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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(4): 445-452

www.thepharmajournal.com
Received: 06-02-2019
Accepted: 10-03-2019

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Profiling of phytochemical, antioxidant properties and antimicrobial activity of marine red seaweed *Jania rubens*

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Abstract
To evaluate the phytochemical screening, *in vitro* antioxidant activity and antibacterial activity of marine red algae such as *Jania rubens*. The marine red algae *Jania rubens* was collected from Kilakarai region located between 9.23135° N, 78.7844° E Ramanathapuram District, Tamil Nadu, India. The algal extract was prepared from different solvents namely aqueous, ethanol, methanol, acetone and was tested for their primary phytochemical screening, antioxidant activity such as DPPH radical scavenging activity and ABTS radical scavenging activity and antibacterial activity against human pathogens viz., *Escherichia coli*, *Pseudomonas aeruginosa* and *Stephylococcus aureus* and fish pathogens viz., *Aeromonas hydrophila* and *Vibrio vulnificus* bacteria using disc diffusion methods. Preliminary phytochemical screening of sixteen different chemical compounds were carried out. The maximum biochemical compounds were present in the ethanol extract and the minimum compounds were present in aqueous extract. The ethanol extract was significantly higher in DPPH radical scavenging activity (43.23±0.7 µg/ml) and ABTS radical scavenging activity (33.66±1.5 µg/ml). In human pathogen the highest antibacterial activity was present in *S. aureus* (22.00±1.0mm) likewise the fish pathogen the antibacterial activity was increase in *Aeromonas hydrophila* (22.66±0.5mm). The present work shows a comparable therapeutic potency of the tested seaweed members *Jania rubens* extracts in treating human and fish microbial pathogens to synthetic chemical antibiotics. The present study showed that the ethanol extract of marine red seaweed *Jania rubens* contains bio active constituents with highest antibacterial activity.

Keywords: *Jania rubens*, DPPH, ABTS, antibacterial activity, *A. hydrophila*, *S. aureus*

1. Introduction
The microbial infection causes a high rate of mortality in human and aquaculture organisms. Preventing infection outbreaks or treating the disease with drugs or chemicals tackles these problems [1].

The progress of numerous vaccines, mostly against human and fish pathogens, and the use of different antimicrobial agents have reduced the impact of various infective diseases. However, there is presently an increasing demand for more environment-friendly infection control schemes and many researchers have examined alternative approaches. Among these approaches, the use of various natural products that derive from different living organisms, such as plants, animals, and seaweeds has received a lot of attention [2].

Macroalgae contain various kinds of inorganic and organic substances which probably benefit human health in proteins, minerals, vitamins, antioxidants, phytochemicals, polyunsaturated fatty acids [3].

Seaweeds, also recognized since macroalgae, are photosynthetic multicellular aquatic organisms that can be established in almost every aquatic environment, in all geographical areas [4]. Seaweeds are highly productive components of the coastal ecosystem releasing dissolved organic carbon into surrounding waters thus harboring suitable living substrate for microbial colonization [5].

The current scientific trends focus on investigating phytochemicals from marine algae due to their several health-promoting effects, as well as antioxidant, anti-inflammatory, antimicrobial, and anti-cancer [6].

As a result of this strong competition, an elevated percentage of species have evolved chemical means by which to defend against predation. These chemical adaptations commonly take the form of so-called secondary metabolites and occupy such well-known chemical classes as terpenoids, alkoloids, polyketoides, peptides, sugars, steroids, polysaccharides and a multitude of mixed biogenesis metabolites.

A number of the marine compounds show pharmacological activities and are helpful in the development and detection of bioactive compounds, primarily for deadly diseases like urinary tract infections, cervicitis vaginitis, gastrointestinal disorder, respiratory diseases, cutaneous affections, helminthic infections, parasitic protozoan disease and inflammatory processes [7, 8].

In the most recent 20 years, there has been an increasing interest in using a variety of seaweed extracts as prophylactic and/ or therapeutic agents in aquaculture industry [9].

Therefore, the aim of the present study was to investigate the antimicrobial activity of extracts of marine algae against human and fish pathogenic bacteria that are often the cause of bacterial diseases in human and aquaculture. The present study was undertaken to evaluate the extract of red seaweed *Jania rubens* using different solvents like aqueous, ethanol, methanol and acetone. Herein we report the preliminary phytochemicals present in the selected seaweed, *in vitro* antioxidant activity of 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) and 2, 2 -azino-bis (3-ethylbenzthiazoline)-6-sulfonic (ABTS), and their antibacterial efficacy of human and fish pathogens.

2. Material and Methods

2.1 Seaweed material and extraction

Red algae used in this study *Jania rubens*, were freshly collected from the Kilakarai region located between 9.23135° N, 78.7844° E Ramanathapuram District, Tamil Nadu, India. Seaweed taxonomically identified and the voucher specimen was stored in the department of Marine Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. The collected samples were washed in running water for 10 min, transported to the laboratory and shade dried (35±3 °C) for 36 h. The shade dried seaweeds were powdered and used for further experiments.

2.2 Preparation of seaweed extracts

The dried seaweed materials were blended into a coarse powder before extraction portions of the powdered samples (5 g) and packed in Soxhlet apparatus and extracted successively with aqueous, ethanol, methanol and acetone for 10 h [10]. The crude extracts were weighed and deep frozen (-20 °C) until tested.

2.3 Preliminary phytochemical analysis

The extracts from different solvents were tested for Steroids, Tannins, Terpenoids, Flavonoides, Saponins, Alkaloides, Reducing sugar, Cardiac glycosides, Coumarins, Phlobatannins, Anthraquinones, Quinones, Glycosides, Phenols, Anthocyanin, Betacyanin. Phytochemical screening of the extract was carried out according to the standard methods [11, 12].

2.4 *In vitro* antioxidant activity

2.4.1 DPPH radical scavenging activity

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was estimated using the method of Liana-Pathirana and Shahidi, [13]. Appropriate dilutions of the extract (1 mg/mL) was mixed with, 1 mL of 0.135 mM methanolic solution of DPPH radical. Absorbance was measured at 517 nm after 30 min of reaction. BHT was used as reference standard and the inhibition percentage was calculated using the following formula:

Percentage of inhibition= [(Abs control-Abs sample/Abs control)] × 100

2.4.2 ABTS radical scavenging activity

Determination of 2, 2 -azino-bis (3-ethylbenzthiazoline)-6-sulfonic (ABTS) radical scavenging ability of plant extracts was carried out by the method of Re *et al.* [14]. Previously, 7 mM ABTS solution and 2.4 mM potassium persulphate solution were prepared separately. Equal amount of two stock solutions were mixed and allowed to stand for 12 h in dark at room temperature. About 1 mL of diluted ABTS.+ solution react with plant extract (1mg/mL) after 10 min the absorbance was measured UV-spectrophotometrically at 734 nm against the blank solution. ABTS free radical inhibition was calculated by following

Percentage of inhibition= [(Abs control-Abs sample/Abs control)] × 100

2.5 Collection of bacteria

The human pathogenic bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa*, and *Stephylococcus aureus* and fish pathogens *Aeromonas hydrophila* and *Vibrio vulnificus* were used for this experiment. The human pathogenic bacteria were obtained from the Laboratory of Microbiology, KAP Vishwanathan, Government Medical College, Tiruchirappalli, Tamil Nadu, India. Fish pathogens were obtained from microbial type culture collection (MTCC), Indian Institute of Microbial Technology, Chandigarh, India. Mueller-Hinton broth (MHB) was obtained from Hi-Media while solvents used were of HPLC grade.

2.5.1 Disc diffusion method

The antimicrobial activity of *Jania rubens* solvent extract was assessed by the disc diffusion technique [15]. Mueller Hinton agar (MHA) plates were prepared and individually swabbed with pathogenic bacteria. The sterile discs (6mm) were placed over the surface of the agar plates. Seaweed extract (1 mg/mL) was added on the discs at various concentrations (50, 100, 250 and 500 µg/mL). A disc containing standard concentrations of the antibiotic Ciprofloxacin (20 µg/disc) was used as positive control. The agar plates were incubated for 24 h at 37°C, and the inhibition zones were measured in millimeter and the experiment was repeated thrice for concordant results. All the data were statistically analyzed.

2.5.3 Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was carried out according to the methods of National Committee for Clinical Laboratory Standards (NCCLS). The seaweed extracts were selected for the solvents aqueous, ethanol, methanol and acetone. The initial test concentration of extract was 1 mg/mL. Each tube containing 2 mL of broth was inoculated with 5µl of bacterial suspension containing 10^8 CFU/mL of bacteria. Ciprofloxacin was used as positive control. The test tubes were incubated for 24 h at 37°C. MIC was determined as the lowest concentration of extract showing OD of 600nm of spectrophotometer. All the data were statistically analyzed.

2.6 Statistical analysis

All the values were expressed as Mean ± Standard Deviation (SD). The statistical significance was evaluated by two-way Analysis of Variance (ANOVA) using SPSS version 20 (SPSS, Cary, NC, USA) and the individual comparisons were

obtained by Post-hoc analysis, Duncan [16].

3. Results

3.1 Preliminary phytochemical analysis

Preliminary phytochemical screening of sixteen different chemical compounds (Steroids, Tannins, Terpenoids, Flavonoides, Saponins, Alkaloides, Reducing sugar, Cardiac glycosides, Coumarins, Phlobatannins, Anthraquinones, Quinones, Glycosides, Phenols, Anthocyanin, Betacyanin) was tested in four different solvents (Aqueous, Ethanol, Methanol and Acetone) of species *Jania rubens* (Table 1). The maximum biochemical compounds were present in the ethanol extract and the minimum compounds were present in the aqueous extract. When comparing to other biochemical compound, flavonoides and phenol were present in all the selected solvents followed by alkaloids present in three solvents. Tannins, Saponins, Flavonoides, Anthraquinones, Glycosides and Anthocyanin were not present in the selected solvents.

Table 1: Preliminary phytochemical screening of red seaweed *Jania rubens*

Phytochemical compounds	Solvents			
	Aqueous	Ethanol	Methanol	Acetone
Steroids	-	+	-	-
Tannins	-	-	-	-
Terpenoids	-	+	+	-
Flavonoides	+	+	+	+
Saponins	-	-	-	-
Alkaloides	-	+	+	+
Reducing sugar	-	-	-	-
Cardiac glycosides	-	-	-	+
Coumarins	-	+	+	-
Phlobatannins	+	-	-	-
Anthraquinones	-	-	-	-
Quinones	-	+	-	+
Glycosides	-	-	-	-
Phenols	+	+	+	+
Anthocyanin	-	-	-	-
Betacyanin	-	+	-	-

Note: +, present; -, absent

3.2 Antioxidant activity

3.2.1 DPPH radical scavenging activity

The DPPH radical scavenging activity of *J. rubens* extracts and the synthetic chemical BHT compounds are shown in Fig.1. The ability of red seaweed extracts to scavenge the reactive metabolites would inhibit the formation of primary and secondary amines oxidation products. In this analysis, *J. rubens* showed highest DPPH free radical scavenging activity of all extracts increased as the concentration increased ($P < 0.05$). However, significantly higher in ethanolic extract (43.23 %) compared to methanol (33.16 %), acetone (35.66 %) and aqueous extract (32.33 %).

3.2.2 ABTS+ radical scavenging activity

ABTS+ radical scavenging activities of the extracts of *J. rubens* compared with BHT showed 20-100 µg/ml (Fig.2). ABTS+ radical scavenging activity of all extracts increased as the concentration increased ($P < 0.05$). It was observed that the ethanolic extract showed higher activity than that of the methanol, acetone and aqueous extract. The percentage of inhibition of ABTS+ radicals by ethanolic extract of the leaves reached up to 39.00±1.0 % at the concentration of 100 mg/mL, whereas the methanol, acetone and aqueous extract showed 33.33 %, 34.36 % and 30.66 % at the same concentration respectively.

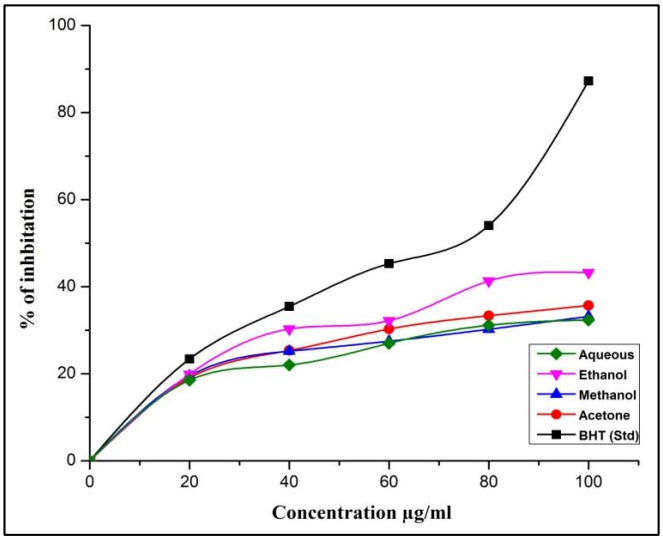

Fig 1: 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of *Jania rubens*

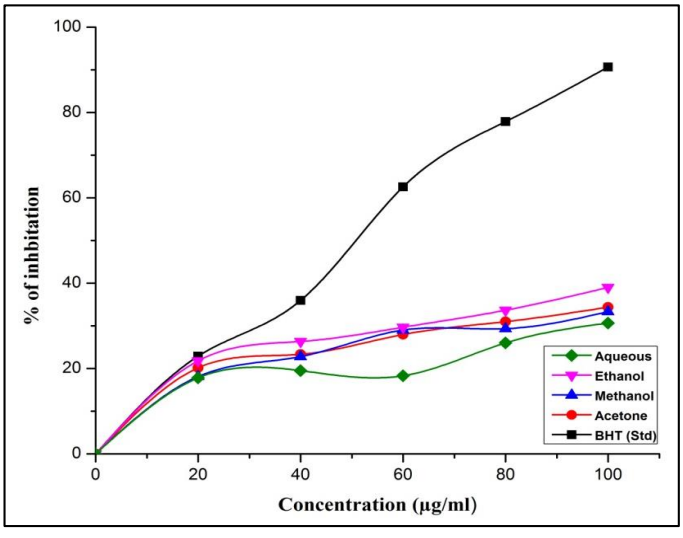

Fig 2: 2, 2 -azino-bis (3-ethylbenzthiazoline)-6- sulfonic (ABTS), radical scavenging activity *Jania rubens*

3.3 Antibacterial activity human and fish pathogenic bacteria

The four different solvent extracts and various concentration of *J. rubens* were tested for its antibacterial activity and their zone of inhibition are shown in Table 2. The aqueous, ethanol, methanol and acetone extract of *J. rubens* the maximum activity showed the ethanolic extract against human pathogenic bacteria *S. aureus* (22.00±1.0mm), the acetone extract exhibit the maximum activity of *P.aeruginosa* (19.66±0.5mm), the methanolic extract exhibited maximum activity (18.66±0.5 mm) against *E. coli*, and aqueous extract exhibit the maximum activity of against *P. aeruginosa* (13.66±0.5 mm) at 500 µg/ml concentration.

The antibacterial activity and their zone of inhibition are shown in Table 3. The aqueous, ethanol, methanol and acetone extract of *J. rubens* showed the fish pathogenic bacteria acetone extract exhibit the maximum activity of (24.66±0.5mm), ethanolic extract (22.66±0.5mm) and methanolic extract (18.66±0.5mm) against *A. hydrophila* and aqueous extract exhibit the maximum activity against *V. vulnificus* (12.33±0.5 mm) at 500 µg/ml concentration.

3.4 Minimum Inhibitory Concentrations (MIC)

The extracts that showed any antibacterial activity in this assay were subjected to the minimum inhibitory concentration assessment and the results are presented in Table 4. The MIC of seaweed were determined by serial broth dilution method and *J. rubens* showed the inhibitory range of (33.31 -76.97). The highest percentage of inhibitory concentrations was found in ethanol extract (76.97%), while the lowest percentage was in aqueous extract.

Table 2: Antibacterial activity of *Jania rubens* extract against human pathogens

Solvents	Concentration (µg/mL)	Bacterial Species (human pathogens)		
		E. coli	***P. aeruginosa***	***S. aureus***
Aqueous	50	8.33 ± 0.5^{dB}	7.33 ± 0.5^{dA}	NA
	100	8.66 ± 0.5^{dB}	8.66 ± 0.5^{dA}	NA
	250	10.33 ± 0.5^{dB}	10.00 ± 1.0^{dA}	NA
	500	11.66 ± 0.5^{dB}	13.66 ± 0.5^{dA}	8.66 ± 0.5^{dC}
	C	25.00 ± 1.0^{dB}	25.33 ± 0.5^{dA}	18.33 ± 0.5^{dC}
Ethanol	50	7.33 ± 0.5^{aB}	7.00 ± 0.0^{aA}	7.33 ± 0.5^{aC}
	100	9.66 ± 0.5^{aB}	7.66 ± 0.5^{aA}	11.66 ± 0.5^{aC}
	250	18.66 ± 0.5^{aB}	12.66 ± 0.5^{aA}	20.33 ± 0.5^{aC}
	500	21.66 ± 0.5^{aB}	19.66 ± 0.5^{aA}	22.00 ± 1.0^{aC}
	C	24.66 ± 0.5^{aB}	24.00 ± 1.0^{aA}	22.33 ± 2.0^{aC}
Methanol	50	NA	10.66 ± 0.5^{cA}	NA
	100	8.66 ± 0.5^{cB}	11.66 ± 0.5^{cA}	7.33 ± 0.5^{cC}
	250	12.33 ± 0.5^{cB}	12.66 ± 0.5^{cA}	8.66 ± 0.5^{cC}
	500	18.66 ± 0.5^{cB}	13.33 ± 1.1^{cA}	11.66 ± 0.5^{cC}
	C	21.00 ± 1.0^{cB}	22.66 ± 0.5^{cA}	21.00 ± 1.0^{cC}
Acetone	50	9.00 ± 1.0^{bB}	11.33 ± 0.5^{bA}	NA
	100	12.66 ± 0.5^{bB}	16.33 ± 0.5^{bA}	8.33 ± 0.5^{bC}
	250	7.66 ± 0.5^{bB}	16.66 ± 0.5^{bA}	10.33 ± 0.5^{bC}
	500	15.66 ± 0.5^{bB}	19.33 ± 0.5^{bA}	12.66 ± 0.5^{bC}
	C	23.6667 ± 0.5^{bB}	25.66 ± 0.5^{bA}	21.00 ± 1.0^{bC}

*__NA__: Not Available, **C**: Ciprofloxacin, ANOVA ($P<0.05$)
Duncan test: Identical lower case superscripts denote similar values vertically
Identical upper case superscripts denote similar values horizontally

4. Discussion

In the present study, red seaweed *J. rubens* with a variety of solvents such as aqueous, ethanol, methanol and acetone were tested for their yield, phytochemicals, *in vitro* antioxidant activity and antibacterial properties.

The marine algae are among the richest sources of known and novel bioactive metabolites, compounds of interest in the pharmaceutical industry [17, 18].

In this study, phytochemicals of seaweed showing the presence of steroids, terpenoids, flavonoids, alkaloids, Cardiac glycosides, coumarins, phlobatannins, quinine, phenols, betacyanin in all samples tested. They have great potential as sources of natural bioactive compounds [19, 20]. Previously reported that three marine red seaweeds alkaloids, flavonoids, triterpenoids, steroids, tannins, coumarins, terpenoids, quinine, phytosteroids and phlobatannins present in all samples [21, 22].

Marine algae are known to produce a variety of secondary metabolites [23, 24]. Secondary metabolites are phytochemical that play a role in the maintenance of the human body [25]. The presence of phyto constituents, such as phenols, flavonoids, and tannins present in the seaweeds indicates the option of antioxidant activity and this activity will help in preventing a number of diseases through free-radical scavenging activity [26].

Table 3: Antibacterial activity of *Jania rubens* extract against fish pathogens

Solvents	Concentration (µg/mL)	Bacterial Species (Fish Pathogens)	
		A. hydrophila	***V. vulnificus***
Aqueous	50	7.00±0.0^{d}	7.33±0.5^{d}
	100	8.00±0.5^{d}	10.33±0.5^{d}
	250	9.33±0.5^{d}	11.66±0.5^{d}
	500	9.66±1.5^{d}	12.33±1.1^{d}
	C	21.33±0.5^{d}	25.00±1.0^{d}
Ethanol	50	10.33±1.5^{a}	7.66±0.5^{a}
	100	13.00±1.0^{a}	12.66±0.5^{a}
	250	18.66±0.5^{a}	17.33±1.5^{a}
	500	22.66±0.5^{a}	18.00±1.0^{a}
	C	25.66±0.5^{a}	26.00±1.0^{a}
Methanol	50	8.66±0.5^{c}	7.66±0.5^{c}
	100	10.66±0.5^{c}	8.66±0.5^{c}
	250	15.66±0.5^{c}	10.00±1.0^{c}
	500	18.66±0.5^{c}	12.33±0.5^{c}
	C	25.00±1.0^{c}	24.00±1.0^{c}
Acetone	50	10.33±0.5^{b}	NA
	100	13.66±0.5^{b}	NA
	250	19.66±0.5^{b}	11.66±0.5^{b}
	500	24.66±0.5^{b}	19.33±0.5^{b}
	C	29.00±1.0^{b}	20.66±0.5^{b}

Antioxidant activity of marine algae may well occur from pigments such as chlorophylls, carotenoids, vitamins and vitamin precursors, including cophenol, carotene, niacin, thiamine, ascorbic acid and phenolic compounds, such as polyphenols, hydroquinones and flavonoids. Phospholipids, particularly phosphatidylcholine, terpenoids, peptides, and other antioxidative substances, directly or indirectly contributed to the reserve or control of oxidation processes [27, 28].

The present study shows the ethanol extract of seaweed to have strong antioxidant activity. The antioxidant mechanisms of seaweed extract maybe credited to their free radical scavenging ability. The ability of a compound to scavenge DPPH radicals is dependent on their ability to pair with the unpaired electron of a radical [29].

The present study indicate DPPH scavenging activity for *J. rubens*, ethanolic extract (43.23±0.7 %) have a higher antioxidant activity. Gheda *et al.* [30] the *in vitro* assay of the antioxidant activity of eight marine seaweed species showed that the red seaweed *J. rubens* had the highest DPPH (2,2 diphenyl-1-picrylhydrazyl) free radical scavenging activity.

Many species of seaweed possess scavenging ability for hydrogen peroxide [31, 32]. The previous reports in the literature of the antioxidant ability of algae, alcoholic and aqueous extracts of seaweeds have been evaluated for antioxidant activity, DPPH assay [33, 34]. Devi *et al.* [35] reported that DPPH radical scavenging ability differed very much between the different varieties and ranged between 5% and 72.5% for *C. hornemanni* and *G. acerosa*, respectively. Souza *et al.* [36] reported that the ethanolic extracts of *G. birdiae* and *G. cornea* exhibit the best performance showing a high scavenging activity of 60%. Ethanolic extracts from *E. compressa* and *C. fulvescens* were the most efficient DPPH scavengers [37].

Although the DPPH and ABTS radical scavenging are based on the same principle, the ethanol extract and its fractions from red seaweeds, *J. rubens* showed higher ABTS radical scavenging activity compared to other three extracts (Table 3). Sachindra *et al.* [38] discussed both butanol and ethyl acetate fractions from the crude extract of brown seaweeds showed scavenging activity above 90%. Among ABTS radical scavenging activity showed highest in red seaweed *A. spicifera*,

The seaweed bioactive compounds have potential therapeutic interest. The creations of bioactive compounds have antibacterial activity because marine standard products has a broad range of novel bioactive compounds and antibiotics with characteristic complex structure because they developed the unique metabolic and physiological capability. The marine macroalgae have a high effective antibacterial activity against most of the human pathogenic bacteria [39].

Several numerous studies have focused on the study of the direct antibacterial properties of seaweed extracts against human bacterial pathogens, for example: *B. subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Clostridium spp.*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus spp.*, *Salmonella typhimurium*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes* and *Vibrio cholerae* [40-42].

The majority of the bacterial species that can cause diseases in fish and shrimp are relatively everywhere in the aquatic atmosphere, as for example many members of the genus *Aeromonas* and *Vibrio* species [43, 44] can affected both fish and shrimp and in many cases the manifestation and the progress of the associated diseases are affected by the presence of various stressful conditions. In comparison to human bacterial pathogens, fewer studies have been conducted to identify the antibacterial potential of seaweed metabolites against fish pathogens.

Table 4: MIC value of *Jania rubens* extracts against the human and fish pathogens

Bacteria species	Aqueous		Ethanol		Methanol		Acetone		Ciprofloxacin % of Inhibition
	Con. (mg/ml)	% of Inhibition	Con. (mg/ml)	% of Inhibition	Con. (mg/ml)	% of Inhibition	Con. (mg/ml)	% of Inhibition	
E. coli	50	42.23	50	45.94	50	55.01	50	54.53	93.20
	40	41.68	40	44.71	40	53.70	40	52.26	
	30	41.62	30	44.50	30	50.48	30	44.78	
	20	38.25	20	41.75	20	45.05	20	41.75	
	10	35.64	10	33.31	10	43.88	10	41.27	
P. aeruginosa	50	57.74	50	51.06	50	60.45	50	53.11	97.74
	40	53.69	40	52.86	40	54.59	40	50.54	
	30	53.31	30	48.23	30	52.09	30	46.49	
	20	50.35	20	45.01	20	51.44	20	46.43	
	10	49.13	10	35.36	10	49.83	10	45.91	
S. aureus	500	52.46	50	52.20	50	53.09	100	48.05	96.54
	400	50.47	40	51.69	40	52.58	80	43.25	
	300	49.45	30	50.03	30	50.67	60	42.81	
	200	48.24	20	38.33	20	49.20	40	42.49	
	100	47.92	10	35.14	10	43.25	20	42.10	
A. hydrophila	50	62.77	50	54.07	50	58.97	50	53.53	95.83
	40	60.48	40	50.39	40	55.10	40	53.11	
	30	58.97	30	50.33	30	54.80	30	52.44	
	20	56.55	20	50.15	20	52.68	20	49.66	
	10	56.37	10	46.04	10	52.14	10	49.06	
V. vulnificus	50	44.05	50	76.97	50	55.88	50	56.52	96.78
	40	40.90	40	49.71	40	48.48	40	46.94	
	30	40.25	30	49.06	30	44.50	30	45.20	
	20	38.77	20	46.55	20	44.18	20	44.88	
	10	35.88	10	46.49	10	41.92	10	43.66	

In the present study, agar disc diffusion method was carried out to test the antibacterial activities of four different organic extracts of marine red algae in *Jania rubens* (Table 4). The ethanol, extract showed maximum inhibition activity against human pathogenic bacteria *S. aureus.* Previously reported Ethanol extracts of *S. vulgare* show inhibition activity against *P. aeruginosa* and high activity against *K. pneumoniae* [45]. Vallinayagam *et al.* [46] have reported that the red algae showed higher activity than the brown and green algae when tested against seven human pathogenic bacteria. Silva *et al.* [47] found that *E. coli* and *P. aeruginosa* were affected only by the ethanolic extract of the brown seaweed *Padina gymnospora*. Ethanol extract of *Sargassum tenerrimum* showed the highest activity against *S.aureus* and aqueous extract shows less activity [48]. Our previously reported that findings that the different extract of *S. swartzi* ethanol extract shows the potential antimicrobial activity against human and fish pathogens [22].

Hanniffy and Kraan, [49] described that the macroalgal species, *Ulva, Porphyra,* and *Palmararia palmata* showed strong antibiotic activity against fish and human pathogens. Previously reported crude extracts from the seaweeds, *Gracilaria edulis, Calorpha peltada,* and *Hydroclothres sp*. screened for their antibacterial activity against six fish pathogens [50]. Lavanya and Veerappan, [51] discussed the extracts of six seaweed samples that were screened for antibacterial activity against fish and human pathogens.

5. Conclusion

In our study results showed that seaweeds of all extracts have shown moderate activity against the selected pathogens. It could be concluded that ethanol extract of *J. rubens* was effective as antioxidant agents, and potential antimicrobial activity against human and fish pathogens. Further studies could be done to explore new compounds from seaweeds to develop alternative therapeutic drugs.

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