1.Introduction

1. Seaweed

Seaweeds or marine macro algae are different species of marine plants and algae that grow in water bodies such as rivers, lakes and oceans. They are macroscopic multicellular eukaryotic photosynthetic organisms and have the potential to produce many valuable compounds such as proteins, carbohydrates, fatty acids, amino acids, phenolic compounds, pigments etc. They are considered as one of the rich sources of biologically active ingredients having powerful biological activities. Many seaweed species have antioxidant, anti-inflammatory, anticancer, antiviral and antimicrobial activities. Therefore, they have several industrial applications in food and dairy, pharmaceutical, medicinal, cosmeceutical, nutraceutical etc.

The majority of marine macroalgal species found in oceans is brown seaweeds. Seaweed may provide a source of innovative medications with relatively low toxicity due to their abundance of bioactive chemicals. Many studies have demonstrated the link between a diet high in natural phenols and the presence of antioxidants such phenols and flavonoids, which are typically present in plants and seaweeds, and a lower risk of acquiring chronic diseases, a longer life expectancy, and various cancer types (Asha, *et al*., 2021).

1.2 Brown Seaweed

Brown seaweeds, especially those from the genus Fucales, are abundant in the Gulf of Mannar on the Southeast coast of India. They are vital to preserve the marine ecology in the vast coastal zones. Compared to red and green seaweed, brown seaweed has a greater consumption rate. Every year, more seaweed is harvested globally, mostly for food and for its polysaccharides in the cell walls. Due to the lack of chemical contaminants unique antioxidants found in brown seaweeds are more acceptable than those found in synthetic antioxidants. (Arumugama, *et al*., 2017)

as in biotechnology, pharmacologists have recently focused their attention on secondary metabolites. The first marine organism to be studied for medicine was macroalgae. Acyclic or cyclic short chain hydrocarbons, terpenoids, acetogenins, polyphenols, and terpenoid-aromatic chemicals are all products of brown algae.

1.2.1 *Turbinaria conoides*

Turbinaria conoides is one such brown seaweed which has been used as food since ancient times and is more popular in Asia than in Europe and North America. *Tubunaria conoides* belongs to the family of Sargassaceae (brown algae) is coming under the order of Fucales. It has historically been used as a fertiliser, insect repellent, pesticide, and antibacterialcidal in addition to treating children's fever. Brown seaweed sodium alginate has a variety of biological features, including the ability to remove heavy metals and have antitumor and anti-inflammatory effects. Therefore, natural antioxidants are considered as a safe ingredient in medicine, dietary supplements, nutraceuticals and cosmetics preparation

With the objective of improving consumer health, reducing the effects of harmful diseases and other broader aspects of immune functions. Turbinaria has a charming disposition and is abundant in phytochemicals. Phytochemicals such as steroids, phenolics, flavonoids, reducing sugars, fucosterol, sulfated polysaccharides including fucoidan, neutral glucan, guluronic and alginic acid were reported to be presence in *T. conoides*. (Arumugama, *et al*., 2017). Only 22 species of *Turbinaria* have been identified to date, with 14 species recorded in south-west Asia, from India to Sri Lanka, where there is the most diversity. *T. turbinate* was only found in the Atlantic Ocean, while *T. conoides*, *T. decurrens*, and *T. ornate* were all found in the South Pacific Ocean (Ponnan *et al*., 2023).

1.3 Phytochemicals

Plants play a crucial role in the human diet and are a major source of nutrients. They give us vital sources of carbs, protein, vitamins, chemicals that decrease cholesterol, antioxidants, and other biologically useful compounds. There have been numerous discussions in the literature about the nutritional benefits of plants, but there has been very little research on the biologically active substances they contain. Biologically active substances like this are referred to as phytochemicals. (Vishnu, *et al*., 2019).

Due to their individual, additive, or synergistic effects on health, phytochemicals are helpful in the treatment of some illnesses. For the creation of therapeutic agents and the development of new medications, phytochemicals are essential in the pharmaceutical business. The first step in creating new medications is to identify the active ingredients in natural sources. A novel method for discovering therapeutically effective chemicals in various plant species is the screening of plant extracts. Numerous biological features of phytochemicals, including antioxidant, anti-

inflammatory, anti-diarrheal, anti-ulcer, and anticancer activity, can be found in flavonoids, tannins, saponins, alkaloids, and terpenoids. (Njoku, *et al*.2021). In this study, both qualitative and quantitative analysis of phytochemicals were carried out in *Turbinaria conoides*.

1.4 GC-MS

A combined analytical method called gas chromatography-mass spectroscopy (GC-MS) is used to find and identify the chemicals in a plant sample. The phytochemical analysis and chemotaxonomic investigations of medicinal plants with physiologically active constituents depend heavily on GCMS (Njoku, *et al*., 2021). The discovery of therapeutic agents is desirable, but it is also important to understand the chemical constitution of plants because this knowledge may be crucial for identifying new sources of valuable phytocompounds for the synthesis of complex chemicals and for understanding the true value of folk remedies. Higher plants continue to play a significant role in the preservation of human health as sources of bioactive chemicals. As this approach has proven to be a useful tool for the analysis of nonpolar components including volatile essential oils, fatty acids, lipids, and alkaloids, GC-MS investigations have been used more frequently in recent years for the research of medicinal plants (Abeer *et al.,* 2017).

1.5 Antioxidant

An antioxidant is something that fights oxidation. Antioxidant refers to any substance that, when present in small amounts compared to an oxidizable substrate, considerably slows down or stops the oxidation of that substrate. In order to preserve food quality and protect human health, antioxidants are essential. Compounds that can stabilize ROS are antioxidants. These compounds are free radical scavengers and are quickly oxidized. The most significant group of non-enzymatic antioxidants are vitamins. Endogenous ROS are created by human metabolic activity; exogenous ROS are created by smoking, air pollution, radiation, ozone, and industrial chemicals. ROS are stabilised by processes that result in cellular harm and the creation of cancer-causing DNA adducts. Human disorders of the heart, brain, and many malignancies are primarily brought on by ROS. It has been demonstrated that antioxidant consumption lowers the likelihood of contracting various diseases (Samina *et al*., 2019).

Brown seaweeds are potential source of antioxidants and are more acceptable than synthetic antioxidants due to the absence of chemical contaminants (Liu *et al.,* 2012). Different compounds of marine macroalgae are believed to exhibit antioxidant activity. Especially,

phenolic compounds and other compounds such as carotenoids, ascorbic acid and sulphated polysaccharides present in them are potent antioxidant molecules (Lee *et al.,* 2013; Gamze *et al.,* 2011). The antioxidant activity of the *T. conoides* was determined using DPPH radical scavenging assay, FRAP activity, ABTS radical scavenging assay and total antioxidant assay. This study focusses on identifying the phytochemicals present in the ethanolic extract of *T. conoides* extract and determine the antioxidant activity of the extract.

2. AIM AND OBJECTIVES

Aim

The aim of the present study is to explore the phytochemical constituents present in the brown algae *Turbniaria conoides* and to evaluate its *in vitro* antioxidant potential.

Objectives

- To qualitatively analyse the phytochemicals, present in *Turbniaria conoides.*
- To estimate the phenols and alkaloids present in the brown algae *Turbinaria conoides.*
- To analyse the compounds, present in the ethanol extract of brown algae *Turbinaria conoides* by using GC-MS (Gas Chromatography- Mass Spectrometry) technique.
- To estimate the antioxidant potential of the brown algae *Turbinaria conoides*.

3. MATERIALS AND METHODS

3.1 Collection of seaweed

Turbinaria conoides – a brown seaweed belongs to the family Sargassaceae was collected from the coastal area at Mandapam native to Rameshwaram district, Tamil Nadu, South eastern India (9° 22′ N, 78° 52′ E). After removing the epiphyte and sand, the seaweed samples were washed with clean sea water and transferred to polythene bag.

3.2 Identification of seaweed

The collected seaweed was identified with the help of seaweed taxonomist Dr. U. Elaya Perumal in Annakili Amma Research Institute (AARI), Chennai, Tamilnadu, India.

3.3 Preparation of sample

The collected seaweeds were washed gently with tap water followed by distilled water to remove debris and dust particles. The samples were cut into small pieces and shade dried under normal room temperature for about 15 days. The dried sample is pulverized using a mixer grinder. The powdered sample is stored in an air tight container at room temperature for further use.

Figure 1 - Dried sample of *Turbinaria conoides*

Figure 2 - Powdered sample of *Turbinaria conoides*

3.4 Preparation of extract

The ethanolic extraction of the dried sample of *Turbinaria conoides* was performed by the Soxhlet method using the Soxhlet apparatus. About 100g of powdered sample is weighed accurately in a weighing balance, About 250ml of 99.9% ethanol (solvent) was taken and was added to the distillation flask of Soxhlet apparatus. The weighed powders were added in the extractor portion of the apparatus. Then the apparatus was assembled and the water inlet and outlet was fixed. The electric heat source was put on and the temperature was adjusted frequently according to the quantity of the solvent in the flask. The approximate temperature set was around 400°C. The extraction went on continuously for about 2 days and at the end of two days all of the seaweed contents present were dissolved in the ethanol. The ethanolic extracts were then transferred into separate beakers, labelled and used for further analysis.

Figure 3 -Soxhlet Extraction of *T. conoides*

3.5 PHYTOCHEMICAL ANALYSIS

The phytochemical analysis of ethanol extract of *T. conoides* was carried out to determine the presence or absence of active secondary metabolites. The phytochemicals of the extracts were determined qualitatively as reported by (Trease and Evans,1989; Sadasivam and Manickam, 1996).

1. **Test for Saponins:**

   Froth test:

To 1 ml of the extract, 1 ml of deionised water was added and shaken vigorously for 30 seconds. Then the tube is allowed to stand for 15 minutes. The persistent frothing indicates the presence of saponins.

2. Test for Glycosides:

Keller-Killiani test:

To the test tube containing 2ml of extract 1 ml of glacial acetic acid, 3 drops 5% W/V ferric chloride and concentrated sulphuric acid were added and observed, disappearance of reddish-brown colour at the junction of two layers and bluish green in upper layer indicates the presence of glycosides.

3. Test for Alkaloids:

Mayer's test:

To 3ml of the extract, 1 ml of Mayer's reagent (potassium mercuric iodide – 1.36g mercuric chloride + 5g potassium iodide) was added. The appearance of white precipitate indicates the presence of alkaloids.

Hager's test:

To 3ml of the extract, 1 ml of the Hager's reagent (saturated picric acid solution-l g of picric acid in 100 ml of distilled water) was added. The appearance of yellow precipitate indicates the presence of alkaloids.

Wagner's test:

To 3ml of the extract, 1 ml of the Wagner's reagent (Iodine in potassium iodide – 5g of iodide + 15g of potassium iodide in 250ml of water) was added. The appearance of reddish-brown precipitate indicates the presence of alkaloids.

4. Test for Tannins:

To a 1 ml of plant extract, 1 ml of 1% ferric chloride is added. The appearance of brown colour indicates the presence of tannins.

5.Test for Terpenoids:

To 0.5 ml of plant extract, 2 ml of the chloroform is added and followed by the addition of 3ml of concentrated sulphuric acid to form a layer. Formation of a reddish-brown interface indicated the presence of terpenoids.

Chloroform test:

To 5ml of extract, 2ml of chloroform was added and evaporated on boiling water bath. Then boiled with 3ml of concentrated sulphuric acid. The appearance of grey colour shows the presence of Terpenoids.

6.Test for Phenolic compound

Ferric Chloride test:

To 3ml of extract, 3ml of 5% w/v Ferric chloride solution was added. The blue-black colour indicates the presence of phenolic compound. **Lead Acetate test:**

To 3mI of extract, 3ml of Lead acetate solution was added. The occurrence of white precipitate indicates the presence of tannin and phenol.

7.Test for Quinones:

To a 1 ml of the plant extract, 1 ml of the concentrated sulphuric acid were added. A prominent red colour indicates the presence of quinones.

8.Test for Vitamin C:

DNPH test:

To 2ml of extract, DNPH (di-nitro phenyl hydrazine) was added and mixed well. Then concentrated sulphuric acid was added. The formation of yellow precipitate indicates the presence of vitamin C.

9. Test for Flavonoids:

Sodium Hydroxide test:

To 2ml of extract, few drops of sodium hydroxide was added. The change in colour blue to violet indicates the presence of Flavonoids. The yellow colour indicates the presence of flavones and the change in colour yellow to orange indicates the presence of flavonoids.

10. Test for Steroids:

To 1 ml of the extract, 1 ml of acetic acid is added and few drops of concentrated sulphuric acid is added. The appearance of bluish green indicates the presence of steroids.

3.6 ESTIMATION OF TOTAL PHENOLIC CONTENT

Total phenolic content of different extracts was estimated using Folin–Ciocalteu method described by Alhakmani *et al.* [11] with few modifications. A calibration curve was constructed with different concentrations of gallic acid (20– 100 µg/ml) as the standard. 0.3 ml of plant extract (1000 µg/ml) or gallic acid was treated with 1 ml of Folin–Ciocalteu reagent (1:10 v/v). After 5 min, 2 ml of sodium carbonate solution (7.5%, w/v) was added to the mixture. After 30 min of incubation at room temperature in the dark, the absorbance of the sample and the standard was measured at 765 nm. Distilled water was used as the reagent blank. The total phenolic content of a sample was determined using linear regression equation obtained from the calibration curve of gallic acid. The content of total phenolic compounds was calculated as mean ± standard deviation (SD) (n=3) and expressed as mg gallic acid equivalent (GAE)/g dry extract.

3.7 ESTIMATION OF TOTAL ALKALOID CONTENT

The plant extract (1mg) was dissolved in dimethyl sulphoxide (DMSO), added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1,2, 3and 4 ml chloroform by vigorous shaking and collected in a 10 ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The experiments were carried out in triplicates and the data were presented as mean ± S.D. (Standard deviation) and the total alkaloid content was expressed as mg of AE/g of extract.

3.8 ANTIOXIDANT ACTIVITY

3.8.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay

Aim:

To determine the radical scavenging activity of ethanolic extract of *Turbinaria conoide* using DPPH.

Principle

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical, which is purple in colour. When the extracts are incubated, the antioxidant molecules react DPPH and convert it into di-phenyl hydrazine, which is yellow in colour. The degree of discolouration of purple to yellow is measured at 517 nm, which is the measure of free radical scavenging potential of extracts. (Contreras-Guzman and Srong, 1982) **Materials required**

- Pipettes
- Spectrophotometer
- Standard flasks

Reagents

0.2 mM DPPH reagent

Standard – Ascorbic acid solution

0.1 g of L-ascorbic acid were dissolved in freshly prepared 0.05M oxalic acid solution (to protect oxidation of ascorbic acid) and made up to 100 ml and stored at room temperature.

Preparation of control

To 1 ml of ethanol, 2 ml of DPPH reagent was added, mixed well and incubated in dark at room temperature for 30 minutes.

Preparation of standard

0.1, 0.2, 0.3, 0.4 and 0.5 ml of standard ascorbic solution which corresponds to the concentration of 100, 200, 300, 400, 500 µg respectively was pipetted out into a series of test tubes. The volume in all the test tubes were made up to 1 ml using ethanol. To all the tubes, 2 ml of DPPH reagent was added and mixed well. The tubes were incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured at 517 nm in a spectrophotometer. A standard curve was plotted with the concentration of ascorbic acid on the x-axis and the optical density on the y-axis.

Preparation of sample

To 10 mg of algal extract, 10 ml of ethanol was added and vortexed.

Concentration: 1000 µg/ml

Procedure

- The DPPH radical scavenging assay was performed according to the method of Burits and Bucar (2000)
- 0.1, 0.2, 0.3, 0.4 and 0.5 ml of the extract which corresponds to the concentration of 100, 200, 300, 400, 500 µg respectively was pipetted out into a series of test tubes.
- The volume in all the test tubes were made up to 1 ml using ethanol.
- To all the tubes, 2 ml of DPPH reagent was added and mixed well.
- The tubes were incubated in the dark at room temperature for 30 minutes.
- After incubation, the absorbance was measured at 517 nm in a spectrophotometer.
- Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.
- The percentage inhibition of the samples and the standards were calculated by the following equation:

% Inhibition of DPPH = (Ac-Ao)AC × 100

where, Ac was the absorbance of control

A_0 was the absorbance of sample

3.8.2 FRAP assay (Ferric ion Reducing Antioxidant Power)

Principle

The method is based on the reduction of Fe^{3+} TPTZ complex (colourless complex) to Fe^{2+}tripyridyltriazine (blue colored complex) formed by the action of electron donating antioxidants at low pH.

Reagents

i.300 mM Acetate Buffer

ii.10 mM 2,4,6-Tris(2-pyridyl)-s-Triazine (TPTZ) in 40mM Hydrochloric acid (HCl)

iii.Standard: Iron(II) sulfate heptahydrate ($FeSO_4.7H_2O$) - 2000 micro molar (µM)

Working solution

Mix the following reagents in a proportion of 10:1:1

- Acetate buffer - 15 ml
- TPTZ- 1.5 ml
- Iron(III) chloride hexahydrate ($FeCl_3.6H_2O$) - 1.5 ml

Procedure

Freshly prepared working FRAP reagent was pipetted using 1-5 ml variable micropipette (3.995 ml) and mixed with each 1 ml of samples (Unfermented and Fermented). An intense blue colour complex was formed when ferric tripyridyl triazine (Fe^{3+} TPTZ) complex was reduced to ferrous (Fe^{2+}) form and the absorbance at 593 nm was recorded against a reagent blank (FRAP reagent + distilled water) after 30 min incubation at 37°C. The experiments were performed in duplicates. The calibration curve was prepared by plotting the absorbance at 593 nm versus different concentrations of $FeSO_4$.

3.8.3 ABTS- 2,2´-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay

Principle

Free radical scavenging activity of plant samples was determined by ABTS radical cation decolorization assay. ABTS·+ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12-16 h before use. **Reagents**

- 7mM ABTS
- 2,45 mM Potassium persulfate **Procedure**

ABTS·solution was diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After the addition of samples: Unfermented and Fermented each 1 ml to 3.995 ml of diluted ABTS·+ solution, the absorbance was measured at 30 min after the initial mixing. An appropriate solvent blank was run in each assay. All the measurements were carried out at least three times. Quercetin was used as standard substance.

Percent inhibition of absorbance at 734 nm was calculated using the formula,

ABTS·+ scavenging effect (%) = (Ac-Ao)AC × 100 where, Ac was the absorbance of control A_0 was the absorbance of sample

3.8.4 Total Antioxidant Assay

The total antioxidant activity of the EOC was evaluated by the phosphomolybdenum assay as described previously (Aliyu *et al.,* 2013). Briefly, 0.3 ml of EOC ranging between 25100µg/ml was added to 3ml reagent solution (0.6 M H_2SO_4, 28 mM of sodium phosphate, 4mM of Ammonium molybdate) and incubated at 95°C for 90 min. The absorbance was measured at 695 nm using a UV- visible spectrophotometer. **Statistical analysis**

All the assay were carried out in triplicates and the values were expressed as mean ± SD (Standard deviation). Statistical analysis was done using Microsoft excel 2016.

3.9 GAS CHROMATOGRAPHY–MASS SPECTROMETRY (GC-MS) ANALYSIS

Aim

To identify the bioactive compounds, present in the ethanolic extract of *T. conoides*.

Principle

Gas chromatography-mass spectrometry is a combination of analytical techniques to separate and identify various substances present in the mixture. Gas chromatography is used to separate the components and mass spectrometry is used to identify the separated components. The sample mixture which is injected into the inlet of gas chromatography is vapourised, carried by inert gases such as helium and passes through the column. The components are separated based on their interaction with the stationary phase which is the coating of the column and the mobile phase which is the carrier gas. Different compounds travel at different speeds through the column and reaches the end of the column at a particular retention time. At the end of the column, mass spectrometer is attached to it which detects the compounds based on its concentration and produces a plot of intensity vs. time in a chromatogram. Depending upon the number of components present in the sample mixture, a series of peaks in a chromatogram is generated.

Procedure

The ethanolic extract of *T. foenum-graecum* was analysed by GC-MS method. GC-MS technique was done by GC Shimadzu QP2010 system and Gas Chromatography interfaced to a mass spectrometer equipped with Elite-1 fused silica capillary column. For GC-MS detection, an electron ionization energy system with ionization energy of 70 eV was used. Helium was used as carrier gas at a constant flow rate of 4 ml/min and an injection volume of 2 µl was employed. The oven temperature was programmed from 100°C [isothermal for 5 minutes] with a temperature of 4°C/min to 240°C with column flow rate of 1.21ml/min. The sample was run for 47 minutes with solvent out time of 9.50 minutes. Mass spectra were taken with scan interval of 10 minutes.

Identification of bioactive compounds

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute of Standard and Techniques (NIST17 LIB) for different bioactive compounds. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST17 library. The name, molecular weight, molecular formula and structure of the component of the test material were identified.

4. RESULTS AND DISCUSSION

4.1 PHTOCHEMICAL ANALYSIS

The major phytoconstituents present in the ethanolic extract of *T. conoides* are shown in the table 1.

Table 1: Phytochemical screening in ethanolic extract of *T. conoides*

S.No.	Phytochemical compounds	Ethanolic extract of *T. conoides*
1	Saponin	+
2	Glycosides	++
3	Alkaloids	+
4	Tannins	+
5	Terpenoids	++
6	Phenolic compounds	++
7	Quinones	+
8	Vitamin C	+
9	Flavonoids	-
10	Steroids	+

Phytochemical analysis helps in identifying new source of therapeutically and industrially valuable compounds. Phytochemical analysis of *T. conoides* shows the presence of Secondary phytochemical compounds such as saponin, glycosides, alkaloids, tannins, terpenoids, phenolic compounds, quinones, vitamin C and steroids. Flavonoids were found to be absent in the extract. Mohideen and, Sujatha (2019), reported the presence of steroids, terpenoids, saponins, alkaloids, quinones, glycosides and phenol and absence of flavonoids and tannins in *T. conoides.*

4.2 TOTAL PHENOLIC CONTENT AND ALKALOID CONTENT

The total phenolic content was estimated using gallic acid as standard and it was found to be 478.16 ± 3.15 mg/g. The total alkaloid content was estimated using atropine as standard and was found to be 3.95 ± 0.01 mg/g. Table - 2 shows the concentration of total phenols and alkaloid present in *T. conoides.*

Table -2 Concentration of total Phenols and Alkaloid present in *T. conoides.*

Phytochemical constituent	Concentration (mg/g)
Total phenolic content (mg of Gallic acid equivalent/g of extract)	478.16 ± 3.15
Total alkaloid content (mg of atropine equivalent/g of extract)	3.95 ± 0.01

The presence of more concentration of phenolic content may be responsible for the antioxidant activity in *T. conoides*. Phenolic compounds act as an antioxidant by reacting with a variety of free radicals.

4.3 ANTIOXIDANT ACTIVITY

4.3.1 DPPH Assay

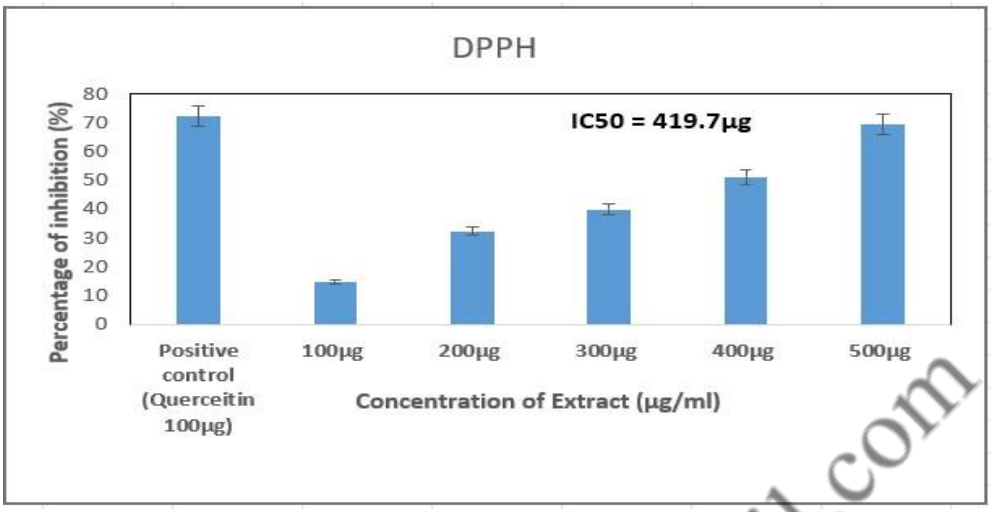
The mean absorbance, % inhibition and IC50 value of the ethanolic extract of *T. conoides* against DPPH at their concentrations ranging from 100 µg to 500 µg are depicted in Table 3 and Figure 5.

Table 3 – DPPH radical scavenging activity of *T. conoides*

Concentration (µg)	Mean Absorbance	% inhibition	IC_{50}
100	0.78 ± 0.003	14.83	**419.7 µg**
200	0.62 ± 0.001	32.39	
300	0.55 ± 0.005	39.91	
400	0.45 ± 0.033	51.09	
500	0.28 ± 0.003	69.41	

Values are mean ± SD; n = 3.

Figure 4 – DPPH radical scavenging activity of *T. conoides*



The DPPH radical scavenging assay was carried out to determine the antioxidant activity of the ethanolic extract of *T. conoides*. The extract has shown a maximum of about 69.41% of inhibition against at a maximum concentration of 500 µg with the IC_{50} value of 419.7 µg. It may be attributed to the presence of bioactive compounds present in the plant extract especially phenolic compounds. The effect of antioxidants on DPPH radical scavenging is thought to be due to hydrogen donating ability. DPPH is a stable free radical and it accepts an electron or hydrogen radical to become a stable diamagnetic molecule. In accordance with our study Ganesan et al. (2008) also reported higher percentage DPPH radical scavenging activity in methanol extract of brown seaweed *T. conoides*.

4.3.2 FRAP assay (Ferric ion Reducing Antioxidant Power)

The absorbance and FRAP activity of the ethanolic extract of *T. conoides* at their concentrations ranging from 100 µg to 500 µg are given in Table 4 and Figure 6.

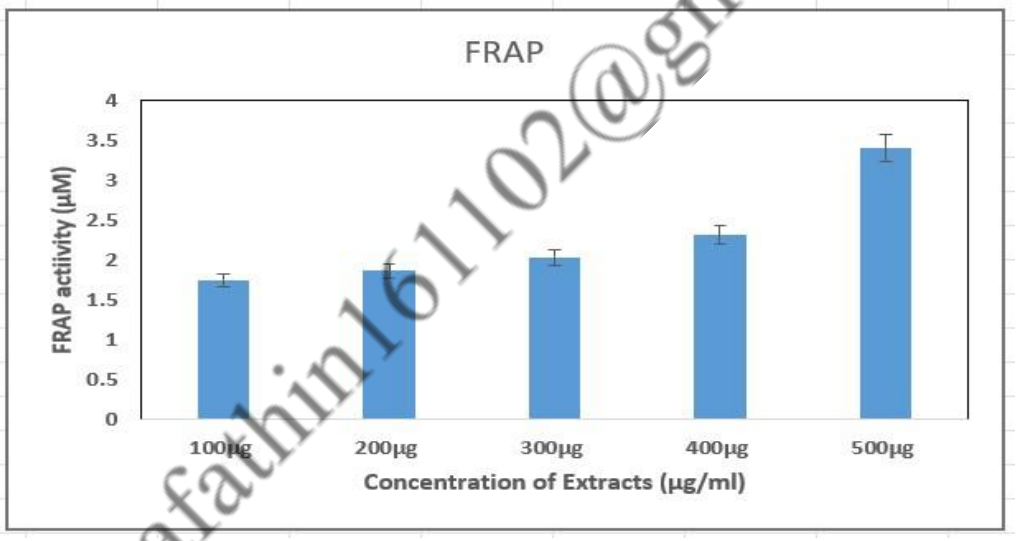
FRAP values are calculated by measuring the increase in the absorbance at 593 nm and expressing them as µM ferrous ion (Fe^{2+}) equivalents. The activity of FRAP was found to higher (3.42µM) in concentration of 500 µg. As the concentration of extract increased from 100 µg to 500 µg, the FRAP activity also increased. This shows that *T. conoides* extract has antioxidant activity that increases when its concentration is increased.

Table – 4 FRAP assay (Ferric ion Reducing Antioxidant Power)

Concentration (µg)	Absorbance	FRAP activity
100	0.55 ± 0.017	1.75
200	0.44 ± 0.001	1.87
300	0.49 ± 0.029	2.04
400	0.58 ± 0.013	2.31
500	0.66 ± 0.013	3.42

Values are mean ± SD; n = 3.

Figure 5 - FRAP assay (Ferric ion Reducing Antioxidant Power)



4.3.3 ABTS- 2,2´-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay

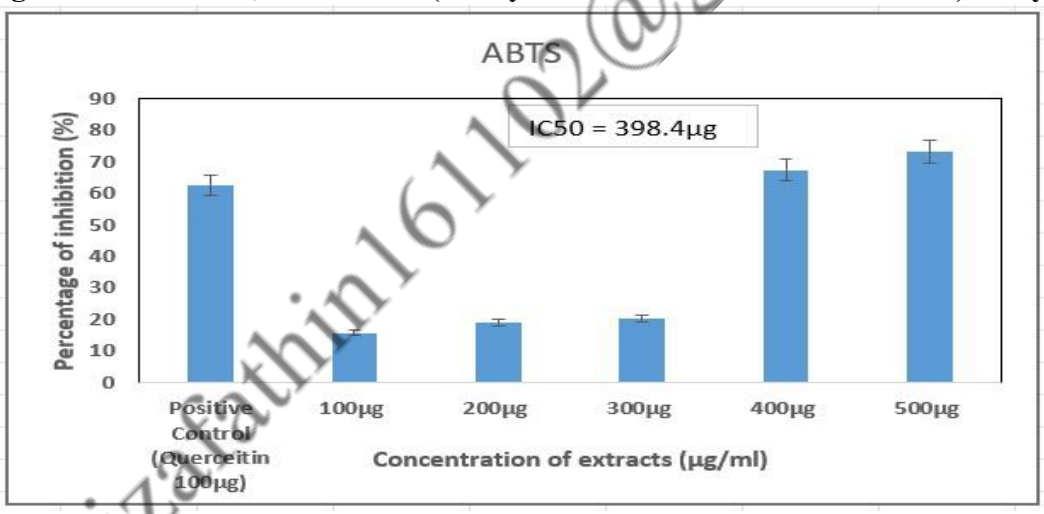
The absorbance, % of ABTS inhibition and IC50 value of the ethanolic extract of *T. conoides* at their concentrations ranging from 100 µg to 500 µg are shown in Table 5 and Figure 8.

Table – 5 ABTS- 2,2´-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay

Concentration (µg)	Absorbance	% inhibition	IC_{50}
100	0.638 ± 0.0045	15.78	**398.4 µg**
200	0.613 ± 0.0005	19.08	
300	0.603 ± 0.0020	20.34	
400	0.247 ± 0.0060	67.37	
500	0.204 ± 0.0005	73.11	

Values are mean ± SD; n = 3.

Figure – 6 ABTS- 2,2´-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay



The ABTS assay was carried out to determine the antioxidant activity of the ethanolic extract of *T. conoides*. The extract has shown a maximum of about 73.11% of inhibition against at a maximum concentration of 500 µg with the IC_{50} value of 398.4 µg. In a study conducted by Boonchum et al., 2011 the Aqueous extract and Ethanolic extract of T. *Conoides* showed IC50 of 5.290 ± 0.088 and 96.242 ± 1.643.

4.3.4 Total antioxidant assay

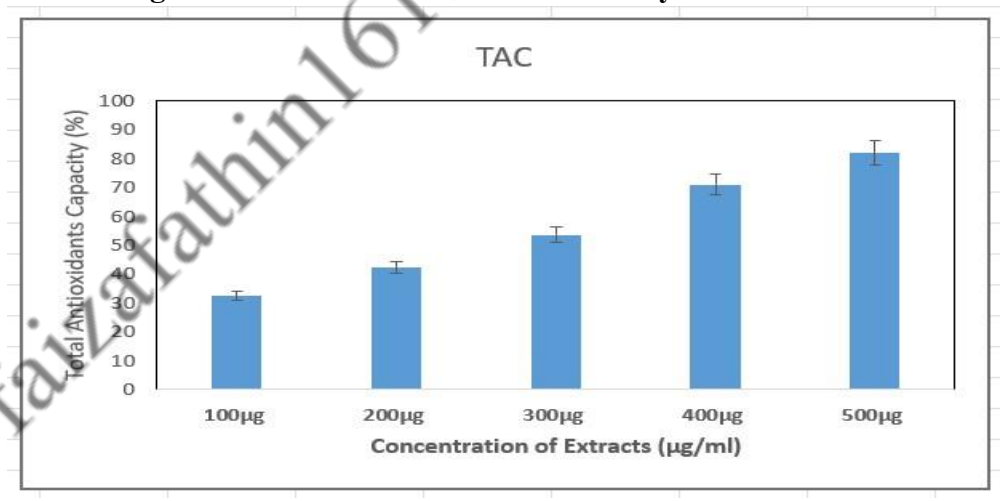
The total antioxidant activity of *T. conoides* was evaluated and the results are presented in table 6 and figure 8.

Table 6 - The total antioxidant activity of *T. conoides*

Concentration (µg)	Absorbance	Total Antioxidant capacity
100	0.54 ± 0.013	32.43
200	0.46 ± 0.001	42.48
300	0.37 ± 0.025	53.71
400	0.23 ± 0.016	70.99
500	0.14 ± 0.017	82.15

Values are mean ± SD; n = 3.

Figure 7 - The total antioxidant activity of *T. conoides*



The total antioxidant activity of *T. conoides* was found to be 82.5 percent at the concentration of 500µg. The total antioxidant activity exerted by *T. conoides* could be due to the presence of phenols and alkaloids present in it.

Phenolic compounds are commonly found in plants, reportedly having several biological activities including antioxidant properties. Earlier reports have revealed that marine

seaweed extracts, especially polyphenols, have antioxidant activity (Yan *et al.,* 1999; Kuda *et al.,* 2005; Lim *et al.,* 2002). Phenolic compounds are regarded for their important dietary roles as antioxidants and chemo preventive agents (Bravo, 1998). Previous reports suggested that there is a direct relationship between the antioxidant activity and the total phenolic content in some herbs, vegetables, and fruits (Velioglu *et al.,* 1998). A number of studies have been focused on the biological activities of phenolic compounds, which are potential antioxidants and free-radical scavengers (Rice-Evans et al., 1995). Studies also have shown that *T. conoides* could be a potential source of phenolic compounds (Gallyot *et al.,* 2018)

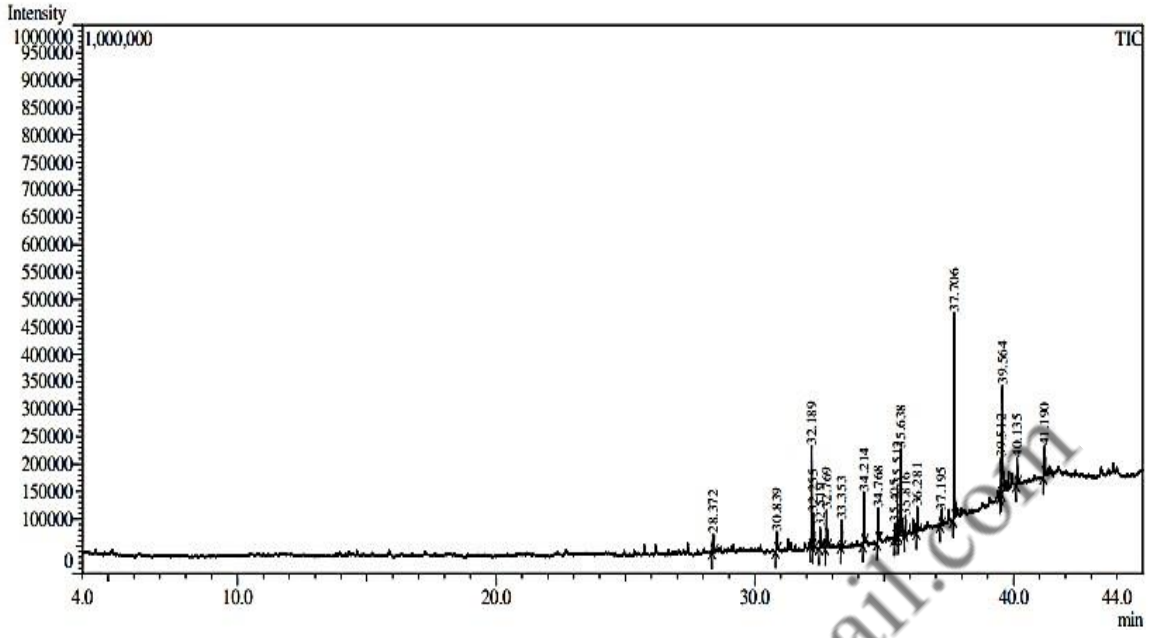
The major active compounds in different seaweed extracts have been reported to be phlorotannins and fucoxanthin (Xiaojun *et al.,* 1996; Yan *et al.,* 1999;). Phlorotannins (Burtin, 2003), is bipolar in nature, and mostly found in brown seaweeds (Targett and Arnold, 1998), such as T. conoides. The phlorotannin function as an antioxidant component due to the presence of multiple phenolic groups which could assist the algae to overcome oxidative stress as well as play a putative adaptive role in defense against grazers, such as marine herbivores (Van Altena and Steinberg, 1992) due to their plasticity characters. The presence of antioxidative phlorotannins in *T. conoides* could cause to have higher TPC.

4.4 GAS CHROMATOGRAPHY–MASS SPECTROMETRY ANALYSIS (GC–MS)

Gas Chromatography–Mass Spectrometry analysis (GC–MS) is a potent tool for qualitative and quantitative investigation of several compounds present in natural products. The compounds present in the ethanol extract of brown algae *T. conoides* were identified by GCMS.

The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the *T. conoides*. The active principals with their retention time (RT), molecular formula, molecular weight and concentration (%) in the ethanol extract of *T. conoides* are presented in Table 7.

Twenty components were identified and the results revealed that Hexadecanoic Acid, 2Hydroxy-1-(Hydroxymethyl) (20.18%) and 9-Octadecenoic acid,1,2,3-propanetriyl ester (13.13%) were found to be the major compounds. Some of the activities of the compounds identified in the current study are given in Table 8.



GC-MS Chromatogram of *Turbinaria conoides*

Table 7 – Bioactive Compounds identified in the brown algae *T. conoides*

S.No.	Name of the Compound	Molecular Weight	Molecular Formula	RT (min)	Peak Area	% Peak area
1	Bis(tridecyl) phthalate 1,2Benzenedicarboxylic acid, ditridecyl ester phthalic acid	530	$C_{34}H_{58}O_4$	28.372	92987	2.56
2	2(4h)-Benzofuranone,5,6,7,7a-Tetrahydro-6-Hydroxy-4,4,7a-Trimethyl, (6s-Cis)	196	$C_{11}H_{16}O_3$	30.839	78171	2.15
3	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne $$ Neophytadiene	278	$C_{20}H_{38}$	32.189	356404	9.82
4	2-Pentadecanone,6,10,14trimethyl	268	$C_{18}H_{36}O$	32.255	160384	4.42
5	2,6,10-Trimethyl,14-Ethylene-14-Pentadecene	278	$C_{20}H_{38}$	32.519	73889	2.04
6	3,7,11,15-Tetramethyl-2hexadecen-1-ol	296	$C_{20}H_{40}O$	32.769	132937	3.66

7	Hexadecanoic Acid, Methyl Ester	270	$C_{17}H_{34}O_2$	33.353	89903	2.48
8	Tetradecanoic Acid, Ethyl Ester	256	$C_{16}H_{32}O_2$	34.214	173277	4.78
9	Arachidonic acid	304	$C_{20}H_{32}O_2$	34.768	111263	3.08
10	Hexadecanoic acid,1-(hydroxymethyl)-1	568	$C_{35}H_{68}O_5$	35.405	79320	2.19
11	9-Octadecenoic Acid (Z)-, Methyl ester	296	$C_{19}H_{36}O_2$	35.512	175368	4.83
12	Phytol Isomer	296	$C_{20}H_{40}O$	35.638	351484	9.69
13	Methyl stearate	298	$C_{19}H_{38}O_2$	35.816	63417	1.75
14	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	294	$C_{19}H_{34}O_2$	36.281	88373	2.44
15	Hexadecanoic Acid,2-Hydroxy-1-(Hydroxymethyl)	330	$C_{19}H_{38}O_4$	37.195	48616	1.34
16	Hexadecanoic Acid,2-Hydroxy-1-(Hydroxymethyl)	330	$C_{19}H_{38}O_4$	37.706	732116	20.18
17	9,12-Octadecadienoyl chloride,(Z,Z)-	298	$C_{18}H_{31}C_{10}$	39.512	131995	3.64
18	9-Octadecenoic acid,1,2,3propanetriyl ester	884	$C_{57}H_{104}O_6$	39.564	476204	13.13
19	Bis(2-ethylhexyl) phthalate	390	$C_{24}H_{38}O_4$	40.135	97582	2.69
20	5,8,11,14-Eicosatetraenoic acid, methyl ester	318	$C_{21}H_{34}O_2$	41.190	113753	3.14

TABLE 8 - Biological activities of some compounds identified in ethanol extract of *Turbinaria Conoides*

S.No.	Name of the compound	Compound Nature	Activity
1	Bis(tridecyl) phthalate 1,2-Benzene dicarboxylic acid, ditridecyl ester phthalic acid	Diesters of phthalic acid	Antimicrobial and antibacterial activity **(** Sathish *et al.,* 2018)
2	2(4h)- Benzofuranone 5,6,7,7a- tetrahydro-6Hydroxy-4,4,7a- Trimethyl, (6s-cis)	Fatty acid ethyl ester	Antimicrobial and antitubercular activity **(**Fadipe *et al.,*2014**)**
3	2,6,10- Trimethyl , 14-ethylene-14-Pentadecene $$ neophytadiene	Alkene and a diterpene	Anti-inflammatory agent and antimicrobial agent (NCBI Database- PubChem)
4	2-Pentadecanone,6,10,14trimethyl	A sesquiterpene isolated from impatiens parviflora	Antibacterial, antinociceptive and antiinflammation (PubChem Database- Neophytadiene database)
5	2,6,10-Trimethyl,14-Ethylene-14-Pentadecene	Olefins	phytochemical screening, antibacterial activity (Sekaran *et al.,* **2018**)
6	3,7,11,15-Tetramethyl-2hexadecen-1-ol	a natural product found in echinophora tournefortii, juniperus and other organisms	Anxiolytic cytotoxic, metabolism modulating, antioxidant, antinociceptive, antimicrobial and anti-inflammatory (NCBI Database)
7	Hexadecanoic Acid, Methyl Ester	fatty acid methyl ester, hydrophobic	Antioxidant . hypocholesterolemic, antiandrogenic, hemolytic, alpha reductase inhibitor (Kavitha and Uduman 2017)
8	Tetradecanoic Acid, Ethyl Ester	long chain saturated fatty acid, ethyl ester	Antimicrobial activity (Babu, *et al.,2*014)
9	Arachidonic acid	Linoleic acid	ntioxidative functions, visual ctivity and

			attenion (Kotani *et al.,* 2003)
10	Hexadecanoic acid,1(Hydroxymethyl)	Fatty acid ethyl ester	Antimicrobial activity (Mohammad *et al., 2*016)
11	9-Octadecenoic Acid (Z)-, Methyl ester	Monounsaturated fatty acid	Anti-bioflim, antibacterial, antifungal and antioxidant activity (Yahya Hadi, *et al.,* 2015)
12	Phytol Isomer	Acyclic diterpenoids	Antioxidant, anti-inflammatory, anti-microbial, immune modulating, autophagy and apoptosis (Tahreen *et al., 2*021)
13	Methyl stearate	Fatty acid methyl esters	Antifungal and antioxidant activity (Maria *et al.,2*017)
14	Hexadecanoic Acid,2Hydroxy-1(Hydroxymethyl)	Amino acid	Anti-oxidant, Anti-microbial activity (Tulika and Agarwal 2017)
15	Hexadecanoic Acid,2Hydroxy-1(Hydroxymethyl)	Amino acid	Anti-oxidant, Anti-microbial activity (Sunita and Kumar 2017)
16	9,12-Octadecadienoyl chloride,(Z,Z)-	Fatty acid nature	Anti-microbial and anti-diabetic activity (Roshani *et al.,*2018)
17	9-Octadecenoic acid,1,2,3-propanetriyl ester	Unsaturated fatty acid	Antibacterial and anticancer activity (Dr. Duke's phytochemical and Ethnobotanical Databases)
18	Bis(2-ethylhexyl) phthalate	Ester of phthalic acid	Antimicrobial, antioxidant, antiviral and antitumour activity (Momen *et al.*.2018)
19	5,8,11,14-Eicosatetraenoic acid, methyl ester,	Arachidonic acid	Antiviral activity (Manjula and Selvaraju, 2020)

5. SUMMARY AND CONCLUSION :

The brown algae *Turbinaria conoides* were collected from the Mandapam coastal area native to Rameshwaram district, Tamil Nadu, India. The samples were washed, dried, powdered, and extracted using ethanol. Qualitative phytochemical screening and estimation of total phenol and alkaloids were carried out in the ethanol extract. The extract showed the presence of secondary metabolites such as saponin, glycosides, alkaloids, tannins, terpenoids, phenolic compounds, quinones, vitamin C and steroids. Flavonoids were found to be absent in the extract. The total phenolic content and alkaloid content were found to be 478.16 ± 3.15 mg/ and 3.95 ± 0.01 mg/g respectively. The *in vitro* antioxidant activity was evaluated by DPPH, ABTS, and FRAP assays. In the DPPH radical scavenging assay, the algal extract showed a maximum of about 69.41% of inhibition at the concentration of 500 µg. ABTS assay recorded 73.11% inhibition and FRAP assay showed 3.42% inhibition at 500 µg concentration. The total antioxidant capacity was estimated to be 82.15%. In the present study, *Turbinaria conoides* were found to possess strong antioxidant activity. The antioxidant mechanisms of seaweed extracts may be attributed to their free radical-scavenging ability. In addition, phenolic compounds appear to be responsible for the antioxidant activity of seaweed extracts. In GCMS analysis twenty components were identified and the results Hexadecanoic Acid, 2-Hydroxy-1-(Hydroxymethyl) (20.18%) and 9-Octadecenoic acid,1,2,3-propanetriyl ester (13.13%) were found to be the major compounds. Many of the compounds identified were found to possess a broad spectrum of biological activities. Further studies are required for a better understanding of the bioactive constituents of seaweed and their pharmacological potential.

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