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# THR-α and THR-β gene profiling and characterization of *Gelidiella acerosa* extract binding and uptake by 3,3′,5-TRIIODO-L-THYRONINE Stimulated NIH3T3 cell line

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**ABSTRACT**

The marine resources, macro-algae raw materials have gained much attention as they exhibit a great applicability as dietary ingredients and being highlighted as a potential source of bioactive compounds. Thyroid issues in India are likely due long standing iodine deficiency in the diet. This investigation was to assess the antithyroid activities of the macroalgae *Gelidiella acerosa.* Macroalgae extract characterized for the presence of antithyroid compound confirmed the presence of pharmaco-bioactive compounds were confirmed. Further, the highly sensitive NIH3T3 fibroblast cell line was stimulated with 3,3′,5-Triiodo-L-thyronine (T3) - an active thyroid prohormone to confirm the binding and expressing capacity of the active alga extract fraction for the THR-α and β gene expression. The results indicate that *Gelidella acerosa* treatment led to higher expression levels of thyroid hormone receptors compared to the control. Thyroid hormone receptor expression in the cell line, contributing to its potential as a regulatory agent in thyroid function.

## 1. Introduction

Thyroid hormones play an important role in regulating various physiological functions, encompassing metabolism growth and development [1,2]. Triiodothyronine (T3), recognised as the most biologically active form of thyroid hormone, mediates its physiological effects via interaction with thyroid hormone receptors, predominantly (THRA) Thyroid hormone receptor alpha and (THRB) thyroid hormone receptor beta Nuclear receptors operate as transcription factors that are dependent on ligands, modulating gene expression in reaction to T3 stimulation [3,4]. The intricate mechanisms underlying thyroid hormone action have been progressively elucidated in recent

decades, uncovering a complex array of molecular interactions that coordinate cellular responses [5,6]. Thyroid hormone receptors, which are part of the nuclear receptor superfamily, emphasise distinct features of tissue distribution and developmental expression, indicating their specialised functions in diverse physiological contexts [7]. THRA exhibits predominant expression in cardiac and skeletal muscle, bone, and the central nervous system, whereas THRB demonstrates elevated expression levels in the liver, kidney, and retina [8,9].The gene expression regulated by thyroid hormone receptors comprises various molecular mechanisms, which include the induction of coactivators and corepressors, chromatin remodelling, and interactions with various other transcription factors [10]. The complexity of this system is augmented by the inclusion of various receptor isoforms, each exhibiting unique functional characteristics and patterns of expression that are specific to particular tissues [11]. The THRA gene produces multiple isoforms via alternative splicing, with THRα1 identified as the principal T3-binding variant, whereas THRα is characterised by an absence of hormone-binding ability and may function as a natural antagonist [12].

The NIH3T3 cell line, derived from mouse embryonic fibroblasts, is widely utilised as a model for examining cellular responses to various stimuli, including hormones [13]. This cell line serves as a critical resource for investigating the molecular mechanisms governing T3-mediated gene regulation and the resulting cellular effects [14,15]. The NIH3T3 cells exhibit a well-characterized nature and demonstrate a robust response to thyroid hormones, thereby establishing them as an optimal model system for the investigation of the molecular mechanisms underlying thyroid hormone signalling [16,17].Recent investigations have increasingly concentrated on the potential of natural products, particularly those derived from marine environments, to modulate endocrine activity and influence cellular mechanisms [18,19]. The marine ecosystem constitutes a substantial reservoir of bioactive compounds that may possess therapeutic applications, especially concerning endocrine regulation [20]. Macroalgae, distinguished by their rich array of bioactive compounds, are garnering considerable attention owing

to their potential therapeutic applications [21,22].

*Gelidiella acerosa*, a species of red algae, demonstrates potential in various biomedical studies; however, its effect on thyroid hormone signalling remains to be closely studied [23]. This marine species is characterised by the presence of diverse bioactive compounds, such as polyphenols, sulfated polysaccharides, and additional secondary metabolites, which have the potential to affect cellular signalling pathways [24,25]. Earlier research has shown that it has antioxidant, anti-inflammatory, and immunomodulatory characteristics, which might be used in therapeutic applications [26,27].

The interaction between marine- derived compounds and nuclear receptors has emerged as an important area of research, with implications for drug discovery and therapeutic development [28]. A variety of marine natural products have demonstrated the ability to modulate the function of nuclear receptors, acting either as agonists or antagonists. This modulation presents potential avenues for therapeutic intervention in a range of diseases [29,30]. The capacity of compounds derived from marine sources to affect thyroid hormone signalling pathways signifies a promising area of exploration within the field of endocrine research [31].

This research endeavours to elucidate the impact of T3 stimulation on gene expression that occurs in NIH3T3 cell line, concentrating particularly on the expression profiles of THRA and THRB [32]. In addition, this study aims to clarify the possible modulatory effects of *Gelidiella acerosa* extract on alterations in gene expression induced by T3. The integration of T3 stimulation with seaweed treatment is anticipated to elucidate new understandings regarding the interaction between thyroid hormone signalling and bioactive compounds derived from marine sources[33,34].

The elucidation of the molecular mechanisms that govern thyroid hormone action, as well as their modulation by natural compounds, is essential for the advancement of novel therapeutic strategies aimed at addressing thyroid-related disorders [35]. The analysis of THR gene expression profiles in response to T3 stimulation, alongside the examination of the effects of *Gelidiella acerosa* extract, has the potential to uncover new regulatory mechanisms and identify

possible therapeutic targets [36]. This investigation has the potential to enhance our comprehension of the impact of marine-derived compounds on nuclear receptor signalling pathways [37].

Assessing the molecular mechanisms that regulate these interactions would offer substantial insights into the function of thyroid hormones and possibly identify innovative approaches for the modulation of thyroid hormone-responsive genes [4,8]. This investigation could illuminate the potential therapeutic implications of *Gelidiella acerosa* concerning thyroid-associated disorders and other conditions influenced by thyroid hormone signalling [23,33,34].

## 2. Materials and Methods

### 2.1. Collection and processing of macroalgae

Red algae *Gelidiella acerosa* was collected from the Valinokkam coastal area in the Gulf of Mannar, Tamilnadu, South India. Algal collection took place in March 2023 at low tide, with a water temperature of 25◦ C, air temperature of 26◦ C, and a salinity of 42ppt. The geographical coordinates of the collection site are latitude 9° 9' 24.1308" N and longitude 78° 39' 4.1976" E. The sample was obtained using the hand-picked method and scraped from the calcareous substrates such as coral line rocks. The obtained specimen was recognized and verified by BSI Coimbatore with the authentication code BSI/SRC/5/23/2023-24/Tech-282.

### 2.2. Preparation of algal extract

The algal sample was collected by handpicking method by using a collection bag and then rinsed with distilled water to remove the debris. After bringing to the laboratory samples were dried in the shade at room temperature. It was then powdered using a pulveriser at room temperature. The powdered sample was stored in an airtight container at -20◦C in the laboratory for extraction. The crude was extracted using a solvent called methanol. The extraction process was carried out by using the Soxhlet method. The crude extract of the algae processed through rotary evaporated to separate the solvent and extract. Then it was refrigerated at a temperature of -20°C for further study after evaporating the solvent by using rotary evaporator.

### 2.3. Preliminary screening

The preliminary phytochemical analysis carried by the procedure of collected algal extract by this method [38].

### 2.4. UV-Vis and FTIR Characterization

UV- Vis is used to evaluate the metal composition of the sample. The extracts were centrifuged at 3000 RPM for 10 minutes. By using the Whatman No.1 filter paper the sample was extracted with a high-pressure vacuum pump. By using the same solvent, dilute the sample 10 times. The materials were examined using a Perkin Elmer spectrophotometer from 260 to 900 nm. The instrument identified specific spectra peaks. FT-IR analysis was performed on a Perkin Elmer Spectrophotometer to find 400-4000 cm-1 peaks and their functional groups. The highest UV and FTIR values were recorded. This examination was performed twice to confirm the spectrum [39].

### 2.5. GC-MS Analysis of macroalgae

By using the Clarus 680 GC with helium the components were separated at a continual flow rate of 1ml/min on a fused silica column which is packed with Elite-5MS (5% biphenyl, 95% dimethylpolysiloxane; dimensions: 30 m × 0.25 mm ID × 250 µm df). At 260°C injector temperature the chromatography analysis was conducted. For 1µL of extract sample introduced into the instrument, the oven temperature was first set at 60 °C for 2 minutes, then increased to 300°C at a rate of 10 °C per minute and held for 6 minutes. The mass detector parameters included a transfer line temperature of 240 °C, an ion source temperature of 240°C, electron impact ionization at 70 eV, a scan length of 0.2 seconds, and a scan interval of 0.1 seconds, with a mass range of 40-600 Da. Component spectra were compared to the GC-MS NIST (2008) library database of identified components.

### 2.6. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

Free radical scavenging activity was determined according to the method of [40]. For testing, 500 µL of 0.3 mM DPPH alcoholic solution (Himedia, India) was added to 2.5 ml at 250–1000 µg/mL doses, and it was incubated in the dark for 30 minutes, samples were measured at 518 nm using an ultraviolet (UV)-visible spectrophotometer (Systronics AU-2700, India) with methanol as a blank

and BHT as a positive control. Scavenging activity as % inhibition in triple trials is calculated below.

$$\%Inhibition = \frac{Abs\ of\ control\ -\ Abs\ of\ test\ sample}{Abs\ of\ control} \times 100$$

The IC 50 values were also calculated using Graph Pad Prism 8 software.

**2.7. Ferric Reducing Antioxidant Property Assay**

The FRAP assay was carried out according to reference [41]. FRAP reagent was freshly prepared by combining acetate buffer (300 mM), TPTZ (10 mM in HCl (40 mM)), and FeCl3 (20 mM in distilled water) in a volumetric ratio of 10:1:1, followed by incubation at 37 °C for 10 minutes.3 ml of FRAP reagent was combined with 0.1 ml of the sample solution (0.5 mg mL-1 lignin in a 90:10 dioxane-water ratio) and incubated at 37°C for 10 minutes prior to detecting absorbance at 593 nm. Ascorbic acid is used as a as the positive control.

**2.8. Metal Chelating**

Metal chelating activity indicates the extract's ability to bind harmful metal ions, reducing oxidative stress. This evidence supports its protective role in thyroid regulation under T3 stimulation conditions. The metal chelating activity was measured by using the methodology outlined in [42]. Different type of solvent extracts of *Gelidiella acerosa* at concentrations ranging from 100 to 500 g/ml were added to a 0.15 mM ferrous sulfate solution, and the reaction proceeded with the addition of ferrozine at a concentration of 0.5 mM. The solution was agitated briskly and incubated for 20 minutes at ambient temperature; the absorbance was measured at 562 nm. Ascorbic acid is used as a as the positive control.

$$\%Inhibition = \frac{Abs\ of\ control\ -\ Abs\ of\ test\ sample}{Abs\ of\ control} \times 100$$

**2.9.**

**Enzymic activity assay**

The activity of SOD was analysed according to the methodology of [43]. The GSH of the macroalgae is determined by [44] with some modification. The GST was measured using a mixture of 5 mM GSH, 2.5 mM CDBN, and a 0.1M phosphate buffer, and the results were taken at an absorbance of 340nM. Thyroid peroxidase helps add iodine to tyrosine parts of thyroglobulin and links iodotyrosines together, which are important steps in making thyroid hormones. We used the TPO assay as directed [45].

$$\%Inhibition = \frac{Abs\ of\ control\ -\ Abs\ of\ test\ sample}{Abs\ of\ control} \times 100$$

The TPO assay was carried by [43] modifications of the macroalgae. By using the spectrophotometer (BioTek, Model Epoch2TC, Winooski, Vermont, VT, USA) in 96 well plate at the wavelength of 470 nm. Assay performed by using the components 50µL of buffer, 40µL of solution and 50µL of guaiacol, 20 µL of TPO enzyme and 50µL of TPO enzyme, and 50µL of $H_2O_2$. The total volume of mixture of 210 µL. The extracts were suspended with the buffer. The reading was taken after 30 minutes at 37◦C.

$$\%Inhibition = \frac{Abs\ of\ control\ -\ Abs\ of\ test\ sample}{Abs\ of\ control} \times 100$$

**2.10.**

**Cell culture**

NIH3T3 Fibroblast mouse cell lines were procured from the National centre for cell science (NCC) in Pune and cultured as per the instruction of NCC. The cell line was cultured in the DMEM medium and grown by 10% of FBS and 1% antibiotics (streptomycin/penicillium). The cultured cells were sustained in an environment with 5% $CO_2$ at 37◦ C.

**2.11. Cell viability of macroalgae**

Invitro analysis for the cytotoxic effects of the extract was carried when the cell attains 70 – 80% proliferation [45]. The stock solution of the macroalgae extract diluted in the medium to attain the proper working concentrations. 200µl of suspended cells were introduced into the 96 well plate at 20,000 cells in each well, not

including the test agents. After 12 hours if proliferation. The cells were treated with 10 to 100µg/ml of the samples for 24 hours at 37◦C in 5% $CO_2$. 20µl of MTT solution (5mg/ml total volume) added to each well and maintained for 3 hours at 37◦C in 5% of $CO_2$ with humidified air. Eventually after the transformation of the live cells with mitochondrial dehydrogenase, 100µl of DMSO is used to dissolve the pieces of formazan crystal produced by MTT. Cells which are viable was measured by 570 nm at the absorbance with 655nm.

**% Cell viability = NIH3T3 of treated cells / NIH3T3 of control cells × 100%**

**2.12. Hormonal stimulation of 3,3',5'-Triiodo-L-Thyronine**

3,3',5'-Triiodo-L-Thyronine sodium salt (T3, Sigma) was dissolved in 1N NaOH at 1 mg/ml and diluted in $dH_2O$ at 200µM. 3,3′,5-Triiodo-L-thyronine (Reverse T3) was dissolved in 100% ethanol at 50 mM. The chemicals were decontaminated by passing through a 0.2m filter, aliquoted, and stored at −20 ◦C. Repeated freezing and thawing was avoided for T3 stocks. For cell treatment, stock solutions were diluted in dH2O at concentrations of 1nM to 10nM. For 96-well tray assays [46] modified.

**2.13. Cell viability of the 3,3′,5-Triiodo-L-Thyronine**

In vitro assay for cytotoxic activity of studied marcro algal extract was carried when the cells reached 70–80% of growth [46]. Macroalgae extract stock solution was dissolved in the corresponding medium which consist of the drug 3,3′,5-Triiodo-L-Thyronine sample was dissolved in the medium at 1-10nM as the working concentration. 200µl of cell suspension was seeded in 96 well plate at 20,000 cells in each well. The proliferation of the cells proliferates during 12 hours. The cells were treated with 1-10nM at 37◦C in 5% of $CO_2$ of 24 hours. 20µl of MTT solution (5mg/ml of total volume), each well was incubated for 3 hours at 37◦C in 5% of $CO_2$ and humidified air. The MTT assay solution were added after the mitochondrial dehydrogenase of the live cells.100µl of DMSO Was used to dissolve the formazan crystals and it was observed under 570 nm.

**% Cell viability = NIH3T3 of treated cells/ NIH3T3 of control cells × 100%**

**2.14. Cell viability of 3,3′,5-Triiodo-L-Thyronine treated with macroalgae extract**

Invitro experiment for the cytotoxic effect of the cells which attained 70-80% confluence [46]. The macroalgal extract stock solution was prepared in the medium which contains the drug 3,3',5-Triiodo-L-Thyronine at the working concentration.200µl of the cells which was introduced into a 96-well plate at the mentioned cell density of 20,000 cells per well, which eliminates the test agent. The cells were allowed to proliferate for approximately 12 hours. The cells were cultured with different concentrations of the materials, about the concentration of 1nM to 5 nM at 37◦C in 5% $CO_2$ environment. 20µl of MTT solution (5mg/ml total volume) was introduced to each well and incubated for 3 hours at 37◦C in a 5% $CO_2$ atmosphere with humidified air.

**% Cell viability = NIH3T3 of treated cells / NIH3T3 of control cells × 100%**

**2.15. Gene expression analysis**

The total RNA was isolated by using Trizol reagent (TaKaRa). The RNA was separated and reverse transcribed with the Yaeasen Biotechology's $1^{ST}$ standard cDNA synthesis supermix. The expression level of mRNA was quantified by using the easen Biotechnology's qPCR SYBR Green Master Mix. The mRNA levels were measured by using the technique of $2^{-\Delta\Delta}$Ct [47].

## 3. Result And Discussion

The presence of different phytoconstituents in the macroalgae crude extract identified through preliminary analysis. The presence of crude extract was indicated by the positive sign (+) and the absence by negative sign (-) as show in the table (1). Alkaloids are the primary and vast component which functions as the molecules that derived from the amino acids [48]. The presence of Terepnoids indicates that macroalgae has antimicrobial, anti-inflammatory and antioxidant properties which may leads to discover the pharmacological targets [49]. Saponins are many biological activates which has a capacity to control the digestive system and creates

metabolic process and health in general
[50]

| Phytochemicals | *Gelidiella acerosa* |
|---|---|
| Alkaloids | ++ |
| Terepnoids | ++ |
| Steroids | ++ |
| Tannins | ++ |
| Saponin | ++ |
| Flavonoids | ++ |
| Phenol | - |
| Coumarins | - |
| Quinones | + |
| Glycosides | - |

**Table 1.** Phytochemical analysis of Macroalgae extract of *Gelidiella a*cerosa.

The UV-vis spectroscopy analysis of the macroalgae extract carried to identify various bioactive compounds. The result showed the strong peak absorbance in 200-300 nm. The secondary peaks which are below 400 nm shows the presence of bioactive compounds (Figure 1).

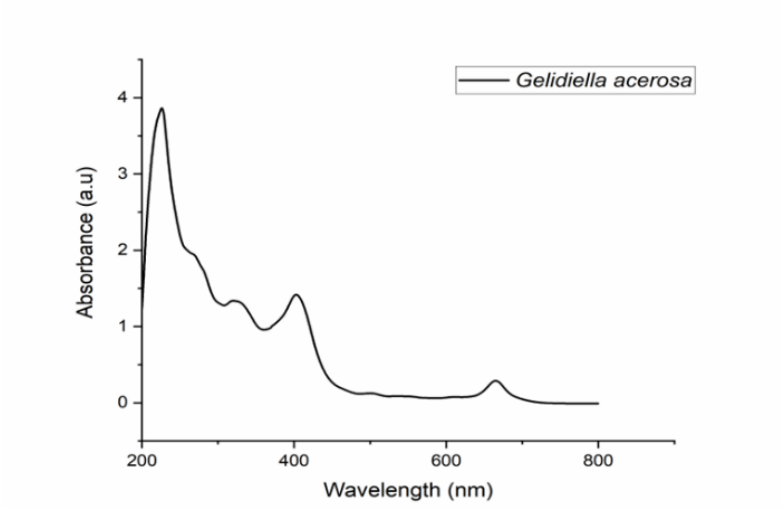

**Figure 1.** UV-visible spectroscopy analysis of the macroalgae extract *Gelidiella acerosa.*

Fourier transform infrared spectroscopy used to detect various functional groups. In the result showed 15 peaks in the macroalgae extract sample 3967.82 $cm^{-1}$, 3325.64 $cm^{-1}$, 2936.09 $cm^{-1}$, 2831.95 $cm^{-1}$, 2517.61$cm^{-1}$, 2362.37 $cm^{-1}$, 2223.52 $cm^{-1}$, 2041.28 $cm^{-1}$, 1651.73 $cm^{-1}$, 1452.14 $cm^{-1}$, 1410.67 $cm^{-1}$, 1314.25 $cm^{-1}$,1112.73 $cm^{-1}$, 1021.12 $cm^{-1}$, & 704.855 $cm^{-1}$. The FTIR analysis of the macroalgae extract shows functional groups. The peak shows the significance of O-H Stretching, while 2925 $cm^{-1}$ which indicates the presence of C-H bonding and polysaccharides and phenolics with antioxidant characters are present [51,52]. The peaks at 1650.73 $cm^{-1}$ and above 1410.67 $cm^{-1}$, which is supportive of C=O bonds in carboxylic acids and amides, which is combined with the bio stimulant effects on plants [53,54].The C– O bonds which has the polysaccharides such as alginate, are the good for texture and health [55]. The peaks lie between 1020 $cm^{-1}$ and 700 $cm^{-1}$ that confirms the presence of antioxidant properties and sensory of flavours of consumers [56,57] (Figure 2).

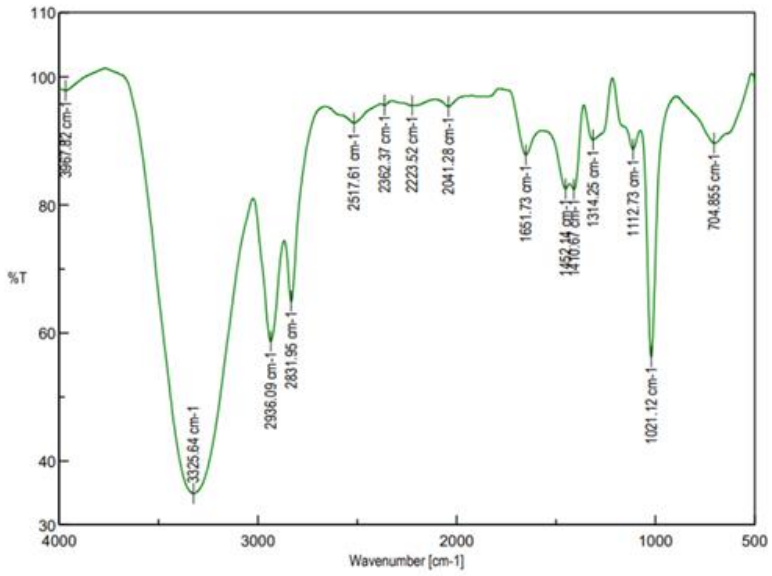


**Figure 2.** Fourier transform infrared spectroscopy (FTIR) of the macroalgae extract *Gelidella acerosa.*

The results of GC-MS included the compounds which is related to thyroid disorder are recorded in the Retention time of 1.018 is Mercaptamine, Methyl hydrogen disulfide, Cysteine, S-Methyl-, Retinoic acid, Carbonic acid, Bis(1-Methylethy) Ester and alcohols like Pentanediol, Hydrocarbons and unsaturated alcohols like Z-1,8- Dodecadiene & 3-Octyn-1-ol. Oxirane, Octyl & Oxirane, observed in the Retention time of 15.359 Decyl-, Recorded at the retention time of 15.599 Penta-Decanoic acid. The bioactive compounds are identified by various retention time by using NIST library (Figure 3).

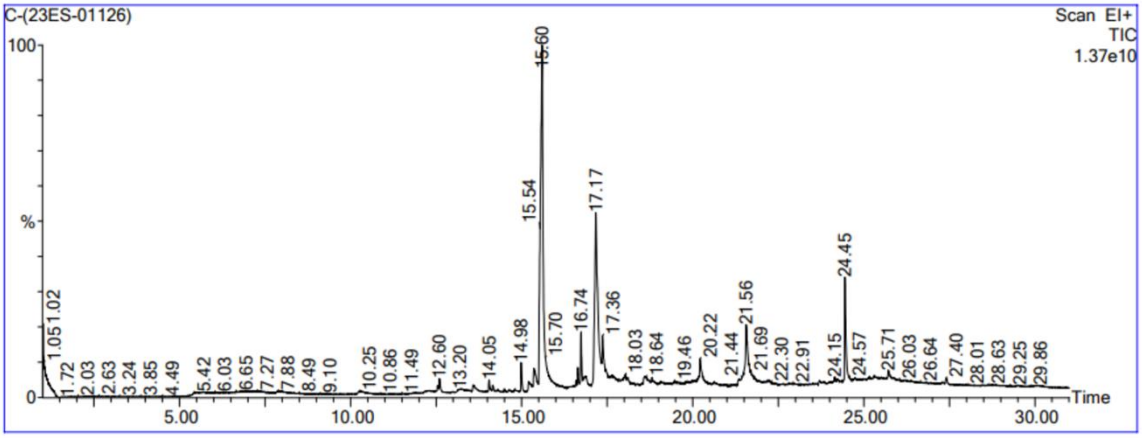


**Figure 3.** Gas Chromatography-mass spectrometry analysis of macroalgae extract *Gelidella acerosa.*

Cysteamine, involves in catabolism of cysteine which detoxify the mechanisms of the thyroid tissues, which the oxidative stress with thyroid diseases such as autoimmune disorder. This result coincides with the early report [58] cysteamine has a potential as a growth and health promotor which enhancing the hormonal secretion and oxidative stability in the grazing lambs. Retinoic acid which significant on thyroid disorder which inhibits the activity of T cells, which balances the immune response. The previous study reported that retinoid plays a supportive role in goitre and thyroid cancers [59]. The carbonic acid, Bis (1-Methylethyl) Ester which is bicarbonate transport and availability of ions which helps to maintain the pH which effect which is tangled in iodine uptake and hormone synthesis its ability to iodinate tyrosine

residues on thyroglobulin which plays a major role in production of T3 and T4. Pentanediol provides the presence of alcohol group which creates an effect on the lipid metabolism, when it's dysregulated in hypothyroidism. Z-1,8-Dodecadienel, 3-Octyn-1-ol generally involves in lipid mobilization which is thyroid dysfunction. Oxirane groups are hormone receptors that will interact with thyroid hormone transport proteins and principal to the disruption in normal thyroid signalling. The GC-MS analysis data shows the fraction and bioactive compounds shows pharmacological efficiency.

The antioxidant assays of macroalgal extract *Gelidiella acerosa* were carried out in the concentration of 12.5, 25, 50, and100 µg/ml. DPPH assay result shows that *Gelidiella acerosa* extract, the concentration is 12.5, 25, 50, and 100 µg/ml with ascorbic as control. It showed minimum as 64% inhibition on 12.5 µg/ml and as higher shows 95% inhibition at 100 µg/ml. The extract of *Gelidiella acerosa* shows minimum inhibition of 58% at 12.5 µg/ml and the maximum inhibition as 83% at 100 µg/ml. This shows that the algal extract has the antioxidant capacity. (Figure 4).

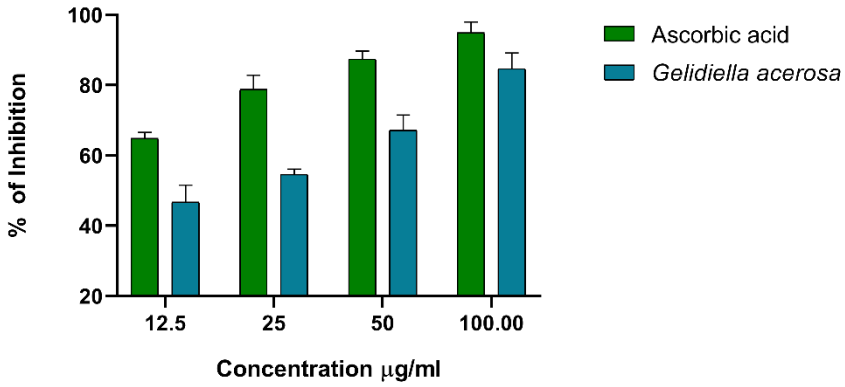


**Figure 4.** DPPH analysis of the macroalgae extract *Gelidella acerosa*, ascorbic acid is used as the control.

In previous study *Gelidella acerosa* has a natural antioxidant property, which reduce oxidative stress [60]. FRAP assay result finding showed the antioxidant activity compared with ascorbic as a control. Ascorbic acid shows the higher inhibition of 90% at 100 µg/ml, and the minimum inhibition about 60%. When compared to the control the minimum inhibition of 40% which is near to the ascorbic acid and the maximum inhibition about 60%, the

finding indicates that the macroalgae extract *Gelidiella acerosa* has a markable antioxidant property. The reducing power characterise the antioxidant function as electron donors, capable the reducing oxidized metabolites in lipid peroxidation process, it functioning as two types that is primary and secondary antioxidants [61] (Figure 5).

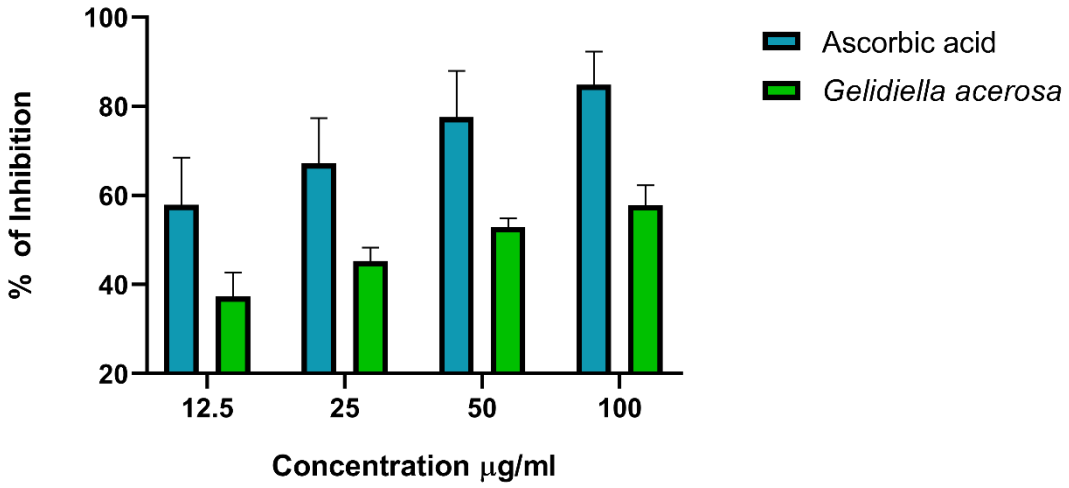


**Figure 5.** Ferric Reducing Antioxidant Power Assay of the algal extract *Gelidiella acerosa.*

The metal chelating result showed the minimum 52% at 12.5µg/ml and 79% at 100% at the standard of ascorbic acid. The extract of macroalgae showed the minimum value of 49% at 12.5µg/ml and at the maximum of 100µg/ml it showed 75% of concentration (Figure 6). The result states that it has an ability to bind the harmful metal ions.

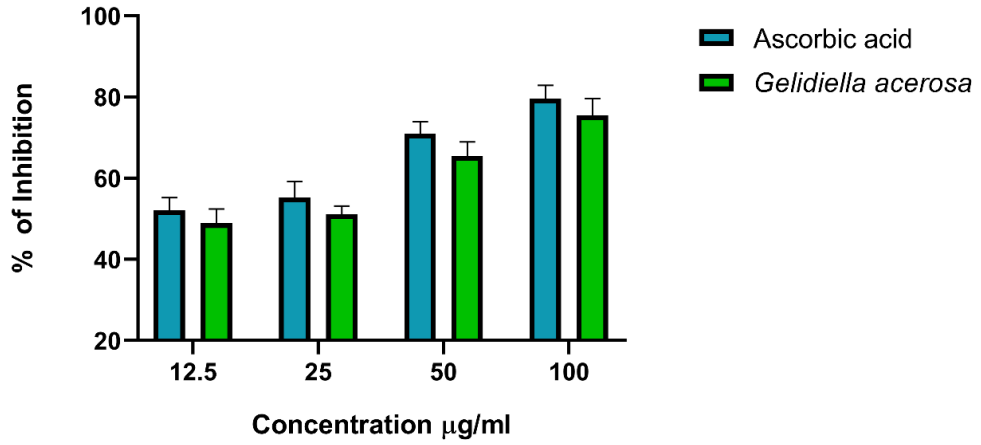


**Figure 6.** Metal chelating assay of the algal extract *Gelidiella acerosa.*

The enzymatic antioxidant assays carried out in the concentration of 25%,50%,75% and 100% to assess the antioxidant and protective. The value of standard ascorbic which is used as a positive control is 3.1 U.mg$^{-1}$ at the concentration in the SOD analysis. At the concentration of 100% it showed 2.03 U.mg$^{-1}$ which is nearly to the control value. The result shows that the macroalgae *Gelideilla acerosa* has the antioxidant capacity. The GPx assay the control has a value of 2.2 U.mg$^{-1}$. The *Gelidella acerosa* at 100% of concentration it has a value about 1.8 U.mg$^{-1}$ which is nearly shows the antioxidant capacity. The GSH activity proves the presence of activity were the ascorbic acids shows the value of 0.361 mmol/mg protein as a positive control and the extract of *Gelidiella acerosa* showed 0.311 mmol/mg at 100% concentration which is approximately nearer to the control.

The positive control of GST activity ascorbic acid shows the value of 0.3 mmol/mg protein. When it compared to the macroalgae crude extract it gives the result of 0.2 mmol/mg protein which is slightly differs from the control this shows that the presence of GST activity in the species *Gelidiella acerosa.* The control value of TPO activity is 0.6 mmol/mg protein when it compared to the extract t the highest concentration of 100% it showed the value about 0.4 mmol/mg it is partial result compared the control. The figure 7 showed the enzymatic antioxidant assays. Macroalgae extract improved the oxidative stress endurance because of the antioxidant enzymes SOD which helps in scavenging superoxide radicals [62].

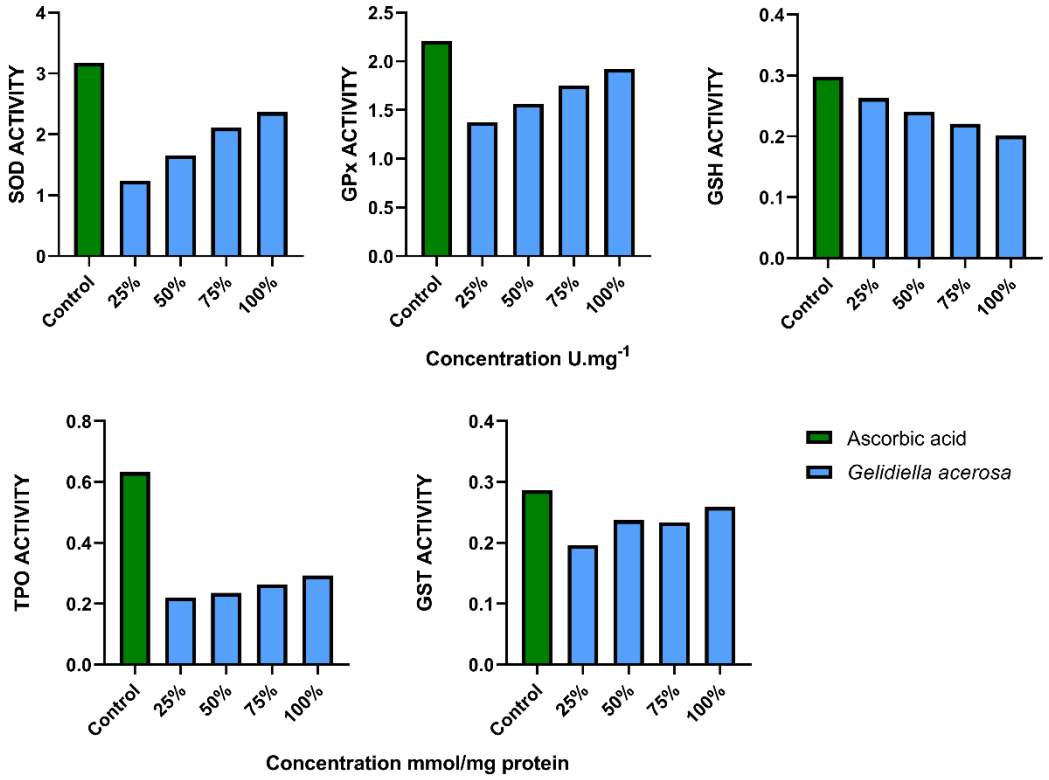


**Figure 7.** The antioxidant enzymic assays SOD, GPx, GSH, GST & TPO of macroalgal extract of the *Gelidiella acerosa.*

The cytotoxicity effect of the *Gelidiella acerosa* algal extract showed 99% cell viability at the concentration of 10 µg/ml, 96% assessed 50 µg/ml and at the maximum concentration of 100 µg/ml is about 94%. Hence it doesn't show any cytotoxicity effect until the 100 µg/ml and the value shows its significant. Therefore, the $Ic_{50}$ concentration was not determined (Figure 8).

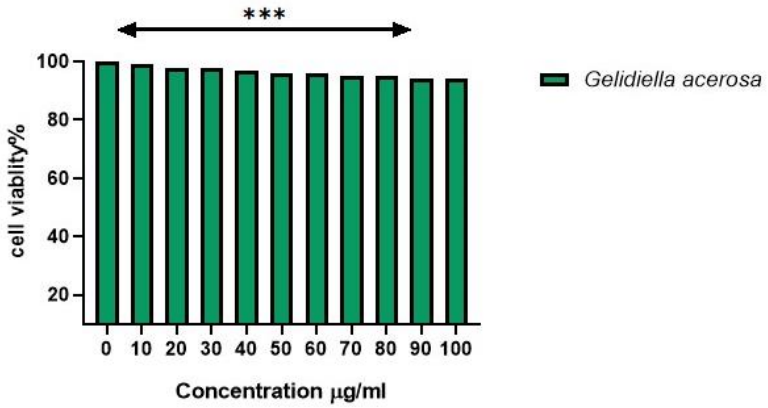


**Figure 8.** Cytotoxicity effect of macroalgae extract *Gelidiella acerosa.* The (***) suggests that it is highly significant. The (***) suggests that the results are highly significant. The significant p value is <0.001.

The hormonal stimulation carried out by using the drug 3,3',5'-L-Triiodothyronine (T3), and the cell viability assessment was taken in the NIH3T3 cell line. This displayed the effect of 95% of cells being viable at the maximum concentration of 10nM and in the minimum concentration of 1nM it showed 99%, 5nM about 97% cells are viable (Figure 9 & 9a).

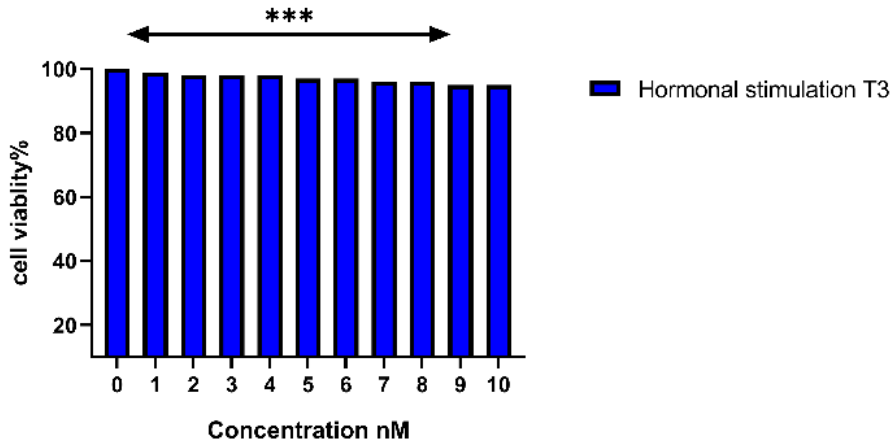


**Figure 9.** Hormonal stimulation of 3,3',5'-L-Triiodothyronine (T3) in NIH3T3 cell line. The (***) suggests that the results are highly significant. The (***) suggests that the results are highly significant. The significant p value is <0.001.

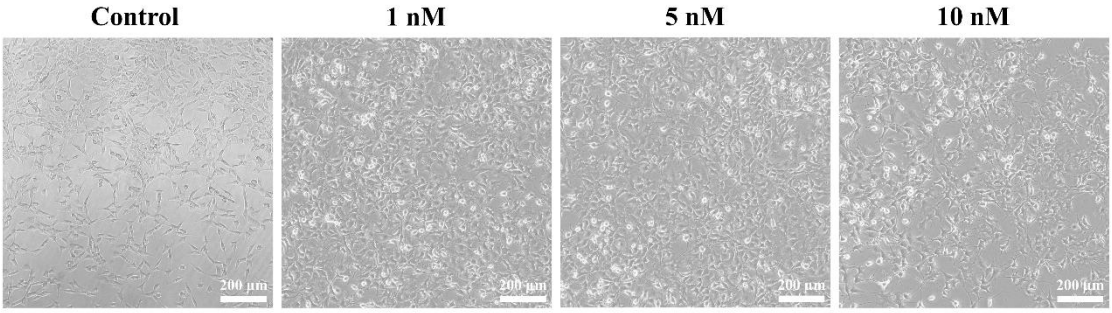


**Figure 9a.** Hormonal stimulation of 3,3',5'-L-Triiodothyronine (T3) in NIH3T3 cell line. The scale bar value is 200µm.

The drug 3,3',5'-L-Triiodothyronine induced in cell line was treated with macroalgae extract about 1nM to 10Nm. The result showed that no cytotoxicity occurred by 99% of cells viable in 1nM and 97% in 5nM and it showed 96% of cell viability at the maximum concentration of 10nM (Figure 10 and 10a).

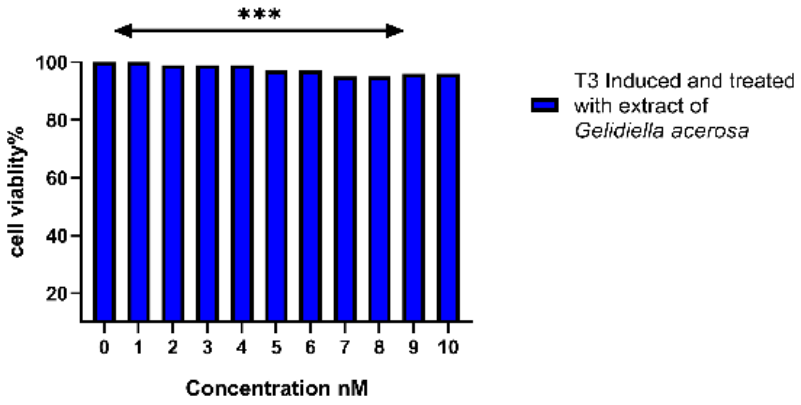


**Figure 10.** Hormonal stimulation of 3,3',5'-L-Triiodothyronine (T3) treated with macroalgae extract in NIH3T3 cell line. The (***) suggests that the results are highly significant. The significant p value is <0.001.

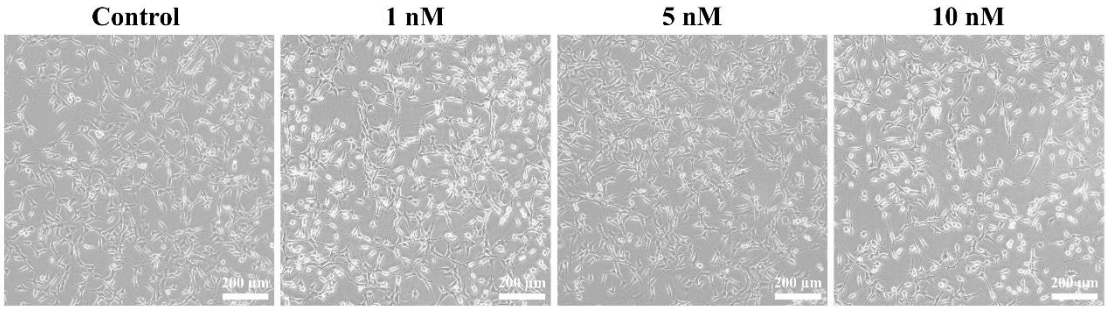

**Figure 10a.** Hormonal stimulation of 3,3',5'-L-Triiodothyronine (T3) in NIH3T3 cell line treated with the extract of macroalgae *Gelidiella acerosa*. The scale bar value is 200µm.

The bioactive substances which is present in the macroalgae extract could be responsible for the determined cell proliferation activities. The gene expression study of partial concentration of 5nM treated with the macroalgae extract in cell line showed the downregulation expression of the thyroid hormone receptor THR-α and THR-β than the control which is induced with the drug T3 (Figure11).

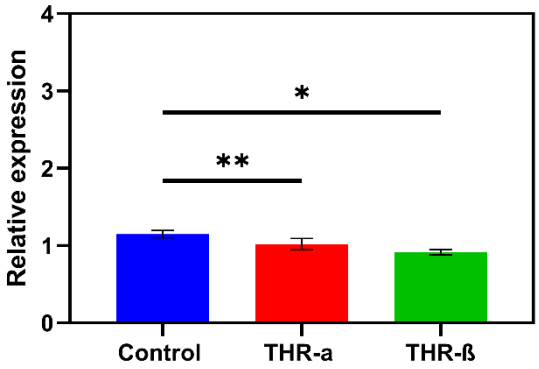


**Figure 15.** Gene expression study of the 5nM concentration of macroalgae extract treated in the cell line NIH3T3. The error bars indicate the biological triplicates (n=3) and the result shows significance.

This shows that the macro algae *Gelidiella acerosa* has the bioactive compounds which has the potential to control the regulation. The downregulation shows that the macroalgae can modulate thyroid hormone signalling activity in the cell lines that is treated to enhance the thyroid hormone levels.

## 4. Conclusion

The study shown that the crude extract of macroalgae *Gelidiella acerosa* has different bioactive compounds and the characterization proved the presence of functional groups and the compounds that related to thyroid regulation mainly cystamine and retinoic acid. The antioxidant and enzymatic antioxidant assays showed that *Gelidiella acerosa* extract has free radical scavenging activity and

oxidative stress. The cytotoxicity assays proved that there is an increased cell viability in both stimulated and control. The gene expression study showed that THR-α and THR-β both are down regulated when it is treated with macroalgae than the control which is thyroid induced. This indicates that the macroalgae extract of *Gelidella acerosa* has a potential to control the expression of thyroid hormone signalling.

**Declaration of competing interest**

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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**Credit authourship contribution statement**

Darsana Panchalingam: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft

Dhanalakshmi Balasubramanium: Data curation, Methodology, Formal analysis, Writing – Review editing, Supervision.

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Jegatheeswaran Sonamuthu: Writing – Review editing, Investigation, Supervision, Methodology, Validation.

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