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ORIGINAL ARTICLE

In Vitro antioxidant and antimicrobial activity of phlorotannin from brown seaweed *Turbinaria decurrens* [(Bory de Saint-Vincent, 1828)]

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ABSTRACT
In recent decades, marine organisms are highly investigated by several scientific communities due to their potential biological properties includes antioxidant, anti-inflammatory, and anti-microbial activities. Among the marine species, brown seaweeds have a crucial role in the construction of diverse bioactive compounds. The present study was mainly focused on the isolation of phlorotannin (PHT) from brown seaweed Turbinaria decurrens*. The phlorotannin was extracted through n-hexane solvent solid/liquid ratio 50/250, w/v, and the yield of phlorotannin was obtained as 6.48g (14%) on a dry weight basis. Total phlorotannin content was obtained as 1.97 ± 0.188mg phloroglucinol equivalent/g phlorotannin dry weight. Initially, the compound was confirmed by the absorption in a UV–Visible spectrum at 280nm. P-HPLC was performed for the separation and purification of the chemical compounds. Whereas, phlorotannin and standard phloroglucinol produced a single peak at 4.1 and 3.9 min retention time respectively. Furthermore, those peaks were collected as a fraction and which were subjected to an antioxidant, anti-inflammatory, and antimicrobial test for the evaluation of their biological potential, which they are all exhibit the strong inhibition activity which correlation with phenolic content. Positive results were further analyzed through UHPLC–ESI/MS analysis for compound identification which reveals the dimer form of dieckol phlorotannin. The actual mass of dieckol was 743m/z. This investigation illuminates Turbinaria decurrens as an alternative source of PHT (polyphenolic) compound with highly potential biological properties.*
Keywords: *Phlorotannin, Antioxidant activity, Anti-inflammatory activity, antimicrobial activity, HPLC, UHPLC–ESI/MS, and FTIR spectroscopy*

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INTRODUCTION

In recent decades, marine organisms are investigated by several scientific communities worldwide for their potent biological activities such as antioxidant, anti-inflammatory, and anti-microbial activity [1]. Among the marine organisms, brown seaweeds play a crucial role in diverse bioactive compound production. Phlorotannin is one of the chemical compounds which is metabolites of polyphenols. Moreover, it's synthesized on the earth only by brown algae [2].

Phlorotannins are hydrophilic nature phloroglucinol-based compounds and they exist in a wide range of molecular sizes. They are mainly synthesized by acetate malate pathway [3].Phlorotannins are classified according to their linkages between phloroglucinol units. The main groups are 1. Phloroethols and fuhalols are ether linkage phlorotannin, 2. Fucols-phenyl linkage phlorotannin, and 3. Eckols-dibenzodioxin linkage phlorotannin. Moreover, phloroetholsare varied from the tannin of fuhalos by the presence of additional hydroxyl groups at the end [2].With a unique chemical structure, several phlorotannins have isolated from various brown algae in past years. This polyphenol has a significant role in brown seaweed metabolic activity that could help to protect themselves from UV radiation [4].

Laminariacea and *lessoniacea* are brown seaweeds that have frequently been investigated for the isolation of phlorotannins, due to their strong biological activities are anti- antibacterial, inflammatory, antioxidant, and antiallergic activity [2]. Phlorotannins are polyphenolic compounds and also known as secondary metabolites of brown seaweeds. Moreover, they produce overall antioxidants. Phlorotannins are formed by eight phenol rings interconnected phloroglucinol units. This unique structure is a strong reason for the higher antioxidant activity when compared to monophenols[5].

Polyphenolic compounds are known to be rich dietary antioxidants which help to scavenge free radicals. Moreover, ROS (Reactive oxygen species) production would be inhibited by polyphenols during cell metabolism. In general, phlorotannin is produced as natural antioxidant in brown seaweed and may have a potential to replace synthetic antioxidants for commercial purposes. The antioxidant potential of phlorotannin that has derived from brown seaweed has exhibit strong antioxidant activity than synthetic antioxidants [6]. In contrast, much research has reported synthetic antioxidant additives in food and medicine might cause side effects and toxicity. Therefore, natural antioxidant compounds get in the focus of increased attention. The availability of chromatography linked with MS (Mass spectrometry) techniques can be helpful for the tentative identification of phlorotannins[7]. Hence, the present study has applied those analytical techniques for the identification and characterization of phlorotannin extracted from the edible brown seaweed *Turbinaria decurrens*. It was used to obtain a crude extract, which was fractionated as a phlorotannin rich fraction by P-HPLC. Furthermore, Electrospray Ionization (ESI) coupled MS/MS analysis technique was used for phlorotannin tentative identification. The purified phlorotannin was evaluated through an in-vitro (colorimetric assays) study to gain information about the capacity of biological activities includes antioxidant, anti-inflammatory, and antimicrobial activity. The present study illuminates *Turbinaria decurrens* as an alternative source of PHT (polyphenolic) compound with highly potential biological properties.

MATERIAL AND METHODS

Sample collection

The marine algae *Turbinaria decurrens* was collected at the Mandapam coast (latitude 9°28'N and Longitude 79°15'E). The collected seaweed samples were washed thoroughly multiple times using tap water to remove the salt, epiphytes and other dirt particles. Then, the seaweed was shadow dried, ground as a fine powder and kept for further use.

Extraction of phlorotannin

The phlorotannin was extracted from brown algal powder by the slightly modified method Koivikko et al. [8]. The pretreatment comprised of washing the seaweed powder by using the organic solvent n-hexane (solid/liquid ratio of 50:250 W/V) and centrifugation at 2000rpm for 20min. This procedure was done triplicate to remove pigments and lipids. The resulting pellet was taken and kept for air drying to evaporate the n-hexane solvent. An acetone: water (10:3) mixture which contained 0.3% ascorbic acid was added to the solvent free pellet and was kept in a shaker for 2 hrs at 200rpm (40ºC). The mixture was centrifuged thrice at 2000rpm for 6 min. Acetone solvent was evaporated from the supernatant via air-drying. Finally the residue was lyophilized and stored for further use.

Total phlorotannin content

The total content of phlorotannin was estimated in the lyophilized extract followed by Folin-ciocalteu method of Swanson and Druehl *et al*. [9]. The crude extract (1mg/ml) was dissolved with distilled water (different concentration as 100µl, 200µl, 300µl, 400µl and 500µl). 1ml of 10% Folin-ciocalteu reagent was added into each test tube and left 5min for incubation followed by further addition of 2ml of 10% sodium carbonate. After that, test tubes were incubated for 1.5h at room temperature in the dark. The absorbance of the blue color produced was measured at 750nm. Phloroglucinol was used as the standard. The results were expressed as milligram of phloroglucinol equivalents (PGEs) per g dry weight of the sample.

Preparative RP-HPLC Analysis of phlorotannin

The RP-HPLC analysis method was followed by Ferreres *et al*. [2].Phenolic contents were eluted with using of ODS Hypersil column (250 mm × 4.6 mm × 5 µm, Phenomenex, Torrance, CA, USA), Gradient solvents A,comprises (1% formic acid with water) and gradientB contain acetonitrile alone. The elution profile was 0–10 min, 0% B; 30 min, 30% B; 35 min, 80% B; 40 min, 80% B; 42 min, 0% B; 52 min, 0% B. A 20µL injection volume was fixed with 1mL/min flow rate. Spectral data were determined within the range of 240–400 nm, and which were recorded at 280 nm. The phlorotannin peak was eluted through preparative RP- HPLC.

UHPLC-ESI/MS Analysis of Phlorotannin

UHPLC–ESI/MS analysis was performed using a LC–MS-Triple-Quadra pole instrument (Shimadzu, Tokyo, Japan) equipped with a Prominence UHPLC system (SIL-20A HT autosampler, LC-20AD pump system,

SDP-M20A UV detector). The LC separation was performed using a method adapted from Ferreres et al. [2].

FT-IR Analysis of phlorotannin

Purified Phlorotannin of a freeze dried sample (spectral data) was analyzed by FT-IR. 1mg of sample was added in 200 mg KBr (FT-IR grade) and converted as a pellet. The pellet was directly placed into the sample holder and allowed to record FT-IR spectra data within the range from 4000 to 400 cm^{-1} [10].

Antioxidant activity

Total Antioxidant Activity

Total antioxidant activity (TAA) of phlorotannin extracts was determined by following the method of Prieto et al. [11]. The various concentrations of (100 - 500µg/ml) phlorotannin were added into 1ml of reagent that consist of 0.6m sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate and filled up with distilled water for final concentration. The samples were incubated at 95ºC for 90min in a water bath. The absorbance reading was measured at 695nm. Ascorbic acid was used as a standard. The total antioxidant activity was calculated using the obtained OD value.

DPPH radical scavenging

DPPH radial scavenging activity was measured by following the method of Duan et al. [12]. Briefly, 1ml of 0.16mM DPPH was poured into 1ml of a purified sample of (20 - 100 µg/ml) in different concentrations. The blank solution (blank sample) was composed only of methanol without DPPH. Whereas, in control only DPPH solution was applied without sample. Then the mixture was subjected to homogenize for 1min by using vortex shaker and left for 30min at 30ºC in a dark condition. The absorbance was read at 517nm. DPPH free radical scavenging activity was calculated according to the following equation.

$$\text{Scavenging effect (\%)} = [1 - (\text{Absorbance of sample} - \text{Absorbance of blank}) / \text{Absorbance of control}] \times 100$$

Ferric reducing power activity

The ferric reducing power activity was calculated by using method of Oyaizu et al. [13]. Various phlorotannin concentrations (100-500µg/ml) were added to 2.5ml of 0.2M sodium phosphate buffer with pH-6.6 and 2.5ml of 1% $K_3Fe(CN)_6$ solution. The test tubes were well homogenized and allowed to incubate at 50ºC for 20min in the vortex shaker. After the incubation, 2.5ml of trichloroacetic acid (10%) was added into each tube and centrifuged at 650 rpm for 10 min. 2.5ml of supernatant (upper layer) was collected and transferred to the test tube that contain 2.5ml of distilled water along with 0.5 ml of 0.1% ferric chloride. The colored solution was read at 700nm against the blank with reference to a standard using UV-spectrophotometry. Here, ascorbic acid was used as a reference standard.

Anti-inflammatory activity

Inhibition of albumin denaturation

Anti-inflammatory activity of the purified pholorotannin was evaluated by inhibition of albumin denaturation according to the method of Mizushima and Kobayashi [14] with slight modifications. The reaction mixture solution (0.5 ml; pH 6.3) contains distilled water - 0.05 ml, BSA 0.45 ml, and 5% aqueous solution. Sample solutions of variable concentrations (100-500 µg/ml) were poured into the reaction mixture, incubated at 37ºC for 20 min and then subjected to heat the samples at 57ºC for 5 min. Then, the test tubes were cooled and 2.5 mL of phosphate buffer saline was added. Turbidity was measured at 600 nm, by spectrophotometrically. The percentage inhibition of protein denaturation was calculated as follows

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Antimicrobial Activity

Test Organism

Human pathogen strains were collected from the Department of Medical Microbiology (Raja Muthiah Medical College and Hospital), Annamalai University, Annamalai Nagar, Tamilnadu, India. The following pathogens were used as test organism: *Proteus vulgaris, Salmonella paratyphi A, Proteus mirabilis, Bacillus subtilis* and *Shigella* sp.

Screening of antibacterial activity

Antibacterial activity of phlorotannin from brown seaweed was determined by the agar well diffusion method of Kirby-Bauer et al. [15]. A sub cultured 24hr bacterial suspension was spread on Muller Hinton Agar plates with sterile cotton swab. Subsequently, wells of 8mm diameter were punched into the agar medium plate and then loaded the 20µl (1mg/ml) of phlorotannin extract and allowed to diffuse at room temperature for 2 hr. These plates were then incubated in the upright position at 37ºC for 24hr. Wells containing the same volume of distilled water served as negative control while antibiotics of Amoxicillin

(1mg/ml) concentration of 10µl were used as the reference positive controls. After the incubation, the diameters of the inhibition growth zones were measured in mm. The samples were carried out triplicates times against each of the test pathogens.

Statistical analysis

The results were expressed as mean ± standard error of mean (Mean ± SEM). The data were analyzed by one- way ANOVA followed by Tukey's multiple comparison tests.

RESULTS

Yield and Spectral analysis

The phlorotannin extract was obtained as powdered substances with green colour. A yield of about 6.48g (14%) was obtained from *T. decurrens* extract on a dry weight basis. The UV-Visible spectrum was recorded at 280nm for phlorotannin from *T. decurrens*. The spectral property is given in Figure 1.

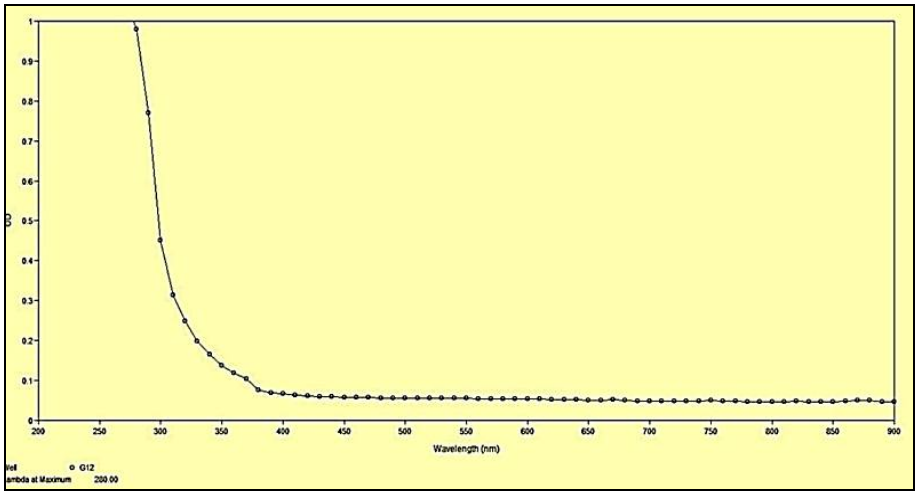

Figure 1. UV–Visible spectrum of phlorotannin extract at 280nm

Estimation of total phlorotannin content

The total phlorotannin content in the extract of *T. decurrens* was found to be 1.94 ± 0.188 mg phloroglucinol equivalent/g of phlorotannin dry weight. However, an OD value of the sample was compared with standard phloroglucinol, shown in (Fig. 2).

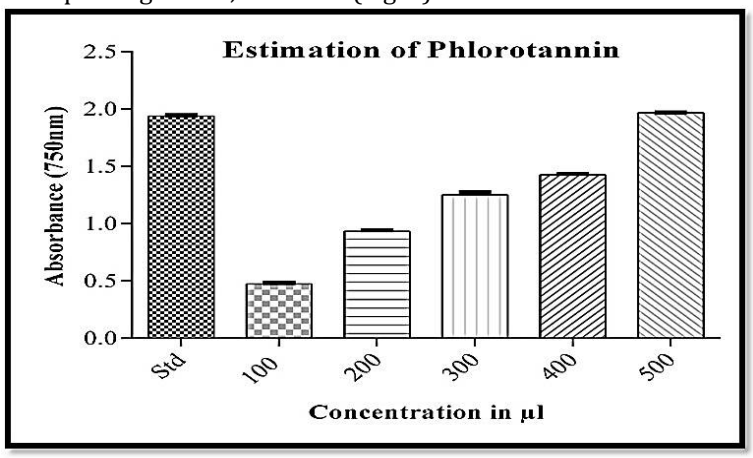

Figure 2. Estimation of total phlorotannin content

Preparative RP-HPLC Analysis

Preparative RP- HPLC was performed to analyze and collect the purified phlorotannin from the partially purified extract. However, only a single peak was observed at the retention time of 4.110 min which was compared with the standard phloroglucinol of R_T at 3.904 minutes, respectively shown in (Fig. 3).Thus, the single peak was collected in the preparative HPLC and used for further analysis.

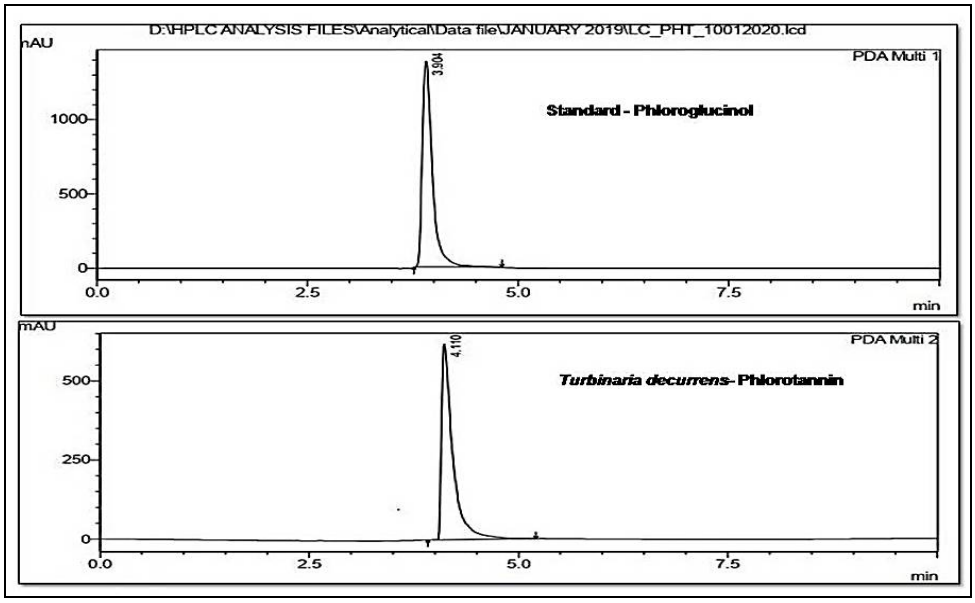

Figure 3. RP- HPLC analysis of phlorotannin from *T. decurrens*and Standard Phloroglucinol

UHPLC-ESI/MS for Purified phlorotannin

Phlorotannins profiling was assessed by UHPLC-ESI/MS and the particular phlorotannin was found to be dieckol of the molecular formula $C_{36}H_{22}O_{18}$ at 1488m/z of the mass pattern $[2M+2H]^+$. This result revealed that the obtained phlorotannin belongs to the dimer form of dieckol and the actual mass of dieckol was 743 m/z. Additionally, there has been an ion loss of 102m/z in the same spectrum, which shows the pattern $[2M+CH_2CO_2C_2H_7+H]$ for 1386m/z respectively (Fig.4).

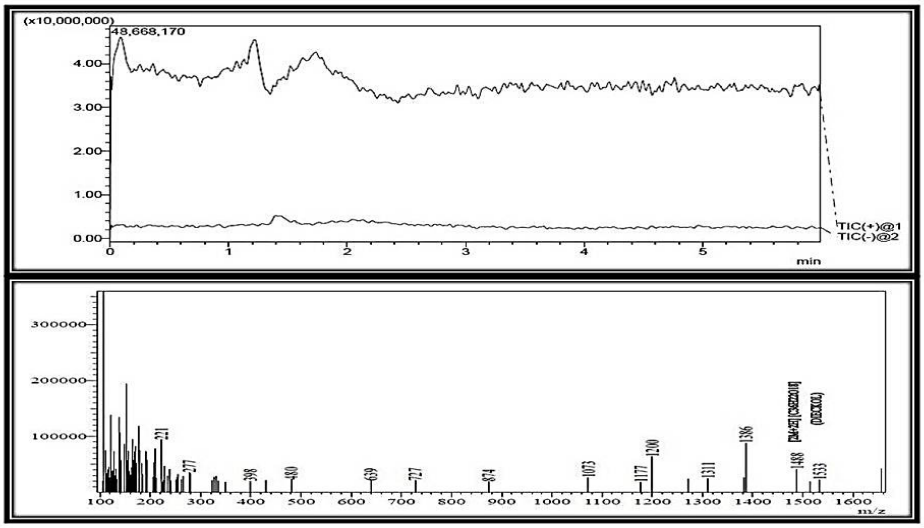

Figure 4. Image shows UHPLC-ESI/MS of purified Phlorotannin from*T. decurrens*

3.5 FT-IR analysis

FTIR spectral data confirmed the occurrence of many chemical functional groups within the phlorotannin compound. The IR spectrum of purified phlorotannin is shown in (Fig. 5) and the peak assignment is shown in table 1 and their analytical data well agreed with their compounds detected in LC-MS. There has broadband at 3369.37cm-1 that, might be phenol hydroxyl group (OH-), 2923.63 cm-1 and 2850.12 cm-1 range vibration stretches could be (C-H) bond, The peaks observed at 2006.99 cm-1, 1789.29 cm-1, 1639.96 cm-1, 1407.85 cm-1, 1206.97 cm-1 are maybe carbon double bond presence (C=C), and also peaks observed at 1139.02 cm-1, 1024.09 cm-1 are represented carbon, oxygen (C-O) bond, The peaks at 720.63 cm-1, 599.57 cm-1 are more probablyC-Br bond.

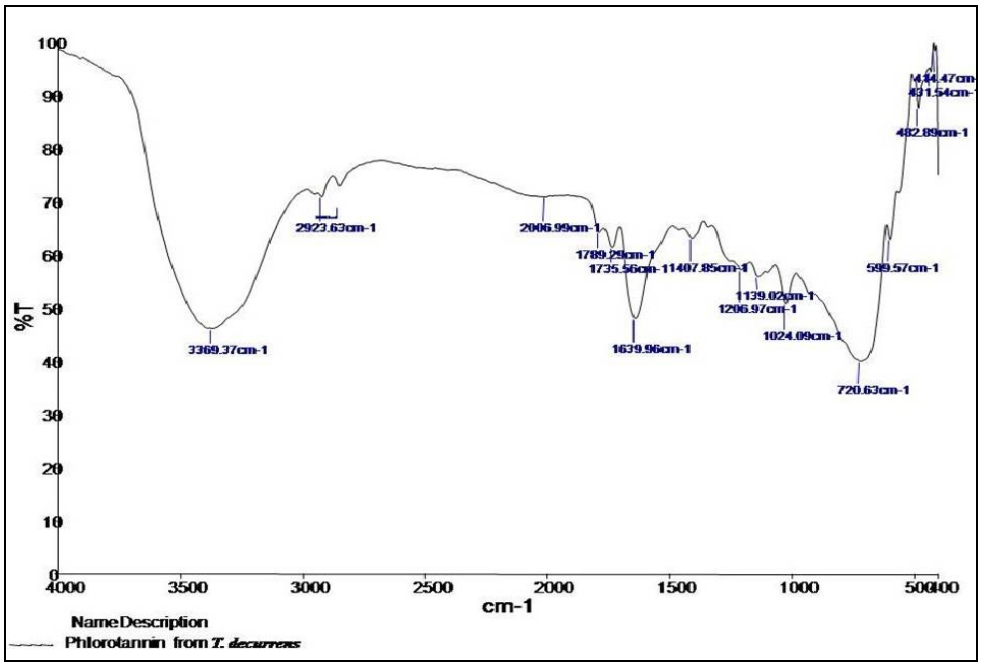

Figure 5. Image shows FT-IR Spectrum of Phlorotannin

Table 1. Peak assignment of FT-IR spectra of Phlorotannin from *T.decurrens*

Major Functional groups	Absorption frequency region
Phenols Ar O H Bonded (OH)	3369.37cm-1
Alkane CH_2	2923.63cm-1
Alkane CH_2	2850.12cm-1
Misc R-N=C=S	2006.99cm-1
1,3,5 Substitute benzene C_6H_6	1789.29cm-1
Alkenes C=C stretch	1735.56cm-1
Alkenes R CH_2CH_3	1639.96cm-1
Phenol Ar OH C-O Stretch	1407.85cm-1
Phenol Ar OH C-O Stretch	1206.97cm-1
Aryl – o- CH_2	1139.02cm-1
Alkanes C-H	1024.09cm-1
Alkyl halides R-Br stretch	720.63cm-1
Alkyl halides R-Br stretch	599.51cm-1

In-vitro bioactivity of phlorotannin

Antioxidant activity

The antioxidant activity of the phlorotannin of *T. decurrens* was tested by different in in-vitro methods. The results revealed that the free radicals scavenged by the phlorotannin are dose depended manner for all the assays.

Total antioxidant activity

The phlorotannin was evaluated for total antioxidant capacity. The TAA of phlorotannin was determined as 0.513 ± 0.000882, 0.055 ± 0.00115, 0.0603 ± 0.00120, 0.0663 ± 0.000667, 0.072 ± 0.00153 for the concentration (100 -500µg/ml) respectively. Whereas, ascorbic acid was standard which showed 0.823 ± 0.00353 at 500µg/ml (Fig. 6(a)). This clearly shows that the antioxidant dependeds on the concentration.

DPPH activity

DPPH inhibition potential of the phlorotannin was expressed as a percentage. As illustrated in Fig. 6(b), disparate concentrations of phlorotannin (20 -100 µg/ml) showed as 49.0 % ± 3.79%, 56.3% ± 3.53%, 75.0% ± 2.65%, 89.0%± 2.65%, 92.3% ± 1.20% respectively. Marked DPPH radical scavenging activity almost comparable with standard ascorbic acid evaluated as 93.7 ± 1.86% at 100 µg/ml concentrations.

Ferric reducing power assay

As shown in Fig. 6(c), different concentrations of phlorotannin (100-500 µg/ml) exhibited OD values of 0.371 ± 0.0156, 0.898 ± 0.0304, 1.21 ± 0.0197, 1.58 ± 0.0496, 1.72 ± 0.0140, respectively. The sample 500

µg/ml showed the highest value but the standard ascorbic acid showed the lowest value as 1.68 ± 0.00606 at same the concentrations. Values are presented as mean ± SEM.

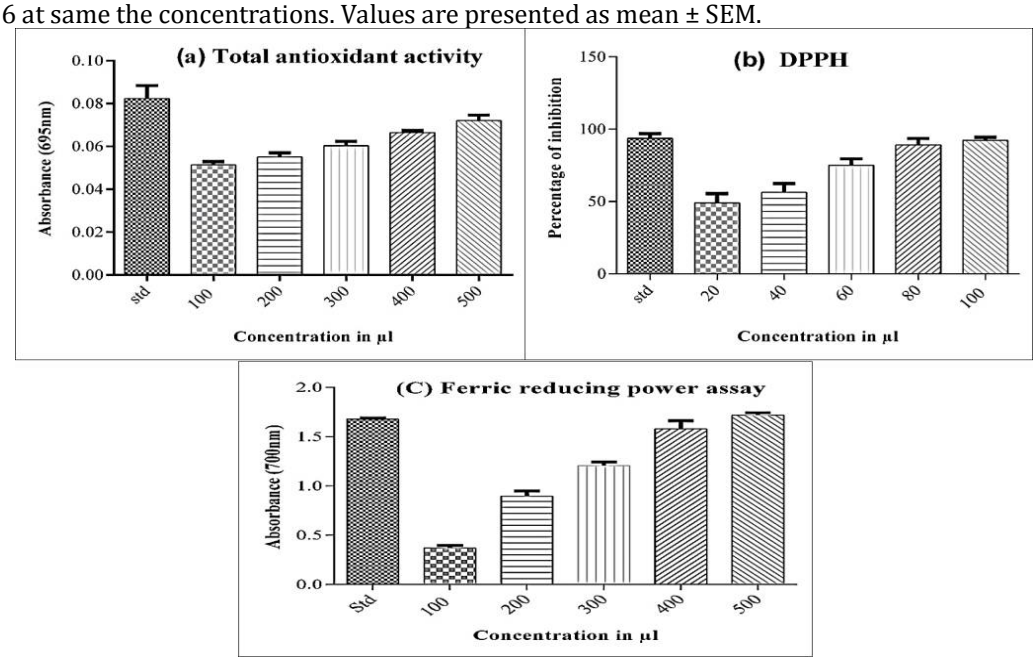

Figure 6. In vitro antioxidant activity of purified phlorotannin from *T. decurrens*and ascorbic acid. (a) Total antioxidant activity, (b) DPPH radical scavenging activity and (c) Ferric Reducing power activity. Values are presented as triplicate (n=3), mean ± SEM, statistical significance was performed by one-way ANOVA followed by Tukey's multiple comparison test.

Anti-inflammatory assay

Inhibition of albumin denaturation

The phlorotannin extract was tested for anti-inflammatory activity with bovine serum albumin. The phlorotannin showed significant activity, which was found to be 87.3 ± 0.667, 88.5 ± 0.764, 89.3 ± 0.636, 90.5 ± 0.491 and 91.4 ± 0.721% for the concentration (100-500 µg/ml), respectively. However, standard Aspirin showed 91.4 ± 0.721% inhibition at 500 µg/ml (Fig. 7).

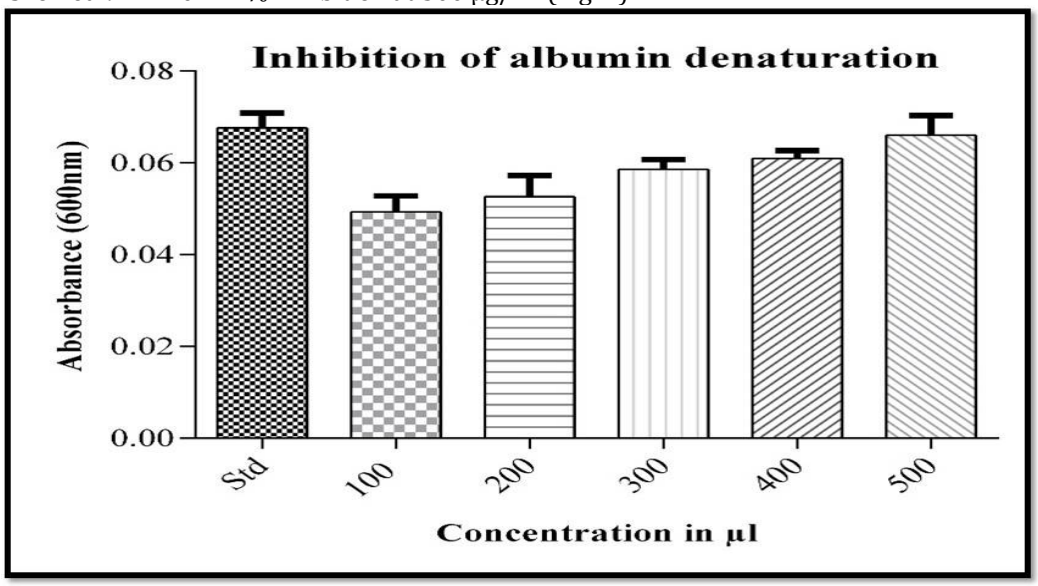

Figure 7. In vitro anti-inflammatory activity of phlorotannin and Aspirin.Values are presented as triplicate (n=3), mean ± SEM, statistical significance was performed by one-way ANOVA followed by Tukey's multiple comparison test.

Antibacterial activity

The phlorotannin was screened for the antibacterial activity with using of well diffusion method against gram positive and gram negative human pathogens. The phlorotannin was tested in a concentration of 10

and 20µg/ml, which has exhibited potent inhibitory activity against all tested bacterial strains. The inhibition results are given in table 2 and the zone of inhibition is illustrated in Figure 8.

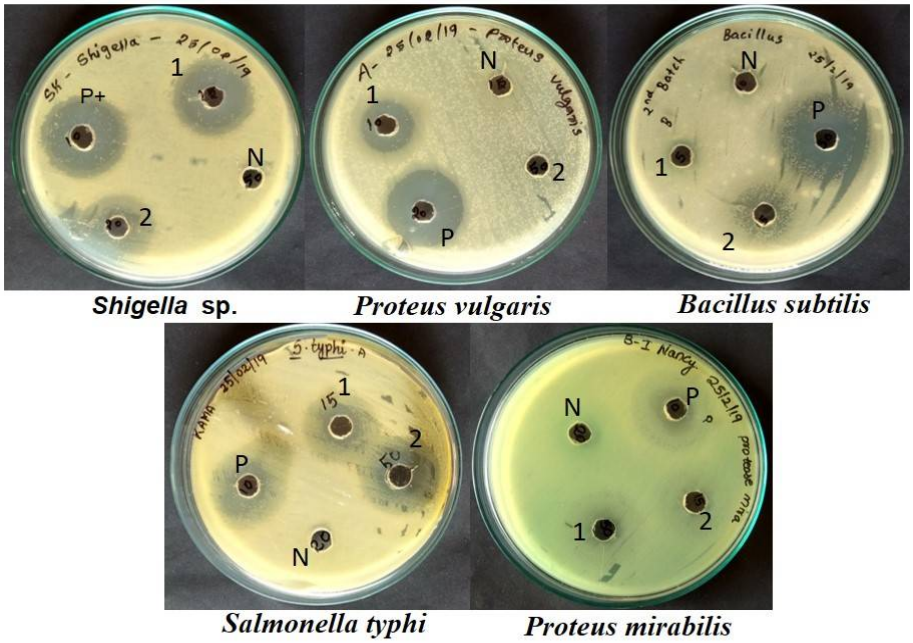

Figure 8. Plate shows antibacterial activity of phlorotannin against human pathogen.

Table 2. Zone of Inhibition (mm) against Gram Positive and Negative pathogen

Pathogens	Positive control (10µl)	Negative control (10µl)	PHT (10µl)	PHT (20µl)
Proteus vulgaris	20.7mm	0	10.8mm	17.3mm
Salmonella typhi	20.9mm	0	20.4mm	20.5mm
Bacillus subtilis	30mm	0	11.7mm	14.8mm
Shigella sp.	19mm	0	17.5mm	20.7mm
Proteus mirabilis	20.5	0	14.9mm	20.5mm

DISCUSSION

Seaweeds have been highly available in the market as functional foods and nutraceuticals. They contain various bioactive compounds (phytochemicals) that have many pharmacological properties [16].In the current investigation, the purified phlorotannin of *T. decurrens* was evaluated for their biological activities includes antioxidant, anti-inflammatory and antimicrobial activity. The yield of phlorotannin extracted from *T. decurrens* was found to be 6.48g (14%). This yield was higher than the *Macrocystispyrifera*, obtained as 2.20% [17].An earlier report showed a yield of 5.452 g (21 %) from the methanolic extract of *T. ornata*[18].which is a higher percentage than observed in our present study. On the other hand *Sargassum fusiforme* species showed significant yield for different percentage of ethanolic extract which found to be 63.6% (30%) and 62.17% (50%) [19].Ethanol is considered as a low-polar solvent when compared with the strong polar solvent water, hence the polarity of the extraction solvent might continuously decrease with the addition of ethanol solvent in water. In most extraction methods, extraction time, solvent type and temperature are well-known and important factors that cause variation in phlorotannin yield. The present study observed a strong impact of solvent polarity on yield percentage.

Generally, each compound has specific absorption characteristics that can be observed in a UV spectrum, which provides information about the particular absorption range. In this study, the presence of phlorotannin extract was confirmed by UV spectrum at 280nm**.** The previous study reported that phlorotannin from *T. ornata* shown by a characteristic absorption pattern (λmax at 35.90 and 261.06 nm) which can be confirmed by the present result [18].Therefore, the UV spectrum indicated that the purified compound was phlorotannin.

The total phlorotannin content of *T. decurrens* obtained here was lower than that in *Fucus vesiculosus*, found as 2.92 ± 0.05 mg [19].According to previous study Yajing et al. [20] the total phlorotannin content

from *S. fusiforme* was 88.48 ± 0.30 mg, which was much higher than the content determined in the present study. The reasons for the low content of phlorotannin could be the choice of significant extraction parameters, including the solvent concentration, solvent-solid ratio, extraction temperature, and extraction time.HPLC chromatogram result revealed that the extracted phlorotannin was free of impurities by showing a prominent single peak at RT 4.1min, which is similar to the standard phloroglucinol at RT 3.9 min. On the other hand, the previous study revealed that *Cystoseirasedoides* phlorotannin was eluted at 3.655 min retention [21].However, the purified phlorotannin was eluted through preparative RP-HPLC. Subsequently, purified phlorotannin was characterized by UHPLC-UV-ESI/MS.

The MS study of the ions allowed the detection of one dimer form of phlorotannin named dieckol, in *T. decurrens* with molecular ions [(2M + 2H) +] at m/z 1488. These compounds showed similar fragmentation patterns in which some ions are characteristic of phlorotannins fragmentation with losses of 102 m/z, which reveals the [2M+CH2CO2C2H7+H] pattern for 1386m/z respectively. This result revealed that the obtained phlorotannin belongs to the dimer form of dieckol and the actual mass of dieckol was 743 m/z. The same phlorotannin subunit component dieckol was detected in the *Fucus spiralis* extracted phlorotannin [2].FT-IR is an important tool for the identification of specific compounds based on the presence of functional groups. In the present study functional groups obtained in FTIR spectrum are confirmed with the previous studies [18].

Anti-radical activity is highly significant when there is an abandoned production of reactive species in the body and the endogenous antioxidants cannot conquer their harmful effects. Oxidative stress occurred in the cells because of disparity between the pro-oxidant/antioxidant systems, causing injury to biomolecules, such as nucleic acids, proteins, structural carbohydrates and lipids [2].In order to test the anti radial activity, the purified phlorotannin was evaluated for its antioxidant capacity using TAA, DPPH and FRP assays. The TAA of phlorotannin from *T. decurrens* showed the highest activity at 500µg/ml concentration. However, several reports showed various ranges of TAA at a concentration of 245 mg/g for *Phyllanthus* sp. and 30 µmol/mg for *S. pallidum,* respectively Kumaran et al.[22], [23].The solvents used for extraction that might be influence the chemical composition of the extracts [24].

The DPPH assay is usually accomplished for measurement of free radical scavenging indicating thepotential of an antioxidant molecule. It is well considered as one of the standards and effortless colorimetric methods for the assessment of antioxidant properties of pure compounds and natural antioxidants [25].DPPH activity of phlorotannin from *T. decurrens* showed the highest activity as 92.3% at 100µg/ml concentration. Earlier studies [26],[27] revealed the lowest DPPH inhibition activity as 87.53% for phlorotannin of *S. serratum* and 74.66% for the DCM fraction of phlorotannin from *Cystoseiratrinodis*. According to Kang et al. [28] the dieckol, isolated from *E.cava*showed a strong scavenging effect when compared to standard ascorbic acid. This indicates that the dieckol of *T. decurrens* possesses a stronger scavenging activity.

In addition, the Ferrous Reducing Antioxidant power (FRAP) assay is based on the ability of antioxidant components to reduce Ferric (III) to Ferrous(II) iron in a redox reaction that involves a single electron transfer [29].However, the present study exhibit a strong reducing activity that due to the presence of total phenolic content in phlorotannin. The Ferric Reducing Power assay obtained as 910.7 ± 27.5 (%) from *F. vesiculosus*, significantly less activity when compared with the present study [30].The crude phlorotannin *S. tenerrimum* shows the reducing power of 0.098 ± 0.095 (%) [31]. This finding reveals that the antioxidant activity of the extracts was related to the content of phlorotannins and to their molecular weight.

Denaturation of proteins is playing a major role for inflammation. Anti-inflammatory activity drugs arebeen dose-dependent capacityfor thermally-induced protein denaturation. While Bovine serum albumin is warmed, it will denature and express antigens with overly sensitive response which are associated with illnesses, for example, serum infection, glomerulonephritis, rheumatoid joint inflammation and systemic lupus erythematosus. Thus, it is important to conduct assays applied for the discovery of those drugs which can stabilize the protein from the denaturation process [32]. As a part of the investigation, the ability of the phlorotannin extract of *T. decurrens* to inhibit protein denaturation was calculated as a 91.4 ± 0.721 which is equivalent to Aspirin (standard drug) 91.67% at 500 µg/ml concentration. Similarly, previous research also reported anti-inflammation activity to inhibit heat induced albumin denaturation to be 74.4 % [33]. In past decades, marine algal-based remedies are becoming more general and widely used for healthcare and inflammation remedies. Several researchers are been reported that the anti-inflammatory properties of phlorotannins derived from edible brown seaweed such as eckol, phlorofucofuroeckols, dieckol, phloroglucinol, and bieckols [4], [34].

The antimicrobial activity of brown seaweed species could be attributed to the phlorotannin content. Antibacterial activity of the phlorotannin against both gram positive and gram negative bacteria has been established by several scientists. Such a variation in antimicrobial activity could be the presence of different antibacterial substances. The current study showed a strong zone of inhibition against pathogens of Gram positive and Gram negative which was higher than the *E. kurome* brown algae phlorotannin reported by Nagayama et al. [35].Moreover, the bactericidal effect of the phlorotannins has a mode of action that could be related to its ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins, and also to complex with polysaccharide [36].

CONCLUSION

The present investigation focuses on the extraction and purification of phlorotannin from the marine brown algae, *T. decurrens*. It is a key bioactive ingredient in the algal system and also plays a vital role in human health and nutrition. Therefore, it can be suggested that due to its' valuable biological functions and health beneficial effects, phlorotannin has a greater potential to be developed as an active ingredient for the preparation of nutraceutical, cosmeceutical and pharmaceutical products. In this study, the purified phlorotannin was characterized through analytical techniques such as UV analysis, FTIR, HPLC and LCMS. In addition, the dieckol of *T. decurrens*displayed the strongest antioxidant, anti-inflammatory and antibacterial property. Thus, the results of the present investigation support that the *T. decurrens* dieckol could be developed as a promising therapeutic candidate for inflammatory and bacterial diseases.

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