



Comparison of Physico-Chemical and Antioxidant Activity of *Sargassum henslowianum* J. Agardh by Different Extraction Methods

N.T. VIET^{1,2,*}, L.T. LE³, N.N. QUY³, N.M. TIEN³, T.C. THAI⁴ and T.K.N. TRAN^{1,2,*}

¹Institute of Applied Technology and Sustainable Development, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam

²NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam

³Faculty of Pharmacy, Nguyen Tat Thanh University, Ho Chi Minh City 700000, Vietnam

⁴Research and Development Institute of Advanced Agribiology, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam

*Corresponding authors: E-mail: ntviet@ntt.edu.vn; ttkngan@ntt.edu.vn

Received: 26 February 2025;

Accepted: 16 June 2025;

Published online: 30 June 2025;

AJC-22048

Sargassum henslowianum is a seaweed that shows promise for extracting valuable compounds used in various fields. This study focused on the extraction of nutrients from *S. henslowianum* to identify biologically active compounds and assess acetylcholinesterase inhibitory activity in different extracts. The seaweed was extracted using water and EDTA and the extracts were analyzed for active compounds and AChE inhibitory activity using Ellman's spectrophotometric method. The results indicated that the water extract had higher levels of polyphenols, flavonoids and antioxidant activity, with IC₅₀ values of 1977.54 µg mL⁻¹ (DPPH) and 68.82 µg mL⁻¹ (ABTS). The water extract also showed a MIC of 2.813 mg mL⁻¹ against *E. coli* and *S. aureus*, while the EDTA extract had a MIC of 5.625 mg mL⁻¹. This study highlights the potential of seaweed as a source of acetylcholinesterase enzyme inhibitors. Extracting high-quality compounds from seaweed could lead to new opportunities in the pharmaceutical and cosmetic industries, supporting sustainable development in the marine sector.

Keywords: *Sargassum henslowianum*, Physico-chemical, Antioxidant, Antibacterial, Extraction.

INTRODUCTION

Sargassum henslowianum is a species of brown algae in the genus *Sargassum*. It is found in marine environments and is known for its potential health benefits and applications in various fields, including food, pharmaceuticals and cosmetics [1-3]. *Sargassum* has long, branched stems with a brown or dark brown colour. They have air sacs that help maintain buoyancy. *Sargassum henslowianum* is commonly found in temperate and tropical regions of the oceans, especially in the Pacific and Indian Oceans, in areas such as Southeast Asia, including Vietnam, Thailand, Indonesia and the Philippines. *Sargassum* plays an important role in marine ecosystems. It forms dense algal mats, providing habitat and food for many marine species. These algal mats are also capable of producing oxygen through photosynthesis, maintaining atmospheric balance and supporting marine life.

S. henslowianum contains many important pharmacologically active biocompounds, including polysaccharides, polyphenols, flavonoids, sterols and antioxidants [4-8]. These com-

pounds provide pharmacological properties such as anticancer, antibacterial, anti-inflammatory, antioxidant and immune-enhancing properties [9-11]. Alginate, a polysaccharide found in *Sargassum henslowianum*, has gel-forming properties and is widely used in the pharmaceutical industry.

Phlorotannin, a polyphenol typical of brown algae, not only has strong antioxidant effects but also has the ability to inhibit AChE. Some sterols in seaweed have also been found to have inhibitory activity against this enzyme [2]. Studies on the ability of seaweed extracts to inhibit the enzyme acetylcholinesterase are often aimed at combating Alzheimer's disease and other neurological disorders [12]. Extracts from seaweed may contain compounds such as polyphenols, flavonoids and other bioactive compounds that may have inhibitory effects on the enzyme acetylcholinesterase. The choice of the appropriate extraction method (reflux, ultrasound, enzymatic, microwave assisted, *etc.* or using solvents) depends on the type of compound to be extracted, the intended use and economic and technical factors [13,14]. Each method has its advantages and disadvantages and combining methods can optimize the extrac-

tion process. Studies have shown that seaweed extracts can inhibit the growth of a wide range of bacteria, including both Gram-positive and Gram-negative bacteria. Methanol extracts of two brown seaweeds, *Sargassum latifolium* and *Sargassum platycarpum*, have stronger activity against Gram-positive bacteria than Gram-negative bacteria. However, the acetone extract of *S. latifolium* exhibited the highest inhibitory activity against *Salmonella* sp. [15]. Lim & Aida [16] evaluated the antioxidant activities of extracts from *S. serratifolium* using various solvents and identified the major antioxidant components. Ethyl acetate, ethanol and methanol extracts showed strong activity in DPPH and ABTS free radical scavenging assays.

The study of seaweeds not only opens up opportunities in the medical and pharmaceutical fields but is also an attractive research area for scientists and experts in marine biology, chemistry and biotechnology. Therefore, the study was conducted to verify the presence of several bioactive compounds and acetylcholinesterase inhibitory activity of different extracts from seaweeds. Successful research will help increase the value of using high-value aquatic algae and contribute to developing the potential of aquatic resources in Vietnam.

EXPERIMENTAL

S. henslowianum sample (Fig. 1) was collected from the South China sea at Khanh Hoa province in Vietnam during the summer season when seaweed growth is at its peak. Upon arrival at the laboratory, the seaweed was washed with clean water to remove sand, mud and other impurities. To preserve the seaweed after collection, it was dried at 45 °C to maintain its quality and nutritional content.

Extraction process: *S. henslowianum* was cleaned and analyzed for moisture and ash content. It was then dried at a temperature below 50 °C until completely dry and the moisture content was determined. The sample was crushed or chopped to increase the surface area, making the extraction process easier. *S. henslowianum* was stirred with ethanol for 24 h at approximately 30 °C to remove colorants and small molecular compounds [17]. The mixture was then centrifuged for 10 min at 6000 rpm and the residue was collected and dried at room temperature.

Method A: *S. henslowianum* (50 g) was stirred in distilled water at 65 °C for 1 h before being centrifuged at 6000 rpm

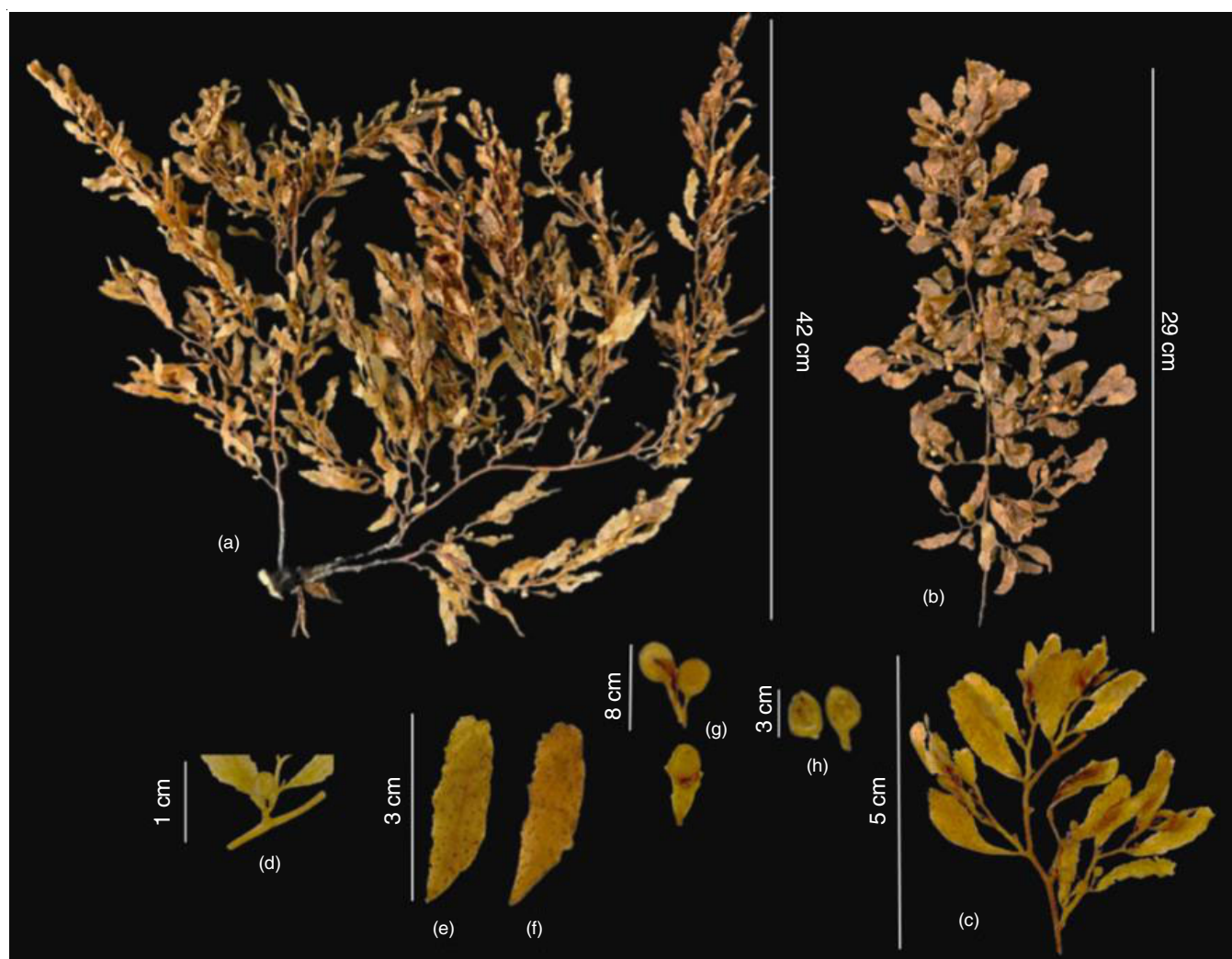


Fig. 1. Image of *Sargassum henslowianum* J. Agardh. Note: (a) whole plant; (b) long branches; (c) branches; (d) float-bearing stem; (e) anterior leaves; (f) posterior leaves; (g) fruit-like part (spherical float); (h) cross section of float

for 10 min. The supernatant was then mixed with 1% CaCl_2 and left overnight at 4 °C. The precipitate was recovered by centrifugation at 6000 rpm for 10 min and the residue was dried at 45 °C.

Method B: *S. henslowianum* powder (50 g) was soaked in 0.5% EDTA (1:30, w/v) and stirred in a water bath for 3 h at 70 °C. The solution was then cooled to room temperature and filtered to remove the residue by centrifugation at 6000 rpm for 10 min. The residue was recovered and dried at 45 °C.

Phytochemicals identification: The identification of compounds was carried out using different reagent methods to identify the presence of chemical components in the extract. Specifically, phenolics (ferric chloride reagent), alkaloids (Mayer reagent), saponins (foaming test), tannins (ferric chloride reagent), flavonoids (aluminum chloride reagent) and terpenoids (Salkowski reagent). The results of the chemical reactions were recorded by the symbol '+' (positive) indicating the presence of the compound, whereas the sign '-' (negative) indicates the absence of the compound.

Determination of total polyphenol content: The Folin-Ciocalteu method was used to determine the polyphenol content in the sample. Prepared the standard gallic acid solutions with different concentrations to construct a standard curve. Added 0.1 mL of sample or gallic acid solution to the test tube, then added 2.5 mL of Folin-Ciocalteu reagent (10%). After 5 min, add 0.4 mL of 7.5% Na_2CO_3 solution to the test tube. Shake well and leave at room temperature (about 20-25 °C) for 1 h to complete the reaction. Measured the absorbance of the solution at 765 nm wavelength using a UV-vis spectrophotometer. Repeated the measurement three times for each sample and standard solution. From there, determine the standard curve equation ($y = 0.0078x + 0.1377$), where y is the optical absorbance and x is the concentration of gallic acid, $R^2 = 0.09988$. Use the standard curve equation to calculate the polyphenol content in the sample, expressed as GAE (mg GAE g^{-1} DW).

Determination of total flavonoid content: The spectrophotometric technique with AlCl_3 allows one to ascertain the flavonoid content of the sample. To construct a standard curve with quercetin, a series of different concentrations were prepared. The sample or quercetin standard solution (5.0 mL) was added to solution containing 0.1 mL of 1 M CH_3COOK and 0.1 mL of 10% AlCl_3 were added to test tube and the reaction mixture was allowed to stand for 30 min at room temperature. The absorbance of the solution was measured at 415 nm using a UV-vis spectrophotometer. The experiment was repeated three times. The equation of the standard curve ($y = 0.0056x - 0.0063$), where y is the absorbance and x is the concentration of quercetin, $R^2 = 0.998$, was determined. Use the equation of the standard curve to calculate the flavonoid content of the sample, expressed as QE (mg QE g^{-1} DW).

DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging ability: The extract samples were dissolved in ethanol at a concentration of 5000 $\mu\text{g mL}^{-1}$. The concentrations tested were 2500, 1250, 750 and 350 $\mu\text{g mL}^{-1}$. Vitamin C was used as a control. A 6×10^{-4} M DPPH reagent solution was prepared. The reaction was carried out by mixing 0.5 mL of each sample concentration with 1.5 mL of the DPPH solution.

The mixture was allowed to stabilize for 30 min in dark. The absorbance of the reaction solution was measured at 517 nm using a UV-vis spectrophotometer. Free DPPH has a deep purple colour and will lose its colour (turning pale yellow or colourless) when reduced by antioxidants. The percentage of free radical scavenging of the sample was calculated using the following formula:

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

where A_{control} is the absorbance of the DPPH solution without sample and A_{sample} is the absorbance of the DPPH solution after reacting with the sample.

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate)) free radical scavenging ability: The ABTS solution was diluted with water to a concentration of 7 mM. It was then activated by mixing with 2.6 mM $\text{K}_2\text{S}_2\text{O}_8$ solution. The mixture was allowed to react in the dark at room temperature for 24 h to form the ABTS^+ cation. The ABTS^+ solution was further diluted to approximately 1.1 ± 0.02 with an absorbance at 734 nm. A mixture of 1.5 mL of ABTS^+ solution with 62.5-1000 $\mu\text{g L}^{-1}$ of sample solution was prepared. The reaction solution was left at room temperature in the dark for about 30 min. The absorbance of the reaction solution was measured at 734 nm using a UV-vis spectrophotometer. ABTS^+ has a deep blue colour and will lose its colour (turning lighter) when reduced by antioxidants. The ABTS free radical scavenging ability (%) of the sample was calculated using the formula:

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

where A_{control} is the absorbance of the ABTS solution without the sample and A_{sample} is the absorbance of the ABTS solution after reacting with the sample.

Antibacterial activity: To evaluate the antibacterial activity of the extract, the disc diffusion method was used. The Mueller-Hinton medium was used to culture Gram-negative *Escherichia coli* ATCC 8739 and Gram-positive *Staphylococcus aureus* ATCC 6538. The bacterial solution was prepared by diluting the bacterial sample in saline solution to achieve a density of 10^6 - 10^7 CFU mL^{-1} . Chloramphenicol (1 mg mL^{-1}) was used as a positive control. The petri dishes were placed in an incubator at 37 °C for 24 h. After incubation, the diameter of the inhibition zone was observed and measured. Minimum inhibitory concentration (MIC) was determined based on the colour change of the resazurin reagent. The extract solution was diluted in multiples of two in 96-well microplates. Each well contained 150 μL of bacterial medium and 50 μL of the diluted sample in medium. After incubation, 20 μL of 0.01% resazurin solution was added to each well and the MIC value was recorded.

Acetylcholinesterase (AChE) inhibitory activity: The AChE enzyme activity was determined based on the absorbance of sample at 412 nm. Measured the optical density (OD) at 412 nm immediately after adding ATChI and continue to measure for a certain period of time (usually 5 min). The reaction mixture consists of 700 μL of sodium phosphate buffer (pH 8.0),

100 μL of test solutions at different concentrations and 100 μL of 0.5 IU mL^{-1} AChE enzyme solution. Mixed well and incubated for 15 min at 25 $^{\circ}\text{C}$. Then, added 50 μL of 2.5 mM DTNB and 50 μL of 2.5 mM ACTI, mixed well and then continued to incubate the mixture for 10 min at 25 $^{\circ}\text{C}$. Then, measured the solution for absorbance at 412 nm. All experiments were repeated three times. Berberine chloride dissolved in 10% dimethyl sulfoxide was used as a positive control.

RESULTS AND DISCUSSION

The identification features described overview of the structure and morphology of *S. henslowianum*. The height and dense structure suggest its adaptation to dense habitats, while its long branches and branches help in the even distribution of leaves and floats, optimizing photosynthesis and buoyancy. The leaves have distinct anterior and posterior surfaces, with the glossier and darker green anterior surface aiding in light absorption. The spherical and small fruit-like part plays a crucial role in the reproduction and dispersal of plant. The structure of float, with its air-containing ability, helps the plant stay buoyant on the water surface and supports photosynthesis and gas exchange. This information not only helps in the identification and description of plant but also serves as a scientific foundation for further research on the ecology and physiology of this plant species.

Qualitative identification of groups of substances by chemical methods: The results revealed that the *Sargassum* seaweed comprised alkaloids, saponins, flavonoids and terpenoids, with no tannins identified (Table-1). Alkaloids were identified through a reaction with Mayer's reagent, resulting in a reddish-brown precipitate. Alkaloids are soluble or amorphous compounds, often bitter in taste and show promise for pharmaceutical development, particularly in malaria treatment [18]. Saponins, when vigorously shaken with water, produce stable foam, possess antioxidant properties and impact neuronal activity through the GABA system, reducing adrenaline and noradrenaline levels in the brain [19]. Terpenoids, which exhibit a brick red colour when reacted with chloroform and concentrated H_2SO_4 , are derived from isoprene units and contri-

Parameters	Results	Phenomenon
Alkaloid	+	Reddish-brown precipitate
Saponin	+	Durable foam
Flavonoid	+	Yellow precipitate
Terpenoid	+	Brick-red colour
Phenolic	+	Blue precipitate
Tannin	–	Blue-black precipitate

bute to plants aroma and colour. Flavonoids, which yield a yellow precipitate when reacted with lead(II) acetate (10%), are recognized for their various health benefits, including antioxidant, anti-inflammatory and anti-cancer properties [20]. These findings offer valuable insights into the presence of phytochemical constituents in seaweed and their potential applications in the pharmaceutical and medical fields.

Total polyphenol and flavonoid content: Polyphenols and flavonoids are recognized for their potent antioxidant properties, which play a crucial role in preventing cardiovascular diseases, inflammation and reducing the risk of cancer development [21]. Fig. 2 illustrates that the extraction of polyphenols using water as the solvent resulted in a content of 77.740 mg GAE g^{-1} extract, which is double the amount obtained with EDTA. Similarly, the flavonoid content extracted with water was 70.7239 mg QE g^{-1} extract, 2.5 times higher than with EDTA. The superior efficiency of water extraction in recovering polyphenols and flavonoids compared to EDTA can be attributed to various chemical and biological factors related to the properties of these compounds and their interactions with different solvents. Polyphenols and flavonoids are polar compounds with high solubility in water, which enhances their extraction efficiency from plant sources. In contrast, EDTA, a chemical chelating agent, may not be as effective as water due to its less polar nature and its primary function of chelating metal ions rather than extracting polar compounds. Furthermore, Wang & Weller [22] demonstrated that hot water extraction significantly enhances the efficiency of extracting polar compounds like polyphenols compared to non-polar solvents.

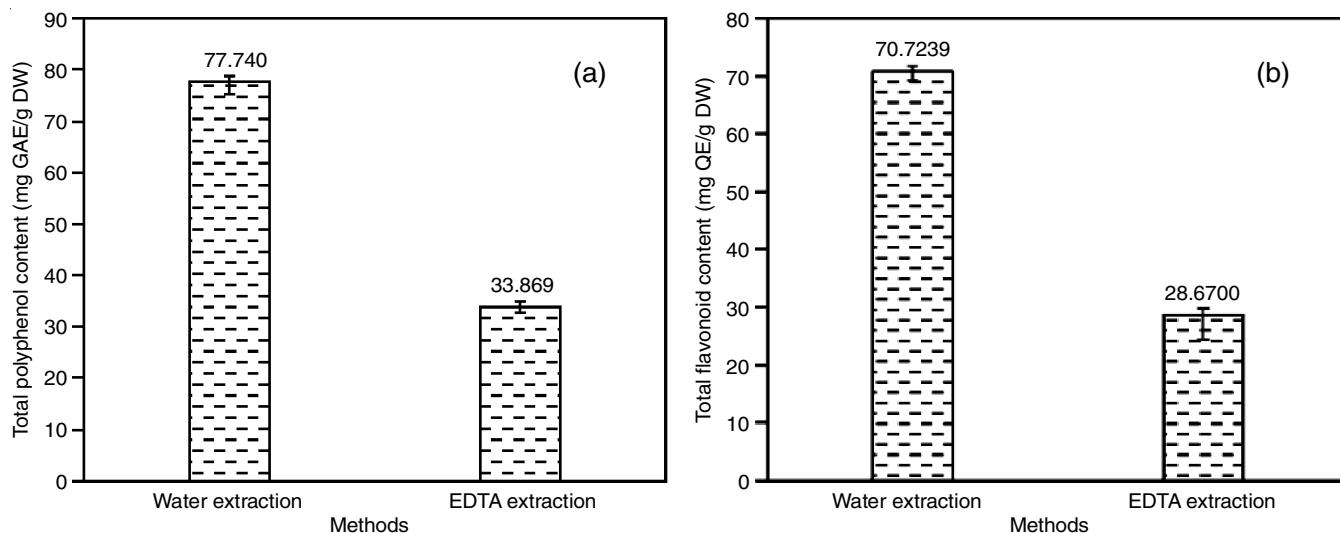


Fig. 2. Effect of (a) water and (b) EDTA extraction methods on polyphenol and flavonoid contents

This underscores the importance of selecting the appropriate extraction method to optimize the recovery of these compounds. These findings support the preference for aqueous extraction for polyphenols and flavonoids and emphasize the use of water in extraction processes for compounds with similar characteristics.

Antioxidant capacity: The aqueous extraction method proved to be more effective than the EDTA extraction in recovering antioxidant compounds from seaweed, as evidenced by the significantly lower IC_{50} values in both the DPPH and ABTS methods when water was used as the extraction solvent (Table-2). Specifically, the IC_{50} value of the aqueous extract for DPPH was $1977.54 \mu\text{g mL}^{-1}$, lower than that of the EDTA extract at $3610.11 \mu\text{g mL}^{-1}$, indicating that the aqueous extract had a greater ability to inhibit DPPH free radicals. Similarly, for the ABTS method, the aqueous extract had an IC_{50} of $68.82 \mu\text{g mL}^{-1}$, also lower than that of the EDTA extract at $296.48 \mu\text{g mL}^{-1}$, confirming the superior antioxidant activity of the aqueous extract. This suggests that the aqueous extract is more effective in inhibiting free radicals compared to the EDTA extract. Water, being a polar solvent, can dissolve phenolic compounds and flavonoids more efficiently, resulting in higher antioxidant capacity. Velioglu *et al.* [23] also demonstrated that polar solvents like water tend to extract more phenolic compounds, thereby enhancing antioxidant capacity. Ascorbic acid, used as a stan-

dard, exhibited the lowest IC_{50} values in both methods ($4.47 \mu\text{g mL}^{-1}$ for DPPH and $18.88 \mu\text{g mL}^{-1}$ for ABTS), indicating its superior antioxidant efficacy.

Antibacterial activity: Water and EDTA extracts were analyzed at two different concentrations (1.5 mg and 3.0 mg) to assess their antibacterial activity by measuring the diameter of inhibition zone (Fig. 3). The water extract at 3.0 mg exhibited superior antibacterial activity compared to the 1.5 mg concentration (Table-3), with inhibition zone diameters of 7.23 mm for *E. coli* and 8.16 mm for *S. aureus*. This suggests that the water extraction method is more effective than the EDTA extraction method at higher concentrations. The EDTA extract showed lower antibacterial activity, with inhibition zone diameters of 6.2 mm for *E. coli* and 6.13 mm for *S. aureus* at 3.0 mg concentration. Phenolic compounds have been shown to inhibit bacterial growth, similar to the antibiotic chloramphenicol, which acts by inhibiting bacterial protein synthesis. The minimum inhibitory concentrations (MIC) of the aqueous and EDTA extracts were compared to chloramphenicol against *E. coli* ATCC 8739 and *S. aureus* ATCC 6538, with the aqueous extracts having lower MIC (2.813 mg mL^{-1}) compared to the EDTA extracts (5.625 mg mL^{-1}) (Table-4). Chloramphenicol exhibited a significantly lower MIC of 0.2 ppm for both bacterial strains, indicating its potent antibacterial activity. While the seaweed extracts were not as effective as chloramphenicol, they still show promise for natural antibacterial applications in personal care products or dietary supplements.

Acetylcholinesterase inhibitory activity: The inhibitory activity of the acetylcholinesterase enzyme is crucial for potential treatment of Alzheimer's disease, a neurodegenerative condition linked to decreased acetylcholine levels in the brain. Research by Marucci *et al.* [24] demonstrated that acetylcholinesterase inhibitors can enhance memory and learning in Alzheimer's

Methods	IC_{50} DPPH ($\mu\text{g mL}^{-1}$)	IC_{50} ABTS ($\mu\text{g mL}^{-1}$)
Water extraction	1977.54	68.82
EDTA extraction	3610.11	296.48
Acid ascorbic	4.47	18.88

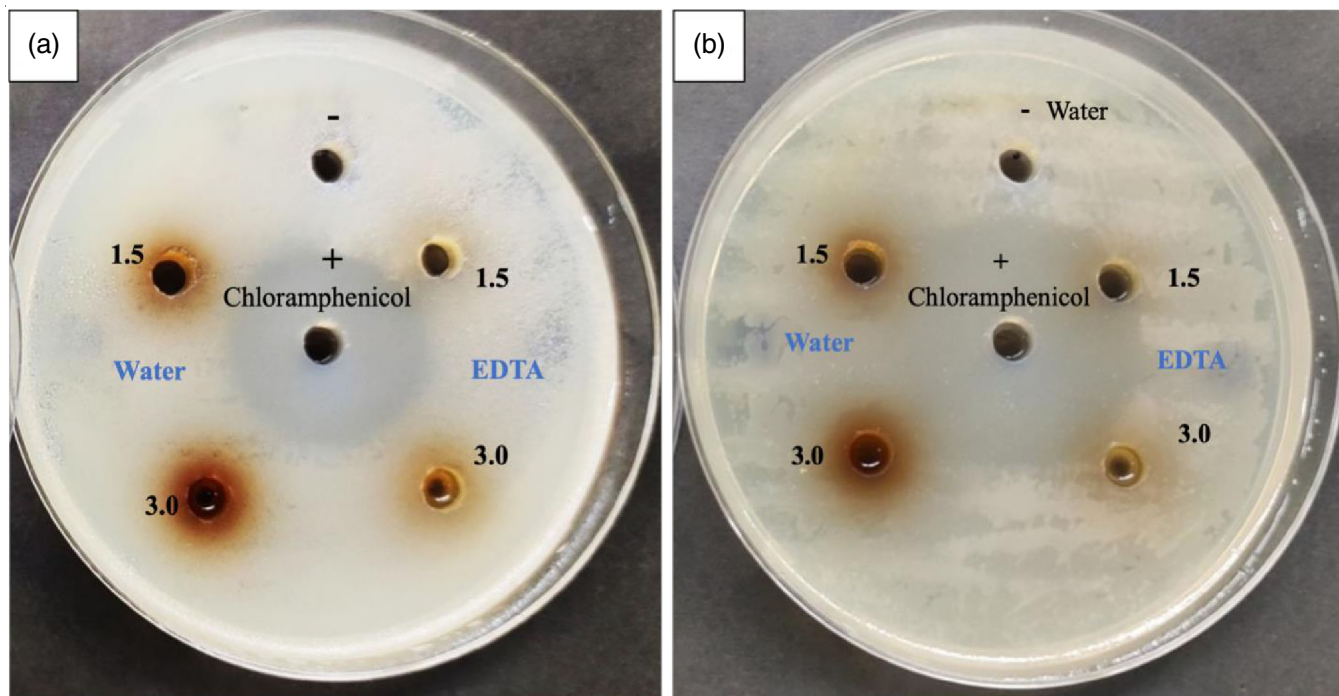


Fig. 3. Antibacterial activity of seaweed extract sample on agar test plate

TABLE-3
ANTIBACTERIAL EFFICACY OF THE EXTRACTS ON
GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

Samples		Resistance index (sterile ring diameter, mm)	
		<i>E. coli</i>	<i>S. aureus</i>
		ATCC 8739	ATCC 6538
Water	1.5	6.20 ± 0.21	6.03 ± 0.04
	3.0	7.23 ± 0.20	8.16 ± 0.12
EDTA	1.5	6.03 ± 0.04	6.10 ± 0.08
	3.0	6.20 ± 0.08	6.13 ± 0.04
Chloramphenicol (20 µg mL ⁻¹)		26.20 ± 0.08	32.26 ± 0.20

Note: the diameter of the agar well is 6 mm.

TABLE-4
MINIMUM INHIBITORY CONCENTRATIONS
(MIC) OF WATER AND EDTA EXTRACTS

Samples	Minimum inhibitory concentrations (MIC, mg mL ⁻¹)	
	<i>E. coli</i>	<i>S. aureus</i>
	ATCC 8739	ATCC 6538
Water	2.813	2.813
EDTA	5.625	5.625
Chloramphenicol (µg mL ⁻¹)	0.2	0.2

patients. The study found that the aqueous extract exhibited an inhibitory activity of 24.19% at a concentration of 312.5 µg/mL, while the EDTA extract showed 21.37% inhibition and berberine showed 35.54% inhibition (Fig. 4). The inhibitory effect increased gradually with higher concentrations of the extract (312.5–5000 µg mL⁻¹). The results indicated that the aqueous extract of seaweed had a stronger inhibitory effect on the acetylcholinesterase enzyme compared to the EDTA method, especially at higher concentrations, with no significant difference compared to berberine. This difference may be attributed to the chemical properties of the solvents and their compatibility with the bioactive compounds in seaweed. The choice of solvent during the extraction process significantly impacts the biological activity of the extracts [17].

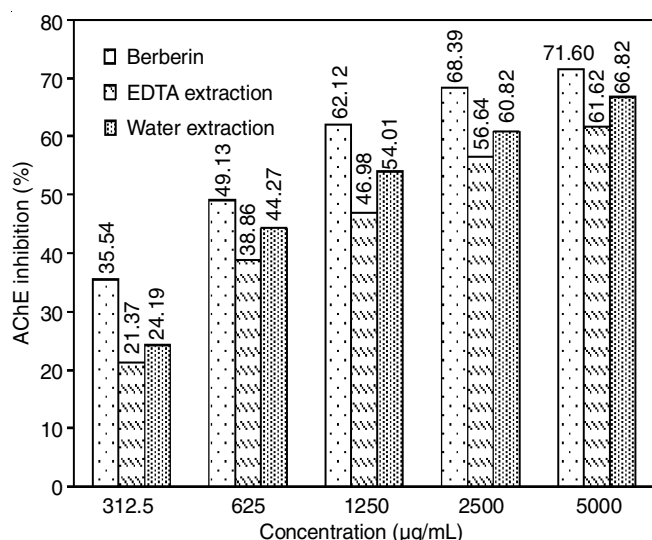


Fig. 4. Percentage of inhibition of acetylcholinesterase enzyme at different concentrations of the extract

Conclusion

The study offered an examination of the structure and morphology of a seaweed species, facilitating its identification. The water extraction yielded 23.523%, while the EDTA extraction yielded only 15.458%. The water extraction showed higher polyphenol and flavonoid content compared to the EDTA method. Overall, the water solvent was found to be more effective in evaluating the free radical scavenging ability (DPPH and ABTS) and acetylcholinesterase enzyme inhibition. The water extract exhibited the stronger antibacterial properties than the EDTA extract, with a minimum inhibitory concentration of 2.813 mg mL⁻¹ for the water extract and 5.625 mg mL⁻¹ for the EDTA extract against both *E. coli* and *S. aureus*.

ACKNOWLEDGEMENTS

This research is funded by Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam under grant number SPUD.2024.01.15/HĐ-KHCN.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

- B. Minh Ly, N. Quoc Buu, N. Duy Nhut, P. Duc Thinh and T. Thi Thanh Van, *ASEAN J. Sci. Technol. Dev.*, **22**, 371 (2017); <https://doi.org/10.29037/ajstd.173>.
- M.I. Rushdi, I.A.M. Abdel-Rahman, H. Saber, E.Z. Attia, W.M. Abdelraheem, H.A. Madkour, H.M. Hassan, A.H. Elmaidomy and U.R. Abdelmohsen, *RSC Adv.*, **10**, 24951 (2020); <https://doi.org/10.1039/D0RA03576A>.
- S. Al-Hashdy, S. Raweh, A. El-Shaibany, A. Humaid and M. El-Aasser, *Int. J. Res. Dev. Pharm. Life Sci.*, **8**, 2 (2022).
- J.K. Patra, S.K. Rath, K. Jena, V.K. Rathod and H. Thatoi, *Turk. J. Biol.*, **32**, 119 (2008).
- A.E. Abd El-Wahab, D.A. Ghareeb, E.E.M. Sarhan, M.M. Abu-Serie and M.A. El Demellawy, *BMC Complement. Altern. Med.*, **13**, 218 (2013); <https://doi.org/10.1186/1472-6882-13-218>.
- A. Husni, N. Izmi, F.Z. Ayunani, A. Kartini, N. Husnayain and A. Isnansetyo, *Int. J. Food Sci.*, **2022**, 1 (2022); <https://doi.org/10.1155/2022/3689724>.
- A.A. Ageeli and S.F. Mohamed, *Chemistry*, **5**, 2756 (2023); <https://doi.org/10.3390/chemistry5040176>.
- B. Luo, Z. Wang, J. Chen, X. Chen, J. Li, Y. Li, R. Li, X. Liu, B. Song, K.L. Cheong and S. Zhong, *Molecules*, **28**, 2610 (2023); <https://doi.org/10.3390/molecules28062610>.
- Q.-L. Sun, Y. Li, L.-Q. Ni, Y.-X. Li, Y.-S. Cui, S.-L. Jiang, E.-Y. Xie, J. Du, F. Deng and C.-X. Dong, *Carbohydr. Polym.*, **229**, 115487 (2020); <https://doi.org/10.1016/j.carbpol.2019.115487>.
- D. Al-hashdy, S. Raweh, A. El-shaibany, A. Humaid and M. El-aasser, *Int. J. Res. Dev. Pharm. Life Sci.*, **8**, (2022); <https://doi.org/10.4172/2278-0238.1000130>.
- M. Sanjivkumar, S.T. Selvan, B. Velramar, K. Nagajothi, S.S.M. Sophia and A. Parameswari, *Biotherapeutic Potential and Properties of Seaweeds; In: Whole-Cell Biocatalysis*. Apple Academic Press, pp. 199–220 (2024).
- M.D.N. Meinita, D. Harwanto and J.-S. Choi, *Appl. Sci.*, **12**, 2638 (2022); <https://doi.org/10.3390/app12052638>.
- J. Liu, S.-Y. Wu, L. Chen, Q.-J. Li, Y.-Z. Shen, L. Jin, X. Zhang, P.-C. Chen, M.-J. Wu, J. Choi and H.-B. Tong, *Int. J. Biol. Macromol.*, **155**, 1385 (2020); <https://doi.org/10.1016/j.ijbiomac.2019.11.113>.

14. P.L. Thao My, V.V. Sung, T.D. Dat, H.M. Nam, M.T. Phong and N.H. Hieu, *ChemistrySelect*, **5**, 4371 (2020); <https://doi.org/10.1002/slct.201903818>
15. N.M.S. Moubayed, H.J. Al Hourri, M.M. Al Khulaifi and D.A. Al Farraj, *Saudi J. Biol. Sci.*, **24**, 162 (2017); <https://doi.org/10.1016/j.sjbs.2016.05.018>
16. S.J. Lim and W.M.W. Aida, Extraction of Sulfated Polysaccharides (Fucoidan) from Brown Seaweed; In: *Seaweed Polysaccharides*. Elsevier, pp. 27–46 (2017).
17. K. Rafińska, P. Pomastowski, J. Rudnicka, A. Krakowska, A. Maruska, M. Narkute and B. Buszewski, *Food Chem.*, **289**, 16 (2019); <https://doi.org/10.1016/j.foodchem.2019.03.025>
18. M. Heinrich, J. Mah and V. Amirkia, *Molecules*, **26**, 1836 (2021); <https://doi.org/10.3390/molecules26071836>
19. B. Tan, X. Wu, J. Yu and Z. Chen, *Molecules*, **27**, 3956 (2022); <https://doi.org/10.3390/molecules27123956>
20. R. Lailatussifa, A. Husni and A.E. Nugroho, *Food Sci. Biotechnol.*, **25**, 589 (2016); <https://doi.org/10.1007/s10068-016-0082-y>
21. M. Rudrapal, S.J. Khairnar, J. Khan, A.B. Dukhyil, M.A. Ansari, M.N. Alomary, F.M. Alshabrm, S. Palai, P.K. Deb and R. Devi, *Front. Pharmacol.*, **13**, 806470 (2022); <https://doi.org/10.3389/fphar.2022.806470>
22. L. Wang and C.L. Weller, *Trends Food Sci. Technol.*, **17**, 300 (2006); <https://doi.org/10.1016/j.tifs.2005.12.004>
23. Y. Velioglu, G. Mazza, L. Gao and B.D. Oomah, *J. Agric. Food Chem.*, **46**, 4113 (1998); <https://doi.org/10.1021/jf9801973>
24. G. Marucci, M. Buccioni, D.D. Ben, C. Lambertucci, R. Volpini and F. Amenta, *Neuropharmacology*, **190**, 108352 (2021); <https://doi.org/10.1016/j.neuropharm.2020.108352>