

Turbinaria: Exploring the Untapped Potential of Bioactive Compounds from Nature's Marine Brown Algae

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Abstract: The study of seaweeds has garnered significant scientific attention for over seven decades, revealing their abundant composition of enzymes, polysaccharides, trace minerals, essential vitamins, and a variety of bioactive compounds. These compounds possess certain health-promoting activities, including antiviral, antibacterial, and antioxidant effects, which contribute to their potential therapeutic value. Among Marine algae, the genus *Turbinaria* stands out for its robust antioxidant, antidiabetic, and anticancer activities, as well as a range of other pharmacological activities. This review will elucidate and emphasize the roles of algae, notably *Turbinaria*, in drug development, their ability to substitute for synthetic drugs, and provide insights into the treatment of anticancer and other neurological disorders. To facilitate effective use of seaweeds, further research will be required to understand the mechanisms of action of these bioactive compounds, to enable their use in certain aspects of public healthcare and commercial applications.

Keywords: *Turbinaria*; bioactive compounds; seaweed; drug development; pharmaceutical applications; biological activities.

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1. Introduction

Marine organisms harbor vast potential as rich reservoirs of highly bioactive secondary metabolites, offering valuable prospects for the development of novel pharmacological agents [1]. Over the past seven decades, considerable progress has been made in identifying new compounds from marine species, many of which exhibit intriguing biological properties. More than 14,000 distinct products have been identified since the discovery of sponge-derived chemicals, many of which are currently participating in clinical studies to treat conditions such as cancer, pain, and other conditions [2]. Algae, scientifically known as photosynthetic eukaryotic microorganisms, comprise a diverse and ubiquitous group found in various aquatic habitats. They constitute a polyphyletic assemblage, comprising a wide array of taxonomic groups. The varieties of algae encompass both microalgae and macroalgae, representing distinct categories within the algae taxonomy. Microalgae are microscopic unicellular or

colonial algae, often characterized by their small size, rapid growth rates, and high photosynthetic efficiency. While macroalgae encompass larger, multicellular, more complex organisms found in both freshwater and marine habitats, they are typically visible to the naked eye. Macroalgae, commonly known as seaweeds, are anchored to substrates such as rocks and shells at depths of up to 180 meters, exhibiting a wide range of sizes, shapes, colors, and textures. Seaweeds play vital ecological roles as primary producers and habitat providers, contributing to nutrient cycling and biodiversity in marine ecosystems. They are abundant in bioactive substances, such as polysaccharides, proteins, lipids, vitamins, minerals, and secondary metabolites, which possess various biological activities, including antioxidant, antimicrobial, and anti-inflammatory properties [3]. India's extensive coastline, stretching 8,100 kilometers, supports a variety of coastal ecosystems, including beaches, coral reefs, estuaries, and mangroves [4]. This vast coastline is home to a wide variety of marine life. The country's Exclusive Economic Zone (EEZ) spans approximately 2.17 million square kilometers, with about 30% of the global population relying on marine and coastal resources [5]. India's diverse coastal ecosystems provide a conducive environment for the growth of economically significant seaweed populations. For instance, the Central Salt and Marine Chemicals Research Institute (CSMCRI) has documented seaweed diversity along the coasts of Gujarat and Kerala, identifying 366 species, representing approximately half of the seaweed diversity in India [6].

Marine seaweeds are renewable resources, increasingly recognized for their various applications, regenerative capabilities, and abundance. They are rich in vital nutrients, encompassing vitamins, proteins, carbohydrates, minerals such as phosphorus, sulfur, calcium, magnesium, potassium, chlorine, and sodium. Additionally, they provide significant micronutrients, including iodine, iron, zinc, copper, selenium, molybdenum, fluoride, manganese, boron, nickel, and cobalt [3]. Based on their pigmentation, seaweeds are categorized into three taxonomic groups: green algae (Chlorophyta), red algae (Rhodophyta), and brown algae (Ochrophyta) (Figure 1). Green algae get their color mainly from the presence of chlorophyll a and chlorophyll b pigments. They exhibit a range of forms, from microscopic single-celled entities to large, complex multicellular structures. Red algae are multicellular [7], lack centrioles and flagella, and the few unicellular red algae are predominantly extremophiles that thrive in acidic hot springs.

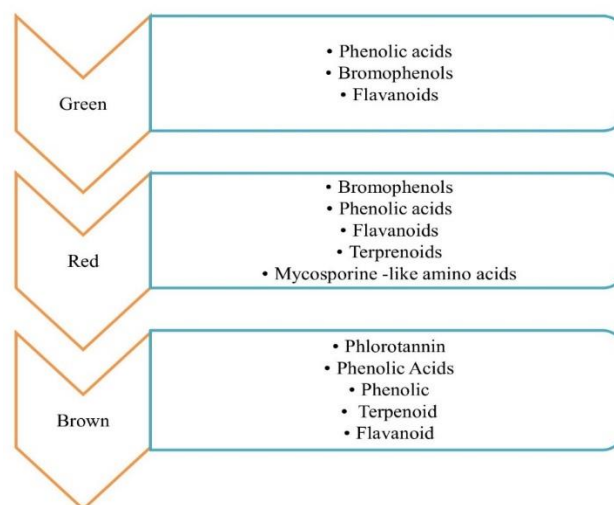


Figure 1. Classification of seaweeds and their phenolic compounds.

These algae vary in color from red to pink to purple [8], although their growth often takes multicellular forms across a range of depths from the surface to the deep sea. Brown algae occur in several of the largest seaweeds and are mostly marine. The algae have a significant ecological impact and are a major food source for marine life [9]. The brown algae are particularly nutritious and have medical potential. Their specific features are attributed to their high polysaccharide content, which confers numerous diverse physical and chemical properties, enabling advantageous interactions with biological systems [10].

1.1. Genus *Turbinaria* and its importance.

The *Turbinaria* genus was first described by J. V. Lamouroux in 1825. Mainly found within Tropical and subtropical marine regions, *Turbinaria* usually grows on stony reefs. This genus includes 33 species in the database, of which 22 are taxonomically recognized [11]. Some of the common species include *Turbinaria capensis*, *Turbinaria conoides*, *Turbinaria costata*, *Turbinaria crateriformis*, *Turbinaria decurrens*, *Turbinaria denudate*, *Turbinaria elatensis*, and *Turbinaria filamentosa*. This genus of brown algae mainly lives on rocky surfaces in tropical marine environments [12], but is also found in temperate and subtropical regions, besides living on tropical coral reefs. The greatest diversity of the genus is observed in Southeast Asia and the Indian Ocean. According to Zubia *et al.* [13], *Turbinaria* can survive in a wide range of environments, including rocky intertidal zones, fore reefs, and tide pools. It can grow at depths of about 30 meters and is tolerant to a variety of exposures. The genus is iron-rich and shows a preference for nickel and arsenic, depending on the concentrations in the environment [13]. The carotenoid pigment contained by *Turbinaria* is fucoxanthin. Thus, in all the brown algae, this pigment participates in energy transfer and light harvesting [14]. The pattern of growth in *Turbinaria* consists of side branches emerging laterally from the central axis of the thallus, giving rise to a branching structure from a single central axis. The leaves bear various morphologies, viz, shield-shaped (peltate), top-shaped (turbinate), and other hard structures, usually with toothed or serrated edges [14]. Depending on the species, the outer leaf surfaces may be flat, concave, triangular, or rectangular. Variation in leaf morphology distinguishes different species of *Turbinaria*. The herbarium of *Turbinaria decurrens* is represented in Figure 2. Various species are identified by complex leaf forms, branching patterns, and adaptations that help them to survive in marine environments [15]. In this alga, the individual plant body, the thallus, is connected by an intricate lattice of spreading branches arising from the main axis, providing overall stability and structure to the alga. The receptacles are located on the upper sides of the leaf stalks. These structures play a very significant role in the algae's reproductive cycle. In fact, many species of *Turbinaria* contain air vesicles within the peltate portion of the leaves. This imparts the thallus, a tendency to float closer to the surface of the water, and allows sunlight access for photosynthesis. Although compounds derived from *Turbinaria* have received little attention from researchers, they possess potential medicinal activities, including antiviral, antibacterial, cytotoxic, anti-pyretic, anti-inflammatory, hypolipidemic, hepatoprotective, and cardioprotective [16]. Several metabolites, including oxygenated fucosterols, tanacetol A, and embelin, have attracted significant interest owing to their unique, intriguing pharmacological properties and intricate structures. This literature review presents significant compounds and biological functions in chemistry and medicine of the genus *Turbinaria*, along with other seaweeds. Relevant publications from 2020 to 2023 were identified through searches of online bibliographic databases such as Scopus, PubMed, and Google Scholar, using keywords including brown

algae, antioxidant activity, antidiabetic activity, anticancer activity, and secondary metabolites in *Turbinaria* seaweeds.



Figure 2. Herbarium of *Turbinaria decurrens*.

1.2. Taxonomical classification of *Turbinaria*.

The genus *Turbinaria* belongs to the Kingdom Chromista, class Phaeophyceae, family Sargassaceae, order Fucales (Table 1). Morphological features such as the form of the vesicles, receptacles, and leaves, as well as the development of the axis, serve as the basis for the taxonomy classification [12]. Nevertheless, these characteristics may vary significantly among various species.

Table 1. Taxonomic classification of the seaweed *Turbinaria*.

Domain	Eukaryota
Kingdom	Chromista
Subkingdom	Harosa
Class	Phaeophyceae
Subclass	Fucophycidae
Family	Sargassaceae
Order	Fucales
Genus	<i>Turbinaria</i>

1.3. Compounds of *Turbinaria*.

Compounds isolated from *Turbinaria* have shown significant potential as anticancer agents, inhibiting cancer cell growth and potentially lowering blood sugar levels, aiding in diabetes management. Some of the compounds (Figure 3) have exhibited antiviral and antimicrobial activity, showing promising natural alternatives to synthetic antimicrobial agents [17]. The hepatoprotective properties of some compounds may benefit individuals with liver diseases by helping protect the liver from damage [18]. For instance, *Turbinaria* fucose constitutes most of the fucoidan, a sulfated polysaccharide, and also contains simple sugars such as galactose, xylose, and glucuronic acid. The potential health benefits of fucoidan, particularly its anticancer properties, have garnered considerable interest among medical scientists [19]. Fucoidan exhibits anticoagulant properties, with the majority of sulfate groups at the C3 and C6 positions of galactose, while fucoidan with anticoagulant activity has been identified at the C4 and, to a lesser extent, C2 positions of the (1–3)- α -l-fucopyranosyl residue. Silver nanoparticles (AgNPs) with potent anticoagulant properties that are produced with isolated fucoidan efficacy and high antimicrobial activity against gram-negative pathogens [20]. Among the various pharmacological properties of fucoidans, their anti-proliferative efficacy against the human lung cancer cell line A549 has been emphasized in studies by Boo

et al. [21] and Jin *et al.* [22]. Researchers have also identified fucoxanthin from *Turbinaria decurrens* (TD) as a potential treatment for colon cancer. Cytotoxicity assays using the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) method and the Cell Counting Kit-8 (CKK-8) have shown that TD inhibits proliferation and triggers apoptosis in human colorectal cancer cell lines [23,24]. Potential use of fucoxanthin for colorectal cancer treatment has been supported by various research findings [25]. Studies have demonstrated that natural phlorotannins extracted from *Turbinaria ornata* (TO) hinder the expression of a chemical, namely histamine release from histamine-releasing cell line RBL-2H3 mast cells [26]. These phlorotannins have also shown anti-allergic activity against DNP-BSA (2, 4-Dinitrophenyl-Bovine Serum Albumin) and compound 48/80-induced anaphylaxis [27]. Other phlorotannins isolated from TO exhibit antioxidant properties, modulating the transcriptional levels of specific genes [(SOD1 (superoxide dismutase type 1), GPX (Glutathione Peroxidase), CAT (catalase), Nrf2 (nuclear factor erythroid 2-related factor 2), BCL2 (-cell leukemia/lymphoma 2 protein), and BAX (Bcl-2-associated protein x)] that mediate reactive oxygen species (ROS) activity in RAW 264.7 cells [28]. Phlorotannins also activate the Nrf2 signaling pathway in primary mouse splenocytes [29]. In addition to TO's anticancer, antioxidant, antidiabetic, and anti-allergic activities, its metabolites have shown antiviral and antimicrobial properties, indicating the genus's wide-ranging potential for disease management [30].

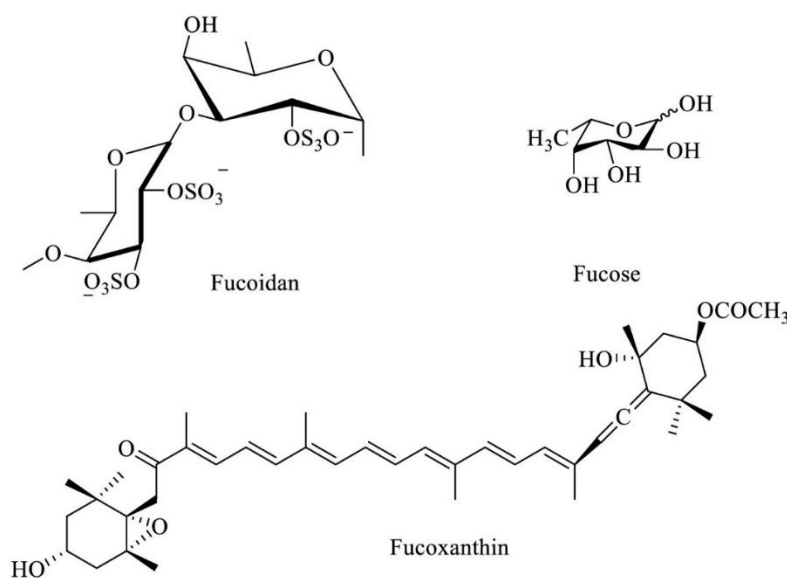


Figure 3. Chemical structures of Fucoidan, Fucose, Fucoxanthin (ChemDraw Ultra 8 software was used to draw the chemical structures).

2. Secondary Metabolites of *Turbinaria*

Turbinaria synthesizes many secondary metabolites, such as phlorotannins, fucosterol, and polyphenols, which serve crucial roles in defense mechanisms against pathogens. These metabolites are instrumental in the alga's ability to counteract various biotic stresses, offering substantial potential for pharmaceutical and therapeutic applications (Figure 4).

2.1. Phlorotannins.

Phlorotannins, a subset of phenolic compounds, are synthesized in seaweeds through the polymerization of phloroglucinol through the acetate-malonate pathway. These are hydrophilic compounds that exhibit molecular weights ranging from 126kDa to 650kDa [25].

Phlorotannins are known for their diverse biological processes, namely photoprotection, radioprotection, anti-allergic, anti-diabetic, antioxidant, anti-proliferative, and anti-HIV [27]. According to recent studies by Kapilan *et al.* [28], the molecular weight and total phlorotannin content in *TD* were identified by using UV-visible spectroscopy and RP-HPLC (Reverse Phase High Performance Liquid Chromatography). The isolated phlorotannin fractions displayed the extract's important antibacterial, anti-inflammatory, and antioxidant activities. Another research conducted by Girija *et al.* [29] confirmed the antioxidant capacity of phlorotannins present in the *TO* methanolic extract on the basis of Thin Layer Chromatography (TLC), UV-Visible, and Fourier-Transform Infrared (FT-IR) spectroscopy spectral analysis of the F5 fraction.

2.2. Fucosterol.

Fucosterol, a marine algae-specific sterol abundant in brown seaweeds, is known for its several bioactivities, including anti-inflammatory, anticancer, and antiphotaging properties, immunostimulating, hepatoprotective, neuroprotective, antioxidant, algicidal, anti-obesity, and antibacterial effects. This sterol is a major constituent of the *Turbinaria* species and exhibits substantial health-beneficial effects. Kumar *et al.* [30] isolated fucosterol from *T. conoides* (*TC*) gathered from the Gulf of Mannar, India, that showed potent antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger*, and *Candida albicans*. Sheu *et al.* [31] demonstrated the cytotoxicity of fucosterol isolated from *TC* against several cancer cell lines, and the structures were supported by spectral analysis. Other sterols, such as 24 ξ -hydroperoxy-24-vinylcholesterol, were found in *T. tricostata* (*TT*) extracts, with cytotoxicity against the cancer cell line CC50, ranging from 14.8 to 41.2 $\mu\text{g/mL}$. Fucoidans extracted from *TO* using enzyme-assisted extraction also displayed strong *in vivo* anti-inflammatory activity, as assessed by FTIR spectroscopy and tested in RAW 264.7 macrophage cell lines. The results of the experiment demonstrated that fucoidan effectively inhibited inflammation induced by lipopolysaccharides, suggesting potential applications in both the pharmaceutical and cosmeceutical industries [32].

2.3. Polyphenols.

In brown algae, polyphenols, including phlorotannins, are usually produced under extreme environmental conditions, enabling them to absorb UV radiation and aid wound healing [33,34]. Such polyphenols play a vital role in cosmeceutical and nutraceutical formulations owing to their broad health implications [35,36]. Vijayabaskar *et al.* [37] also demonstrated that *TO* contains high levels of phenolic compounds, as confirmed by FTIR and TLC, and exhibits strong free-radical-scavenging activity. Moreover, ABTS and DPPH free radical assays of the *TD* methanolic extract demonstrated polyphenol content, indicating broad-spectrum antioxidant activity. These extracts also exhibited a pronounced ability to reduce copper ions [38]. The antibacterial properties of fucoidan extracted from *TO* extract were examined against a range of pathogenic microorganisms, both Gram-positive and Gram-negative, with polyphenols being identified as the key active agents. These phenolic compounds, based on their composition, significantly influence bacterial growth and metabolism, supporting the use of *TO* methanol extracts as potent antimicrobial agents [39].

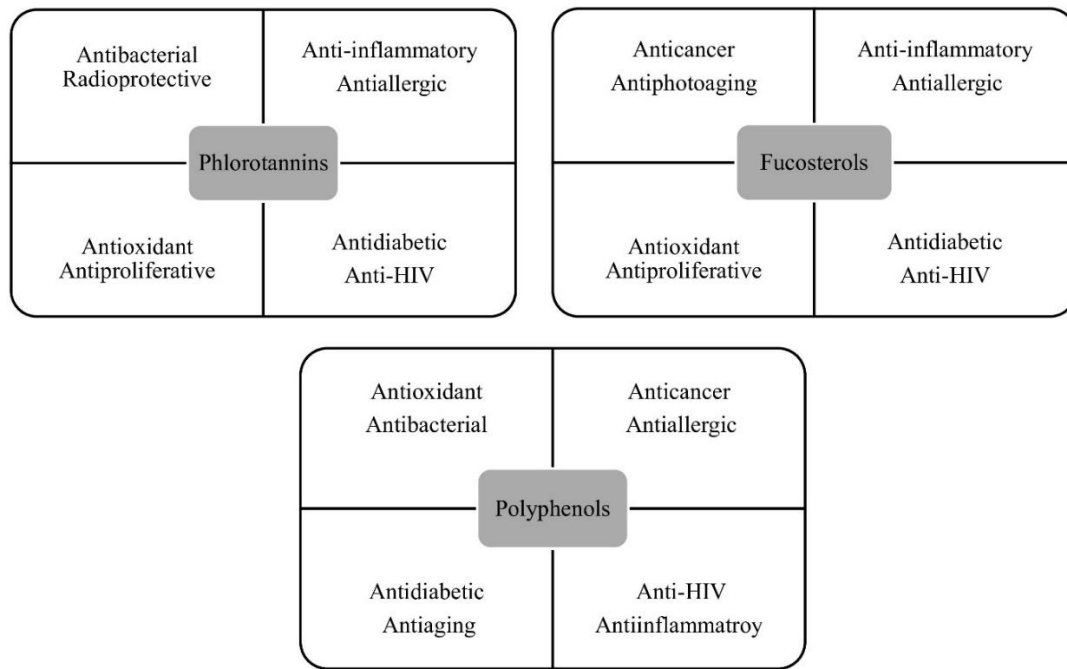


Figure 4. Bioactivities of phlorotannins, fucosterols, and polyphenols.

3. Biological Properties of Algae

3.1. Anticancer activity.

Cancer continues to be among the most prevalent diseases worldwide, with existing therapies often accompanied by significant side effects. Therefore, developing novel treatments with fewer adverse effects is crucial. Seaweed-derived polysaccharides offer a promising avenue for anticancer therapy due to their potential to treat various cancer types. These polysaccharides function via multiple mechanisms, including disrupting mitochondrial membranes, inducing DNA damage, initiating cell cycle arrest, and stimulating nitric oxide production [40]. Several anticancer properties of fucoidan have been demonstrated, including stimulation of apoptosis and inhibition of tumor-induced angiogenesis [41]. It has been shown to be effective against various cancers such as the bladder, breast, colon, liver, prostate, and melanoma. In addition to its potential benefits for gastrointestinal health, fiber from polysaccharides may also help prevent colon cancer. Marudhupandi *et al.* [42] documented the anti-proliferative activity of fucoidan from *TC* on cancer cell lines in a dose-dependent manner. Fucoidan induces stress signals in cancer cell lines, triggering the release of cytochrome c and activating initiator caspase-9. This activation triggers the activation of executioner caspases, which cleave essential structural and repair proteins, ultimately leading to DNA fragmentation and apoptotic cell death. Another researcher reported that the hentriacontane compound from the *TO* hexane (*TO*-HE) extract had the highest anti-proliferative and antioxidant activity. Hentriacontane inhibited ERK/MAPK (mitogen-activated protein kinase) signaling, thereby preventing the proliferation of cancer cell lines. It also suppresses NF- κ B, leading to decreased levels of anti-apoptotic proteins and inflammatory cytokines. Furthermore, it reduces oxidative stress to prevent DNA damage, promotes apoptosis, and stabilizes cell membranes, ultimately disrupting the survival and growth of cancer cells [43]. Further, antioxidants, including β -carotene, have been shown to help prevent precancerous conditions such as oral leukoplakia [44]. Green synthesis of gold nanoparticles (AuNPs) via the hydromethanolic extract (HME) of *TD* was used to carry out <https://nanobioletters.com/>

the antioxidant and anticancer properties of both HME and its AuNPs. The biosynthesized AuNPs induced substantial production of reactive oxygen species (ROS) in cancer cell lines, causing significant oxidative damage. ROS-mediated mitochondrial membrane depolarization resulted in the release of cytochrome c into the cytosol, where it interacted with apoptotic protease-activating factor 1 (Apaf-1) to form the apoptosome, activating initiator caspase-9, ultimately leading to DNA fragmentation and programmed cell death [45].

3.2. Neuroprotective activity.

A study by Meenakshi et al. [46] indicated that fucoidan from TD has neuroprotective effects against MPTP (1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-induced Parkinson's disease in a rat model. MPTP intoxication triggers ROS overproduction, mitochondrial dysfunction, and activation of microglial inflammatory pathways, leading to dopaminergic neuronal apoptosis. Fucoidan reduces ROS overproduction, stabilizes mitochondrial membrane potential, and thereby prevents cytochrome c release. Additionally, fucoidan suppresses NF- κ B signaling and downregulates proinflammatory cytokine release. Myricetin, a flavonoid compound in various plants, including *TO*, also exhibits neuroprotective effects. Rotenone-treated *Drosophila melanogaster* models of Parkinson's disease are used to evaluate the efficacy of myricetin on neurodegenerative conditions. Myricetin has been shown to delay neurodegeneration, maintain dopamine levels, prevent apoptosis, and reduce oxidative stress by regulating the balance between oxidants and antioxidants [47]. An experiment was conducted to check the inhibitory potential of Acetylcholine (ACh) and Butyrylcholine (BuCh) esterase enzymes in a fraction of *TO*. ACh and BuCh are important neurotransmitters required for the proper performance of brain signal transmission, as well as the electrical impulses that go between neurons in the synaptic cleft. The methanol-water fraction from *TO* (MWTO) suppresses ACh and BuCh, leading to elevated acetylcholine concentrations in the synaptic cleft. This elevation enhances signaling through muscarinic and nicotinic receptors, activating pathways such as the PI3K/Akt (phosphoinositide 3-kinase/protein kinase B) signaling pathway, thereby facilitating synaptic plasticity and neuronal differentiation with no harmful effects. MWTO, therefore, can serve as an excellent protective drug for healthy neurons while replacing damaged cells, thereby slowing the progression of neurodegenerative diseases [48].

3.3. Tissue engineering.

A naturally occurring polymer called alginate is derived from brown seaweeds such as *Turbinaria*, *Macrocystis*, *Sargassum*, *Ecklonia*, *Laminaria*, *Lessonia*, and *Ascophyllum*. These polymers consist of linear chains of D-mannuronic acid and L-guluronic acid units linked by β (1 \rightarrow 4) bonds [49]. Alginate's unique physicochemical properties, particularly its ability to form gels in aqueous environments, have attracted increasing attention in biomedicine and pharmaceuticals. Crosslinked alginate hydrogels have been developed for tissue engineering and the delivery of bioactive materials. These scaffolds are crucial for regulating the shape and functionality of engineered tissues, facilitating tissue growth and regeneration. Fucoidan-enriched seaweed extracts have shown potential for treating osteoarthritis by enhancing osteocalcin levels and alkaline phosphatase activity, thereby promoting bone mineral deposition [50]. Alginate-based biocompatible hydrogels are significantly impactful in

addressing various organ, bone, and cartilage disorders and are utilized in stem cell transplantation [51].

3.4. Other biological activities.

Algae contain a significant amount of soluble dietary fiber, known for its capacity to lower plasma cholesterol, increase viscosity, and reduce glycemic response. Additionally, these fibers help reduce the risk of cardiovascular disease and triglyceride levels [52,53]. A study by Krishnamurthy *et al.* [54] suggested that the sulfated polysaccharides of *TC* have membrane-stabilizing activities that protect against isoproterenol-induced myocardial damage, possibly by reducing lipid peroxidation. It may also revive the interest in using *TC* fucoidan as a possible inhibitor of myocardial injury. Moreover, the anti-HIV potential of *TD* has recently been well documented [55], in which RP-HPLC and FT-IR analyses were employed to assess the bioactivity of the sulfated polysaccharide fucoidan obtained from two macroalgae: *Dictyota bartayesiana* (*DD*) and *TD*. The inhibitory activity of the fucoidan extracts was 90.5% and 89% in *TD*, and 89.7% and 92% in *DD*; it would therefore require extensive scientific work to develop this bioactive substance for HIV clinical treatment. In addition, antibacterial activity against uropathogens was reported using silver nanoparticles extracted from *Turbinaria ornata*. The agar well diffusion technique was employed to assess various microbial strains, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Klebsiella pneumoniae*. The silver nanoparticles showed increased antibacterial and antioxidant activity, suggesting they may constitute a new class of antibiotics for the treatment of UTIs [56]. Furthermore, in vivo studies of the acetone extract of *TD* demonstrated its antidiabetic potential in alloxan-induced diabetic rats. Treatment of diabetic rats with different doses of *TD*-AE showed antihyperglycemic activity and recovery of body weight loss, total protein, and haemoglobin levels compared with untreated diabetic rats [57].

4. Conclusion

Turbinaria and other brown algae have been extensively documented in the literature with rich phytochemical and pharmacological diversity, as illustrated in Table 2. The diversification of the phytochemical and pharmacological profile of the *Turbinaria* genus has motivated the global scientific community to explore its potential for novel drug discovery further. Polysaccharide fractions (SPs) that encompass fucoidan and glucosamine have demonstrated various therapeutic effects, including neuroprotective, cardioprotective, anti-inflammatory, antioxidant, anticoagulant, mosquitocidal, and anti-proliferative. In addition, phenolic compounds and total flavonoids also contributed significantly to the pharmacological efficacy of *Turbinaria species*.

Table 2. Phytochemical and pharmacological profile of *Turbinaria* species.

Species	Bioactive molecules	Bioactivities	Reference
<i>Turbinaria conoides</i>	Sulphated polysaccharides (fucoidan, alginate)	Anticancer, cardioprotective, antioxidant	[42, 54]
<i>Turbinaria decurrens</i>	Fucoidan, phlorotannins, sterols	Antioxidant, anticancer, anti-HIV, antibacterial	[45,46,57]
<i>Turbinaria ornata</i>	Hentriacontane, fucoidan, flavonoid (myricetin)	Anticancer, neuroprotective, antibacterial, antioxidant	[43,47,56]
<i>Turbinaria turbinata</i>	Fucoxanthin, flavonoids, polyunsaturated fatty acids	Antiprotozoal, anti-inflammatory	[58, 59]

Additionally, the development of synthetic analogs of these bioactive metabolites extracted from *Turbinaria* should be directed toward enhancing the efficacy and effectiveness of the phytochemicals, thereby broadening their potential applications in therapeutic interventions.

Author Contributions

Conceptualization, J.K., L.C., and V.S.; writing—original draft preparation, J.K., L.C., and V.S.; writing—review and editing, J.K. and A.S.; supervision, L.C. and A.S. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

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Data Availability Statement

No new data were created or analyzed in this study. Data sharing is not applicable.

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Conflicts of interest

The authors declare no conflict of interest.

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