In vitro and in silico analysis for acetylcholinesterase (AChE) inhibitors of some seaweed: Insights into their biological activity

Shymaa A. Abdelkader, Elham E. Moustafa and Afaf H. Ali
Botany Department, Faculty of Women for Arts, Science and Education, Ain Shams University, Egypt

Corresponding Author: Shymaa A. Abdelkader
Botany Department, Faculty of Women for Arts, Science and Education, Ain Shams University, Egypt
Email: shymaa.qader@women.asu.edu.eg

Submitted: May 07, 2025
Accepted: July 21, 2025
DOI: 10.21608/egyjs.2025.382778.1047

ABSTRACT: Marine algae, unlike terrestrial plants, produce biologically active molecules as a defense mechanism against the adverse environment in which they live and may include various physiologically active substances with medical benefits. The phytochemical contents of *Ulva lactuca* and *Corallina officinalis* in various solvents are examined in this paper. The aqueous extract of seaweed was studied for antioxidants, antibacterial, antibiofilm, anticancer, and anti-Alzheimer effects, as well as best-pose compounds for their interaction against acetylcholinesterase (AChE-targeted) Alzheimer disease (AD). *U. lactuca* extract had the highest inhibitory efficiency against all bacterial biofilms when compared to C. officinalis, and it had better antibacterial activity against Gram-positive bacteria than Gram-negative bacteria. *U. lactuca* had greater anticancer activity against hepatic carcinoma (HepG-2) compared to *C. officinalis*, with IC_{50} values of 182 ± 4.61 µg/ml and 222 ± 5.98 µg/ml, respectively. The last one was the most effective in inhibiting AChE, with an IC_{50} of 319.1 ±1.045 µg/ml. In GC-MS analysis of *C. officinalis*, the most concentration compounds found were found, there were five, were studied in molecular docking with AChE. The result was the Tricyclo most effective of the AChE domain binding conformation with a binding energy of -5.7 kcal/mol and 7 hydrophobic residues which may be effective in treating Alzheimer disease.

Keywords: *Ulva*, *Corallina*, pathogenic organisms, hepatic cancer (HepG-2), anti-Alzheimer's disease and molecular docking

INTRODUCTION

Alzheimer's disease is predicted to be the fourth-leading cause of mortality in the industrialized world (Estrada *et al.,* 2019), with 19.3 million new cancer cases and 10 million cancer deaths documented globally in 2020 (Sung *et al.,* 2021). Alzheimer's disease (AD) and cancer have grown to be two of the most pressing global public health issues. Most cancer treatments include several medications that permeate bodily tissue and alter the mechanisms of action of both malignant and healthy cells (El-Sheekh *et al.,* 2022). Many investigations have been conducted to find therapeutic substances derived from natural sources that combat cancer. Similarly, given the serious side effects of commercial drugs, there is an urgent need for innovative natural medicine alternatives to treat Alzheimer's disease (Syad *et al.,* 2012).

Seaweeds, also known as marine macroalgae, have chemical structures and features that vary from those of terrestrial species. Their presumably bioactive components exhibit a wide range of nutritional, pharmacological, and nutraceutical effects (El Nemr *et al.,* 2021). Marine algae produce a wide range of biologically active secondary metabolites, including alkaloids, polyketides, cyclic peptides, polysaccharides, phlorotannin, diterpenoids, sterols, fatty acids, vitamins, minerals, quinones, lipids, glycerol (Abd El Hafez *et al.,* 2022), which can be used to treat chronic diseases such as cancer, cardiovascular disease, osteoporosis, neurodegenerative diseases, and diabetes mellitus (Meinita *et al.,* 2022). Furthermore, seaweed includes bioactive components that inhibit some Gram-positive and Gram-negative bacterial infections from multiplying (Vinodkumar and Packirisamy, 2024). Using marine natural chemicals with the ability to inhibit bacterial growth has tremendous therapeutic potential (Labes, 2023). The antibacterial properties of marine algae against many ailments are particularly interesting. Seaweed extracts may have immune-modulating effects, increasing their anticancer efficacy *in vivo* (El-Sheekh *et al.,* 2022).

Many studies have revealed that algal extracts, both in vitro and in vivo, exhibit anti-proliferative effects that limit tumor growth. These findings suggest that compounds derived from algae may have anticancer properties. According to Moussavou *et al.* (2014), eating a variety of seaweed is linked to a lower incidence of cancer in Asian countries where people consume a lot of seaweed. Certain studies show that bioactive compounds derived from seaweed have antitumor effects through various modes of action, such as inhibiting cell growth, carcinogenicity, incursion, and metastases, and stimulating programmed cell death in cancer cells (Farooqi *et al.,* 2012; Liu *et al.,* 2019). Mofeed *et al.* (2021) collected a list of marine macroalgal species from Egypt, including *Amphiroa anceps* and *Corallina mediterranea* (Rhodophyta), *Ulva fasciata* and *U. lactuca* (Chlorophyta), and reported that the substances were studied as anticancer therapies. Abd El Hafez *et al.* (2022) and Kalasariya *et al.* (2024)

investigated the antioxidant, antimicrobial, anti-cancer, anti-inflammatory, anti-diabetic, and anti-acetylcholinesterase activity of methanolic extracts of *Hypnea cornuta* (red algae) and *Ulva prolifera* (green algae) *in vitro*. *Ulva lactuca*, according to Pappou *et al.* (2022), is a rich source of marine bioactive compounds with several applications in the biomedical field. Khalil Mohamed *et al*. (2023) demonstrated that ethanol extract of *Ulva lactuca* acts as apoptotic agent and participates in the inhibition of colorectal cancer cells.

A reduction in acetylcholine (ACh) levels in the hippocampus and cortex of the brain signifies the most remarkable biochemical change in Alzheimer's disease patients. Currently, the most effective established treatment for Alzheimer's disease (AD) is the inhibition of acetylcholinesterase (AChE) (Chen *et al.,* 2022). Experimental research suggests that bioactive chemicals from seaweed may penetrate the brain and modify many forms of neuronal activity. Through epigenetic mechanisms, they may directly disrupt specific neuronal molecules or indirectly modulate the transcription of proteins associated with neurotransmission, hence impacting neuronal survival and plasticity (Strandwitz, 2018; Frozza *et al.*, 2018; Rosa *et al.*, 2019). A study conducted in Japan revealed that elderly individuals who regularly consumed seaweed had a reduced risk of developing dementia compared to non-consumers (Meinita *et al*., 2022).

Even though they seem to be different, new information suggests that AD and cancer may be linked in the opposite way (Ording *et al.*, 2019). The possible failure of a system that controls both cell death and survival could help explain this backward connection and play a part in the development of both diseases (Behrens *et al.*, 2009). Another study found that people with AD had a 61% lower chance of getting cancer (Driver *et al.,* 2012). Kang *et al.* (2023) looked at the link between AD and the risk of getting cancer. Molecular docking is an important part of both computer-based drug design and structure-based molecular biology. It shows how very small chemicals can associate with proteins or receptors at the nanoscale level. This important method is needed to predict how ligands and receptors will interact (Grigoriadis, 2020). More research needs to be done on this method before it can be used to test new drugs.

This study aims to compare the potential therapeutic roles of two seaweed algae in the treatment of hepatic cancer (HepG-2) and Alzheimer's disease by evaluating their phytochemical compounds and potential medical applications, including antimicrobial, antibiofilm, cytotoxicity, anticancer, and anti-acetylcholinesterase activities. To underscore the effectiveness of anti-Alzheimer's pharmaceuticals, silico research was conducted, which included molecular dynamic modeling, molecular docking, and intermolecular interactions. The objective was to identify the chemical components of seaweed that could be targeted for the treatment of Alzheimer's disease. The role of seaweed algae in the treatment of conditions such as hepatic malignancy (HepG-2) and Alzheimer's is underscored by this investigation.

MATERIALS AND METHODS

Algal samples collection

The following algae were collected from Alexandria in the Mediterranean Sea at a depth of 0.5 m: *Ulva* (Chlorophyta) at 31°13'29.7"N 29°56'07.5"E and *Corallina* (Rhodophyta) at 31°12'24.2"N (Figure 1), during winter 2022. The materials were subsequently transported to the phycology laboratory of the Department of Botany, Faculty of Women, using plastic sacks. The samples were rinsed three times with distilled water to remove grime and debris from the seaweed.

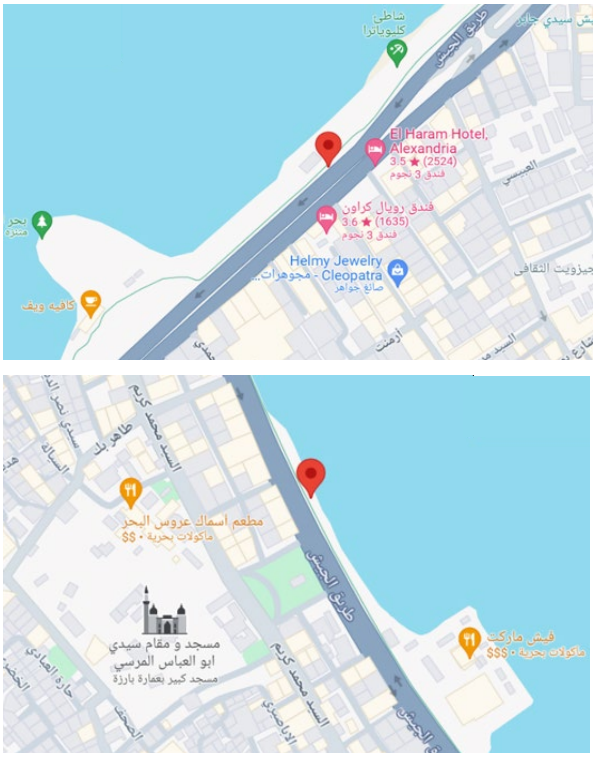


Figure 1. Location of collected samples, Alexandria, Egypt.

Identification and preparation of algal samples

According to Jha *et al*. (2009), the algae were identified based on their morphological characteristics to *Ulva lactuca* and *Corallina officinalis.* The algal samples were oven-dried for 48 hours at 50°C after being air-dried for 10 days at 26°–30°C under indirect light. A mixer grinder was used to crush and powder the dried seaweed. After that, the powdered samples were kept for later use at room temperature (Sivasankari *et al.,* 2006).

Preparation of seaweed extracts and fractions

According to Hasan *et al.* (2019), the extracts using a soaking technique based on a conical flask, 5 gm of seaweed powder samples each were submerged in 50 ml distilled water and then covered with aluminum foil. The samples were softly shaken and then left at room temperature for 72 hours. The samples were softly shaken and then left at room temperature for 72 hours. After filtration, the liquid was then allowed to concentrate to get specific solvent extracts. The same process above was carried out by using hot distilled water and solvents including acetone, benzene, butanol, chloroform, ethyl acetate, ethanol, methanol, and petroleum ether. Secondary metabolites were tested qualitatively using the crude extracts.

Preliminary qualitative phytochemical analysis

Acetone, benzene, butanol, chloroform, ethyl acetate, ethanol, methanol, petroleum ether, cold water and hot water extracts were subjected to phytochemical analysis to detect the presence of the following biomolecules using the standard qualitative procedures as described by Trease and Evans (1989). Phytochemical screening was carried out to identify the major natural chemical groups such as alkaloids, terpenoids, tannins, saponins, flavonoids, phenols, quinones and glycosides. General reactions in these analyses revealed the presence or absence of these compounds in the algal extracts tested.

Quantification of algal phytochemical compounds

According to qualitative phytochemical analysis, hot aqueous extracts were selected for their quantification as they contain many active substances. The Folin–Ciocalteu colorimetric method is used in the total phenolic content (TPC) test (Singleton *et al.*, 1999; Singleton and Rossi, 1965). Total soluble fat and total carbohydrate contents were determined by Van Handel (1985). Total flavonoid content (TFC) assay, Chang *et al.* (2002). Polysaccharide content is based on Evstigneyev (2017) estimate and Pawar and Mello (2011) estimate. Total protein content using the Bradford method (Bradford, 1976).

Tested bacteria

The tested microorganisms were obtained from MERSEN, The Agriculture Faculty, Ain Shams University, Cairo, Egypt.

Gram-positive bacteria
Bacillus subtilis ATCC6633
Staphylococcus aureus NRRLB-767

Gram-negative bacteria
Escherichia coli ATCC25922
Pseudomonas aeruginosa ATCC10145.

Antibacterial activity

The antibacterial activity of algal extracts by hot water (the concentrations ranged from 62.5 to 1000 mg/ml), was performed in a 96-well flat polystyrene plate by El-Bendary *et al*. (2021), and Qader *et al*. (2021) methodology. Clearance in the wells indicated that the studied extracts had a positive antibacterial activity. The control is the pathogen without any treatment, the positive control for bacterial strains was Ciprofloxacin. After roughly 20 hours, the absorbance at OD 600 nm was measured using a Spectrostar Nano Microplate Reader (BMG LABTECH GmbH, Allmendgrun, Germany)**.** The experiment was performed in triplicate.

Antibiofilm activity

The biofilm inhibitory activity of the tested extracts was assessed using the microtiter plate assay (MTP) method in a sterile 96-well polystyrene microtiter plate with a flat bottom. According to Antunes *et al.* (2010), each well was filled with 180 µl of LB broth, 10 µl of overnight growing bacterial isolates (0.5 McFarland), 10 µl of each algal extract, whose concentrations from 62.5 to 1000 mg/ml, along with the negative control (i.e., LB and bacteria without extracts) and then the plate was incubated at 37 °C for 24 h. Following the removal of each well's contents, 200 µl of phosphate buffer saline pH 7.2 was added to each well to wash off any floating bacteria. Following a one-hour staining process with crystal violet (0.1%, w/v) in each well, the plate was washed with 200 µl of distilled water and allowed to dry in a laminar flow. Finally, 200 µl of 95% ethanol was added to the dried plate, and then OD was measured at 570 nm using (Spectrostar Nano absorbance plate reader – BMG LABTECH). The

experiment was performed in triplicate. The inhibition percentage of biofilm formation was calculated according to the following formula:

% Inhibition = 1- (OD_{600} of tested / OD_{600} of the non-tested x 100 (Siddique *et al.*, 2020).

Anticancer assay and Cytotoxicity

The cytotoxicity of the algal extract (concentrations ranged from 0-500 µg/ml) was evaluated against HepG-2 cells (human hepatocellular carcinoma) at the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University using the MTT cell viability assay, according to Mosmann (1983) and Gomha *et al.* (2015).

Acetylcholine esterase inhibitor assay

The assay was conducted in the NAWAH scientific laboratory following the methodology of Ellman *et al.* (1961) and Osman *et al.* (2014).

Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical composition of the selected extract was determined using a Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) equipped with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven's temperature was raised from 50°C to 250°C at a rate of 5°C per minute and maintained at that temperature for two minutes. The temperature was then raised to 300°C at a rate of 30°C per minute and maintained for two minutes. The temperatures of the MS transfer line and injector were maintained at 270°C and 260°C, respectively. Helium served as the carrier gas at a steady flow rate of one milliliter per minute. Samples diluted to 1 µl were automatically injected in split mode using the Autosampler AS1300 in conjunction with GC, after a 4-minute solvent delay. Electron impact mass spectra were acquired over the m/z range of 50–650 using full scan mode at 70 eV ionization voltage. The temperature of the ion source was established at 200 °C. The components were identified by comparing their mass spectra with those in the WILEY 09 and NIST 14 mass spectral databases (Abd El-Kareem *et al.,* 2016).

***In silico* molecular docking study**

According to the study, the best-pose compounds will interact with AChE's AD targets. The potential lead compounds that have the highest binding affinity for the receptors are chosen.

Protein sequence retrieval and preparation

The biological information and annotation data of the AChE protein were acquired from the Protein Data Bank (PDB) (PDB ID: 4PQE) (Thandivel *et al*., 2024). The 3D structure of the AChE enzyme should be prepared and edited before docking. The preparation process was done by deleting water molecules, adding hydrogen atoms' polar type, removing atomic clashes, checking for missing atoms if so, repairing the missing ones, and finally saving them into a pdbqt file format (Elmezayen *et al*., 2021).

Ligands selection and preparation

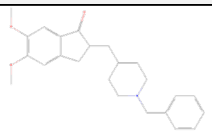
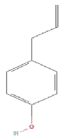
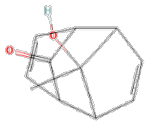
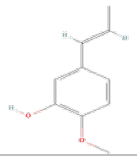
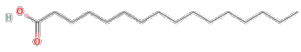
Ligands tested were obtained from the PubChem database listed in Table 1 to evaluate their inhibitory effect with the AChE enzyme (https://pubchem.ncbi.nlm.nih.gov/). Three-dimensional (3D) coordinates for all ligands acquired as Structured Data Format (SDF) files for each ligand then were converted to pdbqt file format via the Open Babel program (Version 2.3.1). The energy minimization process was ensured for all chosen ligands and AChE proteins by using the Swiss pdb viewer version (4.1.0).

Molecular docking procedure

The AutoDock Vina program was operated to estimate the position of the grid box. The grid was positioned with a box size of 126 × 126 × 126 angstrom (Å) and a center x, y, and z center of 25.528, 7.545, and 37.453 Å respectively with a spacing of 0.375 Å. The blind docking was to search for all possible ligand conformations in the entire protein space.

Docking runs were conducted using the Autodock Vina software under a Lamarckian Genetic Algorithm (LGA). Based on the top 5 postures of ligand binding sites, the one with the highest score was chosen. Considering the farthest most suitable binding position chosen depending on the lowest free energy of binding and optimal chemical interactions, the conformations generated were clustered (Díaz *et al.,* 2020). Using the software visualization tool for BIOVIA's discovery studio 3.5 (https://discover.3ds.com/discovery-studio-visualizer download), the Docking data of molecular interactions between the target protein and ligands could be visualized implying 2D chemical interactions, hydrogen bonds, hydrophobicity, ionizability and solvent-accessible surface (SAS) interactions for every domain-ligand complex.

Table 1. Ligands and their CID Number, molecular formula, molecular weight and chemical structure.

Compound name	PubChem CID	Molecular formula	Molecular weight(g)	Chemical structure
Donepezil (Standard)	3152	$C_{24}H_{29}NO_3$	379.5	
4-Allylphenol	68148	$C_9H_{10}O$	134.17	
Tricyclo [4.2.1.1(2,5)] deca-3,7-dien-9-one, 10-hydroxy-10-methyl-stereoisomer	569218	$C_{11}H_{12}O_2$	176.21	
Phenol, 2-methoxy-5-(1-propenyl)-, (E)- (Isochavibetol)	1781947	$C_{10}H_{12}O_2$	164.20	
Hexadecenoic acid (Palmitic Acid)	985	$C_{16}H_{32}O_2$	256.42	
9,12-Octadecadienoic acid (Z,Z)- (Linoleic Acid)	5280450	$C_{18}H_{32}O_2$	280.4	

Statistical Analysis

Statistical analyses were carried out using SPSS software. The data obtained were analyzed statistically to determine the degree of significance using one-way analysis of variance (ANOVA) at probability level $P \leq 0.05$. A post-hoc test was applied according to Duncan's test, when differences are significant (SPSS, 2012).

RESULTS

Preliminary qualitative phytochemical analysis

Table 2 shows the preliminary phytochemical analysis of the seaweed used in the present study for their presence or absence. The acetone, benzene, butanol, chloroform, ethyl acetate, ethanol, methanol, petroleum ether, cold water, and hot water extract of *U. lactuca* and *C. officinalis* shows alkaloids, terpenoids, tannins, saponins, flavonoids, phenols, quinones, and glycosides. The cold and hot water extracts of both algae showed the highest content of active ingredients except flavonoids in cold water and alkaloids in hot water extract. Phenols and terpenoids were the most abundant active ingredients in almost all solvents. The intensity of colors was observed with the naked eye.

Quantitative analysis of phytochemical substances in algal extracts

Two algal extracts showed significant difference in all detected biochemical components except lipid. The phenolic concentration was higher in *C. officinalis* (44.30±1.64mg/g) than *U. lactuca* (35.01± 1.95mg/g) (Figure 2). The lipid concentration was relatively close while the amounts of flavonoid, polysaccharides and protein were different simply in both species. The most notable difference was the carbohydrate content, *C. officinalis* recording a higher value of 30.11 ± 2.95mg/g than *U. lactuca* (23.07 ± 0.37mg/g).

Antimicrobial activity

Hot water extracts of two algae were tested against 4 pathogenic bacteria and results are present in Figure 3. *U. lactuca* and *C. officinalis* extracts had dose-dependent antibacterial activity with significant difference. *U. lactuca* extract had higher significant

Table 2. The preliminary phytochemical analysis of *U. lactuca* and *C. officinalis*

solvent	algae	Alkaloids (organic compounds)	Terpenoids	Tannins	Saponins	Flavonoids (organic compounds)	Phenols	Quinines (organic compounds)	Glycosides (carbohydrate)
Acetone	*U*	-	-	+	++	++	+	+	+
	C	-	-	-	++	++	+	++	++
Benzene	*U*	-	+	+	++	-	+	-	-
	C	+	-	+	++	-	-	-	-
Butanol	*U*	+	+	-	+	+	-	-	-
	C	+	+	-	+	+	-	+	-
Chloroform	*U*	-	++	-	-	-	++	-	-
	C	+	++	-	-	-	++	+	-
Ethyl acetate	*U*	-	++	++	+	+	+++	-	-
	C	-	+	-	+	+	++	-	-
Ethanol	*U*	+	++	-	++	+	+	-	+
	C	+	+	-	++	++	++	-	-
Methanol	*U*	-	-	+	+	++	+	++	++
	C	-	-	-	+	++	+	+	+
Petroleum ether	*U*	+	-	-	+	-	-	-	-
	C	+	-	-	+	-	-	-	-
Cold water	*U*	++	+++	++	+	-	+	++	-
	C	+	+	-	+	-	++	+	-
Hot water	*U*	-	+++	-	++	+	+	++	-
	C	-	+++	-	++	+	+	++	-

U: *U. lactuca* C: *C. officinalis* - absent + Trace presence ++ Moderate presence +++ high presence

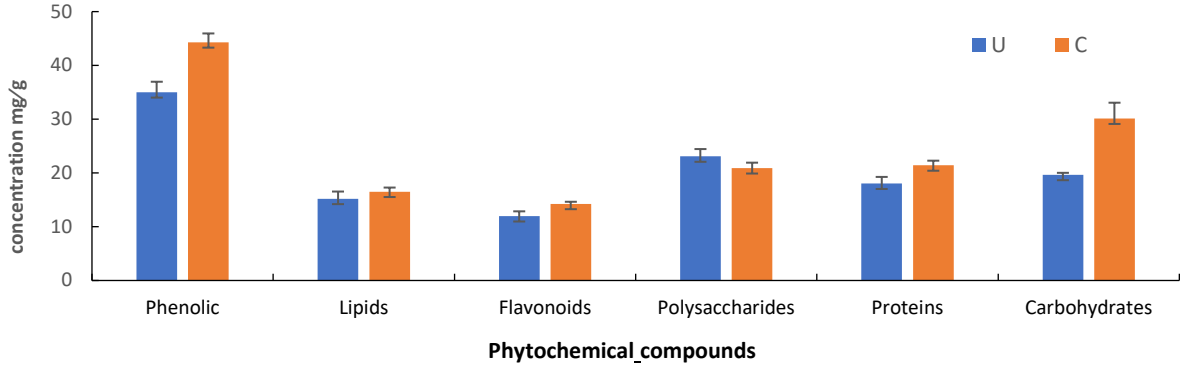


Figure 2. Quantitative analysis of phytochemical substances presents in *U. lactuca* and *C. officinalis*

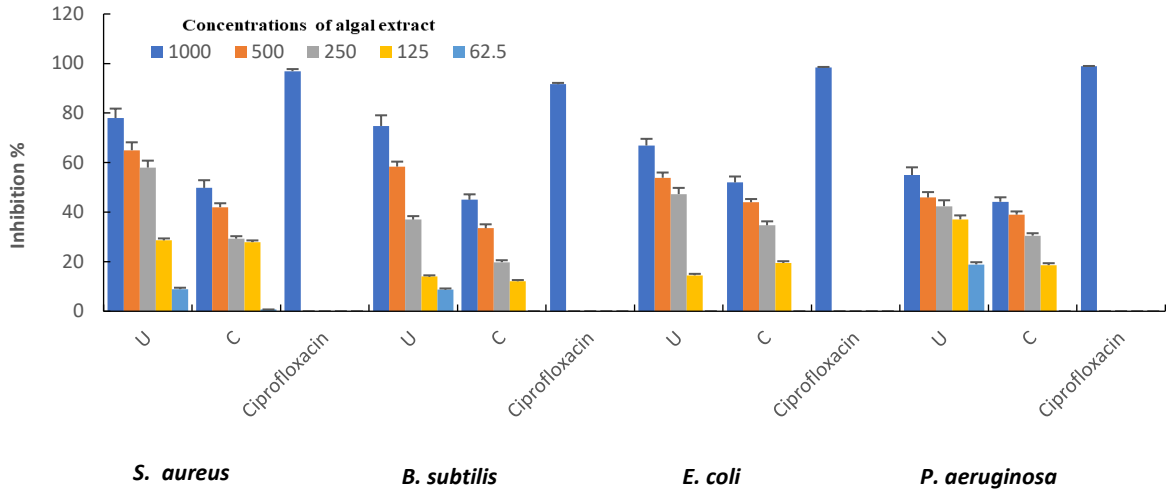


Figure 3. Antibacterial activity of tested algae against pathogenic bacteria (U: *U. lactuca* C: *C. officinalis*)

antibacterial activity against Gram +ve bacteria than Gram -ve bacteria compared to *C. officinalis.* The *U. lactuca* extract showed activity towards the bacterial strains in the given order: *S. aureus* (78%) > *B. subtilis* (74.8%) > *E. coli* (66.9%) > *P. aeruginosa* (55%) at 1000 mg/ml.

Biofilm inhibition

As represented in Figure 4. *U. lactuca* and *C. officinalis* extracts had concentration-dependent biofilm inhibition activity with significant difference. Results revealed that *U. lactuca* extract had the highest inhibitory activity towards all bacteria strains but the highest values of 67.6% and 60% against biofilm formed by *E. coli* and *S. aureus,* respectively, at 1000 mg/ml, while *C. officinalis* extract had the highest ability to inhibit biofilm formation by *S. aureus* and *B. Subtilis* strains with 29.5% and 26%, respectively, at 1000 mg/ml. Generally, the highest inhibitory activity of *U. lactuca* extract towards bacteria strains was *E. coli* > *S. aureus* > *P. aeruginosa* > *B. subtilis* with significant difference.

Anticancer activity

U. lactuca and *C. officinalis* were testing its anticancer activity as an inhibitor of cell growth of HepG-2 cells (human hepatocellular cancer). Initially, cytotoxicity testing was conducted as the first step in the screening of potential anticancer compounds by cell line. Indices of anticancer effectiveness come from the percentage of inhibition and IC_{50} value, more anticancer activity was lower the IC_{50} value. Figure (5a) exhibits Human amnion {normal Liver cells (WISH) cytotoxic impact of algae extracts. Results revealed that *U. lactuca* and *C. officinalis* extracts had no cytotoxic effect on WISH cells with CC_{50} values of 258 ± 9.82 µg/ml and 357 ± 11.74 µg/ml, respectively. Both *U. lactuca* and *C. officinalis* extracts exhibited substantial anticancer activity on HepG-2 cell line with IC_{50} values of 182 ± 4.61µg/ml and 222± 5.98 µg/ml, respectively, (Figure 5b) but *U. lactuca* was best.

Anti- Alzheimer activity

Figure 6 indicates that the two algal extracts inhibited AChE. Greater inhibitory effects follow from increased extract concentration. With the lowest IC_{50} value of 319.1 ±1.045 µg/ml, the extract from C*. officinalis* was the most efficient AChE inhibitor; in the case of *U. lactuca*, it was 366.3±1.016 µg/ml.

GC-MS analysis of *C. officinalis*

The presence of thirty-four (34) bioactive compounds was demonstrated in the GC-MS chromatogram of the hot water extract of *C. officinalis* (Figure 7). Table 3 displays the chemical constituents, including their retention time (RT), molecular formula, concentration (area %) and structure. The following components are present in high concentrations: 9,12-Octadecadienoic acid (Z,Z)- (RT 30.55, Area % 18.53), Phenol, 2-methoxy-5-(1-propenyl) (E)- (RT 13.48, Area % 13.5), Tricyclo[4.2.1.1(2,5)] deca-3,7-dien-9-one, 10-hydroxy-10-methyl-, stereoisomer (RT 10.44, Area % 10.71), Hexadecanoic acid (RT 27.40, Area % 10.34) and 4-Allylphenol (RT 11.14, Area % 5.70).

The *silico* docking analysis for each ligand complex's domain, 2D chemical interactions, interpolated charges, hydrophobicity, ionizability and solvent-accessible surface (SAS) interactions help understand how a ligand binds to a protein (Figures 8-12). Hydrophobic interactions, driven by the tendency of nonpolar molecules to avoid water, play a crucial role in stabilizing the binding interface. Ionizable groups on both the ligand and protein can form electrostatic interactions, like ionic bonds or salt bridges, which can be either attractive or repulsive depending on the charges. The solvent-accessible surface (SAS) provides a measure of the surface area exposed to the solvent, offering insights into the burial of hydrophobic patches and the potential for polar interactions.

The 2D of the chemical interaction with inhibitors as the number of conventional hydrogen bonds was three in Palmitic acid and Linoleic acid. The other tested ligands-AChE complexes had no hydrogen bond regions. The AChE bound strongly to the first and second compounds only because it was in the pocket (Figure 8c-12c). The hydrophobic interactions between the inhibitors and the AChE domain, however, varied from one inhibitor to another, as seen in Figures 8d–12d, and were represented by the colors brown for the hydrophobic areas and blue for the hydrophilic areas. The hydrophobic interaction residues were mentioned in Table 4 and only 4-Allylphenol, Tricyclo and Isochavibetol had this type of interaction. The ionization surface tends to acidity and basicity. The basic complex's ionizability was in blue and the acidic complexes were in red (Figures 7f–11f). Most complex interaction sections were acidic except Palmitic acid and Linoleic acid docked complexes were neutral and the charges were represented by blue for positive charges and red for negative charges, as shown in Figures 8e–12e. The surface zone of the receptor is the solvent accessibility surface (SAS), Figures 8g-12g.

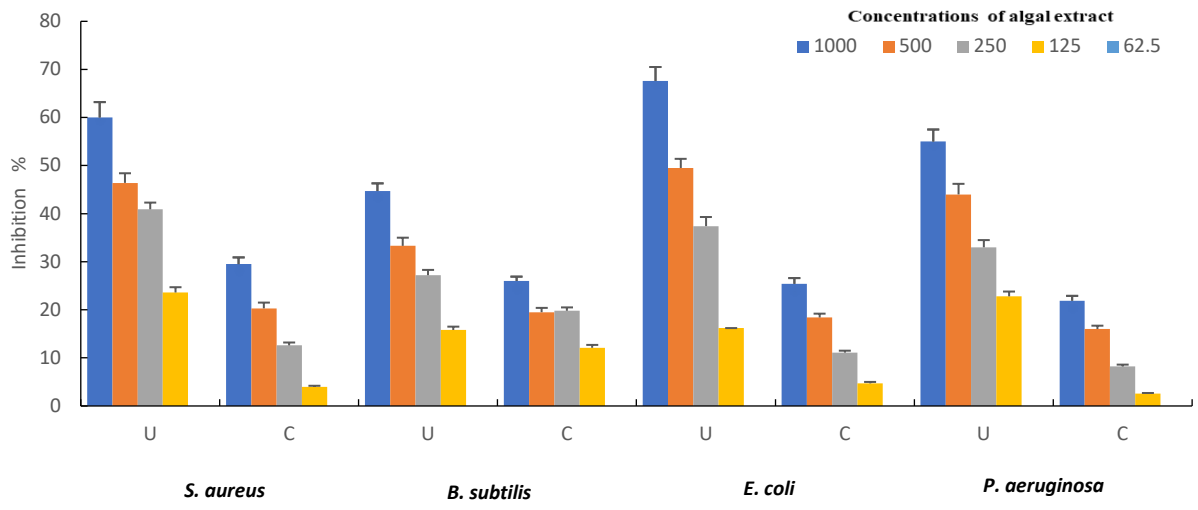


Figure 4: Effect of *U. lactuca* (U) and *C. officinalis* (C) extracts on the bacterial biofilm formation.

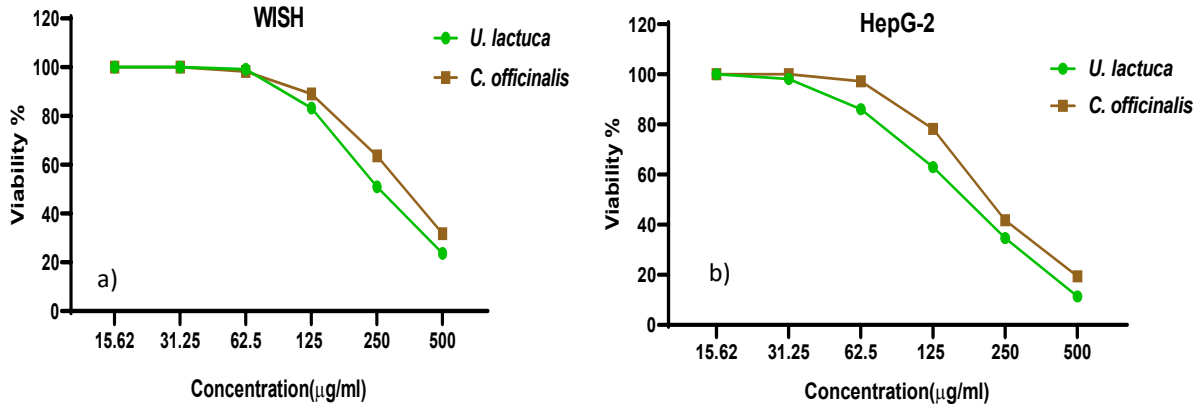


Figure 5. a) Cytotoxic assay of two algae on Human ammunition (normal Liver cells) and b) Anticancer activity of two algae on HepG-2 cell lines.

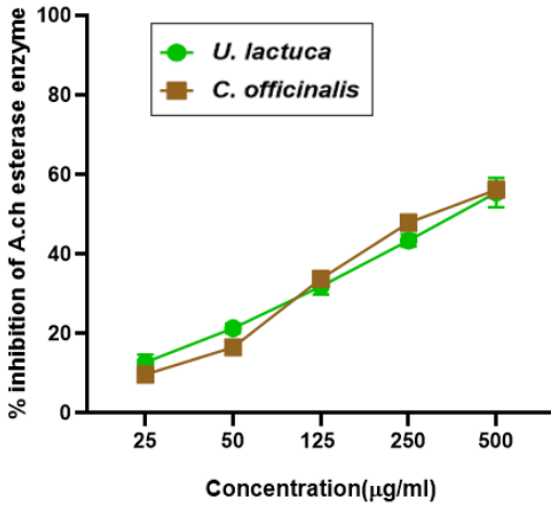


Figure 6. The anti-acetylcholinesterase activity of algal extracts.

The zone with low accessibility was shown in green and the area with excellent accessibility was shown in blue. Its intensity was nearly high and excellent accessibility in 4-Allylphenol, Tricyclo, Palmitic acid and Linoleic acid docked complexes, unlike Isochavibetol, which had low accessibility.

In silico molecular docking study

To investigate the in silico AChE inhibition action of 5 highest concentration compounds resulting from the GC-MS analysis of *C. officinalis*, the docking study was applied and listed in Table 4. All compounds were molecular weight below 500 and all the identified compounds were bound to enzyme AChE with different energy binding (values in the range of -3.9 to - 5.9 kcal/mol). Donepezil was also analyzed for comparison and showed a value of energy binding equal to -6.9 kcal/mol. Compounds one (4-Allylphenol), two (Tricyclo) and three (Isochavibetol)

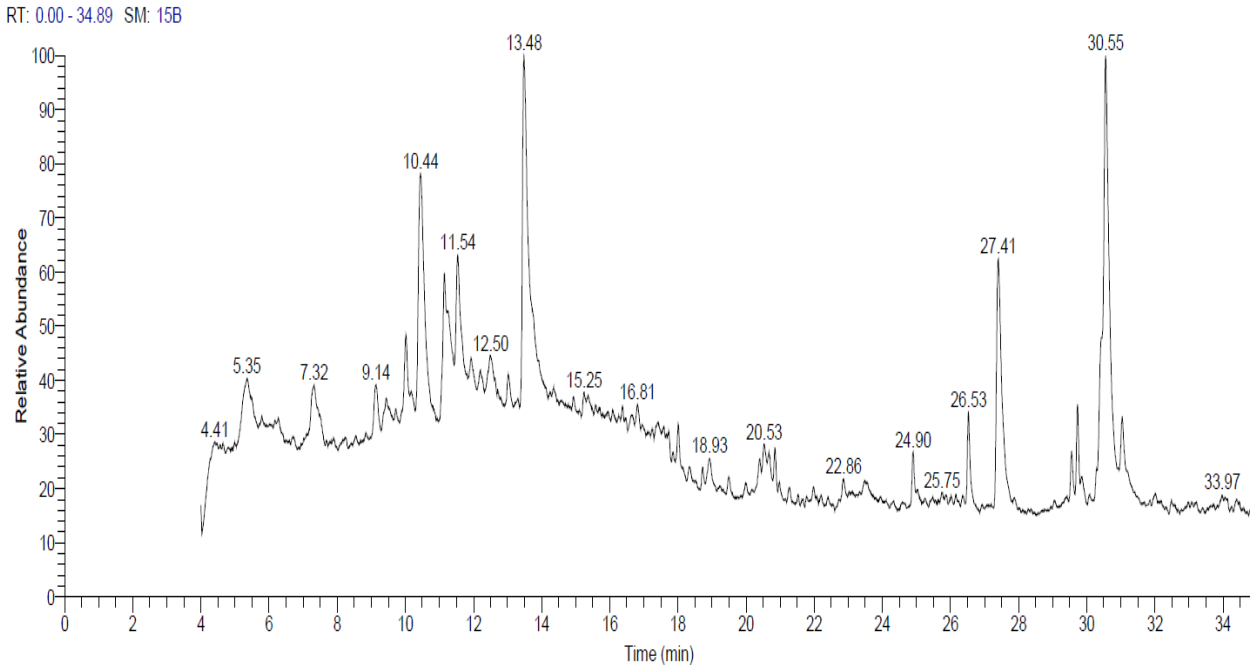
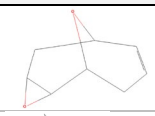
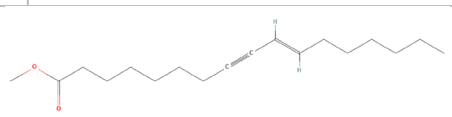
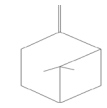
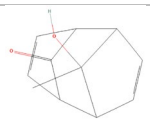
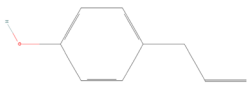
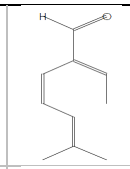
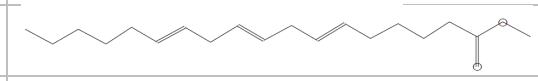
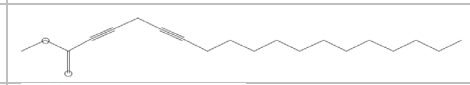
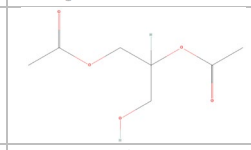
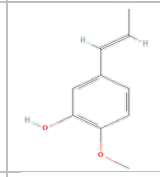
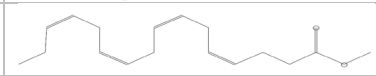
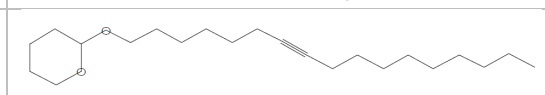
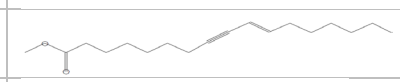
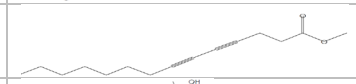
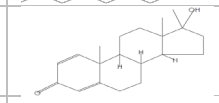
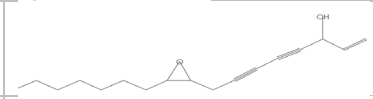
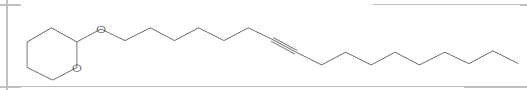
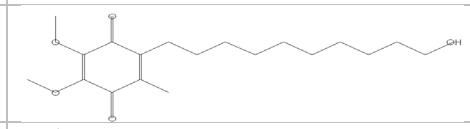
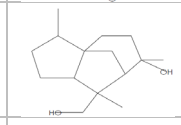
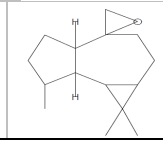


Figure 7. GC-MS chromatogram of aqueous extract of *C. officinalis*

Table 3. Compounds identified from the aqueous extract of *C. officinalis* using GC-MS

RT	Compound	Molecular formula	Area	Structure
5.36	3,10 Dioxatricyclo [4.3.1.0 (2,4)] dec-7-ene	$C_8H_{10}O_2$	3.72	
7.31	Bicyclo 3.1.1] heptane,6,6-dimethyl--2-methylene-	$C_{10}H_{16}$	2.86	
7.50	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	$C_{18}H_{30}O_2$	0.68	
9.13	Z,Z,Z-1,4,6,9 Nonadecatetraene	$C_{19}H_{32}$	1.64	
10.02	1,5,5-Trimethyl-6-methylene-cyclohexene	$C_{10}H_{16}$	2.35	
10.17	6,6-dimethyl-2 methylene bicyclo [3.1.1] heptane	$C_{10}H_{16}$	0.54	
10.44	Tricyclo[4.2.1.1(2,5)]deca-3,7-dien-9-one, 10-hydroxy-10-methyl-, stereoisomer	$C_{11}H_{12}O_2$	10.71	
11.14	4-Allylphenol	$C_9H_{10}O$	5.70	

11.55	3,5-Heptadienal, 2-ethylidene-6-methyl-	$C_{10}H_{14}O$	3.84	
11.93	5,7-Dodecadiyn-1,12-diol	$C_{12}H_{18}O_2$	0.72	
12.49	6,9,12-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	1.79	
12.65	2,5-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	0.22	
13.01	2-(acetyloxy)-1-(hydroxymethyl) ethyl acetate	$C_7H_{12}O_5$	0.85	
13.48	Phenol, 2-methoxy-5-(1-propenyl) (E)-(Isochavibetol)	$C_{10}H_{12}O_2$	13.50	
15.23	Methyl 4,7,10,13 hexadecatetraenoate	$C_{17}H_{26}O_2$	0.44	
16.38	2H-Pyran,2-(7-heptadecynyloxy) tetrahydro-	$C_{22}H_{40}O_2$	0.38	
16.81	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	$C_{18}H_{30}O_2$	0.78	
17.59	Methyl 4,6-tetradecadiynoate	$C_{15}H_{22}O_2$	0.57	
17.66	Androsta-1,4-dien-3-one,17-hydroxy -17-methyl-, (17à)-	$C_{20}H_{28}O_2$	0.32	
17.73	Panaxydol	$C_{17}H_{24}O_2$	0.45	
18.02	2H-Pyran,2-(7-heptadecynyloxy) tetrahydro-	$C_{22}H_{40}O_2$	1.12	
18.91	Idebenone	$C_{19}H_{30}O_5$	1.61	
20.41	Cedran-diol, 8S,13-	$C_{15}H_{26}O_2$	0.79	
20.53	Alloaromadendrene oxide-(1)	$C_{15}H_{24}O$	1.45	

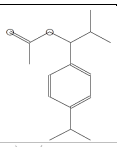
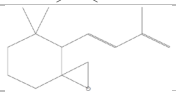
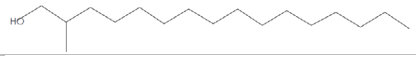
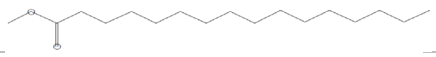
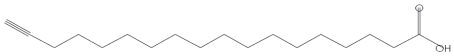
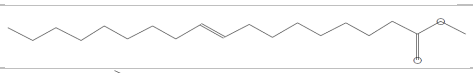
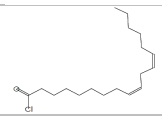
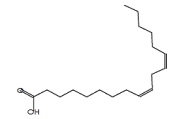
20.66	1-(4-isopropyl phenyl)-2-methyl propyl acetate	$C_{15}H_{22}O_2$	1.11	
20.85	1-Oxaspiro[2.5]octane,5,5-dimethyl-4-(3-methyl-1,3-butadienyl)-	$C_{14}H_{22}O$	0.78	
22.85	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	0.76	
26.52	Hexadecenoic acid, methyl ester	$C_{17}H_{34}O_2$	2.40	
27.40	Hexadecenoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	10.34	
29.56	17-Octadecynoic acid	$C_{18}H_{32}O_2$	1.02	
29.73	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	1.88	
29.85	9,12-Octadecadienoyl chloride,(Z,Z)-	$C_{18}H_{31}ClO$	0.75	
30.55	9,12-Octadecadienoic acid (Z,Z)- (Linoleic acid)	$C_{18}H_{32}O_2$	18.53	
31.04	Oleic Acid	$C_{19}H_{34}O_2$	1.85	

Table 4. The docking study results of ligands (5 compounds) binding with the predicted AChE domain

Compound name (ligand)	Molecular weight	Binding energy (kcal/mol)	H- bond residues (H- bond donors)	No. of H-Bond	Distance (A°)	Hydrophobic residues (amino acids with nonpolar side)	Distance (A°)
Donepezil (Standard)		-6.9	ARG 475 ARG 475 TYR 479	3	2.81546 2.94363 2.605	ARG 475 ALA 497	4.41805 4.84129
4-Allylphenol	134	-5.9	-	-	-	TRP 286 TRP 286 TYR 341	4.64636 3.8742 4.97545
Tricyclo[4.2.1.1(2,5)]deca-3,7-dien-9-one, 10-hydroxy-10-methyl-stereoisomer	176	-5.7	---	---	---	TYR 72 TRP 286 TRP 286 TRP 286 TRP 286 TRP 286 TYR 341	4.57494 5.18766 4.43309 4.98397 4.57027 4.65699 5.49672
Phenol, 2-methoxy-5-(1-propenyl)-, (E)-(Isochavibetol)	164	-5.2	---	---	---	TYR 382 ALA 397	4.19054 4.52232
Hexadecanoic acid (Palmitic acid)	256	-4.1	HIS 287 ASN 283 TRP 286	3	2.23775 2.94136 3.50875	---	---
9,12-Octadecadienoic acid (Z,Z)-(Linoleic acid)	280	-3.9	ARG 219 HIS 322 PHE 321	3	2.15137 2.42879 3.58014	ARG 219	3.99992

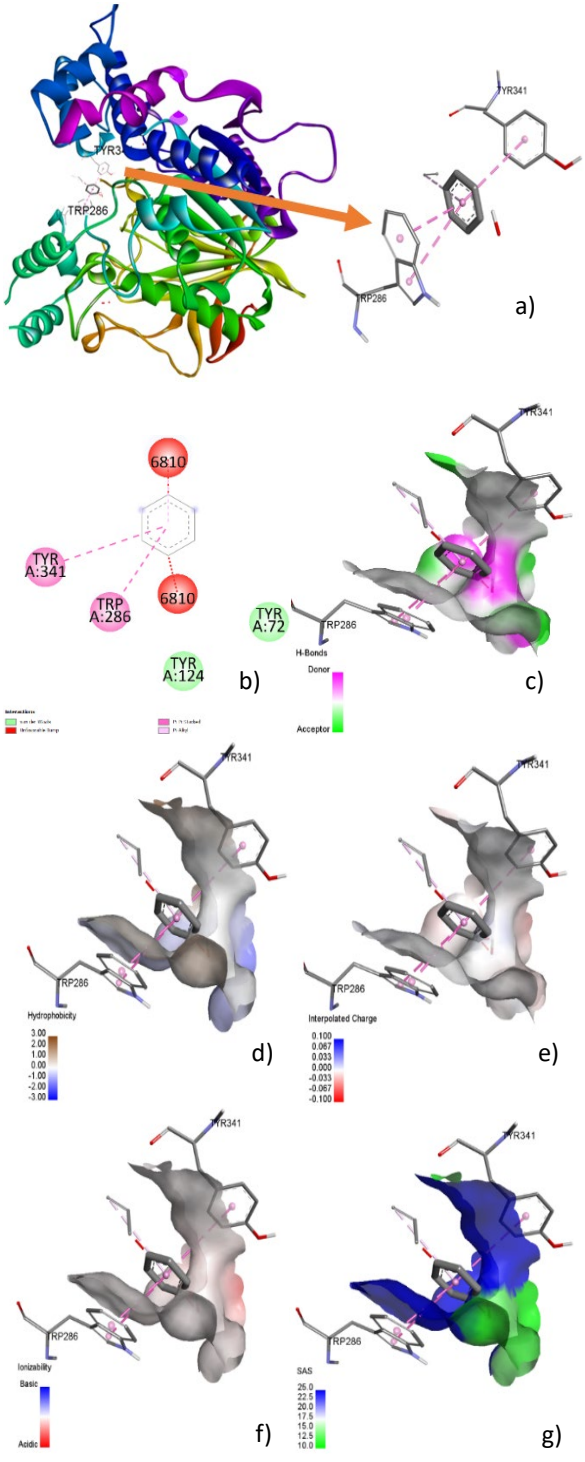


Figure 8. (a) PyMOL image of ribbon demonstrates the 4-Allylphenol and AChE domain docked complex (binding energy -5.7). (b) 2D chemical interaction of 4-Allylphenol with AChE domain. (c) Surface view showing the hydrogen bond interaction. (d) Hydrophobicity (e) Interpolated charges. (f) Ionizability. (g) SAS interactions of the docked complex.

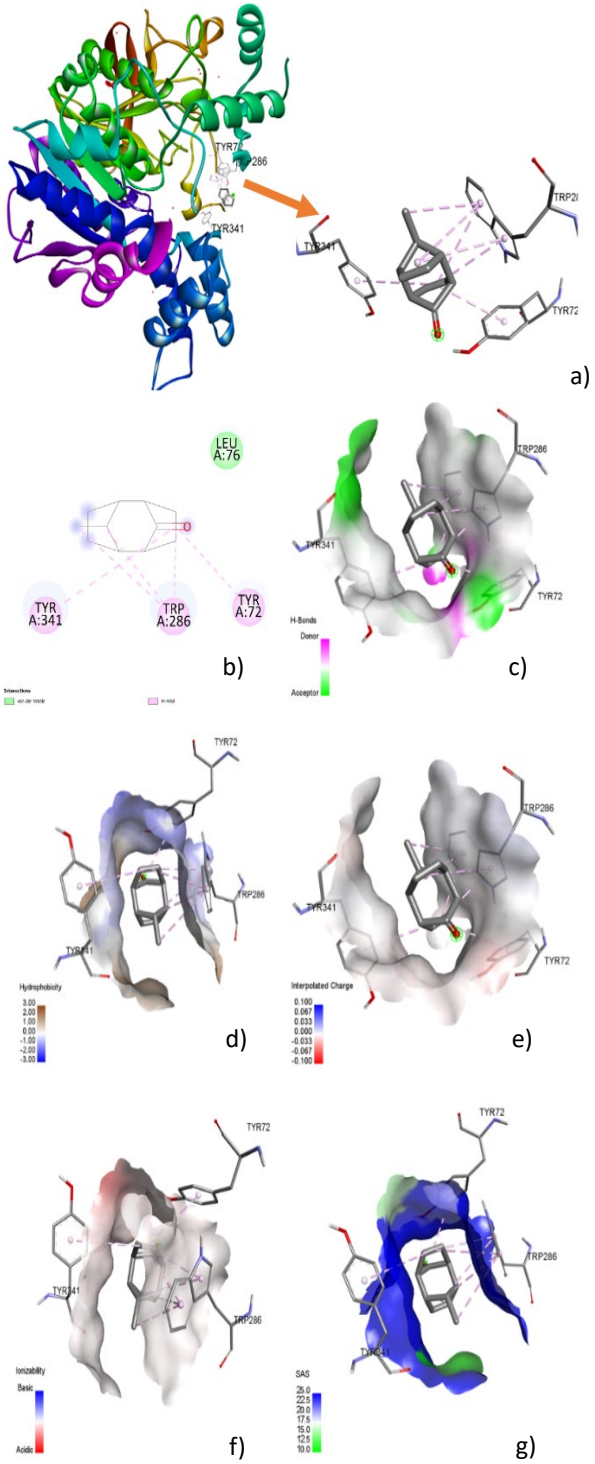


Figure 9. (a) PyMOL image of ribbon demonstrates the Tricyclo and AChE domain docked complex (binding energy -5.7). (b)2D chemical interaction of Tricyclo with AChE domain. (c) Surface view showing the hydrogen bond interaction. (d) Hydrophobicity (e) Interpolated charges. (f) Ionizability. (g) SAS interactions of the docked complex.

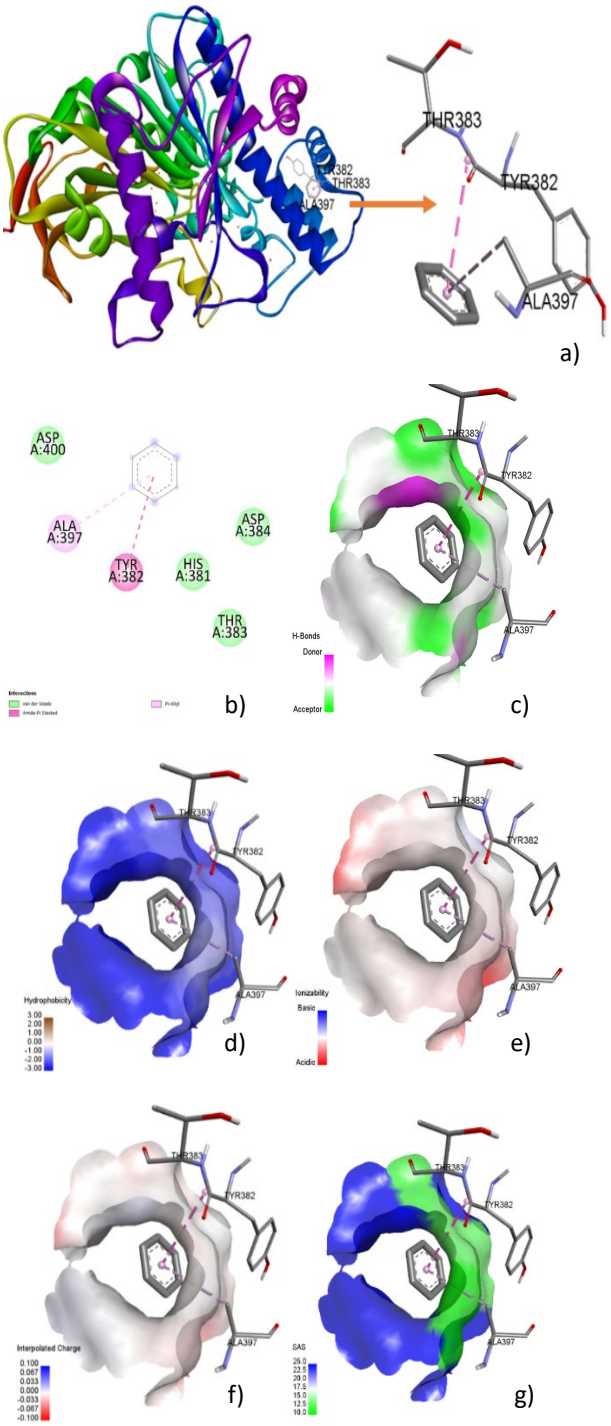


Figure 10. (a) PyMOL image of ribbon demonstrates the Isochavibetol and AChE domain docked complex (binding energy -5.2). (b)2D chemical interaction of Isochavibetol with AChE domain. (c) Surface view showing the hydrogen bond interaction. (d) Hydrophobicity (e) Interpolated charges. (f) Ionizability. (g) SAS interactions of the docked complex.

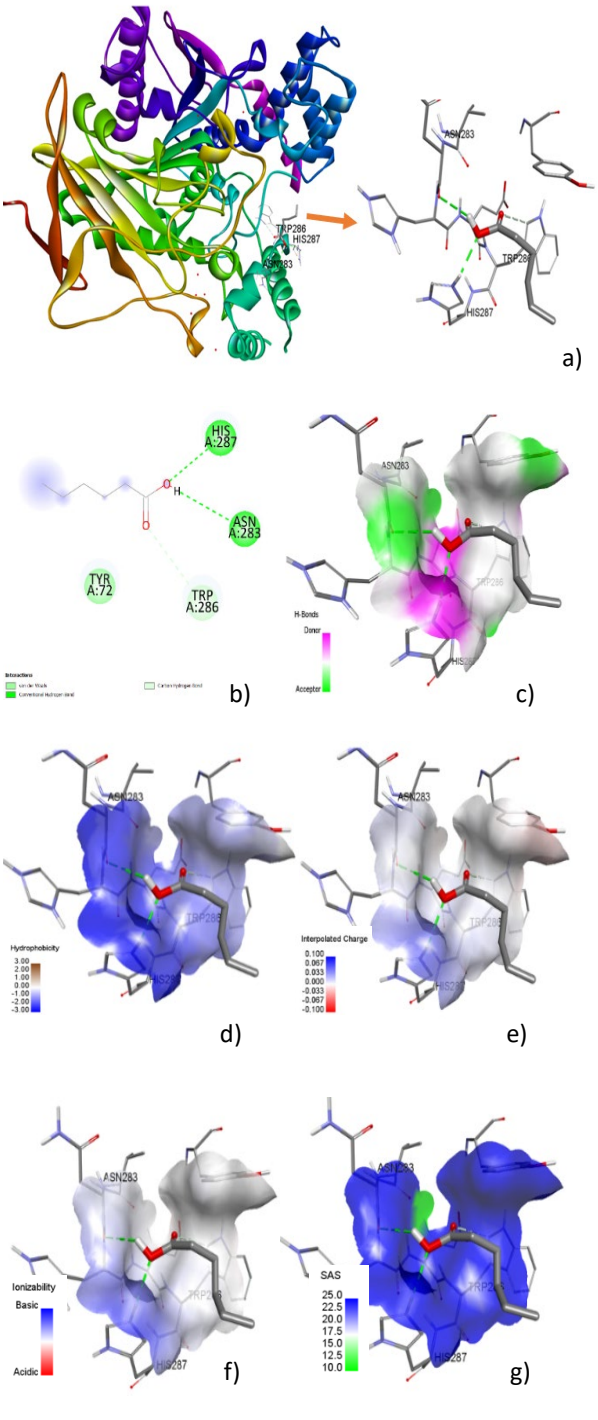


Figure 11. (a) PyMOL image of ribbon demonstrates the Palmitic Acid and AChE domain docked complex (binding energy -4.1). (b)2D chemical interaction of Palmitic Acid with AChE domain. (c) Surface view showing the hydrogen bond interaction. (d) Hydrophobicity. (e) Interpolated charges. (f) Ionizability. (g) SAS interactions of the docked complex.

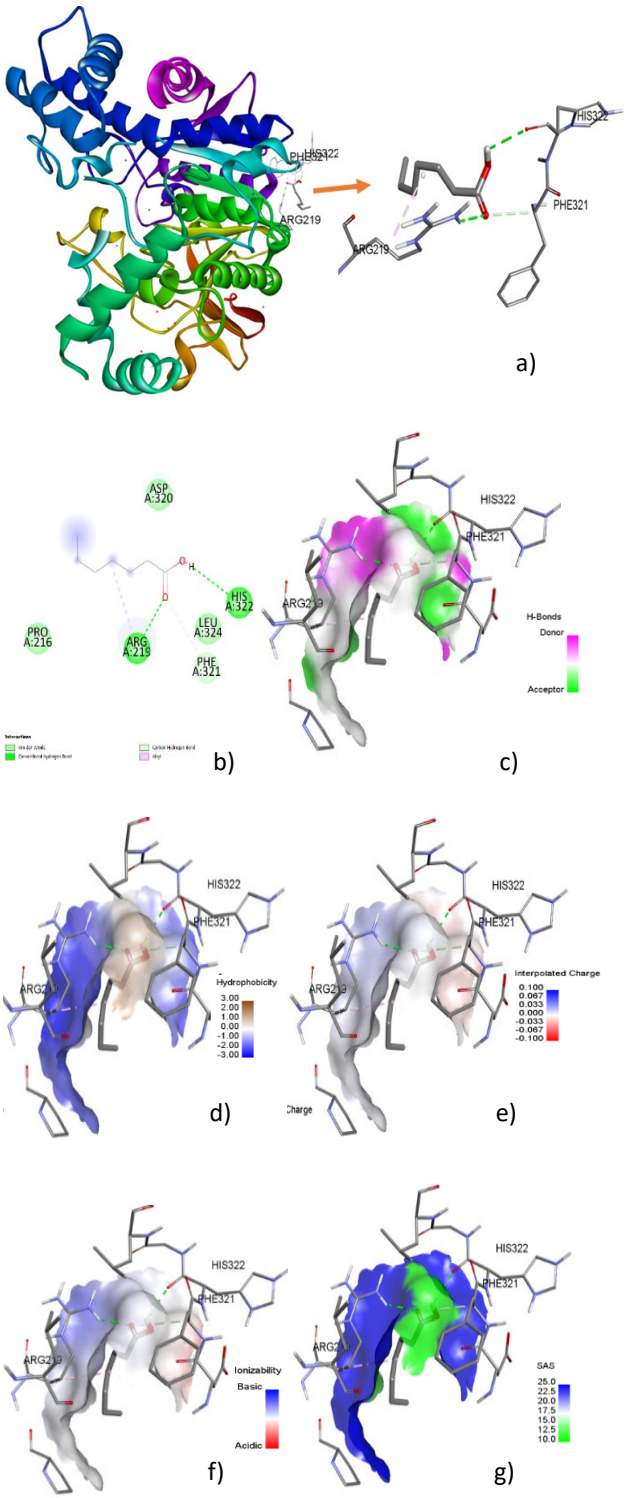


Figure 12. (a) PyMOL image of ribbon demonstrates the Linoleic Acid and AChE domain docked complex (binding energy -3.9). (b)2D chemical interaction of Linoleic Acid with AChE domain. (c) Surface view showing the hydrogen bond interaction. (d) Hydrophobicity. (e) Interpolated charges. (f) Ionizability. (g) SAS interactions of the docked complex.

exhibited the highest binding energy (-5.9, -5.7, and -5.2 Kcal/mol, respectively). Palmitic acid (4) followed with a binding energy of -4.1 kcal/mol and Linoleic acid (5) was the lowest binding energy with a value of -3.9 kcal/mol. 4-Allylphenol was anti-AChE activity via the interaction with 3 amino acids such as TRP 286, TRP 286 and TYR 341 forming 3 linkages while Tricyclo, the best ,was interacted with 7 amino acids (TYR 72, TRP 286, TRP 286, TRP 286, TRP 286, TRP 286 and TYR 341) forming 7 linkages. Isochavibetol interacted with 2 amino acids (TYR 382 and ALA 397), creating two bounds. Linoleic acid bound to AChE by interacting with only one amino acid ARG 219. Palmitic acid was no interaction with any amino acid. The Donepezil bound to AChE via an interaction with 2 amino acids (ARG 475 and ALA 497).

DISCUSSION

The research of traditional medicine and natural chemicals has become quite important as ailments become more common and worries about synthetic drugs develop. Though it presents some difficulties, this road also has interesting prospects (Bharathi *et al.,* 2019). Extensive resources for finding new molecules with unique modes of action might lead to a new generation of medications for treating illnesses with creative means including marine species (Montaser and Luesch, 2011). Algae make up 25% of the approximately 7000 recognized marine natural things, according to Kijjoa and Sawangwong (2004). Various phytochemical constituents including alkaloids, terpenoids, flavonoids, tannins, and phenols are revealed by phytochemical analysis of aqueous and other solvent extracts in investigated marine algae. Several factors affect the extraction yield such as material size, solvent type, solvent concentration, extraction technique and extraction duration (Aprillia *et al.,* 2023). This shows in our findings that for every solvent, active chemical elements like flavonoids, alkaloids, phenol and so on are either present in varying levels or absent. Furthermore, seasonal and geographical factors, nutrient content of the surrounding environment, quantity and quality of light, photoperiod and temperature affect the phytochemical content of algae (Zucchi and Necchi, 2001). *U. lactuca* and *C. officinalis* were found to contain elevated levels of protein and carbohydrates. Matanjun *et al.* (2009) and Omar *et al.* (2018) have previously reported a comparable result for certain maritime macroalgae. Dawczynski *et al.* (2007) reported that marine algae typically contained 10–30% dried weight protein. Red seaweeds typically exhibit the highest protein

content, which can reach up to 47% of their dried weight (García-Vaquero and Hayes, 2016). *Gelidium latifolium* and *Hypnea musciformis*, the red algae, as well as *C. officinalis*, were found to contain higher levels of phenolic compounds and flavonoids than *U. lactuca*, in accordance with Alghazeer *et al.* (2018). The use of liquid water at high temperatures is a valuable tool for the extraction of medium-low polarity compounds Herrero *et al.* (2006). An environmentally friendly substitute for traditional extraction techniques that are used on organic solvents is hot water extraction. It makes it possible to extract bioactive substances from a variety of natural sources such as proteins, polysaccharides and phenolic compounds (Plaza and Marina, 2023).

The increase of water temperature will produce a series of effects, including an improved mass transfer because of the increment of the solubility of the compounds present on the sample being extracted, as well as a decrease on the surface tension of water that allows a better penetration into the sample (Castro-Puyana *et al.,* 2013). It is also interesting to note the differences among the tested solvents, which can give an idea of the main compounds found in the extracts (according to the different solvent polarities tested). In both cases, the higher the polarity of the solvent employed the highest content of active ingredients (Plaza *et al.,* 2010).

U. lactuca extract had higher antimicrobial activity against Gram +ve bacteria (*S. aureus* and *B. subtilis*) than Gram -ve bacteria (*E. coli* and *P. aeruginosa*) compared to *C. officinalis,* this agreement with Kolanjinathan and Stella (2011) who reported that the *U. lactuca* extract showed antibacterial activity against *S. aureus*. Taskin *et al.* (2007) found that the extract of *C. officinalis* showed no effect against *S. aureus* and the extract of *U. rigida* was the most effective but in the case of *E. coli*, *C. officinalis* inhibition more than *U. rigida*. Mofeed *et al.* (2022) recorded that the antibacterial inhibition activity of *C. officinalis* on *S. aureus* was more than *E. coli* and *Salmonella*. The results showed that the extract of *C. officinalis* possesses an effective antibacterial activity against these pathogenic bacteria. Hamad *et al.* (2023) recorded that *C. officinalis* displayed considerable antibacterial activity against *B. cereus* more than *E. coli.* In our results, *U. lactuca* has more polysaccharide compounds compared with *C. officinalis*, Kumar *et al.* (2015) suggested that polysaccharides can invade the pathogens and damage the nucleus effectively. After entering the nucleus, it affects entire bacteria and arrests the cell cycle growth, leading to cell death. The more antibacterial activity effect of *U. lactuca* extract against pathogenic bacteria may be due to that. Or it may be because those tested algae having secondary bioactive substances, such as phenol and flavonoid compounds, which are known to possess antibacterial properties, this agreed with El-Din and El-Ahwany (2016) and Abou Gabal *et al.* (2021) who stated that the flavonoid composition of algae extracts responsible for the antimicrobial activities. The mechanism of each of these phytochemical compounds may be synergizes with each other to increase their effectiveness and activity in inhibiting growth or killing bacteria (Marselia *et al.,* 2015). The increased biological activities of marine sources depend on environmental factors like temperature, carbon, nitrogen content, pH, salinity and other organic nutrients (Rajivgandhi *et al.*, 2020). Generally, results revealed that *U. lactuca* extract had the highest inhibitory activity towards all bacteria strains than *C. officinalis* and this is contrary to Rao (1991) who reported that red algal extracts its greater antibacterial activity than green algae.

In the current work, green alga (*U. lactuca*) was found to be more effective against biofilm forming bacteria compared to red alga (*C. officinalis*). Our finding was consistent with Balasubramanian *et al.* (2011) who found that *U. lactuca* was more effective against biofilm forming bacteria compared to brown alga (*Padina* sp.). According to Rajivgandhi *et al.* (2021), phytochemical compounds from seaweed may reduce virulence genes including quorum sensing and biofilm development using internal inhibition of infections.

Among the most important uses of marine algae are those against cancer cell lines. Much earlier research has shown different algae's anticancer effects (Senousy *et al.,* 2020). The cytotoxicity test represents a rapid to evaluate the toxicity of extracts from natural sources such as seaweeds. The results of this assay revealed that the extract of *U. lactuca* and *C. officinalis* had promising cytotoxic activity against HepG-2 cancer cell lines but *U. lactuca* was more effective as an anticancer than *C. officinalis*. This was read in line with Zandi *et al.* (2010) and Ranahewa *et al.* (2019), who found encouraging suppression of cancer (lymphocytes and breast) from aqueous extracts of *Gracilaria corticata* (red algae) and *Ulva fasciata*. El-Kassas and El-Sheekh (2014) reported a cytotoxic effect of gold nanoparticles with an aqueous extract of *C. officinalis* against human breast cancer cells while Alghazeer *et al.* (2018)

reported a significantly high cytotoxic effect of *U. lactuca* extract against human colorectal carcinoma at a concentration range of 50–200 µg mL^{-1}. In this study, the values of IC_{50} from *U. lactuca* and *C. officinalis* extracts were a 182 ± 4.61µg/ml and 222± 5.98 µg/ml, respectively against hepatic cancer, that is compatible with Ryu *et al.* (2013) found that the IC_{50} of *U. fasciata* against colon cancer is 200 µg/ml while Moussavou *et al.* (2014) demonstrated that IC_{50} of *Gracilaria tenuistipitata* (red algae) against cancer was 309 µg/ml.

The computer technique known as molecular docking accurately forecasts the orientation and interactions between target enzymes and ligand molecules. Assessing the bonds created within the complex is the primary objectives of molecular docking. When evaluating a molecule's potential as a therapeutic candidate, it is essential. They help predict how a molecule will behave in the body, influencing its efficacy and safety (Adelusi *et al.,* 2022). Many investigations have previously attempted to develop ChEIs for the treatment of AD, either synthetically or via the exploitation of plants and fungi used in traditional medicine. In this work, the extract from *C. officinalis* was the most effective of AChE inhibitor, this agreement with Natarajan *et al.* (2009) which indicated that the extracts of *Gracilaria gracilis* (red algae) had high inhibitory activities on AChE. Suganthy *et al.* (2010) showed that *Hypnea valentiae* and *Ulva reticulata* inhibited this enzyme. Syad *et al.* (2012) indicated that the extract of *Gelidiella acerosa* (red algae) has a powerful AChE inhibitory activity under in vitro conditions. Bianco *et al.* (2015) represented that the species of red algae *Hypnea musciformis*, *Laurencia translucida* and *Palisada perforata* exhibited high inhibition against AChE activities while El Nemr *et al.* (2021) found that neither of the tested extracts of *C. mediterranea* or *U. lactuca* exhibited noticeable AChE inhibitory activity. Pereira and Valado (2023) called that red algae extracts had the potential to alleviate the pathophysiology associated with AD. According to Olasehinde *et al.* (2019), phenolic compounds can inhibit the enzyme acetylcholinesterase (AChE) and affect neurodegenerative diseases such as Alzheimer's disease.

In the current work GC-MS and molecular docking results all 5 tested compounds were molecular weight ranged from 134 to 280 and hydrogen bond donors below 5. According to Lipinski's rule, compounds have hydrogen bond donors fewer than 5 and molecular weights under 500 can have greater vivo absorption and bioavailability (El-Sayed *et al.,* 2023).Also, Hydrophobic residues contribute to binding affinity through non-specific interactions that drive the release of water molecules from the binding site, increasing the overall stability of the complex (Desantis *et al.,* 2022).

In this docking study of compounds into targeting protein enzyme, the compounds or ligands were able to contact and bind to the enzyme at various sites with binding energy. To gain a deeper understanding of the most effective inhibitors, the first and second compounds are the most effective based on the number of binding sites. Although 4-Allylphenol showed the best docking score against AchE (binding energy value of -5.9 kcal/mol) via interaction with 2 amino acids, creating 2 linkages but Tricyclo was binding with 7 amino acids, therefore 7 linkages. According to Phan *et al.* (2023), the most stable ligand-enzyme structure, the most binding sites, so Tricyclo was the best interactive inhibitor of AChE.

CONCLUSION

Our research indicates that marine algae extracts include potentially physiologically active compounds for the pharmaceutical industry to develop anti-pathogenic medications .*Ulva lactuca* has demonstrated promising antibacterial properties and potential benefits against HepG-2 cancer cells. The red algae, *C. officinalis*, has shown promise as a source of acetylcholinesterase inhibitors. Molecular docking studies have revealed that the alga's chemical Tricyclo is a potent inhibitor of the acetylcholinesterase (AChE) enzyme. Therefore, this may open promising horizons for its use in treating Alzheimer's disease and need to conduct applied clinical trials by pharmacology researchers.

REFERENCES

Adelusi, T. I., Oyedele, A. Q. K., Boyenle, I. D., Ogunlana, A. T., Adeyemi, R. O., Ukachi, C. D., and Abdul-Hammed, M. (2022). Molecular modeling in drug discovery. *Informatics in Medicine Unlocked*, *29*, 100880.

Abd El Hafez, M. S., AbdEl-Wahab, M. G., Seadawy, M. G., El-Hosseny, M. F., Beskales, O., Saber Ali Abdel-Hamid, A., and Ghareeb, D. A. (2022). Characterization, in-silico, and in-vitro study of a new steroid derivative from *Ophiocoma dentata* as a potential treatment for COVID-19. *Scientific Reports*, *12*(1), 5846. https://doi.org/10.1038/s41598-022-09809-2.

Abd El-Kareem, M. S., Rabbih, M. A. E. F., Selim, E. T. M., Elsherbiny, E. A. E. M., and El-Khateeb, A. Y. (2016). Application of GC/EIMS in combination with semi-empirical calculations for identification and

investigation of some volatile components in basil essential oil. *International Journal of Analytical Mass Spectrometry and Chromatography*, *4*(1), 14-25.

Abou Gabal, A., A Khaled, A., M Aboul-Ela, H., M Aly, H., and Kh Shalaby, O. (2021). Variation of photosynthetic pigments and biochemical screening in some seaweeds from Eastern Harbor, Alexandria, Egypt. *Egyptian Journal of Aquatic Biology and Fisheries*, *25*(1), 213-226.

Alghazeer, R., Enaeli, M., and Howell, N. K. (2018). Anticancer and Antioxidant Activities of Some Algae from Western Libyan Coast." Natural Science 10: 232-246.

Antunes, A. L. S., Trentin, D. S., Bonfanti, J. W., Pinto, C. C. F., Perez, L. R. R., Macedo, A. J., and Barth, A. L. (2010): Application of a feasible method for determination of biofilm antimicrobial susceptibility in staphylococci. *Apmis*, *118*(11), 873–877.

Aprillia, T. I., Adam, M. A., Supii, A. I., Mahardika, K., and Kusnadi, J. (2023). Antibacterial activity of crude extracts *Ulva lactuca* against *Staphyloccocus aureus and Propionibacterium acne*. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1273, No. 1, p. 012034). IOP Publishing.

Balasubramanian, V., Palanichamy, S., and Rajaram, R. (2011). Effects of certain seaweed extracts on the primary biofilm forming bacteria. *J. Mar. Biol. Assoc. India*, *53*(1), 94-100.

Behrens, M. I., Lendon, C., and Roe, C. M. (2009). A common biological mechanism in cancer and Alzheimer's disease. *Current Alzheimer Research*, *6*(3), 196-204.

Bharathi, N. P.; Jayalakshmi, M.; Amudha, P. and Vanitha, V. (2019): Phytochemical screening and in vitro antioxidant activity of the seagrass *Cymodocea serrulata*. Indian J. Geo-Marine Sci., 48(8).

Bianco, É. M., Krug, J. L., Zimath, P. L., Kroger, A., Paganelli, C. J., Boeder, A. M., and Rebelo, R. A. (2015). Antimicrobial (including antimollicutes), antioxidant and anticholinesterase activities of Brazilian and Spanish marine organisms–evaluation of extracts and pure compounds. *Revista Brasileira de Farmacognosia*, *25*(6), 668-676.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, *72*(1-2), 248-254.ist, with over

Castro-Puyana, M., Herrero, M., Mendiola, J. A., and Ibáñez, E. (2013). Subcritical water extraction of bioactive components from algae. *In Functional ingredients from algae for foods and nutraceuticals (pp.534-560). Woodhead Publishing.*

Chang, C. C.; Yang, M. H.; Wen, H. M. and Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of food and drug analysis*, *10* (178–182)

Chen, Z. R., Huang, J. B., Yang, S. L., and Hong, F. F. (2022). Role of cholinergic signaling in Alzheimer's disease. *Molecules*, *27*(6), 1816.

Dawczynski, C., Schäfer, U., Leiterer, M., and Jahreis, G. (2007). Nutritional and toxicological importance of macro, trace, and ultra-trace elements in algae food products. *Journal of agricultural and food chemistry*, *55*(25), 10470-10475.

Desantis, F., Miotto, M., Di Rienzo, L., Milanetti, E., and Ruocco, G. (2022). Spatial organization of hydrophobic and charged residues affects protein thermal stability and binding affinity. *Scientific Reports*, *12*(1), 12087.

Díaz, K., Espinoza, L., Carvajal, R., Conde-González, M., Niebla, V., Olea, A. F., and Coll, Y. (2020). Biological activities and molecular docking of brassinosteroids 24-norcholane type analogs. *International Journal of Molecular Sciences*, *21*(5), 1832. https://doi.org/10.3390/ijms21051832

Driver, J. A., Beiser, A., Au, R., Kreger, B. E., Splansky, G. L., Kurth, T., and Wolf, P. A. (2012). Inverse association between cancer and Alzheimer's disease: results from the Framingham Heart Study. *Bmj*, *344*.

El Nemr, A.; Shobier, A.H.; El Ashry, E.H.; Kandil, S.H. and Kenawy, E. (2021). Pharmacological screening of some Egyptian marine algae and encapsulation of their bioactive extracts in calcium alginate beads. *Egyptian Journal of Aquatic Biology and Fisheries*, *25*(2), 1025-1052.

El-Bendary, M. A.; Afifi, S. S.; Moharam, M. E.; Abo El-Ola, S. M.; Salama, A.; Omara, E. A., and Gawdat, N. A. (2021). Biosynthesis of silver nanoparticles using isolated Bacillus subtilis: characterization, antimicrobial activity, cytotoxicity, and their performance as antimicrobial agent for textile materials. *Preparative Biochemistry & Biotechnology*, *51*(1), 54-68. https://doi.org/10.1080/10826068.2020.1789992

El-Din, S. M. M., and El-Ahwany, A. M. (2016). Bioactivity and phytochemical constituents of marine red seaweeds (*Jania rubens, Corallina mediterranea and Pterocladia capillacea*). *Journal of Taibah University for Science*, *10*(4), 471-484. https://doi.org/10.1016/j.jtusci.2015.06.004

El-Kassas, H. Y., and El-Sheekh, M. M. (2014). Cytotoxic activity of biosynthesized gold nanoparticles with an extract of the red seaweed Corallina officinalis on the MCF-7 human breast cancer cell line. *Asian Pacific journal of cancer prevention*, *15*(10), 4311-4317. https://doi.org/10.7314/apjcp.2014.15.10.4311

Ellman, G. L., Courtney, K. D., Andres Jr, V., and Featherstone, R. M. (1961): A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical pharmacology*, *7*(2), 88-95.

Elmezayen, A. D., Al-Obaidi, A., Şahin, A. T., and Yelekçi, K. (2021). Drug repurposing for coronavirus (COVID-19): in silico screening of known drugs against coronavirus 3CL hydrolase and protease enzymes. *Journal of Biomolecular Structure and Dynamics*, *39*(8), 2980-2992. https://doi.org/10.1080/07391102.2020.1758791

El-Sayed, H., Hamada, M. A., Elhenawy, A. A., Sonbol, H., and Abdelsalam, A. (2023). Acetylcholine esterase inhibitory effect, antimicrobial, antioxidant,

metabolomic profiling, and an in silico study of non-polar extract of the halotolerant marine fungus *Penicillium chrysogenum* MZ945518. *Microorganisms*, *11*(3), 769.

El-Sheekh, M. M., Nassef, M., Bases, E., Shafay, S. E., and El-Shenody, R. (2022). Antitumor immunity and therapeutic properties of marine seaweeds-derived extracts in the treatment of cancer. *Cancer Cell International*, *22*(1), 267.

Estrada, L. D., Ahumada, P., Cabrera, D., and Arab, J. P. (2019). Liver dysfunction as a novel player in Alzheimer's progression: looking outside the brain. *Frontiers in aging neuroscience*, *11*, 174.

Evstigneyev, E. I. (2017): Quantification of polysaccharides in vegetable raw materials and lignin preparations. Russian Journal of Bioorganic Chemistry, 43(7), 732-736.

Farooqi, A. A., Butt, G., and Razzaq, Z. (2012). Algae extracts and methyl jasmonate anti-cancer activities in prostate cancer: choreographers of 'the dance macabre'. *Cancer cell international*, *12*(1), 1-6.

Frozza, R. L., Lourenco, M. V., and De Felice, F. G. (2018). Challenges for Alzheimer's disease therapy: insights from novel mechanisms beyond memory defects. Front Neurosci. 2018; 12: 37.

García-Vaquero, M., and Hayes, M. (2016). Red and green macroalgae for fish and animal feed and human functional food development. *Food Reviews International*, *32*(1), 15-45.

Gomha, S., Riyadh, S. M., Mahmmoud, E. A., and Elaasser, M. M. (2015). Synthesis and anticancer activities of thiazoles, 1, 3-thiazines, and thiazolidine using chitosan-grafted-poly (vinylpyridine) as basic catalyst. *Heterocycles*, *91*(6), 1227-1243.

Grigoriadis, I. (2020). Chern-Simons Topology Geometrics for the generation of the Roccustyrna TM molecule, a ligand targeting COVID-19-SARS-COV-2 SPIKE D614G binding sites.

Hamad, G. M., Samy, H., Mehany, T., Korma, S. A., Eskander, M., Tawfik, R. G., and Khalifa, E. (2023). Utilization of Algae Extracts as Natural Antibacterial and Antioxidants for Controlling Foodborne Bacteria in Meat Products. *Foods*, *12*(17), 3281.

Hasan, M., Imran, M. A. S., Bhuiyan, F. R., Ahmed, S. R., Shanzana, P., Moli, M. A., and Dabi, S. B. (2019): Phytochemical constituency profiling and antimicrobial activity screening of seaweeds extracts collected from the Bay of Bengal Sea coasts. bioRxiv, 680348.

Herrero, M., Ibáñez, E., Cifuentes, A., Reglero, G., and Santoyo, S. (2006). *Dunaliella salina* microalga pressurized liquid extracts as potential antimicrobials. *Journal of food protection*, *69*(10), 2471-2477.

Jha, B.; Reddy, C. R. K.; Thakur, M. C. and Rao, M. U. (2009): *Seaweeds of India: the diversity and distribution of seaweeds of Gujarat coast* (Vol. 3). Springer Science & Business Media.

Kalasariya, H. S., Maya-Ramírez, C. E., Cotas, J., and Pereira, L. (2024). Cosmeceutical Significance of Seaweed: A Focus on Carbohydrates and Peptides in Skin Applications. *Phycology 4*(2), 276-313.

Kang, H. S., Kim, J. H., Lim, H., Kim, J. H., Noh, H. M., Choi, H. G., and Kwon, M. J. (2023). Alzheimer's Disease and Different Types of Cancer Likelihood: Unveiling Disparities and Potential Protective Effects in a Korean Cohort Study. *Cancers*, *15*(18), 4615.

Khalil Mohamed, A. A., Mohamed, E. A. M., and Mohamed, A. F. (2023). Evaluation of Anticancer Activities of *Ulva Lactuca* Ethanolic Extract on Colorectal Cancer Cells. *Egyptian Journal of Chemistry*, *66*(13), 531-539.

Kijjoa, A., and Sawangwong, P. (2004). Drugs and cosmetics from the sea. *Marine Drugs*, *2*(2), 73-82.

Kolanjinathan, K., and Stella, D. (2011). Comparative studies on antimicrobial activity of Ulva reticulata and Ulva lactuca against human pathogens. *Int. J. Pharm. Biol. Arch*, *2*(6), 1738-1744.

Kumar, S.; Sahoo, D. and Levine, I. (2015): Assessment of nutritional value in a brown seaweed *Sargassum wightii* and their seasonal variations. *Algal Research*, *9*, 117-125.

Labes, A. (2023). Marine resources offer new compounds and strategies for the treatment of skin and soft tissue infections. *Marine Drugs*, *21*(7), 387.

Liu, Z., Gao, T., Yang, Y., Meng, F., Zhan, F., Jiang, Q., and Sun, X. (2019). Anti-cancer activity of porphyran and carrageenan from red seaweeds. *Molecules*, *24*(23), 4286.

Marselia, S., Wibowo, M. A., and Arreneuz, S. (2015). Aktivitas antibakteri ekstrak daun soma (ploiarium alternifolium melch) terhadap propionibacterium acnes. *Jurnal Kimia Khatulistiwa*, *4*(4).

Matanjun, P., Mohamed, S., Mustapha, N. M., and Muhammad, K. (2009). Nutrient content of tropical edible seaweeds, *Eucheuma cottonii*, *Caulerpa lentillifera* and *Sargassum polycystum*. *Journal of Applied Phycology*, *21*, 75-80.

Meinita, M. D. N., Harwanto, D., and Choi, J. S. (2022). Seaweed exhibits therapeutic properties against chronic diseases: An overview. Applied Sciences, 12(5), 2638.

Mofeed, J., Deyab, M., Sabry, A. E. N., and Ward, F. (2021). In vitro anticancer activity of five marine seaweeds extract from Egypt against human breast and colon cancer cell lines.

Mofeed, J., Deyab, M., Mohamed, A., Moustafa, M., Negm, S., and El-Bilawy, E. (2022). Antimicrobial activities of three seaweeds extract against some human viral and bacterial pathogens. *Biocell*, *46*(1), 247.

Montaser, R., and Luesch, H. (2011). Marine natural products: a new wave of drugs. *Future medicinal chemistry*, *3*(12), 1475-1489.

Mosmann, T. (1983): Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods, 65(1-2), 55-63.

Moussavou, G., Kwak, D. H., Obiang-Obonou, B. W., Maranguy, C. A. O., Dinzouna-Boutamba, S. D., Lee, D. H., and Choo, Y. K. (2014). Anticancer effects of different seaweeds on human colon and breast cancers. *Marine drugs*, *12*(9), 4898-4911.

Natarajan, S., Shanmugiahthevar, K. P., and Kasi, P. D. (2009). Cholinesterase inhibitors from *Sargassum* and *Gracilaria gracilis*: seaweeds inhabiting South Indian coastal areas (Hare Island, Gulf of Mannar). *Natural Product Research*, *23*(4), 355-369.

Olasehinde, T. A., Olaniran, A. O., and Okoh, A. I. (2019). Macroalgae as a valuable source of naturally occurring bioactive compounds for the treatment of Alzheimer's disease. *Marine drugs*, *17*(11), 609.

Omar, H. H., Al-Judaibiand, A., and El-Gendy, A. (2018). Antimicrobial, antioxidant, anticancer activity and phytochemical analysis of the red alga, Laurencia papillosa. *International Journal of Pharmacology*, *14*(4), 572-583.

Ording, A. G., Veres, K., Horváth-Puhó, E., Glymour, M. M., Rørth, M., Henderson, V. W., and Sørensen, H. T. (2019). Alzheimer's and Parkinson's diseases and the risk of cancer: A cohort study. *Journal of Alzheimer's Disease*, *72*(4), 1269-1277.

Osman, H., Kumar, R. S., Basiri, A., and Murugaiyah, V. (2014): Ionic liquid mediated synthesis of mono-and bis-spirooxindole-hexahydropyrrolidines as cholinesterase inhibitors and their molecular docking studies. *Bioorganic & medicinal chemistry*, *22*(4), 1318-1328.

Pappou, S., Dardavila, M. M., Savvidou, M. G., Louli, V., Magoulas, K., and Voutsas, E. (2022). Extraction of bioactive compounds from Ulva lactuca. *Applied Sciences*, *12*(4), 2117.

Pawar, H. A., and Mello, P. M. (2011). Spectrophotometric estimation of total polysaccharides in Cassia tora gum. Journal of Applied Pharmaceutical Science, (Issue), 93-95.

Pereira, L., and Valado, A. (2023). From the ocean to the brain: harnessing the power of marine algae for neuroprotection and therapeutic advances. *Exploration of Neuroprotective Therapy*, *3*(6), 409-428.

Phan, T. K. P., Wang, S. L., Nguyen, Q. V., Phan, T. Q., Nguyen, T. T., Tran, T. T. T., and Doan, M. D. (2023). Assessment of the Chemical Profile and Potential Medical Effects of a Flavonoid-Rich Extract of Eclipta Prostrata L. Collected in the Central Highlands of Vietnam. *Pharmaceuticals*, *16*(10), 1476.

Plaza, M., and Marina, M. L. (2023). Pressurized hot water extraction of bioactives. *TrAC Trends in Analytical Chemistry,* 166, 117201.

Plaza, M., Santoyo, S., Jaime, L., Reina, G. G. B., Herrero, M., Señoráns, F. J., and Ibáñez, E. (2010). Screening for bioactive compounds from algae. *Journal of pharmaceutical and biomedical analysis*, *51*(2), 450-455.

Qader, M. M.; Hamed, A. A.; Soldatou, S.; Abdelraof, M.; Elawady, M. E.; Hassane, A. S. I.; Belbahri, L.; Ebel, R. and Rateb, M. E. (2021). Antimicrobial and antibiofilm activities of the fungal metabolites isolated from the marine endophytes *Epicoccum nigrum* M13 and *Alternaria alternata* 13A. *Marine Drugs*, 19(4), 232. https://doi.org/10.3390/MD19040232

Rajivgandhi, G. N.; Kanisha, C. C.; Ramachandran, G.; Manoharan, N.; Mothana, R. A.; Siddiqui, N. A., and Almarfadi, O. M. (2021): Phytochemical screening and antioxidant activity of *Sargassum wightii* enhances the anti-bacterial activity against *Pseudomonas aeruginosa*. *Saudi Journal of Biological Sciences*, *28*(3), 1763-1769.

Rajivgandhi, G. N.; Ramachandran, G.; Maruthupandy, M.; Manoharan, N.; Alharbi, N. S.; Kadaikunnan, S., and Li, W. J. (2020): Antioxidant, anti-bacterial and anti-biofilm activity of biosynthesized silver nanoparticles using *Gracilaria corticata* against biofilm producing K. pneumoniae. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *600*, 124830.

Ranahewa, T. H., Premarathna, A. D., Wijesundara, R. M. K., Wijewardana, V., Jayasooriya, A. P. and Rajapakse, R. P. V. J. (2019). Biochemical composition and anticancer effect of different seaweed species (in-vitro and in-vivo studies). *Sustainable Marine Structures*, *1*(2), 5-11.

Rao, P. P. S. (1991). Biological investigation of Indian marine algae, screening of some green, red and brown seaweeds for their antimicrobial activity. *Seaweed Res. Util*, *14*(1), 37-43.

Rosa, G. P., Tavares, W. R., Sousa, P. M., Pagès, A. K., Seca, A. M., and Pinto, D. C. (2019). Seaweed secondary metabolites with beneficial health effects: An overview of successes in vivo studies and clinical trials. *Marine drugs*, *18*(1), 8.

Ryu, M. J., Kim, A. D., Kang, K. A., Chung, H. S., Kim, H. S., Suh, I. S., and Hyun, J. W. (2013). The green algae Ulva fasciata Delile extract induces apoptotic cell death in human colon cancer cells. *In Vitro Cellular & Developmental Biology-Animal*, *49*, 74-81. https://doi.org/10.1007/s11626-012-9547-3

Senousy, H. H., Abd Ellatif, S., and Ali, S. (2020). Assessment of the antioxidant and anticancer potential of different isolated strains of cyanobacteria and microalgae from soil and agriculture drain water. *Environmental Science and Pollution Research*, *27*, 18463-18474. https://doi.org/10.1007/s11356-020-08332-z

Siddique, M.H.; Aslam, B.; Imran, M.; Ashraf, A.; Nadeem, H.; Hayat, S. and Muzammil, S. (2020). Effect of silver nanoparticles on biofilm formation and eps production of multidrug-resistant Klebsiella pneumoniae. Biomed Res Int.:9.

Singleton, V. L., and Rossi, J. A. (1965): Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, *16*(3), 144-158.

Singleton, V. L., Orthofer, R., and Lamuela - Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in enzymology*, *299*, 152-178.

Sivasankari, S., Venkatesalu, V., Anantharaj, M., and Chandrasekaran, M. (2006). Effect of seaweed extracts on the growth and biochemical constituents of *Vigna sinensis*. *Bioresource technology*, *97*(14), 1745-1751.

SPSS. (2012): IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.

Strandwitz, P. (2018). Neurotransmitter modulation by the gut microbiota. *Brain research*, *1693*, 128-133.

Suganthy, N., Pandian, S. K., and Devi, K. P. (2010). Neuroprotective effect of seaweeds inhabiting South Indian coastal area (Hare Island, Gulf of Mannar Marine Biosphere Reserve): Cholinesterase inhibitory effect of *Hypnea valentiae* and *Ulva reticulata*. *Neuroscience letters*, *468*(3), 216-219.

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., and Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, *71*(3), 209-249.

Syad, A. N., Shunmugiah, K. P., and Kasi, P. D. (2012). Assessment of anticholinesterase activity of *Gelidiella acerosa*: implications for its therapeutic potential against Alzheimer's disease. *Evidence-Based Complementary and Alternative Medicine*, *2012*(1), 497242.

Taskin, E., Ozturk, M., and Kurt, O. (2007). Antibacterial activities of some marine algae from the Aegean Sea (Turkey). *African journal of Biotechnology*, *6*(24), 2746.

Thandivel, S., Rajan, P., Gunasekar, T., Arjunan, A., Khute, S., Kareti, S. R., and Paranthaman, S. (2024). In silico molecular docking and dynamic simulation of anti-cholinesterase compounds from the extract of *Catunaregam spinosa* for possible treatment of Alzheimer's disease. *Heliyon*, *10*(7). https://doi.org/10.1016/j.heliyon.2024.e27880

Trease, G.E. and W.C Evans (1989): Phytochemical screening. In: Textbook of Pharmacognosy, Trease, G.E. and W.C Evans (Eds.). 10th Edn. Bailliere Tindal Limited, London, pp: 541. microbiomes as asou

Van Handel, E. M. I. L. E. (1985): Rapid determination of glycogen and sugars in Mosquitoes. J. Am. Mosq. Control Assoc, 1(3), 299-301.

Vinodkumar, M., and Packirisamy, A. S. B. (2024). Exploration of Bioactive Compounds from *Sargassum myriocystum*; A Novel Approach on Catalytic Inhibition Against Free Radical Formation and Glucose Elevation. *Topics in Catalysis*, *67*(1), 60-73.

Zandi, K., Ahmadzadeh, S., Tajbakhsh, S., Rastian, Z., Yousefi, F., Farshadpour, F., and Sartavi, K. (2010). Anticancer activity of *Sargassum oligocystum* water extract against human cancer cell lines. *European review for medical and pharmacological sciences*, *14*, 669-673.

Zucchi, M. R., and Necchi Jr, O. (2001). Effects of temperature, irradiance and photoperiod on growth and pigment content in some freshwater red algae in culture. *Phycological Research*, *49*(2), 103-114.