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Research Article

Efficacy of Bioactive Compounds Extracted from Marine Algae

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Abstract

For the past few decades, bioactive compounds from marine organisms have been utilized for their therapeutic activity. Among algae, microalgae are extensively studied and reported to exhibit more diversity in their pharmacological action. Our attempt is to explore the profile of the biologically important active molecules in macroalgae from the marine environment. Simultaneously we also investigated the therapeutic potential of algal extracts. The results may open new avenues for medical research and offer substitute treatments for illnesses linked to oxidative stress and inflammatory diseases. The methodologies consist of two phases, the first stage consists of algal culturing and staining, extraction of phytonutrients and their estimations. We have compared three marine algal species, Padina pavonica, Ulva fasciata and Hypnea valentiae. The phytochemical profile including alkaloids, tannin, phenol, saponin, and flavonoids along with major biomolecules (protein, carbohydrate, and lipid) was analyzed in all three algal extracts. The second phase consists of the assessment of the therapeutic potential of marine algal extract. Anti-inflammatory, anti-arthritic, and anti-oxidant potential along with the antiproliferative effect on UV-exposed mammalian cells was assessed. Its rich nutritional profile has been highlighted by examining its biochemical composition, which positions algae as a sustainable source of protein and other essential nutrients for human and animal feed. Study indicates that the compounds derived from algae can regulate inflammatory pathways, prevent oxidative stress, and mitigate arthritic symptoms. This makes marine algae a viable and sustainable substitute for traditional pharmaceutical treatments. Furthermore, the safety profile of algal elements offers minimal side effects compared to synthetic medications. Further investigation into the phytochemical composition and therapeutic mechanisms of algae can reveal their potential in healthcare and quality of life.

Keywords: Protein; Carbohydrate; Algae

Introduction

As per the popular studies cancer and to a certain extent diabetes are considered as the top worldwide health problems. But still, inflammatory diseases triggered by reactive oxygen species (ROS) such as arthritic problems are also gaining importance. Epidemiological studies have shown that higher intake of unhealthy "fast food" and overconsumption of medicines such as paracetamols and antibiotics along with a recent increase in alcoholism are the reasons for oxidative stress-related inflammatory diseases. Annually many synthetic drugs are released on the market, but all these anti-inflammatory drugs result in adverse effects, such as toxicity, drug tolerance, and metabolic impairments. This has led to the search for alternative drugs with better characteristics and natural products proved to be a better choice. It is assumed and expected that supplementation of a regular diet with these natural pharmacologic agents in limited dosage can prevent inflammatory diseases.

Normally terrestrial plant-derived bioactive compounds attract attention as they are easily available. However, the marine environ-

ment is a vast resource for novel unexplored bioactive compounds. Many of these compounds may have medicinal properties ranging from anti-cancer to anti-diabetic effects. They are expected to possess anti-microbial and anti-inflammatory activities that also bring about their pharmacological effect. In this study, attention is given to marine algae because they are included not only in diet but also used extensively in traditional medicine in Asian countries. They are a rich source of proteins, lipids, minerals, and many other bioactive compounds, such as phlorotannins, fucoidans, alginic acid, tripeptides, etc. As they are endowed with many medicinal properties, they are assumed to be promising biocatalysts for new and sustainable life.

These features enable an opportunity for several biotechnological applications. The extensive and rich biodiversity of the marine environment and medicinal potential of marine organisms; offer a novel role in developing new therapeutics with innumerable benefits.

Phytonutrients are naturally occurring chemicals or bioactive compounds produced by plants that have antioxidant and anti-inflammatory properties. Many phytonutrients are generally found in plants, many microbial systems help to exploit them. Some of the most common phytonutrients are reported to have antioxidant and anti-inflammatory properties [1]. Phytonutrients modulate the immune system, helping to maintain the delicate balance between an overactive immune system usually associated with autoimmune diseases, and an underactive immune system in general observed in infections or cancer [2].

Evaluation of phytochemical constituents in algal extracts has revealed their significant role in the prevention of many diseases. The presence of compounds such as alkaloids, flavonoids, phenols, tannins, terpenoids, glycosides, and steroids in algae was thought to act as a defense mechanism against reactive oxygen species (ROS). The presence of antioxidant phytochemicals in algae is proven to protect against oxidative damage [3].

Assuming the same properties are also inherent to marine algal species, this study was conducted to analyze the presence of phytonutrients in marine algae and their anti-inflammatory and antioxidant effects.

Materials and Methods

Procurement of algae

The marine algae, *Ulva fasciata*, *Hypnea valentiae*, and *Padina pavonica*, were procured from an algae collection center located in the district of Ramanathapuram, Tamil Nadu. Using a modified version of Schreiber's medium [4], the dried form of algae were revived. A combination of vitamins comprising thiamine, and biotin were used in revival medium.

Soil extract, sodium chloride, magnesium sulphate, calcium chloride, potassium nitrate, sodium orthophosphate, and EDTA were used to make the customized seawater [5]. The solution was autoclaved before the vitamin mixture was added. It was then poured to clean autoclaved petri dishes and algae and they were cultured and revived in the presence of sufficient light and air.

Staining of algae

The three algal species: *Ulva fasciata*, *Hypnea valentiae*, and *Padina pavonica*, were stained using safranin stain to focus is using the microscope [6]. The cells were observed using a microscope under a magnification of 10x and 40x.

Homogenization and extraction

One of the most common and popular laboratory procedures used to break down the cell walls and remove intracellular components from algae samples with a mortar and pestle [7]. Homogenization is performed in specific buffers with additional factors like detergents, to liberate the intracellular components like proteins, lipids, and phytonutrients. Here the main objective was to rupture the cell walls [7].

The homogenized content was then centrifuged at 5000rpm for 10 minutes and the supernatant was collected to perform further tests [8].

Estimation of biomolecules

All the estimations were conducted 3-5 times to get consistent result and the average of the observed results with standard deviations are used for expressing the data.

Estimation of protein

Estimation of protein from Algae

Algal samples were treated with Tween 20 [9] in 250 mM Tris buffer at pH 8.3 and homogenized at 4°C in the presence propanediol and incubated for 2 hours at 4°C. After centrifugation, the supernatant is used for Biuret estimation [10].

Estimation of lipid

The lipid present in Tween 20 treated homogenized algal extract was separated with the solvent mixture chloroform: methanol. After centrifugation to remove debris, the supernatant is subjected to saponification [11].

Estimation of polysaccharides

Polysaccharide concentration was estimated in the same algal extract by the phenol-sulphuric acid method [12].

Quantitative assessment of phytochemical composition

The quantitative content of phytochemicals such as alkaloids, phenolics, flavonoids, Tannins, and saponins in three different marine algae species was assessed.

Alkaloid content

Alkaloid levels were assessed in algal samples by treating with acetic acid and subsequent precipitation with ammonium hydroxide [13]. The percentage of total alkaloid content was computed using the formula:

Percentage of total alkaloids (%) = (Weight of residues/Weight of sample taken) ×100

Total Phenolic content

Total phenolic content was assessed using the Folin-Ciocalteu reagent method [14]. Total phenolic content in the algae extract was expressed as mg of gallic acid equivalent (mg GAE/g extract) utilizing the standard curve.

Total Flavonoid content

Total flavonoid content was determined by the protocol outlined by [15]. It was done by treating algal extract with sodium nitrate, sodium chloride and sodium hydroxide sequentially as per the protocol. The appearance of a pink color indicated the presence of flavonoid content. The total flavonoid content was quantified as rutin equivalent mg RE/g extract on a dry weight basis using the standard curve.

Tannin content

Tannin levels in each algae extract are estimated by Polypyrrolidone treatment [16].

Saponin content

Total saponin content was assessed using a modified version of the vanillin-sulphuric acid colorimetric reaction [17]. The results were quantified as diosgenin equivalents (mg DE/g extract) utilizing a standard curve.

Estimation of antioxidant activity

The radical scavenging potential of *Hypnea valentiae*, *Ulva fasciata,* and *Padina pavonica* was evaluated using DPPH assay [18]. (The efficacy of DPPH radical scavenging activity was calculated using the following formula

DPPH scavenging effect (% of inhibition) = (A0 – A1) × 100/A0

Here, A0 represents the absorbance of the control and A1 represents the absorbance of the sample extract.

Estimation of anti-inflammatory activity

The *in-vitro* anti-inflammatory effects of extracts from marine algae *Hypnea valentiae*, *Ulva fasciata,* and *Padina pavonica* were evaluated using the HRBC (human red blood cell) membrane stabilization method [19], with fresh chicken blood mixed with an equal volume of Alsever's solution to cause hemolysis. After incubation at 37˚c for 30 minutes, the mixture was centrifuged, and the hemoglobulin content in the suspension was calculated using the formula

Percentage of Hemolysis = (OD of test solution/OD of control) × 100

The anti-inflammatory activity represented was calculated using the formula:

Percentage of hemolysis = 100 - Hemolytic percentage.

The OD(optical density) of the test corresponds to the optical density of the test sample's absorbance, and OD of control refers to the optical density of the negative control, consisting of Alsever's solution with blood.

Anti-arthritic studies by protein denaturation method

The anti-arthritic assay was performed *in vitro* using the protein denaturation method [20]. The reaction mixture comprised egg albumin in phosphate-buffered saline (PBS with pH 6.4) and 2ml of Algae extracts. The mixture was then incubated at 37˚c for 15 minutes. The percentage of inhibition of protein denaturation was calculated using the formula:

The percentage of inhibition = 100 – (optical density of test solution-optical density of control)/ optical density of test solution) x 100)

Determining the effect of algae on uv-exposed cells

The efficiency of algal extracts on cell viability was assessed after treating the primary culture of bovine liver cells with UV.

Primary culture of bovine liver cells in Hank's Balanced Salt Solution was maintained in culture for one week. The proliferation confluency was assessed under phase contrast microscopy. The cells were sub-cultured. After subculturing, the cells were exposed to UV for 30 minutes and further maintained in culture, for 2 days along with unexposed cells as control. A phase contrast microscope initially checked the visual observation of cell proliferation and confluency. The UV-exposed cells were treated with algal extract and incubated for 2 days at 37°C. The cell viability was checked using trypan blue staining [21] using a formula:

Percentage of cell viability = (No. of alive cells / Total No. of cells) × 100

Results and Discussion

Procured algal species of marine origin were revived and maintained in a culture medium (Figure 1).

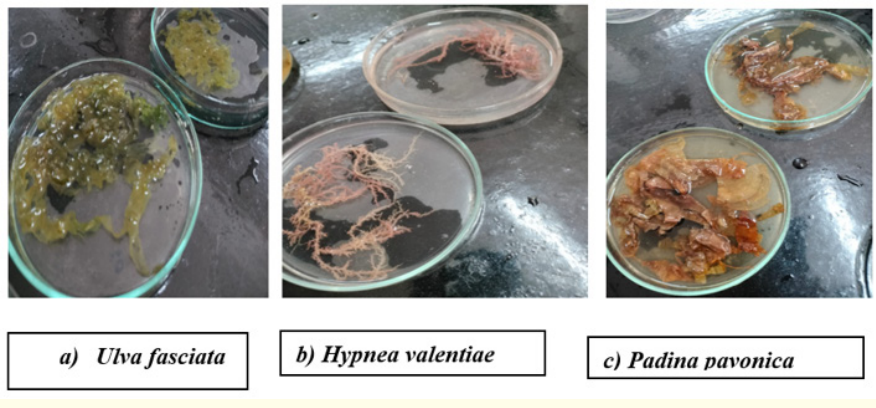

Figure 1: Revived Algal species in artificially made seawater.

Estimation of proteins

Here, based on the results obtained from the biuret test, *Padina pavonica* has the highest protein content when compared to other two species, followed by *Ulva fasciata* and *Hypnea valentiae*.

These findings suggest that *Padina pavonica* could be a valuable source of protein for various applications, including food, feed, and biotechnology. The potential uses of *Padina pavonica* protein in various industries have to be explored further (Figure 2).

Estimation of lipids

The purpose of this study is to examine the capacity for lipid accumulation in three species of marine algae. According to the results obtained *Padina pavonica* showed the highest lipid content followed by *Hypnea valentiae* and *Ulva fasciata* (Figure 3). Different genetic variables, environmental factors, and metabolic process that are unique to each species of algae could be responsible for the variations in lipid content of the three species.

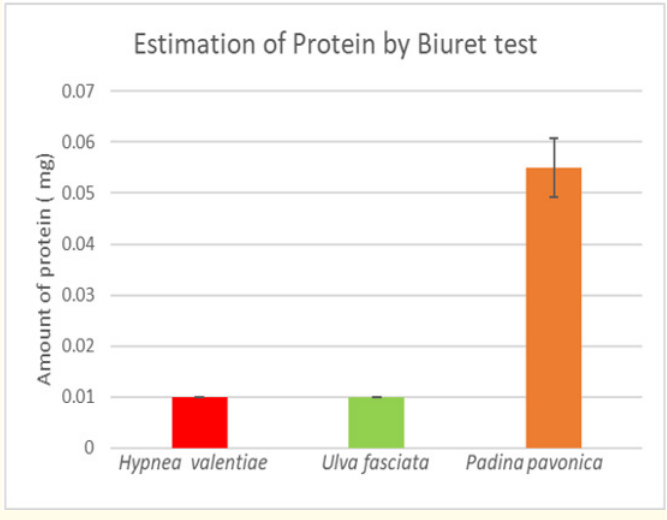

Figure 2: Estimation of protein in different algal species.

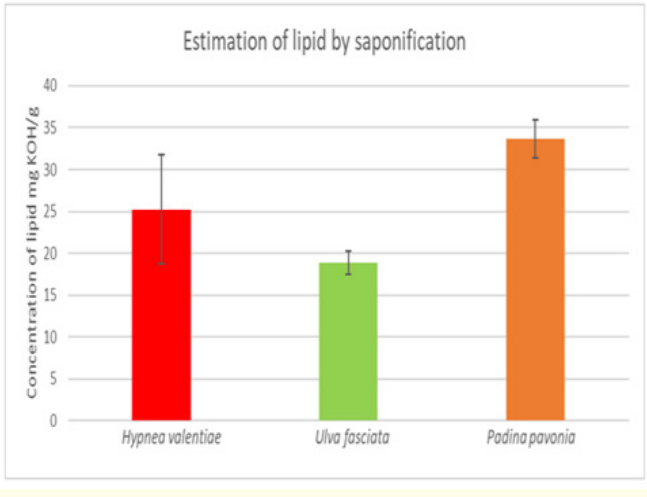

Figure 3: Estimation of saponification efficiency of algal species.

Estimation of polysaccharides

It is essential to estimate the polysaccharide content of various algal species to comprehend its nutritional value and possible applications in various industries like the food, and pharmaceutical industry. According to the results obtained three different algal species have different polysaccharide contents, the polysaccharide content of *Padina pavonica* is the highest, followed by *Hypnea valentiae* and *Ulva fasciata* (Figure 4). Genetic variables, environmental factors, and metabolic processes that are unique to each species of algae could be responsible for variations in polysaccharide contents. Further research could help in exploring various functional properties of polysaccharides and could lead to the development of novel polysaccharide-based health products with potential health benefits.

The above results showed that *Padina pavonica* is the species with higher nutritional value with respect to protein, lipid, and carbohydrate content.

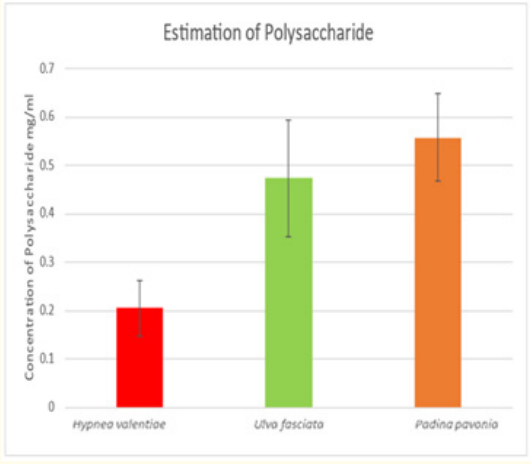

Figure 4: Estimation of polysaccharide in algal species.

Quantitative assessment of phytochemical composition

The study revealed that the extract from algae *Hypnea valentiae*, *Ulva fasciata* and *Padina pavonica* contains alkaloids, phenol, flavonoids, tannic acid and saponin (Figure 5). This indicates the presence of compounds with potential bioactive properties. The presence of these compounds in algae extracts suggests their potential for various pharmacological applications.

Alkaloids

Alkaloids encompass a range of naturally occurring chemical compounds with varied pharmacological effects on the human body and various biological activities, including antimicrobial, antiviral, anti-inflammatory, and even cytotoxic effects [22]. In algae, alkaloids may play roles in defense against predators. Using gravimetric analysis alkaloid levels in different algal species such as *Hypnea valentiae*, *Ulva fasciata,* and *Padina pavonica* is determined. The results obtained from gravimetric analysis show that *Hypnea valentiae* contains the maximum alkaloid content followed by *Padina pavonica* and the least for *Ulva fasciata*. This indicates that *Hypnea valentiae* holds potential for therapeutic applications owing to its elevated alkaloid content (Figure 5).

Total phenolics

Antioxidant properties characterize phenolic compounds and can scavenge free radicals, thereby protecting cells from oxidative damage [23]. Phenols also have antimicrobial and anti-inflammatory activities and are implicated in various health benefits [24]. The total phenolic content of the algae showed that *Padina pavonica* had the highest phenol content, followed by *Ulva fasciata* and the least amount was found *Hypnea valentiae*. The potential health benefits of consuming seaweed rich in phenolic compounds and incorporating them into our diet could provide protection against oxidative stress and reduce the risk of chronic diseases.

Tannins

Polyphenolic chemicals called tannins are present in many different plant species, including marine algae. They provide defense against infections against invading microbes including viruses [25]. Tannins are known for their capacity to bind and precipitate proteins in addition to their anti-bacterial and anti-oxidant qualities. Determining the amount of tannin present in various algae species is crucial for comprehending their uses in sectors like medicine, cosmetics and tanning. According to the results obtained *Hypnea valentiae* had the highest tannin content, followed by *Ulva fasciata* and *Padina pavonica* (Figure 5).

Flavonoids

Flavonoids are the secondary metabolites that are found in various plants and algae, it is known for their antioxidant, anti-inflammatory and its bioactive properties [26]. According to the results obtained *Padina pavonica* exhibited the highest flavonoid content, followed by *Ulva fasciata*, and *Hypnea valentiae*. the results suggest that *Padina pavonica* have a higher flavonoid accumulation potential (Figure 5).

Saponins

A class of naturally occurring substances called saponins is present in many different plant species including marine algae. Saponins possess a range of biological functions, such as antibacterial, antifungal, and anti-inflammatory activity [27]. Due to their variety of biological functions, they also can be used in pharmaceutical, cosmetic, and agricultural industries. According to the results obtained, *Hypnea valentiae* had the highest saponin content followed by *Ulva fasciata* and *Padina pavonica* (Figure 5). A variety of factors including genetic factors, environment and species-specific factors may contribute to the variations in saponin content.

In vitro antioxidant activity

Antioxidants play a crucial role in protecting our body from oxidative stress and damage caused by free radicals. In this study we utilized DPPH assay to evaluate the antioxidant activity of three marine algae *Hypnea valentiae, Ulva fasciata* and *Padina pavonica*. Since these algae shows a significant amount of compounds endowed with antioxidant properties the antioxidant activity assay supports our observation. Many of the polyphenolic compounds in algae, target multiple inflammatory components and results anti-inflammatory response, anti oxidant properties of algae are responsible for their anti-inflammatory and immunomodulatory functions [28]. Since limited studies are conducted in marine macroalgae, this observations will help make use of marine macroalgae in clinical interventions.

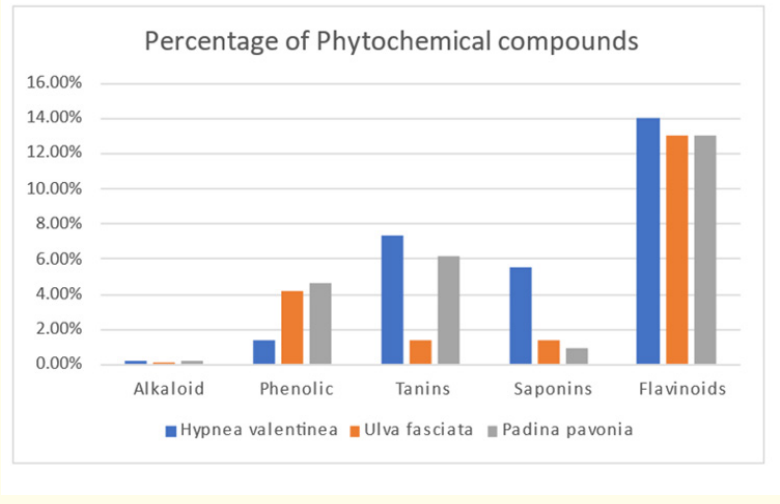

Figure 5: Percentage of Phytochemical compounds.

The results revealed that *Padina pavonica* exhibited the highest antioxidant activity among the three species (Figure 6). This finding suggests that *Padina pavonica* possess rich bioactive compounds capable of scavenging free radicals and inhibiting oxidative processes. The elevated antioxidant activity of *Padina pavonica* could attributed to its unique chemical composition, which may include phenolic compounds, flavonoids, alkaloids etc. *Hypnea valentiae* exhibited moderate antioxidant activity, indicating its potential utility in scavenging free radicals, and mitigating oxidative stress. On the other hand, *Ulva fasciata* displayed the lowest antioxidant activity among three species. This may indicate a relatively lower concentration of antioxidant compounds or a differ-

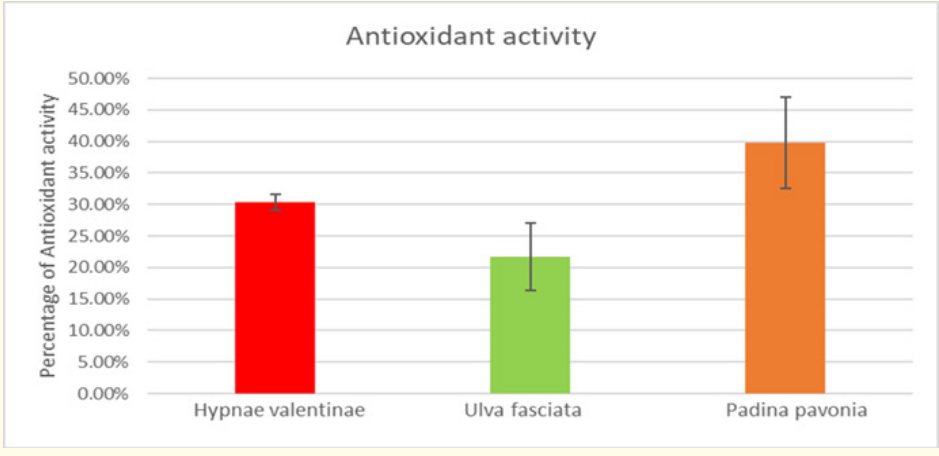

Figure 6: Estimation of the percentage of antioxidant activity.

ent antioxidant profile compared to the other algal species tested. Literature survey had shown that in plant extracts, with high phenolic compounds exhibit maximum antioxidant properties, which is true for *Padina sps*. But in the case of *Hypnea,* even a low concentration of phenolic compounds does not reduce its antioxidant capacity, as it has a higher content of tannins and saponins. The observed difference in antioxidant activity among the three algae species highlights the importance of species-specific variation in bioactive compound profiles correlating to free radical scavenging efficiency. These findings signify the potential of marine algae as a source of natural antioxidants with implications for various applications, including pharmaceuticals, functional foods, and cosmetics. Further research into the specific antioxidant compounds present in each algal species and their mechanism of action will reveal methods to fully understand their potential health-promoting effects and industrial applications.

Anti-inflammatory studies- hrbc membrane stabilization method

The anti-inflammatory potential of three algae species, *Hypnea valentiae*, *Ulva fasciata,* and *Padina pavonica* was investigated using HRBC (Human Red Blood cell) membrane stabilization method. A hypotonic solution was used to induce hemolysis in HRBC (Figure 7). Control HRBC were kept in an isotonic solution. The three other tubes with HRBC incubated in hypotonic solution showed significant membrane damage of 78-72% of membrane damage.

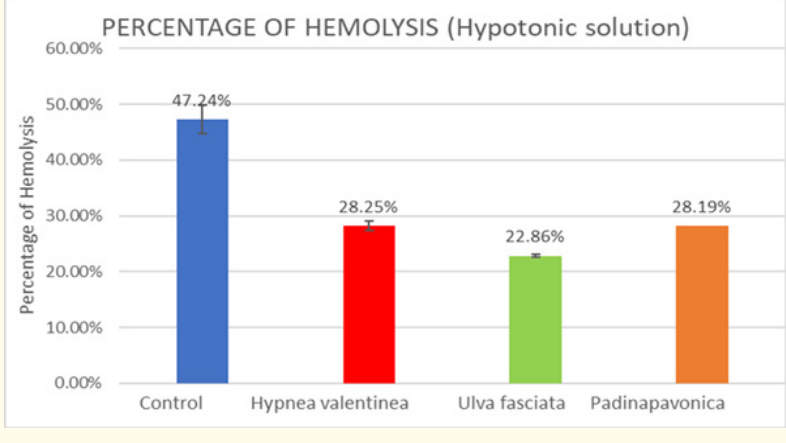

Figure 7: Percentage of Hemolysis of HRBC in Hypotonic solution.

The anti-inflammatory activity of algal extract in preventing membrane damage was observed in all three species of algae. The results demonstrated significant anti-inflammatory effects for all three algae species, with no significant variation in their potency. Even then, *Ulva fasciata* exhibited the highest percentage of retrieval from hemolysis induced membrane damage, with a value of 77% followed by *Padina pavonica* and *Hypnea valentiae* (Figure 8). The control in normal isotonic solution retained the membrane stability between 47-52%.

Bioactive constituents such as phenolic compounds, flavonoids, or alkaloids are known for their anti-inflammatory properties. Their presence in algal cells resulted in significant membrane stabilization, from 22-28% membrane stabilization an increase of 70-77% was observed. This suggests the presence of bioactive compounds with anti-inflammatory properties.

Anti-arthritic study by protein denaturation method

Arthritis is a common inflammatory condition affecting millions worldwide, necessitating the search for novel anti-arthritic agents. Tissue proteins are denatured in inflammation, leading to autoantigen formation and inflammatory diseases like arthritis [29]. Bioactive compounds from algal populations with antioxidant and anticancer compounds are reported already. Secondary metabolites like phenolics, flavonoids, and tannins from marine algae are active against several disease condirtions. Various studies are conducted focusing on efficacy of functional compounds extracted from algae and their beneficial effects against cancer, diabetes, and many other inflammatory diseases [30].

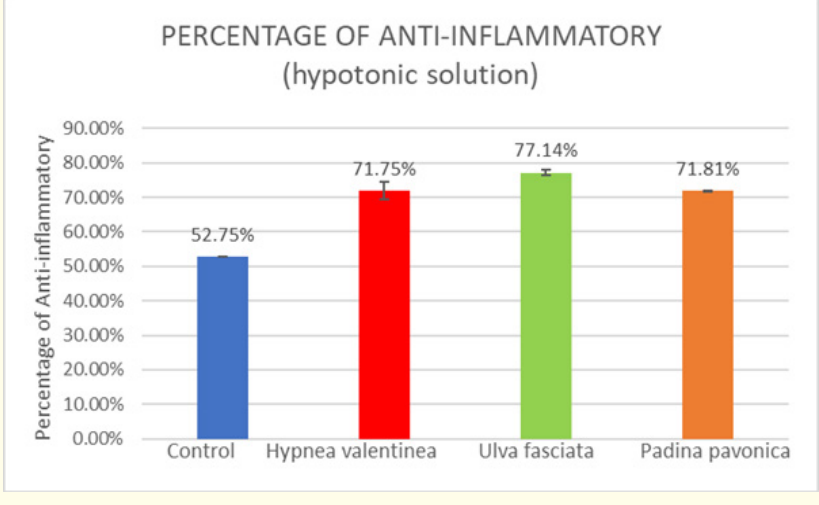

Figure 8: Percentage of anti-inflammatory activity.

In this study, we investigated the anti-arthritic potential of extracts from three macro algae species such as *Hypnea valentiae*, *Ulva fasciata,* and *Padina pavonica* using the protein denaturation method as a screening tool, to find their potential as an anti-inflammatory agent against arthritis. The ability to inhibit protein denaturation is taken as antiarthritic effect.

Hypnea valentiae exhibits the highest percentage of inhibition of protein denaturation, with a value of 44%, followed closely by *Padina pavonica* with 37%. In contrast, *Ulva fasciata* displayed the lowest percentage of inhibition at 16%. The observed difference in the anti-arthritic activity of *Ulva fasciata*, *Padina pavonica,* and *Hypnea valentiae* extracts highlights the importance of species-specific variations in bioactive compound composition (Figure 9). *Hypnea valentiae* and *Padina pavonica* extracts showed promising inhibitory effects on protein denaturation, suggesting their utility in arthritis management. However, additional studies are needed to validate these findings and identify specific bioactive compounds responsible for the observed anti-inflammatory and antiarthritic activity.

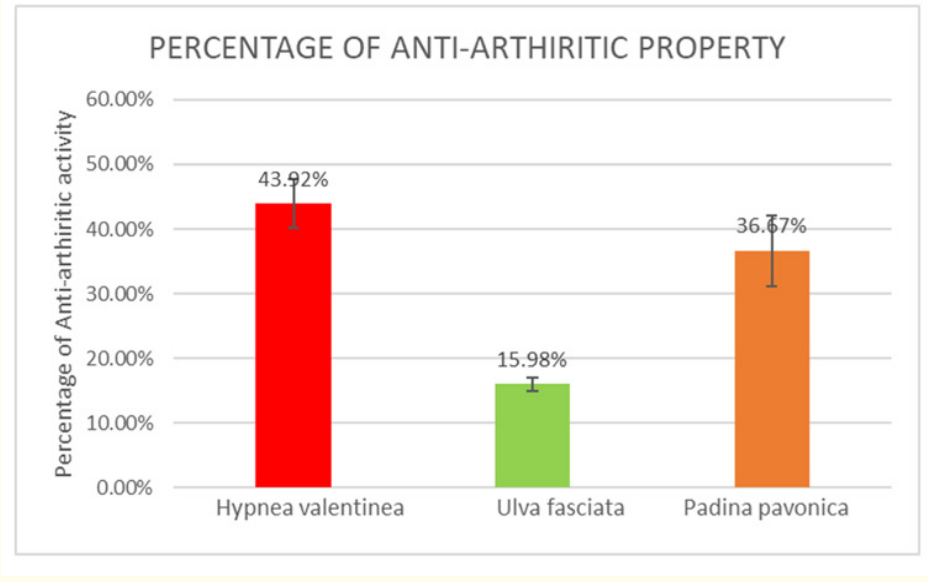

Figure 9: Estimating the percentage of anti-arthritic activity.

The effect of algal extracts on uv-exposed cells

Ultraviolet (UV) radiation is identified as a tumorigenic agent, but UVB radiation is responsible for such an effect. Although the 254 nm (UVC) emission of low-pressure vacuum lamps has an antimicrobial effect, at the same time, this radiation also causes harm to human cells [31]. The mechanism behind it may be either mutagenic or any other specific damaging properties [32]. In this study, we investigated the efficacy of algal extracts from three different species in the retrieval of UV-exposed cells (Figures 10 a and b).

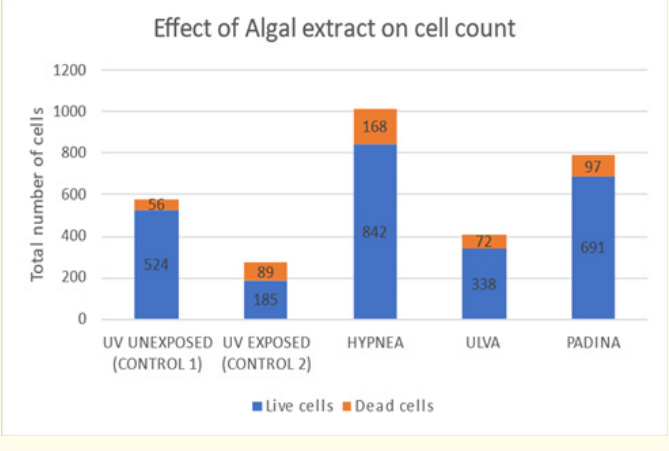

Figure 10a: Effect of algal extract on cell count.

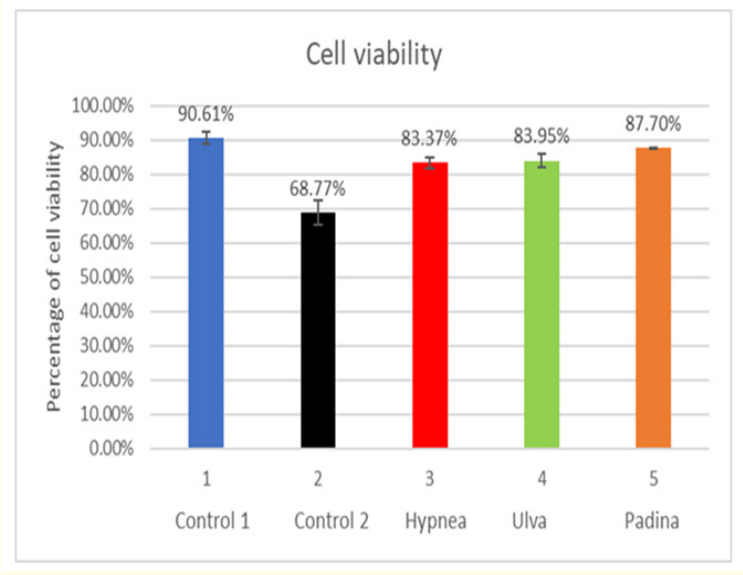

Figure 10b: Effect of algal extract on cell viability.

UV exposure at 254 nm brings a significant reduction in cell number and viability. The algal extracts were effective in retrieving cell viability in UV-exposed cells, thereby mitigating UV-induced cellular damage. Interestingly, while all three algae extracts exhibited similar cell retrieval efficacy, *Padina pavonica* extract demonstrated the highest efficiency. This superior performance may be attributed to the unique biochemical compounds present in *Padina pavonica*, which possess potent cellular repair and protective properties. The observed cell retrieval properties suggest the presence of bioactive compounds capable of promoting cellular repair and regeneration.

UV light of 250-254 nm comes under UVC, a very potent form of UV radiation. Its range is 100-280 nm. Sun is the natural source of UVC, but Earth's ozone layer absorbs it and the potential harmful effects are nullified. UVC light has germicidal properties and it is used for various disinfection purposes. The same source can be used to study the effect of UV radiation on biological samples including cells. UV can induce reactive oxygen species generation, by impairing the natural antioxidant defense mechanism [33]. Our attempt is to find out whether algal compounds are capable of nullifying the damage caused by UV exposure.

UV exposure at 250nm has resulted in a decrease in cell count and approximately 50% of cells were found to be nonviable compared to the control value of 10% nonviable cells. Algal extract-treated cell populations showed a cell count increase and more or less only 20% of cells were nonviable after exposure to UV light and subsequent algal extract treatment. This significant decrease in nonviable cells shows a positive impact of algal phytochemicals on protecting the cells (Figure 10a). The trypan blue exclusion assessment of cell viability also supports the same observation (Figure 10b), ascertaining the protective role of algal bioactive compounds in reversing oxidative-induced damage to cells by UV exposure.

Including these natural bioactive factors called phytonutrients as a part of daily diet can positively impact human health. Our studies have shown the antioxidant and anti-inflammatory properties of algal extracts. The assumption is that once ingested they may improve physiological mechanisms of metabolism and thus regulate metabolic diseases. Another interesting finding [34] is that the microbial flora of the gut has an important role in metabolizing phytonutrients. It is done by breaking complex phytonutrients to small absorbable molecules. Thus their bioavailability will be improved. They also suspected to have a role in maintaining the gut microbiota. The gut microbial and algal phytonutrients interaction is an area to be studied further.

Conclusion

This study is to explore the range of phytochemicals found in macroalgae as many research attempts were concentrated on microalgal populations. Our results highlight the significance of algae as a priceless natural source with a wide range of biologically active compounds inherent to them. These phytochemicals in algae are with a wide range of health benefits and therapeutic potentials, from antibacterial actions to antioxidant and anti-inflammatory capabilities. Our research offers strong evidence that algal phytochemicals are effective in fighting against oxidative stress, arthritis, and inflammation. The purpose was to identify the wide range of bioactive compounds in algae by careful investigation and analysis and many of these compounds have outstanding anti-inflammatory, anti-arthritic, and antioxidant qualities. This study can be extended to unravel the mechanism underlying gut health improvement by algal phytonutrient and gut microbial interaction.

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Authors and Affiliations

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Contributions

- Conceptualization, visualization, design of methodology, manuscript correction and supervision by Preetha Nair.
- Experimental execution, data collection, and manuscript preparation by Anila Joseph and Nancy Ann Joseph S.
- All authors have read and approved the publishable version of the manuscript.

Ethical Approval

The authors followed all applicable international, national, and/or institutional guidelines for conducting the study.

All the experiments were replicated 3-5 times.

Since no clinical trial has been conducted, Clinical Trial no: not applicable.

Conflict of Interest

The authors declare no competing interests.

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