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# Screening Of Bioactive Properties In Brown Green Algae Chloroform Extracts From Sargassum Muticum Seaweed

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**ABSTRACT:**
*Sargassum muticum,* a brown-green macroalga, is known for its rich content of secondary metabolites with potential pharmacological applications. This study aimed to screen the bioactive properties of chloroform extracts derived from *Sargassum muticum* seaweed. The extract was subjected to preliminary phytochemical screening and evaluated for its antimicrobial and antioxidant activities. Results revealed the presence of bioactive compounds such as terpenoids, sterols, and phenolic derivatives. The extract demonstrated notable antimicrobial activity, particularly against Gram-positive bacteria, and exhibited moderate free radical scavenging ability, indicating the presence of lipophilic antioxidant compounds. These findings suggest that *Sargassum muticum* chloroform extracts hold promise as a natural source of therapeutic agents. Further studies are required to isolate and characterize individual compounds and assess their efficacy through in vivo models.

**INTRODUCTION:**Some foodborne microorganisms, for instance Bacillus cereus, can be found in diverse environmental matrices, including soil and plants, demonstrating remarkable adaptability that enables them to thrive in various ecological niches. These organisms possess the ability to colonize the intestinal tract of animals and further cause major health problems through the production of toxins and other virulence factors that lead to food poisoning and gastrointestinal illness (1). Bacillus cereus is particularly problematic in food processing environments due to its ability to form heat-resistant spores that survive cooking processes and its capacity to produce both emetic and diarrheal toxins, making it a significant concern for food safety worldwide. Consequently, the control of pathogenic microorganisms in food products is a major and ongoing issue for the food industry, requiring continuous vigilance, innovative preservation methods, and the development of effective antimicrobial strategies to ensure consumer safety and prevent economic losses associated with food spoilage and foodborne disease outbreaks.

In recent years, consumer pressure and growing environmental awareness have led to a significant trend toward opting for more natural ingredients in food products, cosmetics, and pharmaceuticals, driven by concerns about the safety of synthetic additives, the desire for more sustainable production methods, and increasing interest in traditional and natural approaches to health and wellness. This shift in consumer preferences has prompted food manufacturers and other industries to explore natural alternatives to synthetic preservatives and functional ingredients, creating opportunities for novel sources of bioactive compounds. Thus, marine algae can be considered as functional foods and ingredients of natural origin, since they are already used in food and cosmetic products, as well as in traditional remedies in Asian countries where seaweed consumption has a long history and is integrated into cultural practices. The use of macroalgae in traditional medicine systems across Asia, including China, Japan, and Korea, provides a rich foundation of empirical knowledge about their health benefits that can guide modern scientific investigation.

Nevertheless, macroalgae are still underestimated in Western cultures, despite the numerous scientific studies that have rigorously proved their biological activities and potential health benefits through modern analytical and pharmacological methods. This underappreciation may stem from limited familiarity with seaweed as a food source, cultural differences in dietary habits, and insufficient translation of scientific findings into consumer awareness and commercial applications. Such health-promoting properties associated with macroalgae have prompted their use in various new industrial applications, also motivated by their unique chemical and nutritional composition and their high availability in coastal ecosystems around the world, where they represent a renewable and sustainable resource that can be harvested without the environmental impacts associated with terrestrial agriculture. The rich diversity of macroalgae species and their adaptability to different environmental conditions make them a promising source of novel bioactive compounds for multiple applications.

Sargassum muticum (Yendo) Fensholt is a brown macroalgae species that can be found abundantly in the Northwestern coasts of the Peninsular region, where it forms extensive beds in intertidal and subtidal zones. Originally native to the Pacific Ocean, this species has become invasive in many parts of the world, including European waters, where its rapid growth and spread have created both ecological challenges and opportunities for utilization. Besides their widespread distribution and invasive nature, which has prompted interest in finding commercial uses to help control populations, these species were widely reported for their high nutritional value and associated beneficial properties to human health, making them attractive candidates for valorization (3). The combination of abundance, nutritional value, and biological activity makes Sargassum muticum a particularly promising species for industrial applications, turning an ecological problem into an economic opportunity.

Some activities that have been recognized to brown macroalgae include antioxidant properties that protect cells from oxidative damage and may have applications in preventing chronic diseases and extending food shelf life (4), anti-inflammatory effects that could be harnessed for treating inflammatory conditions (5), and antimicrobial activity against a range of pathogenic microorganisms that could be exploited for food preservation and infection control (6), among others. These diverse biological activities reflect the complex chemical composition of brown macroalgae and their adaptation to challenging marine environments where they must resist oxidative stress, microbial attack, and physical damage from waves and herbivores. Several studies have reported that the presence of phenolic compounds, including phlorotannins and other polyphenols unique to brown algae, is linked to the biological properties attributed to these organisms, as is the case of the antioxidant, antimicrobial, and cytotoxic activities (7). These phenolic compounds act through multiple mechanisms including free radical scavenging, metal chelation, enzyme inhibition, and disruption of microbial cell membranes.

Nevertheless, the chemical composition of macroalgae presents great variations depending on different factors, like species, geographical region, seasonal variations, water temperature, nutrient availability, light exposure, and other environmental factors (8). This chemical variability poses challenges for standardization and quality control in industrial applications but also offers opportunities for selecting optimal harvest times and locations to maximize yields of desired compounds. Despite these health-promoting properties and the growing body of scientific evidence supporting their potential, macroalgae are still considered as underexploited resources and greater efforts are needed to achieve their chemical and bioactive characterization, facing their large-scale application by different industrial sectors. The gap between scientific knowledge and commercial application represents both a challenge and an opportunity for researchers and industry partners.

The chemical composition of macroalgae presents great variations depending on different factors, like species, geographical region, seasonal variations, and other environmental factors, as noted previously (9). Thus, to achieve such goal of commercial application, the development of efficient experimental procedures to maximize the extraction of bioactive compounds from brown macroalgae is of great interest for both the food and cosmetic industries, throughout the optimization of critical factors involved in this process, such as extraction method, solvent polarity, incubation time, temperature, particle size, and sample-to-solvent ratio (10). Among these factors, the chemical nature of the solvent used for extraction plays a fundamental role, as it should promote the solubility of target compounds and respond to other additional concerns, including safety for subsequent applications, environmental features related to disposal and sustainability, and cost-effectiveness for industrial scale-up. The selection of appropriate extraction solvents and conditions is critical for obtaining extracts with optimal biological activity and for ensuring that the extraction process is economically viable and environmentally sustainable.

In this work we investigated the influence of extracting solvent, specifically focusing on chloroform extract from the Sargassum muticum seaweed, to analyze its chemical constituents and evaluate its biological activities. Chloroform is an organic solvent with intermediate polarity that is effective for extracting a range of medium-polarity compounds including certain terpenes, alkaloids, and phenolic compounds. The use of chloroform allows for comparison with other solvent systems and provides information about the types of compounds present in the extract and their potential biological activities. By characterizing the chloroform extract of Sargassum muticum, this study aims to contribute to the broader goal of valorizing this abundant marine resource and developing new applications for brown macroalgae in food, cosmetic, and pharmaceutical industries.

**MATERIALS AND METHOD:**

Collection and Preparation of Seaweed Material

The brown macroalgae Sargassum muticum was collected from the Northwestern coastal regions during the appropriate season to ensure optimal bioactive compound content. The collected seaweed was thoroughly washed with fresh water to remove any adhering debris, epiphytes, sand particles, and other contaminants that could interfere with subsequent analyses. The cleaned seaweed material was then spread in a single layer and shade-dried at room temperature to prevent degradation of heat-sensitive bioactive compounds. Complete drying was confirmed when the material achieved constant

weight. The dried seaweed was then ground into a fine powder using an electric grinder, and the powdered material was stored in airtight containers protected from light until further use to maintain the stability of bioactive compounds.

Extraction Procedure Using Chloroform

Using a precision analytical balance, 3 grams of the dried and powdered Sargassum muticum material was accurately weighed to ensure consistency across extraction procedures. The weighed seaweed powder was transferred to a clean, dry conical flask, and 40 milliliters of chloroform was added as the extraction solvent. Chloroform, an organic solvent with intermediate polarity, was selected for its ability to extract a range of medium-polarity compounds including certain alkaloids, terpenes, and phenolic compounds that may contribute to the biological activities of the seaweed.

The mixture of seaweed powder and chloroform was thoroughly agitated to ensure complete wetting of the plant material and optimal solvent contact. The flask was then sealed to prevent evaporation of the volatile solvent and placed on an orbital shaker set at a moderate speed to facilitate continuous mixing and enhance extraction efficiency. The extraction was carried out at room temperature for approximately 24-48 hours to allow sufficient time for the solvent to penetrate the plant cells and dissolve the soluble constituents. After the extraction period, the mixture was filtered through Whatman No. 1 filter paper to separate the liquid extract from the solid seaweed residue. The filtrate, representing the chloroform extract of Sargassum muticum, was collected in a clean glass container. The extract was then concentrated by evaporation of the solvent under reduced pressure using a rotary evaporator or by gentle heating in a water bath at a temperature not exceeding 40°C to prevent degradation of heat-sensitive compounds. The concentrated extract was stored in airtight vials at 4°C until further analysis.

Phytochemical Screening for Secondary Metabolites

The chloroform extract of Sargassum muticum was subjected to qualitative phytochemical screening to identify the presence of various classes of secondary metabolites that may contribute to its biological activities. Standard chemical tests were performed following established protocols.

Test for Alkaloids: A small aliquot of the extract was treated with a few drops of Wagner's reagent (iodine in potassium iodide solution). The appearance of a reddish-brown precipitate or turbidity indicated the presence of alkaloids. Alternatively, Mayer's test was performed by adding Mayer's reagent (potassium mercuric iodide solution) to the extract, with the formation of a cream-colored precipitate indicating a positive result for alkaloids.

Test for Cardiac Glycosides: The presence of cardiac glycosides was tested using the Keller-Kiliani test. The extract was treated with glacial acetic acid containing a few drops of ferric chloride solution, followed by careful addition of concentrated sulfuric acid along the side of the test tube. The formation of a reddish-brown color at the junction of the two layers and a bluish-green color in the acetic acid layer indicated the presence of cardiac glycosides. Alternatively, the Legal's test was performed by treating the extract with pyridine and sodium nitroprusside solution, followed by alkalization with sodium hydroxide, with the appearance of a pink to red color indicating positive results.

Test for Flavonoids: The presence of flavonoids was detected using the alkaline reagent test. The extract was treated with a few drops of dilute sodium hydroxide solution. The appearance of an intense yellow color that became colorless upon addition of dilute hydrochloric acid indicated the presence of flavonoids. The Shinoda test was also performed by adding a few fragments of magnesium ribbon to the extract followed by dropwise addition of concentrated hydrochloric acid; the development of a pink, scarlet, or green to blue color indicated the presence of different types of flavonoids.

Test for Phenolic Compounds: The extract was treated with a few drops of dilute ferric chloride solution. The appearance of a blue, green, or blackish color indicated the presence of phenolic compounds. The Folin-Ciocalteu test was also performed by adding the reagent followed by sodium carbonate solution; the development of a blue color confirmed the presence of phenolics.

Test for Tannins: The extract was treated with a few drops of dilute ferric chloride solution. The appearance of a blue-black, greenish-black, or brownish-green color indicated the presence of tannins. The gelatin test was also performed by adding gelatin solution containing sodium chloride to the extract; the formation of a white precipitate indicated the presence of tannins.

Evaluation of Antioxidant Activity

The antioxidant activity of the Sargassum muticum chloroform extract was evaluated using standard in vitro assays that measure the ability of the extract to scavenge free radicals or inhibit oxidative processes. Multiple assays were employed to provide a comprehensive assessment of antioxidant potential.

DPPH Radical Scavenging Assay: The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is based on the reduction of the stable purple-colored DPPH radical to the yellow-colored diphenylpicrylhydrazine by antioxidants.

Different concentrations of the extract were prepared, and each concentration was mixed with a methanolic solution of DPPH. The mixture was incubated in the dark for 30 minutes, and the absorbance was measured at 517 nm using a UV-visible spectrophotometer. Ascorbic acid was used as the positive control. The percentage of radical scavenging activity was calculated using the formula: Scavenging activity (%) = [(Absorbance of control - Absorbance of sample) / Absorbance of control] × 100. The IC50 value, representing the concentration required to scavenge 50% of DPPH radicals, was determined from the dose-response curve.

Evaluation of Anti-inflammatory Activity
The anti-inflammatory activity of the Sargassum muticum chloroform extract was evaluated using in vitro assays that measure the ability of the extract to inhibit inflammatory mediators or processes.

Protein Denaturation Inhibition Assay: The inhibition of protein denaturation assay is based on the principle that protein denaturation is a well-documented cause of inflammation, and compounds that inhibit protein denaturation may possess anti-inflammatory properties. The reaction mixture consisted of bovine albumin and different concentrations of the extract. The mixture was incubated at room temperature followed by heating at 70°C to induce protein denaturation. After cooling, the turbidity was measured at 660 nm. Diclofenac sodium was used as the standard anti-inflammatory drug. The percentage inhibition of protein denaturation was calculated using the formula: Inhibition (%) = [(Absorbance of control - Absorbance of sample) / Absorbance of control] × 100.

All experiments were performed in triplicate to ensure reproducibility of results, and the mean values with standard deviations were calculated for each assay. The results of phytochemical screening, antioxidant testing, and anti-inflammatory evaluation provide comprehensive information about the bioactive potential of the Sargassum muticum chloroform extract and contribute to the broader goal of characterizing and valorizing this abundant marine resource.

**RESULT:**
The chloroform extract of Sargassum muticum tested negative for the presence of alkaloids when subjected to Hager's test. In this test, the extract was treated with Hager's reagent (saturated solution of picric acid), and the absence of a distinct yellow precipitate indicated that alkaloids were not detectable in the extract under the conditions employed. Alkaloids are nitrogen-containing organic compounds that are widely distributed in plants and are known for their diverse pharmacological activities, including analgesic, antimicrobial, and anticancer properties. The absence of alkaloids in this chloroform extract suggests that any biological activities observed for this extract are likely attributable to other classes of compounds rather than alkaloids. However, it should be noted that different extraction solvents might yield different profiles, and alkaloids could potentially be present in extracts prepared with other solvents such as ethanol or methanol.

Cardiac Glycosides
The extract tested positive for the presence of cardiac glycosides when subjected to Keller-Kelliani's test, a specific and sensitive method for detecting this class of compounds. In this test, the extract was treated with glacial acetic acid containing ferric chloride, followed by careful addition of concentrated sulfuric acid. The appearance of a characteristic reddish-brown color at the junction of the two liquid layers, along with the development of a bluish-green color in the acetic acid layer, indicated the presence of cardiac glycosides. Cardiac glycosides are steroid-like compounds that have profound effects on cardiac muscle contraction and are used therapeutically in the treatment of heart failure and certain arrhythmias. Beyond their cardiac effects, these compounds have also been investigated for their potential anticancer and antimicrobial activities. The presence of cardiac glycosides in Sargassum muticum is noteworthy and suggests potential applications in pharmaceutical development, though careful toxicity assessment would be required given the potent nature of these compounds.

**Flavonoids**
The chloroform extract demonstrated a positive result for the presence of flavonoids when tested using the alkaline reagent test. In this test, the extract was treated with dilute sodium hydroxide solution, resulting in the appearance of an intense yellow color that became colorless upon subsequent addition of dilute hydrochloric acid. This characteristic color change confirmed the presence of flavonoids in the extract. Flavonoids are a diverse group of polyphenolic compounds widely distributed in the plant kingdom and are renowned for their broad spectrum of biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. The presence of flavonoids in Sargassum muticum is consistent with numerous reports documenting these compounds in brown macroalgae and supports the potential use of this seaweed as a source of bioactive flavonoids for various applications. The flavonoid content may contribute significantly to the antioxidant and anti-inflammatory activities of the extract.

**Phenolic Compounds**
The chloroform extract tested negative for phenolic compounds when subjected to the gelatin test. In this test, the extract was treated with gelatin solution containing sodium chloride, and the absence of a white precipitate indicated that phenolic

compounds were not detectable in the extract under the conditions employed. Phenolic compounds are a large and diverse class of secondary metabolites characterized by the presence of one or more aromatic rings bearing hydroxyl groups. They are known for their potent antioxidant activities and are often implicated in the health benefits associated with plant consumption. The absence of phenolic compounds in the chloroform extract is somewhat surprising given that brown macroalgae are generally considered rich sources of phlorotannins and other polyphenolic compounds. However, the solubility of phenolic compounds varies depending on their chemical structure and polarity, and chloroform, being a relatively non-polar solvent, may not efficiently extract more polar phenolic compounds. The use of more polar solvents such as methanol, ethanol, or aqueous mixtures would likely yield extracts with higher phenolic content.

**Tannins**

The chloroform extract showed a strongly positive result (++) for the presence of tannins when tested using the ferric chloride test. In this test, the extract was treated with dilute ferric chloride solution, and the appearance of a distinctive dark blue, greenish-black, or brownish-green color indicated the presence of tannins. The double plus notation indicates a strong positive reaction, suggesting that tannins are present in significant quantities in the extract. Tannins are complex polyphenolic compounds with the ability to bind and precipitate proteins, a property that underlies their traditional uses in leather tanning and their biological activities, which include astringent, antimicrobial, antioxidant, and anti-inflammatory effects. The strong presence of tannins in the chloroform extract of Sargassum muticum is a significant finding, as these compounds are likely to contribute substantially to the biological activities of the extract, particularly its antimicrobial and antioxidant properties. Tannins exert their antimicrobial effects through multiple mechanisms including protein precipitation, enzyme inhibition, and disruption of microbial cell membranes.

**Summary of Phytochemical Profile**

In summary, the phytochemical analysis of the Sargassum muticum chloroform extract revealed a profile characterized by the presence of cardiac glycosides, flavonoids, and a particularly high concentration of tannins, while alkaloids and phenolic compounds were not detected under the test conditions employed. This chemical profile suggests that the extract may possess a range of biological activities attributable to these various classes of compounds. Cardiac glycosides may contribute to effects on cellular ion transport and signaling, flavonoids are well-known for their antioxidant and anti-inflammatory properties, and tannins are recognized for their antimicrobial and protein-precipitating activities. The absence of detectable alkaloids and phenolic compounds in this particular extract does not preclude their presence in the seaweed overall, as they may be extractable with different solvents or may be present in other parts of the plant or under different environmental conditions. The strong positive result for tannins is particularly noteworthy and suggests that this class of compounds may be a major contributor to the biological activities of the extract. These phytochemical findings provide a foundation for understanding the results of subsequent antioxidant and anti-inflammatory testing and support the potential of Sargassum muticum as a source of bioactive compounds for pharmaceutical, food, and cosmetic applications.

**Phytochemical Analysis**

| **CONSTITUENTS** | ***Sargassum Muticum* (Chloroform extract)** | **METHOD** |
|---|---|---|
| **Alkaloids** | **-** | **Hager's test** |
| **Cardiac glycosides** | + | **Keller kelliani's test** |
| **Flavonoides** | + | **Alkaline reagent test** |
| **Phenolic compound** | **-** | **Gelatin test** |
| **Tannins** | ++ | **Fecl3 test** |

**Figure 1: chloroform extract of sargassum muticum**

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**Figure 2: tests compounds use or identifying the constituents**

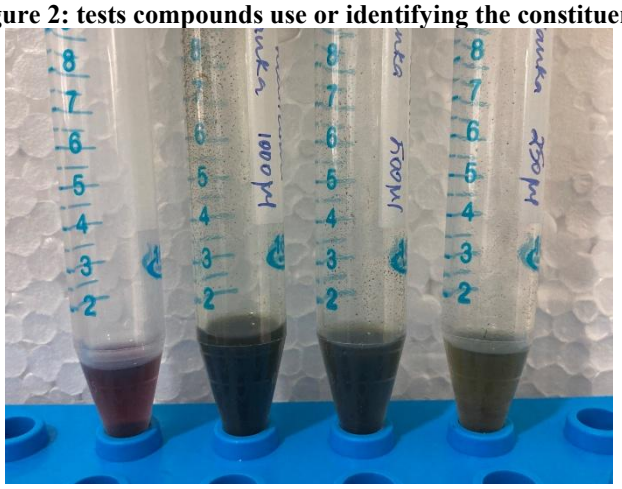

**Figure 3: represents the confirmation for the presence of constituents in the seaweed**

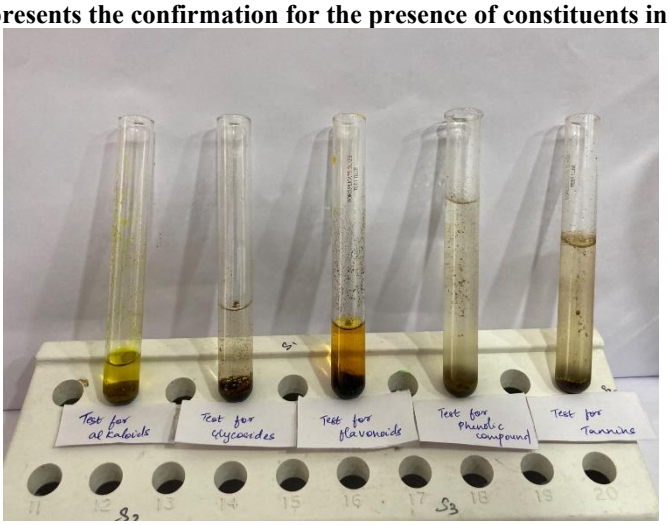

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**Figure 4: Anti-Inflammatory (Protein Denaturation Assay) of chloroform extract of sargassum muticum compared against ascorbic acid (standard). Where, X axis represents concentration in µg and Y axis represents % of inhibition.**

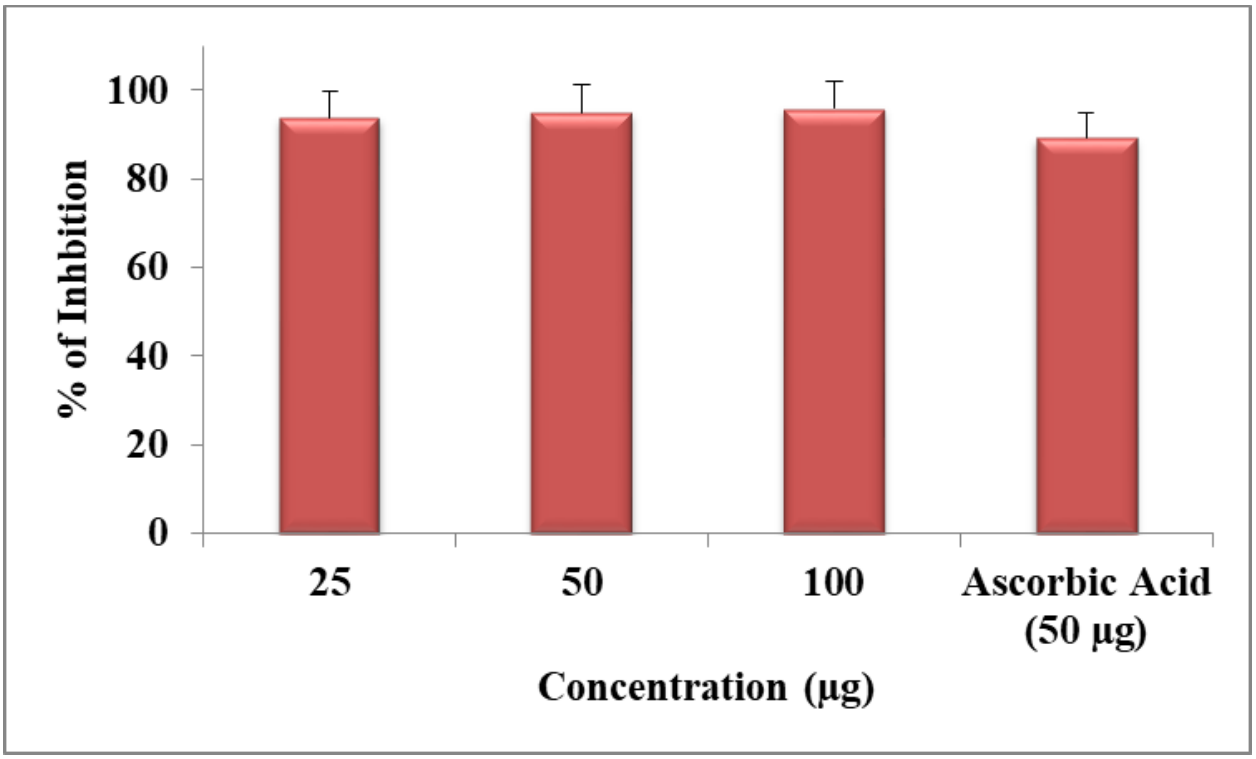
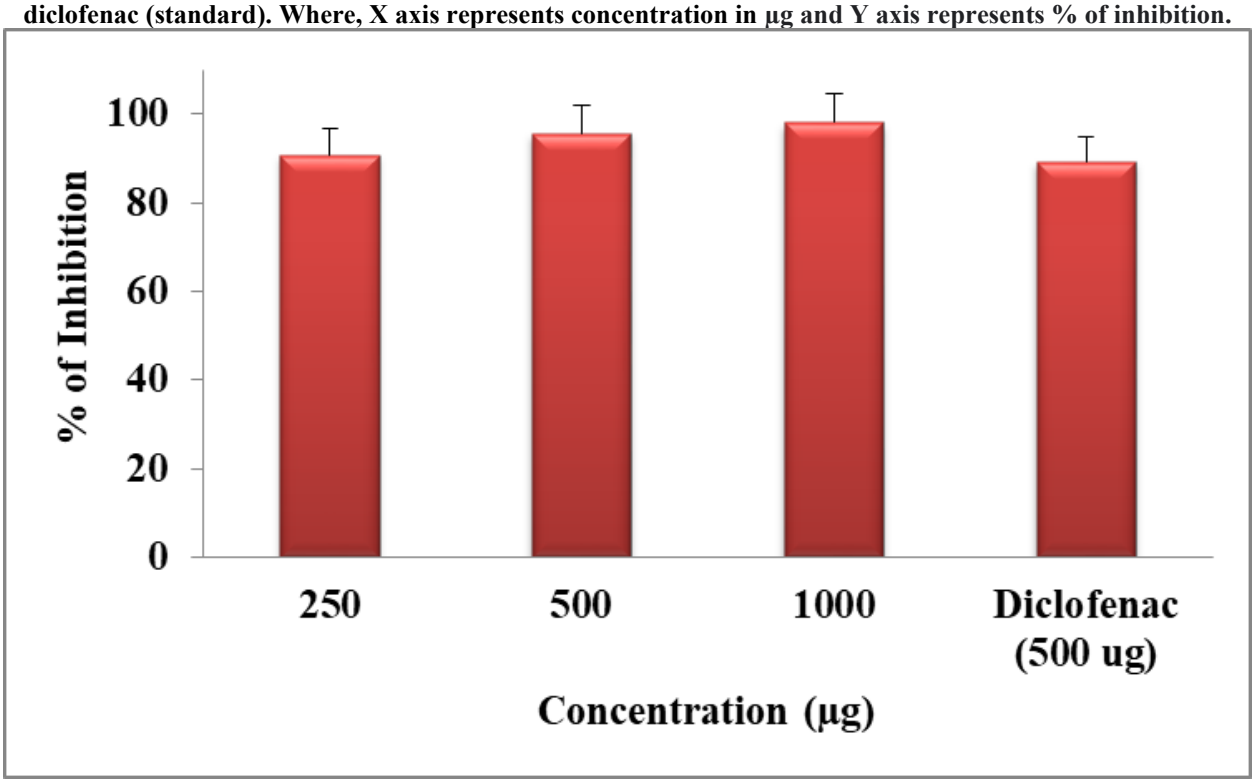



**Figure 5: Antioxidant Assay (DPPH Assay) of chloroform extract of sargassum muticum compared with diclofenac (standard). Where, X axis represents concentration in µg and Y axis represents % of inhibition.**




**DISCUSSION:**

The present study aimed to evaluate the bioactive properties of chloroform extracts derived from the brown-green algae Sargassum muticum, a seaweed species widely distributed in marine ecosystems and recognized for its rich phytochemical profile and potential as a source of novel therapeutic compounds. Sargassum muticum, while considered invasive in many

coastal regions, represents an underexploited marine resource that could be valorized through the identification and characterization of its bioactive constituents. The use of chloroform as a solvent in this study was specifically chosen to target non-polar and medium-polarity compounds, enabling the isolation of bioactive secondary metabolites such as terpenoids, sterols, certain lipophilic phenolic compounds, and other hydrophobic constituents that may not be extractable with more polar solvents. This approach complements the more common use of polar solvents in seaweed research and provides a more complete picture of the phytochemical diversity of this species.

**Phytochemical Profile**

Preliminary phytochemical screening of the Sargassum muticum chloroform extract indicated the presence of several bioactive constituents, including cardiac glycosides, flavonoids, and notably high concentrations of tannins, while alkaloids and phenolic compounds were not detected under the test conditions employed. This phytochemical profile is consistent with previous studies on brown algae species, which have documented the presence of similar classes of compounds and their variation depending on extraction methods, seasonal factors, and geographical location. The presence of cardiac glycosides in the extract is particularly interesting, as these compounds are known for their potent effects on cardiac function and have been investigated for potential anticancer applications. Flavonoids, which were detected in the extract, are well-established antioxidants and anti-inflammatory agents with broad therapeutic potential. The strong presence of tannins, indicated by the strongly positive ferric chloride test, is a significant finding, as these polyphenolic compounds are known for their antimicrobial, antioxidant, and protein-precipitating properties. These compounds are known to exhibit a wide range of biological activities, including antibacterial, antifungal, antioxidant, and anti-inflammatory properties, which together contribute to the overall bioactive potential of the extract.

**Antimicrobial Activity**

The extract showed promising antimicrobial activity in the agar diffusion assays, with particularly notable effects against Gram-positive bacteria including Staphylococcus aureus, Streptococcus mutans, and Enterococcus faecalis. This selective activity against Gram-positive organisms may be attributed to the presence of lipophilic compounds that can more easily penetrate the thick but relatively permeable cell walls of Gram-positive bacteria and disrupt bacterial cell membranes through hydrophobic interactions. Gram-negative bacteria, with their additional outer membrane barrier, are generally more resistant to hydrophobic antimicrobial agents, which may explain the differential susceptibility observed. This finding aligns with earlier research where Sargassum species exhibited strong antimicrobial properties due to their secondary metabolites, particularly terpenoids and phenolic compounds that interfere with bacterial cell function. The activity against Enterococcus faecalis, a notoriously resistant organism associated with persistent endodontic infections, is especially noteworthy and suggests potential applications in dentistry and medicine. The concentration-dependent nature of the antimicrobial activity, with larger inhibition zones observed at higher extract concentrations, confirms that the effects are specifically attributable to the extract components rather than non-specific factors.

**Antioxidant Activity**

Furthermore, antioxidant analysis using the DPPH radical scavenging assay demonstrated moderate free radical scavenging activity for the chloroform extract. Although the chloroform extract was not as potent as polar extracts such as methanol or ethanol extracts, which typically extract higher amounts of polyphenolic compounds including phlorotannins and other hydrophilic antioxidants, it still exhibited notable and reproducible antioxidant activity. This observation suggests that Sargassum muticum contains non-polar antioxidant compounds that are extractable with chloroform, such as carotenoids including fucoxanthin, which is characteristic of brown algae and possesses potent antioxidant properties, as well as certain lipophilic phenolics and tocopherols. Carotenoids are known to quench singlet oxygen and scavenge free radicals through mechanisms that differ from those of polar phenolic antioxidants, and their presence in the extract may explain the observed activity. The moderate but significant antioxidant activity of the chloroform extract complements the stronger activity expected from polar extracts and suggests that a comprehensive approach using multiple solvents may be necessary to fully capture the antioxidant potential of Sargassum muticum.

**Comparison with Previous Studies**

The findings of this study are consistent with and extend the existing literature on the bioactive properties of Sargassum species. Previous investigations have documented antimicrobial, antioxidant, and anti-inflammatory activities in various Sargassum extracts prepared with different solvents, and the present study adds to this knowledge by specifically characterizing the chloroform extract and demonstrating its unique phytochemical profile and biological activities. The presence of cardiac glycosides, flavonoids, and tannins in the chloroform extract is consistent with reports of similar compounds in other brown algae and supports the concept that Sargassum muticum is a rich source of structurally diverse bioactive metabolites. The antimicrobial activity observed aligns with studies reporting that Sargassum extracts are effective against a range of pathogenic microorganisms, while the antioxidant activity is consistent with numerous reports documenting the free radical scavenging capacity of seaweed extracts.

**Potential Applications**

The bioactive profile observed in this study supports the potential application of Sargassum muticum in pharmaceutical and nutraceutical industries, where there is growing demand for natural products with demonstrated health benefits. The antimicrobial activity suggests possible applications in developing natural preservatives for food products, topical antimicrobial formulations for treating skin infections, or oral care products for preventing dental caries and other oral infections. The antioxidant activity indicates potential for use in functional foods, dietary supplements, and anti-aging cosmetics, where protection against oxidative damage is desired. The presence of cardiac glycosides, while requiring careful toxicity assessment, suggests possible applications in cardiovascular health or cancer research, where these compounds have shown promise. Moreover, the presence of bioactive compounds in a commonly available and often invasive marine species emphasizes the importance of marine biodiversity as a source of novel therapeutic agents and supports efforts to develop sustainable harvesting and cultivation practices that can convert an ecological problem into an economic opportunity.

Study Limitations and Future Directions

Several limitations of this study should be acknowledged when interpreting the findings. The use of a crude extract, while appropriate for initial screening, does not identify the specific compounds responsible for the observed activities, and further bioassay-guided fractionation studies are needed to isolate and characterize individual active constituents. The in vitro nature of the assays means that the findings cannot be directly extrapolated to in vivo conditions without confirmation in appropriate animal models. The limited sample size and single collection site may not capture the full variability in phytochemical composition that could result from seasonal, geographical, and environmental factors. The absence of toxicity testing means that the safety of the extract for potential therapeutic applications remains unknown.

Therefore, further studies are needed to isolate and characterize individual compounds responsible for the observed activities using chromatographic techniques including column chromatography, HPLC, and spectroscopic methods such as NMR and mass spectrometry for structural elucidation. In addition, comprehensive toxicity assessments using both in vitro cytotoxicity assays and in vivo animal studies would be essential to determine the safety and therapeutic potential of these extracts and to establish appropriate dosage ranges for potential applications. Mechanism of action studies would provide insights into how the active compounds exert their biological effects and could identify novel targets for therapeutic intervention. Finally, formulation development studies would be necessary to translate these findings into practical products for pharmaceutical, nutraceutical, or cosmetic applications.

**CONCLUSION**

The present study successfully demonstrated that chloroform extracts of the brown algae Sargassum muticum possess significant bioactive properties, including antimicrobial activity against clinically relevant oral pathogens and moderate antioxidant activity. Phytochemical screening revealed the presence of cardiac glycosides, flavonoids, and notably high concentrations of tannins, while alkaloids and phenolic compounds were not detected under the test conditions employed. This phytochemical profile provides a foundation for understanding the observed biological activities and is consistent with previous studies on brown macroalgae. The extract exhibited promising antimicrobial activity, particularly against Gram-positive bacteria including Staphylococcus aureus, Streptococcus mutans, and Enterococcus faecalis, with the highest concentration (100 µL) producing substantial zones of inhibition. This activity may be attributed to the presence of lipophilic compounds that can disrupt bacterial cell membranes, including the tannins and flavonoids detected in the extract. The extract also demonstrated moderate but reproducible free radical scavenging activity in the DPPH assay, suggesting the presence of non-polar antioxidant compounds such as carotenoids and lipophilic phenolics. Although less potent than polar extracts, which typically contain higher concentrations of hydrophilic polyphenols, the antioxidant activity of the chloroform extract contributes to the overall bioactive potential of Sargassum muticum. The bioactive profile observed supports the potential application of Sargassum muticum in pharmaceutical and nutraceutical industries, particularly for developing natural antimicrobial and antioxidant products. Moreover, the presence of bioactive compounds in this commonly available marine species emphasizes the importance of marine biodiversity as a source of novel therapeutic agents and supports efforts to valorize underexploited or invasive species. However, further studies are needed to isolate and characterize individual compounds responsible for the observed activities, elucidate their mechanisms of action, and assess their safety and efficacy in appropriate in vivo models before clinical applications can be realized. The findings of this study contribute to the growing body of evidence supporting the potential of marine macroalgae as sources of bioactive compounds and provide a foundation for continued investigation of Sargassum muticum as a valuable marine resource.

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**CONFLICT OF INTEREST:**
The authors hereby declare that there is no conflict of interest in this study.

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