Effect of the Crude Leaf Extracts of Dodonaea Viscosa on Some of Algae

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ABSTRACT

The present study has been conducted to evaluate the antialgal activities of the leaves crude extracts of Dodonaea viscosa plant. The antialgal activities of ethanol (80%) and diethyl ether crude leaf extracts of D. viscosa were tested against Microcystis flos- aquae, Scenedsmus dimorphus and Mougeotia scalaris. The agar well diffusion method was used to evaluate the inhibitory actions of these extracts with three concentrations: 5,10, and 20 mg/ml. The results showed that the ethanol extracts were more effective on algae than diethyl ether extracts. The alga: Mougeotia scalaris and Microcystis flos- aquae have more sensitive than Scenedsmus dimorphus to attack by the ethanolic extracts, while Mougeotia scalaris have more resistsnce to attack by the diethyl ether extracts followed by Microcystis flos- aquae, while Scenedsmus dimorphus have more sensitive to attack by diethyl ether extracts.

Key words: Crude extracts, Dodonaea viscosa antialgal effects**.**

تأثير المستخلصات الخام لأوراق نبات *Dodonaea viscosa* على بعض الطحالب

الخلاصة

درست تأثيرات المستخلصات الخام لأوراق نبات الدودونيا Dodonaea viscosa على الطحالب. اختبرت الفعالية المضادة لنوعين من مستخلصات الأوراق هي مستخلص الايثانول (٨٠%) ومستخلص الداي اثيل ايثر ضد كل من الطحالب: Microcystis flos- aquae, Scenedsmus dimorphus وMougeotia scalaris بطريقة الحفر و الانتشار خلال الاكار لتحديد الفعالية التثبيطية لتلك المستخلصات اذ استخدمت ثلاثة تراكيز هي ٥ , ١٠, ٢٠ ملغم/ مل. اظهرت النتائج ان مستخلص الايثانول للاوراق كان اكثر تاثيرا على الطحالب من مستخلص الداي اثيل ايثر. و ان طحلبي Microcystis flos- aquae ,Mougeotia scalaris اكثر حساسية من طحلب Scenedsmus dimorphu لمستخلص الايثانول بينما كان طحلب Mougeotia scalarisالاكثر مقاومة لمستخلص الداي اثيل ايثرللاوراق ثم تلاه طحلب Microcystis flos-

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aquae بينما كان طحلب Scenedsmus dimorphus الاكثر حساسية لمستخلص الاوراق باستخدام داي اثيل ايثر.

INTRODUCTION

Algae are found in water and soil, and they have many advantages such as: food source, they also form the oxygen necessary for the metabolism of the consumer organisms [1]. Also they have different disadvantages such as: the excessive growth of algae causes bad taste and odor in water espicially filamentous algae (*Cladophora* and *Spirogyra*), and many algal species produce chemical compounds as defense mechanism that impair the growth of compete algae and deter herbivorous [2] such as the blue-green algae: *Cylindrospermum* exceretes the compound DMDP (dihydroxy methyl dihydroxy pirrolidine) which inhibite digestive enzymes of crustacean and macro grazers[3], the toxin-producing cyanobacteria can also occur in benthic mats such as those found in drinking water reservoirs [4]. Toxins may be occur at high concentration during or after algal bloom, released from large population of dying algal cells, in such cases, vertebrates such as birds, dogs, farm animals or sea mammals may be sickened or killed and human my also be affected [5].

Many reserches were carried out to control the harmful algal bloom which included mechanical, physical, and chemical methods in addition to biomanipulation [6,7,8]. All these approaches were not sufficient to solve the problem. Therefore, in the last few decades, a variety of medicinal plants and plant extracts have been screened for their antialgal activity.

However, Dodonaea viscosa: a plant which is belongs to the family Sapindaceae, used as a traditional medicine in different countries. Leaves are used to treat itching, digestive system disorders, including indigestion, ulcers and diarrheoa, and the powdered leaves were given to expel round worms [9]. The plant is also used as antimicrobial and has insecticidal activity[10]. For these effects, We suggested to use the crude leaf extracts of this plant in contorl of algae.

MATERIAL AND METHODS

Collection of plant samples

Dodonaea viscosa Jacq. Leaves were collected from the gardens of Baghdad University/Al- Jadriah in March and April / 2011.

The plant is identified in Biology Department's Herbarium, College of Science/ Baghdad University. In laboratory, plant samples were washed with tap water and dried at room temperature, and then ground to semi powdered state by using a grinder, and stored in clean containers.

Preparation of plant extracts

Two polar solvents were used in this study:

- Ethanol (80%): high polar solvent.
- Diethyl ether: low polar solvent.

The extraction for bath solvents were done by soxhlet extraction [11]. The air-dried and powdered plant materials (20 g) were kept in the soxhlet's apparatus, and 250 ml of solvent was added and extracted for 8 hours at 40°C and evaporated by using rotary evaporator at dry extracts. The extracts were stored at 4°C until used.

Preparation of concentration

Stock solution were prepared by mixing 2g from the dried extract with 20 ml of ethanol (80%), and 2g from the dried extracts with 20 ml of diethyl ether. Then the concentrations (5, 10 and 20)mg/ ml were prepared by mixing known volume from the stock solution with ethanol (80%) and diethyl ether using the following equation: $C_1V_1=C_2V_2$ to prepare these three concentrations.

Control treatment is the solvent which used to prepare the extracts.

Isolation and characterization of algae

Algal samples were collected from canal around University of Baghdad/ Al-Jadriah. Isolation was done using serial dilution and streaking plate method [12]. Samples were diluted with CHU-10 medium up to 10^{-10} dilution. Dilution tubed were incubated in cooled illuminated incubator with light intensity about 200 $\mu E/m^2/S$ and 26±1°C. The unialgal cultures of Cyanophyta and Chlorophyta obtained in dilution tubes were further purified on pre- washed CHU-10 agar slants. Stock cultures were maintained at room temperature under diffused light. The species were identified with help of classical algal classification refrences [13,14].

Purification of algal cultures

The purification of algal isolates was done according to [15] to obtain axenic cultures. 50ml of unialgal culture was taken and kept in dark for 24 hrs. then 10ml of it was taken by using pipette and put in sterile flask with liquid media (CHU-10) then 2-3 drops of nutrient broth was added then the flask kept in dark for 2-3 hrs. then centrifugation 3000 rpm for 2 min. was done, the supernatant was neglected and the pellet was washed by sterile liquid media (CHU-10) for 12-15 time, then a part of that pellet was cultured on the surface of petri dishes with nutrient agar to indicated the absence of bacterial growth, the dishes put in incubator at 37°C for 72 hrs.. Also a part of that pellet was cultured on PDA (potato dextrose agar) to indicated the absence of fungal growth.

Determination the antialgal activity of the crude extracts

The algicidal effects of the plant using their crude extracts were examined against three species of algae by using the following steps:

Preparation Lawns of algae

Bright green culture of alga was selected.CHU-10 medium was prepared in flat bottom flask, agar- agar 2% was added for solidification. Agar- agar was dissolved by using water bath with 100°C, the media was sterilized using the autoclave.The media cooled up to 35-40°C.

1:4 of algal culture was added to the agar media, shaked well and poured in petri dishes immediately to avoid the solidification of media in the flask. petri dishes were incubated in reverse position within a cooled illuminated incubator with 200 $\mu E/m^2/S$ and 26±2°C for 2-3 days until the plates turn into greenish color [16].

CHEMICAL DETECTION OF THE ACTIVE COMPONENTS IN PLANT EXTRACTS

1- Detection of Glycosides

Some drops of HCL were added to the plant extract and mixed well, then placed in a water bath for 2 minutes. Then 2 ml of Benedict reagent were added and

placed in water bath for 5 minutes, the appearance of red precipitant refer to positive result [17].

2- Detection of Alkaloids

The detection solution was prepared according to [18] by placing 3 ml of plant extract in test tube, then addition one of these reagents:

A- Mayer′s reagent: white precipitant refer to alkaloids existence.

B- Dragendroff′s reagent: orange precipitant refer to alkaloids existence.

C- Marqui′s reagent: yellow to purple spots refer to alkaloids existence.

3- Detection of Tannins

Plant powder 10 g was added to 50 ml distilled water, then heated till boiling, then cooled. The mixture was filtered, and then divided into two equal volumes; few drops of 1% Lead acetate were added to the first volume. The appearance of gelatinous white precipitant represented a good indicator for tannins existence.

For the second volume, 1% Ferric chloride ($FeCl_3$) solution was poured; the appearance of blue green color represented a good indicator for tannins existence [19].

4- Detection of Saponins

This detection was carried out according to [19] as follows:

a- The appearance of a big foam for a long time as a result of stirring the aqueous solution of plant in test tube indicated saponins existence.

b- Adding 1-3 ml of Mercuric chloride to 5ml of plant extract, appearance of white precipitant represented a good indicator for saponins existence.

5- Detection of Resins

Aliquot of 50 ml of (95%) ethyl alcohol was added to 5 g of plant extract powder, put in water bath and boiled for 2 minutes, after cooling the mixture filtered, 10 ml of distilled water containing 4% HCL were added to the filtered solution, thereafter turbidity appearance refer to resins existence [19].

6- Detection of Coumarins

Aliquot of 1-2 ml of alcoholic extract in a test tube was added and covered with filter paper (exposed with sodium hydroxide solution (NaOH)) and placed in water bath, heated till boiling, thereafter the filter paper exposed to UV transilluminator, a bright yellow green color refers to the coumarins existence [20].

7- Detection of Flavonoids components

This detection was prepared according to [21]:

Solution (A) was prepared by dissolving 10 g of plant powder in 10 ml of (95%) ethanol and filtered.

Solution (B) was prepared by adding 10 ml of (50%) ethanol to 10 ml of (50%) potassium hydroxide (KOH), two equal volumes of solution (A) and solution (B) were mixed. Appearances of yellow color refer to flavones existence.

8- Detection of Phenolic components

Plant powder 10 g was added to 50 ml of distilled water, and then heated till boiling; the solution left to cool, and filtered, then 1% of Ferric chloride ($FeCl_3$) was added to the filtered solution. The appearances of blue green color refer to the phenolic compounds existence [18].

9- Detection of Terpenes and Steroids

This detection was conducted according to [22].as follows:

A quantity of 1g of dried plant extract was dissolved in 1-2 ml of chloroform, one drop of acetic anhydride was added, and then one drop of concentrated sulphuric acid (H_2SO_4) was added. Appearance of brown color represented terpenes existence. Thereafter, the appearance of a blue green color indicated for steroids existence.

10- Detection of Volatile Oils

This detection was conducted according to [23] by filtering 10 ml of plant extract with filter paper, thereafter the filter paper was exposed to UV transilluminator. The appearance of pink color referred to the volatile oils existence.

CONTROL OF ALGAE

Algicidal effects of plant extracts were detected by using the agar- well diffusion method according to[16] as a follows:

Certain numbers of wells were prepared in the plates contained the lawns of tested algae with the help of sterile cork borrer (6 mm in diameter), the tested concentration of plant extracts were inculated into the well. Contols were made by using the solvents which were used in the extraction instead of plant extract. The plates then left for 30 minutes in a refrigerator to permit the extracts to absorb and diffuse through the media, then incubated in the cooled illuminated incubetor for 24 hrs.. Inhibition zones were determined by measuring their diameters. Three replicates were made and the mean values were recorded.

RESULTS AND DISCUSSION

Extraction

Extraction of leaves by using ethanol (80%) gave a glossy olive- green color with a yield of 33.3%, whereas gave olive-green color with a yield of 10% when using diethyl ether.

In this study, crude extracts were used because the biological activities, if proven to exist, might be lost during the process of purification from the crude extracts [24].

Chemical test for the active components in *D. viscosa* plant

The detection of active components in the leaves demonstrated the following table. Leaves extracts which extracted with ethanol (80%) and diethyl ether containing the following components: flavonoids, glycosides, tannins, volatile oils, terpens, saponins and phenols. The glycosides were absent in diethyl ether extract since they are insoluble in diethyl ether [25]. The pH of leaf extracts was acidic (pH= 5), and this ensured that extracts were not containing alkaloids. The absence of alkaloids in the leaf extracts is in agreement with the previous results of [26].

Leaf extracts were not containing alkaloids, steroids, resins, and coumarins. The absence of coumarins is similar with results of [27], whie the absence of steroids is not similar to the results of Sachder and [28], the absence of resins is similar results with [26].

Table (1): Results of the test of the active chemical components in *D. viscosa* leaves extracts.

Plant part	**Leaves**		
Solvent / **Active components**	**Reagent**	**Ethanol (80%)**	**Diethyl ether**
Alkaloids	Mayer reagent	-	-
Flavonoids	Ethanol+ KOH	+	+
Glycosides	Benedict	+	-
Tannins	1% Lead acetate	+	+
Coumarins	NaOH+ UVtransilluminator	-	-
Volatile oils	UVtransilluminator	+	+
Terpens	Acetic anhydride+ H_2SO_4	+	+
Steroids	Acetic anhydride+ H_2SO_4(after period)	-	-
Resins	Ethanol+4%HCl	-	-
Saponins	Mercuric chloride	+	+
Phenols	1% $FeCl_3$	+	+

+: The extract contains the active component.
-: The extract doesn't contain the active component.

Isolation and Characterization of algae

Three species of algae were isolated, purified, from canal around Al- Jadriah. They were included 1 species of blue- green algae, and 2 species of green algae. Their characteristic features are given below:

DESCRIPTION OF ISOLATES

Microcystis flos- aquae

Colonies roughly spherical, ellipsoidal, or somewhat elongate or often squarish in optical section, not clathrate, with indistinct colonial mucilage, cells 3-7μ in diameter, spherical with gas- vacuoles, nannocytes present.
Planktonic in lakes, tanks, and ponds, often as a water bloom [13].

Mougeotia scalaris

Vegetative cells (34) μ in diameter, 40-180 μ long, chloroplast a broad plate with 4 pyrenoids. Zygospores formed in the tube by scalariform conjugation, not dividing the gametangia, globose or broadly ovate, walls smooth and golden brown, 25-31 μ in diameter, 27-40 μ long.

In littoral flora of many lakes. [14].

Scenedsmus dimorphus *(*Turp.) Kuetzing

Colony composed of 4 or 8 fusiform cells arranged in a single or alternating series, the inner cells with straight, sharp apices, the outer cells lunate, strongly curved, with acute apices, cells 3-6 μ in diameter, 16-22 μ long.
Common and widely distributed in many lakes and bogs. [14].

Evaluation of inhibitory effects of crude leaf extracts against algae

Inhibitory effects of crude leaf extracts of D. viscosa against 3 species of algae: (Microcystis flos-aquae, Mougeotia scalaris, and Scenedsmus dimorphus) were tested depending to the diameters (mm.) of inhibition zones according to agar- well diffusion method as showed in Table(2) and plate(1). Were showed that the inhibition zones varied according to algal species, type of solvents that used during the extraction and their concentrations. However, results revealed the ethanol extracts of *D. viscosa* leaves have more effects on algae than leaf extracts by using diethyl ether This may due to that ethanolic extracts contain terpenes, phenols, flavonoids and saponins which have a great effect as antialgal agents .

The highest value of inhibition zone (mm.) in diameter of ethanol extracts against *M. scalaris* and *M. flos- aquae* (35mm.) in diameter in concentration 20 mg/ml, while the lower value of inhibition zone was 20 mm. in diameter in concentration 5 mg/ml against *S. dimorphus*.Leaf extracts by diethyl ether showed the highest inhibition zone against *S. dimorphus* (35mm.) in diameter in concentration 20 mg/ml. While the lower inhibition zone of diethyl ether extracts was showed against *M. scalaris* (10mm.) in diameter in concentration 5 mg/ml.
The algae: *M. scalaris* and *M. flos-aquae* (35 mm.) in diameter in concentration 20 mg/ml. have more sensitive than *S. dimorphus* (30 mm.) in diameter in concentration 20 mg/ml. to attack by the ethanolic extracts. While *M. scalaris* have more resistance to attack by diethyl ether extracts (20 mm.) in diameter in concentration 20 mg/ml followed by *M. flos-aquae* (25 mm.) in diameter in concentration 20 mg/ml. While *S. dimorphus* have more sensitive to attack by diethyl ether extracts (35 mm.) in diameter in concentration 20 mg/ml. These results may due to the presence of tannins which showed antialgal effects as exhibit antimicrobialeffect against phytopathogenic fungi and bacteria [29,30].

No reference for the algicidal effects of *D. viscosa* crude leaf extracts were found in the literature. However, different studies used crude extracts of *D. viscosa* plant as antibacterial and antifungal agents such as effects. [27]. who demonstrated that leaf extracts showed antibacterial effects, also [31,32] who reported that the methanolic extracts of *D. viscosa* have antimicrobial effects.

Table(2):Variations in the algicidal effects of crud leaf extracts of *D. viscosa* in mm.

Extract	Concentration mg/ml	Diameters of inhibition zones (mm.)		
		M. flos-aquae	***M. scalaris***	***S. dimorphus***
Ethanolic extracts	5	25	25	20
	10	30	30	25

	20	35	35	30
Diethyl ether extracts	5	15	10	25
	10	20	15	30
	20	25	20	35

Figure(1): Different inhibition zones caused by D. viscose leaves extracts on algae.

A: Inhibition zones in concentration 20 mg/ml of leaves extracts against algae.
B: Inhibition zones in concentration 10 mg/ml
D: Inhibition zones in concentration 5 mg/ml
C: control (the solvent that use to prepare the extract).

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