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# Evaluation Antimicrobial Activity of Cladophora Algae Ethanolic and Hexane Extracts Against Uropathogenic Bacteria

**Conflict of interest:** nothing to declare.

**Authors' contribution:** Salah Faiz Shnaa – conceptualization, data curation, investigation, methodology, project administration, resources, software, writing (original draft, review & editing); Roaa J. Elkheralla – conceptualization, data curation, investigation, methodology, supervision, validation, visualization, writing (original draft, review & editing); Mohammed Hashim AL-Yasiri – conceptualization, data curation, methodology, supervision, validation, visualization, writing (original draft, review & editing).

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## Abstract

**Introduction.** In recent years, there has been a growing inclination in using Algae extracts to treat various illnesses. This is predominantly due to the rise in resistance of pathogenic bacteria, which affectedness a significant health anxiety for individuals in both developed and developing countries, to overcome this, must searching for new antimicrobial agents, especially of natural origin.

**Purpose.** To highlight the ability of Cladophora extracts to provide advanced solutions for combating harmful microorganisms such as *E. coli* and *Staphylococcus aureus* isolated from urinary tract infections.

**Materials and methods.** Cladophora extracts were prepared by successive maceration in two solvents; namely, ethanol, and hexane by using Ultrasonic Bath. The extracts were filtered, intense, and stored in sterile conditions. Antimicrobial activity was evaluated using varying concentrations (25, 50, 75, and 100 mg/mL) against bacterial strains *Staphylococcus aureus* and *E. coli* by the Kirby-Bauer disk diffusion method. The bioactive compounds in each extract were also identified using a GC-MS device.

**Results.** All extracts showed a high inhibitory ability on the growth of *E. coli* and *S. aureus* the highest inhibition zone was recorded in Ethanol extract at a concentration of 100% on *E. coli* with a mean of  $16 \pm 1$  mm, while *S. aureus*  $11.8 \pm 0.44$  mm, and recorded in Hexane extract at a concentration of 100% on *S. aureus*  $13.4 \pm 1.14$  mm, while *E. coli*  $14.6 \pm 0.89$  mm, then the activity decreased for both extracts with decrease concentration. The ethanol extract showed activity against *E. coli* and *Staph. aureus* at all concentrations. On the other hand, the hexane extract showed activity against *E. coli* and *Staph. aureus* at all concentrations. Several active chemical compounds in each extract were also identified using GC-MS.

**Conclusion.** These findings present new insights into the ability of the green algae *Cladophora* spp. as a valuable source of antibacterial.

**Keywords:** Cladophora algae, ethanol, hexane, DMSO, GC-MS, *E. coli*, *S. aureus*

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## Оценка антимикробной активности этанольных и гексановых экстрактов водорослей кладофоры против уропатогенных бактерий

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### Резюме

**Введение.** В последние годы наблюдается тенденция к увеличению использования экстрактов водорослей для лечения различных заболеваний. Это обусловлено главным образом ростом резистентности патогенных бактерий, что вызывает серьезную обеспокоенность по поводу здоровья людей как в развитых, так и в развивающихся странах. Для преодоления этой проблемы необходим поиск новых антимикробных препаратов, особенно природного происхождения.

**Цель.** Подчеркнуть способность экстрактов кладофоры обеспечивать передовые решения в борьбе с вредными микроорганизмами, такими как кишечная палочка и золотистый стафилококк, выделенными при инфекциях мочевыводящих путей.

**Материалы и методы.** Экстракты кладофоры были получены путем последовательной мацерации в двух растворителях: этаноле и гексане, с использованием ультразвуковой ванны. Экстракты были отфильтрованы, подвергнуты интенсивной обработке и хранились в стерильных условиях. Антимикробную активность оценивали, используя различные концентрации (25, 50, 75 и 100 мг/мл) в отношении штаммов бактерий *Staphylococcus aureus* и *E. coli* диско-диффузионным методом Кирби-Бауэра. Биоактивные соединения в каждом экстракте также идентифицировали с помощью прибора ГХ-МС.

**Результаты.** Все экстракты продемонстрировали высокую ингибирующую способность в отношении роста *E. coli* и *S. aureus*. Наибольшая зона ингибирования была зарегистрирована в этанольном экстракте при концентрации 100% для *E. coli* со средним размером  $16 \pm 1$  мм и *S. aureus*  $11,8 \pm 0,44$  мм, а также в гексановом экстракте при концентрации 100% для *S. aureus*  $13,4 \pm 1,14$  мм и *E. coli*  $14,6 \pm 0,89$  мм. Затем активность снижалась для обоих экстрактов с уменьшением концентрации. Этанольный экстракт проявил активность против *E. coli* и *S. aureus* во всех концентрациях, как и гексановый экстракт. Несколько активных химических соединений в каждом экстракте были также идентифицированы с помощью ГХ-МС.

**Заключение.** Полученные результаты продемонстрировали, что зеленые водоросли *Cladophora* spp. являются ценным источником антибактериальных веществ.

**Ключевые слова:** водоросли Cladophora, этанол, гексан, ДМСО, ГХ-МС, E. coli, S. aureus

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## ■ INTRODUCTION

Urinary tract infection (UTI) is a common bacterial and fungal infection that affects individuals of all ages and both of sex in the worldwide [1]. It is one of the most common infections in local primary care [2]. UTI is a common contagion among men and women but the incidence is quite high among women due to their physiology [3–5]. The examination of the clinical symptoms of UTI in a patient depends on the anatomical region, microorganisms, severity of infection and the patient's immune system [6].

The emergence of drug-resistant strains of bacteria and fungi has further complicated the management of UTIs [7]. The extensive, frequent and inappropriate use of antimicrobial agents have invariably resulted in the development of antibiotic resistance which, in recent years, has become a major problem international [8]. In recent years, there has been a growing trend in using Algae extracts to treat various ailments, this is particularly due to the rise in resistance of pathogenic bacteria [9]. Algae-derived chemicals possess distinctive pharmacological characteristics, including cost-effectiveness, reduced toxicity, diminished side effects, and a lower likelihood of resistance development [10].

Cladophora Algae is capable of producing different kinds of antioxidant, carotenoid, enzyme polymer, lipid, natural dye, polyunsaturated fatty acid, peptide, toxin and sterols [11]. Some of the high-value bioactive compounds produced by Algae are acetylic acids,  $\beta$ -carotene agars, agarose, alginates, carrageenan's, polyunsaturated fatty acids, vitamin B, keto-carotenoid astaxanthin, and lutein that can using in synthesis of antimicrobial, antiviral, antibacterial and anticancer drugs [12]. The chemical composition of Algae includes lipid at 20–30%, protein around 50%, carbohydrate 20–30% and other compounds account for approximately 5%, the market value depends on the concentration and amount of the essential amino acids, poly saccharides, polyunsaturated fatty acid and number of essential vitamins, also, the value depends upon the application, or what is the purpose of the use of algae and compared to similar alternative sources of this product [13].

### **Cladophora spp.**

Cladophora is a group of macroscopic green algae that includes more than 183 species and widely distributed in both marine and freshwater ecosystems [14]. Also called blanket weed (Kelly and King, 2007). The common name of this species is Mekong weed [15]. The genus Cladophora includes filamentous green algae sparsely, or densely branched, and often taking a bushy cushion-like or globular form [16]. This alga contains a high amount of carbohydrates, minerals, and proteins and is characterized by high moisture – typically around 90% [17]. Also, this moss contains abundant flavonoids, alkaloids phenols, tannins, fatty acids, sterols, and terpenoids. [18]. Polysaccharides, polyphenols, terpenes, carotenoids, and phycobiliproteins are some of the anticancer bio-constituents present in algae [19] They contain photosynthetic pigments common chlorophyll a, chlorophyll b, carotene, xanthophyll. Quantitative and qualitative analysis of basic protein amino acids in Cladophora cells showed the presence of 18 amino acids [20]. Cladophora are rich in phytochemical compounds that can be exploited for sustaining both human and

animal health [18]. *Cladophora* possesses numerous biologically active properties and is regarded as one of the most essential medicinal algae as an antibacterial, antifungal, and anti-inflammatory agent, anti-diabetic, anti-oxidation, anticancer, anti-hypertensive, and cytotoxicity activities [19]. The use of *Cladophora* in medicine and pharmacology is one of the most profitable promising ways to utilize the biomass. *Cladophora* biomass is an unlimited, low cost and valuable resource for medicine and pharmacology [21].

## ■ MATERIALS AND METHODS

### **Algae samples collection and processing**

The study involved the collection and preparation of *Cladophora* Algae from the marshes of Suq Al-Shuyukh, Nasiriyah city, Dhi Qar Governorate, Iraq. The Algae were cleaned, dried, and ground into powder. Twenty grams of the dried powder were mixed with 200 mL of ethanol and hexan solvents, respectively, and subjected to ultrasonic extraction. The extracts were filtered, concentrated, and stored in sterile conditions. Antimicrobial activity was assessed using varying concentrations (25, 50, 75, and 100 mg/mL) against bacterial strains *Staphylococcus aureus* and *E. coli* using the Kirby-Bauer disk diffusion method.

### **Collection and classification of study stations**

The Algal specimens used in this study were obtained from the marshes of Suq Al-Shuyukh, located in the Thi-Qar Province in southern Iraq, province for the period from 15 October to 15 February, 2023–2024. Specifically, *Cladophora* Algae were collected. Dr. Roaa Jafar khudhayer, a professor at the University of Thi-Qar, College of Science, identified the Algae. The impurities were completely removed from them, and they were transformed into a fine powder using an electric mill. After that, they were kept in sterile glass bottles until they were ready to be used [22].

### **Preparation of algal extract**

#### **Preparation of Hexan and ethanol extract**

The chemical components were qualitatively screened by subjecting a mixture of 20 grams of Algal powder and 200 milliliters of Hexan (Hexan Extract), as well as 200 milliliters of ethanol (Ethanol Extract), to an ultrasonic bath. The resulting solution was filtered using multiple layers of Whatman 0.22 filter paper and then concentrated at 50 °C under reduced pressure using a rotary evaporator. Afterwards, it was subjected to a drying procedure at a temperature of 25 °C. The extract was ultimately gathered in sterilized glass tubes that are now prepared for utilization [23]. Preparation Concentration to obtain different concentrations, we dilute the stock solution (100%) with DMSO dissolving solution.

### **GC-MS analysis of extracts**

Gas chromatography-mass spectrometry (GC-MS) conditions the GC-MS analysis was performed using the GCMS-QP2010 plus instrument (Shimadzu, Kyoto, Japan), equipped with an autoinjector and a 5 ms capillary column of 30×0.25 mm with a film thickness of 0.25 µm. The carrier gas used is helium, with a flow rate of 1.15 ml/min. The 70eV ionized charge system was used to do mass spectroscopic scanning. The temperature was initially set at 80 °C for 2 minutes, and then increased steadily at a pace of 10 °C per minute until it reached 280 °C for 5 minutes. The samples that were injected were exposed to splitting

mode at a temperature of 250 °C. Two databases contain mass spectral data. The National Institute of Standards and Technology (NIST14) and Wiley 10th/NIST 2014 mass spectral library (W10N14) was utilized to characterize the isolated components based on them.

### **Culturing of samples and antibiotic susceptibility**

Bacterial cultures of *E. coli* and *S. aureus* were maintained at 4° C in Brain Heart Infusion agar with glycerol and subculture on MacConkey and blood and then on Muller Hinton agar were used Disc diffusion methods to determine the sensitivity of isolates to antibiotics [24].

### **Antimicrobial activity of algal extracts against bacteria**

The researchers employed the Kirby-Bauer disk diffusion susceptibility test to assess the sensitivity and resistance of Algal extracts to bacteria recovered from UTI patients [25]. To accomplish this, they evenly distributed 100 µL of the bacterial inoculum obtained from an 18–24-hour broth culture onto the surface of Muller Hinton agar media plates. Subsequently, the researchers placed antibiotic discs on the inoculated plates, followed by the Algal extract at concentrations of 25, 50, 75, and 100 mg/ml. Furthermore, several antibiotics were examined in this investigation. The plates were placed in an incubator at a temperature of 37 °C for a period of 18–24 hours, following a chilling period of 2 hours at 4 °C. The inhibitory zones on each plate were then measured in terms of their diameter.

### **Statistical analysis**

The data was analyzed by using SPSS (Statistical Package of Socio Science) by using one-way ANOVA for variation, LSD and independent sample t test at p. value <0.05 [26].

## **■ RESULT**

### **GC-MS**

The GC-MS analysis of Hexane and Ethanol extracts showed the presence of bioactive chemicals. GC-MS analysis of chemical compounds in Hexane extract of Cladophora Algae, shows 9 compounds. The 6-Octadecenoic acid compound is the most abundant with 95.1% of the total area, while the Trifluoroacetic acid, 2-tetrahydrofurylmethyl ester compound is the least area with 0.01% (Table 2). Whereas the GC-MS analysis of chemical compounds in the Ethanolic Extract of Cladophora Algae, showed 8 compounds. The Dichloroacetic acid, undec-2-enyl ester compound is the most abundant with 88.57% of the total area, while the 1,5-Heptadiene, 2,6-dimethyl-compound is the least area with 0.15 % (Table1).

### **Activity of Cladophora ethanol extract with different concentrations against *E. coli* and *S. aureus***

The present study was showed a significant difference at p. value <0.05, in the activity of Cladophora ethanol extract according to extract concentration, was showed the high activity of Cladophora ethanol against both *E. coli* and *S. aureus* in 100% concentrations, then the activity decreased with decrease concentration. In the other hand, the study showed a non-significant difference between *E. coli* and *S. aureus* in all concentration with except 100%, furthermore the study noted a non-significant difference between concentration of 75% and 50% *S. aureus* as in Table 3.



**Table 1**  
**Chemical compounds in the Hexane extract of Cladophora**

Seq	R. Time	Area%	Common Name	Formula
1	2.040	0.37	Hexane, 2,2,3-trimethyl-	C <sub>9</sub> H <sub>20</sub>
2	16.34	0.01	1H-Pyrazole, 3-methyl-5-(trifluoromethyl)-	C <sub>5</sub> H <sub>5</sub> F <sub>3</sub> N <sub>2</sub>
3	18.62	0.10	d-Glucohexodialdose	C <sub>6</sub> H <sub>10</sub> O <sub>6</sub>
4	19.13	0.01	(+)-3,4-Dehydroproline amide	C <sub>5</sub> H <sub>8</sub> N <sub>2</sub> O
5	20.94	4.31	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>
6	22.15	0.01	Trifluoroacetic acid, 2-tetrahydrofurylmethyl ester	C <sub>7</sub> H <sub>9</sub> F <sub>3</sub> O <sub>3</sub>
7	22.93	95.1	6-Octadecenoic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>
8	23.48	0.09	Cyclohexaneethanol, acetate	C <sub>10</sub> H <sub>18</sub> O <sub>2</sub>
9	25.62	0.00	Oxygen difluoride	F <sub>2</sub> O
Total		100		

**Table 2**  
**Chemical compounds in the ethanol extract of Cladophora**

Seq	R. Time	Area%	Common Name	Formula
1	16.341	0.15	2,2-Dimethylpropanoic anhydride	C <sub>10</sub> H <sub>18</sub> O <sub>3</sub>
2	17.472	0.27	Bicyclo[4.1.0]heptane, 3-methyl-	C <sub>8</sub> H <sub>14</sub>
3	19.136	0.40	Tridecanoic acid	C <sub>13</sub> H <sub>26</sub> O <sub>2</sub>
4	20.802	7.83	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	C <sub>21</sub> H <sub>40</sub> O <sub>4</sub>
5	22.701	88.57	Dichloroacetic acid, undec-2-enyl ester	C <sub>13</sub> H <sub>22</sub> Cl <sub>2</sub> O <sub>2</sub>
6	22.833	2.44	1,3-Dioxolane, 2-phenyl-	C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>
7	23.034	0.19	2-Butene, 1-bromo-3-methyl-	C <sub>5</sub> H <sub>9</sub> Br
8	23.386	0.15	1,5-Heptadiene, 2,6-dimethyl-	C <sub>9</sub> H <sub>16</sub>
Total		100		

**Table 3**  
**Activity of Cladophora ethanol extract with different concentrations against E. coli and S. aureus**

Concentration	E. coli	S. aureus	p. value
	Mean±S.D.		
25%	8.80±0.83 <sup>c</sup>	8.40±1.14 <sup>c</sup>	0.546
50%	11.4±1.14 <sup>c</sup>	10.6±0.89 <sup>b</sup>	0.252
75%	13.0±0.70 <sup>b</sup>	11.6±0.89 <sup>ab</sup>	0.025
100%	16.0±1.00 <sup>a</sup>	11.8±0.44 <sup>a</sup>	<0.01
p. value	<0.01	<0.01	
LSD	1.24	1.16	

### Activity of Cladophora hexane extract with different concentrations against E. coli and S. aureus

The present study was showed a significant difference at p. value <0.05, in the activity of Cladophora hexane extract according to extract concentration, was showed the high activity of Cladophora hexane against both E. coli and S. aureus in 100% concentrations, then the activity decreased with decrease concentration. In the other hand, the study showed a non-significant difference between E. coli and S. aureus in both 50% and 75%, furthermore the study noted a non-significant difference between concentration of 50% and 25% in E. coli as in Table 5.

**Table 5**  
**Activity of Cladophora hexan extract with different concentrations against E. coli and S. aureus**

Concentration	E. coli	S. aureus	p. value
	Mean±S.D.		
25%	9.20±0.83 <sup>b</sup>	8.80±0.84 <sup>d</sup>	0.471
50%	11.8±0.83 <sup>b</sup>	10.0±0.70 <sup>c</sup>	<0.01
75%	13.6±0.89 <sup>a</sup>	12.2±0.83 <sup>b</sup>	0.034
100%	14.6±0.89 <sup>a</sup>	13.4±1.14 <sup>a</sup>	0.101
p. value	<0.01	<0.01	
LSD	1.15	1.18	

## ■ DISCUSSION

The importance of freshwater algae has increased significantly in recent times due to their antimicrobial activity. There is a great concern of the World Health Organization about bacterial resistance to many antibiotics and antibiotics, which has led to increased mortality rates, especially in immune compromised individuals. There are many studies that prove the importance of algae-derived bio active compounds as antimicrobial agents and can serve as an alternative to traditional antibiotics. The diversity of secondary receptors present in algae makes them attractive candidates, as many of these receptors exhibit antibacterial effects by preventing metabolic activity or damage to the cell walls of microbes [27]. Accordingly, in this research, Cladophora algae was selected and the antimicrobial activities were evaluated and the bio active compounds were extracted from this alga using (GC-MS). According to the results, both ethanol and hexan extracts of Cladophora algae exhibit antibacterial activity, with the ethanol extract being more effective against both E. coli and S. aureus. Both extracts demonstrated antibacterial properties, but their efficacy varied depending on the concentration and the type of bacteria tested. The ethanol extract showed a significant increase in antibacterial activity for E. coli with increasing concentration, from 8.80 ± 0.83 mm at 25% to 16±1 mm at 100%.

The p-value for this increase (<0.01) indicates a statistically significant effect. The Least Significant Difference (LSD) of 1.24 suggests that differences in means between concentrations are meaningful. Similar to E. coli, the extract exhibited increased activity with higher concentrations for S. aureus, starting at 8.40±1.14 mm at 25% and reaching 11.8±0.44 mm at 100%. The p-value for S. aureus is also significant (<0.01). However, the differences in means are less pronounced than for E. coli. The hexan extract showed antibacterial activity for E. coli as well, with values increasing from 9.20±0.83 mm at 25% to 14.6±0.89 mm at 100%. The p-value for the 50% concentration is significant (<0.01), indicating effective inhibition. The LSD of 1.15 reinforces the significance of differences between concentrations.

The activity was slightly lower on S. aureus compared to E. coli, showing a maximum inhibition of 13.4±1.14 mm at the highest concentration. The p-value of 0.101 for the 100% concentration indicates no significant difference from lower concentrations, suggesting that hexan extract may not be as effective against S. aureus as the ethanol extract. Both extracts demonstrated increased antibacterial activity with higher concentrations, but the ethanol extract showed overall greater efficacy, especially against E. coli.

The differences in susceptibility between the two bacterial strains indicate that E. coli is more sensitive to Cladophora extracts than S. aureus. This could be due to differences

in cell wall structure and resistance mechanisms. The p-values indicate that both extracts have significant antibacterial properties, particularly at higher concentrations. The LSD values provide confidence that the observed differences are not due to random variation. The effectiveness of the Cladophora Algae can be attributed to its content of active compounds such as Hexadecanoic acid methyl ester has Antioxidant, Antimicrobial, Hypocholesterolemia, Hemolytic, Alpha reductase inhibitor [28]. In addition, 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester has (Antibacterial, anti-candidal, anti-inflammatory, hypocholesterolemia, cancer preventive, hepatic protective, nematicide, insect Fuge anti histaminic, anti-arthritic, ant coronary, antieczemic, anti-acne, 5-Alpha reductase inhibitor and anti-androgenic activities [29].

Furthermore, this current study's identification of octadecenoic acid (Oleic acid) in Cladophora extract is corroborated by the prior work of Al-Khafaji and Saeed [30], where they found that octadecenoic acid is one of the active compounds present in Cladophora extract. Stabili also noted in their study that since Oleic acid is the most abundant among the fatty acids of Cladophora extract, it is likely responsible for the antibacterial activity [31].

## ■ CONCLUSION

Given the numerous side effects associated with most antibiotics employed in the medical profession, our current study aimed to investigate the effects of ethanol and hexane extracts of Cladophora Algae on pathogenic bacteria that cause (UTI), demonstrating superior inhibition compared to some medicines. The Ethanol and Hexane extracts exhibit antimicrobial properties due to their chemical composition, which includes compounds such as Hexadecanoic acid, ethyl ester, 9,12-Octadecadienoic acid (Z,Z),9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester, Heptadecane Pentadecanoic acid, 14-bromo, positioning them as potential alternative medicinal treatments.

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