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## Phytochemical analysis and antimicrobial activity of *Chorella vulgaris* isolated from Unkal Lake

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### ABSTRACT

**Objective:** To determine the presence of bioactive molecules and to check their antimicrobial activity from green algae *Chlorella vulgaris* (AS-3) (*C. vulgaris*) isolated from Unkal Lake in Dharwad District, Karnataka, India.

**Methods:** Based on the polarity, benzene, chloroform, ethyl acetate, ethanol, hexane, methanol, petroleum ether and distilled water were the solvents used for the preparation of algal extracts using Soxhlet apparatus, which were further subjected to phytochemical analysis and screening of antimicrobial activity. Human pathogens such as *Staphylococcus aureus*, *Corynebacterium*, *Bacillus subtilis*, *Streptococcus*, *Escherichia coli*, *Salmonella* Paratyphi B, *Klebsiella pneumoniae*, *Aerobacter aerogenes*, *Candida albicans* and *Aspergillus niger* were used for antimicrobial assay. Standard methods were followed for qualitative estimation of phytochemicals.

**Results:** Phytochemical determination of bioactive molecules showed the presence of alkaloids, flavonoids, glycosides, carotenoids, phenols, lignins, saponins, sterols, tannins, reducing sugars, volatile oil, fats, amino acids and carbohydrates. *In vitro* analysis of organic solvent extracts of *C. vulgaris*, a green microalgae, showed an activity by suppressing the proliferation of bacterial, fungal and human pathogens. Four extracts (chloroform, ethyl acetate, hexane and methanol) showed effective inhibitory activity against the tested pathogens. Depending on the percentage of bioactive molecules present in each of the organic extracts, different extracts showed different inhibition zone diameters against the pathogens. Among the eight organic extracts used for the study, excellent inhibitory effects were shown by chloroform and methanol extracts.

**Conclusions:** The present study indicates that green algae *C. vulgaris* is rich in natural compounds which are highly important in pharmacology and nutraceuticals. Although the presence of bioactive molecules is very less in the algae, excellent effect on the microbial pathogens was observed.

## 1. Introduction

A recent research has proved that there is a vast range of naturally occurring bioactive molecules with a broad range of biological activities in algae, such as antimicrobial, antioxidant, anti-inflammatory, antiviral and antitumor activities[1]. The ability of algae to exhibit such vast range of activities is due to the presence of bioactive molecules and their concentrations in these organisms[2].

Phytochemical and pharmacological studies and literatures have suggested the availability of terpenoids and steroids in most algal species. Algae are a good source of proteins, carbohydrates, lipids, phenols, steroids, ketones and alkenes[3]. Both marine and fresh water algae produce a wide variety of chemically active secondary metabolites which act as a chemical defence against predators and herbivores under environmental stress and competition for space[4]. These bioactive metabolites are synthesized by certain species of marine and fresh water microalgae. These molecules have antibacterial, antialgal and antifungal properties which are effective in the remediation of various diseases, bio-fouling and are employed in other aspects such as in therapeutics[5]. There is an increasing demand for the use of antibiotics and other important

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pharmacological bioactive molecules isolated from algae. In the field of research involving bioactive substances from plants, a greater interest has now arisen in algae. The first investigation on antibiotic activity of algae was carried out by Pratt *et al.* in the year 1944[6]. There is a long history of the use of algae for the therapy practices in disease control and the extraction and use of biologically active substances from algae, of which most antibiotics were stated during early 1950s. Various solvent and aqueous extracts obtained from algae were analysed for the effective inhibitory action against some Gram-positive and Gram-negative bacterial strains[7-10]. The important molecules which have good antimicrobial activities are fatty acids such as saturated, unsaturated and monounsaturated acrylic acid, halogenated aliphatic compounds, terpenes, sulphur containing hetero cyclic compounds, carbohydrates and phenols[11]. Several products of the algal origin such as alginate, carrageenan and agar such as phycocolloids have been used for decades in medicine, pharmacy and researches. Products of commercial and industrial importance such as varnishes, perfumes, cosmetics, lubricants, biopolymers, polyhydroxyalkanoate, polyhydroxybutyrate, surface active agents, shampoos, toilet soaps, household cleaners and detergents can also be manufactured by using algae. In recent times, algae have received a lot of attention as an excellent source of biomass for the production of renewable energy such as bio-diesel, bioethanol and bio-hydrogen.

Various studies have identified microalgae as an ideal candidate producing hundreds of thousands of diversified bioactive and chemical molecules which have vast range of biological activities. These chemical substances have the capacity to stop the growth and proliferation of microorganisms and destroy them. The need of the hour is to develop new molecules with high antimicrobial activities and determine novel mechanism of action and chemical structures of isolated compounds, as the microorganisms are becoming resistant to the current drugs used during clinical administration. The use of herbal algal antibiotics will help us to get rid of synthetic antibiotics which have harmful side effects on body organs. Therefore, the present study was to investigate *Chlorella vulgaris* (AS-3) (*C. vulgaris*), a kind of freshwater green microalgae, which has been employed in isolation and determination of bioactive molecules and to study the antimicrobial effect of organic extracts on human pathogens.

## 2. Materials and methods

### 2.1. Isolation and culture

*C. vulgaris* was collected and screened from Unkal Lake, a fresh water lake in Hubli-Dharwad[12]. Culturing the collected algal strain was done using 100 mL BG-11 media for inoculum preparation, and mass culture was carried out by using BG-11 media with sodium nitrate as nitrogen source in 20 L pet jar made of poly vinyl chloride

which was supplied with sterile air under fluorescent light intensity of 20  $\mu\text{mol}/\text{m}^2/\text{s}$  with 16 h of photoperiod and at 25 °C for 20 days. Wet biomass was obtained by centrifugation was done at 5000 r/min for 6 min. Obtained biomass was cleaned to remove the present epiphytes and necrotic parts. Then, the samples were rinsed with sterile water to remove the associated debris.

### 2.2. Preparation of algal crude extracts

The obtained algal biomass was subjected to centrifugation (2500 r/min) for the time duration of 10 min so as to partially dehydrate it. About 25 g algal biomass was subjected to extraction for a time period of 30 min using Soxhlet apparatus, using 150 mL of each organic solvent, *i.e.* benzene, chloroform, diethyl ether, ethyl acetate, ethanol, hexane, methanol and distilled water. Respective solvents were reduced/recovered from the extracts using rotary evaporator. Each extract was completely dried and weighed.

### 2.3. Preliminary phytochemical screening

The dried powdered extract samples were subjected to qualitative tests for the identification of phytochemical constituents such as carotenoids, alkaloids, flavonoids, glycosides, phenols, lignins, saponins, sterols, tannins, reducing sugars, volatile oil, fats, amino acids and carbohydrates according to standard procedures[13-16].

### 2.4. Chemical composition analysis

All extracts of *C. vulgaris* were used for the determination of bioactive compounds by gas chromatography-mass spectroscopy (GC-MS) in University Science Instrumentation Centre at Karnatak University, Dharwad. Analysis was done using GC-MS-QP2010S instrument.

### 2.5. Tested microorganisms

Gram-positive bacterial strains *Staphylococcus aureus* (*S. aureus*), *Corynebacterium*, *Bacillus subtilis* (*B. subtilis*), *Streptococcus*, Gram-negative bacterial strains *Escherichia coli* (*E. coli*), *Salmonella* Paratyphi-B (*S. Paratyphi-B*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Aerobacter aerogenes* (*A. aerogenes*), and fungus *Candida albicans* (*C. albicans*), *Aspergillus niger* (*A. niger*) were employed for this study purpose. The parent cultures were maintained in the Department of Microbiology, Karnatak University, Dharwad.

### 2.6. Disc diffusion method

The obtained organic extracts were diluted using their respective

solvents at the concentration of 10 mg/mL for preparation of stocks. To evaluate the antimicrobial activity, disc diffusion method was used. The bacterial suspension culture was made by diluting in distilled autoclaved water, 1 mL of which was poured into the Muller-Hinton agar plates for uniform distribution of microorganisms. Sterile disc with 3 mm diameter was used. Organic crude extracts (25 µg/disc) from the stock were poured on each disc using a sterile micropipette and dried. Streptomycin, gentamycin and erythromycin (25 µg/disc) were used as standards. The plates were incubated for 18 h at 37 °C. At the end of the incubation period, the inhibition zone was measured.

### 3. Results

#### 3.1. Preliminary phytochemical determination

Different crude organic extracts of *C. vulgaris* were different in colour based on the components present in the extracts. High concentrations of carotenoids referred to as colouring pigments and various fatty acids are present. Sterols, phenols and saponins are also present in good concentrations. Sugars, amino acids and flavanoids are also identified. Tannins were absent in all extracts. Chloroform and hexane extracts showed the presence of a larger group of molecules followed by ethyl acetate and methanol extracts. The presence of carbohydrates, amino acids and lipids was confirmed in almost all the organic extracts. Petroleum ether extract showed less significance in the preliminary phytochemical analysis as compared to other solvent extracts. The tested phytochemicals are listed in Table 1.

#### 3.2. GC-MS profiling of various solvent extracts of *C. vulgaris*

Various molecules of pharmaceutical, commercial and industrial importance were identified by GC-MS. The peaks obtained from

various extracts of *C. vulgaris* are given in Table 2. GC-MS results of aqueous extract could not be obtained because there was no solubility of molecules in water/ dimethyl sulfoxide. The list of molecules identified by refering National Institute of Standards and Technology library based on the retention time of the compound and molecular weight, the International Union of Pure and Applied Chemistry name of the compound, common name, molecular weight, molecular formula and their uses are listed in Table 2. Several values added molecules which are raw materials for various industries are also determined. These can be used for the production of commercial products such as cosmetics, perfumes, detergents, etc. Molecules which are precursors for biofuel production are also been found. Fatty acids such as butyric (C4), valeric (C5), lauric (C12), myristic (C14), pentadecanoic (C15), palmitic (C16), palmitoleic (C16:1), stearic (C18), ω-3 fatty acids such as α-linoleic (C18:3), ω-6 fatty acid linoleic (C18:2), ω-7 fatty acid palmitoleic (C16:1), ω-9 fatty acid oleic (C18:3), oleic acid, valeric acid, palmitic acid, perfluoroacetic acid, palmitoleic acid, linoleic acid, malonic acid, ascorbic acid, etc. were present in chloroform, ethyl acetate, hexane and methanol extracts. Most of these fatty acids have high antibacterial activities which are justified by further results.

#### 3.3. Antimicrobial activity of solvent extracts of *C. vulgaris*

The antimicrobial activity or zone of inhibition was observed in most of the human bacterial pathogens. Solvent extracts of *C. vulgaris* showed a good activity towards Gram-positive bacteria and some Gram-negative bacteria. Poor zone of inhibition was observed with fungal strains. The maximum zone was of 7 mm with aqueous extract against *A. niger*. The zones observed with control antibiotics was more than that with solvent extracts using these fungal strains. Extracts of chloroform, ethyl acetate, hexane and methanol showed a maximum activity against Gram-positive strains *S. aureus*, *Streptococcus*, *Corynebacterium* and *B. subtilis*. The highest zone of

**Table 1**

Phytochemicals presenting in various solvent extracts of *C. vulgaris*.

SL No.	Name of compounds	Extracts of <i>C. vulgaris</i>							
		Benzene	Chloroform	Ethyl acetate	Ethanol	Hexane	Methanol	Petroleum ether	Aqueous
1	Carotenoids	+	+	+	NP	+	+	NP	+
2	Alkaloids	NP	+	NP	NP	+	NP	+	NP
3	Flavonoids	+	NP	+	+	+	+	NP	+
4	Glycosides	NP	+	+	NP	+	+	+	+
5	Phenols	NP	+	+	NP	+	+	NP	NP
6	Lignins	+	+	+	NP	+	NP	NP	NP
7	Saponins	NP	+	NP	+	NP	+	NP	+
8	Sterols	NP	NP	+	+	+	+	NP	+
9	Tannins	NP	NP	NP	NP	NP	NP	NP	NP
10	Reducing sugars	NP	+	NP	NP	NP	+	NP	+
11	Volatile oil	+	+	+	NP	+	NP	NP	NP
12	Fatty acids	+	+	+	+	+	+	NP	+
13	Amino acids	+	+	+	+	+	+	+	+
14	Carbohydrates	+	+	+	+	+	+	+	+

+: Present; NP: Not present; All readings were taken in triplicates.

**Table 2**

Compounds obtained by GC-MS profiling (as referred to NIST library) in various solvent extracts of *C. vulgaris*, their molecular weight, molecular formula, retention time and application.

Solvent extracts	The compound IUPAC name	Common name	Retention time	Molecular weight	Formula	Applications	
Benzene extract (S-1)	<i>n</i> -Hexadecanoic acid	Palmitic acid	14.143	256	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	Cosmetics, antioxidant, antibiotic	
	13-Docosenoic acid	Erucic acid	15.814	338	C <sub>22</sub> H <sub>42</sub> O <sub>2</sub>	Biodiesel	
	Pentanoic acid	Valeric acid	15.899	102	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	Perfumes, cosmetics, antiviral	
	Octadecanoic acid	Stearic acid	15.977	284	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	Lubricants, softening, release agents	
Chloroform extract (S-2)	1-Dodecene	Lauric acid	7.584	168	C <sub>12</sub> H <sub>24</sub>	Production of detergents, antibiotics	
	Phenol,2,4-bis (1,1,dimethyl ethyl) -	-	9.250	206	C <sub>14</sub> H <sub>22</sub> O	Antioxidants, antibiotic	
	1-Tetradecene	Cyclotetradecane	10.008	196	C <sub>14</sub> H <sub>28</sub>	Surface active agents	
	Polysisobutylene	Butyl rubber	12.231	224	C <sub>16</sub> H <sub>32</sub>	Fuel and lubricant additive	
	Tetradecanoic acid	Myristic acid	14.155	228	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	Cosmetic and topical medicinal preparations	
	1-Octadecene	Stearic acid	14.254	250	C <sub>18</sub> H <sub>34</sub>	Antimicrobial	
	Octadec-9-enoic acid	Oleic acid	15.795	282	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	Antibiotic, emulsifying agent	
	Pentanoic acid	Valeric acid	15.880	102	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	Perfumes, cosmetics and antiviral	
	Ethyl hexadecanoate	Ethyl palmitate	15.989	284	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	Skin-conditioning agent	
	1-Tetracosanol	Lignoceryl alcohol	16.099	354	C <sub>24</sub> H <sub>50</sub> O	Antibiotic	
	Trifluoro acetic acid	Perfluoroacetic acid	17.787	324	C <sub>2</sub> HF <sub>3</sub> O <sub>2</sub>	Peptide synthesis, antibiotics	
	Ethyl acetate extract (S-3)	1-Dodecene,	Lauric acid	7.559	168	C <sub>12</sub> H <sub>24</sub>	Production of detergents
		1-Tetradecene	Cyclotetradecane	9.990	196	C <sub>14</sub> H <sub>28</sub>	Surface active agents, antimicrobial
		2-Tridecene	Tridec-2-ene	12.215	182	C <sub>13</sub> H <sub>26</sub>	Antitumor
<i>n</i> -Hexadecanoic acid		Palmitic acid	14.135	256	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	Cosmetics, antioxidant, antibiotic	
Trifluoro acetic acid		Perfluoroacetic acid	14.240	324	C <sub>2</sub> HF <sub>3</sub> O <sub>2</sub>	Peptide synthesis, antibiotics	
(9Z)-Hexadec-9-enoic acid		Palmitoleic acid	15.892	254	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	Anti-inflammatory, antibiotic	
Ethanol extract (S-4)	1-Octadecyne	Octadecylene	12.733	250	C <sub>18</sub> H <sub>34</sub>	Antimicrobial	
	Tetradecanoic acid	Myristic acid	14.338	228	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	Cosmetic and topical medicinal preparations	
	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Phytol	15.539	296	C <sub>20</sub> H <sub>40</sub> O	Cosmetics, shampoos, toilet soaps, household cleaners, and detergents	
	9,12-Octadecadienoic acid	Linoleic acid	16.058	280	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	Antioxidant	
Hexane extract (S-5)	3-Propyloxirane methanol	Propyloxirane methanol	14.105	116	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	Solid oxidation catalysts	
	2,4,6,8-Tetramethyl-1-undecene	Pentadecanoic acid	15.493	210	C <sub>15</sub> H <sub>30</sub>	Antibiotic	
	1,1-Bicyclooctyl	Bicyclooctyl	15.920	222	C <sub>16</sub> H <sub>30</sub>	Fuel oil additives and compositions	
	1,2-Dihydro- $\psi$ , $\psi$ -Caroten-1-ol	Rhodopin	19.956	554	C <sub>40</sub> H <sub>58</sub> O	Carotenoid, antibiotics	
	Octadecane	Octadecyl vinyl ether	20.478	296	C <sub>20</sub> H <sub>40</sub> O	Fuel and lubricant additives	
Methanol extract (S-6)	Ascorbyl	Ascorbic acid	15.440	176	C <sub>6</sub> H <sub>8</sub> O <sub>6</sub>	Fragrance, antidiabetic	
	Proanedioic acid	Malonic acid	15.725	104	C <sub>3</sub> H <sub>4</sub> O <sub>4</sub>	Polyesters, alkyd resins	
	Dibutyl ester	Dibutyl sebacate	16.416	314	C <sub>18</sub> H <sub>34</sub> O <sub>4</sub>	Desensitizer in otto fuel II	
	1,2-Dihydro- $\psi$	Rhodopin	17.552	554	C <sub>40</sub> H <sub>58</sub> O	Carotenoid	
Petroleum ether extract (S-7)	Butyric acid hydrazide	Butyrohydrazide	14.208	102	C <sub>4</sub> H <sub>10</sub> N <sub>2</sub> O	Cosmetic and pharmaceutical	
	9-Octadecene	Octadec-9-ece	15.439	252	C <sub>18</sub> H <sub>36</sub>	Photographic material	

IUPAC: International Union of Pure and Applied Chemistry.

**Table 3**

Antimicrobial activity of different solvent extracts (25  $\mu$ g/disc) of *C. vulgaris* (mm).

Name of the organism	Inhibition zone in diameter of various solvent extracts of <i>C. vulgaris</i>										
	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	Streptomycin	Gentamycin	Erythromycin
<i>S. aureus</i>	6	18	17	6	18	15	4	10	22	18	17
<i>Streptococcus</i>	8	22	16	8	17	16	NA	6	15	18	20
<i>Corynebacterium</i>	5	18	8	6	9	10	NA	6	22	18	20
<i>B. subtilis</i>	8	22	18	8	18	18	NA	10	18	22	20
<i>E. coli</i>	NA	NA	4	6	6	NA	NA	8	22	20	20
<i>K. pneumoniae</i>	7	22	18	6	19	18	NA	16	20	22	20
<i>A. aerogenes</i>	5	21	20	6	21	20	8	16	18	18	18
<i>S. Paratyphi-B</i>	5	8	7	7	8	7	NA	8	15	13	15
<i>C. albicans</i>	NA	5	4	NA	NA	4	NA	5	12	10	12
<i>A. niger</i>	NA	6	5	NA	5	4	NA	6	10	12	10

S-1: Benzene extract; S-2: Chloroform extract; S-3: Ethyl acetate extract; S-4: Ethanol extract; S-5: Hexane extract; S-6: Methanol extract; S-7: Petroleum ether extract; S-8: Aqueous extract; NA: No activity.

inhibition was of 22 mm by chloroform extract against *B. subtilis*. The zone diameter was similar with that of control antibiotic gentamycin against the same bacterial strain. Certain Gram-negative bacteria such as *K. pneumoniae* and *A. aerogenes* also showed good activity with the same extracts, and the zone of inhibition was quite similar with that of Gram-positive bacteria. *E. coli* showed resistance to most of the extracts. The highest zone of inhibition was 8 mm with aqueous extract. The extracts showed marginal activity against *S. Paratyphi-B*, and the zones were between the diameter of 5–8 mm. Antimicrobial activities of various extracts of *C. vulgaris*, and measured zone of inhibition against different human bacterial and fungal pathogens are depicted in Table 3.

#### 4. Discussion

In recent years, the use of algae for extraction of metabolites, bioactive molecules and other industrial important products has been gaining lots of importance. Some algal species have been used for therapeutically practices against various pathogens both in medicine and agriculture for decades. The current study is to determine the existence of natural bioactive molecules along with fatty acids presenting in *C. vulgaris* isolated from fresh water lake, and to determine its antimicrobial activity using various human pathogenic strains. In the present study, preliminary phytochemical screening of *C. vulgaris* showed the presence of alkaloids, saponins, lignins, flavanoids, phenols, proteins, carotenoids, sugars, fatty acids, carbohydrates, volatile oils and glycosides in most of the organic solvent extracts, hexane and chloroform extracts showed the presence of a larger group of molecules followed by ethyl acetate and methanol extracts. The presence of carbohydrates, amino acids and lipids was confirmed in almost all the extracts. Petroleum ether extract showed less significance in the preliminary phytochemical analysis as compared to other solvent extracts. This may be because the hexane solvent was used before petroleum ether and the polarity of both the solvents is nearly similar, hence, most of the molecules which are soluble in petroleum ether have been extracted by hexane. Similar qualitative phytochemical results were obtained [6-8,17,18]. Coumarin, quinine and tannin were absent in *C. vulgaris* extracts.

The actual concentrations of all the phytochemicals is yet to be determined. These phytochemicals are basically used in pharmaceutical, nutraceutical, cosmetic industries. Various molecules of industrial importance were also determined by GC results of different extracts. Value added molecules such as palmitic acid, valeric acid, ethyl palmitate, myristic acid and oleic acid were present which are widely used in preparation of perfumes, cosmetics and antibiotics. Stearic acid, cyclotradecane and  $\alpha$ -dodecene are used for the production of detergents,

lubricants, softening agents and surface active agents. Erucic acid is a good source to manufacture biodiesel, as butyl rubber is used to produce various forms of fuel and lubricants. In this study, chloroform, ethyl acetate, hexane and methanol extracts showed the maximum zone of inhibition in all bacterial cultures. MartinezNadal [13] stated that benzene and diethyl ether were the most desirable and suitable solvents for the extraction of compounds with antibiotic activity. Acetone was used by Hornsey and Hide [14] as a suitable solvent for the extraction of antimicrobial compounds from British marine algae. In the present study, organic extracts of *C. vulgaris* showed a powerful effect against the pathogenic bacterial strains as compared to that of fungal strains *A. niger* and *C. albicans*. Gram-negative *E. coli* and fungal strains of *C. albicans*, *A. niger* showed resistance to the organic extracts as the minimal zone of inhibition was seen. Human pathogenic Gram-positive bacteria such as *S. aureus*, *Corynebacterium*, *B. subtilis*, *Streptococcus* which are the major causative organisms for ailments, such as sepsis caused in wounds and burns, septicemia, pharyngitis, sinusitis, diphtheria and tonsillitis, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteraemia, pneumonia, meningitis, respiratory infections, urinary tract infections, etc. The organic extracts of chloroform, ethyl acetate, hexane and methanol showed an excellent antimicrobial effect on these deadly pathogens, hence, these can be used in the treatment of the above mentioned human and animal diseases. The same organic extracts of *C. vulgaris* also showed a significant inhibitory zone against Gram-negative bacterial strains such as *K. pneumoniae*, *A. aerogenes* which are responsible for causing diseases like pneumonia, diarrhoea or gastroenteritis, pyogenic infections, etc. The antibacterial effect of the extracts was marginal on *Salmonella Paratyphi-B*. The overall results showed that Gram-positive bacteria were more susceptible to extracts of *C. vulgaris* than Gram-negative bacteria. The difference in fatty acid sensitivity between Gram-negative and Gram-positive bacteria is because the outer membrane of Gram-negative bacteria is less permeable to long chain fatty acids [6,15]. The maximum inhibition zone obtained is somewhat comparative or similar with that of synthetic antibiotics which has been used as control. Hence, these synthetic or chemically prepared antibiotics which have major side effects to body organs and tissues can be replaced with the herbal antibiotics produced from the organic extracts of *C. vulgaris*.

Studies have suggested that the antimicrobial activity is because of the presence of fatty acids and is directly proportional to the amount of lipophilic, phenolic molecules and coloured pigments presenting in the extracts. Saturated fatty acids such as butyric (C4), valeric (C5), lauric (C12), myristic (C14), pentadecanoic (C15), palmitic (C16), palmitoleic (C16:1), stearic (C18),  $\omega$ -3 fatty acids such as  $\alpha$ -linoleic (C18:3),  $\omega$ -6 fatty acid linoleic (C18:2),  $\omega$ -7 fatty acid palmitoleic (C16:1),  $\omega$ -9 fatty acid oleic (C18:3) were present in



the organic extracts of *C. vulgaris*. The current results agreed with the previously conducted studies on *Bortryococcus braunii*, where antimicrobial activity was due to the presence of the mixture of free fatty acids including linolenic, oleic, lanolin and hexadecanoic acid [16,19,20]. Studies were also conducted on *Spirulina maxima*, which explain that the concentration of phenol molecules will have a direct effect on the antimicrobial activity. Similar results were obtained for the antibacterial activity of *Spirulina maxima* organic extracts in an assay against six different bacterial strains [21].

The preliminary phytochemical screening of *C. vulgaris* showed the presence of alkaloids, saponins, lignins, flavanoids, phenols, amino acids, carotenoids, reducing sugars, fatty acids, carbohydrates, volatile oils and glycosides in various extracts. Coumarin, quinone and tannin were absent. The actual concentration of all the phytochemicals is yet to be determined. GC-MS analysis revealed the presence of saturated fatty acids such as butyric (C4), veleric (C5), lauric (C12), myristic (C14), pentadecanoic (C15), palmitic (C16), palmitoleic (C16:1), stearic (C18), and unsaturated fatty acids such as oleic acid, linolic acid, linolenic acid, palmitoleic acid, stearidonic acid. Various other valuable added compounds were also determined. Excellent effect was obtained against the pathogenic bacterial strains with the organic solvent extracts of *C. vulgaris*, chloroform, ethyl acetate, hexane and methanol, showed a synthetic or chemically prepared antibiotics which have major side effects to body organs and tissues can be replaced with the herbal antibiotics produced from the organic extracts of various algal species.

### Conflict of interest statement

We declare that we have no conflict of interest.

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