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Research Article

Nutritional profiling and *in vitro* bioactive properties of *Sargassum wightii* extracts for nutraceutical applications

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Abstract

Seaweeds are widely recognized as nutritious foods due to their high protein, mineral, and essential amino acid content, as well as their rich array of bioactive compounds and low-calorie profile. This study aimed to evaluate the nutritional composition of *Sargassum wightii* and compare the *in vitro* anti-cancer, anti-diabetic, anti-inflammatory, and anti-obesity activities of its aqueous and ethanolic extracts. Additionally, the extracts were assessed for their preliminary phytochemical composition, total phenolic and flavonoid content, antioxidant, and antibacterial activity. Phytochemical screening revealed the presence of various bioactive constituents, with the ethanolic extract exhibiting the highest total phenolic content (25.30±0.01 mg GAE/g) and flavonoid content (16.50±0.02 mg QE/g). The ethanolic extract also demonstrated superior antioxidant activity against DPPH radicals (IC_{50}=5.32±0.04 mg/mL), potent anticancer effects against the MDA-MB-231 breast cancer cell line (IC_{50}=20.21 µg/mL), and no cytotoxicity on the L6 rat skeletal myoblast cell line. Furthermore, it showed strong anti-inflammatory (IC_{50}=74.5 µg/mL), anti-obesity (IC_{50}=3.46 µg/mL), and anti-diabetic activity (IC_{50}=111 µg/mL), along with antibacterial effects, exhibiting the highest inhibition zones against *Staphylococcus aureus* (10 mm) and *Bacillus subtilis* (11 mm). FTIR analysis indicated the presence of polyphenols (3169-3904 cm^{-1}), supporting its potential nutraceutical applications. The ethanolic extract of *Sargassum wightii* showed strong multifunctional bioactivities, making it a promising nutraceutical candidate. Its effectiveness is likely due to the rich presence of polyphenolic compounds. Further studies are needed to isolate active compounds and explore therapeutic applications.

Keywords: Anti-cancer, Anti-diabetic, Anti-inflammatory, Antioxidant, *Sargassum wightii*

Highlights

- Ethanolic extract showed superior biological activity over aqueous extract
- High phenolic content correlated with strong antioxidant activity
- Exhibited notable anti-cancer and anti-inflammatory properties
- Safe for normal cells and effective against bacterial pathogens
- FTIR confirmed presence of phenolic and functional bioactive group

INTRODUCTION

The escalating global prevalence of chronic diseases, including diabetes, cancer, obesity, and cardiovascular disorders, has amplified the demand for safe, naturally derived bioactive compounds exhibiting therapeutic efficacy. Marine macroalgae, particularly brown seaweeds, have emerged as promising candidates due to their rich composition of biologically active constituents such as polyphenols, flavonoids, sulfated polysaccharides, terpenoids, and sterols. These compounds demonstrate diverse pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects, while being inherently biocompatible and devoid of the adverse effects associated with synthetic additives like BHA and BHT (Huang & Wang, 2004; Cornish & Garbary, 2010; Gupta & Abu-Ghannam, 2011).

Sargassum wightii, a brown seaweed prevalent in the Indian Ocean, has been utilized in traditional medicine for over two millennia, particularly in the treatment of thyroid-related conditions attributed to its high iodine content (Mattio & Payri, 2011; Liu et al., 2012). Recent research underscores its potential in alleviating metabolic disorders and enhancing animal productivity (Casas-Valdez et al., 2006; Bocanegra et al., 2009; Singh et al., 2017). Its secondary metabolites, particularly phlorotannins, phenolic acids, sterols, and terpenoids, serve critical ecological and defensive functions and have drawn scientific interest for their prospective nutraceutical applications (Mattio & Payri, 2011; Rosa et al., 2019).

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Solvent extraction techniques play a pivotal role in isolating these bioactive compounds, wherein the selection of solvent, temperature, pH, and duration significantly influences the efficiency and integrity of the extracted bioactives (Do et al., 2014; Azwanida, 2015). Ethanol has proven particularly effective in extracting phenolic compounds and is recognized as safe for human applications (Tomsone et al., 2012). The objective of the present study is to evaluate and compare the nutritional composition and *in vitro* bioactivities of aqueous and ethanolic extracts of *S. wightii*, encompassing antioxidant, anti-diabetic, anti-inflammatory, anti-obesity, antibacterial, and anticancer potentials, as corroborated by FTIR spectral analysis.

MATERIALS AND METHODS

Sample collection and preparation: *Sargassum wightii* was collected from the Mandapam coast in Tamil Nadu, India, in October 2023. The collected samples were thoroughly cleaned using both seawater and distilled water to eliminate debris. The cleaned biomass was subsequently oven-dried at 50°C. Following the drying process, the biomass was ground utilizing a mechanical grinder to obtain a powder that passed through a 1-mm sieve, and it was stored in airtight containers at -21°C for further analysis.

Proximate composition: The proximate composition of the seaweed was determined using standard methodologies established by the Association of Official Analytical Chemists. Moisture content was assessed according to (Association of Official Analytical Chemists [AOAC], 2016a); crude protein was quantified using the Kjeldahl method as per (AOAC, 2016b); crude fat was extracted via the Soxhlet method in accordance with (AOAC, 2016c); ash content was determined following (AOAC, 2016d); and crude fiber was analyzed based on (AOAC, 2000). The carbohydrate content was calculated using the phenol-sulfuric acid method (DuBois et al., 1956).

Extraction process: For the extraction of phytochemicals, one gram of the powdered seaweed was extracted with 10 mL of either distilled water or 70% ethanol (Merck, India). The mixture was stirred at 720 rpm for a duration of 72 hours at room temperature. Following the extraction, centrifugation was performed at 2090 rpm for 10 minutes (REMI Instruments Ltd., Mumbai, India), and the resulting supernatant was filtered using Whatman No. 1 filter paper. The filtered extract was then evaporated and stored at 4°C for subsequent analyses (Cho et al., 2007).

Phytochemical analysis: Qualitative screening for major secondary metabolites, which included phenols, flavonoids, tannins, saponins, alkaloids, and terpenoids, was conducted utilizing standard chemical tests as previously described in the literature (Harborne, 1973; Trease & Evans, 1989; Rafiquzzaman et al., 2016; Kancherla et al., 2019).

Determination of total phenolics and flavonoids: The total phenolic content was quantified using the Folin-Ciocalteu reagent (Slinkard & Singleton, 1977), while the flavonoid content was determined employing the $AlCl_3$ colorimetric method (Kumar et al., 2020). The results were subsequently expressed as milligrams of gallic acid equivalents (GAE) and quercetin equivalents (QE) per gram of dry extract, respectively.

DPPH radical scavenging activity: The antioxidant capacity of extracts was evaluated using the 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging activity method, as outlined by Blois (1958). In this analysis, 2 mL of seaweed extract was combined with 2 mL of 0.16 mM methanolic DPPH solution and agitated for 1 minute. The resulting mixture was then left to incubate for 30 minutes in a dark environment at room temperature. Subsequently, the absorbance of the samples was measured spectrophotometrically at 517 nm. The DPPH radical scavenging activity was determined using a specific formula.

DPPH radical scavenging activity (%) =

$$\left(\frac{Abs\ control - Abs\ sample}{Abs\ control}\right) * 100$$

Abs control – blank

Abs sample- ethanol extract, aqueous extract

Cell viability on L6 cells by MTT assay: Cytotoxicity of the extracts on L6 rat skeletal myoblast cells was assessed via the MTT assay (Mosmann, 1983). The cells were seeded in 96-well plates at 2500 cells per well and treated with varying concentrations of the extract (ranging from 6.25 to 100 µg/mL). They were then allowed to acclimate to the culture conditions, which included a 37°C temperature and a 5% CO2 environment in an incubator for 24 h. Test samples were prepared in Dulbecco's Modified Eagle Medium (DMEM) media (10 mg/mL) and were filter sterilized using a 0.2 µm Millipore syringe filter. The samples were then diluted in DMEM media and added to the wells containing cultured cells at the final

concentrations. Untreated wells were kept as controls. All experiments were carried out in triplicate, and average values were taken to minimize errors. After treatment with the test samples, the plates were further incubated for 24 h. Following the incubation period, the media from the wells were aspirated and discarded. Next, 100 µL of 0.5 mg/mL MTT solution in PBS was added to the wells, and the plates were incubated further for 2 h to develop formazan crystals. The supernatant was removed, and 100 µL DMSO (100%) was added per well. The absorbance at 570 nm was measured using a microplate reader- three wells per plate without cells served as blanks. The MTT cytotoxicity was expressed as the concentration of drug inhibiting cell growth by 50% (IC50), calculated by comparing with the control (cells treated with 0.1% DMSO). Cell viability was determined using the following formula:

$$\text{Percentage of cell viability} = \frac{\text{Average absorbance of treated}}{\text{Average absorbance of control}}$$

Anti-inflammatory activity: The anti-inflammatory activity of the extracts was evaluated using the inhibition of albumin denaturation technique as described by Mizushima and Kobayashi (1968), and Sakat et al. (2010) with minor modifications. The reaction mixture consisted of the powdered sample with various concentrations of 1% aqueous solution of bovine albumin fraction, while the pH of the reaction mixture was adjusted using a small amount of 1N HCl. The prepared samples were incubated at 37ºC for 20 minutes and then heated to 51ºC for 20 minutes. After cooling the samples, the turbidity was measured at 660 nm using a UV-Visible Spectrophotometer. The percentage inhibition of protein denaturation was calculated using the following formula.

$$\text{Percentage Inhibition (\%)} = [(\text{Abs of Control} - \text{Abs of sample})/\text{Abs of control}] \times 100$$

Antibacterial activity: The *in vitro* antibacterial activity of the seaweed extracts was assessed against some gram-positive and gram-negative pathogenic bacteria such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis*, and *Bacillus cereus*. The ability of seaweed extracts to inhibit the growth of bacteria was estimated by the disk diffusion method given by Bauer et al. (1966). The sterile cotton swab was dipped into the inoculum tube, and excess fluid was removed by rotating the swab against the side of the tube (above the inoculum level) using firm pressure. The dried surface of the Muller Hinton Agar plates (5 to 6 mm depth) was seeded with 0.1 mL of individual test organisms by streaking the swab three times over the entire surface of the agar plate. In order to ensure the uniform distribution of inoculums, the plate was rotated approximately at an angle of 60 degrees each time. The plate was rimmed with a cotton swab to pick up the excess fluid, and the inoculum was allowed to dry for 3 to 5 min. Then, the sterile antimicrobial disc (Hi-media) was impregnated with 1000 µL of sample and placed on the surface of the inoculated agar plate with the help of sterile forceps. 30 µg ampicillin disc was used as the positive control. The plates were incubated at 37°C for 24 h, and the diameter of growth inhibition was measured in millimeters.

Anti-cancer activity: The cytotoxic effects of the extracts against MDA-MB-231 breast cancer cells were evaluated utilizing the MTT assay (Mosmann, 1983; Alley et al., 1986). The cells were treated with varying concentrations of the extract (6.25 to 100 µg/mL), and absorbance was recorded at 570 nm.

Anti-diabetic activity (α-Glucosidase inhibition assay): In order to evaluate the á-glucosidase enzyme inhibition activity, different concentrations of the seaweed extracts (10-150 µg/mL) were combined with 100 µL of 0.1 M phosphate buffer (pH 6.9) and 100 µL of α-glucosidase solution (1 Unit/mL/min) and then incubated at 25°C for 5 minutes (Worthington, 1993). Subsequently, 100 µL of p-nitrophenyl-α-D-glucopyranoside (5 mM) was added, and the reaction mixture was further incubated at 25°C for 10 minutes. Following the incubation, the absorbance readings were taken at 405 nm and compared to a control sample containing 100 µL buffer instead of the sample extract. The results were presented as the percentage of enzyme inhibition.

Anti-obesity activity (Pancreatic lipase inhibition assay): The anti-obesity activity of *Sargassum wightii* extracts was evaluated by pancreatic lipase inhibition using *p*-nitrophenyl butyrate (p-NPB) as the substrate. Porcine pancreatic lipase (1 mg/mL) was prepared in 50 mM Tris–HCl buffer (pH 8.0) and pre-incubated at 37°C for 10 min. Aqueous and ethanolic extracts (6.25–100 µg/mL) and orlistat (100 µg/mL) were used. The enzyme solution (100 µL) was mixed with extract (100 µL) and pre-incubated at 37°C for 15 min. The reaction was initiated by adding 100 µL of p-NPB (0.1 mM) and incubated at 37°C for 30 min. Absorbance was recorded at 405 nm. A control without extract was included.

The results were presented as the percentage of enzyme inhibition (Kim et al., 2010b).

Fourier Transform Infrared Spectroscopy (FTIR): An FTIR spectrophotometer (ATR-FTIR, Model P-4600, Thermo Scientific, USA) was used to conduct Fourier Transform Infrared Spectroscopy (FTIR) to examine the functional characteristics. The extracts were mixed with KBr powder (Potassium Bromide) in a 1:9 ratio and ground thoroughly. The resulting powder was then pressed using a hydraulic press at a pressure of around 100 kg/m^3 to create a pellet removed from the collar after disassembling the die. The pellet was placed on the sample holder to record the spectrum, and the signal was collected over 32 scans at a resolution of 4 cm^{-1} within the 600-4000 cm^{-1} range (Falcão & Araújo, 2013; Ma et al., 2016).

Statistical analysis: All analyses were conducted in triplicate, and the results were expressed as mean±standard deviation. The recorded data were processed and analyzed using the Statistical Package for Social Science software (IBM-SPSS 22.0 for Windows, SPSS Inc., Chicago, USA). A single factor one-way analysis of variance (ANOVA) was performed at a probability level of $p<0.05$ to analyze the data.

RESULTS

Proximate composition of *S. wightii* powder: The moisture content of fresh *Sargassum wightii* was found to be 65.78±0.14%. The proximate composition of seaweed powder, which is measured in terms of its moisture, protein, fat, carbohydrate, fibre, total ash content, and energy, was found to be 8.69±0.12%, 8.73±0.17%, 0.65±0.07%, 63.2±0.04%, 38.44±0.08%, 18.73±0.14% and 3081±12.02% (on a dry basis) respectively.

Extraction yield: The yield of aqueous and ethanol solvent extracts of *S. wightii* was reported as (9.26±0.05%) and (6.46±0.04%), respectively (Table 1).

Total phenolic content and Total flavonoid content: The total phenolic content of the extracts was estimated using the Folin Ciocalteu reagent method. It was found that the aqueous and ethanolic extracts had 12.60±0.04 and 25.30±0.01 mg GAE/g extract, respectively (Table 1). The Total flavonoid content showed a significant difference among aqueous extract (10.25±0.02 mg QE g-1) compared to ethanol extract (16.5±0.02 mg QE g-1) as illustrated in Table 1.

DPPH activity: In the present study, the DPPH assays were represented as IC_{50} value, and the ethanolic extract exhibited rich scavenging effects on DPPH (5.32±0.04 mg/mL) when compared to aqueous extract with an IC_{50} value of (9.54±0.03 mg/mL) respectively (Table 1).

Preliminary phytochemical screening: The present study used ethanol and aqueous extracts for phytochemical screening, confirming the presence of active chemical components such as terpenoids, saponin, tannin, alkaloids, phenolics, and flavonoids (Table 2). Out of ten tests, seven were positive, and three were negative.

***In vitro* cytotoxic activity of S. wightii against normal cells (L6):** The MTT cytotoxicity assay is a quick and indirect calorimetric way of determining the viability of L6 (Rat skeletal myoblast cell line) cells when exposed to varying concentrations of *S. wightii* extract. The results from the study showed that after 24 h of incubation, concentration-based reduction of cell viability was evident in the cells, as presented in Fig. 1 (a, b, c, and d). To evaluate the potential cytotoxic

Table 1. Antioxidant properties of the *S. wightii* solvent extracts

Solvent extraction	Extraction yield (%)	Total phenolic content (mg gallic acid equivalent/g of extract)	Total flavonoids content (mg quercetin equivalent/g of extract)	DPPH (IC_{50} mg/mL)
Aqueous extract	9.26±0.05%	12.60±0.04	8.25±0.02	9.54±0.03
Ethanol extract	6.46±0.04%	25.30±0.01	16.5±0.02	5.32±0.04

Table 2. Preliminary phytochemical screening of *S. wightii* extracts

Name of the extracts	Terpenoids	Saponin	Tannin	Alkaloids	Phenols	Flavonoids
Aqueous	-	-	+	+	+	+
Ethanol	+	-	+	+	+	+

-indicates absence of the components, + indicates the presence of the components.

impact of *S. wightii* extracts on L6 cells, extracts were prepared at concentrations ranging from 6.25 µg/mL to 100 µg/mL. Results showed that cells treated with *S. wightii* extract at a 100 µg/mL concentration had a survival rate of 93.79%. Similarly, at the lowest concentration of 6.25 µg/mL, cell viability was 98.02% (as illustrated in Table 3).

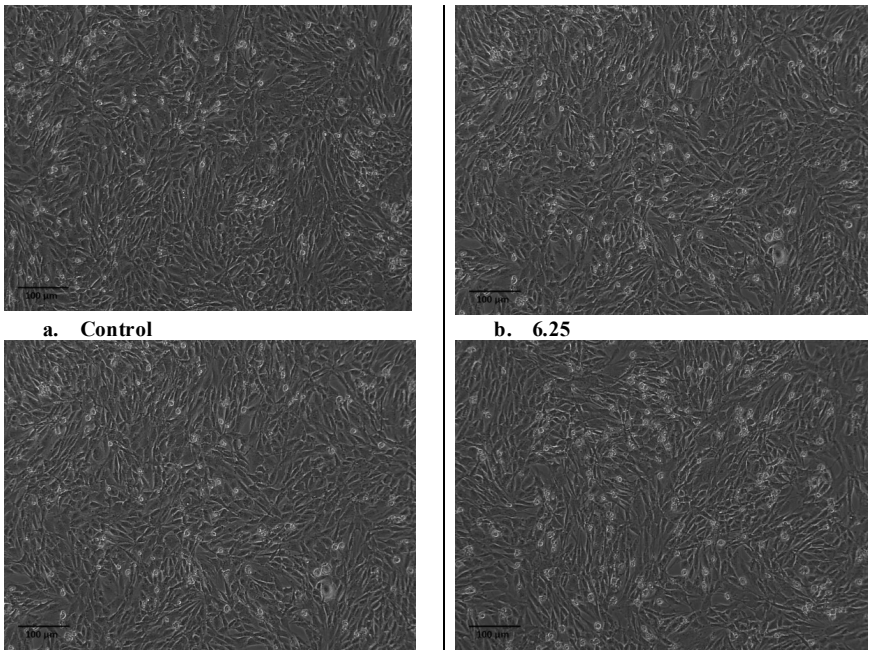


Fig. 1. *In vitro* cytotoxic activity of *Sargassum wightii* at different concentrations against L6 cells

***In vitro* anti-inflammatory activity of *S. wightii* extracts:** The anti-inflammatory activity of *S. wightii* extracts were evaluated in the present study using the egg albumin denaturation method. In the present study, both the ethanolic and aqueous extracts inhibited the denaturation of albumin, a type of protein, when exposed to heat. At a 150 µg/mL concentration, the ethanolic extract showed 98.8±1.23% inhibition while the aqueous extract showed 75.6±1.01% inhibition in comparison with diclofenac sodium produced 92.26±1.41% inhibition (Fig. 2). In the present study, the inhibitory activity of the ethanol extract is higher compared to the aqueous extract.

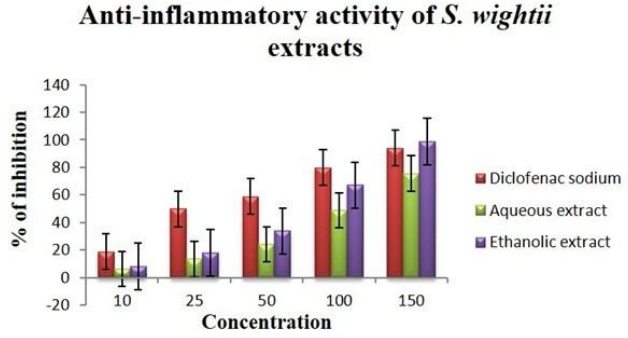
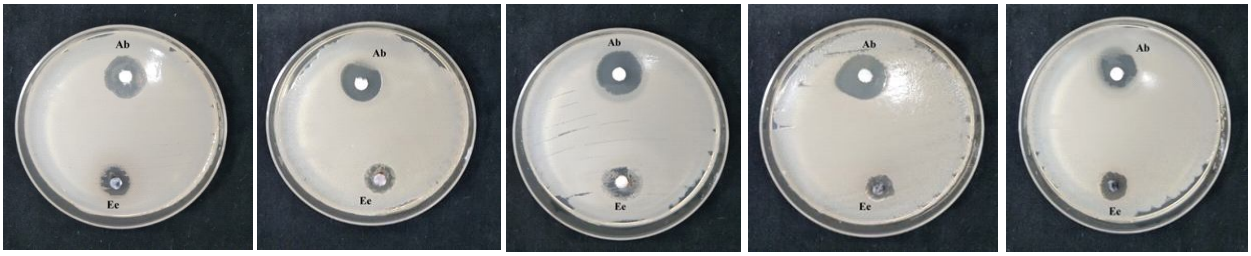


Fig. 2 . *In vitro* anti- inflammatory activity of *S. wightii* extracts

Table 3. % Cell Viability of L6 Cells of *S. wightii* extracts at different concentrations

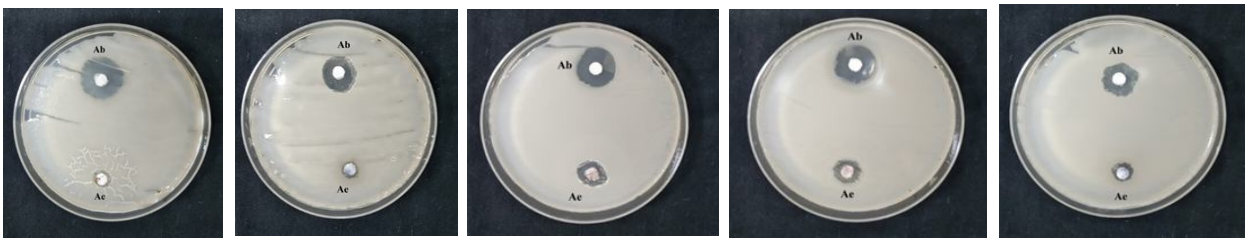
Concentration	% of cell viability
6.25	98.02
12.5	95.61
25	95.53
50	94.09
100	93.79

***In vitro* anti-bacterial activity of *S. wightii* extracts:** The extracts demonstrated significant anti-bacterial activity against the tested bacteria, including *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Bacillus cereus, and Pseudomonas aeruginosa.* The zone



a. E. coli b. *Staphylococcus aureus* c. *Bacillus subtilis* d. *Bacillus cereus* *c. Pseudomonas aeruginosa*

Fig. 3. *In vitro* anti-bacterial activity of ethanol extracts of *S. wightii*



a. E. coli b. *Staphylococcus aureus* c. *Bacillus subtilis* d. *Bacillus cereus* *c. Pseudomonas aeruginosa*

Fig. 4. *In vitro* anti-bacterial activity of aqueous extracts of *S. wightii*

Table 4. Zone of inhibition of Ethanolic extract of *S. wightii* against different bacterial strains

Zone of inhibition (in mm)		
Bacteria	**Ampicillin**	**Ethanol extract**
E. coli	12	8
S. aureus	15	10
B. subtilis	17	11
B. cereus	17	9
P. aeruginosa	13	9

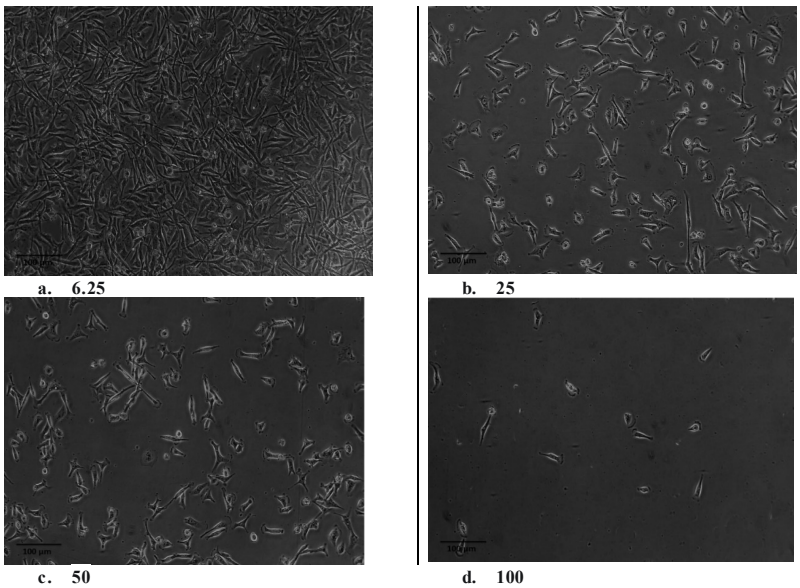
Table 5. Zone of inhibition of Aqueous extract of *S. wightii* against different bacterial strains

Zone of inhibition (in mm)		
Bacteria	**Ampicillin**	**Aqueous extract**
E. coli	11	6
S. aureus	12	5
B. subtilis	12	8
B. cereus	13	8
P. aeruginosa	17	7

of inhibition was calculated for both aqueous and ethanolic extracts, with the ethanol extract exhibiting a more significant inhibition zone than the aqueous extract (Fig. 3 & 4). The ethanolic extract was particularly effective against *B. subtilis, B. cereus, P. aeruginosa, and S. aureus, E. coli*, with inhibition zones of 11 mm, 9 mm, 9 mm, 10 mm and 8 mm, respectively. The aqueous extract was effective against *P. aeruginosa* (7 mm) *B. subtilis* (8 mm) *B. cereus* (8mm), and least effective against *S. aureus* (5mm) and *E. coli* (6 mm) (Table 4 &5).

Table 6. % Viability of *S. wightii* extracts against MDA-MB-231 cells

	% of cell viability	
Concentration	**Aqueous extract**	**Ethanol extract**
6.25	94.16	95.29
12.5	87.88	90.43
25	83.66	43.96
50	73.31	41.41
100	66.29	10.06



a. 6.25 b. 25 c. 50 d. 100

Fig. 5. Cell viability of ethanolic extract of *S. wightii* at different concentrations against MDA 2B cancer cells

***In vitro* anti-cancer activity of *S. wightii* extracts:** Different sample concentrations were administered to cancer cells, resulting in a dose-dependent reduction in cell viability. The highest level of cytotoxicity was observed when the sample was administered at 100 µg/mL, as shown in Table 6 and Fig. 5 (a, b, c, and d). The study found that the ethanol extract had an IC_{50} value of 20.21 µg/mL. These results indicate that the ethanol extract may have a cytotoxic effect on the MDA-MB-231 cancer cell line.

***In vitro* anti-diabetic activity of *S. wightii* extracts:** The anti-diabetic

activity of *S. wightii* extracts were evaluated in the present study using the α-glucosidase inhibitory assay. The anti-diabetic activity of *S. wightii* was tested at different concentrations. The concentration-dependent reduction of glucosidase enzyme activity was observed. The ethanolic and aqueous extracts inhibited the glucosidase enzyme in the present study. At a 150 µg/mL concentration, the ethanolic extract showed 67.5±1.01% inhibition, while the aqueous extract showed 63.8±1.23% inhibition (Fig. 6). In the present study, ethanol extract exhibits higher inhibitory activity than the aqueous extract.

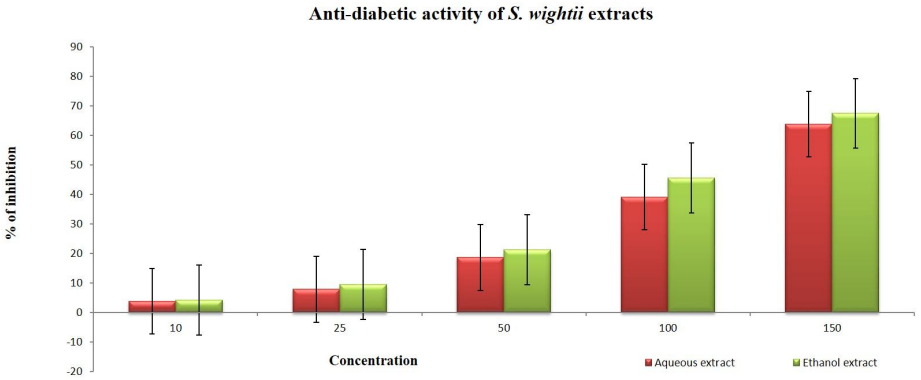


Fig. 6. *In vitro* anti-diabetic activity of *S. wightii* extracts

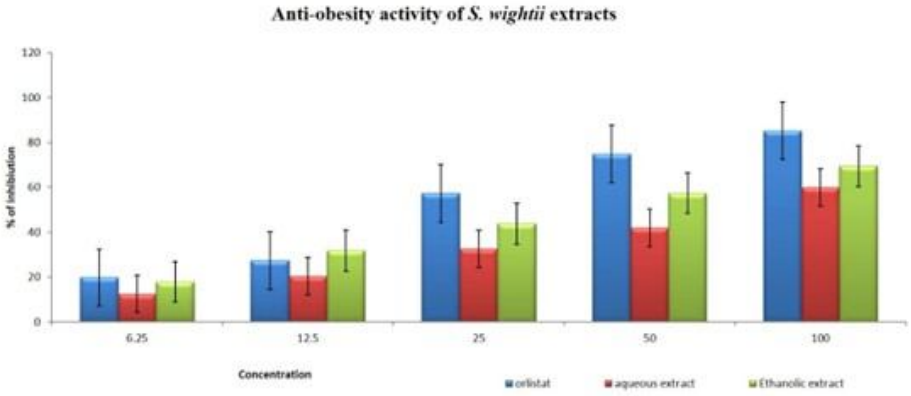


Fig. 7. *In vitro* anti-obesity activity of *S. wightii* extracts

***In vitro* anti-obesity activity of *S. wightii* extracts:** The present study evaluated the potential of *S. wightii* extracts in inhibiting pancreatic lipase. The study examined the anti-lipase activity of different concentrations (6.25, 12.5, 25, 50, 100 µg/mL) of *S. wightii* for PPL inhibition. The results showed that PPL activity was significantly inhibited by *S. wightii's* ethanolic extract (69.39%) and aqueous extract (60.45%) at a concentration of 100 µg/mL. This inhibition was compared to that of the well-known anti-lipase agent orlistat (100 µg/mL), which significantly inhibited PPL activity (85.24%) as shown in Fig. 7. The study also found that the ethanolic extract exhibited greater inhibitory activity when compared to the aqueous extract.

FTIR analysis: This study examined the functional groups of seaweed extracts obtained via different solvents (Aqueous and ethanol). Fig. 8 (a, b) analysed and elucidated the corresponding adsorption bands. In ethanol extract, the results showed strong transmission peaks above 3500 cm^{-1}, which are attributed to the presence of alcohols and phenols. Additionally, strong peaks in the range of 3400 cm^{-1} were observed, which are likely due to the presence of N-H and O-H stretching, indicating the presence of polysaccharides and amino acids native to seaweeds. A weak and moderate transmission peak was also observed in the range of 2064.90-2101.48 cm^{-1}, indicating the presence of alkenes and its derivatives. Furthermore, a strong transmission peak was observed in the carbonyl region of spectra above 1600 cm^{-1}, indicating C=O, N=O stretching. This suggests the presence of ester and amide groups. Peaks from 1400-1500 cm^{-1} and 1500-1600 cm^{-1} indicate several modes of C=C, C-O, O-H stretching, which may be due to the presence of lignin and cutin. Weak peaks above 1300 cm^{-1} were also found, reflecting the presence of alkanes. Peaks in the range of 1100–1000 cm^{-1} indicate several modes, such as C–H deformation or C–O or C–C stretching, on carbohydrates and polysaccharides. Finally, weak transmission peaks observed above 800 cm^{-1} in the aqueous extract correspond to C-H stretching, possibly due to the presence of glucose and galactose.

DISCUSSION

The compositional profile of *S. wightii* highlights its prospective utility as a dietary supplement, characterized by significant carbohydrate and fiber

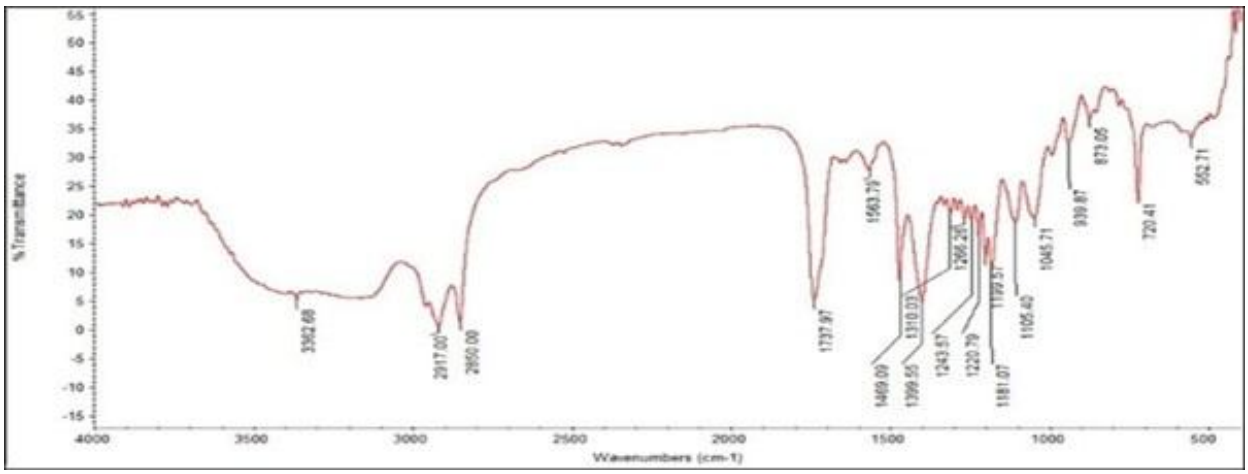


Fig. 8a. FTIR analysis of *S. wightii* extracts (FTIR analysis of aqueous extracts)

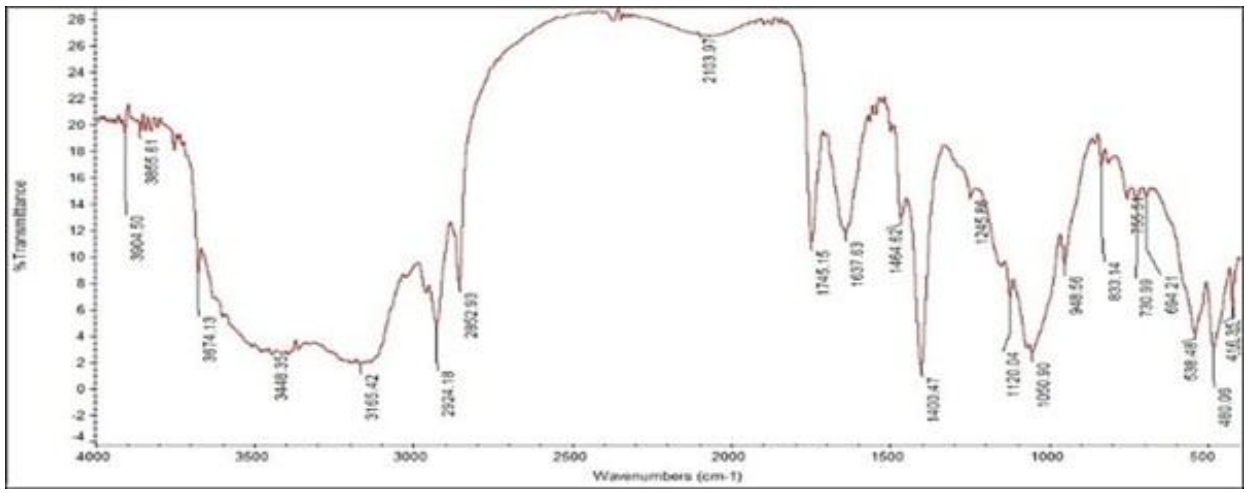


Fig. 8b. FTIR analysis of *S. wightii* extracts (FTIR analysis of ethanol extracts)

content that contributes to digestive health and metabolic regulation (Chawla & Patil, 2010: Al Hammadi, 2017). The protein and mineral concentrations were aligned with previously documented values for brown seaweeds (Dewinta et al., 2020; Premarathna et al., 2022). Extract yield and bioactivity exhibited variations attributed to solvent polarity; while water extraction resulted in higher yields, ethanol demonstrated enhanced efficacy in concentrating polyphenols and flavonoids (Lapornik et al., 2005; Do et al., 2014). Phytochemical screening validated the presence of key secondary metabolites that contribute to the observed biological effects. Results from the DPPH assay corroborate existing literature indicating that phenolic-rich ethanolic extracts from brown algae furnish substantial radical scavenging activity (Matanjun et al., 2008; Syad et al., 2013). Furthermore, the established correlation between phenolic content and antioxidant capacity was well documented (Liu & Ng, 2000; Ye et al., 2009). The low cytotoxicity of *S. wightii* extracts against L6 cells accentuates their safety profile, advocating their incorporation into dietary interventions. Anti-inflammatory effects were observed to be comparable or superior to those of standard drugs such as diclofenac sodium, likely due to the phenolic compounds' capacity to inhibit protein denaturation (Leelaprakash & Dass, 2011; Ali et al., 2019). Notably, the antibacterial activity was most pronounced against Gram-positive strains, attributable to polyphenolic-induced membrane disruption and enzyme inhibition (Sandsdalen et al., 2003; Magesh et al., 2020). The anticancer potential was demonstrated through substantial cytotoxicity against MDA-MB-231 cells, which may involve flavonoid-mediated apoptotic pathways (Cook & Samman, 1996; Luzndberg & Weinberg, 1999; Kim et al., 2010a). The anti-diabetic effect can be attributed to glucosidase inhibition, thereby delaying carbohydrate digestion and glucose absorption;

polyphenol-rich seaweed extracts exhibit comparable activity to synthetic inhibitors (Hamdan & Afifi, 2004; Ravi et al., 2016; Lakshmanasenthil et al., 2018). Similarly, anti-obesity activity via lipase inhibition further supports the therapeutic application of *S. wightii* in addressing metabolic disorders (Armand, 2007; Yun, 2010; Marrelli et al., 2014). FTIR spectra confirmed the presence of phenolic OH, carbonyl, and aromatic groups, substantiating the bioactive potential (Vázquez et al., 2008; Falcão & Araújo, 2013; Ma et al., 2016). Collectively, these findings position ethanolic *S. wightii* extracts as multifunctional ingredients within the nutraceutical sector.

The ethanolic extract of *Sargassum wightii* demonstrates remarkable biological activities across a range of assays, including antioxidant, anti-inflammatory, anticancer, anti-diabetic, anti-obesity, and antibacterial properties. The efficacy of this extract can be attributed to its high concentration of phenolic and flavonoid compounds, which play a crucial role in mediating these effects. Notably, the extract exhibits minimal cytotoxicity while showcasing multi-target efficacy, positioning it as a promising candidate for the development of functional foods and nutraceuticals. However, to fully validate its therapeutic applications, further in vivo studies and formulation investigations are necessary.

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