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Phytochemical evaluation, FT-IR and RP-HPLC Analysis of marine brown algae collected from the coastal area of Okha in Gujarat

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ABSTRACT

Seaweeds are an enormous origin of biologically active components. Brown macroalgae are of tremendous interest at the moment because they contain a variety of bioactive substances that can be used for different therapeutic purposes. This study is focused on the one profusely available brown alga in the coastal area of Okha, Gujarat, Sargassum tenerrimum. Qualitative and quantitative phytochemical analyses were conducted for various bioactive compounds using different types of extracts. Further Fourier transform infrared spectroscopy (FTIR) and Reversed-phase high-performance liquid chromatography (RP-HPLC) analysis was conducted for confirmation of the functional group and the particular compound. An evaluation of qualitative phytochemical analysis revealed the existence of alkaloid, flavonoid, phenol, glycoside, tannin, terpenoid, steroid, coumarin and anthraquinones. Quantitative analysis of phytochemicals revealed that the *S. tenerrimum*. They contain a good amount of total phenolic content (68.13±2.55 mg GAE/gm), total flavonoid content (93.30±2.88 mg QE/gm) and total tannin content (93.13±2.8 mg TAE/gm) in the methanolic extract, which may have several antioxidant properties. The results based on FT-IR analysis revealed the presence of functional groups like alcohol, phenol, aliphatic, anhydrides, Aromatics, and Aliphatic amines. RP-HPLC analysis of methanolic extract confirmed the presence of gallic acid and quercetin. The presence of the phenolic compounds, flavonoids and several functional groups render good DPPH and HRSA activity, indicating this algal extract's medicinal properties. The conclusion put out is that *Sargassum tenerrimum* represents a vast source of biologically active substances with antioxidant potential that may be investigated for the pharmaceutical, cosmetic, and functional food sectors.

Keywords: *Sargassum tenerrimum*; Phytochemical; RP-HPLC; FT-IR; Antioxidant activity

INTRODUCTION

The ocean is prosperous in varied marine resources, including seaweeds (Shobier *et al.,* 2022). Macroalgae have long been recognized as essential to the coastal environment because they are one of the principal ocean contributors (Pakingking *et al.,* 2022). Marine algae are those that grow on the seashore or in saline water, and salt swamps in the sea. For the aquatic environment, they are the main producers. They are essential to the ecosystem because they have been leading to the creation of the aquatic food chain, and nearly all aquatic creatures rely on them (Huynh and Serediak, 2006). They take all their nutrients directly from the water through their tissue, a bit like a sponge absorbs moisture and their holdfast is completely for physically attaching the thallus to the seabed (Jimenez-Escrig *et al.*, 2001).

India has a huge coastal area, so marine algae grow luxuriantly in the coastal regions of Gujarat, Tamil Nadu, Andaman Nicobar Island and Lakshadweep (Chojnacka *et al.*, 2012). Seaweeds have long been perceived as vital, natural renewable sources of bioactive chemicals among all ocean organisms (Shobier *et al.,* 2022). More than 60 elements are present in marine algae in high amounts than in land plants. These include a variety of special bioactive chemicals that are not found in terrestrial plants (Jayabarath *et al.*, 2017).

Currently, 6000 known macroalgae species are classified into green, brown, and red algae (Chandini *et al.,* 2008). Among the three groups of marine algae, brown algae have seemed to be the most influenced by environmental factors, providing a variety of species in different regions (Baweja *et al.,* 2016). Brown algae are an immense source of vitamins, provitamin, iodine calcium, saturated and unsaturated fatty acids, polyphenols, phytosterols and phlorotannin (Mishra and Jha, 2009; Hakim and Patel, 2020). Some brown algae have been introduced as 'natures' medicine chest' because they bear a high concentration of numerous valuable biologically active compounds (Balch *et al.*, 2000). Different extracts of algae are heterogeneous mixtures of compounds with distinct polarities and antioxidant properties that may contribute to synergetic activity (Marimuthu *et al.*, 2012). In the past, the profitable properties for humans, animals and plants were identified and are admired nowadays, in the fields of novel production of biotechnological products (Kähkönen *et al.*, 2001).

There is a modern trend for separating and identifying bioactive compounds from marine algae. To assess the chemical and molecular structure and composition of unidentified mixtures, Fourier Transform Infrared (FT-IR) spectroscopy can be used. HPLC has recently developed as an approved scientific tool for fingerprints and quantification of standard compounds in extracts. The current work thus focuses on the

phytochemical screening, RP-HPLC, and FT-IR analysis of the methanol extracts of *S. tenerrimum* that were used after identification, authentication, and characterization.

MATERIAL AND METHODS

Sampling and extraction:

The brown algae *S. tenerrimum* was collected from Okha, a coastal town in the Dwarka district of Gujarat state in India in December 2021. The collected algae were dried at room temperature, ground in a mechanical grinder and powdered material was stored in an airtight box for further use. In addition, 1 g of the sample was immersed in a conical flask containing 10 ml Water, Methanol, ethyl acetate and benzene as a solvent and put on a shaker for 24 hours after each extract was filtered and stored in the refrigerator for further analysis.

Qualitative and quantitative photochemical analysis:

The qualitative phytochemical analysis of secondary metabolites (alkaloids, coumarin, flavonoids, glycosides, phenols, steroids, saponin, tannins and terpenoid) was tested in four different extracts (water, methanol, ethyl acetate and benzene) using the standard procedure (Harborne, 1998 and Savithramma *et al*., 2011).

Quantitative phytochemical analysis of total phenol, flavonoid and tannin content was measured through a UV-Vis spectrophotometer. Total phenols content (TPC) in different algal extracts was evaluated by a method provided by Sadasivam and Manickam, 1992. The absorbance of the sample was measured at 765 nm. Gallic acid is used as standard. The amount of TPC in standard and extract was expressed as milligram gallic acid equivalents per gram (mg GAE/gm). Total flavonoid content (TFC) in different algal extracts was determined by a method described by Bohm and Kocipai-Abyazan, 1994. The absorbance of the mixture was determined at 510 nm. Quercetin is used as a standard. The amount of TFC in standard and extract was expressed as milligram quercetin equivalents per gram (mg QE/gm). Total tannin content (TTC) in different algal extracts was determined by a method described by Van-Burden and Robinson method, 1981. The absorbance was read at 725 nm after 40 minutes. Tannic acid is used as standard. The amount of TTC in standard and extract was expressed as milligram tannic acid equivalents per gram (mg TAE/gm).

Fourier Transform Infrared (FT-IR) analysis:

The FTIR analysis was carried out to know the functional groups present in the methanolic extracts. The methanolic crude extracts of the selected brown algae were performed by Shimadzu 8400S FTIR spectrophotometer. The measurements were taken in an automatic recording FTIR spectrophotometer in the range of 4000 to 400cm-1. The FTIR signal area was recorded and compared with the standard peak value and interpreted the functional groups.

High-performance liquid chromatography (HPLC) analysis:

Advanced chromatography was employed in the current work to identify the phytoconstituents found in the methanolic extracts of the selected algae. The Shimadzu LC-20AD HPLC system, which includes a model LC-20AT pump, UV-Visible detector SPD-20AT, an injector fitted with a 20-loop, and an auto-injector SIL-20AT, was used to carry out the HPLC method. A Shimadzu C-18 column (4.6×250mm, 5µm size) with a C-18 guard column was used. The condition for HPLC analysis for each standard was given in table 1.

Table 1. HPLC Condition for Standards Gallic Acid and Quercetin

Sr. No.	Parameters	Gallic Acid	Quercetin
1	**Mobile Phase**	Methanol and 0.4% phosphoric acid (49:51)	Methanol and Acetonitrile (50:50)
2	**Flow rate**	1.0 ml/min	1.0 ml/min
3	**Wavelength**	378 nm	370 nm
4	**Temperature**	35°C	35°C
5	**Column**	C18(4.6×250mm,5 µm)	C18(4.6×250mm,5 µm)
6	**Retention time**	11.80 min	4.59 min

Determination of Free Radical Scavenging Activity by DPPH (1, 1-diphenyl-2-picrylhydrazy) method:

The total antioxidant capacity of varying concentrations of the algal extract was evaluated using the 1, 1-diphenyl-2-picrylhydrazy (DPPH) method (Madhu, 2013). At 517 nm, the absorbance of the samples was recorded by a UV-vis spectrophotometer. The equation below calculated each extract's percentage of scavenging activities (AA).

AA%= (A control - A samples/A control) ×100

Where A control is the absorbance of the control (DPPH), A sample is the absorbance of the sample, A blank is the absorbance of the blank (methanol).

Hydrogen peroxide scavenging activity:

The hydrogen peroxide (H_2O_2) scavenging ability of different extracts of the algal sample was determined according to the Ruch *et al*., 1989. Hydrogen peroxide (10mM) was prepared in phosphate buffer (pH 6.6). The crude extract at 1 ml and after makeup 3ml with phosphate buffer. The reaction mixture's absorbance value was measured at 240 nm. The Hydrogen peroxide-free phosphate buffer was regarded as the "control solution". The percentage of scavenging of methanolic extract was calculated according to the following equation:

% Scavenged [H_2O_2] = [(A Control – A Sample) / A Control] × 100

Where A control is an absorbance of the control (phosphate buffer), and A sample is the absorbance of the sample.

RESULTS

Qualitative phytochemical analysis

The qualitative phytochemical results of water, methanol, ethyl acetate and benzene extract were tabulated in Table 2. This demonstrates that methanolic extract had maximum solubility of flavonoid, phenol, glycoside, tannin, terpenoid, steroid, coumarin and anthraquinones. Water extract showed less solubility of bioactive compounds compared to other extracts. The saponin only showed positive results in the water extract. Whereas flavonoid and coumarin were present in all extracts except ethyl acetate extract.

Quantitative phytochemical analysis

The amount of total phenol, total flavonoid and total tannin content were summarised in Table 3. Among all the extracts, TPC was recorded highest in methanolic extract (68.13±2.55 mg GAE/gm), followed by ethyl acetate, water, and benzene extract. TFC showed the maximum result in methanolic extract and the amount was (93.30±2.88 mg QE/gm) whereas TTC showed a maximum amount (93.13±2.82 mg TAC/gm) in methanolic extract. The amount of TPC was (48.13±0.66 mg/gm), TFC was (22.38±0.43 mg/gm) and TTC was (39.07±2.34 mg/gm) in 70% ethanolic extract of *Sargassum podocanthum* (Dang *et al*., 2018). Thus, it can be concluded that *S. tenerrimum* is a good source of all three phytochemicals and other species of this popular genus.

Table 2. Qualitative phytochemical analysis of *S. tenerrimum* in different solvents.

Sr. No.	Phytochemicals	Water extract	Methanol extract	Ethyl acetate extract	Benzene extract
1	**Alkaloid**		■	■	
2	**Flavonoid**	■	■		■
3	**Phenol**		■	■	■
4	**Glycoside**		■	■	■
5	**Saponin**	■			
6	**Tannin**	■	■		
7	**Terpenoid**		■		
8	**Steroids**		■		■
9	**Coumarin**	■	■		
10	**Anthraquinones**		■	■	

■ - Present

□ - Absent

Table 3. Amount of TPC, TFC and TTC in different extracts of *S. tenerrimum.*

Sr. No.	Name of extracts	Total Phenolic content (TPC) (mg GAE/gm)	Total Flavonoid content (TFC) (mg QE/gm)	Total Tannic acid content (TTC) (mg TAE/gm)
1	**Water**	25.16±2.88^{c}	39.01±3.28^{c}	63.19±1.92 ^{c}
2	**Methanol**	68.13±2.55^{a}	93.30±2.88^{a}	93.13±2.82^{a}
3	**Ethyl acetate**	47.14±2.48^{b}	75.13±2.40^{b}	73.44±2.25^{b}
4	**Benzene**	24.31±2.70^{c}	23.67±4.01^{d}	57.01±1.55^{c}

(The experiment was performed in triplicate (mean ± standard error). Different letters within the same column indicate significant differences between treatments according to DMRT. All the value is found Significant at 1% and 5% levels of significance)

FTIR analysis

The FTIR spectrum of methanolic extract of *S. tenerrimum* showed different peaks at 3318cm-1, 2944cm-1, 2832cm-1, 1449cm-1, 1113cm-1, 1021cm-1, 615cm-1, it was confirmed the presence of functional groups such as alcohol and phenol (O-H stretching for H-bonded), C-H antisym and sym stretching for aliphatic, anhydrides (C=O sym stretching), Aromatics (C-C stretching), Aliphatic amines (C-N stretching), Aliphatic amines(C-N stretching for), Alkyne (C-H stretching) respectively (Fig 3and Table 5). By applying the FT-IR spectrum, work fortified the functional components present in the algae extract.

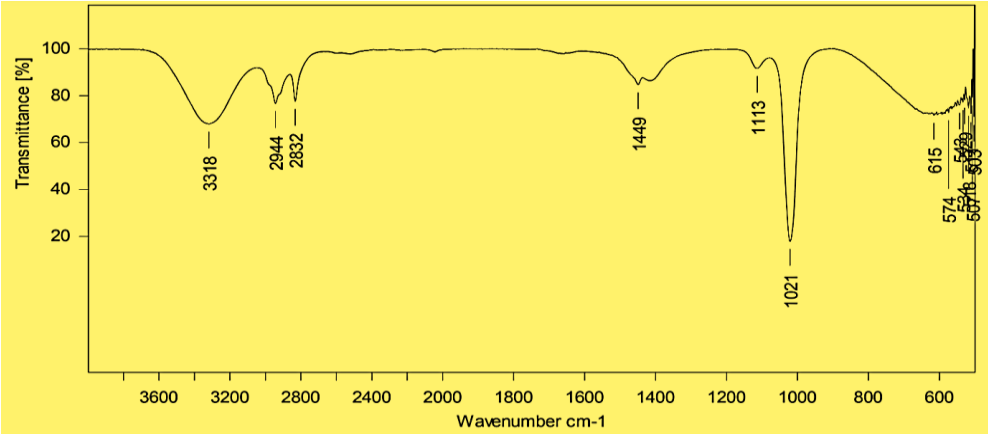

Fig. 3. FTIR spectrum of methanolic extract of *S. tenerrimum.*

The FTIR spectrum of brown algae *Sargassum wightii* and red algae *Gracilaria corticata* showed peck at 3371 cm-1, 2924 cm-1 and 2358 cm-1 corresponding to N-H / O-H, C-H and C-O stretching vibrations respectively in different amines, hydroxyl and Carboxylic groups (Marimuthu, 2014). The FT-IR analysis on algae/seaweeds, extracts showed that the algae surfaces had the toxic interaction sites of carboxyl, amino acid and hydroxyl groups of algae (Mishra and Jha, 2009 and Marimuthu, 2012).

Table 5. Representation of wavelength and characteristic peaks of IR spectrum

Sr.No.	FTIR peaks(cm^{-1})	Functional group assigned	References
1.	3318	O-H stretching for Alcohol and phenol	Fernando *et al.,* 2017
2.	2944	C-H antisym and sym stretching for aliphatic	Kannan, 2014
3.	2832	C=O sym stretching for anhydrides	
4.	1449	C-C stretching for Aromatics	Fernando *et al.,* 2017
5.	1113	Symmetric and asymmetric RO-SO^3 bond for sulfate group	
6.	1021	C-N stretching for Aliphatic amines	Paul and Lawrence, 2017
7.	615	C-H stretching for Alkyne	

HPLC analysis

RP-HPLC profile of methanolic extract was examined and two-component namely gallic acid and quercetin should obtain at distinct retention times (Fig. 2 and Table 4). It was remarked that two main peaks were eluted with a retention time of (11.835 min) and a retention time of 4.457 min identified at 278 nm and 370nm respectively. This major compound was identified as gallic acid and quercetin. The HPLC chromatogram of extract displayed related retention time at a particular wavelength used as a reference compound (Fig 2-A).

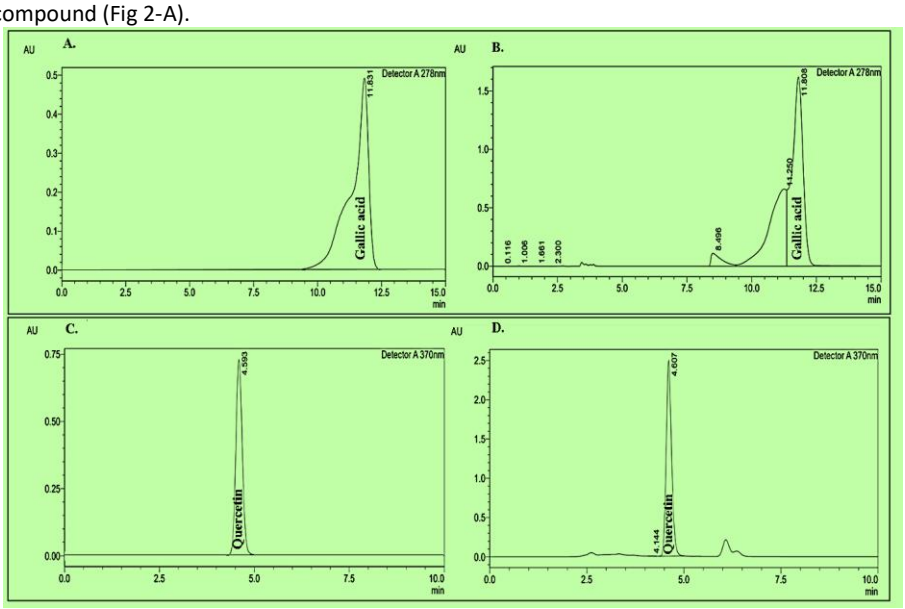

Figure 2. HPLC chromatogram of methanolic extract of *S. tenerrimum* A) Standard gallic acid peck at retention time 11.835 min detected at 278 nm B) *S. tenerrimum* Showed gallic acid peck at retention time 11.808 min detected at 278 nm C) Standard quercetin peck at retention time 4.593 min detected at 370 nm D) *S. tenerrimum* Showed quercetin peck at retention time 4.607 min detected at 370 nm.

Table 4. RP-HPLC Analysis of gallic acid and quercetin in *S. tenerrimum.*

Sr.No.	Retention Time	Area	Height	Amount (ppm)	Phytoconstituents	Compound
1.	11.835	47353640	1618391	11.670447	Gallic Acid	Phenol
2.	4.597	2957683	269142	18.298	Quercetin	Flavonoid

The calibration equation obtained from the calibration curve was y = 2294.8x – 52765 (R^2 = 0.9972) for gallic acid and y = 1196.9x + 320548 (R^2 = 0.9924) for quercetin. Similarly, From the HPLC analysis, it can be proved that the functional components like phenol and flavonoid present in the methanolic extract of *S. tenerrimum.* The findings also confirmed the results of the present research and phytoconstituents like gallic acid and quercetin in methanolic extract of brown algae have shown the appearance of flavonoid and phenolic compounds in *Turbinaria conoides* by using HPLC (Jayabarath and Jeyaprakash, 2017 and Rooban *et al*., 2009).

Antioxidant activity

The results of DPPH and HRSA activity are presented in Table 6. The percentage of inhibition for DPPH activity was in order of methanol> water > ethyl acetate>benzene, the value was 62.17%> 49.19%> 40.37%> 33.71%, respectively and the percentage of inhibition for HRSA activity was in order of methanol> ethyl acetate> water> benzene, the values were 68.43%> 55.60%> 38.82%> 29.07%, respectively. This result indicated that methanolic extracts of algae were potentially active, compared to other extracts. There's also a direct relationship between phenolics and antioxidant activity. *Sargassum swartzii* displayed a good bioactive compound in ethanolic extract (Ravi *et al.,* 2019). The examined results in this research proposed that *S. tenerrimum* is rich in many valuable phytochemical compounds that can be used for medicinal, food and nutritional purposes.

Table 6. Determination of antioxidant activity in various extracts of *S. tenerrimum.*

Sr. No.	Extract Name	Percentage of inhibition for DPPH activity (%)	Percentage of inhibition for HRSA activity (%)
1	**Water**	49.19±0.024^{b}	38.82±0.014^{c}
2	**Methanol**	62.17±0.027^{a}	68.43±0.017^{a}
3	**Ethyl acetate**	40.37±0.024^{bc}	55.60±0.025^{b}
4	**Benzene**	33.71±0.028^{c}	29.07±0.021^{cd}

(The experiment was performed in triplicate (mean ± standard error). Different letters within the same column indicate significant differences between treatments according to DMRT. All the value is found Significant at 1% and 5% levels of significance.)

DISCUSSION

The most varied seaweed habitats can be found in Gujarat and Tamil Nadu (Ganesan *et al*., 2019). The Deccan Tertiary rocks, contemporary alluvial deposits, and limestones containing Pleistocene miliolite fossils made up Gujarat's shoreline (Jha *et al.,* 2009). Seaweeds have evolved some defense mechanisms, including tolerance, physical or structural resistance, and damage tolerance (Baweja *et al*., 2016). Macroalgae produce a range of bioactive molecules, such as phenols, aromatic compounds, and other volatile compounds, as part of their chemical defense systems.

The growing population tends to receive plant-based medicines, and many of them are currently being used extensively in clinical studies. Due to their antioxidant capabilities, numerous plants have been employed extensively over the past few years to cure a variety of ailments. This is a known fact that marine algae are becoming more and more significant as a source of novel compounds. The metabolic and physiological characteristics of marine algae, which enable organisms to thrive in a variety of diverse habitat types, offer considerable potential for the formation of important bioactive compounds that are not present in terrestrial habitats. Therefore, seaweeds are among the greatest sources of new bioactive chemicals (Blunt *et al.,* 2007).

The phytochemical evaluation of several crude extracts indicated a variety of bioactive compounds with variable levels of concentration. Phytochemicals like phenol, flavonoid and terpenoid may be derived from polyphenolics which were having increasing interest because of their prominent antioxidant capacity (Matanjun *et al.,* 2008). The findings of this research showed that methanolic extract of *S. tenerrimum*, which had the highest concentration of phenolic, flavonoid and tannin components has a higher level of antioxidant potential. The various phytoconstituents varied according to the type of seaweed, their chemical composition, the solvents used, the environment, the collection period, and the water's physicochemical parameters (Prabakaran *et al.,* 2018).

It was reported that not only different species of algae but also the environmental conditions and areas changed the number of bioactive compounds in the algae (Jiménez-Escrig *et al.,* 2001). Algal ethanolic and methanolic extracts were subjected to a qualitative phytochemical examination, which revealed a variety of bioactive substances (Hakim and Patel., 2022). Various species of *Sargassum* have beneficial biological activities such as antioxidant, antiviral, antimicrobial, anti-inflammatory, and anticancer properties (Besednova *et al.,* 2014).

Herbal drugs must conduct HPLC studies to validate the existence of active ingredients and any additives (Yamuna Devi *et al.,* 2012). In the present study, the HPLC profile of *S. tenerrimum* showed the presence of important bioactive compounds gallic acid and quercetin. The molecular structure of the various functional groups contained in the methanolic extract of *S. tenerrimum* was determined by FT-IR. The spectrum of FTIR analysis showed characteristic absorbance bands

and confirmed the presence of alcohol and phenol, aliphatic amines (C-N stretching) and Alkyne groups. DPPH is a continuous strong oxidant that transforms a stable diamagnetic particle when it is given a pair of electrons (Elmastaş *et al.,* 2006). Because of the hydroxyl groups present in phenolics, *S. tenerrimum* has a high scavenging capacity.

CONCLUSION

The findings of this study demonstrate that *S. tenerrimum* is a prosperous source of natural compounds. The data of the exploratory studies of the TPC, TFC, TTC, and DPPH activity significantly varied in *S. tenerrimum*. FTIR analysis is a great technique for examining functional groups since it can be used to identify the chemical composition of chemical groups that are present in samples. The developed HPLC method is simple and was used to estimate the bioactive marker compounds gallic acid and quercetin present in selected alga. This wide range of possible bioactivities of algae-derived bioactive molecules or compounds opens up many commercial opportunities in various industries and medicinal fields. HPLC profiling obtained in the study can be evaluated as a basic tool for determining the validity of *S. tenerrimum*. The extract is effective in its antioxidant properties because it contains essential phenolic and flavonoid components. Moreover, these findings may be helpful in future investigations of these chemicals in biological sciences.

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