Evaluation of Codium fragile and Turbinaria conoides seaweeds-extracts as a novel larvicidal agent against the Culex quinquefasciatus

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Abstract

Marine algae possess abundant reserves of bioactive compounds that can host secondary metabolites exhibiting both structural and biological activity. In our present study, the aqueous, ethanol, methanol, and chloroform extract of seaweed such as *Codium fragile* and *Turbinaria conoides*was employed to determine the phytochemical, GC-MS, antioxidant, and larvicidal activity. The phytochemical compounds showed *C. fragile* and *T. conoides* ethanolic extract revealed the existence of fourteen numbers of compounds compared to the others. The GC-MS analysis was performed on the ethanolic extract of *C fragile* and *T. conoides*showed the presence of each of twenty different compounds. *C. fragile* of Hexadecanoic Acid (27.79%), 4-Methanocycloocta[d]Pyridaz (7.39%), 1,1-Dimethoxy-2-Hepten-7-Al (7.04 %) and Octadecanoic Acid, (5.9%). The *T. conaids* of Dibutyl phthalate (69.56%), 1,2-Benzenedicarboxylic acid (14.01%) and Phthalic acid (2.58 %) were present as the major constituent with the highest area percentage. The antioxidant activity of different solvents the *T. conoides* ethanolic extract was found to have the highest DPPH (81.48 ± 1.0%), ABTS (88.29±1.0%) and Hydroxyl radical scavenging (86.51±1.0%) activity followed by other solvent extracts. The chloroform extract of *C fragile* and *T. conoides* showed effective *Cx. quinquefasciatus* larvicidal activity. Therefore, the bioactive substances derived from the ethanolic extracts could be used for the antioxidant and larvicidal activity of chloroform extract which will overcome the synthetic insecticides negative impact on the environment. The seaweed possesses the potential to serve as a promising alternative source for controlling mosquitoes due to its larvicidal properties.

Introduction

Marine products have been identified as potential sources of novel bioactive substances in the last few years. The main sources investigated include seaweed potential bioactive compounds of interest within the therapeutic agents in human and aquaculture (Perez et al. 2016). A wide range of natural antioxidant, antimicrobial, anticancer and other health-promoting compounds have been extracted from algae (Fayaz et al. 2005; Duan et al. 2006; Blunt et al. 2013).

Antioxidants have several functions in biological structures, encompassing protection against oxidative damage and involvement in the primary signaling pathways of cells. A crucial function of antioxidants within cells is to hinder damage caused by reactive oxygen species (Kumar et al. 2008). Free radicals are accountable for the aging process, and their presence in excess constitutes the cause of different diseases in humans. The antioxidant substances that scavenge free radicals play an important function in the prevention of free radical-induced disease (Ismail and Hong 2002).

Reactive oxygen species like hydroxyl, superoxide and peroxyl radicals can cause extensive oxidative damage to human cells, leading to various degenerative conditions, cancer, and other diseases (Reaven and Witzum 1996; Aroma 1999).

Seaweed and Seaweed-derived compounds have a very wide range of potential for natural antioxidants (Boonchum et al. 2011; Saeidnia et al. 2012). Natural antioxidants, such as phenolic compounds as well as other biochemical compounds are responsible for antioxidant activity (Evans et al. 1997; Yogesh et al. 2021).

It's concerning to see that mosquito-borne diseases are still posing a significant threat to public health, especially in tropical and subtropical regions. Experts have predicted that by 2050, almost half of the world's population will be at risk of infection (Kraemer et al. 2019; Lim et al. 2023). To combat this, there has been a shift towards eco-friendly alternatives through biological control methods, rather than relying on chemical insecticides. One promising approach is to explore floral biodiversity to source potential insecticides of marine origin. Unlike conventional insecticides, which often rely on a single active ingredient, algae-derived insecticides are typically composed of seaweed blends containing various bioactive compounds. This has the potential to provide a more effective and sustainable solution to control mosquito populations.

Plants and seaweeds produce secondary metabolites known as phytochemicals as a defense mechanism against herbivorous insects. These phytochemicals have the potential to act as natural insecticides, making them a viable alternative to synthetic products (Beula et al. 2011; Ghosh et al. 2012; Guedes et al. 2014). There are several advantages to using phytochemicals, including their ability to biodegrade quickly, their low cost compared to synthetic insecticides, their minimal harm to non-target organisms, and their multiple target sites that reduce the likelihood of resistance development (Shaalan et al. 2005; Perumalsamy et al. 2015). Currently, phytochemicals make up approximately 1% of the global pesticide market. Mosquitocidal properties have been reported for about 334 plant species from 2000 recognised to be sources of secondary metabolites (Ghosh et al. 2012; Sharma et al. 2015). Although they can also have growth-inhibiting effects, they are typically neurotoxic (Shaalan et al. 2005).

In this study, we evaluate phytochemical compound screening, GC-MS and antioxidant activity in vitro such as aqueous, ethanol, methanol and chloroform extract of *Codium fragile* and *Turbinaria conoides* obtained from Tiruchendur coast, Thoothukudi district, Tamil Nadu was undertaken at present. One mosquito larva evaluates the larvicidal property of seaweed extract against Cx. quinquefasciatus.

Materials and Methods

Seaweed materials

The seaweed species collected from the coastal area of Tiruchendur, Thoothukudi District, Tamil Nadu, India (8.4863° N, 78.1221° E), and were identified as; *Codium fragile* and *Turbinaria conoides* with the help of the Central Marine Fisheries Research Institute (CMFRI), Mandapam Regional Centre (Government of India). Seaweed samples were thoroughly cleaned to remove any unwanted parts, rinsed with sterile water, and then air-dried in the shade before being ground into a fine powder. This process is similar to the method described by Gonzalez del Val et al. (2001).

Preparation of seaweed extracts

The seaweed powder was subjected to consecutive soxhlet extraction with organic solvents with increasing order of polarity starting with aqueous, followed by ethanol, methanol, and chloroform respectively. The resulting extract was then evaporated to a temperature of 50 C and stored in a sterile container in the refrigerator until it was ready for further use.

2.3. Qualitative phytochemical analysis

The study involved identifying sixteen different phytochemicals, including steroids, tannins, terpenoids, flavonoids, saponins, alkaloids, reducing sugar, cardiac glycosides, coumarins, phlobatannins, anthraquinones, quinones, glycosides, phenols, anthocyanin, and betacyanin, as described by Harborne (1973) and Sadasivam and Manikam (1996).

GC-MS analysis

The GC-MS analysis, it is interesting to note that the Shimadzu QP-2010 Plus with Thermal Desorption System TD 20 was used for the analysis. The electron ionization mode with ionization energy of 70 eV was used for MS detection. The mass range for the analysis was set at m/z 50-550, and an HP-5MS capillary column was used for GC-MS. Helium was used as the carrier gas. I understand that vital compounds were identified by their retention times and mass fragmentation patterns using data of standards at WILEY8.LIB.

In vitro antioxidant activity

DPPH radical scavenging activity

DPPH radical scavenging activity was adopted by Singh et al. (2002) with slight modifications.DPPH is a violet coloured stable free radical solution that decolorizes into colourless solution with the addition of a substrate that can donate a hydrogen atom. Briefly, a total of 1 mL of 0.135 mM DPPH prepared in methanol was mixed with 1.0 mL of extract ranging from 20 to 100 µg mL^{-1}. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. Changes in the absorbance of the samples were measured at 517 nm. The inhibition percentage was calculated using the following formula:

% Radical scavenging activity = $[(Abs_{con} - Abs_{sample})/ (Abs_{con})] \times 100$

Where, Abs_{con} is the absorbance of control; Abs_{sample} is the absorbance of test sample/ standard

ABTS radical scavenging activity

The ABTS scavenging activity of the extracts was performed using the method described by Re et al. (1999). The antioxidant agents present in the extract reduces blue- green chromogen of $ABTS^{\cdot+}$ to ABTS and decolourizes it. The working solution was prepared by mixing stock solutions of 7 mM ABTS and 2.4 mM potassium persulphate in equal amounts and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 mL $ABTS^{\cdot+}$ solution with 60 mL methanol to obtain an absorbance of 0.706 ± 0.01 units at 734 nm using a spectrophotometer. Fresh ABTS solution was prepared for each assay. A total of 1

mL of freshly prepared ABTS solution was added to 1 mL of the extracts, the reaction mixture was vortexed for 10 sec and the absorbance was measured at 734 nm after 6 min. The inhibition percentage was calculated using the following formula and compared with that of the standard BHT.

% Radical scavenging activity = $[(Abs_{con} - Abs_{sample})/ (Abs_{con})] \times 100$

where, Abs_{con} is the absorbance of control; Abs_{sample} is the absorbance of test sample/standard

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was determined according to a slightly modified method of the 2-deoxy-ribose oxidation methods (Chung et al. 1997). Hydroxyl radical was generated by Fenton reaction in the presence of $FeSO_4 \cdot 7H_2O$. A reaction mixture containing 0.2 mL each of 10 mM $FeSO_4 \cdot 7H_2O$, 10 mM EDTA and 10 mM 20 deoxyribose was mixed with 0.2 mL of the extract solution, and 0.1 M phosphate buffer (pH 7.4) was added into the reaction mixture until the total volume reached 1.8 mL. Then 0.2 mL of 10 mM H_2O_2 was finally added to the reaction mixture and incubated at 37° C for 4 h. After the incubation 1 mL of 2.8% TCA (Trichloracetic acid) and 1.0% TBA (Thiobarbituric acid) were added. Then, the mixture was placed in a boiling water bath for 10 min. Absorbance was measured at 532 nm.

% Radical scavenging activity = $[(Abs_{con} - Abs_{sample})/ (Abs_{con})] \times 100$

where, Abs_{con} is the absorbance of control; Abs_{sample} is the absorbance of test sample/ standard

Collection of mosquito samples and rearing

The mosquito eggs of *Culex quinquefasciatus* were collected from Tiruchendur (8.500038°N, 78.117141°E), Thoothukudi District, Tamil Nadu, India. Collected egg samples were brought to the testing laboratory and transferred to the enamel trays each containing 300 mL of water and allowed to hatch. Mosquito larvae were fed with 500 mL of deionized water containing glucose and brewer's yeast powder. The larval colonies were maintained in the laboratory at a temperature of 25 C with a relative humidity of 75 ± 5% and 14 hours of photoperiod. The emerging fourth instar stage of the mosquito larvae was used for the experiment.

Mortality assays

The assays were conducted according to the guidelines provided by the World Health Organization (WHO) in 2006 for mosquito *in vivo* testing. We used 10 late 3^{rd} instar / 4^{th} early instar larvae for each cup (disposable plastic cups) and added 99 mL of dechlorinated water with varying concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 µg mL^{-1} of test solution (stock diluted in a concentration of 1000 µg mL^{-1}). Three replicates were made for every experimental condition. Four extracts were tested: *Codium fragile* and *Turbinaria conaids* aqueous, ethanol, methanol, and chloroform extract. One control was used: dechlorinated water, mosquito larvae were maintained with water at a temperature of 26°C ± 2 and a photoperiod of 12 hours light and 12 hours dark. Dead larvae were recorded after 24 and 48 hours of exposure, following the WHO guidelines for mosquito *in vivo* testing.

To calculate the mortality percentage, the number of dead larvae was divided by the total number of larvae and then multiplied by 100. The mortality values were corrected using Abbott's formula (Abbott 1987).

$$\% \text{ Mortality} = \frac{\% \text{ of survival in untreated control-\% of survival in treated sample}}{\% \text{ of survival in untreated control}} \times 100$$

Statistical Analysis

To determine the LC_{50} and LC_{90} (Lethal Concentration) values for the average larval mortality data, a probit regression model was fitted to the observed relationship between the substance's logarithmic concentration and the percentage of larval mortality. The 95% confidence limits of these values were then estimated (Finnely 1971). Version 20.0 of the SPSS (Statistical Package Social Science) program was used for all of the analysis.

Results

Phytochemical analysis

The preliminary phytochemical screening of sixteen different phytochemical compounds in four different solvents, including Aqueous, Ethanol, Methanol, and Chloroform. The seaweed species that were used for the screening were *Codium fragile* and *Turbinaria conaids*. The results from (Table 1, 2) indicate that the maximum phytochemical compounds were present in the ethanolic extract of *C. fragile* and *T. conaids,* with fourteen phytochemical compounds, while the minimum nine compounds were present in the aqueous extract. The phytochemical compounds tested were steroids, tannins, terpenoids, flavonoids, saponins, alkaloids, reducing sugar, cardiac glycosides, coumarins, phlobatannins, anthraquinones, quinones, glycosides, phenols, anthocyanin, and betacyanin.

GC-MS analysis

The identified compounds of the seaweed C*. fragile* and *T. conaids*, along with their retention indices, percentage composition, chemical structure, and activities, are given in Table 3, 4. GC-MS chromatogram of ethanolic extract of *C. fragile* and *T. conaids* indicated the presence of 20 different compounds including Hexadecanoic Acid, Octadecanoic Acid, 1,4-Methanocycloocta[d]pyridazine, Benzoic Acid, Dibutyl phthalate, Benzenedicarboxylic acid, and Phthalic acid as compounds with major biological activities. The results indicate the presence of *C. fragile* of Hexadecanoic Acid (27.79 %), 4-Methanocycloocta[d]Pyridaz (7.39 %), 1,1-Dimethoxy-2-Hepten-7-Al (7.04%) and Octadecanoic Acid, (5.9 %). The *T. conaids* of Dibutyl phthalate (69.56 %), 1,2-Benzenedicarboxylic acid (14.01 %) and Phthalic acid (2.58 %) were present as the major constituent with the highest area percentage (Fig. 1, 2). The individual fragmentation of the components is illustrated as figures.

In Vitro Antioxidant activity

DPPH radical scavenging activity

The DPPH radical scavenging capacity of the aqueous, ethanol, methanol, and chloroform extracts of the seaweed *C. fragile* and *T. conaids* were tested at different concentrations and the findings are presented in Figure 3A, B. The ethanolic extract of *T. conaids* (81.48 ± 1.0 %) exhibited a strong DPPH activity, followed by the ethanolic extract of *C. fragile* (77.76 ± 0.66%), chloroform extracts of *C. fragile* (71.46 ± 1%), methanolic extracts of *T. conaids* (71.33 ± 1.1%), chloroform extracts of *T. conaids* (68.49 ± 0.94%) and aqueous (67.35 ± 0.92%), methanolic extracts of *C. fragile* (64.84 ± 0.35%) and aqueous extracts of (61.99 ± 0.27%) at a concentration of 20-100 µg mL^{-1} compared with the reference synthetic compound BHT having (81.67±0.66 %) activity. As revealed in Table 5, the ethanolic extracts of *C. fragile* and *T. conaids* had the lowest IC_{50} values. Among all samples tested, *T. conaids* showed exhibited higher DPPH activity with ethanolic solvent, compared with BHT.

ABTS radical scavenging activity

ABTS radical scavenging activities of the aqueous, ethanol, methanol, and chloroform extracts of the seaweed *C. fragile* and *T. conaids* showed at a concentration of 20-100 µg mL^{-1} compared with the reference synthetic compound BHT (Fig. 4a,b). ABTS radical scavenging activity of all extracts increased as the concentration increased (P<0.05). However, significantly higher ABTS free radical scavenging activity in ethanolic extracts of (88.29±1.0%) was observed from *T. conaids* than *C. fragile* ethanolic extracts of (84.77±0.53 %) followed by the chloroform, methanolic and aqueous extracts compared with BHT having (90.0±1.0%) free radical scavenging activity. The ethanolic extract of *C. fragile* and *T. conaids* showed the lowest IC_{50} value whilst the IC_{50} value for the aqueous extract of *C. fragile* was the highest as summarized in Table 5.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity from *C. fragile* and *T. conaids* was evaluated in different extracts of aqueous, ethanol, methanol and chloroform at concentrations (20- 100 µg mL^{-1}) as shown in (Fig. 5a,b), and compared with BHT. The ethanolic extract from *T. conaids* showed a significantly (P<0.05) increased H_2O_2 scavenging activity (86.51±1.0%) and *C. fragile* from ethanolic extract of (81.52±1.2%) at the concentration of 100 µg mL^{-1} compared with the other concentrations of 20, 40, 60, 80 µg mL^{-1}. The synthetic standard antioxidant compound BHT (90.0±1.0 %) of H_2O_2 scavenging activity. As shown in Table 5, the ethanolic and methanolic extracts of *C. fragile* and *T. conaids* had the lowest IC_{50} values. Among all samples tested, *T. conaids* showed hydroxyl radical scavenging activity with most of the solvents, compared with BHT.

Mosquito larvicidal activity of mortality percentage

The larvicidal activity test to determine the effectiveness of seaweed derived extracts against certain vectors. They measured the LC_{50} and LC_{90} values after 24 hours and calculated the percentage of mortality after 48 hours. These results were likely presented in Fig. 6a,b. After 24 hours of treatment with *C. fragile*, it caused about the highest chloroform 60-100 % mortality against *Cx. quinquefasciatus* with LC_{50} value of 0.417 and LC_{90} 1.096 µg mL^{-1}. We determined that, *Cx. quinquefasciatus* has shown a higher mortality rate of about 70-100 at 2.5 µg mL^{-1}concentration following 48 hours of treatment with LC_{50} value of 0.375 µg mL^{-1} and LC_{90} 0.837 µg mL^{-1} respectively. *T. conoides* chloroform extracts treated *Cx. quinquefasciatus* larvae showed LC_{50} value of 0.687 and LC_{90} 1.643 µg mL^{-1} at 24 hours of treatment and were comparatively toxic against *Cx. quinquefasciatus* larvae and it has shown a maximum of 40-100% mortality rate. Chloroform extract 48 hours of treatment with LC_{50} value of (0.461 µg mL^{-1}) and LC_{90} (1.023 µg mL^{-1}) against *Cx. quinquefasciatus* larvae and it has indicated a maximum of 60-100% mortality rate respectively (Table 6, 7).

Discussion

Mosquito-borne diseases have caused a significant impact on the health and economy of many countries. The traditional approach of using chemical insecticides to control mosquitoes is no longer effective due to insecticide resistance and long-term negative effects on the environment. Therefore, it is crucial to find alternative mosquito control methods that are safe and effective for everyone(Pavela 2008; Vinayagam and Senthil Kumar 2008).

Seaweeds are remarkable bio-factories that produce an impressive range of secondary metabolites. What's even more fascinating is that these metabolites have diverse biological activities, making them incredibly valuable for various applications. Nowadays researchers are exploring the potential of seaweed in various fields such as agriculture, food, and medicine. Vector control is crucial in reducing the transmission of diseases like malaria, yellow fever, dengue fever, and filariasis. However, it's challenging to control vectors due to various reasons such as the lack of reliable vaccines, anti-cancer and antimicrobial drugs, drug-resistant parasites, and insecticides (Karunamoorthi 2011; Kannan et al. 2013).

In the current investigation, the two seaweeds, *Codium fragile* and *Turbinaria conoides*, have shown the presence of various phytochemicals through screening. The analysis revealed the presence of alkaloids, phenols, flavonoids, triterpenoids, steroids, tannins, coumarins, terpenoids, quinine, phytosteroids, and phlobatannins in all samples.

Phytochemical assessments aid in detecting bioactive principles, which can result in drug discovery and development (Nascimento et al. 2000; Varadarajan et al. 2008). Seaweeds have different phytochemicals that are beneficial in industrial and medicinal uses (Arya et al. 2011; Rebecca et al. 2012; Rajasulochana et al. 2012; El-Beltagi et al. 2022). Phytoconstituents, along with phenol, flavonoids, and tannin compounds, are considered to be the most significant chemical additives of algal cells. These compounds may have either a stimulating or inhibiting effect on microbial growth depending on their concentration and establishment (Rajasulochana et al. 2009, 2012; Athiperumalsamy et al. 2010).

In this study, GC-MS chromatogram has shown that the ethanolic extract of *C. fragile* has appreciable amounts of bioactive compound with notable height % viz., Hexadecanoic acid (27.79 %), 4-Methanocycloocta[d]pyridaz (7.39 %), 1,1-Dimethoxy-2-hepten-7-al (7.04%) and Octadecanoic acid, (5.9 %). The bioactive compound *T. conaids* is present in the ethanolic extract of Dibutyl phthalate (69.56 %), 1, 2-Benzenedicarboxylic acid (14.01 %) and Phthalic acid (2.58 %) was present as the major constituent with highest area percentage which is generally proved for antioxidant and larvicidal activity.

The antioxidant activity of various naturally occurring compounds has been recognized for many years. In recent times, seaweed has emerged as a promising source of inhibitors for reactive oxygen species. In the current study high, DPPH radical scavenging activity was recorded in *C. fragile* (77.76 %), and *T. conoides* (81.48 %) ethanolic extract relatively smaller than the activity of BHT (81.67 %). Kumaran and Karunakaran (2007) have also reported a similar trend in the methanolic extract of *Phyllanthus* species, while the extract from *E. cava* has exhibited higher radical scavenging activities, especially the celluclast extract (Matsukawa et al. 1997). Several researchers have reported a positive correlation between free radical scavenging activities (Oki et al. 2002). Devi et al. (2008) have mentioned that the DPPH radical scavenging ability varied significantly, with a range of 5% to 72.5% for *C. hornemanni* and *G. acerosa*, respectively.

Souza et al. (2011) have reported that ethanolic extracts of *G. birdiae* and *G. cornea* exhibit the best performance, showing a high scavenging activity of 60%. The ethanolic extracts indicate their enhanced level of antioxidant and scavenging activity from *E. compressa* and *C. fulvescens* were the most efficient DPPH scavengers (Cho et al. 2011).

The ABTS assay has become a popular method for studying the free radical scavenging activity of different types of extracts. In this context, the ethanolic extract of *C. fragile* (84.77%) and *T. conoides* (88.29%) of seaweeds have been found to exhibit lower ABTS scavenging activity as compared to BHT (90.0%). The assay used in this study was an improved technique for generating ABTS, which involves the direct production of the blue/green ABTS chromophore through a reaction between ABTS and $K_2S_2O_8$. The higher ABTS radical scavenging ability displayed by the other solvent fraction could be attributed to the presence of carotenes/other pigments with long hydrocarbon chains (Chakraborty and Paulraj 2010; Gupta and Abu-Ghannam 2011). Certain hexane, chloroform, and methanol extracts of *Porphyra yezoensis* have been found to contain antioxidant compounds such as β-carotene, chlorophyll analogues, and amino compounds. These compounds may contribute to the reported antioxidant activity of the seaweed extracts, according to a study by Nakayama et al. (1999). Additionally, other studies have identified a variety of antioxidant compounds in seaweeds, including pigments like fucoxanthin and astaxanthin, polyphenols like phlorotannins, chlorophyll-related compounds, phospholipids, flavonoids, bromophenols, and polysaccharides (Gupta and Abu-Ghannam 2011; Umayaparvathi et al. 2012).

Hydroxyl radical scavenging activity assay was used to determine the potential of different solvent fractions from seaweeds to scavenge short-lived radicals such as HO. radicals. These radicals have been known to abstract H-atoms from lipid membranes, which can lead to peroxide reactions of lipids. The study reported that the brown seaweeds demonstrated HO. scavenging activities due to the presence of polyphenolic compounds like phlorotannins. These compounds can act as electron traps and are responsible for the multifunctional antioxidant properties that include scavenging of hydroxyl radicals, peroxy radicals, or superoxides (Gupta and Abu-Ghannam 2011).

Ascorbic acid is the primary component responsible for scavenging HO. in brown seaweeds (Abe et al. 1992). Furthermore, seaweed extracts have been identified as potential scavengers of HO. (Cho et al. 2011). The study suggests that the ethanolic extract of *C. fragile* and *T. conoides* have novel radical scavenging activities, with percentages reaching 81.52% and 86.51% respectively. These values are higher than the activity of BHT, which was recorded at 76.60%.

Chakraborty et al. (2015), the methanol extract of *J. rubens* was found to have significantly enhanced HO. radical scavenging activity. The current study seems to align with these earlier findings, as about 90% HO scavenging activity was reported in dichloromethane and butanol fractions of red seaweeds *A. spicifera* and *G. edulis* in a study by Ganesan et al. 2008. Additionally, Heo et al. (2006) found that *G. verrucosa, G. textorii, G. filicina,* and *P. japonica* also exhibited potentially high HO. scavenging activity.

To discover the larvicidal efficiency of seaweed extracts, it was tested against *Cx. quinquefasciatus* were treated with aqueous ethanol, methanol and chloroform. We discovered that the *C. fragile* chloroform indicated a greater death rate at different concentrations and time duration. *Cx. quinquefasciatus* has shown (0.5, 1, 1.5, 2 and 2.5 µg mL^{-1}) 70-100 % mortality rate at 24 hr, and 90-100 % 48 hr respectively. Among the seaweed, showed minimum LC_{50} values of, *Cx. quinquefasciatus* larvae were *C. fragile,* chloroform extracts of 24 hr [LC_{50} (0.375 µg mL^{-1})] and 48 hr [LC_{50} (0.375 µg mL^{-1})] and *T. conoides* 24 hr [LC_{50} (0.687 µg mL^{-1})] and 48 hr [LC_{50} (0.461 µg mL^{-1})].

The toxicity of *C. fragile* and *T. conoides* seaweed may be responsible for their mechanism of action on the larvae, which is supported by the findings of previous studies that evaluated the larvicidal activity of ethanol extracts from other types of seaweed against *Ae. aegypti.*

The potential mechanism of action for the seaweed *C. fragile* and *T. conoides* may be attributed to their toxic effects on larvae. This is supported by a previous study that looked into the mosquito larvicidal activity of ethanol extracts from *D. dichotoma, E. intestinalis,* and *A. spicifera* against *Ae. aegypti* by Ravikumar et al. (2011), which is due to the presence of phytochemicals such as flavonoids, phenols, terpenoids, and saponins. Therefore, the above report may potentiate that the shown larvicidal activity of *C. fragile* and *T. conoides* may also be due to the presence of flavonoids, phenols, glycosides, and tannins. Thangam and Kathiresan (1988) reported that *Caulerpa scalpelliformis* and *Dictyota dichotoma* seem to be highly effective in reducing the population of certain vectors. It's fascinating to see how different extracts can have varying levels of effectiveness against these pests. It's also noteworthy that permethrin 10 EC and *Pimpinella anisum* oil have been found to significantly impact the larvae of different mosquito species (Manilal et al. 2011; Ali et al. 2012).

The petroleum ether acetone extract of *Enteromorpha intestinalis* and the ethanol and water crude extract of *Turbinaria decurrens* have shown larvicidal activities against certain vectors. The *Gracilaria corticata* crude extract has also shown activity against *Aedes aegypti, Culex quinquefasciatus,* and *Anopheles stephensi* (Ali et al. 2013; Yu et al. 2014). The chloroform extracts of *C. fragile* and *T. conoides* seaweed have shown promising results in larvicidal activities, according to the current study.

Conclusion

It can be concluded that this study highlights various solvent extracts of *C. fragile* and *T. conoides* seaweed were rich sources of phytohemical compounds, and natural antioxidant compounds and exhibit larvicidal activity. The discovery of bioactive compounds in seaweeds is a significant breakthrough that could have a wide range of medical applications. These natural antioxidants have the potential to be used in the production of pharmaceutical products. The synthetic chemicals are cheap and easy to obtain, and seaweed extracts offer a safer alternative for controlling larvae. The results of the larvicidal activity test could help find newer compounds as these extracts are affordable, easy to handle, and safe to use.

Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

KP: Methodology, Investigation, Conceptualization, Writing – review and editing, Supervision, and Project administration. **SR:** Writing-original draft. **DP:** Writing – review and editing, Formal analysis, Resources, Data curation. **PD:** Draft preparation, review and editing. **VAS:** Writing – review and editing, Data curation. All authors reviewed the manuscript.

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Data availability The corresponding author can provide the datasets upon reasonable request.

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Tables

Table 1 Phytochemical screening of various solvent extracts of *Codium fragile*

S.No.	Phytochemical Compounds	Solvents			
		Aqueous	Ethanol	Methanol	Chloroform
1	Steroids	+	+	-	+
2	Tannins	+	+	+	+
3	Terpenoids	+	+	+	+
4	Flavonoids	+	+	-	+
5	Saponins	-	+	+	+
6	Alkaloids	-	+	+	+
7	Reducing sugar	-	+	-	+
8	Cardiac glycosides	+	+	-	+
9	Coumarins	+	+	+	+
10	Phlobatannins	-	-	-	-
11	Anthraquinones	-	+	+	-
12	Quinones	+	+	+	+
13	Glycosides	-	+	+	-
14	Phenols	+	+	+	+
15	Anthocyanin	+	+	+	+
16	Betacyanin	-	-	-	-

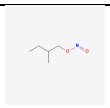
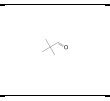
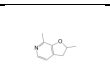
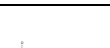
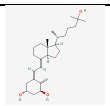
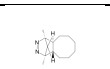
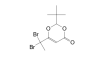
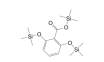
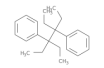
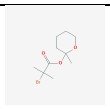
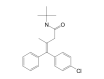
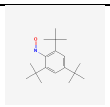
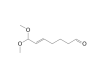
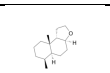
(+) Present (-) Absent

Table 2 Phytochemical screening of various solvent extracts of *Turbinaria conaids*

S.No	Phytochemical Compounds	Solvents			
		Aqueous	Ethanol	Methanol	Chloroform
1	Steroids	+	+	-	+
2	Tannins	-	+	+	+
3	Terpenoids	+	+	+	+
4	Flavonoids	+	-	+	+
5	Saponins	+	+	+	+
6	Alkaloids	-	+	+	+
7	Reducing sugar	-	+	-	-
8	Cardiac glycosides	+	+	+	+
9	Coumarins	+	+	+	+
10	Phlobatannins	-	+	+	-
11	Anthraquinones	-	+	+	-
12	Quinones	+	+	+	+
13	Glycosides	-	+	+	-
14	Phenols	+	+	+	+
15	Anthocyanin	-	-	-	-
16	Betacyanin	+	+	+	+

(+) Present (-) Absent

Table 3 GC-MS spectral analysis of ethanolic extract of *Codium fragile*

Peak#	RT	Compound name	Molecular formula	Molecular weight	Peak area%	Structure	Activity
1	6.909	1-Methylbutyl Nitrite	$C_5H_{11}NO_2$	117	4.71		Antioxidant Antparasitic, Antimicrobial
2	11.623	Disulfide, Dioctyl	$C_{16}H_{34}S_2$	290	2.65		Antimicrobial
3	25.506	Propanal, 2,2-dimethyl-	$C_5H_{10}O$	86.13	3.09		Antibacterial
4	26.571	Furo[2,3-c]pyridine, 2,3-dihydro-2,7-dimethyl-	$C_9H_{11}NO$	149.19	3.35		Antitumor
5	26.665	Hexadecanoic Acid	$C_{16}H_{32}O_2$	256.42	27.79		Antioxidants, Hypocholesterolemic, Nematicide, and Pesticide
6	34.667	Octadecanoic Acid, 2-Hydroxy-1-(Hydroxymethyl)Ethyl Ester	$C_{21}H_{42}O_4$	358.6	5.9		Antioxidant, Anti-inflammatory, and anthelmintic
7	36.38	2-Oxo-6-(Piperidine-1-Sulfonyl)-Benzooxazole-3-Carboxylic Acid Cyclohexylamide	$C_{19}H_{25}N_3O_5S$	407.48	2.32		Anticancer antibacterial and antifungal
8	36.51	(22S,23R,25R)-23,26-Epoxy-5.Alpha.-Furostan-3.Beta.-Ol	$C_{27}H_{44}O_3$	416	5.72		Anti-osteoporotic, Immunomodulatory, Anticarcinogenic, Antipsoriatic, Antioxidant
9	36.585	(1.Alpha.,4. Alpha.,4a.Alpha.,10a.Beta.)-1,4,4A,5,6,7,8,9,10,10A-Decahydro-'1,4,11,11-Tetramethyl-1,4-Methanocycloocta[d]Pyridaz	$C_{15}H_{26}N_2$	234.39	7.39		Antibacterial and Anti-fungal
10	37.285	2-Tert-butyl-6-(1,1-Dibromo-Ethyl)-[1,3]Dioxin-4-One	$C_{10}H_{14}Br_2O_3$	342.03	5.29		Anticonvulsants
11	37.499	Benzoic Acid, 2,6-Bis(Trimethylsiloxy)-, Trimethylsilyl Ester	$C_{16}H_{30}O_4Si_3$	370.67	4.24		Anti-fungal and Anti-bacterial
12	37.595	Benzene, 1,1'-(1,1,2,2-Tetraethyl-1,2-Ethanediyl)Bis-	$C_{22}H_{30}$	294.47	4.19		Antimicrobial
13	37.945	2-(Bromomethyl)-2-Tert-Butyl-6-Methyl-1,3-Dioxan-4-One	$C_{10}H_{17}BrO_3$	264	1.53		Anticonvulsants
14	37.973	3-Butenamide, 4-(4-Chlorophenyl)-N-(1,1-Dimethylethyl)-3-Methyl-4-Phenyl-, (Z)-	$C_{21}H_{24}ClNO$	341.88	1.79		Antimicrobial
15	38.011	5-(5-Hexenyl)-3-(8-Nonenyl) Isoxazole	$C_{18}H_{29}NO$	275.4	2.29		Antifungal
16	38.683	4-(2,3-Dimethoxy-Phenyl)-1-Thiophen-2-Ylmethyl-1,4-Dihydro-Pyridine-3,5-Dicarboxylic Acid Dimethyl Ester	$C_{22}H_{23}NO_6S$	429	1.05		Antitumor, Antifungal, and insecticidal activities
17	38.795	1,1-Dimethoxy-2-Hepten-7-Al	$C_9H_{16}O_3$	172	7.04		Antibacterial Antifungal Anticancer
18	38.825	Naphtho[2,1-B]Furan, Dodecahydro-6,9A-dimethyl-, [3AS-(3A.Alpha.,5A.Alpha.,6.Beta.,9A.Beta.,9B.Alpha.)]-	$C_{14}H_{24}O$	208.18	1.88		Antibacterial and Antioxidant

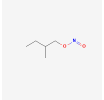
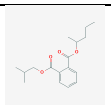
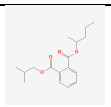
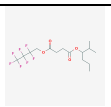
19	38.879	Cis-4-Tert-2-(Methylsulfonyl)Cyclohexanone	$C_{11}H_{20}O_3S$	232.11	4.53		Antimicrobial and Anticancer
20	39.915	D-Homo-18-Norandrosta-7,13,15,17-Tetraene, 17-Methoxy-3-(Methoxymethoxy)-, (3.Alpha.,5.Beta.,10.alpha.)-(.+-.)-	$C_{22}H_{30}O_3$	342	3.25		Antimicrobial and anticancer

Table 4 GC-MS spectral analysis of ethanolic extract of *Turbinaria conaids*

Peak#	RT	Compound name	Molecular formula	Molecular weight	Peak area%	Structure	Activity
1	4.352	(E)-2-(3-Phenylthiopent-2-Enyl)Cyclohexanone	$C_{17}H_{22}OS$	274.44	0.09		Antitumor
2	24.984	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278.35	14.01		Antimicrobial
3	25.781	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.35	6.15		Insect repellent, Antimicrobial, Antioxidant
4	26.074	Phthalic acid, isobutyl 2-pentyl ester	$C_{17}H_{24}O_4$	292.4	1.72		Insecticidal, Antimicrobial Anticancer, Antioxidant
5	26.579	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.35	69.56		Insect repellent, Antimicrobial Anticancer, Antioxidant
6	26.662	Dinonyl Ester of Hexanedioic Acid	$C_{24}H_{46}O_4$	398.61	1.13		Antibacterial
7	26.868	Phthalic acid, butyl 2-pentyl ester	$C_{17}H_{24}O4$	292	2.58		Insecticidal, Antimicrobial
8	27.173	Furo[2,3-c]pyridine, 2,3-dihydro-2,7-dimethyl-	$C_9H_{11}NO$	149	0.35		Antimicrobial
9	27.344	2-Methyl-6-.BETA.-D-Ribofuranosylimidazo[1,2-C]pyrimidin-5(6H)-One	$C_8H_7NO_2$	281.27	0.72		Antifungal, Antibacterial
10	27.438	Furo[2,3-c]pyridine, 2,3-dihydro-2,7-dimethyl-	$C_{12}H_{15}N_3O_5$	149	0.58		Anticancer
11	28.102	2,3-Dihydroindole-4-ol-2-one	$C_8H_7NO_2$	149	1		Antioxidant neuroprotective
12	28.282	Furo[2,3-c]pyridine, 2,3-dihydro-2,7-dimethyl-	$C_9H_{11}NO$	149	0.35		Antimicrobial
13	28.372	Furo[2,3-c]pyridine, 2,3-dihydro-2,7-dimethyl-	$C_9H_{11}NO$	149	0.28		Antimicrobial
14	29.04	1-DODECANOL, 2-METHYL-, (S)-	$C_{13}H_{28}O$	200	0.09		Antimicrobial
15	29.073	Furo[2,3-c]pyridine, 2,3-dihydro-2,7-dimethyl-	$C_9H_{11}NO$	149	0.17		Antimicrobial
16	37.476	Succinic acid, 2,2,3,3,4,4,4-heptafluorobutyl 2-methylhex-3-yl ester	$C_{15}H_{21}F_7O_4$	398	0.41		Antimicrobial
17	37.655	Silane, [3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-1-iodo-1-[[(4-methylphenyl)sulfonyl]methyl]propyl]trimethyl-, (.+-.)-	$C_{20}H37IO_3SSi_2$	540.6	0.13		Antimicrobial
18	37.725	1,1,1,3,5,5,5-Heptamethyltrisiloxane	$C_7H_{22}O_2Si_3$	222	0.12		Antimicrobial
19	37.96	2-(Bromomethyl)-2-Tert-Butyl-6-Methyl-1,3-Dioxan-4-one	$C_{10}H_{17}BrO_3$	265.15	0.29		Antibacterial Antifungal

							Anticancer
20	39.51	Cyclohexanone, 2-Chloro-3-(1,1-Dimethylethyl)-, Trans-(.+-.)-	$C_{10}H_{17}ClO$	188.7	0.28		Antimicrobial

Table 5 The IC_{50} Value of scavenging activity on various solvent extracts of *Codium fragile*, *Turbinaria conaids* and BHT

Species	Antioxidant assay	IC_{50} Value (µg mL^{-1})				
		Aqueous	Ethanol	Methanol	Chloroform	BHT
Codium fragile	DPPH	78.33	47.73	68.58	61.34	63.01
	ABTS	94.48	35.51	45.62	47.18	51.58
	Hydroxyl Radical Scavenging	44.57	52.67	56.86	53.53	27.20
Turbinaria conaids	DPPH	59.04	22.50	29.42	42.64	63.01
	ABTS	75.66	49.44	53.23	34.87	51.58
	Hydroxyl Radical Scavenging	60.97	52.84	41.22	40.66	27.20

Table 6 Observed and expected mortality of *Culex* larvae exposed to *Codium fragile* with aqueous, ethanol, methanol and chloroform extracts. Expected mortality is based on probit regression analysis.

Extract	Duration	LC_{50} (µg mL^{-1})	LC_{90} (µg mL^{-1})	95% Confidence Limit LC_{50} (LC_{90})		Regression equation	X^2 (df = 3)
				Lower (µg mL^{-1})	Upper (µg mL^{-1})		
Aqueous	24 hr	2.785	4.810	2.208 (3.082)	111.8 (84959)	y=5.401+2.403x	0.208
	48 hr	2.581	4.650	2.063 (3.029)	11.167 (318.94)	y=5.012+2.063x	1.251
Ethanol	24 hr	2.134	3.449	1.785 (2.627)	3.035 (11.784)	y=6.144+2.022x	0.644
	48 hr	1.675	3.249	1.310 (2.392)	2.204 (8.558)	y=4.452+0.997x	0.304
Methanol	24 hr	2.212	3.610	1.848 (2.695)	3.423 (15.78)	y=6.023+2.077x	0.378
	48 hr	1.705	2.542	1.407 (2.133)	1.999 (4.020)	y=7.394+1.714x	1.963
Chloroform	24 hr	0.417	1.096	0.048 (0.723)	0.651(2.935)	y=3.051+1.160x	1.042
	48 hr	0.375	0.837	0.005 (0.522)	0.576 (3.799)	y=3.672+1.566x	0.519

Table 7 Observed and expected mortality of *Culex* larvae exposed to *Turbinaria conaids* with aqueous, ethanol, methanol and chloroform extracts. Expected mortality is based on probit regression analysis.

Extract	Duration	LC_{50} ($\mu g\ mL^{-1}$)	LC_{90} ($\mu g\ mL^{-1}$)	95% Confidence Limit LC_{50} (LC_{90})		Regression equation	X^2 (df = 3)
				Lower ($\mu g\ mL^{-1}$)	Upper ($\mu g\ mL^{-1}$)		
Aqueous	24 hr	3.497	11.164	2.190 (4.216)	1.387 (1.065)	y=2.542+1.382x	0.425
	48 hr	2.706	6.854	1.955 (3.534)	23.119 (3546.4)	y=3.175+1.73x	0.432
Ethanol	24 hr	1.613	2.882	1.277 (2.230)	2.018 (5.954)	y=5.083+1.055x	1.759
	48 hr	0.760	1.626	0.474 (1.224)	0.994 (3.006)	y=3.880+0.463x	1.746
Methanol	24 hr	1.870	3.081	1.532 (2.415)	2.366 (7.173)	y=5.909+1.606x	1.549
	48 hr	1.248	2.154	0.949 (1.735)	1.514 (3.515)	y=5.405+0.519x	1.741
Chloroform	24 hr	0.687	1.643	0.358 (1.198)	0.931 (3.491)	y=3.384+0.552x	2.293
	48 hr	0.461	1.023	0.113 (0.723)	0.663 (2.520)	y=3.706+1.245x	1.434

Figures

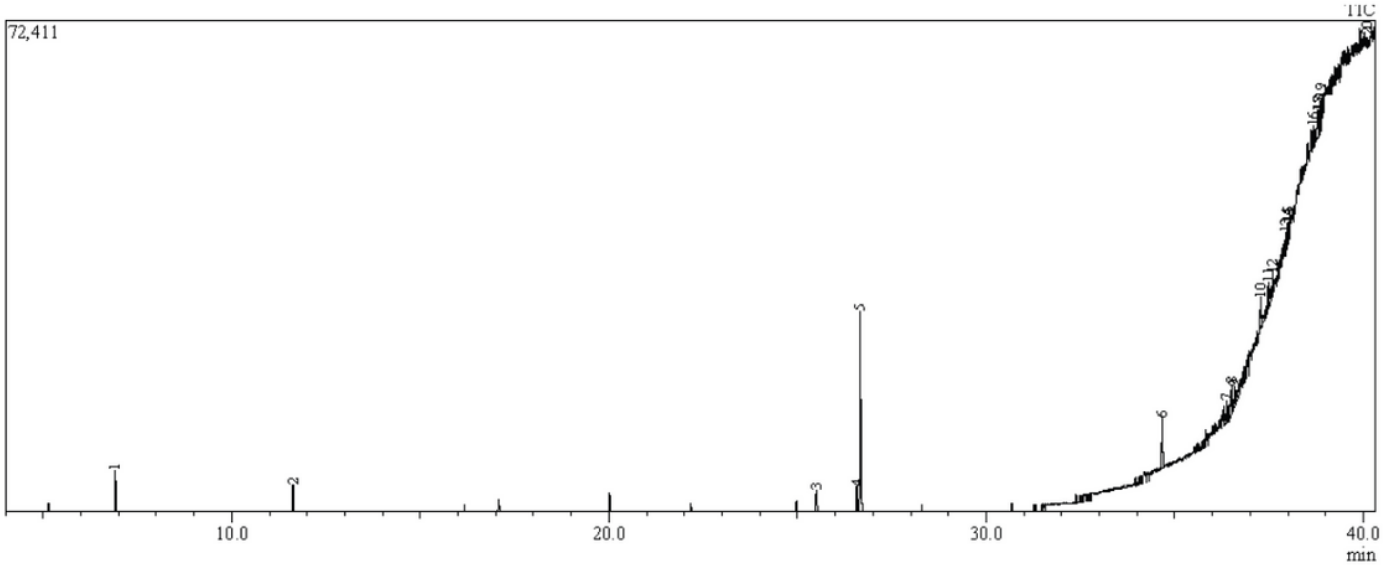


Figure 1

GC-MS chromatogram for seaweed ethanolic extract of *Codium fragile*

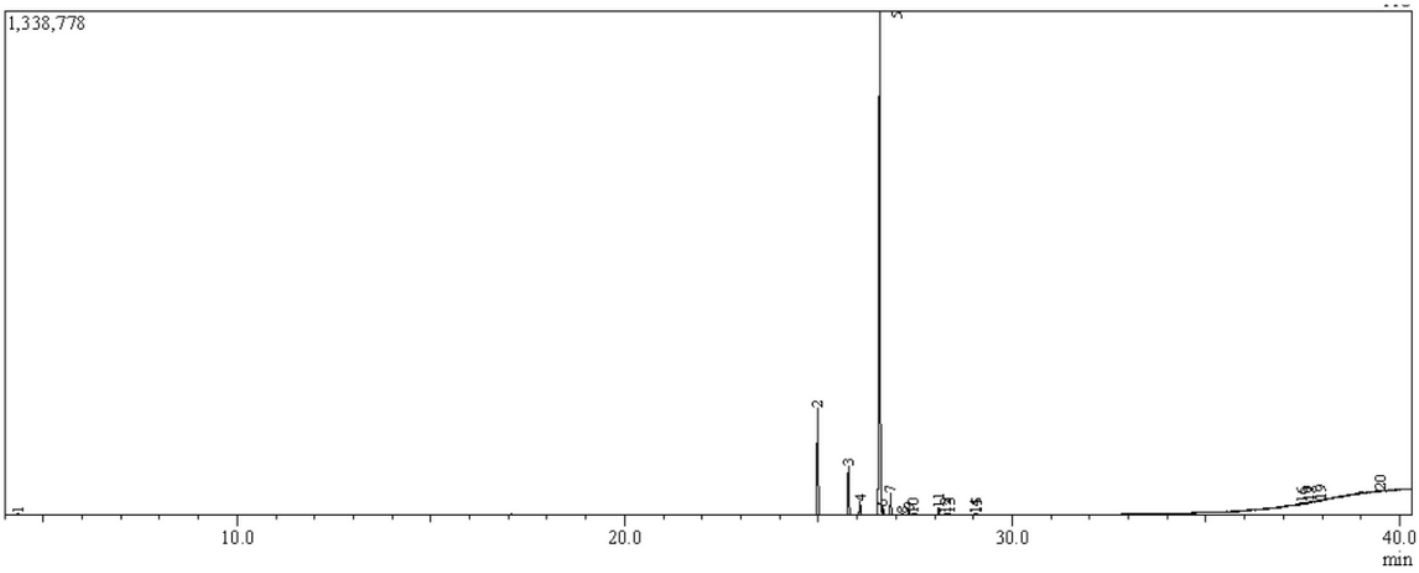


Figure 2

GC-MS chromatogram for seaweed ethanolic extract of *Turbinaria conaids*

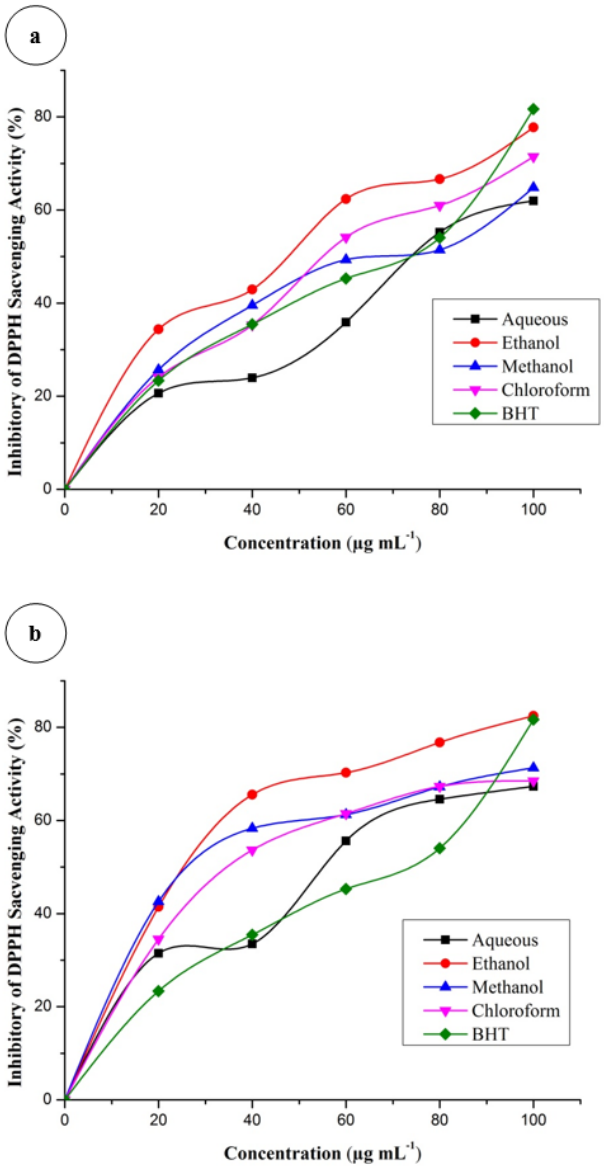


Figure 3

Inhibitory effect of a). *Codium fragile*, b). *Turbinaria conaids* seaweed extracts on 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity compared to butylated hydroytoliene (BHT). Each value is expressed as the (Mean ± Standard Deviation) (N=3)

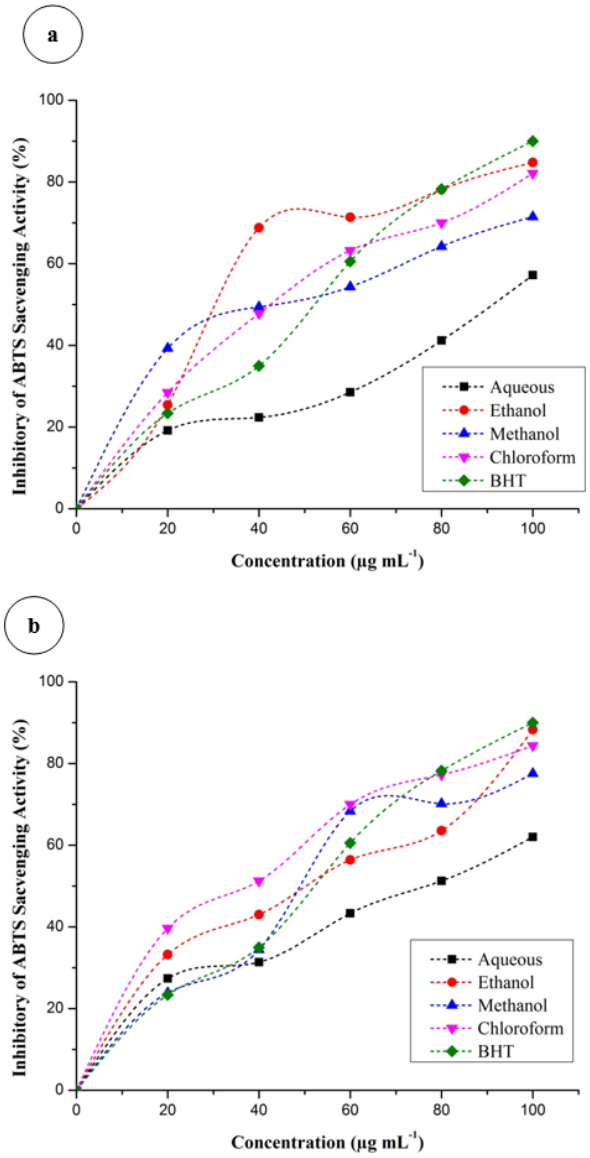


Figure 4

Inhibitory effect of a). *Codium fragile*, b). *Turbinaria conaids* seaweed extracts on 2, 2-azinobis-3-ethylbenzothiozoline-6-sulfonic (ABTS radical scavenging activity compared to butylated hydroytoliene (BHT). Each value is expressed as the (Mean ± Standard Deviation) (N=3)

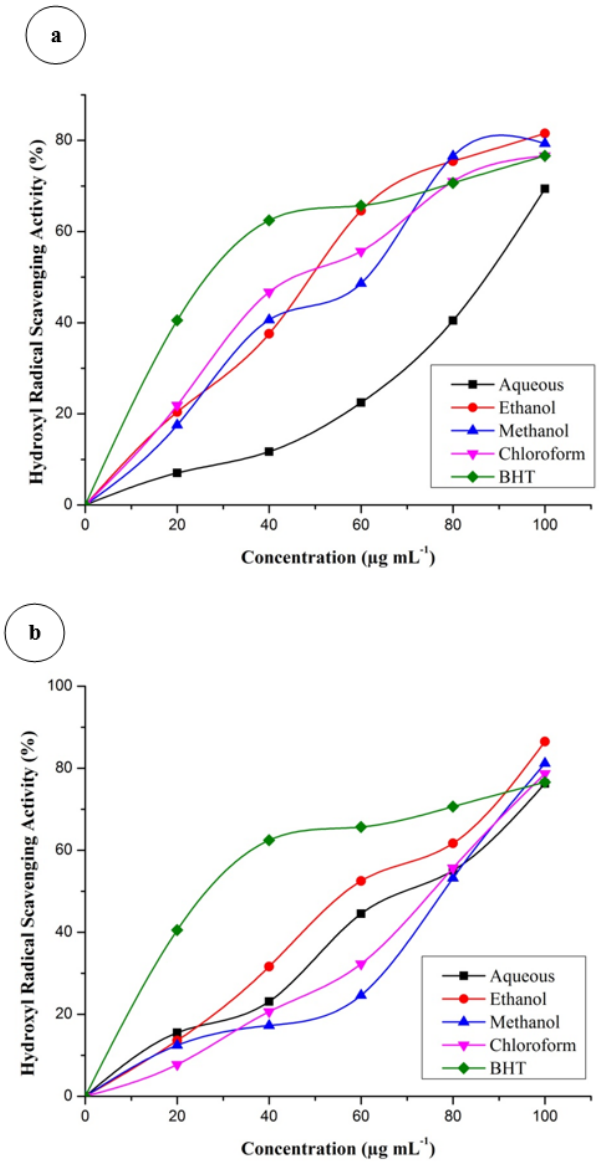


Figure 5

The scavenging activity of a). *Codium fragile*, b). *Turbinaria conaids* seaweed extracts on hydroxyl radical scavenging activity compared to butylated hydroytoliene (BHT). Each value is expressed as the (Mean ± Standard Deviation) (N=3).