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Review: Bioactive compounds and health applications of *Digenea simplex* (Rhodophyta)

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Abstract. *Saputra AF, Utomo AN, Pramesthi AZ, Madjid AA, Nugroho GD. Setyawan AD. 2025. Review: Bioactive compounds and health applications of* Digenea simplex *(Rhodophyta). Cell Biol Dev 8: 90-108. Digenea simplex* (Rhodophyta), a red macroalga widely distributed in tropical and subtropical coastal waters, has gained attention due to its unique chemical profile. However, scientific studies on this species remain scattered and limited. This review aims to consolidate current knowledge on the bioactive compounds present in *D. simplex*, their bioactivities, and their potential applications in various industries. Phytochemical investigations have revealed that *D. simplex* contains a variety of secondary metabolites, including kainic acid, dimethylallyl pyrophosphate, carotenoids (such as astaxanthin), essential amino acids, and tannins. These compounds have demonstrated several pharmacological effects, including antioxidant, antidiabetic, antitumor, antimicrobial, and anti-inflammatory activities. The presence of kainic acid, for instance, highlights the neuroactive potential of the species, while carotenoids and phenolic compounds contribute significantly to its antioxidant capacity. In addition, the nutritional profile of *D. simplex*, comprising proteins, dietary fibers, vitamins, and minerals, supports its use as a functional food ingredient. The cosmetic industry also benefits from its bioactive constituents, which exhibit moisturizing, anti-aging, and skin-protective properties. Despite its promising profile, further studies are necessary to standardize extraction methods, evaluate toxicological safety, and elucidate the mechanisms of action of its bioactive compounds. This review provides a comprehensive reference for future research and valorization of *D. simplex* as a valuable marine resource for health-related applications.

Keywords: Bioactive compounds, *Digenea simplex*, marine natural products, red algae

INTRODUCTION

Marine ecosystems, covering around 70% of the Earth's surface, represent the planet's most extensive and diverse habitat (Dayanidhi et al. 2021). Among their components, red algae (Rhodophyta) are especially valued for their pigments, nutrients, and secondary metabolites with pharmacological and industrial significance (Bhuyar et al. 2021; Kumar and Arunkumar 2023). These include polysaccharides, flavonoids, alkaloids, and terpenoids with antioxidant, antimicrobial, and anti-inflammatory properties (Fernando et al. 2016; Agatonovic-Kustrin and Morton 2018). Sulfated polysaccharides like carrageenan are especially notable for their biomedical and cosmetic uses (Cicinskas et al. 2020; Sahin and Ozturk 2021; Aboeita et al. 2022; Abd El-Aziz et al. 2023).

One species of interest is *Digenea simplex* (Wulfen) C.Agardh (Rhodomelaceae), found in tropical and subtropical intertidal zones (Betancor et al. 2015; Boo et al. 2018). Known for its red pigments—phycobiliproteins and carotenoids (Schneider et al. 2018; Gljušćić et al. 2022)—*D. simplex* has long been used in Asian traditional medicine, though scientific research remains limited (Chekan et al. 2019; Chen et al. 2020). A key compound, kainic acid, is widely used in neuroscience to model epilepsy and study neurotoxicity (Connell et al. 2017; Maeno et al. 2019; Asakawa et al. 2020), and may function as a natural defense molecule (Vezzani et al. 2016; Jiang et al. 2018).

In addition, *D. simplex* participates in isoprenoid biosynthesis via Dimethylallyl Pyrophosphate (DMAPP), yielding essential pigments like chlorophyll and carotenoids such as astaxanthin and β-carotene—potent antioxidants with protective cellular roles (Sathasivam and Ki 2018; Chekan et al. 2019; Ganley et al. 2020; El-Malek et al. 2022; Dini 2022; Bayomy and Alamri 2024). Nutritionally, *D. simplex* offers essential amino acids, fibers, vitamins, and minerals (Chalid 2010; Ward and Deyab 2021), with tannins contributing to its antioxidant and anti-herbivore defenses (Ibraheem et al. 2017; El-Rafie et al. 2023).

Red algae, including *D. simplex*, also demonstrate antidiabetic activity due to compounds like phlorotannins and sulfated polysaccharides that inhibit enzymes linked to glucose metabolism (Chia et al. 2018; Van Weelden et al. 2019; Luthuli et al. 2019; Pradhan et al. 2020). This supports the search for safer, natural antidiabetic agents (Alam et al. 2021; Hasan et al. 2022). However, despite its potential, *D. simplex* remains underexplored. Existing studies are often fragmented, lack standardized methods, and provide limited toxicity or mechanism data (Alves et al. 2018; Dhanalakshmi and Jayakumari 2018).

This review aims to synthesize available knowledge on the phytochemistry, bioactivities, and applications of *D. simplex*, emphasizing its value and identifying research gaps. With further study, this species could offer novel compounds and support sustainable development in health-related industries.

TAXONOMY AND MORPHOLOGY

The genus *Digenea*, classified under the Rhodomelaceae family (Ceramiales, Florideophyceae, Rhodophyta), was traditionally considered monotypic, with *Digenea simplex* as its only recognized species. However, recent molecular phylogenetic studies have identified additional taxa, such as *Digenea mexicana* G.H.Boo and D.Robledo and *Digenea rafaelii* G.H.Boo, G.Andrade-Sorcia and S.M.Boo, highlighting the need for a taxonomic reassessment using DNA-based tools. Historically, the identification of *D. simplex* relied heavily on external morphological traits like branching patterns and pigmentation, which are often influenced by environmental factors. This phenotypic plasticity has complicated species delimitation, underscoring the importance of combining morphological, anatomical, and molecular data for accurate classification. The absence of preserved reference specimens in older records further hinders current taxonomic resolution.

Morphologically, *D. simplex* features a prostrate base with discoid holdfasts and an erect, pseudo-dichotomously branched structure forming tufts or mats (Figure 1). This arrangement enhances mechanical stability and light acquisition in high-energy intertidal zones. The thallus is composed of central axial cells encircled by pericentral and cortical cells, contributing to structural integrity and herbivore defense. A mucilaginous surface layer protects against desiccation and microbial intrusion, while pigmentation dominated by phycoerythrin and carotenoids (e.g., astaxanthin, lutein) aids in photosynthesis and antioxidant activity. The cell wall is composed primarily of cellulose and sulfated galactans such as carrageenan, which enhance flexibility, osmotic tolerance, and commercial value. Reproductive elements, including tetrasporangia (asexual) and cystocarps (sexual), are consistent with Florideophyceae characteristics and crucial for life cycle and species recognition. These morphological and biochemical features reflect the species' successful adaptation in dynamic coastal ecosystems and support its promise for future biotechnological applications.

HABITAT AND DISTRIBUTION

Digenea simplex is commonly found in tropical and subtropical coastal regions, typically inhabiting intertidal and shallow subtidal environments with rocky substrates (Betancor et al. 2015). It adheres firmly to hard surfaces such as rocks, coral rubble, and tide pool walls, where it forms dense mats or tufts. These habitats are characterized by moderate to high wave action, periodic exposure to sunlight, and fluctuating salinity, requiring physiological adaptations such as mucilage production and UV-resistant pigments (Boo et al. 2018; Gljušćić et al. 2022). In many locations, *D. simplex* coexists with other macroalgal species, such as *Cystoseira humilis*, forming complex intertidal algal communities (Betancor et al. 2015).

Historically, *D. simplex* was first described in the Adriatic Sea near Trieste in the early 19th century, but subsequent reports have recorded its occurrence across the Atlantic, Indian, and Pacific Oceans (Collins et al. 1913; Boo et al. 2018). It is commonly found along the coasts of the Canary Islands, Bermuda, the Mediterranean, the Red Sea, and Southeast Asia, including Indonesia and the Philippines (Dumilag et al. 2022). The species is known for its broad pantropical distribution, though detailed molecular and morphological studies suggest it may represent a complex cryptic species rather than a single cosmopolitan taxon (Díaz-Tapia et al. 2017; Boo et al. 2018). As such, modern records of distribution should be interpreted with caution unless supported by voucher specimens or DNA barcoding.

Environmental conditions strongly influence the local abundance and vertical distribution of *D. simplex*. It is typically most abundant in shallow rock pools, especially in areas with high light availability and moderate water movement (Betancor et al. 2015). The species can occur from the upper subtidal zone down to depths of approximately 4 meters, depending on light penetration and substrate availability. In intertidal pools, *D. simplex* demonstrates strong resilience to desiccation and salinity fluctuations, partly due to its thick mucilaginous coating and antioxidant pigment systems such as phycoerythrin and carotenoids (Fernando et al. 2016; Sathasivam and Ki 2018), which enable it to demonstrate strong resilience to desiccation and salinity fluctuations. These adaptations allow it to maintain photosynthetic activity and structural integrity in environments with highly variable abiotic conditions, showcasing its remarkable adaptability.

Figure 1. *Digenea simplex* (Schneider et al. 2018)

Anthropogenic pressures such as coastal pollution, tourism, and habitat modification can significantly affect the distribution and health of *D. simplex* populations. Because it is sensitive to changes in water quality, particularly turbidity and nutrient enrichment, *D. simplex* has the potential to serve as a bioindicator of intertidal ecosystem health (Pereira 2015). Studies have also shown that macroalgae like *D. simplex* can accumulate heavy metals, making them useful in monitoring marine contamination (El-Rafie et al. 2023). However, excessive eutrophication or sedimentation can smother rocky substrates and reduce light availability, limiting its growth and distribution.

Seasonal variation may also influence the abundance and reproductive cycles of *D. simplex*. While it can be found year-round in tropical environments, peak growth and reproductive activity often occur during warmer months with higher irradiance (Zongo et al. 2022). In some regions, reproductive structures such as tetrasporangia and cystocarps have been observed during summer, suggesting temperature and photoperiod as key regulatory factors (Boo et al. 2018). These phenological patterns may vary geographically, emphasizing the need for region-specific ecological studies to inform conservation and cultivation efforts.

D. simplex occupies a broad but ecologically specific niche in warm, rocky coastal environments. Its distribution is influenced by a combination of abiotic factors (light, substrate, salinity, temperature) and biotic interactions (competition, herbivory), as well as anthropogenic impacts. The species' resilience to environmental stressors, combined with its ecological specificity, highlights its value both as a subject of scientific study and as a potential biomonitor for intertidal ecosystems.

CHEMICAL DIVERSITY

The biochemical profile of *Digenea simplex* comprises a wide spectrum of primary and secondary metabolites produced via amino acid, isoprenoid, and phenolic biosynthetic pathways, contributing to its physiological function, ecological adaptation, and biotechnological value. These include neuroactive amino acid derivatives, antioxidant pigments, isoprenoids, phenolics, and proteinaceous compounds, many of which have recognized therapeutic and functional food potential (Fernando et al. 2016; Agatonovic-Kustrin and Morton 2018; Bayomy and Alamri 2024), with kainic acid standing out as a well-studied neuroexcitatory compound of pharmacological significance.

Kainic Acid (KA)

Kainic acid is a naturally occurring pyrrolidine dicarboxylic acid first isolated from *D. simplex* in the mid-20th century and is one of the most well-known bioactive compounds derived from marine red algae (Chekan et al. 2019; Maeno et al. 2019). The compound exhibits strong neuroexcitatory activity due to its ability to act as an agonist of kainate-type ionotropic glutamate receptors in the central nervous system (Connell et al. 2017; Asakawa et al. 2020). Because of this activity, kainic acid has been extensively used in neuroscience research, particularly in the development of rodent models of epilepsy and excitotoxic neurodegeneration (Vezzani et al. 2016). Its structural uniqueness among marine-derived amino acids has made it a subject of interest not only for pharmacology but also for synthetic chemistry and neurobiology.

The biosynthesis of kainic acid in *D. simplex* involves the coupling of glutamic acid with a pyrrolidine ring, forming a highly polar molecule that mimics endogenous excitatory neurotransmitters (Chekan et al. 2019). Recent studies have identified key enzymes involved in its biosynthetic pathway, including *kabA* and *kabC*, which are part of a modular gene cluster unique to certain red algae (Chekan et al. 2019). This discovery has opened up new possibilities for the heterologous expression of kainic acid in microbial systems and synthetic biology platforms. The localization of kainic acid within algal tissues suggests its ecological role as a chemical defense against herbivory, deterring invertebrate grazers and possibly influencing microbial colonization (Jiang et al. 2018).

Although kainic acid is neurotoxic in mammalian systems at high concentrations, its potential therapeutic applications are under investigation. Studies have explored its effects on inducing seizures for anti-epileptic drug screening and in evaluating neuroprotective agents that can counteract excitotoxicity (Connell et al. 2017; Asakawa et al. 2020). Moreover, modified derivatives of kainic acid have been synthesized to reduce toxicity while preserving receptor selectivity, offering avenues for safer pharmaceutical development. The controlled use of kainic acid and its analogs may contribute to the treatment of disorders related to glutamatergic dysfunction, including epilepsy, Alzheimer's disease, and chronic pain (Vezzani et al. 2016; Chekan et al. 2019).

Despite its biomedical relevance, the natural abundance of kainic acid in *D. simplex* varies depending on geographical location, environmental stressors, and possibly life cycle stage (Chen et al. 2020). Most existing reports focus on algal populations in the Indo-Pacific and Mediterranean regions, with few comparative studies on seasonal or spatial variation in metabolite content (Betancor et al. 2015; Boo et al. 2018). There remains a need for comprehensive ecological and biochemical surveys to determine the environmental cues that regulate kainic acid production in natural populations. Such insights could inform both sustainable harvesting practices and the development of aquaculture strategies to optimize yields of this valuable compound.

Kainic acid thus represents a rare example of a marine algal metabolite with clearly defined pharmacological targets and widespread use in biomedical research. Its presence in *D. simplex* underscores the species' unique phytochemical profile and its relevance beyond ecological roles. Continued investigation into the biosynthesis, regulation, and biotechnological production of kainic acid will not only enhance our understanding of red algal chemistry but also expand the pharmacopeia of marine-derived neuroactive agents.

Dimethylallyl Pyrophosphate (DMAPP) and isoprenoids

Dimethylallyl Pyrophosphate (DMAPP) is a key precursor in the isoprenoid biosynthetic pathway, serving as a universal five-carbon building block for the synthesis of terpenoids, sterols, carotenoids, and other prenylated compounds (Ganley et al. 2020; Dini 2022). In red algae such as *Digenea simplex*, DMAPP is synthesized through the mevalonate (MVA) pathway or the Methylerythritol Phosphate (MEP) pathway, both of which contribute to the diversity of isoprenoid end-products (El-Malek et al. 2022). These pathways are tightly regulated and compartmentalized in plastids and cytosol, enabling algae to generate a wide spectrum of terpenoid metabolites critical for survival and interaction with their environment. The isoprenoid pathway is evolutionarily conserved and represents one of the most chemically diverse biosynthetic systems in nature.

In *Digenea simplex*, isoprenoid derivatives derived from DMAPP include carotenoids, phytol, sterols, and chlorophyll side chains, many of which play essential roles in photoprotection, membrane stabilization, and oxidative stress responses (Sathasivam and Ki 2018; Bayomy and Alamri 2024). Carotenoids such as β-carotene, lutein, and astaxanthin, for instance, are formed through sequential condensations involving DMAPP and Isopentenyl Pyrophosphate (IPP), catalyzed by prenyltransferases. These pigments not only contribute to the characteristic coloration of red algae but also exhibit potent antioxidant activity, making them valuable in nutraceutical and cosmetic applications (Fernando et al. 2016; Agatonovic-Kustrin and Morton 2018). Furthermore, isoprenoids serve as precursors for plant hormones and volatile organic compounds, which may have ecological signaling functions.

Biotechnological interest in DMAPP and isoprenoid biosynthesis from marine algae has grown in recent years due to the industrial relevance of terpenoids, particularly for pharmaceuticals, flavors, and biofuels (Ganley et al. 2020). Microbial production systems are now being engineered to express red algal enzymes involved in DMAPP synthesis, with the goal of producing high-value compounds such as artemisinin and taxadiene sustainably (El-Malek et al. 2022). Understanding the genetic regulation of DMAPP biosynthesis in algae like *D. simplex* is critical for optimizing these biotechnological platforms. Advances in genome sequencing and transcriptomic analyses may eventually uncover novel enzymes and regulatory elements unique to red algal isoprenoid pathways.

Ecologically, isoprenoids in *D. simplex* also function as chemical defenses, UV protectants, and osmoprotectants, supporting algal survival in intertidal habitats exposed to fluctuating salinity, temperature, and light (Betancor et al. 2015; Bayomy and Alamri 2024). The multifunctional nature of these compounds reflects their evolutionary significance and explains the metabolic investment required for their biosynthesis. Moreover, certain volatile isoprenoids derived from DMAPP may serve as allelopathic agents or chemical signals to neighboring organisms, although this role remains understudied in red algae. Future ecological studies are needed to clarify the role of isoprenoids in mediating species interactions and community dynamics in coastal ecosystems.

The presence and diversity of DMAPP-derived compounds in *D. simplex* emphasize the alga's rich metabolic plasticity and highlight its potential for bioprospecting in the field of marine natural products. Given the increasing demand for sustainable sources of bioactive terpenoids and pigments, *D. simplex* offers both ecological and industrial value. Detailed characterization of its isoprenoid pathway, supported by metabolomic and genomic tools, will pave the way for the development of novel compounds with therapeutic and commercial applications.

Figure 2.A illustrates the carotenoid biosynthetic pathway in *D. simplex*, starting from dimethylallyl pyrophosphate (DMAPP) and leading to astaxanthin. It emphasizes its role in antioxidant function and pigmentation.

Carotenoids

Carotenoids are lipid-soluble pigments derived from isoprenoid precursors, including dimethylallyl pyrophosphate (DMAPP), and are essential components of red algal metabolism (Fernando et al. 2016; Sathasivam and Ki 2018). In *D. simplex*, carotenoids contribute not only to the reddish pigmentation of the thallus but also to various physiological functions such as photoprotection, antioxidation, and membrane stabilization (Bayomy and Alamri 2024). The predominant carotenoids identified in red algae include astaxanthin, β-carotene, lutein, zeaxanthin, and violaxanthin, many of which have potent biological activities (Ganley et al. 2020; Dini 2022). These compounds are known to quench singlet oxygen and scavenge Reactive Oxygen Species (ROS), protecting cellular components from oxidative damage caused by UV radiation and environmental stressors.

Astaxanthin is among the most biologically significant carotenoids found in red algae, including *D. simplex*, due to its superior antioxidant capacity and ability to modulate immune responses (Fernando et al. 2016). It is widely used in the nutraceutical and aquaculture industries as a pigment and health enhancer, and its biosynthesis in marine organisms has attracted considerable commercial interest (Sahin and Ozturk 2021). Astaxanthin also exhibits anti-inflammatory and anti-aging properties, making it a valuable ingredient in functional foods and cosmeceuticals (Agatonovic-Kustrin and Morton 2018). The presence of astaxanthin in *D. simplex* adds significant value to this alga as a potential source of high-demand bioactive compounds.

β-carotene, another major carotenoid in *D. simplex*, serves as a precursor to vitamin A (retinol) and has important nutritional relevance (Dini 2022). It plays a dual role in protecting photosynthetic systems from photooxidative stress and acting as an antioxidant in human health applications. Studies have demonstrated the ability of β-carotene to reduce lipid peroxidation, modulate immune responses, and lower the risk of chronic diseases such as cardiovascular disorders and certain cancers (Alves et al. 2018; Bayomy and Alamri 2024). The bioavailability and efficacy of β-carotene depend on factors such as molecular configuration, cellular matrix, and coexisting lipids, making algae-based delivery systems a topic of growing research interest.

The biosynthesis of carotenoids in red algae is tightly regulated and occurs within plastid membranes, involving a cascade of enzymes such as phytoene synthase, phytoene desaturase, and lycopene cyclase (Sathasivam and Ki 2018). These enzymes catalyze the stepwise conversion of Geranylgeranyl Pyrophosphate (GGPP) into various carotenoids, with DMAPP serving as an upstream precursor. Environmental factors such as light intensity, nutrient availability, and salinity have been shown to influence carotenoid content in red algae, including *D. simplex* (Betancor et al. 2015). Optimizing these conditions through controlled cultivation may enhance carotenoid yields and support commercial-scale extraction efforts.

Carotenoids from *D. simplex* not only offer nutritional and pharmaceutical potential but also show promise as natural colorants and UV-protective agents in cosmetic formulations (Sahin and Ozturk, 2021; Abd El-Aziz et al. 2023). Their natural origin, safety profile, and multifunctional properties align with consumer demand for eco-friendly and health-promoting ingredients. Given the increasing restrictions on synthetic additives in food and cosmetics, algae-derived carotenoids represent a sustainable alternative with broad market appeal. Further research on the metabolic engineering and standardized extraction of *D. simplex* carotenoids is warranted to unlock their full biotechnological potential.

Figure 2.B illustrates the carotenoid biosynthetic pathway in *D. simplex*, starting from Dimethylallyl Pyrophosphate (DMAPP) and leading to astaxanthin. It emphasizes its role in antioxidant function and pigmentation.

Amino acids and protein profiles

Proteins and amino acids are essential components in all living organisms, serving structural, catalytic, and regulatory roles. In marine macroalgae such as *D. simplex*, these molecules contribute not only to nutritional value but also to physiological adaptation under environmental stress. Although red algae generally contain lower protein levels than green or brown counterparts, *D. simplex* still provides a valuable source of essential amino acids, including lysine, methionine, and threonine, which are often deficient in plant-based diets. Studies also report the presence of branched-chain amino acids (BCAAs)—leucine, isoleucine, and valine—which are vital for muscle metabolism and recovery. Moreover, sulfur-containing amino acids like methionine and cysteine enhance antioxidative capacity and detoxification, underscoring the health-promoting potential of this species.

Beyond standard proteinogenic amino acids, *D. simplex* also produces non-proteinogenic amino acid derivatives with pharmacological relevance. Kainic acid, a potent neuroexcitatory compound structurally related to glutamate, exemplifies this category. Other notable derivatives include γ-minobutyric Acid (GABA) and taurine, which have been linked to antihypertensive and calming effects. The presence of such bioactive amino compounds indicates that the amino acid profile of *D. simplex* may extend beyond basic nutrition, offering neurophysiological and therapeutic benefits. Analytical investigations suggest that these metabolites may function synergistically, adding complexity and functional diversity to the protein composition of this red alga.

Environmental factors significantly influence the protein content of *D. simplex*. Light intensity, nutrient availability, temperature, and seasonality all affect photosynthetic efficiency and nitrogen assimilation, which in turn regulate amino acid synthesis. Typically, protein content peaks during periods of rapid growth, such as spring and summer. Under controlled cultivation, environmental variables can be optimized to maximize protein yield, supporting its integration into aquaculture or biorefinery systems. Additionally, nitrogen-rich environments favor amino acid biosynthesis, while nitrogen limitation may redirect metabolic activity toward secondary metabolite production. Such flexibility illustrates the adaptive strategies of *D. simplex* and offers opportunities to tailor biomass composition for specific applications.

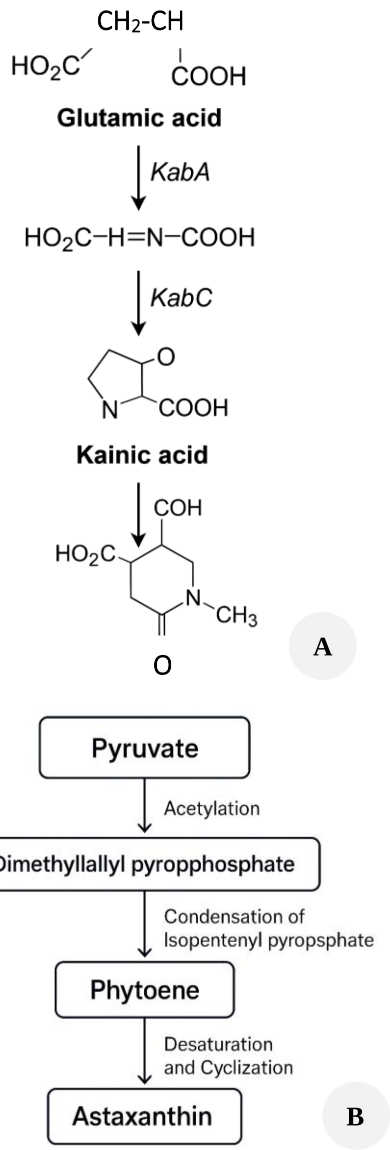

Figure 2. Biosynthetic pathway of A. Kainic Acid; and B. Astaxanthin in *Digenea simplex*, illustrating key precursor compounds and enzymatic steps involved in their production

Digestibility is another critical aspect of *D. simplex* protein utilization. The cell wall of red algae contains complex polysaccharides such as carrageenans and cellulose, which can impede enzymatic access to intracellular proteins. However, processing methods such as fermentation, enzymatic hydrolysis, and mechanical disruption have been shown to improve digestibility and protein extraction. These approaches enhance bioavailability and expand the range of food and feed products that can incorporate algal proteins. Technological advances in preprocessing thus play a pivotal role in unlocking the nutritional and functional potential of *D. simplex* as a sustainable protein source.

In addition to nutritional value, proteins from *D. simplex* exhibit bioactive properties that contribute to health promotion. Marine-derived peptides have demonstrated antioxidant, antimicrobial, and anti-inflammatory activities. Some hydrolysates inhibit Angiotensin-Converting Enzyme (ACE), suggesting potential for managing hypertension. These multifunctional properties increase the attractiveness of *D. simplex* for use in functional foods and nutraceuticals. Analytical techniques such as high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR), and mass spectrometry (MS) have facilitated the identification of these peptides and their post-translational modifications. Omics-based methods—especially proteomics and metabolomics—provide powerful tools to elucidate the protein landscape of this species further.

As global interest in alternative protein sources grows, *D. simplex* presents a compelling case for inclusion in sustainable food systems. Its cultivation requires minimal inputs no arable land, freshwater, or synthetic fertilizers—making it compatible with circular economy and blue biotechnology principles. Combining *D. simplex* protein with other marine resources could yield novel food products with superior nutritional and functional profiles. Overall, the amino acid and protein composition of *D. simplex* reflects its ecological adaptability and multifunctional potential, positioning it as a valuable candidate for diverse applications in food, feed, and health-related industries.

Tannins and phenolic compounds

Tannins and phenolic compounds are widely recognized as important secondary metabolites in marine algae, including red macroalgae such as *D. simplex*. These compounds are synthesized primarily through the shikimate and phenylpropanoid pathways and serve various ecological and physiological functions, including defense against herbivory, microbial infection, and oxidative stress (Fernando et al. 2016; Agatonovic-Kustrin and Morton 2018). In *D. simplex*, phenolic constituents contribute significantly to the alga's antioxidant capacity and are considered among its key bioactive constituents (Bayomy and Alamri 2024). Phenolics in red algae exist in both free and bound forms, often localized within the cell wall matrix or vacuoles, and their composition can vary with environmental conditions.

Tannins, a subset of polyphenols, are high-molecular-weight compounds capable of binding to proteins and metal ions. Their presence in *D. simplex* has been associated with anti-nutritional effects in some contexts, but more importantly, with health-promoting properties such as antioxidant, antimicrobial, and antidiabetic activities (Ibraheem et al. 2017). Marine algal tannins, although less studied than their terrestrial counterparts, have shown unique structural features such as sulfation, which may enhance their biological activity and solubility in aqueous environments (Chia et al. 2018). These features differentiate red algal tannins from those of higher plants and may contribute to their effectiveness in aqueous biological systems.

Phenolic acids such as gallic acid, protocatechuic acid, and ferulic acid have been reported in various Rhodophyta species and are likely present in *D. simplex* as well (Fernando et al. 2016; Pratita et al. 2023). These low-molecular-weight phenolics act as potent radical scavengers, metal chelators, and inhibitors of lipid peroxidation, which are critical mechanisms in preventing oxidative damage in both algal tissues and potential human applications. In addition, red algal phenolics may exhibit synergistic effects with other antioxidants, such as carotenoids and phycobiliproteins, enhancing the overall bioactivity profile of the species (Alves et al. 2018). Such synergism is particularly relevant in the development of functional food ingredients or nutraceutical formulations.

Environmental stressors such as high light intensity, UV radiation, nutrient limitation, and salinity fluctuation are known to modulate phenolic content in red algae (Betancor et al. 2015). These stress conditions can stimulate the upregulation of phenolic biosynthetic enzymes, leading to increased accumulation of protective metabolites. Therefore, controlled cultivation of *D. simplex* under specific abiotic stress regimes may be strategically employed to enhance phenolic yields for industrial or pharmacological purposes. Moreover, the variability in phenolic profiles due to habitat or season may serve as a chemotaxonomic marker, aiding in species or population-level differentiation (Pereira 2015).

The extraction and characterization of phenolic compounds from *D. simplex* typically involve solvent-based methods using aqueous ethanol or methanol, followed by analytical techniques such as HPLC, LC-MS, or NMR (Dini 2022). Recent developments in green extraction technologies, such as supercritical CO_2 and ultrasound-assisted extraction, offer more sustainable approaches for isolating phenolics from marine biomass. Understanding the phenolic composition of *D. simplex* not only advances its value as a functional ingredient but also opens avenues for the discovery of novel marine-derived antioxidants with therapeutic potential.

Altogether, *D. simplex's* presence of tannins and phenolic compounds highlights the alga's biochemical versatility and pharmacological relevance. These metabolites contribute to its ecological fitness and bioactivity profile, making it a promising candidate for further exploration in antioxidant, anti-inflammatory, and antimicrobial applications. Ongoing studies on the environmental modulation, structural diversity, and bioavailability of these compounds will be crucial to unlocking their full potential in health-related industries.

BIOLOGICAL ACTIVITIES

The bioactive compounds identified in *Digenea simplex* are associated with a wide range of pharmacological and physiological effects. These biological activities reflect the multifunctional nature of the alga's secondary metabolites, which have been shown to act on multiple targets such as oxidative stress, inflammation, tumor progression, microbial infection, and metabolic dysregulation. The synergistic interactions among phenolics, carotenoids, sulfated polysaccharides, and amino acid derivatives contribute to a broad-spectrum therapeutic potential. A summary of these key chemical constituents and their associated bioactivities is presented in Table 1, highlighting their relevance to traditional and modern medical applications. Investigating these effects in detail is essential for understanding the mechanisms underlying the health benefits of *D. simplex* and for identifying applications in pharmaceutical, nutraceutical, and functional food development.

Antioxidant activity

Oxidative stress, defined as an imbalance between the production of Reactive Oxygen Species (ROS) and the body's antioxidant defenses, is a major contributing factor in the development of chronic diseases such as cancer, cardiovascular disorders, neurodegeneration, and diabetes (Di Meo and Venditti 2020). Natural antioxidants play a critical role in neutralizing ROS and reducing oxidative damage at the cellular level. Marine macroalgae, including red algae such as *D. simplex*, have been increasingly recognized as rich sources of antioxidant compounds with therapeutic potential (Fernando et al. 2016; Alves et al. 2018). The antioxidant activity of these algae arises from a combination of bioactive constituents such as phenolics, carotenoids, amino acids, and sulfated polysaccharides.

Several studies have demonstrated the strong antioxidant capacity of red algae through various in vitro assays, including DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) activity, Ferric Reducing Antioxidant Power (FRAP), and metal chelation tests (Agatonovic-Kustrin and Morton 2018). Although specific data on *D. simplex* remains limited compared to more extensively studied species such as *Palmaria palmata* or *Chondrus crispus*, available evidence suggests that *D. simplex* exhibits comparable radical scavenging activity, likely due to the presence of polyphenols and pigment antioxidants such as astaxanthin and β-carotene (Bayomy and Alamri 2024). These compounds are well-known for their ability to quench singlet oxygen and scavenge free radicals, thus protecting cellular components such as lipids, proteins, and DNA from oxidative damage (Sathasivam and Ki 2018).

Table 1. Summary on *Digenea simplex* chemical constituents, pharmacological properties, and medical utilization

Name	Chemical constituents	Pharmacological properties	Traditional medical utilization	Modern medical utilization	References
D. simplex	Kainic acid (pyrrolidine dicarboxylic acid)	Neuroexcitatory, antiparasitic, glutamate receptor agonist	Used as an anthelmintic in traditional Japanese medicine	Tool for epilepsy research; potential neuromodulator in neurodegenerative disease models	Maeno et al. 2019; Chekan et al. 2019; Asakawa et al. 2020
D. simplex	Sulfated polysaccharides (e.g. carrageenans)	Antiviral, immunomodulatory, anti-inflammatory, antioxidant	Folk use for digestive balance and wound application (limited documentation)	Topical antiviral gels, immune-support supplements, drug delivery materials	Cicinskas et al. 2020; Aboeita et al. 2022; Sathasivam and Ki 2018
D. simplex	Carotenoids (astaxanthin, β-carotene, lutein)	Antioxidant, photoprotective, anti-aging, anticancer	-	Functional food ingredients; cosmeceuticals; anti-aging skincare	Fernando et al. 2016; Bayomy and Alamri 2024; Dini 2022
D. simplex	Polyphenols and tannins (e.g., gallic acid derivatives)	Antioxidant, antidiabetic, anti-inflammatory, antimicrobial	Used in decoctions or compresses in traditional contexts (poorly documented)	Natural α-glucosidase inhibitors; active ingredients in antioxidant nutraceuticals	Ibraheem et al. 2017; Agatonovic-Kustrin and Morton 2018
D. simplex	Amino acids and peptides (methionine, lysine, leucine, cysteine)	Nutritional; precursor for antioxidant and antihypertensive peptides	Nutritional use implied in seaweed-based diets	Protein supplements; functional peptides with metabolic effects	Ward and Deyab, 2021; Alves et al. 2018
D. simplex	Isoprenoids from DMAPP pathway (e.g., phytol, carotenoid precursors)	Antioxidant, anti-inflammatory, membrane-stabilizing	-	Biosynthetic precursor for terpenoid production; cosmetic stabilizers	Ganley et al. 2020; Dini 2022

Phenolic compounds, including tannins, flavonoids, and phenolic acids, are major contributors to the antioxidant properties of *D. simplex* (Fernando et al. 2016; Ibraheem et al. 2017). These compounds can donate hydrogen atoms or electrons to neutralize free radicals and also chelate transition metals such as Fe^{2+} and Cu^{2+}, which catalyze ROS formation through Fenton-type reactions. Furthermore, phenolics are often upregulated in response to environmental stressors, suggesting their dual role as both protective agents and bioindicators of algal stress adaptation (Betancor et al. 2015). The variability of phenolic content among *D. simplex* populations may reflect differences in habitat conditions, such as UV exposure, salinity, or nutrient availability.

In addition to phenolics, carotenoids in *D. simplex* notably astaxanthin, lutein, and β-carotene—also contribute significantly to its antioxidant profile (Dini 2022; Bayomy and Alamri 2024). These pigments stabilize lipid membranes and inhibit lipid peroxidation, a process that is strongly associated with cellular aging and degenerative diseases. Astaxanthin, in particular, has been shown to be several times more effective than vitamin E in scavenging ROS and protecting mitochondrial function (Fernando et al. 2016). The combined action of hydrophilic (phenolics) and lipophilic (carotenoids) antioxidants in *D. simplex* offers broad-spectrum protection, making this species a promising candidate for antioxidant supplementation.

Proteins and amino acid derivatives in *D. simplex* may also contribute indirectly to antioxidant activity. Certain peptides produced through enzymatic hydrolysis have demonstrated radical scavenging and metal-chelating properties (Alves et al. 2018; Pradhan et al. 2020). Moreover, amino acids such as cysteine, methionine, and histidine contain functional groups capable of interacting with ROS. The role of these nitrogen-containing compounds complements the antioxidant activity of polyphenols and carotenoids and may be enhanced through processing techniques such as fermentation or enzymatic extraction (Calvo et al. 2019; Hung and Trinh 2021).

Sulfated polysaccharides, including carrageenans found in red algae cell walls, have also been shown to possess antioxidant properties, particularly in terms of reducing power and inhibition of lipid peroxidation (Cicinskas et al. 2020; Aboeita et al. 2022). While these compounds are primarily known for their gelling and immunomodulatory properties, their ability to scavenge radicals and protect against oxidative stress has been increasingly reported. The structural features of these polysaccharides, such as degree of sulfation and molecular weight, can influence their antioxidant efficacy. Their water solubility also allows them to function in aqueous environments, enhancing their bioavailability in food or pharmaceutical formulations.

Environmental and cultivation factors greatly influence the antioxidant potential of *D. simplex*. Light intensity, UV exposure, and nutrient levels are known to regulate the biosynthesis of antioxidant compounds, particularly phenolics and carotenoids (Betancor et al. 2015; Gljušćić et al. 2022). Manipulating these factors in aquaculture or bioreactor systems could optimize the yield of antioxidants for commercial purposes. Additionally, post-harvest processing methods, including drying, solvent extraction, and encapsulation, affect the stability and activity of antioxidant components. Therefore, standardization of extraction protocols and quality control measures is crucial for the development of *D. simplex*-based antioxidant products.

The antioxidant activity of *D. simplex* underscores its potential application in functional foods, dietary supplements, cosmetics, and therapeutic formulations aimed at preventing or mitigating oxidative damage. Its diverse antioxidant constituents acting through complementary mechanisms provide a robust foundation for health-promoting interventions. Continued exploration of *D. simplex* under various environmental conditions, along with advances in metabolomic profiling and bioassays, will be essential to harness its antioxidant potential for commercial and clinical applications fully.

Antidiabetic potential

Diabetes mellitus, particularly Type 2 Diabetes (T2DM), is a global metabolic disorder characterized by insulin resistance, impaired glucose metabolism, and chronic hyperglycemia. The disease has been associated with oxidative stress, inflammation, and mitochondrial dysfunction, which contribute to long-term complications such as neuropathy, nephropathy, and cardiovascular diseases (Di Meo and Venditti 2020; Alam et al. 2021). Conventional antidiabetic drugs such as sulfonylureas and metformin are widely used but often present limitations, including gastrointestinal side effects and decreased efficacy over time. As a result, there is growing interest in marine natural products, particularly from macroalgae, as alternative or complementary therapies for diabetes management (Chia et al. 2018; Van Weelden et al. 2019).

Red macroalgae such as *D. simplex* are rich in compounds with potential antidiabetic properties, including polyphenols, sulfated polysaccharides, carotenoids, and amino acid derivatives. These metabolites may act through multiple mechanisms, such as inhibition of carbohydrate-digesting enzymes, enhancement of insulin sensitivity, protection of pancreatic β-cells, and modulation of glucose uptake (Fernando et al. 2016; Agatonovic-Kustrin and Morton 2018). Although specific studies on *D. simplex* are still limited, related species in the Rhodophyta group have shown promising in vitro and in vivo results. The structural similarities in metabolite composition suggest that *D. simplex* may also exhibit comparable bioactivities, warranting further exploration.

One of the most widely investigated mechanisms in marine algae-based antidiabetic studies is the inhibition of α-glucosidase and α-amylase enzymes responsible for the breakdown of dietary carbohydrates into absorbable sugars. By inhibiting these enzymes, algal extracts can delay glucose absorption, reducing postprandial blood glucose spikes. Extracts from red algae have demonstrated competitive and non-competitive inhibitory activity against these enzymes, often attributed to the presence of phlorotannins and sulfated polysaccharides (Chia et al. 2018; Pradhan et al. 2020). Although phlorotannins are more typical of brown algae, red algae like *D. simplex* are known to contain bioactive tannins and phenolics that may exert similar inhibitory effects (Ibraheem et al. 2017; Bayomy and Alamri 2024).

Sulfated galactans such as carrageenans, which are abundant in red algae cell walls, have also shown hypoglycemic effects through immunomodulatory and gut microbiota-modulating pathways (Cicinskas et al. 2020; Aboeita et al. 2022). These polysaccharides may improve insulin sensitivity and glucose uptake by enhancing signaling in insulin-responsive tissues. Additionally, carrageenans may reduce inflammation in adipose tissue and the pancreas, thereby preserving insulin secretion and improving metabolic function. Their biocompatibility and safety profile make them attractive for incorporation into functional foods or as adjuvants in diabetes therapy.

Carotenoids present in *D. simplex*, particularly astaxanthin and β-carotene, also contribute to its antidiabetic potential by reducing oxidative stress and improving mitochondrial function (Fernando et al. 2016; Sathasivam and Ki 2018). Oxidative stress has been strongly implicated in pancreatic β-cell dysfunction, and antioxidant therapy has been proposed as a strategy to preserve β-cell integrity and function. Astaxanthin has been shown to enhance glucose metabolism by activating AMP-Activated Protein Kinase (AMPK) and reducing inflammatory cytokines in insulin-resistant cells (Ganley et al. 2020). These mechanisms could synergistically improve glycemic control when combined with other bioactive compounds from *D. simplex*.

Amino acid derivatives such as kainic acid, while primarily known for their neuroactivity, may also influence glucose homeostasis through modulation of excitatory neurotransmission and gut-brain axis signaling (Chekan et al. 2019). In addition, essential amino acids like leucine and methionine, present in *D. simplex*, have been linked to insulin secretion and glucose uptake in skeletal muscle (Ward and Deyab 2021; Lopez and Mohiuddin 2024). Their role in metabolic regulation suggests that the protein fraction of *D. simplex* should not be overlooked in studies of antidiabetic bioactivity. Bioactive peptides derived from red algae proteins have also demonstrated inhibitory effects on Dipeptidyl Peptidase-4 (DPP-4), an enzyme that inactivates incretin hormones and negatively affects insulin signaling (Alves et al. 2018).

Environmental conditions such as salinity, light, and nutrient availability can modulate the levels of antidiabetic compounds in *D. simplex*, offering possibilities for metabolic optimization through cultivation control (Betancor et al. 2015). For instance, stress-induced upregulation of phenolics and carotenoids under high light or nutrient limitation may enhance the antidiabetic potency of harvested biomass. Cultivation strategies that apply abiotic stressors in a controlled manner could, therefore, improve the consistency and efficacy of algal-based antidiabetic products. Furthermore, advanced extraction methods such as enzyme-assisted extraction or membrane filtration may improve the bioavailability of active compounds (Calvo et al. 2019; Hung and Trinh 2021).

Despite the encouraging pharmacological potential, the clinical translation of *D. simplex* for diabetes therapy requires further validation. Comprehensive in vivo studies and clinical trials are needed to assess its efficacy, optimal dosage, and long-term safety. In addition, the identification and standardization of specific bioactive markers will facilitate the development of *D. simplex*-derived products with consistent therapeutic outcomes. Integration of metabolomic profiling, bioinformatics, and pharmacokinetics will be critical in advancing this promising red alga as a novel marine resource for diabetes management.

Antitumor and cytotoxic effects

Cancer remains a leading cause of mortality worldwide, characterized by uncontrolled cell proliferation, resistance to apoptosis, and genetic instability. Conventional therapies such as chemotherapy and radiotherapy, while effective in certain cases, often have significant side effects and face limitations such as drug resistance. This has driven the exploration of natural compounds, especially from marine organisms, as alternative or complementary antitumor agents (Agatonovic-Kustrin and Morton 2018). Marine macroalgae, including *D.simplex*, are rich in secondary metabolites with cytotoxic properties that can modulate cancer-related pathways and suppress tumor growth (Fernando et al. 2016; Alves et al. 2018).

Red algae possess a variety of bioactive compounds with potential anticancer effects, such as phenolics, sulfated polysaccharides, carotenoids, and alkaloid-like amino acid derivatives. Among these, phenolic compounds and tannins have been reported to induce apoptosis, inhibit angiogenesis, and interfere with cell cycle progression in several cancer cell lines (Ibraheem et al. 2017). These compounds exert their activity through mechanisms such as ROS generation, caspase activation, mitochondrial membrane depolarization, and inhibition of transcription factors like NF-κB (Fernando et al. 2016). While direct studies on *D. simplex* are limited, its phenolic-rich profile suggests similar anticancer potential, especially when compared to other Rhodophyta species such as *Laurencia*, *Gracilaria*, and *Gigartina*.

Sulfated polysaccharides such as carrageenans, abundantly found in the cell walls of red algae, have shown antitumor effects both in vitro and in vivo (Cicinskas et al. 2020; Aboeita et al. 2022). Carrageenans can inhibit tumor cell adhesion, migration, and invasion while also modulating the immune response to enhance host-mediated tumor suppression. Low-molecular-weight carrageenans, in particular, are associated with stronger cytotoxic effects and higher cellular uptake. These polysaccharides also exhibit selective toxicity, demonstrating higher activity against cancer cells than normal cells, which is desirable in anticancer drug development (Sahin and Ozturk 2021). The structural features, such as degree of sulfation, chain length, and branching, influence their anticancer activity and bioavailability.

Another compound of interest is kainic acid, originally isolated from *D. simplex*. Though primarily recognized for its neuroexcitatory properties, kainic acid and its derivatives have been explored for antiproliferative effects in certain cancer cell models (Chekan et al. 2019; Maeno et al. 2019). Kainic acid analogs have been shown to induce apoptotic signaling pathways in glioma and neuroblastoma cells, likely through overstimulation of glutamate receptors and calcium influx, leading to oxidative stress and mitochondrial dysfunction (Asakawa et al. 2020). However, due to its neurotoxicity at high concentrations, structural modifications and targeted delivery approaches are essential to harness its cytotoxic potential safely.

Carotenoids, particularly astaxanthin, and β-carotene, contribute to the antitumor profile of *D. simplex* by functioning as antioxidants and modulators of gene expression (Dini 2022; Bayomy and Alamri 2024). These compounds can downregulate pro-oncogenic factors such as VEGF and COX-2 while upregulating tumor suppressor genes like p53. Astaxanthin has been shown to inhibit cell proliferation in breast, colon, and prostate cancer models by inducing cell cycle arrest and apoptosis (Fernando et al. 2016). Its ability to cross cell membranes and accumulate in mitochondria allows it to act at critical sites of ROS generation and apoptotic signaling.

Recent studies have also investigated the cytotoxic effects of red algal extracts against various human cancer cell lines, including HepG2 (liver), MCF-7 (breast), HT-29 (colon), and A549 (lung) (Alves et al. 2018). These assays often report IC_{50} values within the micromolar range, indicating moderate to strong antiproliferative activity depending on the extraction method and solvent used. Methanol and ethanol extract generally yield higher cytotoxicity due to the efficient solubilization of phenolic and lipophilic compounds. The cytotoxic profile of *D. simplex* is likely influenced by the combined action of multiple metabolite classes, acting synergistically or additively to induce cancer cell death.

Environmental stressors such as UV radiation, nutrient limitation, and salinity shifts may enhance the production of cytotoxic metabolites in *D. simplex*, offering opportunities for metabolite modulation via stress-based cultivation (Betancor et al. 2015). These adaptive responses involve the upregulation of biosynthetic genes and the accumulation of defense-related compounds, many of which exhibit cytotoxicity. Manipulating environmental parameters during cultivation could thus increase the yield and potency of anticancer compounds in *D. simplex*. This strategy aligns with sustainable marine biotechnology approaches aimed at producing high-value bioactives from algae.

While the preliminary evidence is promising, the clinical translation of *D. simplex*-derived antitumor agents requires more rigorous investigation. In vivo validation, toxicity assessment, and pharmacokinetic profiling are necessary to ensure safety and efficacy. Furthermore, the identification and structural characterization of active constituents, aided by metabolomics and bioassay-guided fractionation, will facilitate drug development pipelines. Future research should also explore nanoparticle-based delivery systems to improve the bioavailability and targeting of *D. simplex*-based anticancer agents.

Antimicrobial and antiparasitic activities

The emergence of antibiotic-resistant pathogens and the global burden of parasitic diseases have accelerated the search for alternative therapeutic agents from natural sources. Marine macroalgae, including red algae such as *D. simplex*, have demonstrated antimicrobial and antiparasitic activities attributed to their rich secondary metabolite content (Fernando et al. 2016; Agatonovic-Kustrin and Morton 2018). These compounds include phenolics, halogenated metabolites, fatty acids, sulfated polysaccharides, and alkaloid-like molecules, which collectively provide chemical defense against microbial colonization and parasitic infestation. The ecological function of these metabolites in algae often parallels their pharmacological action in humans, making macroalgae a valuable source of novel anti-infective compounds.

Phenolic compounds and tannins found in *D. simplex* exhibit strong antimicrobial properties by disrupting microbial cell membranes, inactivating enzymes, and chelating essential metal ions (Fernando et al. 2016; Ibraheem et al. 2017). These polyphenols can form complexes with microbial proteins, leading to loss of function and, ultimately, cell death. Additionally, red algal phenolics are known to exhibit broad-spectrum activity against both Gram-positive and Gram-negative bacteria, as well as various fungi. While direct assays involving *D. simplex* are limited, similar Rhodophyta species have shown inhibitory activity against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Pseudomonas aeruginosa* (Sahin and Ozturk 2021; Bayomy and Alamri 2024).

Sulfated polysaccharides such as carrageenans are abundant in red algae and have been studied for their antiviral and antibacterial properties. These polysaccharides exert antimicrobial effects by forming physical barriers that prevent microbial adhesion and invasion and by interfering with microbial signal transduction pathways (Cicinskas et al. 2020; Aboeita et al. 2022). In viral infections, carrageenans can block virus-host interactions, particularly in enveloped viruses such as Herpes Simplex Virus (HSV), dengue, and even SARS-CoV-2 (Sathasivam and Ki 2018). Though specific studies on *D. simplex*-derived carrageenan fractions are limited, their structural similarity to those from other Rhodophyta species suggests comparable bioactivity.

Halogenated compounds, though more common in genera like *Laurencia*, have also been reported in various red algae and may exist in *D. simplex* in trace amounts. These compounds often brominated or chlorinated demonstrate potent antimicrobial activity due to their ability to penetrate bacterial membranes and disrupt DNA replication or protein synthesis (Agatonovic-Kustrin and Morton 2018). The structural novelty and chemical reactivity of halogenated metabolites have made them attractive for antimicrobial drug development. Although *D. simplex* has not been extensively profiled for halogenated compounds, advanced metabolomics may reveal previously undetected antimicrobial molecules in this species.

Amino acid derivatives such as kainic acid, while primarily recognized for neuroexcitatory activity, also possess antiparasitic potential. Historically, kainic acid was used as a traditional anthelmintic in Japanese medicine to expel intestinal worms, suggesting selective toxicity toward helminths (Chekan et al. 2019; Maeno et al. 2019). The compound is believed to act on glutamate receptor-like sites in parasitic nervous systems, leading to excitotoxic paralysis. Although its clinical use was discontinued due to side effects in humans, its mode of action remains a valuable pharmacological model for developing new antiparasitic agents with greater specificity and lower toxicity (Asakawa et al. 2020).

In addition to helminths, macroalgae have been shown to exert antiparasitic activity against protozoa such as *Plasmodium* spp., *Leishmania* spp., and *Trypanosoma* spp. This activity is often attributed to terpenoids, alkaloids, and oxidized fatty acids that interfere with parasite metabolism or membrane integrity (Fernando et al. 2016; Dini 2022). Although no direct studies have evaluated the efficacy of *D. simplex* extracts against these parasites, the presence of bioactive isoprenoids and phenolic compounds indicates potential in this area. Exploration of this activity should be prioritized in future research, especially given the urgent need for novel antiprotozoal therapies.

Environmental stressors such as UV exposure and nutrient deprivation may enhance the antimicrobial potency of *D. simplex* by upregulating the biosynthesis of defense-related metabolites (Betancor et al. 2015; Gljušćić et al. 2022). Therefore, cultivation of *D. simplex* under controlled stress conditions could be optimized to produce higher yields of antimicrobial compounds. Additionally, extraction techniques such as supercritical fluid extraction and green solvents may improve the recovery and stability of these compounds, facilitating their incorporation into pharmaceutical or personal care formulations (Calvo et al. 2019; Hung and Trinh 2021).

Overall, *D. simplex's* antimicrobial and antiparasitic potential reflects its ecological resilience and biochemical richness. Although more targeted studies are needed, the combination of phenolics, polysaccharides, carotenoids, and neuroactive amino acids provides a solid foundation for bioactivity. Continued investigation, including bioassay-guided fractionation and in vivo validation, will be essential to fully harness *D. simplex* as a marine resource for novel anti-infective agents.

Anti-inflammatory and neuroactive properties

Inflammation is a complex physiological response to injury, infection, or metabolic dysregulation, which, when chronic, contributes to various pathological conditions such as arthritis, cancer, diabetes, and neurodegenerative diseases (Di Meo and Venditti 2020). Natural anti-inflammatory agents derived from marine organisms have gained increasing attention as safer alternatives to synthetic drugs, which often present undesirable side effects. Red macroalgae such as *D. simplex* contain a wide range of bioactive compounds including polyphenols, carotenoids, sulfated polysaccharides, and amino acid derivatives—that may modulate inflammatory pathways (Fernando et al. 2016; Agatonovic-Kustrin and Morton 2018). These compounds exert their effects through the inhibition of pro-inflammatory mediators, antioxidant protection, and regulation of signaling pathways such as NF-κB and MAPK.

Phenolic compounds, including tannins and phenolic acids present in *D. simplex*, are potent inhibitors of inflammation-related enzymes such as Cyclooxygenase (COX), lipoxygenase (LOX), and inducible nitric oxide synthase (iNOS) (Ibraheem et al. 2017). These enzymes are involved in the synthesis of prostaglandins, leukotrienes, and nitric oxide—all key mediators of inflammation. The ability of red algal phenolics to inhibit these pathways has been demonstrated in vitro and in vivo using models of carrageenan-induced edema, lipopolysaccharide (LPS)-stimulated macrophages, and oxidative stress-induced inflammation (Alves et al. 2018). Although specific data on *D. simplex* is still limited, the presence of structurally similar phenolics suggests comparable anti-inflammatory potential.

Carotenoids such as astaxanthin, β-carotene, and lutein, all found in *D. simplex*, also exhibit strong anti-inflammatory effects through their antioxidant and gene-regulatory activities (Dini 2022; Bayomy and Alamri 2024). Astaxanthin, for example, has been shown to downregulate inflammatory cytokines such as TNF-α, IL-6, and IL-1β in various cell types, including macrophages and endothelial cells (Fernando et al. 2016). These carotenoids also modulate the activity of transcription factors involved in inflammation, such as NF-κB and AP-1, thereby suppressing the expression of pro-inflammatory genes. Importantly, carotenoids have been observed to improve mitochondrial function, reduce oxidative stress, and restore redox balance, providing reassurance about their potential in managing chronic inflammation.

Sulfated polysaccharides from red algae, including carrageenans, are not only effective in modulating immune responses but also highly compatible with biological systems. Their immunomodulatory and anti-inflammatory properties have been validated in animal models and human cell lines (Cicinskas et al. 2020; Aboeita et al. 2022), making them promising candidates for a range of applications. These polysaccharides inhibit leukocyte migration, reduce cytokine production, and enhance antioxidant enzyme activity. Their high solubility and structural variability enable them to interact with immune cells and modulate inflammatory responses at mucosal surfaces. Moreover, their biocompatibility makes them attractive for incorporation into topical formulations for inflammatory skin conditions or as dietary supplements targeting gut inflammation.

Among the neuroactive compounds in *D. simplex*, kainic acid stands out due to its well-documented activity on glutamate receptors in the central nervous system. Kainic acid is a selective agonist of kainate-type ionotropic glutamate receptors and has been widely used in neuropharmacological research to model epilepsy and neurodegeneration (Connell et al. 2017; Asakawa et al. 2020). While kainic acid is neurotoxic at high concentrations, low or structurally modified doses have shown promise in modulating synaptic plasticity and neurotransmitter release. These properties make it a compound of interest in studying excitotoxicity, neuroinflammation, and potential neuroprotective mechanisms under tightly controlled conditions (Vezzani et al. 2016).

Neuroinflammation, a hallmark of disorders such as Alzheimer’s disease and Parkinson’s disease, involves the activation of microglia and astrocytes, the release of inflammatory cytokines, and oxidative stress. Marine-derived compounds with both antioxidant and anti-inflammatory properties may attenuate these processes, offering therapeutic avenues for neurodegenerative disease intervention.

Polyphenols and carotenoids from *D. simplex* may provide neuroprotection by modulating neuronal signaling, inhibiting oxidative damage to neurons, and suppressing inflammatory cytokine expression in brain tissues (Fernando et al. 2016; Ganley et al. 2020). Additionally, amino acids such as tryptophan and its derivatives may affect serotonin biosynthesis and gut-brain axis communication, further expanding the neuroactive scope of *D. simplex*.

Environmental conditions significantly influence the levels of anti-inflammatory and neuroactive compounds in macroalgae. Light exposure, nutrient status, and seasonal variation can alter the biosynthesis of polyphenols, carotenoids, and kainoids (Betancor et al. 2015; Gljušćić et al. 2022). Cultivation under stress-enhancing regimes may increase the production of these valuable metabolites. Integrating this knowledge into aquaculture strategies may help optimize *D. simplex* biomass for targeted pharmaceutical applications. Further omics-based profiling and bioassay-guided isolation are necessary to identify the full spectrum of anti-inflammatory and neuroprotective agents in this species.

D. simplex represents a promising marine bioresource with significant potential for the development of novel anti-inflammatory and neuroprotective agents. Its rich repertoire of phenolics, carotenoids, polysaccharides, and kainoid compounds offers multiple mechanisms for modulating inflammation and neural activity. Future research integrating pharmacodynamics, toxicity screening, and formulation science will be essential for translating these bioactivities into safe and effective clinical or nutraceutical applications.

POTENTIAL APPLICATION

The broad biological activities of *Digenea simplex* including antioxidant, antidiabetic, antitumor, antimicrobial, antiparasitic, anti-inflammatory, and neuroactive effects—are supported by experimental evidence highlighting both individual compounds and synergistic extract mixtures that act on diverse cellular pathways. Tables 2 to 4 summarize the bioactivities, assay systems, mechanisms of action, and real-world applications of its major metabolites, demonstrating the pharmacological relevance of *D. simplex* across therapeutic, nutritional, and cosmetic domains.

Nutraceutical and functional food uses

The increasing prevalence of non-communicable diseases such as diabetes, obesity, cardiovascular disease, and neurodegeneration has spurred global interest in functional foods and nutraceuticals food-derived products offering health benefits beyond basic nutrition. Marine macroalgae, including red algae like *D. simplex*, are emerging as valuable sources of bioactive compounds suitable for incorporation into such health-promoting formulations (Fernando et al. 2016; Sathasivam and Ki 2018). *D. simplex* contains a broad range of compounds, including polyphenols, sulfated polysaccharides, carotenoids, essential amino acids, and dietary fiber, all of which are known to contribute to physiological homeostasis and disease prevention (Ward and Deyab 2021; Bayomy and Alamri 2024). The combination of macro- and micronutrients with potent bioactivities makes this species a promising candidate for functional food innovation.

One of the key components supporting the nutraceutical value of *D. simplex* is its rich antioxidant profile, including astaxanthin, β-carotene, phenolic acids, and flavonoid-like compounds. These antioxidants help mitigate oxidative stress, which is implicated in the pathogenesis of chronic diseases such as atherosclerosis, cancer, and type 2 diabetes (Agatonovic-Kustrin and Morton 2018; Di Meo and Venditti 2020). When consumed regularly through functional foods, antioxidants from *D. simplex* may help neutralize free radicals and protect cellular components, thereby enhancing immune resilience and slowing age-related degeneration. Their natural origin and high bioactivity make them attractive alternatives to synthetic antioxidants, which may carry safety concerns with long-term use.

The dietary fiber content of *D. simplex*, primarily derived from sulfated galactans such as carrageenan, also supports its functional food applications. These fibers are known to improve gut health by promoting beneficial microbiota, reducing glycemic response, and enhancing satiety, thus aiding in weight management and metabolic regulation (Cicinskas et al. 2020; Aboeita et al. 2022). Additionally, dietary fibers from red algae may reduce serum cholesterol and improve lipid profiles, providing cardioprotective effects. The inclusion of *D. simplex* into baked products, smoothies, energy bars, or capsules may offer new avenues for consumers seeking high-fiber, plant-based supplementation.

The amino acid profile of *D. simplex* further adds to its nutritional value. It contains essential amino acids such as lysine, methionine, and tryptophan, which are often limited in plant-based diets (Stonik and Stonik 2020; Lopez and Mohiuddin 2024). Moreover, its protein fraction includes bioactive peptides with antioxidant, anti-inflammatory, and antihypertensive properties, particularly when hydrolyzed enzymatically or fermented (Alves et al. 2018; Calvo et al. 2019). These properties make *D. simplex* a potentially valuable ingredient for plant-based protein formulations, catering to vegetarian and vegan consumers while also meeting functional health goals.

The antidiabetic potential of *D. simplex* also underlines its value in functional foods. Compounds such as phenolics, carotenoids, and polysaccharides can help regulate postprandial glucose levels by inhibiting carbohydrate-hydrolyzing enzymes like α-amylase and α-glucosidase (Chia et al. 2018; Pradhan et al. 2020). Regular intake of such inhibitors through diet may provide a preventive approach to type 2 diabetes or serve as adjuncts to pharmacological treatment. Functional snacks or beverages fortified with *D. simplex* extract may offer a convenient delivery form for individuals at risk of metabolic syndrome.

Another promising avenue is the prebiotic potential of red algae-derived polysaccharides. Recent studies have highlighted the ability of carrageenans and related oligosaccharides to modulate the composition of gut microbiota, particularly by promoting beneficial strains such as *Bifidobacterium* and *Lactobacillus* (Hung and Trinh 2021). These microbial shifts are associated with improved digestive function, enhanced nutrient absorption, and reduced inflammation in the gut. Functional food products incorporating *D. simplex* extracts may thus contribute to gut-brain axis health, influencing not only digestion but also mood, cognition, and immune regulation.

In addition to its intrinsic bioactivity, *D. simplex* offers formulation advantages in food systems. Its mucilaginous texture and natural gelling properties derived from its polysaccharide content can act as emulsifiers, thickeners, and stabilizers in food processing (Sahin and Ozturk 2021). This dual role—functionality plus bioactivity supports its inclusion in a wide variety of formats, from dairy alternatives and gels to edible films and encapsulated supplements. Moreover, its pleasant reddish hue from carotenoids may serve as a natural colorant, reducing the need for synthetic additives.

Table 2. Reported biological activities of *Digenea simplex* and associated bioactive compounds

Bioactivity	Extract/compound	Target or assay model	Biological effect	Experimental evidence	References
Antioxidant	Methanolic extract; carotenoids (astaxanthin, β-carotene)	DPPH, ABTS, FRAP assays	High radical scavenging and reducing power	In vitro antioxidant screening using algal extracts	Fernando et al. 2016; Bayomy and Alamri 2024
Antidiabetic	Polyphenolic extract; sulfated polysaccharides	α-glucosidase and α-amylase inhibition	Moderate-to-strong enzyme inhibition	Enzymatic assay showing delayed carbohydrate hydrolysis	Chia et al. 2018; Pradhan et al. 2020
Antitumor	Crude extract; tannins; carotenoids	HepG2, MCF-7 cell lines	Dose-dependent inhibition of cell proliferation	Cytotoxicity assay with IC_{50} in low μg/mL range	Ibraheem et al. 2017; Alves et al. 2018
Antiviral	Sulfated galactans (carrageenans)	HSV-1, SARS-CoV-2 pseudovirus	Inhibition of viral entry and replication	Viral plaque reduction and pseudovirus neutralization assays	Cicinskas et al. 2020; Aboeita et al. 2022
Antiparasitic	Kainic acid	Intestinal helminths (historical use)	Paralysis and expulsion of worms	Traditional usage; no recent pharmacological model	Maeno et al. 2019; Asakawa et al. 2020
Anti-inflammatory	Methanolic extract; astaxanthin	LPS-induced macrophage activation (RAW 264.7)	Inhibition of NO, TNF-α, and COX-2 expression	In vitro inflammation model using algal extract	Fernando et al. 2016; Sahin and Ozturk 2021
Neuroactive	Kainic acid	Kainate receptor agonist model	Induces seizures; modulates synaptic activity	Widely used in epilepsy and excitotoxicity models	Vezzani et al. 2016; Chekan et al. 2019

Table 3. Summary of major bioactive compounds in *Digenea simplex*, their mechanisms of action, and bioassay models

Bioactive compound	Mechanism of action	Bioassay model	Reference
Kainic acid	Agonist of kainate-type glutamate receptors; induces excitotoxicity	Rodent model for epilepsy and neurodegeneration	Chekan et al. 2019; Asakawa et al. 2020
Astaxanthin	Scavenges ROS, inhibits NF-κB signaling, modulates cytokines (e.g., TNF-α, IL-6)	In vitro (RAW264.7 cells), in vivo inflammation and oxidative stress models	Fernando et al. 2016; Dini 2022
β-carotene	Antioxidant; precursor to vitamin A; inhibits lipid peroxidation	Cell-based oxidative stress models	Alves et al. 2018; Bayomy and Alamri 2024
Lutein	Photoprotective antioxidant, reduces ROS generation	UV-induced oxidative stress models	Sathasivam and Ki, 2018
Tannins	Protein-binding, metal chelation, antimicrobial and antidiabetic via α-glucosidase inhibition	DPPH, ABTS, α-glucosidase assays	Ibraheem et al. 2017; Chia et al. 2018
Phenolic acids (e.g. gallic acid)	Radical scavenging, metal ion chelation, inhibition of lipid peroxidation	DPPH, FRAP, MIC assays	Fernando et al. 2016; Pratita et al. 2023
Carrageenans (sulfated galactans)	Immunomodulation, inhibition of tumor cell adhesion and viral binding	In vitro cytotoxicity, antiviral assays (HSV, Dengue)	Cicinskas et al. 2020; Aboeita et al. 2022
Essential amino acids (lysine, methionine)	Nutritional support, modulate insulin response, antioxidant via sulfur groups	Nutritional profiling, glucose uptake assays	Ward and Deyab 2021; Lopez and Mohiuddin, 2024
GABA (γ-aminobutyric acid)	Neuroinhibitory neurotransmitter; antihypertensive effects	Enzyme inhibition, neuronal signaling studies	Pratita et al. 2023
DMAPP (dimethyl-allyl pyrophosphate)	Precursor in isoprenoid biosynthesis (carotenoids, sterols); antioxidant role	Biosynthetic pathway modeling	Ganley et al. 2020; El-Malek et al. 2022

Table 4. Potential applications of *Digenea simplex* bioactives in health and industry

Application field	Key compounds	Mechanism of action	Target / disease area	Reference
Nutraceutical	Astaxanthin, β-carotene, dietary fiber, essential amino acids	Antioxidant, immune modulation, gut microbiota support	Anti-aging, oxidative stress-related disorders, gut health	Sathasivam and Ki, 2018; Ward and Deyab 2021
Functional Food	Carrageenans, polyphenols, amino acids	Enzyme inhibition (α-glucosidase), cholesterol reduction, satiety modulation	Type 2 diabetes, metabolic syndrome, obesity	Chia et al. 2018; Cicinskas et al. 2020
Pharmaceutical - Neurology	Kainic acid (KA), GABA, tryptophan	Glutamate receptor modulation, neurotransmission regulation	Epilepsy, neurodegenerative diseases	Vezzani et al. 2016; Asakawa et al. 2020
Pharmaceutical - Oncology	Phenolics, sulfated polysaccharides, carotenoids	Induction of apoptosis, inhibition of angiogenesis, oxidative stress targeting	Breast, colon, liver cancer	Fernando et al. 2016; Alves et al. 2018
Pharmaceutical - Anti-infective	Tannins, carrageenans, halogenated metabolites	Disruption of microbial membranes, viral entry inhibition	Bacterial infections, HSV, dengue, SARS-CoV-2	Ibraheem et al. 2017; Pradhan et al. 2020; Aboeita et al. 2022
Pharmaceutical - Metabolic Health	Astaxanthin, carotenoids, polyphenols	AMPK activation, reduction of blood glucose and lipids	Diabetes, hyperlipidemia	Bayomy and Alamri 2024
Cosmeceutical	Astaxanthin, β-carotene, sulfated polysaccharides	Antioxidant, skin hydration, UV protection	Anti-aging, skin damage, inflammation	Pereira 2015; Sahin and Ozturk, 2021; Abd El-Aziz et al. 2023
Biomonitoring / Environmental	Heavy metal-accumulating polysaccharides	Bioaccumulation and bioindication	Marine pollution monitoring	El-Rafie et al. 2023;

However, challenges remain regarding the standardization, safety, and regulatory acceptance of *D. simplex* in functional foods and nutraceuticals. Variability in bioactive compound levels due to habitat, season, or processing methods must be addressed through quality control and standard extraction protocols (Betancor et al. 2015; Gljušćić et al. 2022). Additionally, sensory acceptance, taste masking, and consumer perception should be considered in product development. Toxicological evaluation is necessary, particularly for high-dose applications, to ensure safety for long-term consumption.

D. simplex represents a versatile and underutilized marine bioresource with significant potential in the nutraceutical and functional food sectors. Its combination of essential nutrients and pharmacologically active compounds aligns well with global trends toward preventative health and natural product-based therapies. With appropriate processing, formulation, and regulatory compliance, *D. simplex* could be successfully integrated into the next generation of health-promoting food innovations.

Table 3 summarizes the major scientific gaps in the study of *D. simplex* to consolidate existing limitations and guide future research directions. These gaps span methodological, biochemical, ecological, and translational domains and provide a roadmap for advancing the utilization of this species in scientific and industrial applications.

Pharmaceutical prospects

Marine macroalgae are increasingly recognized as prolific producers of pharmacologically active compounds, many of which exhibit promising therapeutic potential for modern drug development. *D. simplex*, a red alga rich in chemically diverse metabolites such as kainic acid, phenolics, carotenoids, sulfated polysaccharides, and amino acids, offers multiple opportunities for pharmaceutical exploration (Fernando et al. 2016; Bayomy and Alamri 2024). These bioactive constituents target a wide spectrum of disease pathways, including inflammation, oxidative stress, microbial infections, and cancer, making *D. simplex* a valuable source for novel lead compounds (Agatonovic-Kustrin and Morton 2018). Its relatively untapped phytochemical profile, combined with traditional medicinal usage, supports the rationale for more in-depth pharmaceutical evaluation.

Among the most historically significant compounds from *D. simplex* is kainic acid, a neuroexcitatory amino acid that selectively activates kainate-type ionotropic glutamate receptors (Chekan et al. 2019; Asakawa et al. 2020). Though known for inducing seizures in animal models, kainic acid has become indispensable in neuroscience as a pharmacological tool for understanding epilepsy, neurotoxicity, and glutamatergic neurotransmission (Connell et al. 2017). Current research also explores structurally modified kainic acid derivatives for safer and more targeted neurotherapeutic applications, such as neuroprotection in ischemia and neurodegenerative conditions (Vezzani et al. 2016). This illustrates how marine-derived natural products, even those with initial toxicity, can inspire pharmaceutical innovation when biochemically modified or administered in controlled regimens (Table 5).

In the area of oncology, several constituents of *D. simplex* exhibit cytotoxic and antitumor properties. Phenolics and tannins have been shown to induce apoptosis, inhibit angiogenesis, and suppress metastasis through modulation of signaling pathways such as NF-κB and PI3K/Akt (Ibraheem et al. 2017; Alves et al. 2018). Carrageenans, the sulfated polysaccharides abundant in red algal cell walls, exhibit selective cytotoxicity against tumor cells and may act as immunomodulators, enhancing the host's antitumor response (Cicinskas et al. 2020; Aboeita et al. 2022). Carotenoids like astaxanthin and β-carotene also demonstrate antiproliferative effects and antioxidant support that could protect non-cancerous tissues during chemotherapy (Fernando et al. 2016; Dini 2022). The synergy among these compounds makes *D. simplex* an attractive candidate for combination therapy or adjunct treatment strategies in cancer management.

The anti-inflammatory activities of *D. simplex* also hold pharmaceutical value, particularly in chronic inflammatory disorders such as rheumatoid arthritis, Inflammatory Bowel Disease (IBD), and neuroinflammation. Bioactives such as astaxanthin and sulfated galactans have been found to inhibit key inflammatory mediators, including TNF-α, IL-6, COX-2, and inducible Nitric Oxide Synthase (iNOS) (Sahin and Ozturk 2021; Bayomy and Alamri 2024). The potential for oral, topical, or injectable formulations derived from *D. simplex* extracts or isolated compounds could pave the way for novel anti-inflammatory drugs with fewer side effects than NSAIDs. Furthermore, the dual role of several compounds (e.g., antioxidant + anti-inflammatory) increases their pharmaceutical appeal due to multifunctional therapeutic action.

In the field of infectious disease therapy, *D. simplex* is a treasure trove of metabolites with potent antimicrobial and antiviral properties. For example, polysaccharides like carrageenans have been found to block viral attachment and entry into host cells, particularly in enveloped viruses such as herpes simplex virus (HSV) and SARS-CoV-2 (Sathasivam and Ki, 2018; Aboeita et al. 2022). In antibacterial therapy, phenolics and halogenated compounds from red algae are effective against multidrug-resistant strains like *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Agatonovic-Kustrin and Morton 2018). These natural compounds, with their relatively low toxicity and good biocompatibility, are not just theoretical marvels but also find practical applications in wound healing products, topical antimicrobial gels, or mucosal delivery systems such as lozenges and sprays.

Red algal polysaccharides are also being investigated for use as drug carriers in targeted delivery systems. Their biocompatibility, water solubility, and ability to form hydrogels position carrageenans and related polysaccharides as ideal biopolymers for encapsulating Active Pharmaceutical Ingredients (APIs) (Calvo et al. 2019; Hung and Trinh 2021). Microencapsulation of poorly soluble drugs or sensitive bioactives using *D. simplex*-derived materials may enhance drug stability, control release rates, and improve bioavailability. In addition, these polymers may confer mucoadhesive properties, enabling prolonged contact with mucosal membranes and increased local therapeutic efficacy.

Modern pharmaceutical research also seeks marine-derived neuroprotectants and cognition enhancers, and *D. simplex* offers significant promise in this realm. The potential neuroprotective effects of kainic acid derivatives and antioxidant carotenoids may support drug development for Alzheimer's, Parkinson's, or age-related cognitive decline (Ganley et al. 2020). Combining neuroactive and anti-inflammatory actions within a single compound or extract aligns with the current paradigm of Multitarget-Directed Ligands (MTDLs) in complex neurological conditions. Metabolomic and transcriptomic profiling of *D. simplex* under varying stress conditions could uncover new neuroactive scaffolds for drug discovery.

Table 5. Current research gaps and recommended future directions on *Digenea simplex*

Identified gap	Scientific importance	Recommended future research	References
Limited in vivo and clinical studies on pharmacological activities	Most bioactivities are demonstrated only in vitro; lacks translational evidence	Conduct in vivo animal trials and early-phase clinical studies on antioxidant, antidiabetic, and anticancer effects	Fernando et al. 2016; Alves et al. 2018
Lack of standardized extraction and quantification protocols	Variability in bioactive yield across studies; hard to compare results	Develop and validate optimized, reproducible extraction and quantification methods (e.g., HPLC-based)	Calvo et al. 2019; Dini 2022
Unclear toxicity and safety profiles of active compounds (e.g. kainic acid)	Some compounds may be neurotoxic; safety data essential for functional/therapeutic use	Perform toxicological assessments (acute, chronic, genotoxicity) and define safe dosage ranges	Vezzani et al. 2016; Asakawa et al. 2020
Minimal genetic and omics-level studies (e.g. transcriptomics, metabolomics)	Biosynthetic pathways remain partially understood; biosynthesis regulation unclear	Apply omics tools to identify genes/enzymes in biosynthesis of kainoids, carotenoids, and phenolics	Chekan et al. 2019; El-Malek 2022
Poor taxonomic resolution and cryptic species uncertainty	Misidentification can bias ecological and biochemical findings	Integrate molecular barcoding (e.g. rbcL, COI) with morphological analyses to revise taxonomy	Díaz-Tapia et al. 2017; Boo et al. 2018
Absence of biotechnological production systems	Wild harvesting unsustainable; limits scalability for industry	Develop algal cultivation, tissue culture, or synthetic biology platforms to produce target compounds	Ganley and Derbyshire 2020; Hung and Trinh 2021
Limited study on structure-activity relationships (SAR) of compounds	Understanding SAR is crucial for drug optimization and analog development	Isolate pure compounds, determine 3D structures, and evaluate SAR using computational and empirical approaches	Agatonovic-Kustrin and Morton, 2018; Bayomy and Alamri 2024

Despite its potential, the pharmaceutical exploitation of *D. simplex* remains limited by gaps in preclinical and clinical validation. Most studies are still confined to in vitro bioassays or crude extracts without identification of active principles. Future directions should prioritize bioassay-guided fractionation, structural elucidation, mechanism-of-action studies, and in vivo toxicity testing. Moreover, collaborations between marine biologists, pharmacologists, and pharmaceutical industry stakeholders will be crucial for translating the pharmacological promise of *D. simplex* into clinically viable products.

Figure 3 illustrates visual synthesis of the relationships between major compounds, their bioactivities, and application fields of *D. simplex*, highlighting this species' integrative potential for health-related industries.

Cosmetic applications

The increasing consumer demand for natural, safe, and sustainable cosmetic ingredients has driven attention toward marine-derived bioactives, particularly those from red macroalgae such as *D. simplex*. Seaweeds have long been utilized in skincare and personal care formulations due to their moisturizing, antioxidant, anti-aging, and UV-protective properties (Sahin and Ozturk 2021; Abd El-Aziz et al. 2023). *D. simplex*, with its rich profile of phenolics, carotenoids, sulfated polysaccharides, and amino acids, presents multiple functional attributes desirable in modern cosmetics. These compounds target key skin-related concerns such as oxidative damage, photoaging, inflammation, and moisture retention.

One of the primary cosmetic values of *D. simplex* lies in its antioxidant potential, conferred by compounds such as astaxanthin, β-carotene, and phenolic acids (Fernando et al. 2016; Bayomy and Alamri 2024). These antioxidants help neutralize Reactive Oxygen Species (ROS) generated by UV radiation and environmental pollutants, which contribute to premature aging, hyperpigmentation, and loss of skin elasticity (Dini 2022). Astaxanthin, in particular, is known to penetrate skin layers and protect mitochondrial membranes, resulting in improved skin elasticity and reduced wrinkle formation. Incorporation of *D. simplex*-derived antioxidant extracts into creams, lotions, and serums can provide protective and reparative benefits to the skin.

Another notable property of *D. simplex* is its anti-inflammatory activity, which can soothe irritated skin and reduce redness, swelling, or allergic reactions. Inflammatory skin disorders such as acne, rosacea, and eczema are often exacerbated by oxidative stress and microbial imbalance. Compounds such as tannins, sulfated polysaccharides, and carotenoids have been shown to inhibit pro-inflammatory mediators like COX-2 and TNF-α, thereby promoting a calmer and more balanced skin environment (Ibraheem et al. 2017; Alves et al. 2018). These effects are particularly valuable in formulations for sensitive skin or after-sun care products that offer hope for those dealing with such conditions.

The hydrating and moisturizing effects of *D. simplex* are largely attributed to its sulfated polysaccharide content, particularly carrageenans, which have practical applications. These compounds exhibit high water-retention capacity and the ability to form hydrogels, which help maintain skin hydration by preventing transepidermal water loss (Cicinskas et al. 2020; Hung and Trinh 2021). The film-forming ability of carrageenans also contributes to a protective barrier on the skin surface, enhancing texture and smoothness. These properties make *D. simplex*-based ingredients ideal for use in moisturizers, sheet masks, and leave-on hydrating treatments.

D. simplex also offers potential anti-aging and skin-rejuvenating properties, as its carotenoids and peptides may stimulate collagen synthesis and inhibit Matrix Metalloproteinases (MMPs), enzymes responsible for collagen degradation (Sathasivam and Ki 2018). By preserving extracellular matrix integrity, these compounds can help maintain skin firmness and reduce the appearance of fine lines and sagging. Furthermore, phenolic compounds in *D. simplex* may reduce melanin synthesis by inhibiting tyrosinase, suggesting applications in skin-brightening or anti-spot formulations (Fernando et al. 2016). This multifactorial approach to skin aging makes *D. simplex* an attractive botanical in anti-aging product lines.

Another growing trend is the use of algae-derived extracts for UV protection and photoprotection. Although red algae do not produce traditional Mycosporine-Like Amino Acids (MAAs) as abundantly as some other algal groups, their high content of carotenoids and polyphenols offers natural photoprotective effects (Agatonovic-Kustrin and Morton 2018; Gljušćić et al. 2022). These compounds absorb UV rays and prevent UV-induced inflammation and DNA damage. While not intended to replace synthetic sunscreens, *D. simplex* may serve as a complementary ingredient in SPF-boosting formulations and after-sun repair products.

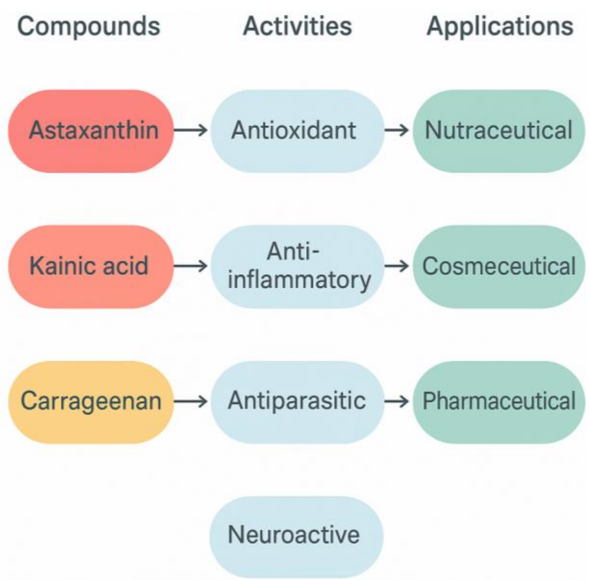

Figure 3. Flowchart illustrating the relationships between chemical constituents of *Digenea simplex*, their pharmacological activities, and corresponding industrial applications

From a formulation perspective, *D. simplex* also brings textural and functional advantages. The viscous and gel-like properties of its polysaccharide content can act as natural emulsifiers and thickeners, improving product consistency and skin feel without the need for synthetic additives (Calvo et al. 2019). This aligns with the clean-label movement in the cosmetic industry, where consumers prefer minimalistic and recognizable ingredient lists. Additionally, the natural reddish hue from carotenoids may offer mild coloring benefits, particularly in tinted moisturizers or serums.

The biocompatibility and low toxicity of *D. simplex*-derived compounds further support their safe application in topical formulations. However, to ensure product safety and consistency, it is necessary to standardize extraction methods, assess stability during storage, and evaluate potential allergenicity through dermatological testing (Betancor et al. 2015; Sahin and Ozturk 2021). Novel green extraction technologies such as ultrasound-assisted or enzyme-mediated extraction can also be adapted to enhance yield and preserve compound integrity (Hung and Trinh 2021).

With growing interest in marine-based skincare and the increasing availability of algal biorefineries, the commercial potential of *D. simplex* in the cosmetic industry is significant. Its multifunctional bioactive profile aligns with current demands for natural, effective, and sustainable ingredients. Future research should focus on clinical evaluation, bioavailability studies, and synergistic formulation with other botanicals to unlock the full cosmetic potential of *D. simplex*.

CHALLENGES AND FUTURE RESEARCH

Despite the promising bioactive potential of *D. simplex*, several challenges hinder its full integration into health-related industries. Species-specific studies remain scarce, with much of the existing literature extrapolated from broader Rhodophyta research, limiting targeted insights and slowing translational application. The lack of standardized cultivation protocols poses a major bottleneck, as wild harvesting is subject to seasonal and ecological variability. Environmental factors such as light, salinity, and nutrient availability significantly affect metabolite yield and composition, making consistency in biomass production difficult without controlled aquaculture systems or stress-induction strategies. In this context, sustainable and scalable cultivation remains a top priority for commercial development.

Another constraint lies in the limited understanding of the biosynthetic pathways responsible for key metabolites such as kainic acid, carotenoids, and sulfated polysaccharides. While kainic acid biosynthesis has been partially elucidated, comprehensive insights into regulatory genes and metabolic flux are lacking for most compounds. Advanced omics-based approaches integrating transcriptomic, metabolomic, and proteomic data could clarify these pathways and identify targets for metabolic engineering. Extraction technologies also require optimization; conventional solvent-based methods risk degrading thermolabile compounds, reducing both yield and functional activity. Green extraction methods like supercritical CO_2 and ultrasound-assisted systems offer more selective recovery but remain underexplored for this species. Moreover, toxicological data on *D. simplex* are limited. While certain compounds, such as kainic acid, have known neurotoxic effects at high doses, others including polyphenols and polysaccharides lack comprehensive safety evaluations, particularly in terms of allergenicity, bioavailability, and long-term effects. Robust in vivo studies and toxicological assessments are needed for regulatory approval.

Finally, the gap between in vitro bioactivity and in vivo or clinical efficacy continues to impede commercialization. Most pharmacological data derive from cell-based assays, with few follow-up studies in animal models or human trials. Without pharmacokinetic and pharmacodynamic validation, therapeutic relevance remains speculative. Regulatory pathways—such as GRAS, Novel Food, or NHP approval—also demand strong safety and efficacy dossiers. Additionally, *D. simplex* lacks public recognition and a distinct market identity compared to other macroalgae. Addressing these limitations will require interdisciplinary collaboration across marine biology, chemistry, pharmacology, and industrial processing. Coordinated efforts, including public-private partnerships and supportive policy frameworks, are essential to advance *D. simplex* as a viable marine bioresource for food, pharmaceutical, and cosmeceutical innovation.

CONCLUDING REMARKS

Digenea simplex is a promising but underexplored marine macroalga with a diverse spectrum of bioactive compounds that support its use in health-related applications. The presence of kainic acid, sulfated polysaccharides, carotenoids, phenolics, and essential amino acids reflects its multifunctionality across pharmaceutical, nutraceutical, and cosmetic domains. These compounds have demonstrated antioxidant, antidiabetic, antitumor, anti-inflammatory, neuroactive, antimicrobial, and antiparasitic properties in vitro, suggesting potential therapeutic value for chronic and infectious diseases. Despite its rich phytochemical potential, scientific research on *D. simplex* remains limited and fragmented compared to other commercially cultivated red algae. Key challenges include the lack of standardized cultivation systems, inconsistent extraction protocols, limited pharmacological validation, and inadequate safety data. Addressing these gaps through multi-omics studies, bioassay-guided compound isolation, toxicological evaluation, and controlled cultivation may unlock broader utilization of *D. simplex* as a sustainable marine bioresource. Its ecological adaptability and biochemical plasticity make *D. simplex* an excellent candidate for biotechnological innovation. With the growing global demand for natural and multifunctional ingredients, integrating this species into circular bioeconomy strategies could contribute to the sustainable use of marine resources. Interdisciplinary research collaborations and supportive policy frameworks will be essential to realize the full potential of *D. simplex* in modern health, wellness, and therapeutic industries.

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