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Biomedical Potential and Preliminary Phytochemistry of the Brown Seaweed *Sargassum wightii* Greville ex J. Agardh 1848

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Abstract

The brown seaweed *Sargassum wightii* Greville ex J. Agardh 1848 was collected from Pamban (south east coast of Tamilnadu, India; Latitude 9°18'N and Longitude 79°12' E) and extracted with different solvents such as acetone, ethanol, benzene and chloroform in a soxhlet apparatus. The antibacterial activity of the extracts were tested against natural pathogens isolated from housefly (*Musca domestica* Linnaeus 1758), such as *Staphylococcus aureus, Enterococcus faecalis, Bacillus cereus, Micrococcus luteus, Pseudomonas aeruginosa* and *Escherichia coli* respectively. The extracts were also subjected to alpha amylase inhibitory, anti-inflammatory and antioxidant activities following standard protocols. Ethanol extract exerted high inhibitory effect on all the microbes and was assertive against *B. cereus* (14.2 mm). Potential and significant ($p<0.05$) alpha amylase inhibition was observed in the chloroform extract (81.24±8.063%). The benzene extract had significantly ($p<0.05$) higher antioxidant activity (74.44±3.27%) and the anti-inflammatory activity was comparatively higher in the acetone extract (65.5±1.21%). However, the control drugs exhibited better activity than all the tested extracts. The qualitative phytochemistry showed the presence of flavonoids, pholobatannins, phenolic compounds, aromatic acids and xanthoproteins. The Fourier transform infra-red (FT-IR) spectrum contained eight major peaks which confirmed the presence of amino, keto, fluoro alkane group and aromatic compounds in the extracts which could be responsible for the bioactivity.

Introduction

The wild bacterial pathogens that infect human beings developed resistance to most of the available antibiotics; hence the need for new drugs becomes mandatory (Anbuselvi et al. 2009). Chemical drugs also lead to adverse effects hence researchers started searching pharmacologically active compounds from natural sources and marine algae serve as an important source of natural bioactive compounds (Kelecom 2002). Inflammation is a consequence of infection, hypersensitivity, tissue damage or driven by certain environmental factors (Jongyoon et al. 2009).

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Commonly used steroidal and non-steroidal anti-inflammatory drugs cause severe side effects, hence the search for novel antimicrobial and anti-inflammatory agents are essential (Ravi et al. 2012). Vairappan et al. (2001) and Xu et al. (2003) reported the antimicrobial and anti-inflammatory activities of bioactive compounds from marine organisms including seaweeds. The harmful effect of reactive oxygen species on human health is well known and the antioxidant research has become more and more popular all over the world (Olga et al. 2004) with bioactive compounds from plants received much attention (Shaha et al. 2008).

Marine organisms contain structurally novel and biologically active secondary metabolites which may be of interest in the drug development process (Bibiana et al. 2012). Seaweeds are sources of pharmacologically active compounds with potential health effects (Meenakshi et al. 2009) and are renewable resource in the marine environment (Kirithika et al. 2011). The brown seaweeds are an excellent source of soluble fibre, iodine,vitamins as well as minerals (Dhargalkar and Pereira 2005). *Sargassum wightii* (*Sargassaceae*), commonly known as brown seaweed is distributed widely across the coast in waters of India, and harbours sulfated polysaccharides responsible for diversified pharmacological properties (Kumar et al. 2012). Hence the present study deals with the preliminary phytochemistry and screening of *S. wightii* extracts for its *in vitro* antibacterial, alpha amylase, antioxidant and anti-inflammatory actions.

Materials and Methods

Collection and preparation of extracts of S. wightii

Fresh samples of *S. wightii* were manually collected from Pamban (south-east coast of Tamilnadu, India; latitude 9^{o}18'N and longitude 79^{o}12'E) and identified by the experts at Central Marine Fisheries Research Institute (CMFRI), Mandapam. The samples were initially washed with sea water, followed by fresh water to remove the adhering macroscopic epiphytes, sand and salt. They were then packed in sealed plastic bags and transported to the laboratory, where they were shade dried for 10 days and then powdered into fine particles using an electric mixer. Fifty grams powdered material were extracted with 200 mL acetone, ethanol, benzene and chloroform separately adopting the procedure of Vijayabaskar and Shiyamala (2011). The obtained crude extracts (5.34, 5.97, 5.62 and 5.18 g for acetone, ethanol, benzene and chloroform, respectively) were stored in a refrigerator at 4 ^{o}C and used for further investigation.

Collection of house fly

Houseflies (*Musca domestica* Linnaeus 1758) were collected from a private hospital, located at Iyyarbangalaw, Madurai, Tamil Nadu and carefully transported to the laboratory in live condition. The house flies were anesthetised by exposing them to 0 ^{o}C for 15 min, then washed twice with distilled water and soaked in 0.85% saline solution.

Isolation and identification of bacteria

The soaked houseflies were taken out, crushed and the extract was serially diluted (up to 10^{-9}dilution). The sample from each dilution was inoculated on to nutrient agar plates following spread plate technique. All the plates were incubated at 37 ^{o}C for 24 h. The colonies developed were sub-cultured until pure cultures were obtained. The pure cultures were maintained in nutrient agar slants. The isolated bacterial strains were subjected to various biochemical tests and identified based on the key characters prescribed in Bergey's Manual of Systematic Bacteriology (Kreig and Holt 1984; Sneath et al. 1986).

Antibacterial assay

The antibacterial activities of acetone, ethanol, benzene and chloroform extracts of *S. wightii* were tested against the isolated organisms following wellcut method (El-Masry et al. 2000). Broth bacterial cultures of 24 h were spread on Muller Hinton Agar plates using sterile'L' rod. The plant extracts were dissolved in their respective solvents (1 $mgmL^{-1}$) and prepared into various concentrations (10, 20, 30, 40, 50 $\mu g \cdot \mu L^{-1}$) and tested for their antibacterial activity. The respective solvents and the antibiotic gentamycin served as negative and positive controls respectively. The plates were then incubated at 37 ^{o}C for 24 h and the inhibition zone (if any) was measured (using graph sheet) in mm. Adequate replications were made for each category.

Alpha amylase inhibition assay

The alpha amylase inhibitory activity of *S. wightii* extract was determined based on the colorimetric assay (Ravi et al. 2012). Soluble potato starch (0.25 g) was boiled in 50 mL distilled water for 15 min to obtain the starch solution. Enzyme solution was prepared by mixing 0.001 g alpha amylase in 100 mL of 20 mM phosphate buffer (pH 6.9) containing 6.7 mM NaCl. Various concentrations (10, 20, 30, 40, 50 $\mu g \cdot mL^{-1}$) of extracts were dissolved in 1 mL dimethylsulfaoxide (DMSO) to give concentrations ranging from 10-50 $mg \cdot mL^{-1}$. A solution containing 96 mM 3,5-dinitosalicyclic acid (20 mL), 5.31 M sodium potassium tartrate in 2 M sodium hydroxide (8 mL) and distilled water (12 mL) served as the colour reagent.

1 mL extract of and 1 mL enzyme solution were mixed in a test tube and incubated at 25 ^{o}C for 30 min. After that, 1 mL starch solution was added and incubated at 25 ^{o}C for 30 min. Then, 1 mL colour reagent was added and placed in a water bath at 85 ^{0}C. After 15 min, the reaction mixture was removed from the water bath, cooled and diluted with 9 mL distilled water. The absorbance value was determined at 540 nm using double beam UV-visible double beam spectrophotometer (Systronics 2203). In order to correct background absorbance, individual blanks were prepared by adding colour reagent alone without the addition of starch. DMSO solution served as the control and acarbose was used as the reference drug.

Three replications were maintained for each category. The alpha amylase inhibition was calculated using the following formula:

Alpha amylase inhibition (%) = $100 \times (\Delta A_{control} - \Delta A_{sample} / \Delta A_{control})$

$\Delta A_{control} = A_{test} - A_{blank}$

$\Delta A_{sample} = A_{test} - A_{blank}$

Anti-inflammatory activity

The anti-inflammatory activity has been performed based on human red blood corpuscles (HRBC) membrane stabilisation method as per Nirmaladevi and Periyanayagam (2010). Blood sample was drawn from healthy human volunteers and immediately mixed with equal volume of sterilised Alsever solution (2 g dextrose, 0.8 g sodium citrate, 0.05 g citric acid and 0.42 g sodium chloride in 100 mL distilled water). The sample was centrifuged at 3,000 rpm for 5 min and the obtained packed cells were washed with isosaline (0.85% NaCl) solution. A 10% (v/v) HRBC suspension was made with isosaline. Two types of media such as hypo saline and distilled water were used in the present experiment. The assay mixture contained 0.5 mL HRBC suspension, 1 mL phosphate buffer (0.15 M, pH7.4), 2 mL hypo saline (0.36%NaCl) solution (2 mL distilled water for distilled water medium) and various concentrations of the extracts (0.2, 0.4, 0.6, 0.8 and 1.0 mL), whereas, hydrocortisone served as the reference drug. The assay mixtures were incubated at 37 ^{o}C for 30 min, centrifuged and the haemoglobin content in the supernatant was estimated using colorimeter (ELICO-CL, 223) at 560 nm. The HRBC membrane stabilisation or protection percentage was calculated using the following formula. Three replications were maintained for each category.

$$\%\text{Protection} = 100 - \frac{\text{OD of drug treated sample}}{\text{OD of control}} \times 100$$

Anti-oxidant activity

DPPH assay

The free radical scavenging activity of the extracts of *S. wightii* was performed based on the method proposed by Hatano et al. (1988) using 2,2-Diphenyl-1-picrylhydrazyl (DPPH). Extracts ranging from 20-100 $\mu g \cdot mL^{-1}$ were added to 0.5 mL 0.25 mM DPPH solution (prepared using 95% ethanol), shaken and kept undisturbed at room temperature for 30 min. To that, 2 mL double distilled water were added and the absorbance measured at 517 nm (Systronics, UV-Vis Double beam spectrophotometer, 2203). Ascorbic acid was used as the antioxidant standard. The inhibition percentage was calculated using the following formula:

$$\text{Scavenging percentage} = [A_0 - A_1/A_0] \text{ X } 100$$

Whereas, A_0 is the absorbance of control and A_1 is the absorbance of sample – turbidity factor.

Reducing power assay

The reducing power was determined according to the method described by Oliveira et al. (2007) with some modifications.Various concentrations of extracts (20-100 $\mu g \cdot mL^{-1}$) were added with 1.0 mL of deionised water, 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%) and incubated at 50 ^{o}C for 20 min. 2.5 mL trichloroacetic acid (10%) was added to the mixture to stop the reaction. Then the reaction mixture was centrifuged at 3,000 rpm for 10 min. The aqueous layer (2.5 mL) was mixed with distilled water (2.5 mL) and 0.5 mL of freshly prepared ferric chloride solution (0.1%). The absorbance of solution was measured at 700 nm using UV-visible double beam spectrophotometer (Systronics 2203). The blank was prepared by excluding the addition of extract. Ascorbic acid at different concentrations (20-100 $\mu g \cdot mL^{-1}$) were used as the standard. The percentage of antioxidant activity was determined (Hendra et al. 2011) by the following formula:

$$\text{Antioxidant activity (\%)} = (A_1 A_0 / A_1)$$

A_0= Absorbance of the control (Potassium phosphate buffer +FRAP reagent)

A_1= Absorbance of the sample

Phytochemistry of S. wightii

Qualitative photochemical analysis of *S. wightii was* carried out following standard protocols (Sofowaran et al.1993). For FT-IR study, 10 mg solid sample of *S. wightii* were mixed with 100 mg dried potassium bromide (standard), compressed into a salt disc and read in the spectrophotometer (Bio-Rad; FT-IR-40 model, USA).

Statistical analysis

All the statistical calculations were worked out using SIGMA STAT (3.5 version) with the aid of computer. The experiments were done in triplicates and result values are expressed as mean±standard deviation. All the data were analysed using one-way ANOVA followed by Tukey test at $P<0.050$ significance level.

Results

Isolation of bacteria from house fly and antibacterial activity

Six bacteria were isolated from *M. domestica*; based on the biochemical characteristics, they were identified as *Pseudomonas aeruginosa* and *Escherichia coli* (Gram negative), *Staphylococcus aureus, Enterococcus faecalis, Bacillus cereus* and *Micrococcus luteus* (Gram positive) (Table 1).

All the solvent extracts of *S. wightii* showed promising inhibitory effect on all the tested bacterial pathogens and the results are depicted in Fig. 1. Among the four extracts tested, ethanol extract exhibited higher inhibitory effect on all the pathogens and it was highest against *B. cereus* (14.2 mm).

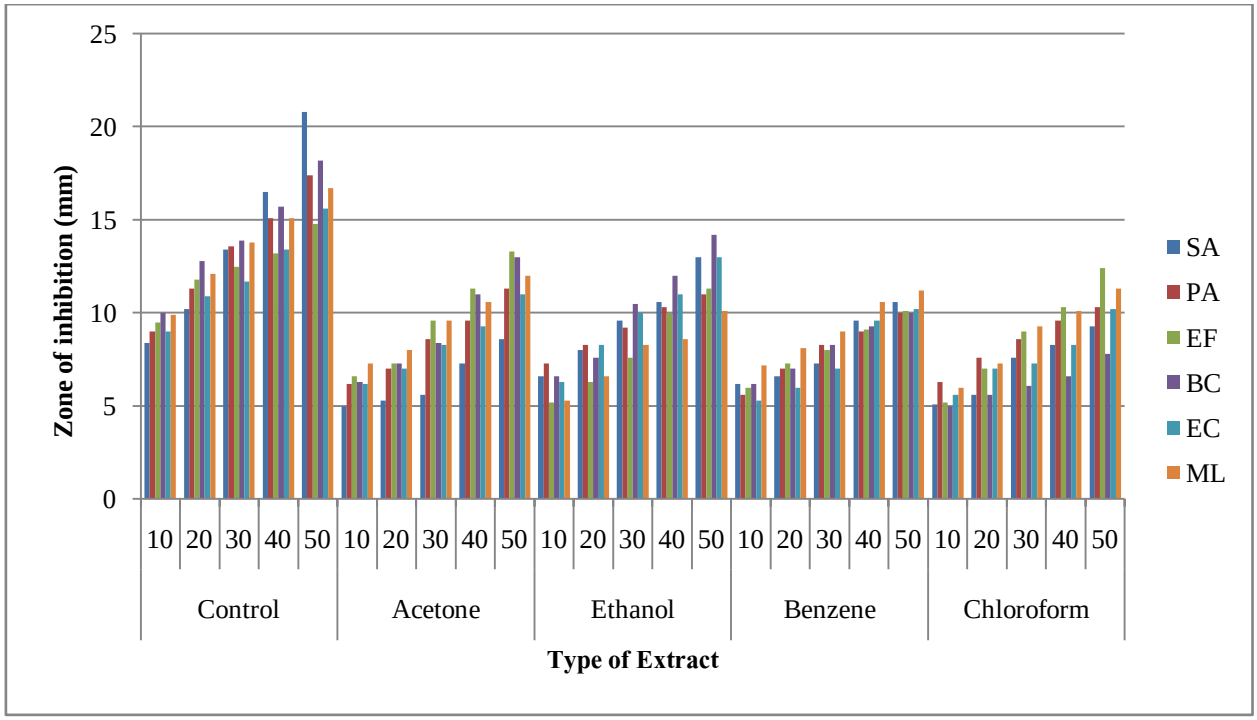

Fig. 1. Zone of inhibition (mm) produced by the different solvent extracts ($\mu g \cdot \mu L^{-1}$) of *Sargassum wightii* against the tested bacterial pathogens.
(SA – *Staphylococcus aureus*; PA – *Pseudomonas aeruginosa*; EF – *Enterococcus faecalis*; BC - *Bacillus cereus*; EC - *Escherichia coli*; ML - *Micrococcus luteus*)

Alpha amylase inhibitory activity

All the solvent extracts of *S. wightii* yielded statistically significant ($p<0.05$) inhibitory action on alpha amylase enzyme, but the control drug acarbose exerted more inhibition than the tested extracts (Fig. 2). High inhibition was observed in the chloroform extract (81.24±8.063%) at 50 µL concentration and low inhibitory activity was observed in the acetone extract (41.82±9.20%).

Table 1. Biochemical characterisation of bacteria isolated from the house fly *Musca domestica*.

Sl. No	Name of test	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6
1	Gram staining	+	-	+	+	-	+
2	Shape	S	R	S	R	R	S
3	Citrate utilization	+	+	+	+	+	+
4	H_2S production	-	-	-	-	-	-
5	Catalase	+	+	-	+	-	+
6	Oxidase	-	+	+	+	-	-
7	Urease	+	+	+	-	+	-
8	Caseinase	-	-	-	-	-	-
9	Litmus	-	-	-	-	-	-
10	Starch	-	-	+	+	-	+
11	Methyl red	-	-	-	-	-	-
12	Voges-Proskaeur	-	-	-	-	-	-
13	Glucose fermentation	+	+	+	+	+	+
14	Lactose	+	+	-	-	-	-
15	Sucrose	+	+	-	+	-	-
16	Indole production	-	+	+	+	+	+
17	NO_2 reduction	+	+	+	+	-	+
Identified Organisms		*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Enterococcus faecalis*	*Bacillus cerus*	*Escherichia coli*	*Micrococcus luteus*

+ = Positive; - = Negative; S = Sphere; R = Rod

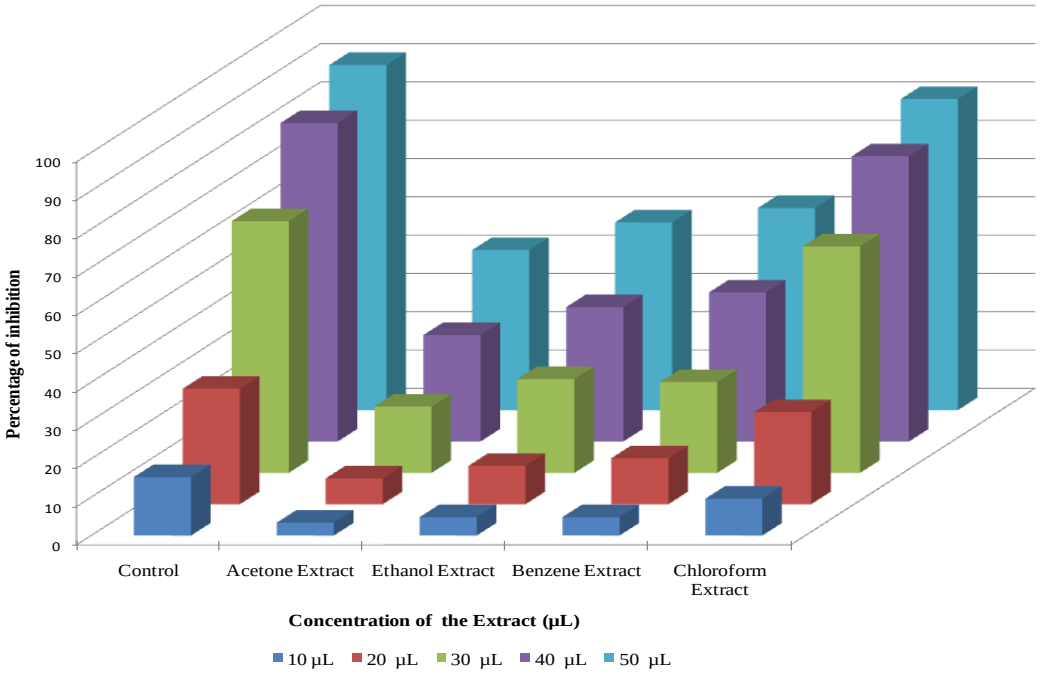

Fig. 2. The alpha amylase inhibitory effect (in %) of the different solvent extracts of *Sargassum wightii*.

Table 2. Anti-inflammatory activity (in percentage) of various solvent extracts of *Sargassum wightii*.
*Data represented as Mean ± SD (n=3); Statistical significant level at $p<0.05$

Medium	Concentration of the extract (mL)	Control (Hydrocortisone)	Acetone Extract	Ethanol Extract	Benzene Extract	Chloroform Extract
Hyposaline	0.2	57.82±1.60	51.16±2.67	31.76±3.36	27.89±2.04	40.37±1.90
	0.4	59.01±7.54	55.66±3.11	36.63±1.68	30.71±1.81	47.12±1.46
	0.6	64.84±1.48	59.95±1.67	38.38±2.42	34.69±0.95	50.84±1.67
	0.8	67.80±2.14	61.62±2.09	44.90±1.71	37.35±1.56	55.47±2.07
	1.0	72.95±2.39	65.5±1.21	50.02±1.18	42.67±2.84	60.89±1.24
Distilled Water	0.2	46.87±1.27	42.03±1.51	25.36±1.51	17.40±1.57	32.91±2.43
	0.4	50.71±1.32	44.08±0.88	27.36±0.88	20.45±1.90	35.48±1.58
	0.6	55.27±2.03	48.64±0.82	29.47±0.82	24.95±1.58	40.90±2.11
	0.8	60.43±2.34	50.51±2.24	32.85±2.24	27.50±1.86	43.54±1.98
	1.0	65.91±1.55	51.32±2.20	38.04±2.20	32.68±2.24	48.15±1.64

Antioxidant activity

In the DPPH assay, the benzene extract of *S. wightii* showed highest DPPH free radical scavenging activity (73.33±4.38%) and was statistically significant (Table 3). The benzene extract exhibited significantly higher reducing power activity (59.62±2.49%) (Table 4).

Table 3. Total DPPH scavenging activity (in%) of various solvent extracts of the *Sargassum wightii*.
*Data represented as Mean ± SD (n=3); Statistical significant level at $p<0.05$

Concentration of the extract mg·mL^{-1}	Control	Acetone	Ethanol	Benzene	Chloroform
20	33.14±1.02	18.66±2.19	29.22±3.06	20.76±2.57	11.11±2.24
40	39.49±0.71	31.86±4.83	35.51±2.13	32.74±5.90	12.09±2.63
60	59.69±2.70	46.2±7.88	54.76±2.49	53.70±6.86	16.77±2.14
80	73.20±2.28	48.29±8.81	61.74±12.55	71.21±5.0	27.87±2.55
100	76.26±3.05	60.59±1.44	65.48±1.74	73.33±4.38	35.47±3.08

Table 4. Reducing power activity (in %) of the various solvent extracts of *Sargassum wightii*.
*Data represented as Mean ± SD (n=3); Statistical significant level at $p<0.05$

Concentration of the extract mg·mL^{-1}	Control	Acetone	Ethanol	Benzene	Chloroform
20	26.76±2.03	6.98±1.54	16.24±1.8	21.76±3.59	12.56±3.72
40	44.31±4.90	12.5±3.35	25.19±3.87	36.12±6.17	15.14±3.48
60	50.59±2.27	24.83±2.98	27.26±2.64	45.24±3.01	19.13±2.02
80	60.94±2.57	34.88±1.75	36.04±3.10	54.78±2.55	23.46±1.61
100	66.84±1.72	43.14±5.56	42.26±1.90	59.62±2.49	35.1±2.10

Phytochemical analysis of S. wightii

Preliminary phytochemical analysis of *S. wightii* revealed the presence of alkaloids, steroids, reducing sugar, tannins, pholobatanins, saponin, flavonoids, cardiac glycosides, phenolic compounds, aminoacids, essential oils, aromatic acids, xanthoprotein and carbohydrate.

The FT-IR spectrum of *S. wightii* yielded eight major peaks (Fig. 3). The peaks at 3,533.2, 3,004.1 and 1,708.2 cm^{-1} correspond to amine group (nitrogen bond), vinyl group (carbon bond) and C=O stretching bond of ketone, respectively. The peaks at 1,420.3 and 1,358.3 cm^{-1} have been attributed to aromatic groups. Peaks at 1,219.9 and 1,091.7 cm^{-1} revealed the presence of fluoroalkene groups, and the peak at 902.5 cm^{-1} was credited to the benzene group of compounds.

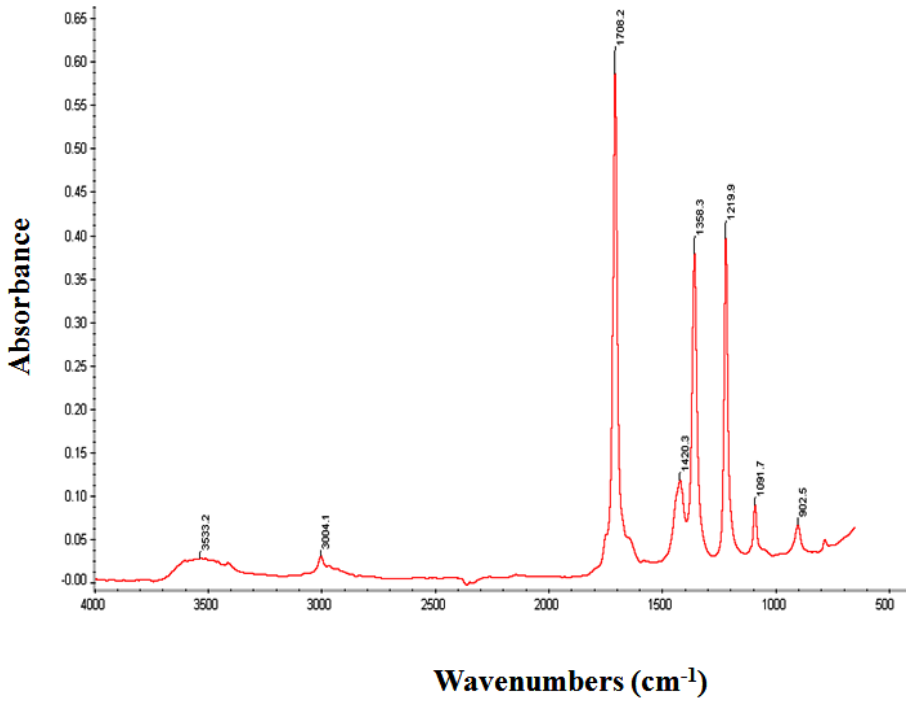

Fig. 3. Peak obtained in the FT-IR analysis of *Sargassum wightii*.

Discussion

Six bacterial pathogens were isolated from *M. domestica* including *S. aureus, E. faecalis, B. cereus, M. luteus, P. aeruginosa* and *E. coli*. Among them, *S. areus, B. cerus* and *E. coli* are associated with food poisonings and diarrhea; *E. faecalis* is responsible for nosocomial infections; *P. aeruginosa* is an opportunistic human pathogen and *M. luteus* is generally non-pathogenic, but causes disease in immune compromised individuals. Banjo et al. (2005) and Barreiro et al. (2013) also isolated *S. areus, P. aeruginosa* and *E. coli* whereas Kassiri et al. (2012) isolated *Staphylococcus* sp., *Pseudomonas* sp., *Bacillus* sp. and *E. coli* from *M. domestica*. The solvent extracts of *S. wightii* inhibited the growth of all pathogens and maximum inhibition was exerted by ethanol extract against *B. cereus* (14.2 mm). The antibacterial activity of the seaweeds may be influenced by the habitat, season, growth stage, time of collection and the solvents used for extraction. Tuney et al. (2006), Kolanjinathan et al. (2009) and Devi et al. (2012) also reported superior activity of ethanol extracts against the tested pathogens. The inhibition of alpha amylase has been considered as treatment strategy for diabetes, obesity and periodontal diseases because of the significant role played by that enzyme in starch and glycogen digestion (Ushasri et al. 2015).

Inhibition of alpha amylase along with alpha glucosidase restricts glucose absorption thus reduces post prandial hyperglycemia (Jayaraj et al. 2013). To stall the performance of both the enzymes by inhibitors might be one of the valuable approaches to control type 2 diabetes (Escandon-Rivera et al. 2012). All the extracts of *S. wightii* possessed alpha amylase inhibition but the reference drug acarbose displayed comparatively better response at concentrations similar to that of the extracts. Similar results were reported by Ravi et al. (2012). Inflammation is the primary physiological defense mechanism in our body (Arunachalam et al. 2011) which may be due to infection, tissue damage, hypersensitivity or imbalanced secretion of cytokines (Yoon et al. 2009). Though all the tested extracts exerted anti-inflammatory activity, the reference drug significantly had a cut above.

The results are in agreement with the earlier findings of Yeshwante et al. (2009) and Boonchum et al. (2011). Free radicals are responsible for oxidative damages to biological systems. Diseases such as cancer and cardiovascular diseases arise mainly due to oxidative stress (Yang et al. 2014). The enzyme inhibitors and metal chelators prevent the generation of free radicals whereas, scavengers clear the radicals directly (Dawidowicz et al. 2013). Seaweeds are always under the stress of high oxygen and light which lead to the formation of free radicals; hence they develop mechanisms and synthesise compounds like anti-oxidants (Cox et al. 2010).

In the present study, benzene extracts have potent antioxidant activity when compared with other solvents but the control drug exerted higher activity than the extracts. In general, the standard drugs may exhibit more activity than the experimental compounds due to the purity of the reference compound (Yeshwante et al. 2009). The qualitative phytochemical analysis of the extracts of *S. wightii* revealed the presence of secondary metabolites such as alkaloids, flavonoids and steroids that may be responsible for the various bioactivities (Akharaiyi 2011). FT-IR analysis revealed the presence of amine, vinyl, ketone, aromatic, fluoroalkanes, and benzene groups of compounds. The results corroborate with the findings of Yang et al. (2014).

Conclusion

The extracts of *S. wightii* possessed antibacterial, alpha amylase inhibitory, anti-inflammatory and antioxidant activities in the *in vitro* conditions. The phytochemical analysis revealed the presence of phenolic compounds and further analysis using FT-IR showed the presence of amino, keto, fluoroalkanes group and aromatic compound. Identification, characterisation and purification of the bioactive principles from *S. wightii* and subsequent testing on *in vivo* models would offer a great promise in the drug design and development process.

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