Journal of Medicinal Herbs and Ethnomedicine 2015, 1(1): 24-31
http://scienceflora.org/journal/jmhe/
doi:10.5455/jmhe.2015-07-09

Original Article

Antibacterial activity of different solvent extracts of *Caulerpa chemnitzia* (Esper) J.V. Lamououx, from Mandapam, Gulf of Mannar Southeast Coast, Tamil Nadu, India

G. Adaikala Raj, M. Chandrasekaran*, S. Krishnamoorthy, V. Venkatesalu

Department of Botany, Annamalai University, Annamalai Nagar - 608 002, Chidambaram, Tamil Nadu, India

Received: 08.07.2015
Revised: 15.07.2015
Accepted: 15.07.2015

***Address for Correspondence:**
Dr. M. Chandrasekaran, Department of Botany, Annamalai University, Annamalai Nagar - 608 002, Chidambaram, Tamil Nadu, India. E-mail: chandruphd@yahoo.co.in

ABSTRACT

Phytochemical analyses and *in vitro* antibacterial activity of different extracts of hexane, chloroform, ethyl acetate, acetone, and methanol extracts of green algae, *Caulerpa chemnitzia* (Esper) J.V. Lamououx, against *Bacillus subtilis, Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella flexneri,* and *Vibrio cholerae*. The extent of the inhibitory zone, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined. The ethyl acetate extract of *C. chemnitzia* showed the presence of phytochemicals, terpenoids, tannins and phenolic compounds strongly 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 13.6 mm. The MIC was between 125 and 500 μg/mL while the MBC were between 250 and 1000 μg/mL. The highest mean zone of inhibition (13.6 mm) and the lowest MIC (125 μg/mL) and MBC (250 μg/mL) values were observed in ethyl acetate extract against *B. subtilis*. These findings suggest that the ethyl acetate extract of *C. chemnitzia* can be used as an antibacterial substance for the treatment of bacteria causing acquired infection.

KEY WORDS: Antibacterial activity, *Caulerpa chemnitzia,* Minimum Inhibitory Concentration, Minimum Bactericidal Concentration

INTRODUCTION

Infectious diseases are the leading cause of death worldwide and at the same time antibiotic resistance has become a global concern (Westh *et al*., 2004). 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 (Chow *et al*., 1991). 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 (MDR) (Nikaido, 2009).

MDR, a microorganism is an emerging serious problem in the health care sector. The improper usage of antibiotics contributes a major role in drug resistance in pathogenic microbes. Microorganisms acquire resistance toward common antibiotics by altering their metabolism and genetic structure (Raghunath, 2008). There is an incessant need to find novel efficient drug molecules against the multi-drug resistant microbes. The emergence of multiple drug resistant bacteria has become a major cause of failure of the treatment of infectious disease (Gibbons, 2005). Most important multi-drug resistant bacteria on the global scale include Gram-positive (methicillin-resistant *Staphylococcus aureus* [MRSA], vancomycin resistant *Enterococci*) and Gram-negative bacteria (members of *Enterobacteriaceae* producing plasmid-mediated extended spectrum β-lactamases and others like *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis* (Sajduda *et al*., 1998). *Klebsiella pneumoniae* carbapenemase (KPC) enzyme and

metallo β-lactamase are the common armamentaria of carbapenem resistance in *Enterobacteriaceae* (Cendejas *et al*., 2010). *Proteus mirabilis* strain has been recorded as showing resistance to a large number of antibiotics, therefore, and their control is very difficult (Doublet *et al*., 2010). As a result, the society is facing one of the most serious public health dilemmas over the emergence of infectious bacteria displaying resistance too many and in some cases, effective antibiotic (Kapil, 2005) much like the situation in human medicine. The use of antibiotics in agriculture, livestock and poultry has accelerated the development of antibiotic-resistant strains of microbial pathogens, potentially complicating treatment for plants, animals, and human (White *et al*., 2002).

Antibiotic chemotherapy is one of the most important medical achievements of the twentieth century. This therapy is widely practiced for the treatment of various microbiological infections; however, Fleming warned that the misuse of antibiotics could lead to the emergence of resistant forms of bacteria. These drug-resistant strains of microorganisms pose a greater threat to the global public health (Kaushik *et al*., 2008). Unfortunately, as we enter the new millennium many of our existing antibacterial agents are under threat due to the widespread emergence of bacterial resistance.

Seaweeds belong to a group of plants known as alga. Seaweeds are classified as *Rhodophyceae* (red algae), *Phaeophyceae* (brown algae) and *Chlorophyceae* (green algae) depending on their nutrient and chemical composition. Like other plants, seaweeds contain various inorganic and organic substances which can benefit the human health (Kuda *et al*., 2002). *Chlorophyceae* seaweeds, popularly known as green algae, are widely distributed in both inter-tidal and deep-water regions of the seas. *Chlorophyceae* are a large and important group of freshwater and marine green algae, which are important both ecologically 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 (Sandsdalen *et al*., 200). 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 (Blunt *et al*., 2006).

The *Caulerpa* genus has a record of stress on marine habitats (Mazumder, 2006), with a great impact on different species and communities of algae, sea grasses, marine invertebrates and fishes. *Caulerpa chemnitiza* green seaweed belongs to the class *Chlorophyceae* among the most abundant species of Gulf of Mannar region were selected for the study. In the present study was made to evaluate the antibacterial activity of different extracts of *Caulerpa chemnitzia* (Esper) J.V. Lamououx, against various bacterial strains.

MATERIALS AND METHODS

Sample Collection

C. chemnitzia (Esper) J.V. Lamououx, (*Chlorophyceae*) were collected from Mandapam, at (Latitude 09°17.417'N; Longitude 079°08.558'E) Ramanathapuram district, the Gulf of Mannar Marine Biosphere, Tamil Nadu, India. The collections were made during the months of November to December 2011 during the low tide. The alga was identified by Dr. R. Selvaraj, Former Professor, Department of Botany, Annamalai University and the museum specimens are deposited in the Department of Botany, Annamalai University, Annamalai Nagar.

Preparation of Extracts

The algal species were handpicked during low tide and washed thoroughly with the sea water to remove all unwanted impurities, epiphytes, animal casting and adhering sand particles etc., morphologically distinct thallus of alga were placed separately in new polyethene bags and were kept in an ice box containing slush ice and transported to the laboratory. Further they were washed thoroughly with the tap water to remove the salt on the surface of the sample. The water was drained off and the algae were spread on blotting paper to remove the excess water. The shade dried samples were again cleaned with the distilled water to remove the salt remaining on the surface of the sample.

The powdered algal materials were extracted by using Soxhlet apparatus and 500 g of plant material was packed inside a Soxhlet apparatus and the successive extraction was carried out using solvent systems like hexane, chloroform, ethyl acetate, acetone, and methanol for 72 h. The solvent was evaporated under vacuum in a rotary evaporator (Heidolph, Germany) and the dried extracts were stored at 4°C for antibacterial assay.

Phytochemical Screening

The hexane, chloroform, ethyl acetate, acetone and methanol extracts of *C. chemnitzia* were used for qualitative phytochemical studies. Screening of phytochemicals like terpenoids, tannins, cardic glycosides, steroids, alkaloids,

phenolic compounds and coumarins were carried out according to the standard method described by Harborne (1973) and Trease and Evans (1983).

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), *Proteus mirabilis* (MTCC 425), *Proteus vulgaris* (MTCC 426), *Pseudomonas aeruginosa* (MTCC 741), *Salmonella typhimurium* (MTCC 98), *Shigella flexneri* (MTCC 1457) and *Vibrio cholerae* (MTCC 3906) were procured from Microbial Type Culture Collection (MTCC), Chandigarh. These strains were maintained on nutrient agar slant at 4°C.

Antibiotic Sensitivity Test

Antibiotic sensitivity of the bacterial strains was determined by standard Clinical Laboratory Standards Institute (CLSI) disc diffusion method (CLSI, 2012) using different classes of antibiotics *viz.,* amikacin (AK, 3 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) (Himedia, Mumbai, Maharashtra, India).

Anti-bacterial Assay

Disc diffusion method

The antibacterial activity of different extracts of *C. chemnitzia* was determined by disc diffusion method according to Bauer *et al*. (1966) with modifications. Petri dishes were prepared by pouring 20 mL of Muller Hinton Agar. Then the plates were allowed to solidify and used in susceptibility test. The standardized inoculum using bacterial suspensions containing 10^8 colony forming units (CFU) per mL were swabbed on the top of the solidified media and allowed to dry for 10 min. The algal extracts was dissolved in 10% dimethyl sulfoxide (DMSO) and under aseptic conditions, sterile discs were impregnated with 20 µl of three different concentrations of the algae extracts (500, 250 and 125 µg/disc). The discs with algae extracts were placed on the surface of the medium with sterile forceps and gently pressed to ensure contact with inoculated agar surface. AMP (10 µg/disc) was used as a positive antibacterial control and 10% DMSO was used as a blind control in all the assays. Finally, the inoculated plates were incubated at 37°C for 24 h for all bacterial strains tested. The zones of inhibitions were observed and measured in millimeters. The assay in this experiment was repeated 3 times.

Microdilution Broth Assay

Determination of the minimum inhibitory concentration (MIC)

The MIC was determined for the algae extracts were determined in Mueller Hinton Broth (MHB) by using a modified reaszurin microtitre plate assay was carried out according to the method of Sarker *et al.* (2007). 50 µl of Sterile MHB were transferred into each well of a sterile 96-well micro titer plate. The algae extracts were dissolved in 10 percent DMSO to obtain 2000 µg/mL stock solutions respectively. A volume of 50 µl of algae extracts stock solution was added to the first well. After fine mixing of the crude extracts and 50 µl of the broth 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 to 15.625 µg/mL of the algae extract in each well. To each well, 10 µL of resazurin indicator solution was added (The resazurin solution was prepared by dissolving a 270 mg tablet in 40 mL of sterile distilled water. A vortex mixer was used to ensure that it was a well dissolved and homogenous solution). Finally, 10 µl of the bacterial suspension was added to each well to achieve a concentration of approximately 5×10^5 CFU/mL. Each plate had a set of controls: a column with all solutions with the exception of the algae extracts; a column with all solutions with the exception of the bacterial solution adding 10 µl of MHB instead and a column with 10% DMSO solution as a negative control. The plates were incubated at 37°C for 24 h for all the bacterial strains tested. The color change was then assessed visually. The growth was indicated by color changes from purple to pink (or colorless). The lowest concentration at which color change occurred was taken as the MIC value.

Determination of the minimum bactericidal concentration (MBC)

The MBC of the algae extracts was determined by plating a loop full of samples 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 tested. The MBC was recorded as the lowest concentration of the extract that did not permit any visible bacterial growth after the period of incubation.

Statistical Analysis

The results are expressed as the mean ± standard deviation. All statistical analyses were performed using SPSS version 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Student's *t*-test was performed to determine any significant difference between different extracts for *in vitro* antibacterial assays. Comparison of means for *in vitro*

antibacterial assessment was carried out using one-way analysis of variance (ANOVA) and Duncan test. $P < 0.05$ was considered statistically significant.

RESULTS

The hexane, chloroform, ethyl acetate, acetone and methanol extracts of *C. chemnitzia* were used for the analyses of phytochemicals, terpenoids, tannins, cardiac glycosides, steroids, alkaloids, phenolic compounds, and coumarins. The ethyl acetate extracts of *C. chemnitiza* showed the presence of phytochemicals terpenoids, tannins and phenolic compounds strongly than the other solvents extracts. Cardiac glycosides were present in all the extracts except acetone and methanol extracts. Alkaloids and coumarins are not present in all the extracts tested.

The MDR profile, of bacterial strains was confirmed by the CLSI-M100-2012 method. The *B. subtilis, K. pneumoniae* and *P. vulgaris* were sensitive to all the antibiotics tested except CFM, AMP and CAZ. The *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 *E. coli* were sensitive to all antibiotics tested except AMP and NA. The *P. aeruginosa* were resistant to CFM, AMP and TE and sensitive to all other antibiotics tested. The *S. typhimurium* were sensitive to all antibiotics except AMP and E. The *V. cholera*e were resistant AMP and intermediate resistant to S and sensitive to all other antibiotics tested.

In the present study, different solvents of hexane, chloroform, ethyl acetate, acetone and methanol extracts of *C. chemnitzia* were studied against the bacterial strains tested. The different extracts were assayed against the test bacteria by disc diffusion assays, the mean zones of inhibition obtained were between 7.1 and 13.6 mm. All the extracts of *C. chemnitzia* significant showed antibacterial activity against all the tested bacterial strains when compared to the available other tested antibiotics. The mean values are presented in Table 1. The highest mean zone of inhibition (13.6 mm) was observed in the ethyl acetate extract of *C. chemnitzia* against *B. substilis* followed by *S. pyogenes* (13.3) *S. flexneri* (13.0 mm) *P. mirabilis* (13.1 mm) and *V. cholerae* (13.0 mm). AMP (10 µg/disc) antibacterial positive control produced mean zone of inhibition ranged from 7.3 to 12.3 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. chemnitiza* ranged between 125 and 500 µg/mL while the MBC values were between 250 and 1000 µg/mL.

DISCUSSION

Marine macroalgae use the targeted antimicrobial chemical defense strategies and secondary metabolites which are 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 (Blunt *et al*., 2006).

In present results indicated that the different solvents *viz.,* hexane, chloroform, ethyl acetate, acetone and methanol extracts of *C. chemnitzia* significant antibacterial activity against all bacterial strains tested. The ethyl acetate extract of *C. chemnitzia* 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 antibacterial activity was displayed by ethyl acetate extract of *C. chemnitiza* against *B. substilis* the mean zone of inhibition (13.6 mm) followed by *S. pyogenes* (13.3) *S. flexneri* (13.0 mm) *P. mirabilis* (13.0 mm) and *V. cholera* (13.0 mm). The MIC values of the different extracts of *Caulerpa racemosa* ranged between 125 and 500 µg/mL, while the MBC values were between 250 and 1000 µg/mL. 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* (Chandrasekaran *et al*., 2014). Chandrasekaran *et al*. (2014) 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*. (2011) reported that the higher antibacterial activity was recorded for the ethyl acetate extracts of *C. racemosa*, *Sargassum dentifolium*, *Padina gymnospora*; methanol extracts of *Sargassum hystrix*, *C. racemosa*, *Codium fragile*, *S. dentifolium,* and *Cystoseria myrica* against *E. coli*, *S. aureus*, *Enterococcus faecalis*, *Salmonella* sp., *Bacillus cereus,* and *P. aeruginosa*.

In the present study, different solvents *viz.,* hexane, chloroform, ethyl acetate, acetone and methanol extracts of *C. chemnitzia* significant antibacterial activity against all the bacterial strains tested. Karthikaidevi *et al*. (2009) reported that the methanol and ethyl acetate extract of *Codium adherens*, *Ulva reticulata* and *Halimeda tuna* possessed antibacterial activirty. Chandrasekaran *et al*.

Table 1: Antibacterial activity of different extracts of *Caulerpa chemnitzia*

Bacterial strains/seaweed extracts prepared with different solvents	Mean zone of inhibition[a] (mm)[b] Concentration of the disc					
	500 (μg/disc)	250 (μg/disc)	125 (μg/disc)	Ampicillin (10 μg/disc)	MIC (μg/mL)	MBC (μg/mL)
Bacillus subtilis (MTCC 441)						
Hexane	11.0±0.50	9.7±0.76	7.3±0.57	10.8±0.76	250	250
Chloroform	13.0±0.28	10.3±0.57	8.1±0.28	10.0±0.50	250	500
Ethyl acetate	13.6±0.76**	10.6±0.76	8.5±0.50	12.3±0.57	125	250
Acetone	12.3±0.57	91±0.28	7.5±0.76	9.1±0.28	500	1000
Methanol	11.1±0.28	9.0±0.50	7.1±0.28	9.5±0.50	500	1000
Streptococcus pyogenes (MTCC442)						
Hexane	11.0±0.50	9.5±0.50	7.3±0.57	7.3±0.28	500	1000
Chloroform	12.6±0.28	10.0±0.76	7.5±0.50	7.8±0.28	250	500
Ethyl acetate	13.3±0.57	10.5±0.28	8.8±0.50	10.3±0.57	250	500
Acetone	12.5±0.50	10.1±0.28	7.3±0.57	11.6±0.50	500	1000
Methanol	11.0±0.50	9.3±0.57	7.1±0.28	12.1±0.50	500	1000
Escherichia coli (MTCC 443)						
Hexane	10.1±0.28	9.0±0.50	7.5±0.50	8.6±0.76	500	1000
Chloroform	12.0±0.50	9.5±0.86	7.6±0.57	9.3±0.57	500	1000
Ethyl acetate	12.8±0.28	11.1±0.28	8.5±0.50	7.3±0.28	250	500
Acetone	11.6±0.76	9.1±0.28	7.5±0.50	11.0±0.50	500	1000
Methanol	10.3±0.57	8.8±0.76	7.1±0.28	9.3±0.57	500	1000
Klebsiella pneumoniae (MTCC 109)						
Hexane	11.1±0.28	9.8±0.28	7.5±0.5	8.0±0.5	500	1000
Chloroform	12.0±0.50	9.5±0.86	7.8±0.76	10.0±0.76	500	1000
Ethyl acetate	12.8±0.28	10.5±0.50	8.3±0.28	7.8±0.76	250	500
Acetone	11.1±0.28	10.0±0.50	7.5±0.50	9.3±0.57	500	1000
Methanol	10.1±0.76	8.8±0.28	7.1±0.28	8.8±0.76	500	1000
Proteus mirabilis (MTCC 425)						
Hexane	11.8±0.76	10.6±0.57	7.8±0.76	8.0±0.50	500	1000
Chloroform	12.6±0.76	10.0±0.50	7.5±0.50	8.8±0.76	500	1000
Ethyl acetate	13.0±0.50	11.6±0.28	9.3±0.57	9.3±0.57	250	500
Acetone	11.5±0.50	9.6±0.76	7.5±0.50	8.8±0.76	500	1000
Methanol	11.0±0.50	9.3±0.57	7.3±0.57	10.3±0.57	500	1000
Proteus Vulgaris (MTCC 426)						
Hexane	11.6±0.76	9.8±0.28	7.5±0.50	11.6±0.76	500	1000
Chloroform	12.5±0.50	10.0±0.50	7.1±0.28	10.3±0.28	500	500
Ethyl acetate	12.8±0.76	10.1±0.28	9.8±0.28	12.1±0.28	250	500
Acetone	12.0±0.50	9.8±0.28	7.5±0.50	10.3±0.57	500	1000
Methanol	10.6±0.76	9.1±0.28	7.1±0.28	8.6±0.57	500	1000
Pseudomonas aeruginosa (MTCC 741)						
Hexane	10.1±0.28	9±0.50	7.1±0.28	8.0±0.50	500	1000
Chloroform	11.8±0.76	9.3±0.28	7.3±0.57	9.3±0.57	500	1000
Ethyl acetate	13.0±0.50	10.5±0.50	8.8±0.76	11.6±0.76	250	500
Acetone	12.1±0.28	10.1±0.76	7.3±0.57	12.1±0.28	500	1000
Methanol	10.3±0.57	8.8±0.28	7.3±0.28	12.8±0.28	500	1000
Salmonella typhimurium (MTCC 98)						
Hexane	12.0±0.50	10.0±0.50	7.3±0.57	8.6±0.76	500	1000
Chloroform	12.8±0.28	10.6±0.76	7.8±0.76	10.8±0.76	250	500
Ethyl acetate	13.3±0.28	11.1±0.28	8.5±0.50	8.6±0.76	250	500
Acetone	12.1±0.28	10.1±0.50	7.6±0.57	10.3±0.57	500	1000
Methanol	12.1±0.28	9.8±0.28	7.3±0.57	7.3±0.57	500	1000
Shigella flexneri (MTCC 1457)						
Hexane	10.0±0.50	9.1±0.57	7.1±0.28	12.0±0.5	500	1000
Chloroform	12.6±0.76	9.5±0.50	7.5±0.28	11.6±0.76	500	1000
Ethyl acetate	13.1±0.28	10.3±0.76	8.6±0.76	10.3±0.28	250	500
Acetone	10.8±0.28	9.3±0.57	7.3±0.57	12.8±0.57	500	1000
Methanol	10.0±0.50	9.1±0.28	7.1±0.28	10.3±0.57	500	1000
Vibrio cholerae (MTCC 3906)						
Hexane	11.0±0.50	9.6±0.57	7.1±0.28	7.8±0.76	500	1000
Chloroform	12.5±0.50	10.0±0.50	7.5±0.50	8.8±0.76	500	1000
Ethyl acetate	13.0±0.50	10.5±0.50	7.8±0.76	9.3±0.57	250	500
Acetone	12.0±0.50	10.0±0.50	7.5±0.50	11.1±0.28	500	1000
Methanol	11.0±0.50	9.1±0.28	7.3±0.57	10.8±0.28	500	1000

[a]Diameter of the zone of inhibition (mm) including the disc diameter of 6 mm; [b]Mean of three assays; ±: Standard deviation; **Significant at $P<0.05$. MTCC: Microbial Type Culture Collection, MBC: Minimum Bactericidal Concentration, MIC: Minimum Inhibitory Concentration

(2006) reported that the highest antibacterial activity were recorded in the brown alga, *Stoechospermum marginatum* against MRSA and vancomycin resistant *E. faecalis* in ethyl acetate extracts when compared to other solvents. The hexane, ethyl acetate and methanol extracts of *Dictyota dichotoma*, *Cystoseira crinita*, *Cystoseira barbata*, *Dictyopteris membranaceae*, *Sargussum vulgare*, *Flabellia petiollata*, *Anadypméne stellata*, *C. fragile*, *H. tuna*, *Ulva rigida*, *Cymodocea nodosa*, *Posidonea oceanica*, *Halophila stipulacea* showed antimicrobial activity against the *E. coli*, *Listeria monocytogéne*, *Salmonella enterica*, *Agrobacterium tumefaciens*, *Pseudomonas aerigunosa*, *S. aureus*, *Micrococcus luteus*, *Candida tropicalis*, *Saccharomyces cerevisiae* and *Aspergillus niger* (Abdallah Kolsi *et al*., 2015).

In the present work, the ethyl acetate extract of *C. chemnitzia* showed significant antibacterial activity may be due to the presence of phytochemicals, terpenoids, tannins and a phenolic compound. A wide range of compounds, particularly terpenes, polyphenolic compounds and steroids, have been reported from various marine green algae (Blunt *et al*., 2006), 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 (Handley and Blackman, 2005). Polyphenols were reported to have microbicidal activity against many pathogenic bacteria (Scalbert, 1991). 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 (Reguant *et al*., 2000). Zapata and McMillan (1979) reported that the role of phenolic compounds present in sea grasses could also enhance the antimicrobial activity.

In the present study, the different solvents *viz.,* hexane, chloroform, ethyl acetate, acetone and methanol extracts of *C. chemnitzia* possessed antibacterial activity against all the bacterial strains tested. The ethyl acetate extract of *C. chemnitzia* showed the highest antibacterial activity than other extracts against *B. subtilis*. The lowest MIC (125 µg/mL) and MBC (250 µg/mL) value were observed in the ethyl acetate extract of *C. chemnitzia* against *B. subtilis*. 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 (Mao *et al*., 2006). The discovery of one new polyacetylenic fatty acid, (8E, 12 Z, 15Z)-10-hydroxy-8, 12, 15- octadecatrien-4, 6-diynoic acid was isolated from the ethyl acetate extracts of *C. racemosa* (Mao *et al*., 2011).

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* (Chandrasekaran and Venkatesalu, 2004) and bark of *Cassia siamea* (Chandrasekaran and Venkatesalu, 2004).

Taskin *et al*. (2001) 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 (Paz *et al*., 1995). In Gram-negative bacteria, the outer membrane acts as a barrier to many environmental substances including antibiotics (Tortora *et al*., 2001). The presence of thick murine layer in the cell wall also prevents the entry of the inhibitors (Martin, 1995). The reason for different sensitivity between gram positive and gram negative bacteria could be ascribed to the morphological differences between these microorganisms (Arias *et al*., 2004).

CONCLUSION

The results of the antibacterial activity suggested that ethyl acetate extract of *C. chemnitzia* would help for the 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. chemnitiza* could be used as a potential natural antibacterial agents against the tested human pathogenic bacterial strains.

ACKNOWLEDGMENT

The authors acknowledge great fully the financial support sanctioned by the University Grants Commission, New Delhi under Major Research Project Programme (F. No.: 40-312/2011(SR) dated: 30.06.2011). The authors are thankful to Dr. K. Arumugam, Professor and Head, Department of Botany, Annamalai University for providing laboratory facilities.

REFERENCES

Abdallah Kolsi RB, Frikha D, Jribi I, Hamza A, Feki L, Belghith, K. Screening of antibacterial and antifongical activity in marine macroalgae and magnoliophytea from the coast of tunisia. Int J Pharm Pharm Sci 2015;7:47-51.

Arias ME, Gomez JD, Cudmani NM, Vattuone MA,

Isla MI. Antibacterial activity of ethanolic and aqueous extracts of acacia aroma Gill. ex Hook et Arn. Life Sci 2004;75:191-202.

Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966;45:493-6.

Blunt JW, Copp BR, Munro MH, Northcote PT, Prinsep MR. Marine natural products. Nat Prod Rep 2006;23:26-78.

Cendejas E, Gómez-Gil R, Gómez-Sánchez P, Mingorance J. Detection and characterization of *Enterobacteriaceae* producing metallo-beta-lactamases in a tertiary-care hospital in Spain. Clin Microbiol Infect 2010;16:181-3.

Chandrasekaran M, Venkatesalu V, Adaikala Raj G, Krishnamoorthy S. Antibacterial activity of *Ulva fasciata* against multidrug resistant bacterial strains. Int Lett Nat Sci 2014;14:40-51.

Chandrasekaran M, Venkatesalu V, Adaikala Raj G, Krishnamoorthy S. Antibacterial properties of various extracts of *Sargassum wightii* against multidrug resistant bacterial strains. Phykos 2014;44:17-28.

Chandrasekaran M, Venkatesalu V, Adaikala Raj G. Antibacterial activity of selected marine macro algae against vancomycin resistant *Enterococcus faecalis*. J Coast Life Med 2014;2:940-6.

Chandrasekaran M, Venkatesalu V, Adaikala Raj G. Anti-MRSA activity of brown and red algae from the gulf of Mannar coast, South India. Int J Life Sci Technol 2014;7:22-31.

Chandrasekaran M, Venkatesalu V. Antibacterial activity of bark of *Cassia siamea*. Indian Drugs 2004;41:298-89.

Chandrasekaran M, Venkatesalu V. Antibacterial and antifungal activity of *Syzygium jambolanum* seeds. J Ethnopharmacol 2004;91:105-8.

Chow JW, Fine MJ, Shlaes DM, Quinn JP, Hooper DC, Johnson MP, *et al*. Enterobacter bacteremia: Clinical features and the emergence of antibiotic resistance during therapy. Ann Intern Med 1991;115:585-90.

Clinical Laboratory Standards Institute (formerly NCCLS): Performance Standards for Antimicrobial Disk Susceptibility Tests: M100-S22, CLSI Vol. 32, No. 3, January 2012.

Doublet B, Poirel L, Praud K, Nordmann P, Cloeckaert A. European clinical isolate of *Proteus mirabilis* harbouring the *Salmonella* genomic island 1 variant SGI1-O. J Antimicrob Chemother 2010;65:2260-2.

Gibbons S. Plants as a source of bacterial resistance modulators and anti-infective agents. Phytochem Rev 2005;4:63-78.

Handley JT, Blackman AJ. Secondary metabolites from the marine alga *Caulerpa brownii* (*Chlorophyta*). Aust J Chem 2005;58:39-46.

Harborne IB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 2nd ed. New York Chapman and Hall; 1973. p. 88-185.

Kapil A. The challenge of antibiotic resistance: Need to contemplate. Indian J Med Res 2005;121:83-91.

Karthikaidevi G, Manivannan K, Thirumaran G, Anantharaman P, Balasubaramanian T. Antibacterial properties of selected green seaweeds from Vedalai coastal waters; the gulf of Mannar marine biosphere reserve. Glob J Pharmacol 2009;3:107-12.

Kaushik P, Chauhan A, Chauhan G, Goyal P. Evaluation of *Nostoc commune* for potential antibacterial activity and UV-HPLC analysis of methanol extract. Int J Microbiol 2008;5:1-5.

Kuda T, Taniguchi E, Nishizawa M, Araki Y. The fate of water soluble polysaccharides in dired *Chorda filum* a brown alga during water washing. J Food Compos Anal 2002;15:3-9.

Mao SC, Guo YW, Shen X. Two novel aromatic valerenane-type sesquiterpenes from the Chinese green alga *Caulerpa taxifolia*. Bioorg Med Chem Lett 2006;16:2947-50.

Mao SC, Liu DQ, Yu XQ, Lai XP. A new polyacetylenic fatty acid and other secondary metabolites from the Chinese green alga *Caulerpa racemosa* (*Caulerpaceae*) and their chemotaxonomic significance. Biochem Syst Ecol 2011;39:253-7.

Martin GJ. Ethnobotany: A Methods Manual. London: Champan and Hall; 1995.

Mazumder S. Industrial polysaccharides from natural sources: Structure and function. Ph.D. Thesis. The University of Burdwan, Burdwan, India. 2006. p. 1-69.

Nikaido H. Multidrug resistance in bacteria. Ann Rev Biochem 2009;78:8.1-.28.

Paz EA, Lacy RN, Bakhtian M. The B-Lactum Antibiotics Penicillin and Cephalosporin in Perspective. London: Hodder Strong; 1995. p. 324.

Raghunath D. Emerging antibiotic resistance in bacteria with special reference to India. J Biosci 2008;33:593-603.

Reguant C, Bordons A, Arola L, Rozès N. Influence of phenolic compounds on the physiology of *Oenococcus oeni* from wine. J Appl Microbiol 2000;88:1065-71.

Sajduda A, Dziadek J, Dela A, Zalewska-Schönthaler N, Zwolska Z, McFadden J. DNA fingerprinting as an indicator of active transmission of multidrug-resistant tuberculosis in Poland. Int J Infect Dis 1998;3:12-7.

Salem WM, Galal H, Nasr El-deen F. Screening for antibacterial activities in some marine algae from the red sea (Hurghada, Egypt). Afr J Microbiol Res 2011;5:2160-7.

Sandsdalen E, Haug T, Stensvag K, Styrvold OB. The antibacterial effect of a polyhydroxylated fucophlorethol from the marine brown alga, *Fucus vesiculosus*. World J Microbiol Biotechnol 2003;19:777-82.

Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. Methods 2007;42:321-4.

Scalbert A. Antimicrobial properties of tannins. Phytochem 1991;30:3875-83.

Taskin E, Ozturk M, Kurt O. Antibacterial activities of

some marine algae from the Aegean Sea (Turkey). Afr J Biotechnol 2001;6:2746-51.

Tortora GJ, Funke BR, Case CL. Microbiology: An Introduction. San Francisco: Benjamin Cummings; 2001. p. 88.

Trease GE, Evans WC. Textbook of Pharmacognosy. 12th ed. London: Balliese Tindall and Company Publisher; 1983. p. 343-83.

Westh H, Zinn CS, Rosdahl VT. An international multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries. Microb Drug Resist 2004;10:169-76.

White DG, Zhao S, Simjee S, Wagner DD, McDermott PF. Antimicrobial resistance of foodborne pathogens. Microbes Infect 2002;4:405-12.

Zapta O, Mc Millan C. Phenolic acid in seagrasses. Aquat Bot 1979;7:307-17.