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PHYTOCHEMICAL ANALYSIS AND COMPARISON OF ANTIBACTERIAL ACTIVITY OF VARIOUS SOLVENT EXTRACTS OF CAULERPA RACEMOSA ON MULTIDRUG RESISTANT BACTERIAL STRAINS

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Abstract. Phytochemical analyses and *in vitro* antibacterial activity of different extracts of hexane, chloroform, ethylacetate, acetone and methanol extracts of green algae, *C. racemosa* against multi-drug resistant standard and clinical bacterial strains *viz., Bacillus subtilis, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Vibrio cholerae, Shigella flexneri, Proteus mirabilis and Proteus vulgaris*. The ethyl acetate extract of the *C. racemosa* showed the strong phytochemicals, terpenoids, tannins, phenolic compounds and steroids than the other solvent extracts. The mean zone of inhibition produced by the extracts in agar diffusion assays against the tested bacterial strains ranged from 7.1 to 17.3 mm. The highest mean of zone inhibitions (17.3 mm) was observed in ethyl acetate extract of *C. racemosa* against *B. subtilis*. The Minimum Inhibitory Concentrations were between 125 μg/ml and 500 μg/ml, while the Minimum Bactericidal Concentrations were between 250 μg/ml and 1000 μg/ml. These finding suggest that the ethyl acetate extract of *C. racemosa* can be used as an antibacterial substance for the treatment of multi drug resistant microbes causing acquired infection.

Keywords: Antibacterial activity, Caulerpa racemosa, MDR Bacterial strain.

1. INTRODUCTION

Infectious diseases are the leading cause of death world-wide and at the same time antibiotic resistance has become a global concern [46]. In developing countries, bacterial infections are widespread, especially in informal settlements, due to poor sanitation and unhygienic conditions. Furthermore, diseases such as AIDS, malaria and tuberculosis, result in higher morbidity and mortality than those caused by susceptible pathogens; the global impact of increasing resistance is a major concern [15]. Drug resistance can be described as a state of decreased sensitivity to drugs that ordinarily cause growth inhibition or cell death. More strains of pathogens have become antibiotic resistant, and some have become resistant to several antibiotics and chemotherapeutic agents, the phenomenon of multidrug resistance [33].

Multi drug resistance (MDR) in microorganism is an emerging serious problem in health care sector. The improper usage of antibiotics contributes a major role for drug resistance in pathogenic microbes. Microorganisms acquire resistance towards common antibiotics by altering their metabolism and genetic structure [36, 38]. There is an incessant need to find novel efficient drug molecules against multi drug resistant microbes. The emergence of multiple drug resistant bacteria has become a major cause of failure of the treatment of infectious disease [19]. Most important multidrug resistant bacteria on the global scale include Gram-positive (methicillin-resistant *S. aureus*, vancomycin resistant Enterococci) and Gram-negative bacteria (members of enterobacteriaceae producing plasmid- mediated extended spectrum β- lactamases and others like *P. aeruginosa, M. tuberculosis* [31, 39]. *K. pneumoniae* carbapenemase (KPC) enzyme and metallo β-lactamase are the common armamentaria of carbapenem resistance in Enterobacteriaceae [26, 10]. *P. mirabilis* strain has been recorded as showing resistance to a large number of antibiotics, therefore, and their control is very difficult [17]. Seaweeds belong to a group of plants known as alga. Seaweeds are classified as Rhodophyta (red algae), Phaeophyta (brown algae) or Chlorophyta (green algae) depending on their nutrient and chemical composition. Like other plants, seaweeds contain various inorganic and organic substances which can benefit human health [27]. Chlorophytan seaweeds, popularly known as green algae, are widely distributed in both inter-tidal and deep-water regions of the seas. Chlorophyceae are large and important group of freshwater and marine green algae. Which are important both ecological and scientifically. More recent reports indicate that in many parts of the world marine algae are still used in folk medicine for the treatment of a variety of disease. The world contribution and use of marine algae as a food source must have contributed to its popularity [1, 41]. These seaweeds are of immense pharmaceutical and agricultural value. A wide range of compounds, particularly terpenes, polyphenolic compounds and steroids have been reported from various marine green algae [8], amongst which terpenoid compounds represent a major share. The previous reported studies have proved that marine macro algae posses broad range of biological activity such as antibiotics, antiviral, antimicrobial, anti-inflammatory, cytotoxic and antimitotis [3, 7, 9, 13, 23, 32]. *Caulerpa racemosa* (Forsska) J. Agardh (Caulerpales, Chlorophyta) is a green marine alga, which is widely distributed in warm waters. The first observation of

C. racemosa in the Mediterranean was reported by Hamel [20] in Sousse Harbor, Tunisia. *C. racemosa* green seaweed belongs to the class Chlorophyceae among the most abundant species of Gulf of Mannar region were selected for the study. *Caulerpa racemosa* belongs to the family Caulerpaceae, plants in larger or smaller groups, conspicuous Rhizome, creeping, cylindrical, simple or divided highly branched forming an intricate system. Rhizoids, forms lower parts of prostrate rhizome, attaching the plants to silt - covered stones, Coral rocks and coral pieces. Erect branches from upper side of rhizome, irregularly scattered, 2-5 cm long and more generally simple, terete, filiform and uniform thickness, naked at base, higher up set up with ramuli. Hence, the present work aimed to evaluate the efficiency of hexane, chloroform, ethyl acetates, acetone and methanol extracts of *C. racemosa* against multi-drug resistant bacterial strains bacterial strains.

2. Materials and Methods

Sample collection

Caulerpa racemosa (Chlorophyceae) were collected from Mandapam, at (Lat. 09° 17.417'N; Long. 079° 08.558'E) Ramanathapuram district, the Gulf of Mannar Marine biosphere, Tamil Nadu India. The collections were made from the month of October 2012. The algae were identified by Prof. R. Panneerselvam, Head and the museum specimens are deposited in the Department of Botany, Annamalai University.

Preparation of extracts

The algal species were handpicked during low tide and washed thoroughly with sea water to remove all unwanted impurities, epiphytes, animal casting, and adhering sand particles etc.,. Morphologically distinct thallus of algae were placed separately in new polythene bags and were kept in a ice box containing slush ice and transported to the laboratory. Then, the samples were blot dried using sterile tissue paper. The seaweed materials dry in one week in room temperature. After getting from all the samples were grounds in to a fine powder. The fine powder with organic solvent with increasing polarity *viz.*, hexane, chloroform, ethyl acetate, acetone and methanol were be prepared using Soxhlet apparatus for 72 hours . The solvent was evaporated under vacuum in a rotary evaporator (Heidolph, Germany) the dried extracts were stored at 4°C until further assay.

Collection of bacterial strains

The standard bacterial strains *viz., Bacillus subtilis* (MTCC 441), *Streptococcus pyogenes* (MTCC 442), *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 741), *Proteus mirabilis* (MTCC 425), *P. vulgaris* (MTCC 426), *Salmonella typhimurium* (MTCC 98), *Shigella flexneri* (MTCC 1457) and *Vibrio cholera* (MTCC 3906) were procured from Microbial Type Culture Collection (MTCC), Chandigarh.

The clinical isolates of bacterial strains *viz., S. pyogenes, E. coli, K. pneumoniae, P. mirabilis,*

P. vulgaris, P. aeruginosa, S. typhimurium, S. dysentriea, S. flexneri and *V. cholerae* were obtained from the Department of Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalai Nagar, Tamil Nadu, India. These strains were maintained on nutrient agar slant at 4°C.

Phytochemical screening

The qualitative phytochemical analyses studies hexane, chloroform, ethyl acetate, acetone and methanol extracts of *C. racemosa*. Phytochemicals like Terpenoids, Tannin, Cardic glycosides, Steroids, Alkaloids, Phenolic compounds, and Coumarins were carried out according to the method described by [22, 45].

Preparation of inocula

Twenty-four hour old culture of selected bacteria was mixed with physiological saline and turbidity was adjusted by adding sterile physiological saline until a McFarland turbidity standard of 0.5 (10^6 colony forming units (CFU) per ml) was obtained.

Antibiotic sensitivity test

Antibiotic sensitivity of the bacterial strains were determined by standard CLSI disc diffusion method [16]. Antibacterial agents from different classes of antibiotics viz., Methicilin (ME 5 μg/disc), Oxacillin (OX μg/disc), Linezolid (LIN 30 μg/disc), Vancomycin (VAN 30 μg/disc) Amikacin (AK 30 μg/disc), Ampicillin (AMP 10 μg/disc), Cefixime (CFM 5 μg/disc), Ceftazidime (CAZ 30 μg/disc), Ciprofloxacin (CIP 5 μg/disc), Chloramphenicol (C 30 μg/disc), Erythromycin (E 15 μg/disc), Gentamycin (GEN 10 μg/disc), Norfloxacin (NX 10 μg/disc), Nalidixic acid (NA 30 μg/disc), Ofloxacin (OF 5 μg/disc), Streptomycin (S 10 μg/disc), and Tetracycline (TE 30 μg/disc), were obtained from Himedia, Mumbai.

Anti-bacterial assay

Disc Diffusion Method

The antibacterial activity was evaluated by agar diffusion method as described [6] was followed for antibacterial susceptibility test. Petri plates were prepared by pouring 20 ml of Mueller Hinton Agar. Then the plates were allowed to solidify and used in susceptibility test. The test culture were swabbed on the top of the solidified media and allowed to dry and uniformly spread. The excess inoculum was drained and the plates were allowed to dry for 10 minutes. The crude extracts were dissolved in 10% DMSO and under aseptic conditions sterile discs were loaded with different extracts impregnated with 20 μl of three different concentrations (500, 250, 125 μg/disc) of crude extracts. The discs with extract were placed on the surface on the medium with sterile forceps and gently pressed to ensure contact with inoculated agar surface. Ampicillin (10 μg/disc) was used as positive control and 10 per cent DMSO was used as blind control in all the assays. Finally, the inoculated plates were incubated at 35°C for 24 h. The zone of inhibition was recorded in millimeters. All the extracts were tested experiment

was carried out replicated three times.

Determination of Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) of the *C. racemosa* crude extracts, a modified reaszurin microtitre plate assay was carried out according to methods of Sarker et al. 27. Sterile Mueller Hinton Broth 100 ml of respective broth was transferred in to each well of a sterile 96-well micro titer plate (Hi-Media TPG 96). The *C. racemosa* extracts were dissolved in 10 per cent DMSO to obtain 1000 °g/ml stock solution. 100 ml of crude extract stock solution was added into the first well. After fine mixing of the crude extracts and broth 100 μl of the solution was transferred to the second well and in this way, the serial dilution procedure was continued to a twofold dilution to obtain concentrations like 1000, 500, 250, 125, 62.5, 31.25, 15.625 °g/ml of the extract in each wells. To each well 10 °L of resazurin indicator solution was added. (The resazurin solution was prepared by dissolving a 270mg tablet in 40mL of sterile distilled water. A vortex mixer was used to ensure that it was a well-dissolved and homogenous solution). Finally, 10 °l of the standardized bacterial suspension were inoculated into all wells. Each plate was set up with a positive control (bacterial suspension adding 10 °l of Mueller Hinton broth and resazurin solution) and negative control (10% DMSO and reaszurin solution without bacterial suspension). The plates were incubated at 37°C for 24 h for all bacterial strains. The color change was then assessed visually. The growth was indicated by color changes from purple to pink (or colorless). In this study, the MIC was the lowest concentration of *C. racemosa* extracts that exhibited the growth of the organisms in the values by visual reading.

Minimum Bactericidal Concentration

MBC of the *C. racemosa* extracts were determined by plating 10 μL of bacterial solution from each MIC assay well with growth inhibition into freshly prepared Mueller Hinton Agar. The plates were incubated at 37°C for 24 h for all bacterial strains. The MBC was recorded as the lowest concentration of the extract that did not permit any visible bacterial growth after the period of incubation.

3. RESULTS

The hexane, chloroform, ethyl acetate, acetone and methanol extracts of *C. racemosa* were used analysed for phytochemicals, terpenoids, tannin, cardiac glycosides, steroids, alkaloids, phenolic compound and Coumarins. The ethyl acetate extracts of *C. racemosa* contained strong phytochemicals terpenoids, tannins, phenolic compounds, and steroids than the other solvents extracts. Cardiac glycosides were present in all the extracts except acetone and methanolic extracts. Alkaloids and coumarins are not present in all the extracts tested.

The multi drug resistance resistance profile, of bacterial strains of both clinical and standard strains was confirmed by CLSI-M100-2012 method. The standard strains of *B. subtilis,*

K. pneumoniae and *P. vulgaris* were sensitive to all the antibiotics tested except CFM, AMP and CAZ. The standard strains of *S. flexneri* and *P. mirabilis* were sensitive to all the antibiotics tested except AMP. The standard strains of *S. pyogenes* were resistant to CFM, AMP, CAZ, NA and E and sensitive to all other antibiotics tested. The standard strains of *E. coli* were sensitive to all antibiotics tested except AMP and NA. The standard strains of *P. aeruginosa* were resistant to CFM, AMP and TE and sensitive to all other antibiotics tested. The standard strains of *S. typhimurium* were sensitive to all antibiotics except AMP and E. The standard strains of *V. cholerae* were resistant AMP and intermediate resistant to S and sensitive to all other antibiotics tested.

The clinical isolates of *S. pyogenes* were sensitive to all antibiotics tested and resistant to CFM, AMP, CAZ, OF and E. The clinical isolates of *E. coli* were sensitive to all the antibiotics tested and resistant to CFM, AMP, CAZ and GEN. The clinical isolates of *K. pneumoniae* were resistant to all the antibiotics tested and sensitive to GEN, S, TE, AK and E. The clinical isolate of *P. aeruginosa* was sensitive to all the antibiotics tested and resistant to CFM, AMP, CAZ and E. The clinical isolate of *S. typhimurium* were sensitive to all antibiotics tested and resistant to AMP, CFM and OF. The clinical isolates of *V. cholerae* were sensitive to all antibiotics tested and resistant to AMP, CFM, CAZ, NX and E. The clinical isolates of *S. flexneri* were sensitive to all antibiotic tested and resistant to AMP, CFM, CAZ, NX, OF and NA. The clinical isolates of *S. dysentriea* were sensitive to all antibiotics tested and resistant to AMP, CFM and OF. The clinical isolates of *P. mirabilis* were sensitive to all antibiotics tested and resistant to AMP, CFM, GEN, NX, NA and E. The clinical isolates of P. mirabilis were sensitive to all antibiotics tested and resistant to AMP, CFM, GEN, NX, NA and OF.

In the present study different solvents of hexane, chloroform, ethyl acetate, acetone, and methanol extracts of *C. racemosa* were studied against multidrug resistant both clinical and standard bacterial strains. The maximum activity was displayed by ethyl acetate extract of *Caulerpa racemosa* against *Bacillus substilis* the mean zone of inhibition (17.3 mm) followed by *S. pyogenes* (14.6) *P. mirabilis* (14.0 mm) and *S. dysentriae* (13.8 mm). All the extracts of marine macro algae possessed significant antibacterial activity against all the bacterial strains tested when compared to the available antibiotics tested. There was no much variation among the clinical and standard bacterial strains towards the algal extracts tested. The mean values are presented in Tables 1 and 2. When the different extracts were assayed against the test bacteria by disc diffusion assays, the mean zone of inhibition obtained were between 7.1 and 18.5 mm. Ampicillin (10 μg/disc) antibacterial positive control produced mean zone of inhibition ranged from 7.1 to 9.0 mm. The blind control (10% DMSO) did not produce any zone of inhibition for all the bacterial strains tested. The MIC values of the different extracts of *C. racemosa* ranged between 125 and 500 μg/ml, while the MBC values were between 250 and 1000 μg/ml.

Table-1: Antibacterial activity of *Caulerpa racemosa* against Multidrug Resistant standard Bacterial Strains

SI.No	Seaweed extracts/ solvents	Mean zone of inhibition$^a(mm)^b$				MIC ($\mu g/ml$)	MBC ($\mu g/ml$)
		Concentration of the disc					
		500(g/disc)	250(g/disc)	125(g/disc)	Ampicillin (10mg/disc)		
1.	*Bacillus subtilis* (MTCC 441)						
	Hexane	13.0±0.50	10.3±0.50	8.0±0.50	8.3±0.28	250	250
	Chloroform	13.5±0.50	10.5±0.50	8.5±0.50	8.5±0.50	250	500
	Ethyl acetate	17.3±0.28	11.5±0.50	9.0±0.50	8.1±0.28	125	250
	Acetone	11.5±0.50	9.5±0.50	7.3±0.57	8.5±0.50	500	1000
	Methanol	10.0±0.50	9.0±0.50	7.1±0.28	9.6±0.76	500	1000
2.	*Streptococcus pyogenes* (MTCC 442)						
	Hexane	12.8±0.57	10.0±0.50	7.1±0.28	9.3±0.28	500	1000
	Chloroform	13.5±0.50	10.5±0.50	7.6±0.57	12.1±0.28	500	1000
	Ethyl acetate	14.6±0.50	10.8±0.28	8.5±0.50	8.8±0.76	250	500
	Acetone	12.5±0.50	10.0±0.50	7.6±0.57	11.0±0.50	500	1000
	Methanol	11.0±0.50	9.3±0.76	7.1±0.28	11.6±0.28	500	1000
3.	*Escherichia coli* (MTCC 443)						
	Hexane	12.0±0.50	9.0±0.50	7.5±0.50	12.0±0.50	500	1000
	Chloroform	12.0±0.50	10.0±0.50	7.5±0.50	12.0±0.50	500	1000
	Ethyl acetate	13.1±0.28	11.1±0.28	8.5±0.50	8.6±0.76	250	500
	Acetone	11.6±0.76	9.1±0.28	7.3±0.57	8.6±0.76	500	1000
	Methanol	10.3±0.28	8.8±0.76	7.1±0.28	9.3±0.57	500	1000
4.	*Klebsiella pneumoniae* (MTCC109)						
	Hexane	11.6±0.28	9.0±0.50	7.3±0.57	9.3±0.57	500	1000
	Chloroform	12.0±0.50	10.1±0.28	8.0±0.50	12.8±0.28	500	1000
	Ethyl acetate	13.1±0.28	11.1±0.28	9.0±0.76	12.0±0.50	250	500
	Acetone	11.6±0.76	9.1±0.28	7.3±0.57	8.6±0.76	500	1000
	Methanol	11.0±0.50	9.1±0.28	7.1±0.28	12.0±0.50	500	1000
5.	*Proteus mirabilis* (MTCC 425)						
	Hexane	11.5±0.50	9.3±0.28	7.1±0.28	12.1±0.28	500	1000
	Chloroform	13.0±0.50	10.3±0.76	8.3±0.57	12.8±0.76	250	1000
	Ethyl acetate	14.0±0.76	11.1±0.28	9.6±0.76	10.8±0.50	250	500
	Acetone	11.8±0.28	9.5±0.50	7.3±0.57	8.8±0.76	500	1000
	Methanol	12.3±0.28	9.6±0.57	7.1±0.28	7.3±0.28	500	1000

Table-1: Continued

SI.No	Seaweed extracts/ solvents	Mean zone of inhibition[a] $(mm)^b$				MIC $(\mu g/ml)$	MBC $(\mu g/ml)$
		Concentration of the disc					
		500(g/disc)	250(g/disc)	125(g/disc)	Ampicillin (10mg/disc)		
6.	*Proteus. vulgaris* (MTCC 426)						
	Hexane	11.0±0.50	9.0±0.50	7.1±0.28	12.8±0.76	500	1000
	Chloroform	11.0±0.50	9.3±0.28	8.0±0.50	11.0±0.50	500	1000
	Ethyl acetate	13.5±0.50	10.8±0.76	8.5±0.50	11.6±0.76	250	500
	Acetone	12.5±0.50	10.3±0.57	7.6±0.57	11.0±0.50	500	1000
	Methanol	10.5±0.50	9.5±0.50	7.1±0.28	12.1±0.28	500	1000
7.	*Pseudomonas aeruginosa* (MTCC 741)						
	Hexane	10.1±0.28	8.6±0.28	7.1±0.28	10.8±0.76	500	1000
	Chloroform	12.0±0.50	9.3±0.28	7.3±0.57	8.6±0.76	500	1000
	Ethyl acetate	13.5±0.50	10.3±0.28	8.3±0.57	10.3±0.28	250	500
	Acetone	12.1±0.76	10.1±0.28	7.8±0.76	12.0±0.86	500	1000
	Methanol	10.6±0.28	9.1±0.28	7.3±0.28	7.3±0.28	500	1000
8.	*Salmonella typhimurium* (MTCC 98)						
	Hexane	12.0±0.50	9.8±0.76	7.1±0.28	11.0±0.50	500	1000
	Chloroform	12.1±0.28	9.8±0.28	7.8±0.76	7.8±0.76	500	1000
	Ethyl acetate	13.5±0.50	10.8±0.76	8.6±0.76	8.8±0.76	250	500
	Acetone	12.0±0.50	9.6±0.28	7.6±0.57	10.3±0.57	500	1000
	Methanol	11.0±0.50	9.1±0.28	7.3±0.57	11.6±0.76	500	1000
9.	*Shigella flexneri* (MTCC 1457)						
	Hexane	10.0±0.50	8.5±0.50	7.1±0.28	8.0±0.50	500	1000
	Chloroform	11.8±0.28	9.6±0.28	7.8±0.76	11.6±0.76	500	1000
	Ethyl acetate	13.5±0.50	9.8±0.76	8.1±0.28	12.1±0.28	250	500
	Acetone	11.8±0.28	9.5±0.50	7.5±0.50	12.8±0.57	500	1000
	Methanol	10.5±0.50	8.6±0.57	7.5±0.5	11.6±0.57	500	1000
10.	*Vibrio cholera* (MTCC 3906)						
	Hexane	10.0±0.50	9.0±0.50	7.1±0.28	7.8±0.76	500	1000
	Chloroform	12.6±0.76	9.6±0.28	7.6±0.57	8.0±0.50	500	1000
	Ethyl acetate	13.5±0.50	10.8±0.76	8.1±0.28	9.3±0.57	250	500
	Acetone	11.8±0.28	9.5±0.50	7.5±0.50	8.6±0.57	500	1000
	Methanol	10.5±0.50	8.6±0.57	7.3±0.57	9.3±0.57	500	1000

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm; [b]-mean of three assays; ± - standard deviation. Positive control: Ampicillin (10 mg/disc) were between 8 and 13 mm.

Table-2: Antibacterial activity of *Caulerpa racemosa* against Multidrug Resistant clinical Bacterial Strains

SI.No	Seaweed extracts/ solvents	Mean zone of inhibition$^a(mm)^b$				MIC ($\mu g/ml$)	MBC ($\mu g/ml$)
		Concentration of the disc					
		500(g/disc)	250(g/disc)	125(g/disc)	Ampicillin (10mg/disc)		
1.	*Streptococcus pyogenes*						
	Hexane	12.8±0.57	10.0±0.50	7.5±0.50	10.0±0.50	500	1000
	Chloroform	13.3±0.28	10.5±0.50	7.5±0.50	12.1±0.28	250	500
	Ethyl acetate	14.0±0.58	10.8±0.28	8.5±0.50	10.3±0.28	250	500
	Acetone	12.5±0.50	9.5±0.50	7.3±0.57	11.0±0.50	500	1000
	Methanol	11.0±0.50	9.3±0.76	7.1±0.28	12.1±0.28	500	1000
2.	*Escherichia coli*						
	Hexane	11.5±0.50	8.8±0.28	7.5±0.50	13.1±0.28	500	1000
	Chloroform	11.8±0.28	10.0±0.50	7.5±0.50	11.8±0.76	500	1000
	Ethyl acetate	13.8±0.28	11.1±0.28	7.8±0.76	11.0±0.5	250	500
	Acetone	11.0±0.50	9.5±0.50	7.3±0.57	10.5±0.50	500	1000
	Methanol	10.5±0.50	9.0±0.50	7.1±0.28	9.3±0.57	500	1000
3.	*Klebsiella pneumoniae*						
	Hexane	11.6±0.76	9.8±0.76	7.3±0.57	7.3±0.57	500	1000
	Chloroform	12.0±0.50	10.0±0.50	7.5±0.50	8.8±0.28	500	1000
	Ethyl acetate	13.5±0.50	10.6±0.76	7.8±0.76	7.8±0.50	250	500
	Acetone	11.5±0.50	9.8±0.57	7.3±0.57	9.3±0.57	500	1000
	Methanol	10.8±0.76	9.1±0.28	7.1±0.28	12.0±0.50	500	1000
4.	*Proteus mirabilis*						
	Hexane	11.5±0.50	9.3±0.28	7.1±0.28	10.3±0.28	500	1000
	Chloroform	13.0±0.50	10.3±0.76	7.8±0.76	9.3±0.57	250	500
	Ethyl acetate	13.8±0.28	11.1±0.28	8.1±0.28	10.8±0.50	250	500
	Acetone	11.8±0.28	9.5±0.50	7.1±0.28	8.8±0.76	500	1000
	Methanol	12.3±0.28	9.6±0.57	7.1±0.28	7.3±0.28	500	1000
5.	*Proteus vulgaris*						
	Hexane	11.5±0.50	9.0±0.57	7.5±0.50	13.1±0.28	500	1000
	Chloroform	12.5±0.50	10.3±0.57	8.0±0.50	11.0±0.50	500	1000
	Ethyl acetate	13.5±0.50	10.8±0.76	8.5±0.58	11.6±0.76	250	500
	Acetone	11.5±0.50	10.0±0.50	7.5±0.50	11.8±0.76	500	1000
	Methanol	10.5±0.50	9.5±0.50	7.1±0.28	12.1±0.28	500	1000

Table-2: Continued

SI.No	Seaweed extracts/ solvents	Mean zone of inhibition[a] $(mm)^b$				MIC $(\mu g/ml)$	MBC $(\mu g/ml)$
		Concentration of the disc					
		500(g/disc)	250(g/disc)	125(g/disc)	Ampicillin (10mg/disc)		
6.	*Pseudomonas aeruginosa*						
	Hexane	11.6±0.28	9.5±0.50	7.1±0.28	11.6±0.76	500	1000
	Chloroform	12.8±0.76	10.0±0.50	7.5±0.50	10.3±0.28	250	500
	Ethyl acetate	13.5±0.50	10.8±0.57	8.5±0.50	12.1±0.28	250	500
	Acetone	12.5±0.50	10.5±0.50	7.3±0.57	13.1±0.28	500	1000
	Methanol	10.6±0.28	9.1±0.28	7.1±0.28	11.0±0.50	500	1000
7.	*Salmonella typhimurium*						
	Hexane	12.0±0.50	9.8±0.76	7.1±0.28	11.0±0.50	500	1000
	Chloroform	12.8±0.28	10.0±0.50	7.8±0.76	11.0±0.50	250	500
	Ethyl acetate	13.5±0.50	10.8±0.76	8.1±0.28	11.6±0.76	250	500
	Acetone	12.0±0.50	9.6±0.28	7.6±0.57	10.3±0.57	500	1000
	Methanol	11.0±0.50	9.1±0.28	7.3±0.57	11.6±0.76	500	1000
8.	*Shigella dysentrieae*						
	Hexane	12.1±0.57	10.3±0.28	7.5±0.50	7.8±0.76	500	1000
	Chloroform	12.5±0.50	10.0±0.50	7.8±0.76	8.0±0.50	500	1000
	Ethyl acetate	13.8±0.28	10.8±0.28	8.6±0.57	11.0±0.50	250	500
	Acetone	12.3±0.57	10.0±0.50	7.5±0.50	12.8±0.28	500	1000
	Methanol	10.8±0.28	8.8±0.76	7.1±0.28	8.6±0.76	500	1000
9.	*Shigella flexneri*						
	Hexane	10.0±0.50	8.5±0.50	7.1±0.28	10.8±0.76	500	1000
	Chloroform	12.0±0.50	9.6±0.28	8.3±0.28	11.6±0.76	500	1000
	Ethyl acetate	13.5±0.50	9.8±0.76	9±0.86	12.0±0.86	250	500
	Acetone	11.8±0.28	9.5±0.50	7.5±0.50	12.1±0.28	500	1000
	Methanol	10.5±0.50	8.6±0.57	7.1±0.28	11.0±0.50	500	1000
10.	*Vibrio cholera*						
	Hexane	10.0±0.50	9.0±0.50	7.1±0.28	9.1±0.28	500	1000
	Chloroform	11.6±0.76	9.6±0.28	7.8±0.76	8.0±0.50	500	1000
	Ethyl acetate	13.6±0.50	9.8±0.76	8.1±0.28	9.3±0.57	250	500
	Acetone	11.8±0.28	9.5±0.50	7.5±0.50	8.3±0.28	500	1000
	Methanol	10.5±0.50	8.6±0.57	7.1±0.28	9.3±0.57	500	1000

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm; [b]-mean of three assays; ± - standard deviation. Positive control: Ampicillin (10 mg/disc) were between 8 and 13 mm.

4. DISCUSSION

Marine macroalgae use targeted antimicrobial chemical defence strategies and secondary metabolites important in the ecological interactions between marine macroorganisms and microorganisms. Therefore, they could be a promising source of novel bioactive compounds. Several metabolites with unusual structures have been isolated from the green marine macroalgae, and some of these metabolites are known to exhibit high order biological activities [8].

In present results indicated of the different solvents *viz.*, hexane, chloroform, ethyl acetate, acetone and methanol extracts of *C. racemosa* possessed antibacterial activity against all the clinical and standard bacterial strains tested. The ethyl acetate extract of *C. racemosa* showed the highest antibacterial activity than other extracts against *B. subtilis, S. pyogenes, E. coli, K. pneumoniae, P. mirabilis, P. vulgaris, P. aeruginosa, S. typhimurium, S. dysentriea, S. flexneri* and *V. cholerae*. The highest activity was displayed by ethyl acetate extract of *C. racemosa* against *B. substilis* the mean zone of inhibition (17.3 mm) followed by *S. pyogenes* (14.6), *P. mirabilis* (14.0 mm), and *S. dysentriae* (13.8 mm). The MIC values of the different extracts of C. racemosa ranged between 125 and 500 μg/ml, while the MBC values were between 250 and 1000 μg/ml. Similar observation The ethyl acetate extracts of *U. fasciata* showed highest antibacterial activity against multi-drug resistant bacterial strains *viz., B. subtilis, S. pyogenes, E. coli, K. pneumoniae, P. aeruginosa, S. typhimurium, V. cholerae, S. flexneri, P. mirabilis* and *P. vulgaris* [14]. Chandrasekaran et al. [13] reported that the ethyl acetate extracts of *Sargassum wightii* showed the highest antibacterial activity against multi-drug resistant bacterial strains *viz., B. subtilis, S. pyogenes, E. coli, K. pneumoniae, P. aeruginosa, S. typhimurium, V. cholerae, S. flexneri, S. dysentriae, P. mirabilis* and *P. vulgaris*.

Salem et al. [40] same reported that higher antibacterial activity was recorded for the ethyl acetate extracts of *C. racemosa, Sargassum dentifolium, Padina gymnospora*; methanolic extracts of *Sargassum hystrix, C. racemosa, C. fragile, S. dentifolium* and *Cystoseria myrica* against *E. coli, S. aureus, E. feacalis, Salmonella sp., B. cereus* and *P. aeruginosa*. These results were in close agreement with those obtained by [35]. It was revealed that the chloroform and ethyl acetate extracts of *Enteromorpha compressa, Chaetomorpha linum* and *Polysiphonia subtilissima* were active against *B. subtilis, Bacillus brevis, E. coli, S. flexneri* and *V. cholerae*.

In the present study, different solvents viz., hexane, chloroform, ethyl acetate, acetone and methanol extracts of *C. racemosa* possessed antibacterial activity against all the clinical and standard bacterial strains tested. Adaikala Raj et al. [3] reported that higher antibacterial activity was recorded for the ethyl acetate extracts of *Stoechospermum marginatum* and *Caulerpa chemnitzia* against *Bacillus subtilis, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Vibrio cholerae, Shigella flexneri, Proteus mirabilis* and *P. vulgaris*. In addition, these results confirmed the evidence in previous

studies reported that the ethyl acetate is a better solvent for more consistent extraction of antimicrobial substances from marine plants compared to other extracts such as hexane, chloroform, acetone and methanol [2].

In the present work, the ethyl acetate extract of *C. racemosa* possed highest antibacterial activity may due to the presence of phytochemicals, terpenoids, tannins, phenolic compound, and steroids. A wide range of compounds, particularly terpenes, polyphenolic compounds and steroids have been reported from various marine green algae [8], amongst which terpenoid compounds represent a major share. For example, *Caulerpa brownii* from Australia was reported to yield a number of bioactive novel diterpenoids and terpenoid esters [21]. The green alga, *C. racemosa*, was reported to yield a bioactive sesquiterpene acid [4]. Polyphenols were reported to have microbicidal activity against many pathogenic bacteria [43]. Phenolic compounds may affect growth and metabolism of bacteria. They could have an activating or inhibiting effect on microbial growth according to their constitution and concentration [37]. Zapata and Mc Millan, [47] reported that the role of phenolic compounds present in seagrasses could also enhance the antimicrobial activity. Steroid glycosides are a class of widespread natural products having either terrestrial or marine origins. Several cardiac glycosides are used therapeutically in the treatment of cardiac failure and atrial arrhytmias, and many glycoside compounds, belonging to other structural groups, show cytotoxic, antimicrobial, hypocholesterolemic and other biological activities [25].

In the present study, the different solvents *viz.*, hexane, chloroform, ethyl acetate, acetone and methanol extracts of *C. racemosa* possessed antibacterial activity against all the clinical and standard bacterial strains tested. The ethyl acetate extract of *C. racemosa* showed the highest antibacterial activity than other extracts against *B. subtilis*. The MIC values of the different extracts of *C. racemosa* ranged between 125 and 500 μg/ml, while the MBC values were between 250 and 1000 μg/ml. The genus Caulerpa has been widely studied, and the structures of many new compounds, such as di, sesqui- and mono-terpenes with the terminal 1,4-diacetoxybutadiene moiety and the nitrogen-containing compounds bisindole alkaloids and caulerpicin [28]. *C. racemosa*, were isolated from ethyl acetate extracts of *C. racemosa*, the discovery of one new polyacetylenic fatty acid, (8E, 12Z, 15Z)-10-hydroxy-8, 12, 15-octadecatrien-4, 6-diynoic acid [29]. Recently, the genus Caulerpa has attracted the attention of researchers due to its important secondary metabolite caulerpenyne (CYN) that is reported to exhibit the antineoplastic, antibacterial and antiproliferative activities. Further, it has also been shown to inhibit the cell division of sea urchin eggs as well as cancer cell lines [18]. Three species of Caulerpa namely *C. racemosa, C. scalpelliformis* and *C. veravelensis* have been found growing luxuriantly in the intertidal region during October February along the Veraval coast of Gujarat (north-western coast of India). Among these three species, *C. racemosa* with a wide distribution over both tropical and subtropical regions of the world has been studied in greater details for its chemical and mineral composition [24] while *C. veravelensis* and *C.*

scalpelliformis remained unexplored.

In the present study, the gram positive bacteria were more susceptible than the gram negative bacteria. The greater resistance of gram negative bacteria to plant extracts has been documented previously for seeds of *Syzygium jambolanum* [11] and bark of *Cassia siamea* [12].

Taskin et al. [44] reported that similar observations, indicating that the more susceptibility of Gram-positive bacteria to the algal extract was due to the differences in their cell wall structure and their composition [34]. In Gram-negative bacteria, the outer membrane acts as a barrier to many environmental substances including antibiotics. The presence of thick murine layer in the cell wall also prevents the entry of the inhibitors [30]. The reason for different sensitivity between gram positive and gram negative bacteria could be ascribed to the morphological differences between these microorganisms [5].

In this respect, the present study was conducted to evaluvate the antibacterial properties of ethyl acetate extract of *C. racemosa* of this plant would help for development of a new alternative medicine system which has no side effects. Although a large number of natural products have been approved as new antibacterial drugs, still there is an urgent need to identify more novel substances that are active towards pathogens of high resistance. The ethyl acetate extract of *C. racemosa* could be used as a potential natural antibacterial agent against the tested human pathogenic MDR bacterial strains.

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