Applied Chemical Engineering (2025) Volume 8 Issue 2 ISSN: 2578-2010 (O)
doi: 10.59429/ace.v8i2.5600

RESEARCH ARTICLE

Phytochemical characterization and anticancer efficacy assessment of *Hormophysa cuneiformis* extract on A549 lung cancer cells

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ABSTRACT

The present study focused on the phytochemical composition of the brown seaweed *Hormophysa cuneiformis* and its anticancer activity in A549 lung cancer cells. Ethanolic and aqueous extracts were prepared and analyzed for proximate analysis and phytochemical screening, followed by evaluation of their antiproliferative effects on A549 cells using the MTT assay. The results indicated that *Hormophysa cuneiformis* extract had a highly significant ash content of 23.4 ± 2.4% and various bioactive compounds, including alkaloids, flavonoids, and terpenoids. The ethanolic extract demonstrated better antiproliferative activity than the aqueous extract, with an IC50 value of 78.475 ± 1.723 µg/mL. Treatment with 200 µg/mL ethanolic extract inhibited cancer cell growth by > 50%. These changes include shrinkage and a reduction in the cell population. The ethanolic extract showed 90.46% DPPH radical scavenging activity at 50 mg/mL, and an H2O2 scavenging effect of 86.7% at the same concentration. These results support the potential anticancer and antioxidant activities of *H. cuneiformis* and support further studies to explore therapeutic possibilities against lung cancer.

Keywords: *Hormophysa cuneiformis*; phytochemical; anticancer; A549 cells; antioxidant

ARTICLE INFO

Received: 13 January 2025
Accepted: 6 June 2025
Available online: 30 June 2025

1. Introduction

Marine algae have recently gained attention as a potential source of bioactive compounds with diverse pharmacological properties and anticancer activity[1]. Brown seaweeds are more promising concerning their rich phytochemical profile and biological effects[2,3]. The marine environment, representing 70% of Earth's surface area, is a poorly exploited resource for the discovery of new bioactive compounds[4].

Hormophysa cuneiformis belongs to the order Fucales, family Cystoseiraceae, and is a brown marine edible seaweed utilized for several years in various regions around the world because of its immense nutritional and medicinal value[5]. Brown seaweeds are among the richest sources of bioactive compounds, such as polysaccharides, including fucoidan and laminarin, and polyphenols, including phlorotannins, carotenoids, and terpenoids[6]. These biomolecules have been reported to exhibit several biological activities, including antioxidant, anti-inflammatory, and anticancer properties[7]. Over the years, it has been increasingly recognized that marine algae

are a potential source of anticancer agents. Notably, several compounds isolated from various seaweeds have shown promising results in preclinical studies. For instance, fucoxanthin is a carotenoid found in brown seaweeds. It has demonstrated anticancer activity against a panel of cancer cell lines in vitro, including lung cancer cells. Similarly, the sulfated polysaccharide fucoidan has also been reported to induce apoptosis and inhibit metastasis in various types of cancers[8].

While *Sargassum* species have been widely studied and shown to inhibit various cancer cell lines with IC50 values ranging from 50-120 μg/mL, and *Laminaria* species have demonstrated moderate antiproliferative effects with IC50 values of 95-200 μg/mL against lung cancer cells[9,10]. The *H. cuneiformis* remains relatively unexplored despite its traditional medicinal applications. Compared to other brown algae, preliminary studies suggest *H. cuneiformis* may contain higher concentrations of fucoxanthin and sulfated polysaccharides, which have demonstrated superior antioxidant properties in DPPH assays (85-95% scavenging activity at 50 μg/mL versus 70-80% for other species)[11]. Additionally, while *Fucus vesiculosus* extracts have shown moderate cytotoxicity against A549 cells, early investigations suggest *H. cuneiformis* may offer enhanced antiproliferative effects due to its unique phytochemical profile rich in terpenoids and flavonoids [12]. This study therefore, focuses on *H. cuneiformis* to determine whether its bioactive compounds provide superior anticancer and antioxidant properties compared to more extensively studied brown seaweeds

Lung cancer is one of the most common causes of cancer-related deaths worldwide, with approximately 85% of patients suffering from NSCLC[13]. Although new therapeutic modalities have been developed, such as targeted therapies and immunotherapies, the overall prognosis for lung cancer patients is still dismal, with a 5-year survival rate of only approximately 19%. The A549 cell line, established from human lung adenocarcinoma, has been used as a model for various potential therapeutic interventions against NSCLC[14]. With drug resistance and serious side effects of conventional therapies continuing to burden lung cancer treatment, the identification of novel anticancer agents with natural ingredients that are much more effective and less toxic is urgently needed. Natural products, especially marine natural products, represent a promising area of study in drug discovery because of their structural diversity and unique chemical properties represent a promising area of study in drug discovery[15].

H. cuneiformis is a potential source of anticancer compounds, considering its traditional use and the bioactive potential of brown seaweeds. However, comprehensive studies related to its phytochemical composition and anticancer effects, especially in lung cancer, have not yet been reported. Phytochemical profiling of *H. cuneiformis* is one of the key ways through which the potential health benefits of this species can be elucidated, and compounds that could be responsible for its biological activities can be identified. Moreover, these antioxidant properties are closely related to their anticancer potential, which is an important cause of cancer development and progression. Many natural compounds possessing anticancer properties also show strong antioxidant activities, supporting the mechanistic link between these two properties[16].

This study aimed to comprehensively characterize the phytochemical composition of *H. cuneiformis* extracts, evaluate their antiproliferative effects in A549 lung cancer cells, and assess their antioxidant properties. We sought to characterize the extract's chemical profile through proximate and phytochemical analysis, determine IC50 values against A549 lung cancer cells, evaluate antioxidant capacity via multiple assays, and examine morphological changes in treated cells. The novelty of this research lies in providing the first integrated investigation combining phytochemical profiling, antioxidant capacity assessment, and direct anticancer evaluation of *H. cuneiformis* extract against A549 lung cancer cells. Through this approach, we analyze the correlations between the aqueous and ethanolicextracts' and their biological activities, providing insights into potential therapeutic mechanisms. The results obtained facilitate the development of innovative marine-derived anticancer agents for lung cancer treatment and establish a foundation for future investigations into specific bioactive compounds.

2. Materials and methods

2.1. Sample collection and extract preparation

Fresh *H. cuneiformis* samples were collected from the coastal region of Tamil Nadu and thoroughly rinsed with distilled water to remove contaminants. The samples were then shade-dried to preserve their bioactive compounds and finely ground using a mortar and pestle. Two types of extracts were prepared: an aqueous extract, in which 5 g of the powdered seaweed was mixed with 50 mL of distilled water, stirred thoroughly and left to stand for 24 h with shaking before being filtered, and an ethanolic extract, in which 5 g of the powdered seaweed was mixed with 25 mL of ethanol, and macerated for 24 h with shaking, followed by filtration to obtain the extract.

2.2. Proximate analysis

Proximate analysis was conducted to determine the moisture, ash, crude protein, crude fiber, and crude lipid content of the extracts. The moisture content was assessed by drying 5 g of each extract at 105°C to a constant weight. The ash content was determined by incinerating 5 g of each extract and measuring the resulting ash weight. The Kjeldahl method was used to measure crude protein content, whereas crude fiber content was calculated from the defatted residue obtained from protein determination. The crude lipid content was extracted using Soxhlet extraction.

2.3. Phytochemical screening

Preliminary phytochemical screening of the extracts was conducted using standard methods described by Harborne (1973) and Trease and Evans (1989). The following tests were performed: Mayer's test for alkaloids; foam test for saponins; ferric chloride test for tannins; Keller-Killani test for cardiac glycosides; alkaline reagent test for flavonoids; lead acetate test for phenols; chloroform and sulfuric acid test for steroids; Salkowski test for terpenoids; alcoholic potassium hydroxide test for quinones and ninhydrin test for proteins.

2.4. Cell culture and antiproliferative assay

A549 human lung cancer cells were obtained from NCCS and cultured in RPMI-1640 growth medium supplemented with fetal bovine serum and antibiotics. Cells were maintained at 37°C in a humidified incubator with 5% CO2. For the antiproliferative assay, A549 cells were seeded in 96-well plates at a density of 5,000 cells/well and allowed to attach. Various concentrations of *H. cuneiformis* extract (10, 25, 50, 100, 150, 200, 250, and 300 µg/mL) were added to the wells, including control wells with only cell culture medium and solvent. After 24, 48, and 72 h of incubation, cell viability was assessed using the MTT assay. The percentage of viable cells was calculated by comparing the treated and control wells.

2.5. Antioxidant assays

2.5.1. DPPH radical scavenging assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was performed according to the method described by Blois (1958), with slight modifications. Briefly, 200 µL of 20 µM DPPH solution in methanol was added to different concentrations (10, 20, 30, 40, and 50 µg/mL) of *H. cuneiformis* extract in a 96-well plate. The mixture was then incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using a spectrophotometer. The percentage DPPH scavenging activity was calculated using the following formula:

$$\% \text{ DPPH Scavenging Activity} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where A control is the absorbance of the control (DPPH solution without the sample) and A sample is the absorbance of the sample (DPPH solution with the extract).

2.5.2. Hydrogen peroxide scavenging assay

The hydrogen peroxide (H_2O_2) scavenging assay was conducted according to the method of Ruch et al. (1989). A 40 mM H_2O_2 solution was prepared in phosphate buffer (pH 7.4). Different concentrations (10, 20, 30, 40, and 50 µg/mL) of *H. cuneiformis* extracts were added to 0.6 mL of the H_2O_2 solution. After 10 min of incubation at room temperature, absorbance was measured at 230 nm. The percentage of H_2O_2 scavenging activity was calculated using the following formula:

$$\% \ H_2O_2 \ Scavenging = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where A control is the absorbance of the control (H_2O_2 solution without the sample) and A sample is the absorbance of the sample (H_2O_2 solution with the extract).

2.6. Statistical analysis

All experiments were performed in triplicate, and the results are expressed as mean ± standard deviation. Statistical analyses were performed using the GraphPad Prism software. One-way ANOVA followed by Tukey's post hoc test was used to determine significant differences between the groups. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Proximate analysis

The proximate composition of *H. cuneiformis* indicates its nutritional potential. The fresh seaweed sample exhibited a moisture content of 63.62 ± 5.2%, highlighting its significant water content, which is typical for marine algae. The ash content of 23.4 ± 2.4% reflects a high mineral content, a key indicator of its potential as a natural source of essential micronutrients. Carbohydrates were the most abundant macronutrient, comprising 23.7 ± 1.6%, indicating their energy-rich nature. On the other hand, the protein content was relatively low (2.4 ± 1.2%) but sufficient to provide supplementary nutrition. The lipid content was minimal at 0.9 ± 0.1%, consistent with the low-fat nature of seaweed.

Table 1. Proximate composition of *H. cuneiformis.*

Content	***H. cuneiformis* (%)**
Moisture	63.62±5.2
Ash	23.4±2.4
Carbohydrate	23.7±1.6
Protein	2.4±1.2
Lipid	0.9±0.1

3.2. Phytochemical screening

Qualitative phytochemical analysis of *H. cuneiformis* ethanolic extract revealed the presence of various bioactive compounds, suggesting its therapeutic potential (**Table 2**). The extract tested positive for alkaloids, flavonoids, glycosides, phenols, terpenoids, steroids, and quinones, which are known for their pharmacological properties. Notably, compounds like flavonoids and phenols are associated with antioxidant and anticancer activities. The absence of tannins, saponins, and proteins suggests a distinct phytochemical profile that could contribute to its specificity in biological applications.

Table 2. Qualitative phytochemical analysis of *H. cuneiformis* ethanolic extract.

Phytochemical	***H. cuneiformis***
Alkaloids	+
Flavonoids	+
Tannins	–
Glycosides	+
Phenols	+
Saponins	–
Terpenoids	+
Steroids	+
Proteins	–
Quinones	+

3.3. Antiproliferative activity

The MTT assay results demonstrated that *H. cuneiformis* extracts exhibited dose-dependent anti-proliferative effects on A549 lung cancer cells (**Figure 1**). The ethanolic extract showed superior antiproliferative activity compared to that of the aqueous extract. At a concentration of 200 µg/mL, the ethanolic extract inhibited cancer cell growth by more than 50%, surpassing the control. The IC50 of the ethanolic extract against A549 cells was 78.475 ± 1.723 µg/mL.

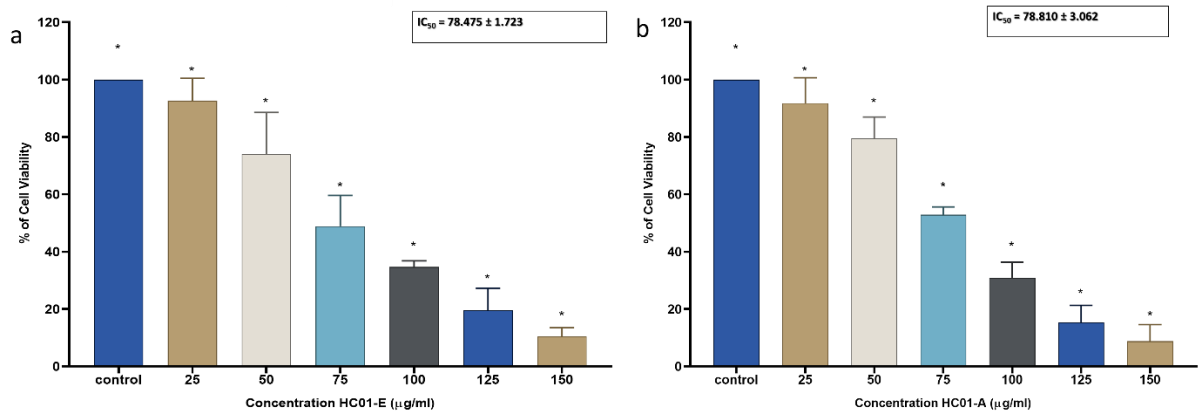

Figure 1. Antiproliferative effects of *H. cuneiformis* aqueous and ethanolic extract on A549 lung cancer cel ls assessed using the MTT assay. Data are shown as mean ± SD (n = 3). '*' denotes statistical significance ($p < 0.05$) between the control and drug treatment groups. a) Ethanolic extract treated group b) Aqueous extract tr eated group

3.4. Morphological changes

Microscopic analysis of A549 cells treated with both the aqueous (HC01-A) and ethanolic (HC01-E) extracts of *H. cuneiformis* revealed notable morphological changes indicative of cytotoxic effects (**Figure 2**). Cells treated with the ethanolic extract exhibited pronounced alterations, including cell shrinkage, reduced cell density, and a higher number of detached cells, when compared to untreated controls. These changes are significant to apoptosis and suggest a more diverse cytotoxic response induced by the ethanolic extract (HC01-E). In contrast, cells treated with the aqueous extract also exhibited morphological changes, but these were less severe than those induced by the ethanolic extract. The superior impact of the ethanolic extract aligns with its

lower IC50 value, reinforcing its greater effectiveness in inducing cancer cell death through cytotoxic mechanisms.

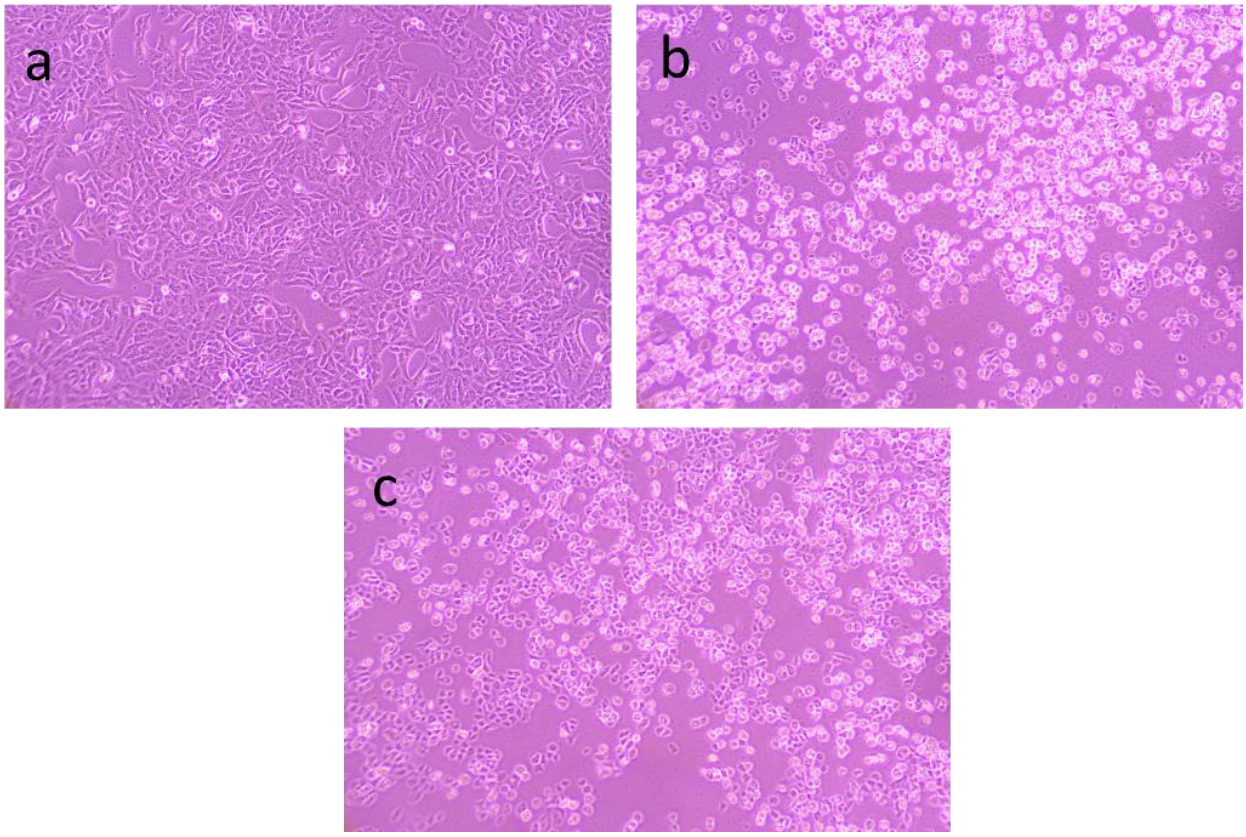

Figure 2. Antiproliferative effects of the *H. cuneiformis* against lung cancer cell line (A549) a) Control b) ethanolic (HC01-E) extracts treated c) aqueous (HC01-A) treated

3.5. Antioxidant activity

3.5.1. DPPH radical scavenging activity

Both the aqueous and ethanolic extracts of *H. cuneiformis* demonstrated dose-dependent DPPH radical scavenging activity, indicating their antioxidant potential. At a concentration of 50 µg/mL, the ethanolic extract showed a maximum DPPH scavenging activity of 90.46%, significantly higher than the aqueous extrac (Figure 3). These results are notably higher than those reported by Gunathilake et al. (2024) for *Ecklonia radiata* extracts, which showed approximately 75-80% DPPH scavenging at similar concentrations[17]. Similarly, our findings exceed the DPPH scavenging activity reported by Nova et al. (2024) for *Fucus vesiculosus* (70-75%)[18]. This suggests that the ethanolic extract exhibits a stronger free-radical neutralising capacity, likely due to its higher concentration of bioactive compounds, such as flavonoids and phenols. The aqueous extract, while effective, displayed comparatively lower DPPH scavenging activity, suggesting a reduced antioxidant potential.

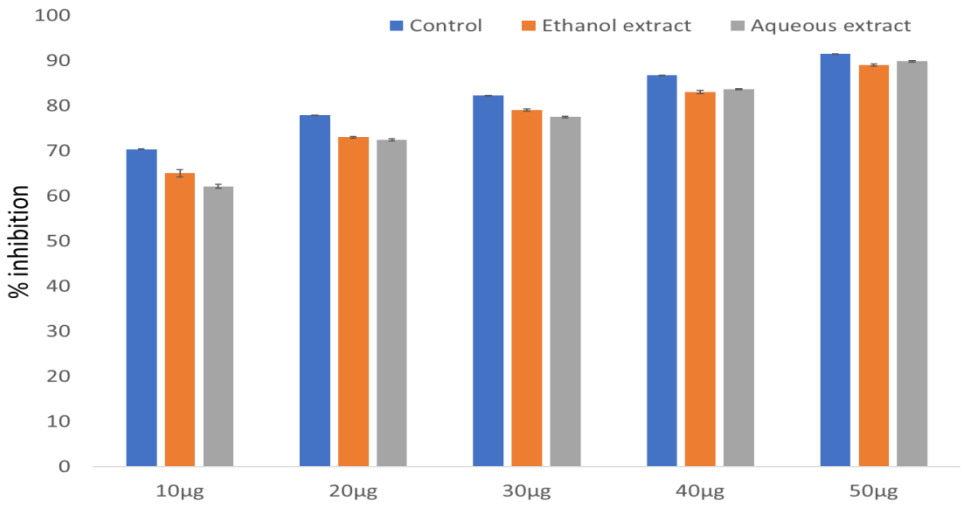

Figure 3. DPPH radical scavenging activity of *H.* cuneiformis (aqueous and ethanolic extract).

3.5.2. Hydrogen peroxide scavenging activity (H_2O_2)

The H_2O_2 scavenging activity of both extracts followed a similar dose-dependent trend, with the ethanolic extract again showing superior performance. At a concentration of 50 µg/mL, the ethanolic extract exhibited an H_2O_2 scavenging activity of 86.7%, surpassing the aqueous extract (Figure 4). The aqueous extract, though effective, demonstrated slightly lower activity levels across all concentrations, further supporting the higher efficacy of the ethanolic extract in scavenging reactive oxygen species (ROS).

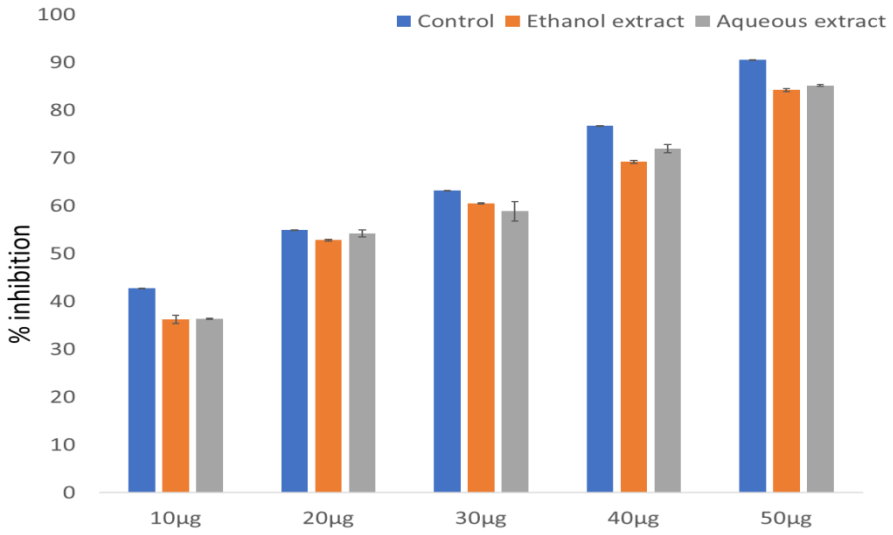

Figure 4. Hydrogen peroxide scavenging activity of *H.* cuneiformis (aqueous and ethanolic extract).

4. Discussion

This study provides comprehensive insights into the phytochemical composition, anticancer potential, and antioxidant properties of *H. cuneiformis* in A549 lung cancer cells. Proximate analysis revealed that *H. cuneiformis* is rich in minerals, as evidenced by its high ash content (23.4 ± 2.4%) and notable carbohydrates (9.68 ± 1.5%). The relatively low lipid content (0.9 ± 0.1%) is consistent with previous reports on brown seaweeds. The protein content (2.4 ± 1.2%), while modest, suggests that *H. cuneiformis* could serve as a complementary protein source in dietary applications [19]. Phytochemical screening of the ethanolic extract revealed the presence of various bioactive compounds, including alkaloids, flavonoids, glycosides, phenols,

terpenoids, steroids, and quinones. These findings are in line with those of previous studies on brown seaweeds, which reported a diverse array of secondary metabolites[20].

The presence of these bioactive compounds not only contributes to the observed anticancer activity but also confers significant antioxidant properties to *H. cuneiformis* extracts[21]. Our study employed multiple assays to evaluate the antioxidant capacity of the extracts, including DPPH radical scavenging andhydrogen peroxide scavenging assays. These diverse methodologies provide a comprehensive assessment of antioxidant potential, as each assay targets different aspects of antioxidant activity[22]. The DPPH radical scavenging assay revealed that both the ethanolic and aqueous extracts of *H. cuneiformis* exhibited dose-dependent free radical-scavenging activity[23]. However, the ethanolic extract showed superior activity, with a maximum inhibition of 90.46 % at a concentration of 50 mg/mL. This finding aligns with previous studies on brown seaweeds, which reported potent DPPH radical scavenging activities due to the presence of polyphenolic compounds[24]. The enhanced activity of the ethanolic extract suggested that the solvent plays a crucial role in extracting the most potent antioxidant compounds from *H. cuneiformis*[25]

Similarly, the hydrogen peroxide scavenging assay showed that the ethanolic extract of *H. cuneiformis* possessed strong H2O2 scavenging ability, with a maximum inhibition of 86.7% at the highest tested concentration. This activity is particularly relevant in the context of cancer prevention, as hydrogen peroxide is a ROS that contributes to oxidative stress and DNA damage, potentially leading to carcinogenesis.

The observed antioxidant properties of *H. cuneiformis* extracts likely contribute to their anticancer potential through multiple mechanisms. Oxidative stress plays a crucial role in the initiation, promotion, and progression of cancers. By neutralizing free radicals and reducing oxidative damage, antioxidant compounds in *H. cuneiformis* may help prevent DNA mutations, inhibit pro-inflammatory signaling pathways, and modulate cell cycle regulation, all of which are important factors in cancer development and progression[29]. The anti-proliferative assay results demonstrated that the ethanolic extract of *H. cuneiformis* exerted potent growth inhibitory effects on A549 lung cancer cells in a dose-dependent manner, with an IC50 value of 78.475 ± 1.723 µg/mL. This level of activity is promising, especially considering that it is a crude extract rather than an isolated compound. The superior activity of the ethanolic extract compared to the aqueous extract is consistent with the trends observed in antioxidant assays, suggesting that most bioactive compounds are more efficiently extracted by ethanol[30].

The observed morphological changes in A549 cells treated with *H. cuneiformis* extracts, including cell shrinkage and detachment, were indicative of apoptosis induction. These findings are consistent with previous reports on the apoptosis-inducing properties of brown seaweed extracts in various cancer cell lines[31]. The ability to induce apoptosis is a crucial characteristic of effective anticancer agents, as it represents a targeted approach for eliminating cancer cells while minimizing damage to healthy tissues.

5. Conclusion

This study demonstrated that the ethanolic extract of the seaweed *H. cuneiformis* possesses potent antiproliferative and antioxidant activities, which is due to its extensive and diverse phytochemical composition. . In the proximate analysis, high content of moisture and ash indicated the presence of a high content of minerals, whereas carbohydrates represented the most abundant macronutrient, followed by crude proteins and crude lipids. Phytochemical screening using qualitative methods indicated the presence of various bioactive compounds such as alkaloids, flavonoids, glycosides, phenols, terpenoids, steroids, and quinones in the ethanolic extract. The antiproliferative assay revealed that ethanolic extracts exhibited a dose-dependent inhibitory action against A549 lung cancer cells and had a higher IC50 value of 78.475 ± 1.723 µg/mL. Moreover, ethanolic extract showed higher DPPH radical scavenging and hydrogen peroxide scavenging activities than those of the aqueous extract. Therefore, *H. cuneiformis* may serve as a good source of bioactive

compounds for therapeutic purposes, particularly lung cancer treatment. Further studies are needed to identify and characterize specific bioactive compounds responsible for the observed activities, investigate the underlying mechanism of action, evaluate *in vivo* efficacy and safety, and study the synergistic effect of conventional treatments against other types of cancers. .

Conflict of interest

The authors declare no conflict of interest

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