, phloroglucinol, phlorofucofuroeckol A, dioxinodehydroeckol, eckol); II: Inhibition of angiogenesis and invasion~~ to other tissues~~ (dieckol, phloroglucinol); III: Sensitization of cancer stem-like cells to drugs (phloroglucinol, eckol); IV: Activation of the innate and adaptative immune responses (eckol). Casp: caspase; $\Delta\psi$m: mitochondria membrane depolarization; Black ~~Dark blue~~ dots: cytochromes C; Blue dots: Th1-type cytokines; ◎: up-regulated by phlorotannins; ⊗: down-regulated by phlorotannins. See the text and the glossary for the meaning of acronyms.

Con formato: Ancho: 21,59 cm, Alto: 27,94 cm

Con formato: Francés (Francia)

Con formato: Sangría: Izquierda: 0 cm, Sangría francesa: 2,5 cm