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Phytochemical and antimicrobial activity of green microalgae from Vellar Estuary, southeast coast of India

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ABSTRACT

Objective: To determine the phytochemicals and screen the antimicrobial potential of *Desmococcus olivaceus* (*D. olivaceus*) and *Chlorella vulgaris* (*C. vulgaris*) against human bacterial pathogens.

Methods: *D. olivaceus* and *C. vulgaris* were collected and confirmed through morphological structures. Phytochemicals were analyzed and confirmed by chemical methods. Thin layer chromatography was performed to evaluate the bioactive compounds present in the solvent extracts. Antimicrobial activity of the extract obtained from dried green microalgae was tested against Gram negative and Gram positive bacteria using agar disc diffusion method. The Gram negative bacteria like *Klebsiella pneumoniae*, *Proteus mirabilis*, *Vibrio cholerae*, *Salmonella typhi*, *Escherichia coli* and Gram positive bacteria including *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus* sp., *Clostridium botulinum* (*C. botulinum*) and *Nocardia* sp. were selected for analysis. The cultures were obtained from Microbial Culture Maintenance Laboratory, Department of Medical Microbiology, Rajah Muthaiah Medical College, Annamalai University, Tamil Nadu, India.

Results: Seven bioactive compounds were detected viz., phenols, tannin, flavanoids, saponins, terpenes, carbohydrates, alkaloids and steroids. In Gram negative bacteria, *Proteus mirabilis* treated with ethanol extract of *D. olivaceus* showed the maximum inhibition zone (17.2 mm), whereas in Gram positive bacteria, *C. botulinum* treated with diethyl ether extract showed inhibition zone of 19.0 mm. In test for *C. vulgaris*, Gram negative bacteria *Salmonella typhi* treated with ethanol extract showed the maximum inhibition zone (13.0 mm), whereas in Gram positive bacteria, *C. botulinum* treated with chloroform extract of *C. vulgaris* showed the maximum inhibition zone of 15 mm.

Conclusions: The results clearly indicated that *D. olivaceus* and *C. vulgaris* contain promising antibacterial compounds.

1. Introduction

Microalgae were considered as natural resource of various biologically and pharmacologically vigorous compounds with structurally composite molecules which are difficult to be produced by chemical synthesis. There is a great demand for beneficial drugs obtained from the vast natural resources. The need to find new bioactive molecules from marine organisms is extremely

increasing. Marine organisms are the major producers of organic matter. More than sixty trace elements including minerals, proteins and many other bioactive substances are present in the ocean[1]. The researchers pay more attention towards marine plants for their potent bioactive secondary or primary metabolites with potential for their use in the development of new pharmaceutical agents[2,3], and many of these substances have been confirmed to possess interesting biological activities[4,5]. Marine organisms were reported to produce a wide range of bioactive secondary metabolites as antimicrobial, antioxidant, antihelmintic and cytotoxic agents, and bioactive substances included alkaloids, polyketides, cyclic peptides, polysaccharides, phlorotannins, diterpenoids, sterols, quinones, lipids and glycerols[6]. Marine macroalgae are considered as the actual producers of some bioactive compounds with high activity[7]. Marine microalgae are a natural source of a variety of compounds for pharmaceutical, food and cosmetic applications including

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carotenoids, terpenoids, steroids, amino acids, phlorotannins, phenolic compounds, halogenated ketones, alkanes and cyclic polysulphides[8,9]. The aqueous and solvent extract from algae was tested against Gram positive and Gram negative bacteria[10-13]. Therefore, the present study was aimed to screen the antimicrobial potential of *Desmococcus olivaceus* (*D. olivaceus*) and *Chlorella vulgaris* (*C. vulgaris*) against human bacterial pathogens.

2. Materials and methods

Two species of microalgae viz., *D. olivaceus* and *C. vulgaris* were collected from Vellar Estuary, Parangipettai, southeast coast of India. The species were identified according to the morphological characters microscopically[14] and used for the preparation of different solvent extracts. The algae samples were cleaned, then rinsed with sterile water to remove any associated debris. These cleaned fresh materials were allowed to air dry and then pulverized with the help of a blender. The powder (5 g) was filled in sterile tubes and extracted with methanol, ethanol, chloroform and diethyl ether by using a rotary evaporator apparatus at 40 °C for 12 h. From the solvent extracts, 5 mL was collected separately, allowed to dry at room temperature and weighed to estimate the concentration in 1 mL. The dry extracts were completely dissolved in 5 mL of 0.5% Tween 80 and preserved at 5 °C in bottles with airtight screw cap until further use[15], and the extracts were used for further antimicrobial studies. Tween 80 was mixed with double distilled water and served as control for all the experiments. All the experiments were carried out in triplicates. Antimicrobial study was carried out by disc diffusion method[16] against the Gram negative bacteria including *Klebsiella pneumonia* (*K. pneumonia*), *Proteus mirabilis* (*P. mirabilis*), *Vibrio cholerae* (*V. cholerae*), *Salmonella typhi* (*S. typhi*), *Escherichia coli* (*E. coli*) and Gram positive bacteria including *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), *Enterococcus* sp., *Clostridium botulinum* (*C. botulinum*) and *Nocardia* sp.

Phytochemical analysis of the extract was carried out using chemical methods and the phytochemicals were confirmed by running thin layer chromatography according to the proposed methodology[17,18].

3. Results

Two marine green algae (*D. olivaceus* and *C. vulgaris*) were extracted with four different solvents viz., methanol, ethanol, chloroform and diethyl ether. The phytochemicals present in the algae were identified as phenols, tannin, flavonoids, terpenes, carbohydrates, terpenoids, alkaloids, and saponins (Table 1). The ethanol extract of *D. olivaceus* contained flavonoids, alkaloids,

carbohydrates and saponins which were present in trace amount, and phenol, tannin, terpenes and terpenoids were absent. Moderate amount of flavonoids and terpenes was noticed in methanol extract of *D. olivaceus*, whereas chloroform extract showed moderate amount of flavonoids. In diethyl ether extract, except terpenoids and alkaloids, all the phytochemicals were absent. In *C. vulgaris*, flavonoids were detected in higher amount in ethanol, methanol and chloroform extracts, and in trace amount in diethyl ether extract. Terpenes, carbohydrates, terpenoids and alkaloids were found in trace amount in ethanol extract of *C. vulgaris*, but phenol content was purely not observed except in methanol extracts. Likewise, tannin was absent in all the extracts and trace amount of saponins was noticed in ethanol extract of *D. olivaceus*. Moderate amount of terpenes and carbohydrates and trace amount of phenols and alkaloids were observed in methanolic extracts of *C. vulgaris*. Carbohydrates were present moderately and trace amount of alkaloids was observed in chloroform extracts, and only flavonoids and alkaloids were present in trace amount in diethyl ether extract of *C. vulgaris*.

The methanolic, ethanolic, chloroform and diethyl ether extracts were tested for antimicrobial activity against ten pathogens. In Gram negative bacteria, the maximum inhibition activity of *D. olivaceus* was found against *P. mirabilis* (17.20 mm), whereas in Gram positive bacteria, *C. botulinum* treated with diethyl ether and ethanol extract of *D. olivaceus* showed inhibition zone of 19.00 and 18.50 mm, respectively (Table 2). In test for *C. vulgaris* solvent extracts, the maximum inhibition zone was recorded in *S. typhi* (13.0 mm) in Gram negative bacteria by ethanol extract followed by *K. pneumoniae* (11.0 mm) by diethyl ether extract. *P. mirabilis* treated with methanolic extracts showed inhibition zone of 11.0 mm. *V. cholerae* treated with chloroform extract showed 11.0 mm inhibition zone and *E. coli* showed the maximum zone of 11.0 mm in ethanol and methanol extract (Table 3).

Table 2

Antibacterial activity of the extracts of *D. olivaceus* from Vellar Estuary. mm.

Bacterial strains	Zone of inhibition			
	Ethanol	Methanol	Chloroform	Diethyl ether
Gram negative				
<i>K. pneumonia</i>	10.00 ± 0.50	12.00 ± 0.50	7.50 ± 0.25	8.50 ± 0.25
<i>P. mirabilis</i>	17.20 ± 0.40	12.20 ± 0.40	8.60 ± 0.43	8.20 ± 0.60
<i>V. cholerae</i>	8.50 ± 0.50	8.50 ± 0.57	8.40 ± 0.62	6.80 ± 0.40
<i>S. typhi</i>	7.00 ± 0.25	8.00 ± 0.50	8.20 ± 0.60	7.00 ± 0.50
<i>E. coli</i>	7.50 ± 0.26	9.50 ± 0.80	8.20 ± 0.60	7.00 ± 0.50
Gram positive				
<i>S. aureus</i>	--	2.00 ± 0.20	11.00 ± 0.40	8.00 ± 0.40
<i>B. subtilis</i>	11.00 ± 0.50	6.00 ± 0.40	5.00 ± 0.30	17.00 ± 0.50
<i>Enterococcus</i> sp.	3.00 ± 0.10	--	--	8.00 ± 0.40
<i>C. botulinum</i>	18.50 ± 0.50	--	--	19.00 ± 0.60
<i>Nocardia</i> sp.	8.10 ± 0.50	3.00 ± 0.20	--	--
Tween 80 (control)	--	--	--	--

All the values were expressed as mean ± SD of three determinations.

Table 1

Phytochemical screening of extracts.

Algal species	Extracts	Phenols	Tannin	Flavonoids	Terpenes	Carbohydrate	Terpenoids	Alkaloids	Saponins
<i>D. olivaceus</i>	Ethanol	-	-	+	-	+	-	+	+
	Methanol	+	-	++	++	+	-	+	-
	Chloroform	-	-	++	+	+	+	+	-
	Diethyl ether	-	-	-	-	-	+	+	-
<i>C. vulgaris</i>	Ethanol	-	-	+++	+	+	+	+	-
	Methanol	+	-	+++	++	++	-	+	-
	Chloroform	-	-	+++	-	++	-	+	-
	Diethyl ether	-	-	+	-	-	-	+	-

+++ : High amount; ++ : Moderate amount; + : Trace amount; - : Absent.

Table 3Antibacterial activity of the extracts of *C. vulgaris* from Vellar Estuary. mm.

Bacterial strains	Zone of inhibition			
	Ethanol	Methanol	Chloroform	Diethyl ether
Gram negative				
<i>K. pneumonia</i>	7.0 ± 0.5	6.0 ± 0.3	7.0 ± 0.4	11.0 ± 0.5
<i>P. mirabilis</i>	10.0 ± 0.6	11.0 ± 0.5	--	8.0 ± 0.5
<i>V. cholerae</i>	--	2.0 ± 0.1	11.0 ± 0.5	--
<i>S. typhi</i>	13.0 ± 0.7	1.0 ± 0.1	3.0 ± 0.1	--
<i>E. coli</i>	11.0 ± 0.6	11.0 ± 0.5	--	10.0 ± 0.6
Gram positive				
<i>S. aureus</i>	8.0 ± 0.4	9.0 ± 0.5	9.0 ± 0.4	3.0 ± 0.1
<i>B. subtilis</i>	9.0 ± 0.5	2.0 ± 0.2	10.0 ± 0.6	3.0 ± 0.1
<i>Enterococcus</i> sp.	7.0 ± 0.3	8.0 ± 0.5	8.0 ± 0.4	7.0 ± 0.4
<i>C. botulinum</i>	14.0 ± 0.7	--	15.0 ± 0.5	--
<i>Nocardia</i> sp.	--	--	3.0 ± 0.1	--
Tween 80 (control)	--	--	--	--

All the values were mean ± SD of three determinations.

4. Discussion

Algae produce numerous primary or secondary metabolites with potential activities acting as chemical defense and gain more attention towards pharmaceutical industries. In addition, many fresh water algae are used as an alternative source for biofertilizer. *P. mirabilis* is a Gram negative bacterium, which causes mainly urinary tract infections and wound infections, and it is also liable for the majority of Proteus infections. The ethanol extract of *D. olivaceus* showed antibacterial activity and was suggested to treat urinary tract infections and wound infections. *E. coli* is a Gram negative, straight and rod shaped bacterium arranged singly or in pairs. It causes mainly urinary tract infections, diarrhea, pyogenic infections and septicemia. It is evident that ethanolic and methanol extracts of *C. vulgaris* showed the antibacterial activity. It is also suggested that both the extracts of *C. vulgaris* may be used to treat urinary tract infections, diarrhea, pyogenic infections and septicemia. *S. typhi* is a Gram negative rod shaped bacterium, and causative agent for enteric fever, sepsis and infectious diarrhea in human beings. The ethanolic extracts of *C. vulgaris* showed the antibacterial activity against the pathogen *S. typhi* and hence, it recommends that the extracts of *C. vulgaris* can be used against the enteric fever, sepsis and infectious diarrhea or gastroenteritis. *C. botulinum* is Gram positive bacterium, causing severe infectious or watery diarrhea with abdomen pain, loss of appetite, fever, blood or pus in the stool and weight loss. The ethanol, diethyl ether, methanol and chloroform extracts of algae *D. olivaceus* and *C. vulgaris* showed antibacterial activity. Hence, the extract from both the species are recommended to treat *C. botulinum* infections.

The present study indicated that the antibacterial property of the two algal species against the selected strains of human pathogenic bacteria varies depending upon the solvent medium used for extraction. The most sensitive bacteria are *C. botulinum* and *P. mirabilis*, which were inhibited by ethanol and diethyl ether extracts of *D. olivaceus*. *S. typhi*, *E. coli* and *C. botulinum* were inhibited by ethanol, methanol and chloroform extracts of *C. vulgaris*. Hence from the present study, it was revealed that the competent antibacterial compounds are present in the marine algae. Phytochemical studies also revealed that it may be due to the presence of metabolites like flavonoids, terpenes and carbohydrates, which are responsible for antibacterial activity of these extracts against bacteria. Further, more research is needed in this particular aspect to throw out synthetic drugs and replace the traditional methods by using plant materials to cure infectious diseases caused by microbes.

Conflict of interest statement

We declare that we have no conflict of interest.

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