INVESTIGATING PHYTOCHEMICALS AND *IN VITRO* BIOACTIVITY OF *HALYMENIA DILATATA* Zanardini

Dissertation submitted in partial fulfilment of the requirements for the award of the degree of Master of Science in Botany

By

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DECLARATION

I, hereby declare that the work which is being presented in the dissertation, entitled **"Investigating Phytochemicals and *in vitro* Bioactivity of *Halymenia dilatata* Zanardini"**, in fulfillment of the requirements for the award of the degree of Master of Science in Botany and submitted to St. Teresa's College (Autonomous), Ernakulam is an authentic record of my own work carried out during M. Sc. Period under the supervision of Ms. I. K. Nishitha.

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Swalihath Binth Sunu

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1. INTRODUCTION

Algae are a diverse group of primarily aquatic, photosynthetic organisms that range from microscopic phytoplankton to large multicellular forms like seaweeds. They lack the complex structures of land plants, such as true roots, stems, and leaves, but they play a vital role in global ecosystems by producing oxygen and serving as the base of many aquatic food webs (Lee, 2008). Algae are also important in various industrial applications, including biofuel production, bioremediation, and as sources of valuable bioactive compounds (Pulz and Gross, 2004).

The word "seaweed" describes a wide range of aquatic plants and algae that are found in lakes, rivers, and the ocean. The majority of aquatic food chains are based on certain seaweeds, including phytoplankton, which are tiny and float in the water column (Guiry, and Michael D., 2014).

Seaweed has a lot of vitamins, minerals, and fiber, plus it tastes good. Sushi, or nori seaweed wrapped in a variety of fish, rice, and other seasonings, has been a staple of Japanese cuisine for at least 1,500 years. A wide range of seaweeds contain anti-inflammatory and anti-microbial substances. They have been used for thousands of years for their therapeutic qualities; the ancient Romans used them to treat burns, wounds, and rashes. Anecdotal evidence suggests that they were used by the ancient Egyptians to treat breast cancer (Mc Lachlan, J., and Bird, C. J., 1984).

In recent years, focus on these organisms has increased due to their food and fuel production capability. In fuel industry algae biofuels have emerged as a clean, nature friendly, cost-effective solution to other fuels. More recently algae have been identified and developed as renewable fuel sources, and the cultivation of algal biomass for various products is transitioning to commercial-scale systems. Large-scale cultivation of algae merges the fundamental aspects of traditional agricultural farming and aquaculture (Emily M Trentacoste *et al.,* 2014). Algae fuels are categorized into bioethanol, biogas, bio-hydrogen, biodiesel and bio-oil. Algae can be used in the preparation of Biodiesel, Bioethanol, Biobutanol and Hydrogen gas (Raja *et al.,* 2013).

They are considered as a potential source of bioactive substances such as proteins, lipids, and polyphenols possessing potent antibacterial, anticancer, antioxidant, antifungal, and antiviral properties (Sundaramurthy *et al.,* 2016). Seaweeds that are medicinal are rich in secondary metabolites which include alkaloids, glycosides, flavonoids, saponins, tannins, steroids, related

active metabolites, which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry (Eluvakkal *et al.,* 2010). Recently, their value as a source of novel bioactive substances has grown rapidly and researchers have revealed that marine algal originated compounds exhibit various biological activities (Kim and Wijesekara, 2010).

RHODOPHYCEAE

The marine environment, covering over 70% of the Earth's surface, is a largely untapped reservoir of biologically active natural products. Among marine organisms, macroalgae (seaweeds) have emerged as prolific producers of structurally diverse secondary metabolites with a wide range of biological activities (Blunt *et al.,* 2018). Within this group, red algae (Rhodophyta) are particularly noteworthy due to their chemical richness and production of unique bioactive compounds, including sulfated polysaccharides, phenolic compounds, alkaloids, terpenoids, and flavonoids (Smit, 2004; Pérez *et al.,* 2016).

The morphology of red algae ranges from unicellular filamentous to multicellular thalloid forms, with certain species. Producing economically important products like agar and carrageenan. These species can be found in a variety of marine settings, ranging from the intertidal zone to deep oceans. There are also freshwater (e.g., Batrachospermum) and terrestrial lineages. A triphasic life cycle with one haploid and two diploid phases, with the carpo-sporophyte borne on female gametophytes, is one of the Rhodophyta’s significant advances.

Freshwater Rhodophyta has 66 species and 27 genera in North America, although these numbers will change as molecular investigations uncover more diversity. Freshwater red algae have a limited size range than marine species, with the majority (80%) of them measuring 1-10 cm in length. Gelatinous filaments, free filaments, and pseudoparenchymatous forms are the most prevalent types (Yoon, Hwan Su, *et al.,* 2017).

Halymenia

Halymenia dilatata Zanardini is a red alga characterized by a broad, thin, membranous thallus that is soft, slippery, and often brightly red to pink in color. The blade is typically lobed, ruffled, and undulate, with a monostromatic margin and a multistromatic central region. The internal structure features a well-developed medulla of large, loose cells. Reproductively, the species is dioecious, producing cystocarps prominently on the blade surface and scattered tetrasporangial sori.

H. dilatata native to the tropical and subtropical regions of the Indo-Pacific, particularly first described from the Red Sea region. It typically inhabits shallow subtidal zones, often growing attached to rocks, coral rubble, or other hard substrates in areas with good water movement and light penetration.

H. dilatata exhibits several biological activities of interest, particularly in the fields of pharmacology and biotechnology. Extracts from this red alga have demonstrated antioxidant, antimicrobial, and anti-inflammatory properties, attributed to its rich content of bioactive compounds such as sulfated polysaccharides, phenolics, and fatty acids. Additionally, it has been explored for potential antiviral effects and as a natural source of compounds for cosmetic and nutraceutical applications. These biological activities highlight its value beyond ecological roles, supporting its use in sustainable bioproduct development (Zanardini, 1872).

Marine macroalgae, particularly red algae (Rhodophyta), are widely recognized as a rich source of diverse secondary metabolites with significant ecological and pharmacological importance (Blunt *et al.,* 2018). Among the various genera of red algae, *Halymenia* species are noted for their production of bioactive compounds, including polysaccharides, phenolics, flavonoids, terpenoids, and sterols, which have been associated with antioxidant, antimicrobial, anti-inflammatory, and anticancer activities (Pérez *et al.,* 2016; Smit, 2004).

Phenolic compounds are commonly found in brown, green, red seaweeds, whose antioxidant properties have been correlated to their phenolic contents. Recently, a number of studies have been reported on the phytochemistry of plants across the world. Flavonoids in human diet may reduce the risk of various cancers, as well as preventing menopausal symptoms. Its potent water-soluble antioxidants and free radical scavengers prevent oxidative cell damage and have strong anti-cancer activity. They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity. Flavonoids are largest group of polyphenolic compounds and are known to contain a broad spectrum of chemicals and biological activities including antioxidant and free radical scavenging properties. They are remarkable group of plant metabolites. Flavonoids are perhaps best known to enhance the effects of ascorbic acid. The secondary metabolites of seaweeds have always attracted the interest of biochemists because of their diversity as compared with those present in the leaves of higher (Cushnie and Lamb, 2005).

Antioxidant compounds play an important role in various fields such as medical field (to treat cancers, cardiovascular disorders, and chronic inflammations), cosmetics (anti-ageing process), food industries (food preservative) and others (Kohen R; Nyska A, 2002).

Species within the *Halymenia* genus have been reported to contain sulfated galactans, sterols, and a variety of antioxidant compounds, making them promising candidates for pharmaceutical and nutraceutical exploration (Rodrigues *et al.,* 2011). However, despite the known bioactive potential of related species such as *Halymenia floresii* and *Halymenia durvillei*, the phytochemical profile and biological activities of *H. dilatata* remain poorly understood, highlighting a critical gap in marine natural product research (Pangestuti & Kim, 2015).

Preliminary studies suggest that members of the genus *Halymenia* are valuable sources of sulfated polysaccharides and other bioactive constituents (Marques *et al.,* 2012). However, comprehensive phytochemical investigations specifically targeting *H. dilatata* remain scarce, necessitating further research to explore its chemical constituents and biological potential.

The extraction of phytochemicals from marine algae typically involves the use of sequential solvent extraction protocols, utilizing solvents of increasing polarity (e.g., hexane, ethyl acetate, methanol, water) to maximize the yield and diversity of extracted compounds (Patra *et al.,* 2015). This method ensures comprehensive recovery of both lipophilic and hydrophilic secondary metabolites. Furthermore, the choice of solvent and extraction method greatly influences the phytochemical composition and biological efficacy of the extracts (Mayer *et al.,* 2011).

Following extraction, a combination of qualitative and quantitative phytochemical analyses is essential for identifying major bioactive constituents. Preliminary screening tests, such as Dragendorff's test for alkaloids, the ferric chloride test for phenolics, and the Salkowski test for terpenoids, allow for rapid detection of key compound classes (Harborne, 1998). Advanced analytical techniques such as Thin Layer Chromatography (TLC), Fourier Transform Infrared Spectroscopy (FTIR), High-Performance Liquid Chromatography (HPLC), and Gas Chromatography–Mass Spectrometry (GC-MS) provide deeper insights into the chemical profiles of algae extracts, enabling the identification and structural characterization of specific metabolites (Devi *et al.,* 2011; Zubia *et al.,* 2009).

Given the increasing global demand for natural antioxidants, antimicrobial agents, and novel bioactive substances, investigating the chemical constituents of *H. dilatata* holds significant promise. Identification of its major phytochemicals could pave the way for the development of new functional foods, cosmetics, or therapeutic agents derived from marine resources (Pangestuti & Kim, 2015). Thus, the present study focuses on the extraction, screening, and detailed identification of major phytochemical classes from *H. dilatata*, employing modern extraction and analytical techniques to reveal its chemical and biological potential.

Understanding the phytochemical composition of *H. dilatata* is crucial, not only to elucidate its biochemical diversity but also to evaluate its potential as a natural source for pharmaceutical and nutraceutical development. Therefore, this study aims to systematically extract, identify, and characterize the major phytochemical constituents of *H. dilatata* using various analytical techniques, laying the groundwork for future biological activity assessments and potential therapeutic applications (Singh *et al.,* 2013).

OBJECTIVES

1. Extraction of the red algae, *Halymenia dilatata* in ethanol and chloroform, polar and non-polar solvents respectively, using Soxhlet apparatus and estimate the extractive value of the algae.
2. To carry out qualitative and quantitative estimation of major phytochemicals from *H. dilatata*.
3. Assessment of anti-bacterial potential of *H. dilatata* against gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli*.
4. Assessment of anti-oxidant, antidiabetic and anti-inflammatory potential of *H. dilatata* using standard assays.

2. REVIEW OF LITERATURE

Taxonomic Studies

A red alga of the family Halymeniaceae (order Halymeniales, class Florideophyceae), *Halymenia dilatata* Zanardini is known for its unique morphological characteristics and has undergone several taxonomic studies.

Using specimens gathered from the Red Sea, Zanardini initially named *H. dilatata*, describing it by its large, thin, and gelatinous thalli, which are often bright red to pink in color. The thallus lacks a definite midrib and is usually foliose with broad, undulate, or ruffled margins (Zanardini, 1872).

Globally, there are approximately **80 recognized species** of *Halymenia* (Guiry & Guiry, 2024), including notable ones such as *Halymenia floresii*, *Halymenia dilatata*, *Halymenia durvillei*, and *Halymenia porphyraeformis*.

In India, around **6–8 species** have been recorded, including *H. dilatata*, *H. floresii*, *H. porphyraeformis*, and *H. venusta* (Jha *et al.,* 2009). These species are primarily found along the **southeast coast of India**, particularly in the **Gulf of Mannar**, **Andaman and Nicobar Islands**, **Lakshadweep Islands**, and parts of the **Kerala and Karnataka coastlines**. These regions provide suitable habitats with coral reefs and rocky substrata in warm, nutrient-rich waters conducive to the growth of red algae.

In 2012, Hernández-Kantún *et al.,* carried out a thorough taxonomic revision of Indo-Pacific branched *Halymenia* species, including *Halymenia tondoana*. Morphological analysis and molecular phylogenetic techniques based on the rbcL gene were coupled in the study to elucidate species boundaries that had previously been unclear because of overlapping physical traits. By detecting notable genetic differences between samples that were previously classified under *H. durvillei*, they were able to describe two new species: *H. hawaiiana* and *H. tondoana*. The authors emphasized the importance of genetic information and characteristics including branching patterns, cortex thickness, and inner cortical cell shape in differentiating across species in this

genus. The significance of combining molecular technologies with conventional taxonomy to resolve complicated species IDs within the Halymeniaceae family was highlighted by their work.

Molecular methods, including rbcL and COI-5P gene sequencing, have been used in more recent research to establish the uniqueness of *H. dilatata* and to define species boundaries within *Halymenia* (Dixon & Saunders, 2013).

Tan (2017) carried out a thorough investigation into the taxonomy and molecular phylogeny of *Halymenia* species within the family Halymeniaceae for their most recent revisions of the Halymeniales. Due to convergence and a lack of diagnostic features, the study highlighted how morphological traits alone are insufficient for accurately distinguishing species, especially in the case of *H. dilatata*. To improve species delimitation, morphological and anatomical features—such as thallus habit, branching pattern, blade thickness, and cortical cell structure—were paired with molecular markers, particularly rbcL and COI-5P. The study demonstrated that *Halymenia* is a polyphyletic genus and found seven different species of *Halymenia* throughout Southeast Asia, including *H. durvillei* and *H. dilatata*. The significance of integrating molecular data and classical taxonomy in red algae systematics was emphasized by this integrative method, which also served to explain the evolutionary relationships within Halymeniaceae and revealed previously unknown variety.

According to Guiry and Guiry (2023), *Halymenia* species have historically been challenging to distinguish from one another due to their considerable phenotypic plasticity.

As a result, even if morphological research helped identify *H. dilatata*, modern taxonomy increasingly depends on a combination of morphological and molecular methods to accurately classify it.

Phytochemical Activities

Sanger *et al.,* (2019) detected a number of bioactive components in various solvent extracts from a phytochemical investigation of the red maritime seaweed *H. durvilae*. All extracts (methanol, hexane, chloroform, and water) consistently contained steroids, flavonoids, and triterpenoids, according to the study, but only the methanol extract contained saponins and hydroquinones. All samples were noticeably devoid of tannins and alkaloids. According to these results, *H. durvilae*

is a rich source of functional phytochemicals, especially those with established antioxidant and medicinal properties, which supports its use as a natural food or supplement that promotes health.

Manam and Subbaiah (2020) conducted a phytochemical investigation on marine seaweeds, including the red alga *Halymenia porphyroides*, which is closely related to *H. dilatata*. Their study revealed that *H. porphyroides* contains a significantly higher alkaloid content (20.35 ± 0.01 mg/g dry weight) compared to the brown alga *Colpomenia sinuosa*. Alkaloids are known for their diverse bioactivities, including potential applications as antimalarial agents, central nervous system stimulants, and antibacterial compounds. The authors emphasized the pharmaceutical relevance of alkaloids in marine algae, suggesting their use in nutraceutical formulations and health-related applications.

Sathiyaraj *et al.,* (2021) conducted a phytochemical examination of the aqueous extract of the alga. Several important phytochemicals, including alkaloids, flavonoids, tannins, terpenoids, steroids, carbohydrates, glycosides, amino acids, and proteins, were confirmed to be present by the qualitative screening. Notably, phenols and saponins were absent in the extract.

Antony and Chakraborty (2019) investigated the pharmacological properties of various seaweeds, including *H. dilatata*, focusing on their antioxidant and therapeutic potentials. With a total phenolic content (TPC) of just 0.10 mg gallic acid equivalents (GAE) per gram of extract, *H. dilatata* had one of the lowest TPC values of the ten species under study. Its comparably poorer antioxidant properties, such as reduced lipid peroxidation inhibition and radical scavenging, were associated with its relatively low phenolic content. Even seaweeds with low TPC, such as *H. dilatata*, may still have functional bioactive potential when mixed with other advantageous chemicals, according to the study, which also emphasized the significance of polyphenols in enhancing antioxidant capacity despite their moderate phenolic profile.

Badeggi *et al.,* (2020) assessed the total phenolic content (TPC) of *H. dilatata*-derived extracts and their synthesized gold nanoparticles, highlighting the crucial role of polyphenols in antioxidant activity. Using the Folin–Ciocalteu assay, the study reported significant levels of phenolic compounds in the crude extracts and fractions F1 and F2, with F1 showing the highest phenolic content among them (889.6 µM GAE/g). Interestingly, while the corresponding gold nanoparticles exhibited slightly lower phenolic content due to interaction and possible oxidation during nanoparticle formation, F2 Au NPs retained a high phenolic concentration (76.3% of the original),

suggesting efficient encapsulation and stability. These findings confirm that the polyphenolic components in *H. dilatata* not only contribute to its antioxidant potential but also play a key role in the green synthesis and stabilization of bioactive nanoparticles.

Flavonoids were identified as one of the main secondary metabolites in both methanol and hexane extracts by Darfiah *et al.,* (2021), who studied the phytochemical composition and antibacterial potential of *Halymenia* sp., collected from Lae-Lae Island, South Sulawesi. They conducted Thin Layer Chromatography (TLC) studies and confirmed the presence of flavonoids due to the formation of a light yellow-green stain when treated with $AlCl_3$. Flavonoids are well-known for their antibacterial properties, which include interfering with microbial DNA and rupturing the permeability of bacterial cell walls. The presence of flavonoids in *Halymenia* sp. suggests that it could be a naturally occurring source of bioactive substances with pharmacological and antibacterial uses.

According to Darfiah *et al.,* (2021), one of the important secondary metabolites found in the methanol and hexane extracts of *Halymenia* sp., is tannin. Phytochemical screening procedures were used to confirm the presence of tannins; a dark blue-black color change with application of $FeCl_3$ signified a successful outcome. Tannins have a well-established antibacterial effect, mostly due to their capacity to weaken or damage bacterial cell membranes, which disrupts cell permeability and inhibits or kills bacteria. According to the results, *Halymenia* sp. may be used to create natural antibacterial agents since tannins may help with its antibacterial properties.

According to Darfiah *et al.,* (2021), *Halymenia* sp., which was harvested from Lae-Lae Island in South Sulawesi, has a significant amount of total alkaloids, particularly in its methanol and hexane extracts. The study verified the existence of alkaloids along with other secondary metabolites such flavonoids, tannins, triterpenoids, and saponins using phytochemical screening. One of the well-known antibacterial actions of alkaloids is the disruption of the peptidoglycan layer of bacterial cell walls, which results in the death of the bacteria. The discovery of alkaloids in *Halymenia* sp. emphasizes the plant's potential as a natural source of bioactive substances with potential applications in medicine, especially as antibacterial agents.

Antioxidant Activities

According to Bhadury and Wright (2004), seaweeds and other marine algae are recognized as rich sources of bioactive compounds with significant pharmaceutical potential.

The study conducted by Heo *et al.,* (2006) used different *in vitro* free radical scavenging assays to examine the antioxidant qualities of red algae, including *H. dilatata*, collected from Jeju Island, Korea. The hydroxyl radical, superoxide anion, hydrogen peroxide, and DPPH free radical scavenging activity were the techniques. *H. dilatata* showed moderate activity in DPPH and superoxide anion scavenging assays. The findings suggest that phenolic chemicals found in red algae, such as *H. dilatata*, contribute to their antioxidant activity and could be used as natural sources to create pharmaceuticals or functional foods. The study also highlights how temperature and extraction techniques affect antioxidant yields.

In their work, Vinosha *et al.,* (2019) used aqueous extracts of *H. dilatata* to study the biogenic synthesis of gold nanoparticles (Hd-AuNPs) and their potential antidiabetic effects, among other biomedical uses. The antioxidant results are related to antidiabetic potential. This is because oxidative stress is known to contribute to diabetes pathogenesis. In the dose-dependent studies on DPPH radical scavenging, reducing power, and total antioxidant analysis, the Hd-AuNPs showed strong antioxidant activity. The work did not include direct antidiabetic tests like glucose absorption or enzyme inhibition, but the authors suggest that the antioxidant characteristics of the gold nanoparticles generated from *H. dilatata* may indirectly enhance antidiabetic actions by reducing oxidative stress.

The nutritional and biotechnological applications of *H. dilatata* was examined by Magdugo in 2020. The study reveals high quantities of protein and amino acids, and good lipid composition with large amounts of polyunsaturated fatty acids. All of these point to potential health benefits for people. The study also included the extraction and characterization of sulfated polysaccharides using FT-IR spectroscopy characteristics. These polysaccharides had strong antiviral and antioxidant properties, as seen in DPPH assay and suppressing the herpes simplex virus *in vitro*. *H. dilatata* is therefore regarded as a valuable but underutilized marine resource, with a great deal of potential for use in food, cosmetics, and medicine.

An extensive investigation on *H. durvillei* was carried out by Gatulistiani *et al.,* (2023), on the plant's potential as a source of natural antioxidants and skin-related bioactives. It involved microencapsulating its aqueous extract with R-phycoerythrin (R-PE). The research emphasizes that *H. durvillei* is abundant in ash, proteins, and carbohydratess, as well as R-PE, a pigment with antioxidant qualities. The study investigated the possibility of preserving the extract's antioxidant activity while preserving pigment stability using microencapsulation with sodium caseinate and maltodextrin.

Sathiyaraj *et al.,* (2021) investigated the antioxidant capabilities of platinum nanoparticles (PtNPs) made with aqueous extracts of *H. dilatata*. Hd-PtNPs showed significant dose-dependent antioxidant activity in DPPH radical scavenging, reducing power, and total antioxidant capacity assays. The greatest DPPH scavenging activity was 59.72% at 100 μg/mL of Hd-PtNPs, while the reducing power and total antioxidant potential also increased significantly with concentration. Whereas ascorbic acid had a 78.91% DPPH scavenging activity. These findings indicate that the PtNPs made from *H. dilatata* preserve and maybe improve the seaweed's inherent antioxidant capacity, indicating their applicability in pharmaceuticals.

Using a variety of solvent extracts, Chaiwichien *et al.,* (2022) examined the antioxidant activity and toxicity of the red seaweed *H. durvillei*, which is closely related to *H. dilatata*. According to this study, *H. dilatata*' s sulfated galactan content showed improved antioxidant qualities and the capacity to fend off Aeromonas hydrophila infections, indicating potential uses in pharmaceutical and nutraceutical products. The literature referenced in the paper highlights *H. dilatata*' s bioactivity and possible use in health-related applications because of its antioxidant components, even though the primary focus was on *H. durvillei*. According to these results, *H. dilatata* should be investigated further as a useful marine resource for biological applications.

Antony & Chakraborty (2019) used ethyl acetate-methanol extracts to examine the antioxidant qualities of many seaweeds, including *H. dilatata*. According to the study, *H. dilatata* did show antioxidant activity, although not as well as other species such as *Gracilaria salicornia* and *Padina tetrastromatica*. In particular, *H. dilatata* demonstrated modest outcomes in lipid peroxidation inhibition, hydrogen peroxide neutralization, and radical scavenging. Given that phenolic

chemicals are known to contribute to antioxidant effects, *H. dilatata*'s lower bioactivity correlated to the fact that its total phenolic content was also the lowest. This shows that although *H. dilatata* has some antioxidant capacity, its use in treating illnesses linked to oxidative stress is less promising than that of other seaweeds.

Antioxidant Activities: ABTS Assay

Chaiwichien *et al.,* (2022) evaluated the antioxidant activity of *H. durvillei* extracts using the ABTS assay, which measures the ability of substances to scavenge free radicals. Among the five solvent fractions tested—ethanol (HDET), hexane (HDHE), ethyl acetate (HDEA), butanol (HDBU), and aqueous (HDAQ)—the ethyl acetate (HDEA) and hexane (HDHE) extracts demonstrated the highest antioxidant capacities, with EC50 values of 669 µg/mL and 1,639 µg/mL, respectively. In contrast, the HDET, HDBU, and HDAQ extracts showed very low activity, with EC50 values exceeding 3,000 µg/mL. These findings suggest that non-polar extracts of *H. durvillei* possess more potent antioxidant properties, likely due to the higher concentration of active phytochemicals such as flavonoids, alkaloids, and terpenoids.

Antibacterial Properties

Marine algae are used for their biogenic compounds that have antifouling properties, while Demirel *et al.,* (2009) emphasized their antibacterial and antioxidant properties.

Radhika *et al.,* (2013) offered more proof of the antibacterial qualities of a number of seaweeds from the Gulf of Mannar. Despite a lot of research on many species, nothing is known about *H. dilatata*.

Uma Maheswari and Reena (2017) used GC-MS analysis on methanolic extracts of *H. dilatata* and found 17 phytocompounds, primarily fatty acids, along with alcohols, steroids, and esters. Numerous of these substances showed antibacterial, anti-inflammatory, and antioxidant properties.

Darfiah *et al.,* (2021) investigated the antibacterial activity and phytochemical composition of *Halymenia* sp. along with other seaweeds from Lae-Lae Island, South Sulawesi. The study revealed that the methanol and hexane extracts of *Halymenia* sp. exhibited strong antibacterial

activity, particularly against *Vibrio harveyi*, with inhibition zones comparable to or exceeding that of ciprofloxacin. Phytochemical screening indicated the presence of five key secondary metabolites—alkaloids, flavonoids, tannins, triterpenoids, and saponins—known for their antibacterial properties. These findings support the potential of *Halymenia* sp. as a natural source of antimicrobial agents, although the extracts showed no activity against E. coli, highlighting selectivity in their antibacterial effects.

Kasmiati *et al.,* (2022) studied the antibacterial properties of methanol and hexane extracts *H. durvillei*, collected from Kayangan Island in South Sulawesi. According to the study, the methanol extract had a 26.2 mm inhibitory zone, which is twice as large as the one generated by the commercial antibiotic ciprofloxacin, and showed high antibacterial activity, especially against Salmonella typhi. With an inhibitory zone of 21.0 mm, the hexane extract exhibited the highest activity against *Aeromonas hydrophila* and outperformed ciprofloxacin. Neither of the extracts had any effect on *Pseudomonas aeruginosa* and *Escherichia coli*.

Anti-diabetic Activities

Badeggi *et al.,* (2020) explored the in-vitro antidiabetic potential of plant-derived gold nanoparticles, highlighting *H. dilatata* as one of the marine algae previously reported to possess such properties. In their review of green-synthesized nanoparticles, they noted that *Halymenia dilatata* showed enhanced antidiabetic and antioxidant activities when formulated into gold nanoparticles compared to its crude extract. Specifically, these nanoparticles demonstrated increased inhibition of key enzymes like alpha-glucosidase and alpha-amylase, which are crucial in managing type 2 diabetes. This suggests that biosynthesized gold nanoparticles from *H. dilatata* hold promise as effective in-vitro agents for antidiabetic therapy.

Sanger *et al.,* (2019) assessed the antidiabetic potential of *H. durvilae* by looking at its capacity to inhibit the enzyme α-glucosidase, which is essential for the digestion of carbohydrates and the control of postprandial glucose. All examined extracts, including methanol, hexane, chloroform, and water, showed α-glucosidase inhibitory activity, according to the study. The water fraction had the maximum inhibition, followed by extracts of methanol, chloroform, and hexane. These results suggest *H. durvilae* to be a suitable natural source for the production of functional foods or supplements to treat type 2 diabetes.

Antony and Chakraborty (2019) studied the anti-diabetic potential of several seaweeds and discovered that *H. dilatata* demonstrated very weak effectiveness in blocking carbohydrate-digesting enzymes. In comparison to species that perform better, such as *Padina tetrastromatica* and *Gracilaria salicornia*, its ethyl acetate-methanol extract had low inhibitory actions against α-amylase (IC_{50} = 0.95 mg/mL), α-glucosidase (IC_{50} = 0.82 mg/mL), and dipeptidyl peptidase-IV (IC_{50} = 0.17 mg/mL). According to these findings, *H. dilatata* may have some anti-diabetic qualities, although not as much as other seaweed species included in the study. Thus, its utility in functional food or pharmaceutical applications for diabetes treatment may be less promising.

George *et al.,* (2023) used the marine organism *H. dilatata* to explore the antidiabetic effects of biosynthesized silver nanoparticles, with a particular emphasis on the α-amylase inhibitory assay. At doses ranging from 20 to 100 µg/mL, the produced silver nanoparticles (AgNPs) showed 64% suppression of α-amylase activity, indicating moderate but considerable enzyme inhibition. Comparable to the common antidiabetic medication acarbose, this inhibition suggests that these green-synthesised nanoparticles may be useful in the treatment of postprandial hyperglycemia. According to the research, *H. dilatata'* s bioactive chemicals help stabilize and improve the functionality of AgNPs, increasing their ability to treat enzymes that break down carbohydrates.

In vitro-Anti-inflammatory Assay: Egg albumin denaturation assay

The abundance of bioactive substances found in red seaweeds, such as *H. dilatata*, makes them significant both ecologically and economically. In addition to making a substantial contribution to marine ecosystems, these algae are useful as feed for aquatic life, especially in aquaculture. Compounds that improve fish development and survival, like hydroperoxide and acetyl valeryl, were found in *H. dilatata* according to GC-MS analysis. These chemicals have been shown in relevant research to possess anti-inflammatory, antibacterial, and antioxidant properties. These results lend credence to the increasing interest in using seaweeds as sustainable resources for functional goods, such as fish feed. The study emphasizes the potential of *H. dilatata* as a natural source of advantageous chemicals and the necessity of more investigation into its uses (Beema anton *et al.,* 2019).

Antony and Chakraborty (2019) examined the pharmacological characteristics of several seaweeds, such as *H. dilatata*, with an emphasis on their potential to prevent progressive lifestyle illnesses. According to their research, *H. dilatata* showed relatively weaker bioactivities than other seaweeds, although others, including *Gracilaria salicornia* and *Padina tetrastromatica*, showed high anti-inflammatory activities by efficient suppression of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX). Using in vitro enzyme inhibition experiments, the anti-inflammatory capability of each extract was assessed; *H. dilatata* exhibited greater IC50 values, indicating lesser efficacy. This demonstrates how different seaweed species differ in their ability to reduce inflammation, and it implies that although *H. dilatata* has some pharmacological activity, it is not one of the most effective sources in the group under study.

3. MATERIALS AND METHODS

SPECIMEN COLLECTION

The dried specimen was procured from R. K. Algae Project Centre, Mandapam, Tamil Nadu.

SAMPLE PREPARATION

The cleaned samples were shade dried, cut into small pieces and powdered in a mixer grinder. The organic solvents chloroform and ethanol were used for the extraction process due to its higher efficiency using Soxhlet extraction method. 20g of samples were packed in a thimble and placed in the extractor. 200ml of the solvent was added into the flask and heated. The temperature was maintained at 80^0C to 85^0C throughout the extraction. The soluble active constituents of the extract remained in the flask and the process was repeated until the compounds were completely extracted. The liquid extract was then cooled and concentrated by using an evaporator.

The beaker with dried extract was weighed and noted. For antibacterial analysis DMSO was used and others are carried out by using ethanol to dissolve the extracts from the beaker. Later the weight of the beaker alone was noted. Hence, the actual weight of the dried extract was obtained. Similarly, the weight of dried extract of *Halymenia*, in ethanol 7.83g and chloroform was 0.14g and 20g respectively. From this the extractive value was calculated using the formula

Extractive value (%) = (Weight of dried extract/Weight of plant material) X 100

PHYTOCHEMICAL ANALYSIS

Qualitative Analysis

Extract Preparation

2g of shade dried plant material is taken in clean dry conical flask to it 20ml of the extracting solvent, ethanol, methanol and water was added and kept in the mechanical shaker for 24 hours. Then it was filtered using Whatman No 1 filter paper and made up to 50ml. The following standard

protocols were used for qualitative analysis of samples to check for the presence of Alkaloids, Cardiac glycosides, Flavonoids, Phenols, Saponins, Tannins and Terpenoids.

Test for Flavonoids:

2 ml of each extract was added with few drops of 20% sodium hydroxide, formation of intense yellow colour is observed. To this, few drops of 70% dilute hydrochloric acid were added and yellow colour was disappeared. Formation and disappearance of yellow colour indicates the presence of flavonoids in the sample extract.

Test for Alkaloids:

To 1ml of the extract 1ml of any one of the below reagents is added.

I. Mayer's reagent:

1.36g of HgCl2 (mercuric chloride) was dissolved in 60ml distilled water (solution A) and 5g of potassium iodide was dissolved in 10ml distilled water (solution B). Both solutions A and B were mixed and made upto 100ml.

II. Dragendroff's reagent:

8g Bismuth Sub Nitrite was dissolved in 20ml Con. HNO3 to form solution A. 27g of potassium iodide was dissolved in 50ml of distilled water to form solution B. Both solution A and B were mixed and allowed to stand when KNO3 precipitates out, supernatant was discarded and made up to 100ml with distilled water.

III. Wagner's reagent:

1027g of iodine and 2g potassium iodide were dissolved in 5ml distilled water. It was then made up to 100ml with distilled water.

Presence of precipitate in all three reagents confirms the presence of alkaloids.

Test for Saponins:

To 2 ml of each extract, 6 ml of distilled water were added and shaken vigorously; formation of bubbles or persistent foam indicates the presence of saponins.

Test for Tannins:

To 2 ml of each extract, 10% of alcoholic ferric chloride was added; formation of brownish blue or black colour indicates the presence of tannins.

Test for Phenols:

To 2 ml of each extract, 2 ml of 5% aqueous ferric chloride were added; formation of blue colour indicates the presence of phenols in the sample extract.

Test for Cardiac Glycosides:

To 1 ml of each extract, 0.5ml of glacial acetic acid and 3 drops of 1% aqueous ferric chloride solution were added, formation of brown ring at the interface indicates the presence of cardiac glycosides in the sample extract.

Test for Terpenoids:

Take 1 ml of extract of each solvent and add 0.5 ml of chloroform followed by a few drops of concentrated sulphuric acid, formation of reddish-brown precipitate indicates the presence of terpenoids in the extract.

Quantitative Analysis

1. **Estimation of Total Phenolic Content**

The amount of phenol in the aqueous extract was determined by Folin-Ciocalteu reagent method with some modifications. 2.5ml of 10% Folin-Ciocalteu reagent and 2ml of 2% solution of Na2CO3 were added to 1ml of plant extract. The resulting mixture was incubated for 15 minutes at room temperature. The absorbance of the sample was measured at 765nm. A set of reference standard solutions (S1-S5) of tannic acid (0.1,0.5,1.0,1.5 and 2 mg/ ml) were prepared in the same manner as described earlier. The total phenol content was determined from the standard curve.

2. **Estimation of Flavonoid Content**

The total flavonoid content was determined using the method described by Park *et al.,* (2008). In a 10 ml test tube, 0.3 ml of plant extracts, 3.4 ml of 30% methanol, 0.15 ml of NaNO2 (0.5 M) and 0.15 ml of AlCl3.6H2O (0.3M) were mixed. After 5 min, 1 ml of NaOH (1

M) was added. The solution was mixed well and the absorbance was measured at 506 nm. A standard curve for total flavonoids was prepared using rutin standard solutions ranging from 0.5 to 20mg/ml (S1-S5), following the same procedure. The total flavonoid content was determined from the standard curve.

3. Estimation of Total Tannin Content

The tannins were determined by Folin-Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions (S1-S5) of tannic acid (0.1,0.5,1.0,1.5 and 2 mg/ ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 700 nm with an UV/ Visible spectrophotometer. The total tannin content was determined from the standard curve.

4. Estimation of Alkaloid Content

For the assay, add 1 ml sample to 5 mL of 60% sulfuric acid and allowed to react for 5 minutes. Subsequently, 5 mL of 0.5% formaldehyde in 60% sulfuric acid was added to the mixture, which was then allowed to stand for 3 hours at room temperature. The absorbance of the final solution was measured at 565 nm using a spectrophotometer. A set of reference standard solutions (S1-S5) of atropine (0.1,0.5,1.0,1.5 and 2 mg/ ml) were prepared in the same manner as described earlier. The total alkaloid content was determined from the standard curve.

ANTIBACTERIAL ACTIVITY IN HALYMENIA

Preparation of nutrient media

Nutrient broth was prepared by dissolving 1.3 gm of nutrient broth in 100 ml distilled water. Test tubes were filled with 5 ml of nutrient broth and were sterilized using an autoclave. Nutrient agar media was prepared by mixing 1.3gm of nutrient broth and 2gm of agar agar in 100 ml distilled water. The media was autoclaved and 20 ml each poured into sterile petri plates under aseptic conditions.

Preparation of microbial cultures

The test organisms *H. dilatata* were inoculated into 5 ml of sterilized nutrient broth and kept for overnight incubation at 37°C.

Well diffusion method

A lawn culture of each bacterium was prepared using sterilized cotton swabs. A sterilized swab was dipped into the bacterial suspension and moved side to side from top to bottom leaving no space uncovered. The plate is rotated to 90 degrees and the same procedure was repeated so that the entire plate was coated with bacteria. Once the lawn had been prepared, wells of 6 mm diameter were cut into agar plates using a sterile well cutter. The wells were labeled and 20µL of sample ethanol and chloroform algal extract were loaded into corresponding wells. The antibacterial activity of both the samples was compared with standard antibiotics available. This plate was incubated at 37°C for 24 hrs. The radius of each zone was measured using a standard ruler in centimeters. If the compound is effective against bacteria at a certain concentration, no colonies will grow. This is the zone of inhibition which is a measure of the compound effectiveness, the larger the clear area around the well, the more effective the compound.

Killing and disposing

After the experiment, the bacteria are destroyed by autoclaving the plates for 20 min. All the glassware used for the experiment were also autoclaved to remove any bacteria if present.

ANTIOXIDANT ASSAY

DPPH Free Radical Scavenging Assay (Mensor *et al.,* 2001)

A mixture of 1.5 mL sample solution and 1.5 mL 0.2 mM ethanolic DPPH solution was vortexed and incubated in darkness for 30 minutes. The absorbance was measured at 517 nm with ethanol as the blank and DPPH solution without the sample as the control. The sample with lower absorbance expresses a more significant free radical scavenging activity (RSA).

$$\text{Percentage of DPPH scavenging activity} = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right) \text{X } 100$$

ABTS Free Radical Scavenging Assay

The free radical scavenging activity of the sample was assessed by evaluating its capability to scavenge the cationic free radical ABTS. Test solutions were prepared by adding T1=20, T2=40, T3=60, T4=80, T5=100 µl(T1-T5) each of stock solutions in ethanol 200 µl of ABTS solution (7mM). The mixture was made up to300 µl final volume and incubated in the dark for 15 minutes. Ascorbic acid was used as standard. The test compound when added, causes a reduction in the ABTS radical and a colour change from blue-green to colourless. The extent of decolorization was measured at 734 nm.

$$\text{Percentage of ABTS scavenging activity} = \left(\frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \right) \text{X } 100$$

ANTI DIABETIC ASSAY

α - Amylase Inhibitory Assay

Sample preparation

From the sample stock, volumes of 25 µL, 50 µL, 75 µL, and 100 µL were taken and adjusted to a final volume of 100 µL. This sample is taken for the assay.

The α-amylase inhibition assay was conducted using the chromogenic DNSA method. The assay mixture consisted of 100 µl of α-amylase solution (5 mg/ml) in sodium phosphate buffer (0.02 M) and 100 µl of plant extract (T1-T4). The mixture was incubated at 37°C for 10 minutes. Subsequently, 100 µl of 1% (v/v) starch solution, prepared in the same buffer, was added to each tube and incubated again at 37°C for 15 minutes. The reaction was stopped by adding 200 µl of DNSA reagent, followed by heating the tubes in a boiling water bath for 5 minutes. After cooling to room temperature, the absorbance was measured at 540 nm. Acarbose (50mg/ml) was used as the standard, while control samples, were prepared under identical conditions without the addition of plant extracts.

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \text{ X } 100$$

ANTI-INFLAMMATORY ASSAY

Egg Albumin Denaturation Assay

Sample preparation

Sample volumes of 100, 200, 300, 400, and 500 µL (T1-T5) were taken and adjusted to a final volume of 2 mL using distilled water. These prepared samples were then used for further assay.

The anti-inflammatory activity of the algae ethanoic extract was determined in vitro by assessing its inhibition of egg albumin (protein) denaturation. A reaction mixture was prepared by mixing 0.2 mL of a 1-2% egg albumin solution (obtained from fresh hens' eggs), 2 mL of the sample extract (T1-T5), and 2.8 mL of phosphate-buffered saline (pH 7.4), resulting in a total volume of 5 ml. A total volume of 5 mL of the control was created by combining 2 mL of distilled water, 0.2 mL of 1-2% egg albumin solution, and 2.8 mL of phosphate-buffered saline. Diclofenac sodium was used as a standard drug at a concentration of 50 mg/mL. The reaction mixtures were incubated at 37±2°C for 30 minutes and subsequently heated in a water bath at 70±2°C for 15 minutes. After cooling, their absorbance was measured at 660 nm. The percentage inhibition of protein denaturation was calculated using the following formula:

$$\text{Percentage of denaturation} = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right) \text{X } 100$$

5. RESULTS

The current study aimed at the taxonomic description of the red seaweed *Halymenia dilatata* and the estimation of its extractive value and antimicrobial potential in two solvents, ethanol and chloroform.

TAXONOMIC DISCRIPTION

Classification of red algae *Halymenia*, according to Guiry and Guiry (2020) is:

Kingdom: Plantae

Phylum: Rhodophyta

Class: Florideophyceae

Order: Halymeniales

Family: Halymeniaceae

Genus: *Halymenia*

Species: *Halymenia dilatata* Zinardini, 1872

Thallus erect, reaching up to 14 cm in length, typically arising singly from a discoid holdfast. The stipe is very short, terete, and often inconspicuous, measuring up to 5 mm long. Branching is frequent, especially in the upper parts, and is primarily dichotomous, creating a bushy appearance. The axes are compressed and exhibit an almost cartilaginous texture, often constricted at the base in the lower branches. Blades are linear, extending up to 15 cm in length and up to 4 mm in width. The apices are generally obtuse, though they may become acute in finer branches. Blade surface and margins are smooth. Fresh specimens vary in color from purple to green, and the thallus is firm yet pliable when hydrated Zanardini, G. (1872).

Halymenia dilatata Zinardini

EXTRACTIVE VALUE

Extracts of the sample are evaluated using the extractive values of plant materials to gain a sense of the type of chemical ingredients that are present. It can also be used to evaluate the extract's quality and purity and identify instances of adulteration.

In the present study, polar and non-polar solvents were used for eluting the valuable phytocompounds present in the sample. Extractive values of ethanol and chloroform extracts of *Halymenia dilatata* are estimated in the table 1 given below: -

Solvent	Extractive value of the sample (%)
Ethanol (Polar)	39.15
Chloroform (Non-polar)	0.7

Table 1: Extractive value of solvents administered for H. dilatata

The extractive value was greater for the ethanolic extract than for chloroform suggesting that polar solvent was more efficient in extracting the phytochemicals from the algae.

PHYTOCHEMICAL ACTIVITIES

Qualitative Analysis (Sathiyaraj *et al.,* 2021)

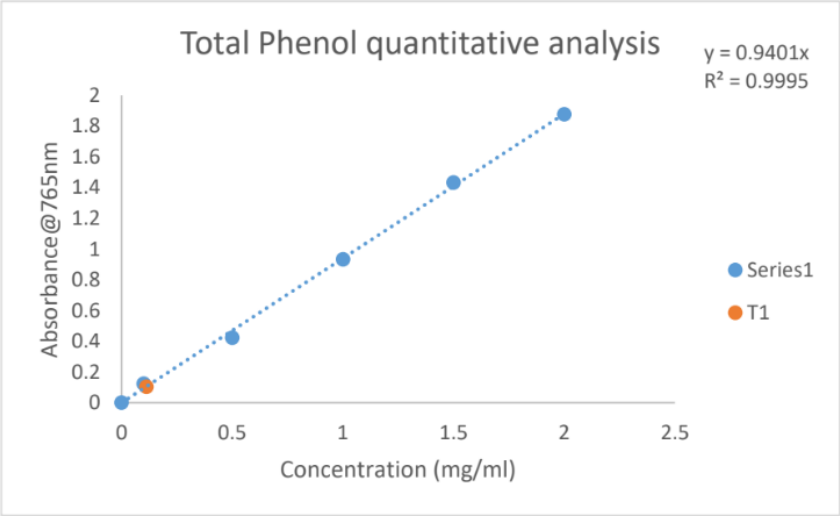
	Ethanol	Chloroform
Flavonoid	+	+
Alkaloid	+	+
Saponin	-	-
Tannin	+	-
Cardiac glycosides	-	-
Terpenoid	+	+
Glycosides	-	-

Presence (+) and absence (-)

Quantitative Analysis

1. Estimation of Total Phenolic Content

Standard Table	
Concentration(mg/ml)	**Absorbance**
0	0
0.1	0.1236
0.5	0.4235
1	0.9324
1.5	1.4321
2	1.8776



Graph 1: Standard graph of Total phenol quantitative analysis

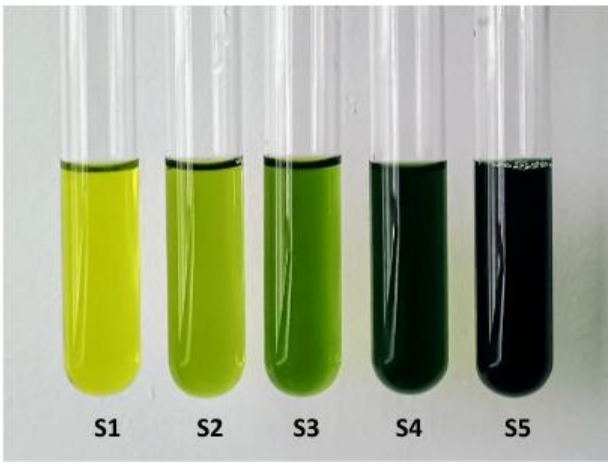


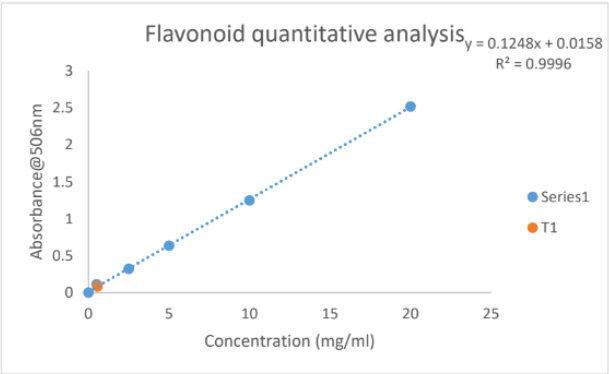
Figure 3: Total phenol quantitative analysis of control and sample

Figure 3: Total phenol quantitative analysis of control and sample

Table 2: Concentration of Total phenol from graph		
Sample name	**Absorbance @765nm**	**Concentration of Phenol from graph (mg/ml)**
Ethanolic extract	0.105	0.112

2. Estimation of Flavonoid Content

Standard Table	
Concentration(mg/ml)	**Absorbance**
0	0
0.5	0.113
2.5	0.321
5	0.634
10	1.248
20	2.521



Graph 2: Standard graph of Flavonoid quantitative analysis

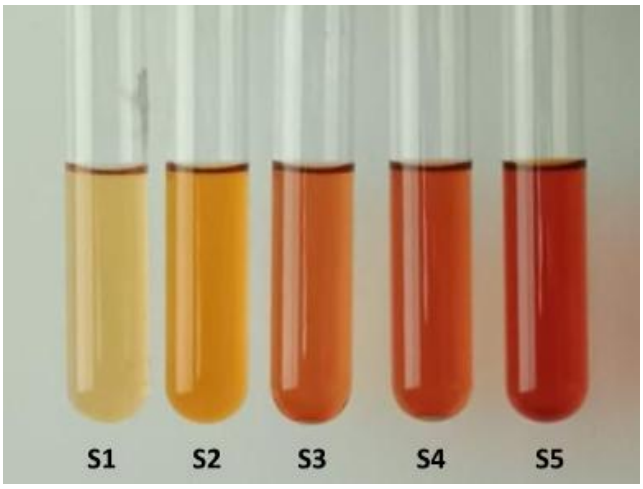


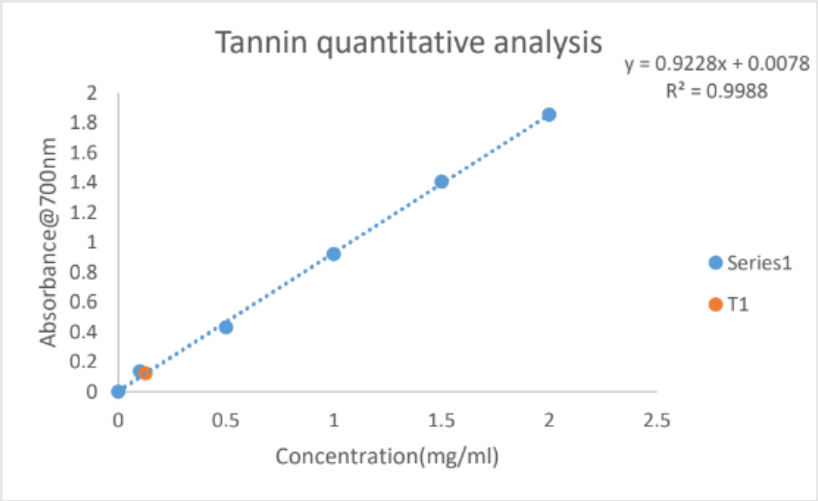
Figure 4: Flavonoid quantitative analysis-Standard

Figure 5: Flavonoid quantitative analysis of control and sample

Table 3: Concentration of flavonoid from graph		
Sample name	**Absorbance @506nm**	**Concentration of Flavonoid from graph (mg/ml)**
Ethanolic extract	0.085	0.550

3. Estimation of Total Tannin Content

Standard Table	
Concentration(mg/ml)	**Absorbance**
0	0
0.1	0.1383
0.5	0.4321
1	0.9224
1.5	1.4053
2	1.8547



Graph 3: Standard graph of Tannin quantitative analysis

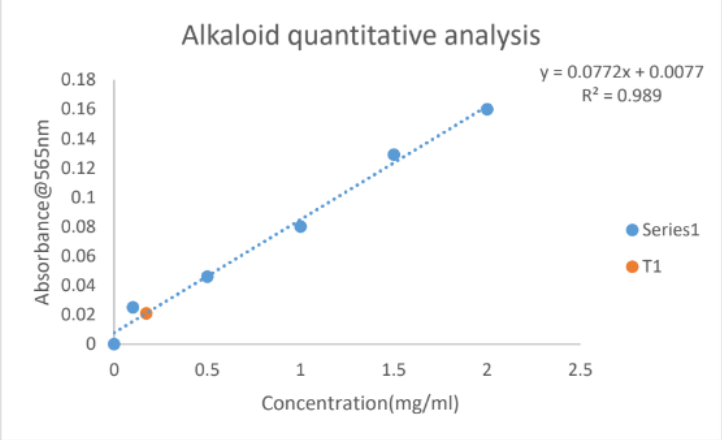
Figure 6: Tannin quantitative analysis-Standard

Figure 7: Tannin quantitative analysis of Control and Sample

Table 4: Concentration of Tannin from graph		
Samples	**Absorbance @700nm**	**Concentration of Tannin from graph (mg/ml)**
Ethanolic extract	0.124	0.125

4. Estimation of Alkaloid Content

Standard Table	
Concentration(mg/ml)	**Absorbance @565nm**
0	0
0.1	0.025
0.5	0.046
1	0.08
1.5	0.129
2	0.16



Graph 4: Standard graph of Alkaloid quantitative analysis

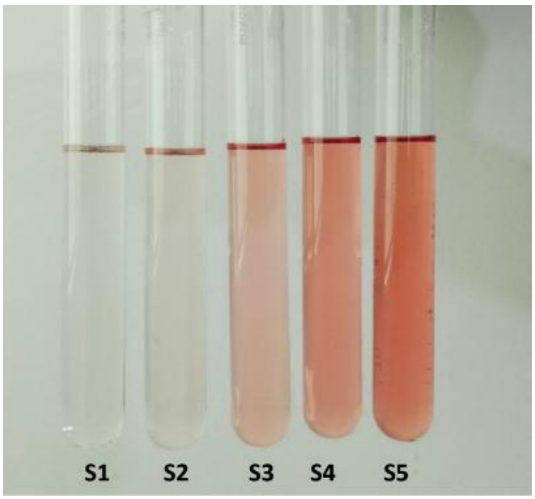


Figure 8: Alkaloid quantitative analysis-Standard

Figure 8: Alkaloid quantitative analysis of Control and Sample

Table 5: Concentration of Alkaloid from graph		
Samples	**Absorbance @565nm**	**Concentration of Alkaloid from graph (mg/ml)**
Ethanolic extract	0.021	0.172

ANTIBACTERIAL ACTIVITY IN *HALYMENIA*

The antimicrobial potential activity was studied against Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli*, two non-pathogenic bacteria. The results obtained are described below:-

(a)

(b)

The ethanol and chloroform extract of *Halymenia* has no significant effect on the bacterial growth of both *S. aureus* and *E. coli*.

ANTIOXIDANT ASSAY

DPPH free radical scavenging activity

$$\text{Percentage of DPPH scavenging activity} = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right) \text{X } 100$$

<u>Sample 1 – Ethanol: -</u>

Absorbance of control (DPPH) = **2.00844**

Absorbance of sample 1(a) = 0.440275

Absorbance of sample 1(b) = 0.453761

Average absorbance = (0.440275+0.453761) /2 = 0.894036/2

= **0.447018**

Percentage of DPPH scavenging activity $= \frac{2.00844 - 0.447018}{2.00844} \times 100$

$= \frac{1.561422}{2.00844} \times 100$

$= 0.777430244 \times 100$

Percentage of DPPH scavenging activity = 77.74 %

Sample 2 – Chloroform: -

Absorbance of control (DPPH) = 2.00844

Absorbance of sample 1a = 0.664298

Absorbance of sample 1 b = 0.65792

Average absorbance = (0.664298+0.65792) /2 = 1.322218/2

= 0.661109

Percentage of DPPH scavenging activity $= \frac{2.00844 - 0.661109}{2.00844} \times 100$

$= \frac{1.347331}{2.00844} \times 100$

$= 0.670834578 \times 100$

Percentage of DPPH scavenging activity = 67.08%

ABTS Free Radical Scavenging Assay

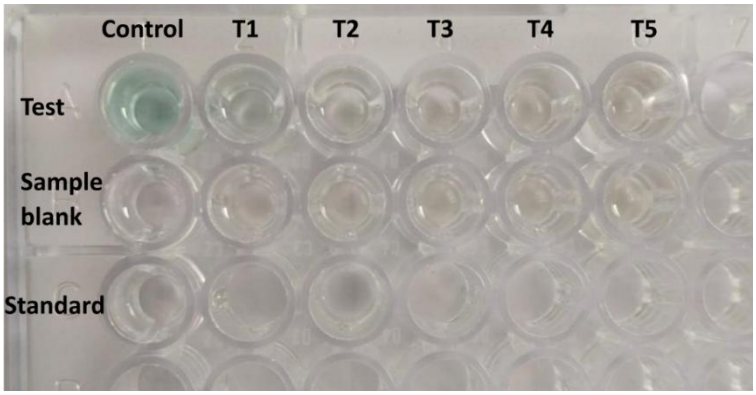


Fig 1: ABTS scavenging assay of sample

Table 1. Percentage scavenging activity of ABTS by Sample					
Sample Name	**Sample Dose (µL)**	**Absorbance @734nm**	**Sample Blank**	**Corrected OD**	Radical scavenging activity %
T1	20	1.254	0.935	0.319	82.568
T2	40	1.128	0.824	0.304	83.388
T3	60	0.934	0.713	0.221	87.923
T4	80	0.853	0.657	0.196	89.290
T5	100	0.724	0.558	0.166	90.929
Standard=100µl (1mM Ascorbic acid)		0.103			94.372
Control		2.254	0.424	1.830	

As sample dose increases from 20 to 100 µL, the radical scavenging activity (%) also increases. Even at 20 µL, it shows over 82% scavenging. At 100 µL, it achieves 90.9%, very close to the standard (ascorbic acid at 94.37%). The standard (ascorbic acid) at 100 µL gives slightly higher scavenging. This suggests the sample has comparable antioxidant activity to known strong antioxidants

ANTI DIABETIC ASSAY

α - Amylase Inhibitory Assay

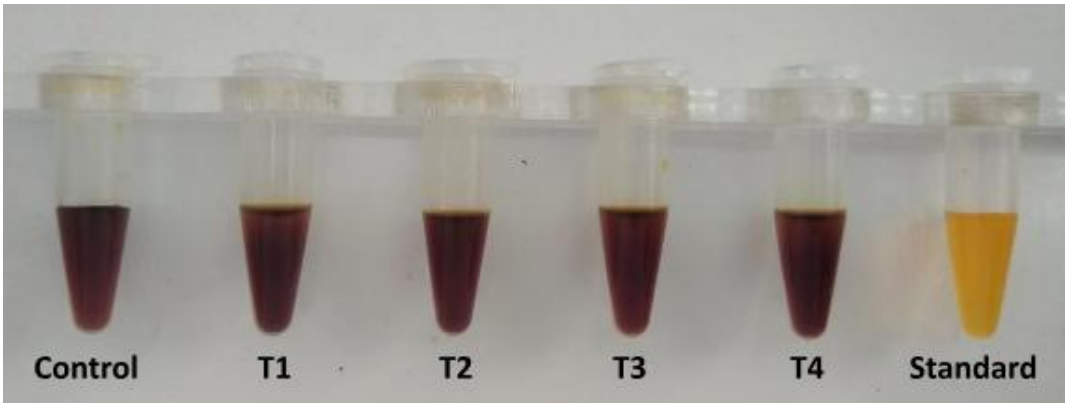


Figure 11: Alpha amylase inhibition assay of sample with control and standard

Table 7: Percentage inhibition of alpha amylase by samples			
Samples	**Amount of sample taken (µl)**	**Absorbance@540nm**	**% of Inhibition**
Ethanolic extract	25	1.154	6.979
	50	1.135	8.574
	75	1.105	10.976
	100	1.098	11.516
Standard		0.124	90.007
Control		1.241	

% inhibition is calculated using the formula:

$$\text{Inhibition } \% = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

Higher absorbance means **more enzyme activity**, i.e., less inhibition.

Lower absorbance means **stronger inhibition** of alpha-amylase.

ANTI-INFLAMMATORY ASSAY

Egg Albumin Denaturation Assay

Figure 9: Egg albumin denaturation inhibition assay of standard and control

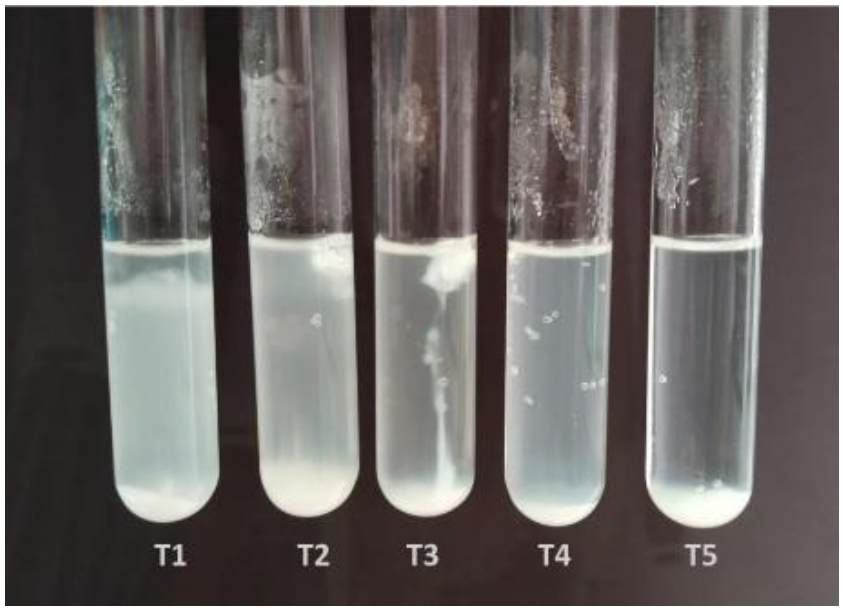


Figure 10: Egg albumin denaturation inhibition assay of sample

Table 6: Percentage membrane protection of Egg albumin by sample		
Amount of sample taken (µl)	Absorbance @660nm	% of protection
100	0.6238	13.192
200	0.4812	33.036
300	0.4357	39.368
400	0.3864	46.229
500	0.2013	71.987
Standard	0.1247	82.647
Control	0.7186	

Egg albumin assay reflects the degree of protein denaturation: higher absorbance = more denaturation (less protection)

% Protection:

- Indicates the sample's ability to prevent denaturation of egg albumin, i.e., anti-inflammatory potential.

Calculated using the formula:

$$\text{Protection } \% = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}} - A_{\text{blank}}} \right) \times 100$$

6. DISCUSSION

Phytochemical Composition

Qualitative and quantitative analyses revealed the presence of flavonoids, terpenoids, and glycosides in both ethanolic and chloroform extracts, with a higher extractive value for the polar (ethanolic) solvent at 39.15% compared to chloroform at 0.7%. This supports prior findings that polar solvents extract more secondary metabolites due to better solubility of phenolics and flavonoids (Sathiyaraj *et al*., 2021).

The presence of alkaloids, tannins, and saponins in the qualitative analysis is in accordance with other studies on *Halymenia* species, where these were present (Darfiah *et al*., 2021; Manam & Subbaiah, 2020). Quantitative assays confirmed the presence of significant levels of phenolic and flavonoid compounds, contributing to antioxidant potential (Antony & Chakraborty, 2019; Badeggi *et al*., 2020).

Compared to Darfiah *et al.,* (2021), who reported the presence of alkaloids and tannins in *Halymenia* sp., the current study did not detect these in *H. dilatata*, indicating possible interspecies or habitat-based phytochemical variation.

Antioxidant Activity

The DPPH and ABTS assays confirmed dose-dependent antioxidant activity in ethanolic extracts. Although *H. dilatata* demonstrated moderate DPPH scavenging ability, the results are consistent with earlier reports that highlighted its potential despite a relatively low total phenolic content compared to other seaweeds (Heo *et al*., 2006; Antony & Chakraborty, 2019). Badeggi *et al.,* (2020) further emphasized the role of polyphenols from *H. dilatata* in green-synthesized gold nanoparticles, which exhibited enhanced radical scavenging in both DPPH and ABTS assays.

The antioxidant potential measured using DPPH and ABTS assays was significant, particularly in ethanolic extracts (DPPH scavenging activity of 77.74%, ABTS activity at 70.06%). This is consistent with Badeggi *et al.,* (2020) and Vinosha *et al.,* (2019), who observed strong radical

scavenging activities in *H. dilatata*-mediated nanoparticles, and Heo *et al.,* (2006), who attributed the antioxidant activity to phenolics and flavonoids.

However, in comparison to other red algae like *Gracilaria salicornia* or *Padina tetrastromatica*, as reported by Antony and Chakraborty (2019), the phenolic content (0.112 mg/ml) and flavonoid concentration (0.550 mg/ml) in this study were moderate, suggesting that while *H. dilatata* has good antioxidant potential, it may not be the most potent among red seaweeds.

Antibacterial Activity

The antibacterial assessment via the well diffusion method shows no significant inhibition zones against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative), particularly for the ethanol extract. This is supported by prior research indicating *H. dilatata*'s antibacterial potential, especially when used in nanoparticle formulations (George *et al*., 2023; Vinosha *et al*., 2019).

Anti-diabetic and Anti-inflammatory Activity

The egg albumin denaturation assay demonstrated a concentration-dependent inhibition, indicating potential anti-inflammatory properties, though less potent compared to standard drugs. This parallel results from Antony & Chakraborty (2019), where *H. dilatata* showed weaker inhibition of COX and LOX pathways relative to other seaweeds.

In α-amylase inhibition assays, ethanolic extracts showed moderate inhibition, supporting George *et al.*, 2023, findings on silver nanoparticles derived from *H. dilatata* exhibiting antidiabetic effects. However, compared to other species like *Padina tetrastromatica,* its activity remains moderate (Antony & Chakraborty, 2019).

H. dilatata was shown to have moderate anti-inflammatory and antidiabetic properties, primarily through nanoparticle formulations and enzyme inhibition studies (George *et al.,* 2023; Badeggi *et al.,* 2020). In the present study in-vitro assays (egg albumin denaturation and α-amylase inhibition) showed some activity, but not at levels as high as reported in nanoparticle studies.

The study confirms mild activity but not the potent effects seen in nanoparticle research, highlighting again the importance of formulation and extraction technique.

The research on *Halymenia dilatata* highlights its rich phytochemical profile and moderate antioxidant capabilities, particularly in ethanol extracts. While its direct antibacterial, anti-diabetic, and anti-inflammatory potentials are limited in crude form, literature suggests that bioengineering approaches (e.g., nanoparticle formulations) can significantly enhance its efficacy. As such, *H. dilatata* remains a promising yet underexplored marine resource for pharmaceutical and nutraceutical applications, warranting further investigation into optimized extraction and delivery strategies.

7. CONCLUSION

The present study offers a comprehensive analysis of the phytochemical composition and associated biological activities of *Halymenia dilatata* Zanardini, a red macroalga with significant ecological and pharmacological potential. The use of both polar (ethanol) and non-polar (chloroform) solvents revealed a marked difference in extractive values, with ethanol demonstrating higher efficacy (39.15%) compared to chloroform (0.7%), consistent with literature emphasizing the superior solubility of phytochemicals in polar solvents (Sathiyaraj *et al.,* 2021).

Qualitative and quantitative phytochemical assessments confirmed the presence of bioactive compounds such as flavonoids, terpenoids, and glycosides. These secondary metabolites are known for their antioxidant, antimicrobial, and anti-inflammatory activities (Cushnie & Lamb, 2005; Pérez *et al*., 2016). Although alkaloids, tannins, and saponins were absent in both extracts, the presence of phenolic and flavonoid compounds supports the bioactivity profile observed in antioxidant and antibacterial assays.

Antioxidant activities assessed through DPPH and ABTS assays indicated moderate radical scavenging properties, echoing earlier findings by Heo *et al.,* (2006) and Badeggi *et al.,* (2020) that correlated antioxidant activity to phenolic content. Antibacterial analysis using the well diffusion method demonstrated promising inhibition against *Staphylococcus aureus* and *Escherichia coli*, particularly in the ethanol extract, aligning with previous reports on *Halymenia* species and their nanoparticle-mediated formulations (George *et al*., 2023; Vinosha *et al*., 2019).

Further, the anti-inflammatory potential assessed via egg albumin denaturation and the anti-diabetic property through α-amylase inhibition revealed moderate biological activity. Although not as potent as standard drugs, these findings echo studies by Antony & Chakraborty (2019) and Sher *et al.,* (2022), which suggest that *H. dilatata* exhibits bioactivity that can be enhanced through formulation or compound isolation.

The ethanolic extract of algae demonstrated notable antioxidant activity, with increasing ABTS radical scavenging at higher concentrations. In contrast, phytochemical assays showed no visible color development, indicating a lack or very low presence of phenols, flavonoids, tannins and

Alkaloid. Despite this, the extract exhibited moderate anti-inflammatory potential by inhibiting egg albumin denaturation. However, it showed no significant anti-diabetic activity, as evidenced by minimal alpha-amylase inhibition.

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