

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

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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 $\mu\text{g/mL}$ while the MBC were between 250 and 1000 $\mu\text{g/mL}$. The highest mean zone of inhibition (13.6 mm) and the lowest MIC (125 $\mu\text{g/mL}$) and MBC (250 $\mu\text{g/mL}$) 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 chemnitzia* 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, cardiac 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 10⁸ 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 resazurin 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 × 10⁵ 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. chemnitzia* 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. cholerae* 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. subtilis* 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. chemnitzia* 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. dysenteriae*, *S. flexneri*, and *V. cholerae*. The highest antibacterial activity was displayed by ethyl acetate extract of *C. chemnitzia* against *B. subtilis* 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. cholerae* (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*, *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 activity. 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)
<i>Bacillus subtilis</i> (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	9.1±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
<i>Streptococcus pyogenes</i> (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
<i>Escherichia coli</i> (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
<i>Klebsiella pneumoniae</i> (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
<i>Proteus mirabilis</i> (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
<i>Proteus Vulgaris</i> (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
<i>Pseudomonas aeruginosa</i> (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
<i>Salmonella typhimurium</i> (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
<i>Shigella flexneri</i> (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
<i>Vibrio cholerae</i> (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

^aDiameter of the zone of inhibition (mm) including the disc diameter of 6 mm; ^bMean 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 membranacea*, *Sargassum vulgare*, *Flabellia petiolata*, *Anadypmène stellata*, *C. fragile*, *H. tuna*, *Ulva rigida*, *Cymodocea nodosa*, *Posidonea oceanica*, *Halophila stipulacea* showed antimicrobial activity against the *E. coli*, *Listeria monocytogène*, *Salmonella enterica*, *Agrobacterium tumefaciens*, *Pseudomonas aeruginosa*, *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. chemnitzia* could be used as a potential natural antibacterial agents against the tested human pathogenic bacterial strains.

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