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Evaluation of *Cladophora vadorum* antimicrobial activity collected from Tabalino lakes in Benghazi city, Libya on human pathogenic bacterial isolates

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Highlights

- ***Cladophora vadorum* algae have many active substances supports their placement among medicinal plants because of their medically-influenced compounds.**
- ***Staphylococcus aureus* was found to be more sensitive by all the organic solvent extracts used.**
- ***E. coli* has resistance and did not give positive results against extracts used except petrolium ether extract**

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ABSTRACT

This study was conducted using *Cladophora vadorum* (green algae), collected from Tabalino lakes in Benghazi to evaluate the effect of this algae on the growth of four strains of bacteria (*Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa*, and *Klebsiella pneumonia*) using 5 types of organic solvents (ethanol, acetone, methanol, petroleum ether, and ethyl acetate). The effect of organic solvent extracts on the growth of the bacteria used was varied. The petroleum ether extract was the most effective against the growth of all the bacteria used. Ethanol, Acetone, and ethyl acetate were active against at least one or two of the tested bacteria. The inhibitory effect on other bacteria was different. *S. aureus* was found to be more sensitive by all the organic solvent extracts used. *E. coli* has resistance and did not give positive results against extracts used except petroleum ether extract, while *K. pneumonia* bacteria was not affected by all types of organic extracts used. Presence of many active substances in *Cladophora vadorum* methanol extract supports their placement among medicinal plants because of their medically influenced compounds.

1. Introduction

Large algae are known as seaweeds and include red algae (Rhodophyta), green algae (Chlorophyta), and brown algae (Phaeophyta), and these herbs contain various organic and inorganic substances that can be a benefit for human health both directly or indirectly (Kuda *et al.* 2002). Green algae are one of the largest groups of algae in terms of the number and distribution of species found in fresh, semi-salty, salty, and highly saline and on the soil surface (Omar *et al.* 2016). The great difference between the types of this group of algae is observed as they range from single-cell microscopy close to the size of bacteria such as *chlorella* to sizes greater than two meters such as *Ulva grandi*.

Libya has a long coastline estimated at about 1,900 kilometers on the Mediterranean Sea. It is very rich in algae, however, the numbers of seaweeds that were studied on the east coast are about 168 species under 96 species (Godeh *et al.,* 1992) and in view of the many studies that carried in intermittent periods, the majority of them were focused on the inventory and definition of algae that located on the Libyan shore (Nizamuddin *et al.,* 1979; Nizamuddin, 1981; Godeh *et al.,* 1992; Nizamuddin and Godeh, 1993). Their results are needed to be used in promoting public health. The coast of Benghazi stretches over 18 kilometers on the Mediterranean Sea and it is very rich in marine green algae, with 54 species of green algae, belonging to 17 genera (Godeh *et al.,* 2010). *Cladophora vadorum* is one of the green algae identified in Libya, and it lives in saltwater and semi-salty and freshwater bodies, it has a simple branching filament; each cell represents a multi-nuclear cellular integrated with a layer of cytoplasm with a thick wall. Most species living on rocks and some species live as a symbiosis with sponges in the seas. This genus of algae reproduces asexually via producing bi or tetraspores, and sexually by bi flagellated gametes. Its life cycle is Isogamy (Haplo-diplonatic type). Other nine species of *Cladophora* were recorded on the Libyan coast, *C. albida*, *C. catenata*, *C. ramulosa*, *C. dalmatica*, *C. globulina*, *C. glomerata, C. nigrescens, C. pellucida, and C. prolifera.*

Algae have a direct effect on many bacteria and fungi where they produce many active compounds that may be present within or outside their cells (extracellular products). These effective compounds can be extracted by different methods, which usually depend on using of various solvents that maybe work singly or mixed with others such as acetone, methanol, ethanol, hexane, methylene chloride, petroleum ether, etc. and may use water in the extraction processes as well (Kim *et al.,* 2006).

Algae have gained extensive attention to their chemical composition and physiological properties, coinciding with the development of chemical analysis techniques, although their chemical compounds have nutritional values (Adedayo *et al.,* 2011; Chronakis and Madsen, 2011; Priyadarshani and Rath, 2012; Samarakoon and Jeon, 2012). Algae have been used as food because they contain high amounts of carbohydrates, proteins and minerals as a good source of many important vitamins such as folic acid, fatty acids, ascorbic acid, nicotinic acid, thiamine, biotin, prodoxin, and riboflavin acid. These compounds are considered a good substance for the treatment of malnutrition diseases and problems (Rupe'rez *et al.,* 2001; Thennarasan and Murugesan, 2015). Using many antibiotics in the production of resistant bacterial strains has led to hospitals to become fertile ground for such as resistant organisms and have posed a threat to human life. So algae and other medicinal plants are an important source of biologically active compounds such as anti-human viruses, viruses, many infectious diseases, and others

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are being developed for use as new drugs, the most important of which are phenols, sugars, peptides, proteins, and terpenes (Pulz and Gross 2004, Chew *et al*., 2007, Mayer *et al*.,2007). Several studies for the inhibitory effects on the growth of pathogenic bacteria and fungi were concerned by secondary metabolic compounds of algae extracts were performed (El-Fatimy *et al*., 2009; Al-saif *et al.,* 2014; Alshalmani *et al.,* 2014; Abdel-Raouf *et al*., 2017; Godeh *et al.,* 2017).

Cladophora vadorum is new record species in Libya, it is identified by Prof. M. M. Godeh, so, the purpose is to study the effect of organic solvent extracts of this species on the growth of some locally pathogenic bacterial isolates from the children's hospital in Benghazi and to identify some of the activity of the effective compound that affects the growth of these bacteria isolates.

2. Materials and methods

2.1. Samples collection:

This study was conducted using *Cladophora vadorum*, collected from the Tabalino lake in Benghazi, a small lake belonging to 23 July Lake in Benghazi. Samples were collected in plastic bags and washed by lake water to remove any phytoplankton, fauna and suspended sand, or any other types of algae. The samples were transferred to the lab and well washed again by tap water, and then distilled water. The parts of *C. vadorum* samples were kept on herbarium sheets (28.75×41.25 cm). In addition, other parts were kept in 4% formalin in special bottles, while the majority of the samples have been dried in shade for 6 to 7 days at room temperature with good ventilation, and then placed in an oven at 40°C temperature for 20-30 minutes to dry the samples until getting a stable weight. The samples were milled using an electric mill until they became fine powder and kept in sealed containers until use.

2.2. Bacterial strains

Four strains of bacteria that cause human diseases were obtained from the Benghazi Children's hospital and identified by a BD Phoenix device. The bacteria were kept on nutrient agar and stored in the refrigerator at 4°C until use. Two types of media, nutrient agar, and Muller Hinton agar were used as a nutritional medium for growth and testing of bacteria's sensitivity to plant extracts (Cheesbrough, 1984).

2.3. Preparation of algae extraction

Five grams of dried algae were extracted by 100 ml of different organic solvents (ethanol, methanol, acetone, petroleum ether, ethyl acetate) in a 250 ml conical flask on a shaker for 21 days at 100 cycles per minute speed and the extract were filtered. The solvent was vaporized and the solid matter was melted in 2.5ml of 50% Dimethyl sulphoxide (DMSO) (Mahadik, 2015). Several dilutions were performed to obtain the lowest concentration (50µl) for use. The antimicrobial activity test of algae extracts was done by the "well diffusion method" (Crasta, 1997). The bacterial culture was inoculated to Muller Hinton agar and planned in three directions by using a sterile cotton swab on the surface of the medium. Then 200 µL of the extracts were poured into holes using a sterile cork borer (8 mm diameter). Another hole was made containing on the solvent without algae extract as a negative control (DMSO), and amikacin antibiotic was added as a positive control. The plates were incubated at 37 °C for 24 h. After incubation, the inhibition zones formed around the holes were measured in millimeters.

2.4. Preliminary phytochemical tests for the methanol extract of Cladophora vadorum

A series of preliminary phytochemical tests were carried out to identify active substances in the methanol extract of *C. vadorum* algae. Alkaloids were detected using Wagner reagent as described in Harborne (1984). Flavonoids were indicated by adding ethanol acidic KOH reagent [5N] to the extract (Newall *et al.,* 1996). Tannins were indicated by adding lead acetate (1%) to the extract (Hussein and Nabeel, 2018). Phenols were indicated by 1-2 drops of FeCl3 chloride solution (1%) as described in Harborne, (1998). Saponins were indicated by adding a mercury chloride detector (5%) to the extract (Haddad, 1965). Detection of sugars was done by the Benedict test and confirmation of the result was done by the Fehling test (Davison and Cheyne, 1974). Detection of resins done by adding ethyl alcohol (95%) to the extract (Mason and Wasserman, 1987).

2.5. Statistical analysis

Two-way variance statistical analysis was used to test differences of means of inhibition zones diameters and the duration of time by SPSS software.

3. Results and discussion

The inhibitory effects of *C. vadorum* extracts on the growth of four pathogenic bacteria (*E. coli, S. aureus, P. aeruginosa* and *K. pneumonia*) using various organic solvents: methanol, ethanol, acetone, petroleum ether, and ethyl acetate were represented in Table 1. Methanol alcohol extracts affected the growth of *S. aureus* only without affecting the growth of other bacteria, while ethanol extracts affected the growth of *S. aureus* bacteria and *P. aeruginosa.* Acetone and ethyl acetate extracts only affected the growth of *S. aureus* bacteria. While petroleum ether extracts have affected most of the bacteria tested except *K. pneumonia* and its highest effect was on *S. aureus* bacteria, where the diameter of the inhibition zone is 14 mm, and it should be noted that *S. aureus* bacteria are considered as the most sensitive to the extracts.

Table 1

The effect of *Cladophora vadorum* extracts on the growth of certain bacteria using five organic solvents in millimeters (mm).

Organic solvent	Methanol	Ethanol	Acetone	Petroleum ether	Ethyl acetate	Amikacin	Control DMSO
Bacterial strains↓	Inhibition zone diameter (mm)						
S. aureus	11	11	11	14	8	38	0
E .coli	0	0	0	4	0	17	0
K. pneumonia	0	0	0	0	0	12	0
P. aeruginosa	0	2	0	4	0	17	0

DMSO=Dimethyl Sulphoxide

Methanol extract does not affect the growth of *S. aureus* only, and this is not compatible with Khalid *et al.,* (2012) who indicated that methanol extract has an inhibitory effect on all types of bacteria used in the current study. As for the effect of the ethanol extract of *C. vadorum* it was clear that the extract did not have an inhibition effect on the activity and reproduction of bacteria, which means that the extract did not penetrate through the plasma membrane or the cell wall, which led to non-inhibition effect on these bacteria.

These results are not agreeing with those obtained by Tuney *et al.,* (2006) who explained that ethanol extract is one of the most effective extracts with pre-mentioned bacteria.

Amikacin 30 was used as a positive control (+ve control) and showed an inhibitory effect on the growth of all used bacteria, especially *S. aureus,* while the negative control (–ve control) consisting of dimethyl sulphoxide solution (DMSO) did not show any inhibitory effect on the growth of the used bacteria (Table 1).

The results also showed that acetone and ethyl acetate have less impact on the growth of the bacteria as they have had an inhibitory effect on the growth of *S. aureus* bacteria only without the rest of the bacterial species. This is in line with many researches that confirm that gram-positive bacteria are more sensitive to the effect of extracts than gram-negative bacteria; this is probably due to the difference in the composition of the cell wall. This is confirmed on *S. aureus* (gram-positive) which has shown a greater sensitivity to the effect of secondary metabolic compounds (Ibtissam *et al.,* 2009; Zbakh *et al.,* 2014; Seenivasan *et al.,* 2010; AL-Ghazeer *et al.,* 2013 a; Salem *et al.,* 2014; Soltani *et al.,* 2014). However, this is not agreed with Alang *et al.* (2009) who confirmed in his study that bacteria *E. coli* (gram-negative) are the most sensitive to secondary metabolic compounds, while in the current study *E. coli* has resistance and did not give positive results against extracts used except petroleum ether extract. *K. pneumonia* was resistant to all the used extracts; this is agreed with Mahasneh *et al.,* (1995). The resistance of *K. pneumonia* may be due to their containing in a capsule that surrounds them from the outside and spread in the surrounding environment is responsible for protecting bacteria from antibiotics (King & Roberts, 2016).

The petroleum ether extract of *Cladophora vadorum* has the highest inhibitory effect on the growth of all the tested bacteria (gram-positive and negative). Among all other solvents, the petroleum ether has a high ability to extract active materials of algae and Mahadik *et al.* (2015) stated this. The study`s results revealed that *P. aeruginosa* was affected by both ethanol extract and petroleum ether for *C. vadorum*, while not affecting by methanol and acetone extracts. *P. aeruginosa* has considered as one of the most resistant bacteria to antibiotics. Green algae are plants with a high content of secondary metabolites such as Mono-and-polycyclic, and Acyclic algal diterpenes, phenolic compounds, and some other bioactive compounds that inhibit the activity and vitality of some vital systems such as bacteria (Holdt and Kraan, 2011).

Table 2

Phytochemical screening for the methanol extract of *Cladophora vadorum*

Active substances concentration						
Alkaloids	flavonoids	Tannins	Saponins	Phenols	Saccharides	Resins
6.23%	30.13%	+	+	36.14%	14%	++

By identifying the concentrations of dissolved substances in the methanol extract of *C. vadorum* (Table 2), these algae species were found to contain alkaloids, flavonoids, phenols, saponins, tannins, saccharides, and resins in methanol extract at a different rate, these results were consistent with Soltani *et al.* (2011) and AL-Ghazeer *et al.* (2013 b). The presence of these active substances in algae supports their placement among medicinal plants because of their medically influenced compounds, which are medically effective in several studies.

4. Conclusion

The current results indicate that the new record species *Cladophora vadorum* collected from Libyan lake represent valuable active substances that can inhibit the growth of some pathogenic bacteria.

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