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Original Article

# Phytochemical profiling of *Turbinaria ornata* and its antioxidant and anti-proliferative effects

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**الملخص**

**أهداف البحث:** لتحليل المواد الكيميائية النباتية، وتقييم القدرات المضادة للأكسدة والمضادة للتكاثر لـ "تربيناريا أورناتا" ( ترنر) أجارد ١٨٤٨.

**طرق البحث:** أجريت التحاليل الكيميائية النباتية للمستخلص الهكساني، والمستخلص المائي لـ "تربيناريا أورناتا". تم تحليل المستخلصات باستخدام تقنيات؛ الطيف الكتلي اللوني للغاز، وجهاز "فورييه" للتحويل الطيفي للأشعة تحت الحمراء، وفاصل الألوان السائل عالي الكفاءة. تم تحديد الخاصية المضادة للأكسدة للمستخلص الهكساني والمستخلص المائي لـ "تربيناريا أورناتا" باستخدام فحص بكرايل هايدرزايل ثنائي الفينايل للكسح الجذري وفحص قوة اختزال أيون الحديديك. بالإضافة إلى ذلك تم تقييم الخاصية المضادة للتكاثر داخل الخلية على الخلايا الظهارية الكلوية للقرد الأفريقي الأخضر (فيرو)، والخلايا الظهارية القاعدية السنخية البشرية السرطانية باستخدام فحص إم تي تي.

**النتائج:** كشف الفحص الكيميائي النباتي لـ "تربيناريا أورناتا" وجود سابونين، وقلويدات وأحماض أمينية، وزيت ودهن ثابتين، ومركبات فينولية (العفص وفلافونيدات والفينول الكلي). وجدت الخاصية المضادة للتكاثر الأعلى في المستخلص الهكساني ويليها المستخلص المائي لـ "تربيناريا أورناتا". كانت قيم فعالية مكافحة التكاثر (بالـ مايكروجرام/ مليلتر) بالنسبة للمستخلص الهكساني والمستخلص المائي على الخلايا الظهارية القاعدية السنخية البشرية السرطانية وخلايا فيرو: ٦٢،٩١ و٩٣،٠٠ و٧٢،٦٤ و١٠٦،٦ على التوالي. وقد أظهر التحليل باستخدام جهاز "فورييه" للتحويل الطيفي للأشعة تحت الحمراء وجود مجموعات وظيفية هي: الكحول، الأميدات والعطريات، والأمينات، وهاليدات الألكيل، والألكاينات، والألكانات، والأحماض الكربوكسيلية. كما كشف الطيف الكتلي اللوني للغاز عند تحليل المستخلص الهكساني لـ "تربيناريا أورناتا" عن وجود ١٣ مركبا نشطا.

**الاستنتاجات:** بناء على الخاصية المضادة للتكاثر المسجلة فإن هناك حاجة للمزيد من الدراسات الدوائية في سبيل تحضير دواء مضاد للسرطان.

**الكلمات المفتاحية:** A549؛ مضاد الأكسدة؛ مضاد التكاثر؛ هينترياكونتان؛ تربيناريا أورناتا

**Abstract**

**Objectives:** To analyse the phytochemicals and evaluate the antioxidant and anti-proliferative ability of *Turbinaria ornata* (Turner) J. Agardh, 1848.

**Methods:** A phytochemical analysis of the *T. ornata*-hexane extract (To-HE) and *T. ornata*-aqueous extract (To-AE) was performed. *T. ornata* extracts were analysed by gas chromatography-mass spectrometry (GC-MS), Fourier transform infrared spectroscopy (FTIR) and high-performance liquid chromatography (HPLC). The antioxidant properties of To-HE and To-AE were determined by 2,2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH) and ferric ion reducing power (FRAP) assays. In addition, the *in vitro* anti-proliferative properties of To-HE and To-AE were assessed in kidney epithelial cells from the African green monkey (*Vero*) and in adenocarcinomic human alveolar basal epithelial cells (A549) using the MTT (3-(4,5-dimethylthiazol- 2yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) assay.

**Results:** The phytochemical screening of *T. ornata* revealed the presence of saponin, alkaloids, amino acids, fixed oil and fat and phenolic compounds (tannins, flavonoids and total phenol). A higher antioxidant ability was found in To-HE than in To-AE. The anti-proliferative efficacy values (μg/mL) of To-HE and To-AE for A549 and *Vero* cells were 62.91 and 93.00 and 72.64 and 106.6, respectively. The FTIR analysis revealed

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the presence of functional groups such as alcohols, amides, aromatics, amines, alkyl halides, alkynes, alkanes and carboxylic acids. The GC-MS analysis of To-HE revealed the presence of 13 active compounds.

**Conclusion:** Owing to its recorded anti-proliferative effect, further pharmaceutical studies on the development of this anticancer drug are merited.

**Keywords:** A549; Antioxidant; Anti-proliferative; Hentriacontane; *T. ornata*



## Introduction

Cancer is a group of diseases characterized by the uncontrolled growth of cells that can lead to death. Despite many therapeutic treatments available for cancer, the survival rate and disease curative percentage are very low. It has become an increasing public health problem that accounts for 6 million cases every year throughout the world.[1] Of the various types of cancer, lung cancer is the second most important cause of death worldwide that accounts for 75–80% of the deaths.[2] Current cancer chemotherapy can damage or kill the rapidly dividing and healthy cells, which causes serious side effects such as anaemia, hair loss, and nausea. In addition, the costs of chemotherapeutic drugs are high compared to those of the natural compounds derived from medicinal plants.[3] Therefore, the use of natural products could be an alternative method to control and eradicate cancer.[4] Natural sources such as plants, microorganisms and marine organisms are potential bioresources for anticancer compounds.[5] Earlier studies reported that the seaweeds *Undaria pinnatifida*,[6] *Gracilaria edulis*,[7] *Turbinaria conoides*,[8] and *Sargassum pallidum*[9] exhibit anti-proliferative effects against human lung cancer cells (A549).

*Turbinaria ornata* (Turner) J. Agardh, 1848 is an extensive species of marine green alga belonging to the family Phaeophyceae and is rich in fucoids and sulphated polysaccharides.[10] It is widely distributed on the southeast coast of Tamil Nadu (India) and is reported to be used as an animal food, food ingredient and fertilizer. This alga is widely distributed in tropical and subtropical areas of the central and western Pacific and in the Indian Ocean.[11] A wide range of biological properties of this seaweed, including antibacterial,[12] anti-coagulant,[13] anti-inflammatory[14] and antioxidant properties, have been reported.[15] In addition, studies on fucoidan isolated from *Turbinaria* spp. have shown therapeutic benefits such as the prevention of myocardial injury,[16] along with hepatoprotective,[17] anticancer[9] and neuroprotective activities.[18] In view of these findings, the present study aims to evaluate the *in vitro* antioxidant and anti-proliferative effects of *T. ornata*-hexane extract (To-HE) and *T. ornata*-aqueous extract (To-AE) against A549 and *Vero*.

## Materials and Methods

### *Collection of seaweed material and processing*

Algal samples of *T. ornata* (Figure 1) were collected from Mandapam, Ramanathapuram District, Tamil Nadu, southeastern India (9° 22′ N, 78° 52′ E). The samples were washed thoroughly with tap water and then distilled water to remove the associated biota and salt debris and then shade dried for 2–3 weeks. Finally, the seaweed was powdered using a kitchen electric blender. The seaweeds were identified based on standard keys[19] and further confirmed by Dr. N. Kaliaperumal, Principal Scientist (Retd.), Central Marine Fisheries Research Institute, Mandapam Camp, Ramanathapuram District (India). The reference specimens have been kept in the Department of Biotechnology, Periyar University (Salem). All the chemicals used in this study were of analytical grade with maximum purity.

### *Preparation of To-HE*

Approximately 10 g of powdered seaweed material was initially soaked in 50 mL of hexane for three days with mild shaking. Then, the extract was filtered through Whatman filter paper and concentrated in a Rotary evaporator. This extract was stored in refrigerator until use.

### *Preparation of To-AE*

Aqueous extract was prepared by mixing 10 g of dry seaweed powder in 100 mL of sterile double distilled water and boiling at 60 °C for 30 min. Finally, the extract was filtered with Whatman no. 1 filter paper and stored in a refrigerator until use.

### *Phytochemical screening*

The samples (To-HE and To-AE) were subjected to preliminary phytochemical screening as described by Harborne (1973).[20]

**Figure 1:** *Turbinaria ornata* (Turner) J. Agardh, 1848.

### Structural characterization of To-HE and To-AE

#### Fourier transform infrared spectroscopy (FTIR)

The FTIR analysis of To-HE and To-AE was recorded in the mid-IR region (4000–400 $cm^{-1}$) at a resolution of 4 $cm^{-1}$ using an FTIR spectrometer coupled with a TGS (Tri-glycine sulphate) detector. In brief, 1 mg of each dried sample was mixed with 100 mg of potassium bromide (KBr) and then compressed to prepare salt-discs (3 mm dia). These discs were analysed with a Fourier transform infrared spectrophotometer (Bruker, D8, Germany model).[8]

#### High-performance liquid chromatography (HPLC)

The HPLC analysis of To-HE and To-AE was performed following the methodology of Eahamban et al. Approximately 1 mg of sample was dissolved in 1 mL of methanol, and 20 µL was injected. Methanol:water (50:50) was used as the mobile phase. The experiment was performed in a Shimadzu LC solution 20 AD, Japan and SPD 20 A, an instrument equipped with a UV detector (254 nm) in order to determine the peak purity. An LCGC C18 column was used for isocratic resolution using the mobile phase at a flow rate of 1.0 mL/min.[21]

#### Gas chromatography-mass spectrometry (GC-MS)

GC analysis of To-HE was performed by injecting 1 µL of sample on a 5MS column of a GC-MS instrument (Perkin Elmer, Massachusetts, USA) with helium as the mobile phase. The qualitative and quantitative analysis of To-HE was carried out using a CP 3800 Saturn 2200 Gas Chromatography-Mass Spectrometer. The temperature programme was 80–350 °C at the rate of 3 °C/min. The ion temperature was 200 °C, and the scan range was 20–500 AMU (Atomic Mass Unit). The identification of components was based on comparison of their mass spectra with those from the Wiley library.[22]

### Antioxidant activity

#### DPPH radical scavenging assay

DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging activity of To-HE and To-AE was measured according to the method of Adeosun et al. In brief, 2-mL (1 mg/mL) samples and standards at varying concentrations (20, 40, 60, 80 and 100 µg/mL) were mixed with 1 mL of 0.1 mM DPPH in methanol. The mixture was instantly shaken and incubated for 30 min in the dark. The absorbance of the samples and control was measured at 517 nm.[23]

#### Ferric ion reducing power (FRAP) assay

FRAP assay of To-HE and To-AE was performed according to the method of Adedayo et al. The FRAP stock reagent was prepared with 300 mM acetate buffer (pH-3.6), 10 mM/L TPTZ (2,4,6-tri[2-pyridyl]-s-triazine) and 20 mM ferric chloride hexahydrate solution. The working solution was prepared with 30 mL of acetate buffer, 4 mL of TPTZ solution and 4 mL of ferric chloride hexahydrate solution. In brief, 500-µL (1 mg/mL) aliquots of varying concentrations (6.25–100 µg/mL) of samples and standard were prepared, and methanol was added to 2.5 mL of FRAP reagent and incubated in the dark for 30 min. The absorbance of the sample and control was measured at 593 nm. All the experiments were performed in triplicate. Ascorbic acid was used as a standard.[24]

#### Statistical analysis

The *in vitro* antioxidant activities of the samples were calculated using the following formula.

$$\%\textbf{Inhibition} = \frac{\mathbf{A_0 - A_1}}{\mathbf{A_0}} \times \mathbf{100}$$

where $A_0$ is absorbance of the control, $A_1$ is absorbance of the sample.

#### Anti-proliferative assay

A549 and *Vero* cells were cultured and seeded on 96-well plates ($\sim 1 \times 10^4$ cells) and incubated for 48 h. The cells were treated with various concentrations of To-HE and To-AE (10–100 µg/mL) and incubated for 6–7 h. Then, the plates were subjected to MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) assay. MTT (100 µL) was added in each well and incubated for 4 h and then dissolved in 100 µL of dimethyl sulphoxide (DMSO). Finally, reaction mixtures were recorded at 620 nm in a multi-well ELISA plate reader. Optical density (OD) was used to determine the percentage of cell viability by using the following formula.[25]

$$\textbf{Percentage growth} = \frac{\mathbf{T - T0}}{\mathbf{C - T0}} \times \mathbf{100}$$

where T is the OD of the sample, C is the OD of the control, $T_0$ is the OD at time zero.

The activity of marine seaweed was expressed by the inhibitory concentration ($IC_{50}$) values that were calculated using Graph pad prism version 5.

## Results

### Phytochemical screening

Flavonoids, saponins, alkaloids and fixed oils were found in both of the extracts (To-HE and To-AE) (Table 1). Carbohydrates, glycosides, proteins and amino acids were absent. In contrast, tannin was found only in the To-HE extract.

**Table 1: Phytochemical screening of To-AE and To-HE.**

| Phytochemical group | To-AE | To-HE |
|---|---|---|
| Flavonoid | + | + |
| Carbohydrate | − | − |
| Saponin | + | + |
| Tannin | − | + |
| Glycoside | − | − |
| Protein | − | − |
| Alkaloid | + | + |
| Fixed oil & fat | + | + |
| Amino acid | − | − |

[+] Present; [−] Absent.

*Fourier transform infrared spectroscopy*

The FTIR analysis of To-HE revealed twelve major peaks (Figure 2a) at 3415.77, 2925.31, 2854.32, 2359.61, 1713.88, 1461.86, 1379.62, 1260.92, 1172.28, 723.71, 618.50 and 476.61 $cm^{-1}$, while To-AE (Figure 2b) displayed ten major peaks at 3448.90, 2360.84, 2337.83, 2053.33, 1635.69, 1406.23, 1213.26, 1106.83, 1045.68 and 602.74 $cm^{-1}$. The corresponding stretches and functional groups are shown in Table 2 and 3.

*High performance liquid chromatography*

The HPLC profile of To-HE and To-AE displayed a sharp intense peak at retention times 5.297 and 2.385,

**Table 2: Chemical constituents of To-HE analysed by gas chromatography-mass spectrometry.**

| Wavenumber ($cm^{-1}$) | Assignment | Functional group |
|---|---|---|
| 3415.77 | O−H | Alcohol |
| 2925.31 | −CH | Alkane |
| 2854.32 | −CH | Alkane |
| 2359.61 | C≡C | Miscellaneous |
| 1713.88 | C═O | Carboxylic acid |
| 1461.86 | C═H | Alkanes |
| 1379.62 | C═H | Alkanes |
| 1260.92 | C═F | Alkyl halides |
| 1172.28 | C═F | Alkyl halides |
| 723.71 | C═H | Alkanes |
| 618.50 | C≡C | Alkynes |
| 476.61 | C−Br | Alkyl halides |

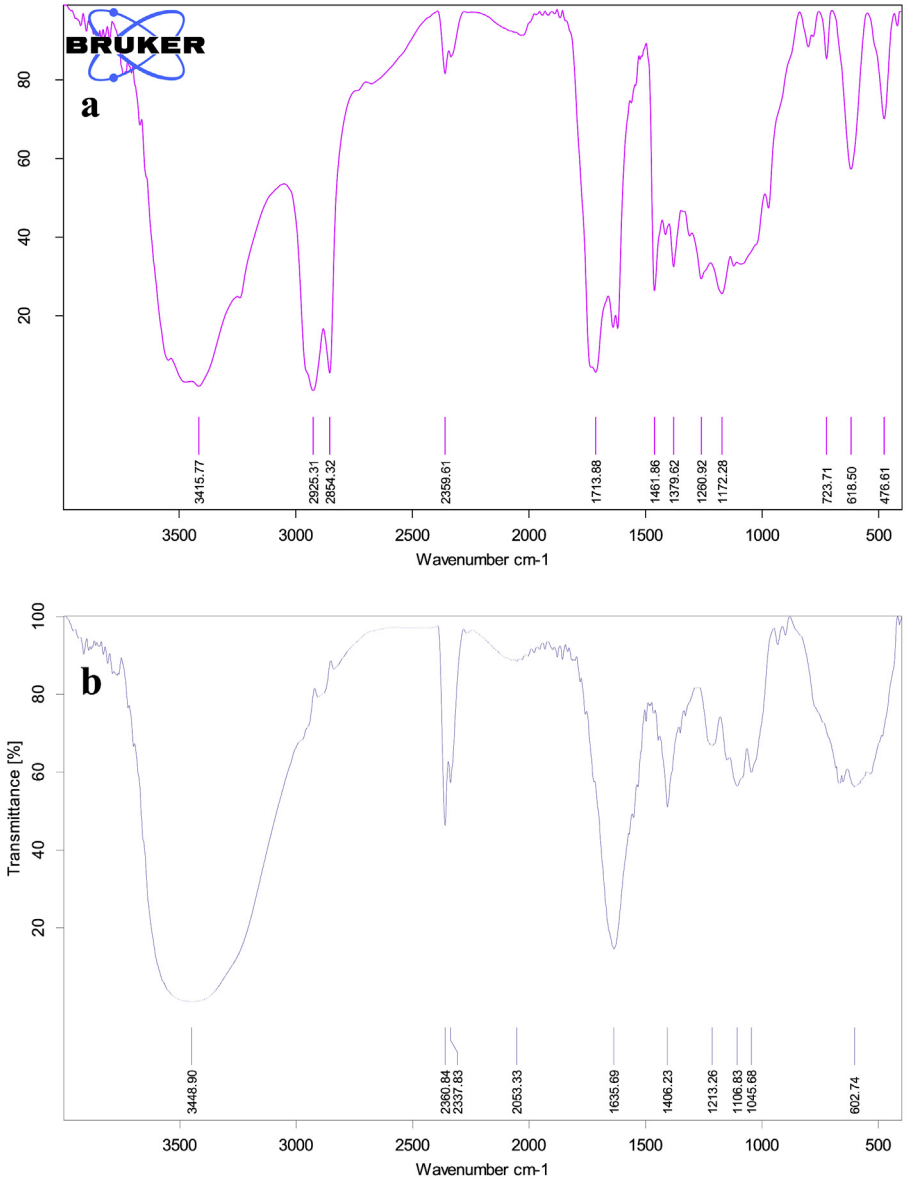


**Figure 2:** FTIR spectrum analysis. (a) To-HE & (b) To-AE.

**Table 3: FTIR analysis of To-HE.**

| Wavenumber ($cm^{-1}$) | Assignment | Functional group |
|---|---|---|
| 3448.90 | O—H | Alcohol |
| 2360.84 | Si—H silane | Miscellaneous |
| 2337.83 | Si—H silane | Miscellaneous |
| 2053.33 | N═C in R—N═C═S | Miscellaneous |
| 1635.69 | C═O | Amides |
| 1406.23 | ArC—C | Aromatics |
| 1213.26 | C—N | Amines |
| 1106.83 | C—N | Amines |
| 1045.68 | C—F | Alkyl halides |
| 602.74 | C≡C | Alkynes |

respectively. In addition, To-AE displayed some minor peaks (Figure 3a and b).

*Gas chromatography-mass spectrometry*

The GC-MS analysis of To-HE indicated the presence of 13 compounds (Figure 4 and Table 4). The major phytochemical constituents present in the To-HE were 1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester (MW: 278, MF: $C_{16}H_{22}O_4$, $R_T$ 0.941), n-hexadecanoic acid (MW: 256, MF: $C_{16}H_{32}O_2$, $R_T$ 2.131), Z,Z-6,28-heptatriactontadien-2-one (MW: 530, MF: $C_{37}H_{70}O$, $R_T$:2.418), 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (MW: 278, MF: $C_{16}H_{22}O_4$, $R_T$ 1.137), cholest-5-En-3-ol, 24-propylidene-, (3 beta.) (MW: 426, MF: $C_{30}H_{50}O$, $R_T$ 3.479) with $R_T$ of 31. The remaining phytoconstituents were present at low concentrations, and the names of the compounds, their retention times and their percentages are listed in Table 2.

*Antioxidant activity*

*DPPH radical scavenging assay*

Among the concentrations of To-HE, To-AE and ascorbic acid used in this study, 100 µg/mL resulted in the highest radical scavenging effect of 73.23%, 45.02% and 93.33%, respectively (Figure 5). The DPPH radical scavenging with $IC_{50}$ values (µg/mL) of the To-HE, To-AE and ascorbic acid, were: 45.38 ± 1.3, 116.5 ± 0.22 and 21.31 ± 0.5, respectively.

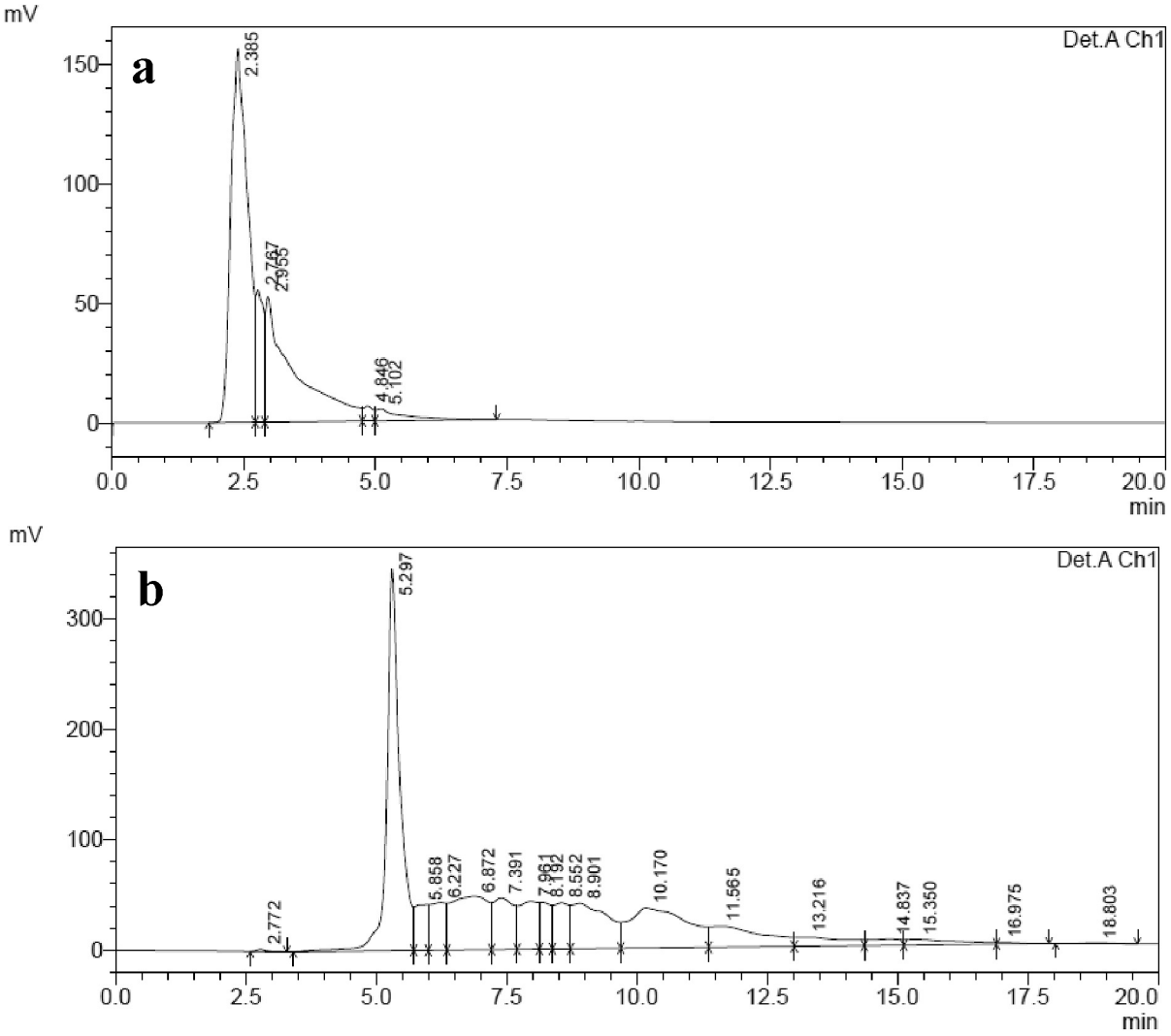


**Figure 3:** HPLC analysis. (a) To-HE & (b) To-AE.

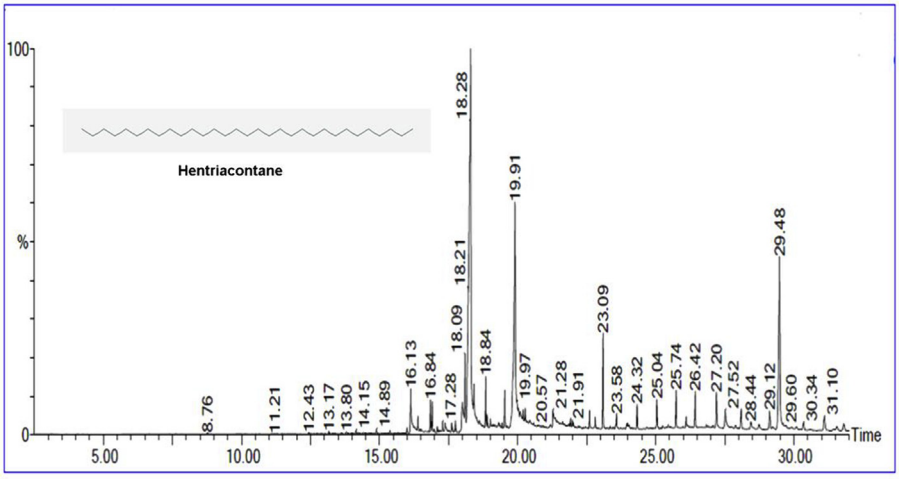


**Figure 4:** GC-MS analysis of To-HE.

**Table 4: FTIR analysis of To-AE.**

| $R_T$ | Name of the compound | MF | MW | Peak area | Biological properties |
|---|---|---|---|---|---|
| 16.13 | Tetradecanoic acid | $C_{14}H_{28}O_2$ | 228 | 2.986 | Antioxidant, hypercholesterolemic, cancer-preventive.[33] |
| 18.09 | 1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester | $C_{16}H_{22}O_4$ | 278 | 0.941 | Antimicrobial, antifouling, antiviral.[34] |
| 18.28 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 2.131 | Antioxidant, pesticide, lubricant, 5-α reductase inhibitor.[35] |
| 19.91 | Z,z-6,28-Heptatriactontadien-2-one | $C_{37}H_{70}O$ | 530 | 2.418 | Vasodilator.[36] |
| 23.09 | 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester | $C_{16}H_{22}O_4$ | 278 | 1.137 | Antiviral, anticancer, antimicrobial, antioxidant, cytotoxic & anti-inflammatory properties.[37] |
| 25.04 | Hentriacontane | $C_{31}H_{64}$ | 436 | 1.528, 1.460, 18.524,1.337 | Antifungal against spore germination, antioxidant, antitumour activity & antibacterial.[35] |
| 29.48 | Cholest-5-en-3-ol, 24-propylidene-, (3-beta.) | $C_{30}H_{50}O$ | 426 | 3.479 | Antifungal activity.[37] |

*Ferric ion reducing power (FRAP) assay*

Among the concentrations of To-HE, To-AE and ascorbic acid used in this study, 100 µg/mL resulted in the highest radical scavenging effect of 70.23%, 56.02% and 95.33%, respectively (Figure 6). The FRAP radical scavenging with $IC_{50}$ values (µg/mL) of the To-HE, To-AE and ascorbic acid were 41.99 ± 1.38, 85.99 ± 0.22 and 12.89 ± 0.52, respectively.

*Anti-proliferative activity*

To-HE treated cells displayed cytoplasmic blebbings, deformed/condensed nuclei and cytoplasmic vacuolation (Figure 7). The cell growth percentage at 100 µg/mL concentration of To-HE was 24.00 and 26.42%, while that of To-AE was 45.75 and 48.51% for A549 and *Vero* cells, respectively (Figure 8). The $IC_{50}$ values of the samples for each cell line were calculated in a dose-dependent manner. The recorded $IC_{50}$ values of To-HE and To-AE were (µg/mL) 62.91 and 93.00 and 72.64 and 106.6, respectively, for A549 and *Vero* cells.

## Discussion

Naturally occurring marine algae are receiving much attention from researchers because they are rich sources of

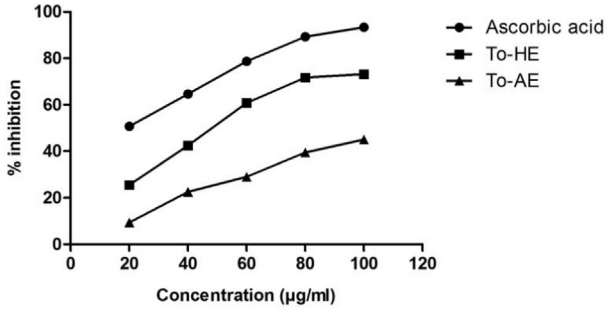


**Figure 5:** DPPH scavenging activity of (a) To-HE & (b) To-AE.

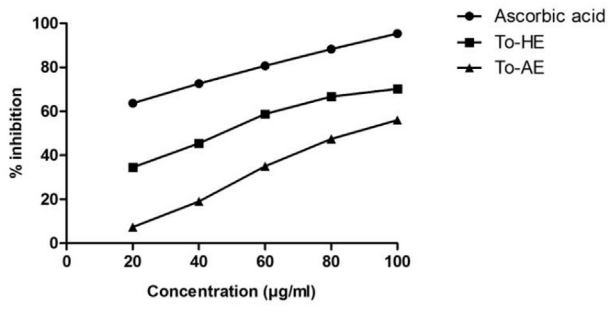


**Figure 6:** FRAP scavenging activity of (a) To-HE & (b) To-AE.

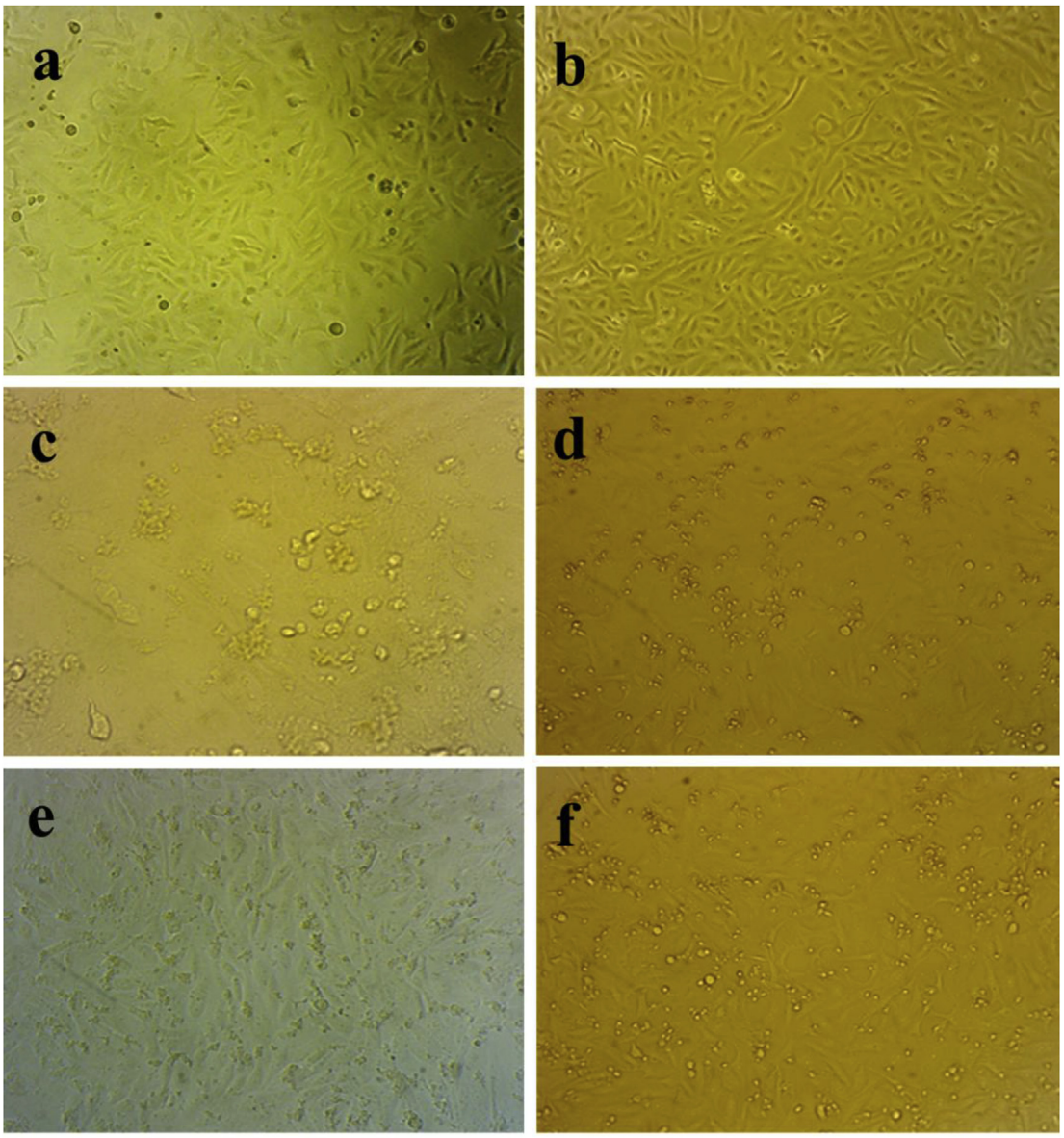


**Figure 7:** Anti-proliferative effect of To-AE and To-HE. (a) A549 control, (b) *Vero* control, (c) A549 treated with To-AE, (d) *Vero* treated with To-AE, (e) A549 treated with To-HE, (f) *Vero* treated with To-HE.

biologically active secondary metabolites. Several studies have shown that seaweed is an excellent source for compounds such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols and carotenoids that have been studied for their various biological activities.[26] Several studies have been carried out using seaweed extracts that exhibited various biological activities such as antibacterial, antioxidant, anti-coagulant, anti-inflammatory, larvicidal, and anti-proliferative effects.[27] Hence, the focus of the present study was to evaluate the antioxidant and anti-proliferative effects of hexane and aqueous extracts of *T. ornata*. The biological activity of any bioorganic compound may be influenced by its functional groups. Thus, the FTIR analysis of the extract reveals the physicochemical properties of a compound. The FTIR analysis of To-HE and To-AE indicated that the various functional groups such as alkanes, alkynes and alkyl halides are similar to those found in an earlier study of Meenakshi et al. (2011).[28] In both types of assays of antioxidant properties (DPPH and FRAP), the To-HE extracts displayed greater levels of inhibition than those of To-AE. These findings are comparable to those observed in a previous study by Chanthini et al.[29] The research of Vijayabaskar et al. revealed that the DPPH scavenging effect of *T. ornata* was 84.27% and is comparable to the values found in the present study.[13] The activity against cancer cell lines is one of the most important specificities of marine algae, and many algae have shown cytotoxic and anti-proliferative activities.[30] The results of this study are consistent with those reported by Marudhupandi et al. who found strong inhibitory potential of *T. conoides*-based fucoidan at the concentrations (µg/mL) of 45 and 325, respectively, on A549 and Vero cell lines.[9] The anti-proliferative effect of the ethyl acetate extract of *G. edulis* on A549 has been found to show the maximum inhibition at a concentration of 24.5 µg/mL.[8] In another study, the polysaccharides extracted

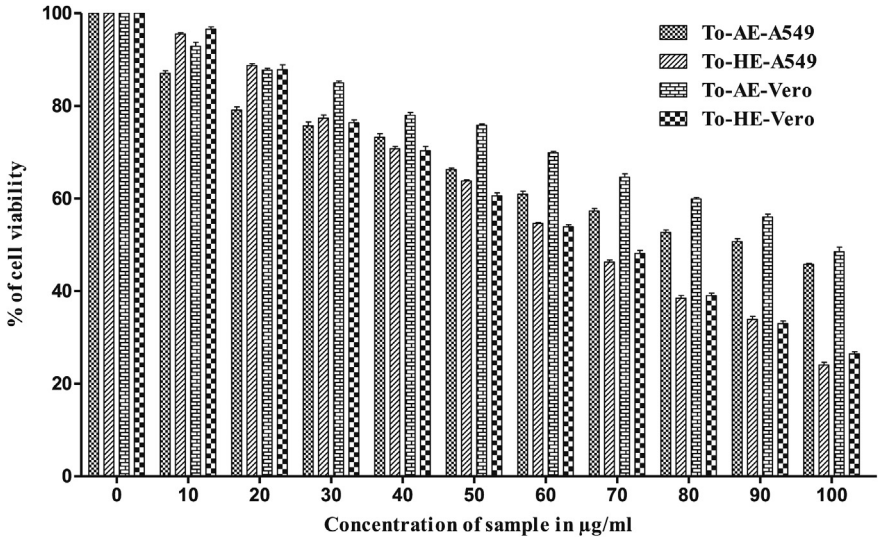


**Figure 8:** Percentage viability of A549 and *Vero* cells against To-AE and To-HE.

from the seaweed *Sargassum pallidum* inhibited A549 cells by 64.8% at the concentration of 1 mg/mL.[10] To identify the phytocomponent responsible for the recorded inhibitory effect on free radicals and cancer cells, To-HE was analysed by GC-MS. Among the compounds identified, tetradecanoic acid was found to have both antioxidant and cancer-preventive properties.[31] The previous research on the GC-MS analysis of methanolic extracts of *Cassia italic* leaf and the endophytic fungi *Alternaria* sp. revealed the presence of some of these compounds such as 1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester, 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester and n-hexadecanoic acid that had anticancer, cytotoxic and anti-inflammatory effects such as those in our present study.[32,33] In this study, hentriacontane was a major component in the hexane extract of *T. ornata* that was previously isolated from *Oldenlandia diffusa*, a natural source used for the treatment of cancer in Asia.[34] Finally, the recorded anti-proliferative effect of To-HE could be due to the hentriacontane compound.

### *Study limitations*

The GC-MS analysis of the To-HE-crude extract confirmed the presence of hentriacontane as a major compound, and it has previously been reported to be a cancer preventive agent.[34] Therefore, future study on the isolation and purification of hentriacontane from To-HE along with its structural confirmation is warranted.

## Conclusion

The present study revealed that the hexane extract of *T. ornata* (To-HE) was more effective than the aqueous extract (To-AE) due to the presence of hentriacontane. In addition, To-HE has shown maximum inhibition effects during the antioxidant and anti-proliferative studies. Therefore, the findings of the present study could form a basis for the development of new pharmaceutical formulations for the treatment of lung cancer.

## Recommendation

Based on the present research work, the use of food/diet supplement of *T. ornata* is recommended.

## Conflicts of interest statement

The authors have no conflict of interest to declare.

## Authors' contribution

PD designed, performed and wrote the article. RS performed the antioxidant studies. The interpretation of HPLC, FTIR and GC-MS studies was conducted by GB. PP supervised the work and performed the careful revision of this article, and all the authors have read and approved the final copy of this article.

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