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Proceeding Paper

Evidence and Perspectives on the Use of Phlorotannins as Novel Antibiotics and Therapeutic Natural Molecules †

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Abstract: Multiple drug-resistant bacterial strains are showing new different mechanisms to overcome the antimicrobial action which reduces the efficacy of conventional antibiotics [1]. Therefore, drug discovery research has focused on developing fast, effective and safe alternatives to prevent this multiresistance. Phlorotannins are a diverse class of polyphenols, secondary metabolites described in brown algae, that are mainly constituted of polymers of phloroglucinol and depending on their linkage and structure can be classified mainly as fucols, fucophlorethols, eckols and phloroethols [2]. These polyphenols have been described in both macro- and microalgae, suggesting that they can be recovered from a great variety of sources [2]. Phlorotannins have been extensively described to possess several biological properties, foremost as antioxidant and antimicrobial compounds. Several in vitro reports have described that phlorotannins showed growth inhibition and bactericidal effects against Gram + (e.g., *Bacillus cereus*, *Streptococcus epidermidis*, *Staphylococcus aureus*) and Gram – bacteria (e.g., *Salmonella* sp., *Campylobacter jejuni*, *Pseudomonas aeruginosa*), also including antibiotic-resistant strains like MRSA [3]. Although the mechanisms of action of this group of compounds has not been fully elucidated, tannins could interact with membrane proteins and key metabolic enzymes, impeding bacterial growth and resulting in membrane lysis [3,4]. Moreover, different phlorotannins were able to inhibit bacterial biofilm formation, production of quorum-sensing molecules, and also viral replication (*e.g.*, influenza) [5,6]. Few in vivo studies support their effectiveness as antibiotics, whereas clinical trials studying other properties, consistently report high bioavailability and null toxicity of phlorotannins [6,7]. Considering current evidence, phlorotannins could be considered as interesting candidates for antibiotic therapy clinical trials. The diversity of these natural compounds provides a promising gateway for researchers and the pharmaceutical industry to develop novel nontoxic, cost-effective and highly efficient antibacterial formulations with a broad scope of applications.

Keywords: algae; phlorotannins; antibiotics; antioxidant

1. Introduction

Antibiotic resistance has become the topmost threat to public health in the 21st century, with more than 40 countries shared reports on antimicrobial resistance, which signifies criticality [8]. As such, there is a need for alternative compounds and treatments that may function as antibiotics in order to deal with this issue. In the last decade, research in natural compounds from plant and algae sources has rapidly increased, with a great number of natural molecules characterized and described. In this context, marine algae, a traditional food and medicinal East-Asian ingredient, have proven to be an excellent source of natural molecule with numerous potential and effective applications in human health [1,3]. In specific, brown algae possess some unique natural compounds with various bioactive properties, such as polysaccharides (laminaran, fucoidan, alginate), lectins, alkaloids, or polyphenols, such as phlorotannins (PT) [1,9]. PT are composed of polymeric units of phloroglucinol (1,3,5-trihydroxybenzene), with molecular weights ranging between 126-650 kDa. They are classified into six major groups, according to the type of linkages between phloroglucinol units and the number of hydroxyl groups (**Error! Reference source not found.**) [2,10]:

(i) *fucols*, with aryl-aryl linkages;
(ii) *phlorethols*, with aryl-ether linkages;
(iii) *fucophlorethols*, with aryl-aryl and aryl-ether units;
(iv) *fuhalols*, with aryl-ether linkages and additional hydroxyl groups in every third ring;
(v) *carmalols*, with a dibenzodioxin moiety and derived from phlorethols;
(vi) *eckols*, which possess at least one three-ring moiety with a dibenzodioxin element substituted by a phenoxyl group at C-4.

Due to their polymeric structure and number of hydroxyl groups, PT are potent free-radical scavengers and can modulate proteins and chelate metals. These capacities explain the wide range of cellular and ecological roles of phlorotannins in seaweeds. Moreover, PT are herbivore deterrents and protect against desiccation, high UVB radiation, and toxic heavy metals, acting as chelators [6,7]. These compounds have been classified as generally regarded as safe (GRAS) substances by both the Food and Drug Administration (FDA) and the European Food Safety Agency (EFSA) [11,12]. In the EU, phlorotannin-rich extracts from *Ecklonia cava* are approved as a "novel food" and considered safe to consume, due to an great number of studies supporting their safety [12].

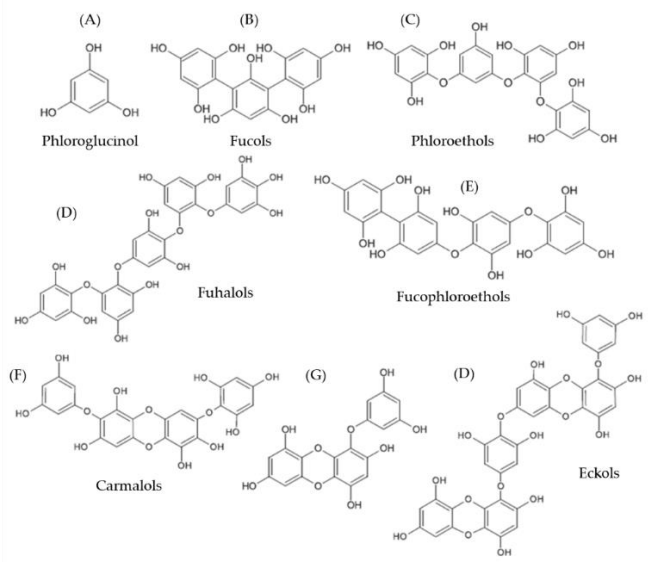

Figure 1. Chemical structure of representative phlrotoannin groups. (**A**) Phloroglucinol monomer; (**B**) Trifucol; (**C**) Tetraphlorethol B; (**D**) Pentafuhalol B; (**E**), Fucodiphlorethol; (**F**) Diphlorethohydroxycarmalol; (**G**) Eckol; (**H**) Dieckol. Adapted from Erpel et al. [10].

Applications of PT, however, are currently limited as antioxidant cosmetic ingredients, or nutraceuticals for metabolic modulation, as there is a growing body of evidence supported by regulators, suggesting that these marine polyphenols are safe and non-toxic. In addition, several reports suggest their antimicrobial potential. Herein, the potential use of PT as antibiotic compounds based on scientific evidence is briefly reviewed.

2. Scientific Studies on Antimicrobial and Antiviral Effects of Phlorotannins

PT antimicrobial potential has mainly been studied through in vitro assays, for which screening studies made on brown algae usually employ representative nosocomial Gram + and Gram- bacteria, such as *Staphylococcus aureus*, or *Pseudomonas aeruginosa*, which usually present antibiotic resistance (Table 1). PT extracts from *Sargassum* species have demonstrated varying microbial growth inhibition on *P. aeruginosa*, *S. aureus*, and also in other pathogenic species such as *Vibrio parahaemolyticus* and the facultative pathogen *Escherichia coli* [6,13,14]. The brown algae *Ecklonia cava* and *Eisenia bicyclis* are also some of the most studied algae as source of PT, since these contain eckols, such as dieckol or phlorofucofuroeckol-A. These purified compounds were reported to display inhibitory and bactericidal effects on relevant gastrointestinal pathogenic bacteria such as *Campylobacter jejuni*, *Salmonella typhimurium*, or *S. typhi* at concentrations below 200 μg/mL [15,16]. Moreover, these eckols have been studied to reduce or nullify antibiotic resistance in some bacterial strains. For example, phlorofucofuroeckol-A from *E. bicyclis* downregulated the Penicillin-binding protein 2a gene, related to methicillin resistance in *S. aureus* exhibiting a minimum inhibitory concentration (MIC) of 16 μg/mL [17]. Likewise, fucofuroeckol-A from the same brown alga reduced biofilm formation and virulence of Streptomycin-resistant *Listeria monocytogenes*, with a MIC of 16 μg/mL [18]. In addition to these results, a few reports indicate that PT may also act as antiviral agents, as phlorofucofuroeckol-A from *E. cava* was described to greatly inhibit various strains of influenza [5], and dieckol to almost nullify human immunodeficiency virus (HIV) replication [19].

Table 1. Antimicrobial and antiviral activity of phlorotannins extracted from marine brown algae.

Algae Source	Compound/Extract	Microbial Strains	Main Findings	Ref.
Sargassum fusiforme	Commercial phlorotannin extract	*Pseudomonas aeruginosa, Staphylococcus aureus, Vibrio parahaemolyticus, Aeromonas hydrophila*	MIC = 97 mg/mL Reduction of *P. aeruginosa* virulence and biofilm formation at 6 mg/mL	[6]
Sargassum thunbergii	PT extract	*Vibrio parahaemolyticus*	MIC = 0.9 mg/mL	[13]
Sargassum muticum	PT extract	*Pseodomonas aeruginosa, Escherichia coli*, HSV-1	5 mg/mL extract achieved 43.7% inhibition in biofilm formation for *P. aeruginosa* and 64.3% for *E. coli*. EC_{50} (HSV-1) = 0.2 mg/mL	[14]
Ascophyllum nodosum	PT extract	*Salmonella agona, Streptococcus suis, Escherichia coli*	MBC (*E. coli, S. agona*) = 3.1 mg/mL MBC (*S. suis*) = 1.56 mg/mL	[20]
Fucus serratus			MBC (All) = 6.25 mg/mL	
Fucus vesiculosus	PT extract	MRSA	100 μg/mL showed a 57.6% inhibition	[21]
	PT extract	*Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Pseudomonas aeruginosa*	20 pg/mL achieved an average 61.3% inhibition, which was higher for Gram+	[22]
Ishige okamurae	Diphlorethohydroxycarmalol	*Staphylococcus aureus, Pseudomonas aeruginosa*	MBC (*S. aureus*) = 512 μg/mL MBC (*P. aeruginosa*) = 256 μg/mL	[23]
Ecklonia cava	Dieckol, 8,8′-dieckol	MRSA, *Bacillus cereus, Campylobacter jejuni, Escherichia coli,*	MBC = 0-03-0.54 μmol/mL against all strains, being *C. jejuni* the most susceptible	[15]

		Streptococcus epidermidis, Salmonella typhimurium, Vibrio parahaemolyticus		
	Phlorofucofuroeckol A	Human Influenza H1N1, A/PR/8/34, H9N2	MIC = 13.48 μg/mL	[5]
	Dieckol	HIV-1	37.1 μg/mL inhibited viral replication by 80%	[19]
	Eckol	MRSA, *Salmonella typhimurium, S. typhi, S. paratyphi, S. gallinarium, S. enteritidis*	MIC = 250 μg/mL; synergy if combined with ampicillin (MIC = 150 μg/mL)	[16]
	Eckol, phlorofucofuroeckol-A, PT extract	VHSV	EC_{50} (eckol) = 1.97 μg/mL EC_{50} (phlorofucofuroeckol-A) = 0.99 μg/mL EC_{50} (PT extract) = 4.76 μg/mL 1 mg/g bw daily oral administration increased survivability of fish by >40%	[24]
Eisenia bicyclis	Phlorofucofuroeckol-A	MRSA	MIC = 16 μg/mL; down-regulation of PBP2a conferring MR	[25]
	Phlorofucofuroeckol-A, fucofuroeckol-A, dioxinodehydroeckol	*Propionibacterium acnes, Staphylococcus aureus, Streptococcus epidermidis, Pseudomonas aeruginosa*	MIC = 126 μg/mL, synergistic bactericidal activity in combination with tetracycline	[17]
	Phlorofucofuroeckol-A, dieckol	Murine norovirus	MIC = 0.9 μg/mL; Phlorofucofuroeckol-A showed a higher selectivity index	[26]
	Fucofuroeckol-A	Streptomycin-R *Listeria monocytogenes*	MIC = 16 μg/mL	[18]

Abbreviations: MIC, Minimum inhibitory concentration; MBC, Minimum bactericidal concentration; EC_{50}, Half-maximal effective concentration; MRSA, Methicillin-resistant *Staphylococcus aureus*; HSV-1, Herpes simplex virus-1; VHSV, Hemorrhagic septicemia virus; PBP2a, Penicillin-binding protein 2a (PBP2a).

3. Discussion and Future Perspectives

Evidence regarding PT as antioxidants and metabolic and inflammatory modulators is vast, with a great number of studies confirming these properties by in vivo studies and clinical trials [10,27]. However, research on their potential applications as antibiotics is still limited, mainly due to the scope of reported studies (number of PT and/or microorganisms tested) and the fact that many of such works have been focused on in vitro studies, which unfortunately may not be translated to effective results in animal models or clinical trials. Nevertheless, the reported inhibitory and bactericidal effects of these molecules, specially purified compounds (e.g., dieckol, phlorofucofuroeckol-A) should be considered.are effective at low concentrations. Since PT have shown antioxidant, photoprotective and anti-inflammatory activities, these could be excellent ingredients for topical antibiotic formulations and cosmetics. Moreover, PT are reported to inhibit *quorum sensing* and limit biofilm formation, which suggests that these compounds could contributed to reduce topical or gut infections [6,15].

Two main factors should be considered regarding potential effectiveness of PT: (1) their generally poor bioavailability and (2) their binding effectiveness to microbial pathogens. Polyphenols and specially highly-polymerized have shown very poor bioavailability (around 5–10% are absorbed), and thus, encapsulation strategies are usually developed [28]. Notably, it has also been described that PT may act as prebiotics but research on this still appears limited, and microbiota alterations based on gut infections and changes due to PT treatment should be further explored [29]. On the other hand, it has been suggested that PTs, similarly to other polyphenols, could interact with cell membrane proteins or metabolic enzymes, precipitating them and disrupting the membrane integrity [3]. This fact was reported as the main factor liable of membrane lysis in MRSA, which also acted in synergy with methicillin [25]. Altogether, despite much needed further research, there

is founding evidence suggesting the potential of PT as an effective antimicrobial and antiviral compounds, among other demonstrated properties.

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