



## Isolation, Purification and Identification of Seven species of Algae in three drinking water plants supply springs in Sulaimani province and growth control of isolated algae by using some plant extracts

Bashar Tareq Ismael AL-Shandah<sup>1</sup>, Riadh Abas Abd Al-Jabar<sup>1</sup>, Trifa K. J. Farkha<sup>2</sup>

<sup>1</sup> Department of Biology, College of Science, University of Tikrit, Tikrit, Iraq

<sup>2</sup> Department of Biology, College of Science, University of Sulaimani, Sulaimani, Iraq

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#### Corresponding Author:

##### Name:

Bashar Tareq Ismael

##### E-mail:

Bashar.alshindah@gmail.com

##### Tel:

##### Affiliation

### Abstract

In this study, three drinking water treatment plant supply springs were selected within different sites in Sulaimani province - Kurdistan region of Iraq. Samples were collected over period of eight months from May to the end of December 2015.

Five stations were selected, Stations 1, 2 and 3 were located at Bestan Sowr drinking water project in Sharazur district, station 4 was located at Saray Subhan Agha drinking water project in Said sadiq district and station 5 was located at Khormal drinking water project in Khormal district within Halabja district.

The study involved an Isolation, purification and identification seven species of algae by using Chu-10 media and Bold Basal Medium (BBM), and evaluation of the effectiveness of plant extracts (as antialgal) on the growth of isolated algae by Agar-Well Diffusion Method.

Seven strains of algae from studying stations were isolated, purified and identified after collection which were: *Ulothrix zonata*, *Desmodesmus lunatus*, *Desmodesmus itascaensis*, *Chlorella vulgaris*, *Calothrix fusca*, *Chroococcus turgidus* and *Navicula riediana*. These isolated algae included 2 species of blue-green algae, 4 species of green algae, and 1 species of diatoms.

In this study, the suggested plants extracts for use as inhibitors belong to different families of plant (5 plants) which were *Hordeum vulgare* (Barley straw), *Peganum harmala*, *Artemisia annua*, *Thymus vulgaris* and *Nasturtium officinale* (Watercress).

On the assessment of the effectiveness of crude plant extracts (as antialgal) on the growth of selected algae by Agar-Well Diffusion Method, the results showed that the greater inhibition zone diameter was obtained 62 mm against *Calothrix fusca* by using crude extracted from *Peganum harmala* in concentration 80 mg/ml, and less inhibition zone diameter was 7 mm against *Ulothrix zonata* by using crud extracted from *Artemisia annua* in concentration 30 mg/ml. *Nasturtium officinale* (Watercress) did not show any effective against any type of isolated algae in all concentrations. Generally inhibition zones diameters differed according to the algal types and types and concentration of plant's extracts.

### 1. Introduction

Algae occur naturally in marine and fresh water, under favorable conditions that include adequate light availability, warm water, and high nutrient levels,

algae can rapidly grow and multiply causing "blooms." Blooms of algae can cause damage to aquatic environments by blocking sunlight and

depleting dissolved oxygen required by other aquatic organisms, restricting their growth and survival. Some species of algae, including golden and red algae and certain types of cyanobacteria, can produce potent toxins that can cause adverse health effects to wildlife and humans, such as damage to the liver and nervous system [1]. When algal blooms impair aquatic ecosystems or have the potential to affect human health, they are known as harmful algal blooms (HABs), in fact, excessive growth of primary producers in water bodies due to high inputs of nutrients, especially phosphorus and nitrogen, has been cited as the most important form of pollution in lentic and lotic systems [2] and [3].

However, research to find ways of controlling their growth was encouraged such as use of algicidal substances and mechanical cleaning for stable tanks and filtration to control algae in water supplies. It also includes development of co-agulation, filtration, and chemical treatment in addition to development methods of storages the water to decrease the growth dense of algae and control of odor and tastes and other algal products [4]. There are some organic chemical compounds such as Quinones and substituted hydrocarbones that act as algicides which are more effective than the inorganic salts such as copper sulphate, potassium permanganate and chlorine, but these organic chemical compounds are very expensive [5].

A variety of methods have already been developed and applied to cope with the problems related to algal blooms, including UV-radiation, nutrient diversion,

oxidation, removal, ultrasonication and bio-manipulation, even though such methods are often effective, many of which are very expensive and sometimes give rise to secondary pollution or act for a short function time. Consequently, more long-term effective and pollution free methods are still required, particularly in developing countries [6]. Medicinal plants have been prescribed and used with a strong belief in their ability to cure diseases for centuries, over the past 20 years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents [7].

Selection of this study was for several reasons, including the lack of research and studies in this area, as well as the lack of data that show the environmental nature of the water project that is sourced from spring water, as well as to study the physical and chemical characteristics and blooms of algae in these projects located outside center of Sulaimani province.

## 2. Studying Area

The study area extends for a distance of over 65 kilometers starting from the Sharazur district through the Said Sadiq district ending in Khurmal district a subsidiary to Halabja district in the province of Sulaimani (Figure 1). Five stations were selected for the study, all outside the city of Sulaimani center. Three of these in a Bestan Sowr drinking water project within Sharazur district, the fourth station lie in Saray Subhan Agha drinking water project in Said Sadiq district, and the fifth station in Khurmal drinking water project.



Picture 2-1: Location of the Studied Sites (Google earth, 2015)

### 2.1 Studying stations

**First station:** The sample of water in this station was taken from the beginning of the assembly basin at Bestan Sowr drinking water project.

#### Second station:

The sample of water in this station was taken from the mid of the assembly basin at Bestan Sowr drinking water project.

**Third station:** The sample of water in this station was taken from the end of the assembly basin at Bestan Sowr drinking water project.

**Forth station:** The sample of water in this station was taken from the assembly basin at Saray Subhan Agha drinking water project.

**Fifth station:** The sample of water in this station was taken from the assembly basin at khurmal drinking water project.

## 3. Materials and Methods

### 3.1 Isolation and Purification of Algae

Isolation and purification of algal strains was done by repeated sub-culturing on solidified and in liquid media (Modified Chu-10 Medium and Bold Basal Medium) by pour plate (solid medium) and dilution technique.

### 3.2 Identification of Algae

Non-diatomc algae were identified by preparing slides and examined under 40 xs by using a compound microscope depending on the following

references which were used for identification of non-diatom algae [8], [9], [10] and [11].

While diatoms were identified after dissolving the organic matter by using nitric acid and examined under 100 xs depending on [12] and [13].

### 3.3 Culture media

Two media were used in this study:

#### 3.3.1 modified Chu-10 medium

Used for culturing blue green algae, the components of this medium were listed in Table 3-1 [14].

**Table (3-1): The components of modified Chu-10 medium and the concentration of each component**

Number of stock solution	Chemical formula of each component	Concentration g/l	Required volume to prepare the media
1	MgSO <sub>4</sub> .7H <sub>2</sub> O	10	2.5 ml
2	K <sub>2</sub> HPO <sub>4</sub>	4	2.5 ml
3	NaNO <sub>3</sub> CaCl <sub>2</sub>	8 16	2.5 ml
4	FeCl <sub>3</sub>	0.32	2.5 ml
5	EDTA-Na <sub>2</sub>	4	2.5 ml
6	NaCl	30	2.5 ml
7	Na <sub>2</sub> CO <sub>3</sub>	8	2.5 ml
8	MnCl <sub>2</sub> .4H <sub>2</sub> O (NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> .4H <sub>2</sub> O ZnSO <sub>4</sub> .7H <sub>2</sub> O CuSO <sub>4</sub> .5H <sub>2</sub> O COCl <sub>2</sub> .6H <sub>2</sub> O H <sub>3</sub> BO <sub>3</sub>	0.02 0.028 0.224 0.08 0.004 0.288	2.5 ml for each one
9	Na <sub>2</sub> SiO <sub>3</sub>	5.7	2.5 ml

Notes:

- Stock solution No.2 autoclaved separately (because PO<sub>4</sub> may be precipitate when sterilized with other components) and added to another stocks after cooling.
- Stock solution No.4 sterilized separately not by autoclave, but has been sterilized by millipore filter 0.2µm (because FeCl<sub>3</sub> may be affected by high temperature)
- Stock solution No.8 sterilized separately by sterile millipore filter 0.2µm not by autoclave (because its damage at high temperature).
- Stock solution No.8 represents a trace elements solution.
- All the stock solutions adjusted at pH (8 - 8.5).

To prepare one liter of the medium, were taken 2.5 ml from each stock solution and completed to one liter with distilled water, then sterilized by autoclave at 121°C, except the stock solutions No.2, No.4 and No.8 which were sterilized alone (stock solution No.2 by autoclave, stock solution No.4 & No.8 by sterile Millipore filter) and added finally to get one liter of

modified Chu-10 and its pH was adjusted to pH (8-8.5) before sterilization using (0.01N) of sodium hydroxide or diluted hydrochloric acid.

#### 3.3.2 Bold Basal Medium

Used for culturing green algae, the components of this medium were listed in Table 3-2 [15].

**Table (3-2): Composition of the Bold Basal Media (BBM)**

Number of stock solution	Chemical formula of each component	Concentration g/100 ml	Required volume to prepare the media
1	NaNO <sub>3</sub>	2.50 g	10 ml
2	MgSO <sub>4</sub> .7H <sub>2</sub> O	0.75 g	10 ml
3	NaCl	0.25 g	10 ml
4	K <sub>2</sub> HPO <sub>4</sub>	0.75 g	10 ml
5	KH <sub>2</sub> PO <sub>4</sub>	1.75 g	10 ml
6	CaCl <sub>2</sub> .2H <sub>2</sub> O	0.25 g	10 ml
7	ZnSO <sub>4</sub> .7H <sub>2</sub> O MnCl <sub>2</sub> .4H <sub>2</sub> O MOO <sub>3</sub> CuSO <sub>4</sub> .5H <sub>2</sub> O Co(NO <sub>3</sub> ).6H <sub>2</sub> O	8.82 g 1.44 g 0.71 g 1.57 g 0.49 g	1 ml
8	H <sub>3</sub> BO <sub>3</sub>	1.14 g	1 ml
9	EDTA.Na <sub>2</sub> KOH	5.0 g 3.1 g	1 ml
10	FeSO <sub>4</sub> .7H <sub>2</sub> O H <sub>2</sub> SO <sub>4</sub> Con.	4.98 g / L 1 ml (to acidify)	1 ml

Notes: - Stock 7 represents a trace elements solution.



To prepare one liter of the medium, were taken from each stock solution required volume as shown in the table above, and completed to one liter with distilled water, then sterilized by autoclave at 121°C under pressure 15 lbs for 15 min, and its pH was adjusted to pH (8 - 8.5) before sterilization using (0.01N) of sodium hydroxide or diluted hydrochloric acid.

### 3.4 Collection and preparation of plant samples

In this study, the suggested plants belong to different families which were leaves and stems of *Hordeum vulgare* (Barley straw) and were collected from an agricultural field located in Al alam district in Tikrit

city. The seeds of *Peganum harmala*, leaves and stems of *Thymus vulgaris*, flower, stems and leaves of *Artemisia annua* were obtained from herbal shops in Sulaimani province. *Nasturtium officinale* (Watercress) was collected from Khormal district, stems and leaves were collected from it.

All the samples of these plants were dried at room temperature then the selected parts of each plant grinded and stored in clean conditions until use.

### 3.5 Plants Identification

Plants were identified in the University of Sulaimani / College of Science / Biological Department.



Picture 3-1: Barley straw



Picture 3-2: Thymus vulgaris



Picture 3-3: Peganum harmala seeds



Picture 3-4: Artemisia annua



Picture 3-5: Nasturtium officinale (watercress)

### 3.6 Crude extraction of plants

In the extraction process of the plants, we used methanol as an organic solvent as follows: Five grams of the powdered plant material was weighed using a top loading balance and transferred to a conical flask. The solvent (methanol) was added to cover the plant material under a fume hood and left to soak in the solvent at room temperature for 24 hours with intermittent shaking. Extracts were filtered through No. 1 Whatman filter paper and the flask containing the filtrate was closed with a stopper. The filtrate was then rotar vaped to concentrate the extracts [15].

### 3.7 Preparation of different concentrations of plant extracts

Different concentrations of crude extracts were prepared by dissolving certain weight of each plant extract according the concentration in methanol. Different concentrations (5, 10, 20, 30, 40, 60, and 80) mg / ml of plant extracts were prepared according to the following equation:

$$\text{Concentration mg/ml} = \frac{\text{Weight} \times 1000}{\text{Volume}}$$

### 3.8 Determination of the effectiveness of plant extracts (as antialgal) on the growth of algae by Agar-Well Diffusion Method

In order to determine the effectiveness of plant extracts in the inhibition of the growth of algae,

which have been isolated and purified in this study, we have chosen the Agar-Well Diffusion Method with some modifications, which included the creation of wells in the Petri dishes containing solid media by Pastor pipette (diameter of well 6mm) and 100 µl of the different concentrations: (5, 10, 20, 30, 40, 60 and 80 mg/ml) of different plant extracts from different plants were added in the wells and the organic solvent (methanol) was added in the middle well as a control. The plates were then incubated in a cooled illuminated incubator at (26± 2°C) for 7-10 days. The antialgal activity was assayed by measuring the diameter of the inhibition zone formed around the well [17].

### 3.9 Statistical Analysis

The results of this study were analyzed statistically using analysis of variance test (F test), and has been compared the means by a polynomial Duncan test in level of 0.05.

## 4. Results and Discussion

### 4.1 Isolation, purification and identification of algae

Seven strains of algae from studying stations were isolated, purified and identified after collection which were: *Ulothrix zonata*, *Desmodesmus lunatus*, *Desmodesmus itascaensis*, *Chlorella vulgaris*, *Calothrix fusca*, *Chroococcus turgidus* and *Navicula riediana*. These isolates included 2 species of blue-

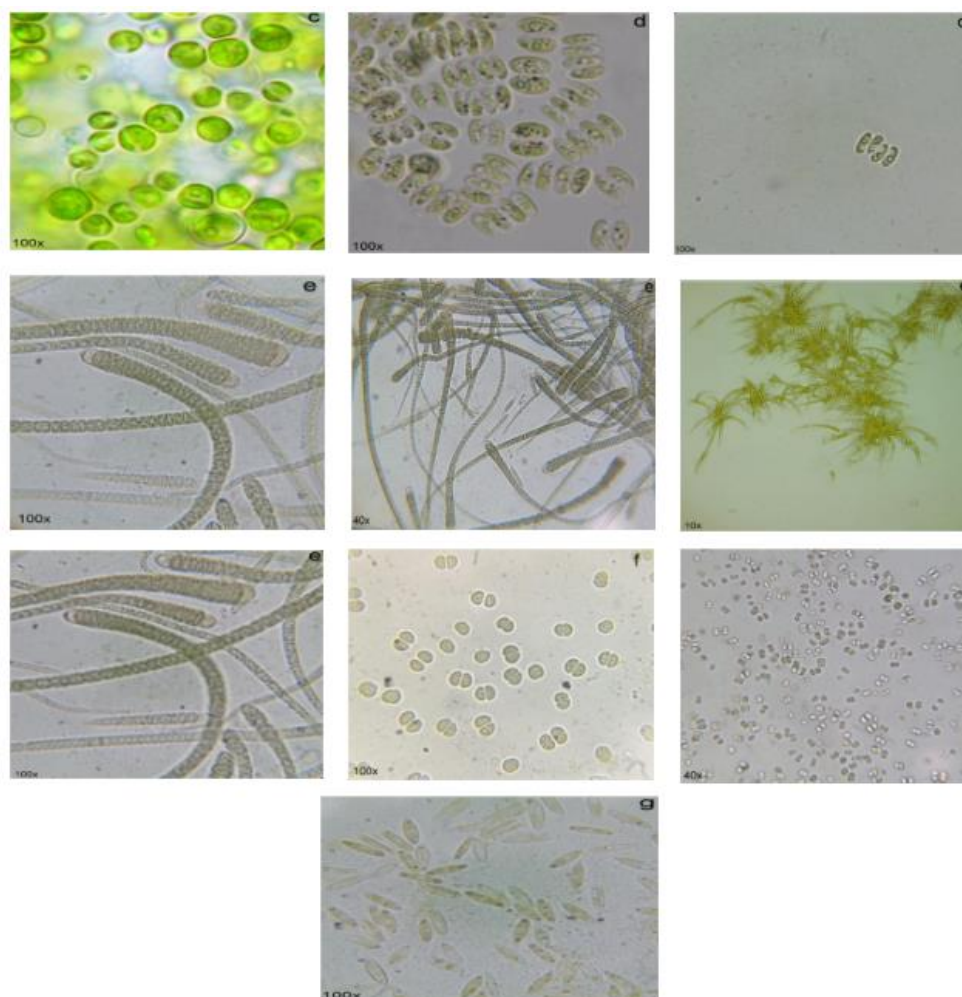
green algae, 4 species of green algae, and 1 species of diatoms. These isolates and their classification are shown in Table (4-1).

**Table (4-1): The Isolated algae in this Study and their Classification**

The isolated algae	Kingdom	Division	Class	Order	Family
<i>Ulothrix zonata</i>	Protista	Chlorophyta	Chlorophyceae	Ulothrichales	Ulothricaceae
<i>Desmodesmus lunatus</i>	Protista	Chlorophyta	Chlorophyceae	Chlorococcales	Scenedesmiaceae
<i>Desmodesmus itascaensis</i>	Protista	Chlorophyta	Chlorophyceae	Chlorococcales	Scenedesmiaceae
<i>Chlorella vulgaris</i>	Protista	Chlorophyta	Chlorophyceae	Chlorococcales	Chlorococcaceae
<i>Calothrix fusca</i>	Monera	Cyanophyta	Cyanophyceae	Nostocales	Rivulariaceae
<i>Chroococcus turgidus</i>	Monera	Cyanophyta	Cyanophyceae	Chlorococcales	Chlorococcaceae
<i>Navicula riediana</i>	Protista	Bacillariophyta	Bacillariophyceae	Pennales	Fragilariaceae

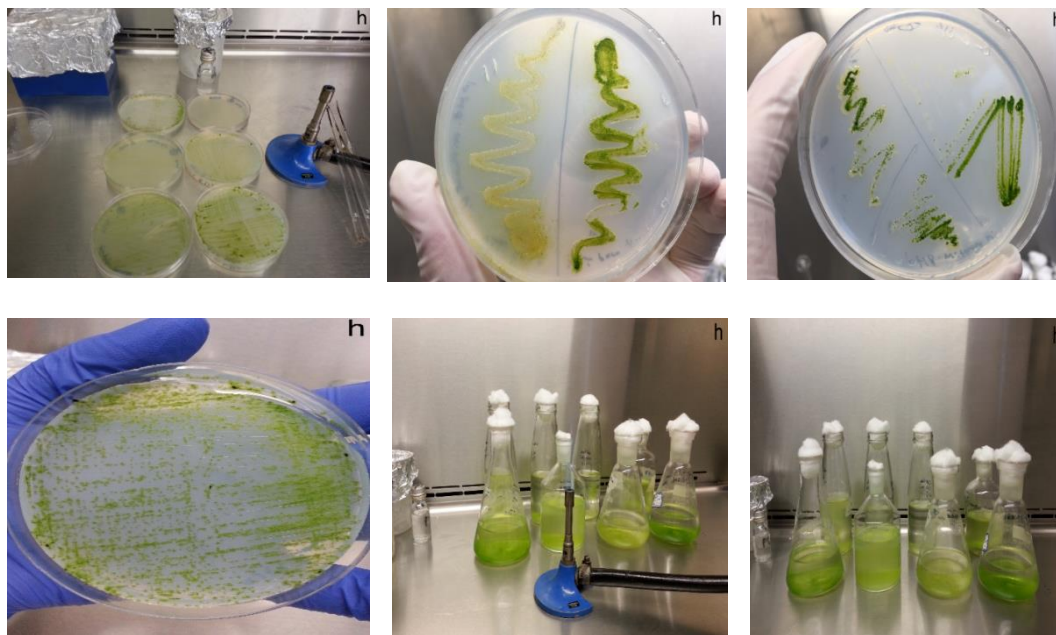


**Pictures 4-1: Pure algae had been isolated and purify from studying stations: a: *Ulothrix zonata*; b: *Desmodesmus lunatus***



**Continued Pictures 4-1 Pure algae had been isolated and purify from studying stations: c: *Chlorella vulgaris*; d: *Desmodesmus itascaensis*; e: *Calothrix fusca*; f: *Chroococcus turgidus* and g: *Navicula riediana*.**





**Pictures 4-2 Models for growth of algae on solid medium and liquid medium**

#### **4.2 Evaluation of the effectiveness of crude plant extracts (as antialgal) on the growth of isolated algae by Agar-Well Diffusion Method**

The inhibitory actions depending on the selected algae, were shown in Table (4-2) and Figures (4-1 to 4-7). The greater inhibition zone diameter was obtained 62 mm against *Calothrix fusca* by using crude extracted from *Peganum harmala* in concentration 80 mg/ml, and less inhibition zone diameter was 7 mm against *Ulothrix zonata* by using crud extracted from *Artemisia annua* in concentration 30 mg/ml. However, the diameters of inhibition zones of *Ulothrix zonata* ranged between (7- 39) mm (Figure 4-1), the diameters of inhibition zones of *Desmodesmus lunatus* ranged between (10-32) mm (Figure 4-2), the diameters of inhibition zones of *Chlorella vulgaris* ranged between (7.5- 31) mm (Figure 4-3), the diameters of inhibition zones of

*Desmodesmus itascaensis* ranged between (9-31.5) mm (Figure 4-4), the diameters of inhibition zones of *Calothrix fusca* ranged between (14-62) mm (Figure 4-5), the diameters of inhibition zones of *Chroococcus turgidus* ranged between (8-37) mm (Figure 4-6), the diameters of inhibition zones of *Navicula riediana* ranged between (7.5-45) mm (Figure 4-7).

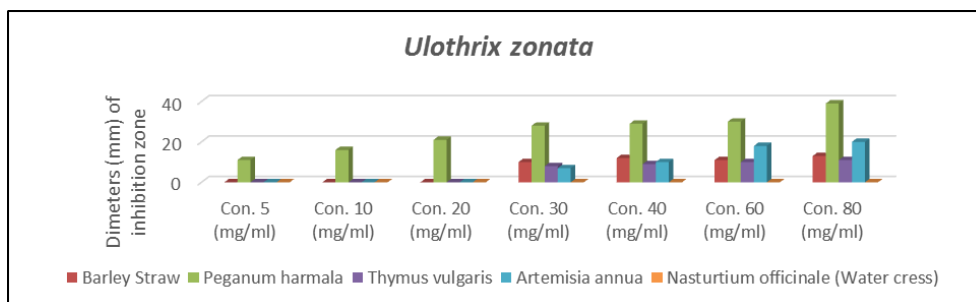
The statistical analysis results of the study revealed according to the analysis of variance that there were a significant differences in inhibition diameters between the species of isolated algae when used the extracts of Barley Straw, *Thymus vulgaris* and *Artemisia annua*, but there were no significant differences between the species of isolated algae when used the extract of *Peganum harmala*, as it is shown in the Table 4-3.

**Table (4-2): Diameters (in millimeter) of Inhibition zone caused by crude extracted from selected plants against isolated algae**

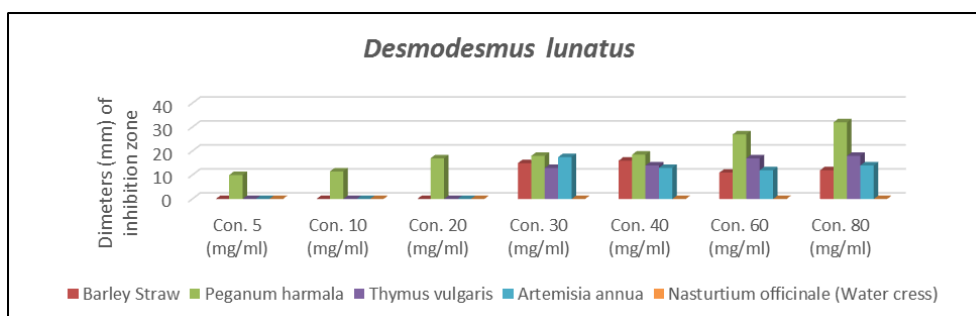
Species of algae	Types of plant	Concentration (mg/ml) of crude extracts from each selected plant						
		5	10	20	30	40	60	80
1. <i>Ulothrix zonata</i>	Barley Straw	-	-	-	10	12	11	13
	<i>Peganum harmala</i>	11	16	21	28	29	30	39
	<i>Thymus vulgaris</i>	-	-	-	8	9	10	11
	<i>Artemisia annua</i>	-	-	-	7	10	18	20
	<i>Nasturtium officinale</i> (Water cress)	-	-	-	-	-	-	-
2. <i>Desmodesmus lunatus</i>	Barley Straw	-	-	-	15	16	11	12
	<i>Peganum harmala</i>	10	11.5	17	18	18.5	27	32
	<i>Thymus vulgaris</i>	-	-	-	13	14	17	18
	<i>Artemisia annua</i>	-	-	-	17.5	13	12	14
	<i>Nasturtium officinale</i> (Water cress)	-	-	-	-	-	-	-
3. <i>Chlorella vulgaris</i>	Barley Straw	-	-	-	7	10	11	12
	<i>Peganum harmala</i>	7.5	9	19	20.5	21.5	29	31
	<i>Thymus vulgaris</i>	9	12	13	13	13	16	17
	<i>Artemisia annua</i>	-	-	10	16	14	17.5	15.5
	<i>Nasturtium officinale</i> (Water cress)	-	-	-	-	-	-	-
4. <i>Desmodesmus itascaensis</i>	Barley Straw	-	-	-	13	14	14.5	15
	<i>Peganum harmala</i>	13	14	17.5	19	23	27	31.5
	<i>Thymus vulgaris</i>	-	-	-	13	13.5	14	15
	<i>Artemisia annua</i>	-	-	-	9	10	13	12
	<i>Nasturtium officinale</i> (Water cress)	-	-	-	-	-	-	-
5. <i>Calothrix fusca</i>	Barley Straw	-	-	15	16	16	16	17
	<i>Peganum harmala</i>	21	27	34	35	40	55	62
	<i>Thymus vulgaris</i>	-	-	-	14	15	25	28
	<i>Artemisia annua</i>	-	-	17	18	23	26	24
	<i>Nasturtium officinale</i> (Water cress)	-	-	-	-	-	-	-
6. <i>Chroococcus turgidus</i>	Barley Straw	-	-	-	9	11.5	12.5	13
	<i>Peganum harmala</i>	8	15	19	25	28	33.5	37
	<i>Thymus vulgaris</i>	-	-	-	14	15	16	20
	<i>Artemisia annua</i>	-	-	-	13	11.5	12	15
	<i>Nasturtium officinale</i> (Water cress)	-	-	-	-	-	-	-
7. <i>Navicula riediana</i>	Barley Straw	-	-	-	7.5	9	10	12
	<i>Peganum harmala</i>	8	22	24	25	27	38	45
	<i>Thymus vulgaris</i>	-	-	-	12.5	13	13.5	16
	<i>Artemisia annua</i>	-	-	-	13	15	12	13.5
	<i>Nasturtium officinale</i> (Water cress)	-	-	-	-	-	-	-

**Table (4-3): Table in the statistical analysis shows the presence or absence of significant differences between the types of isolated algae and effective inhibition of each plant extract**

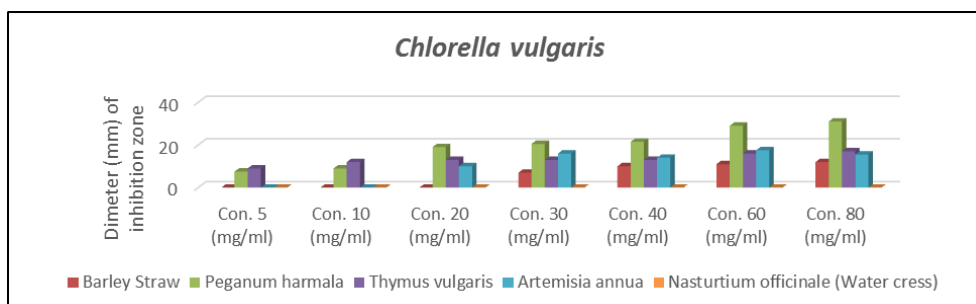
Type of isolated algae \ Plant extract	<i>Ulothrix zonata</i>	<i>Desmodesmus lunatus</i>	<i>Chlorella vulgaris</i>	<i>Desmodesmus itascaensis</i>	<i>Calothrix fusca</i>	<i>Chroococcus turgidus</i>	<i>Navicula riediana</i>
Barley Straw	11.5 b c	13.5 b	10 c	14.125 b	16 c	11.5 c	9.625 c
<i>Peganum harmala</i>	24.85714 a	19.14286 a	19.64286 a	20.71429 a	39.14286 a	23.64286 a	27 a
<i>Thymus vulgaris</i>	9.5 c	15.5 b	13.28571 b c	13.875 b c	20.5 b	16.25 b	13.75 b
<i>Artemisia annua</i>	13.75 b	14.125 b	14.6 b	11 c	21.6 b	12.875 c	13.375 b
<i>Nasturtium officinale</i> (Water cress)	0 d	0 d	0 d	0 d	0 d	0 d	0 d



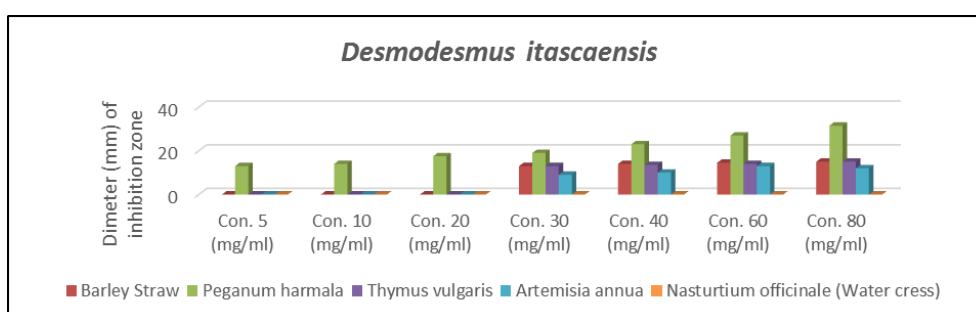
**Figure 4-1: Diameters (in millimeter) of inhibition zone caused by crude extracted from selected plants against *Ulothrix zonata***



**Figure 4-2: Diameters (in millimeter) of inhibition zone caused by crude extracted from selected plants against *Desmodemus lunatus***

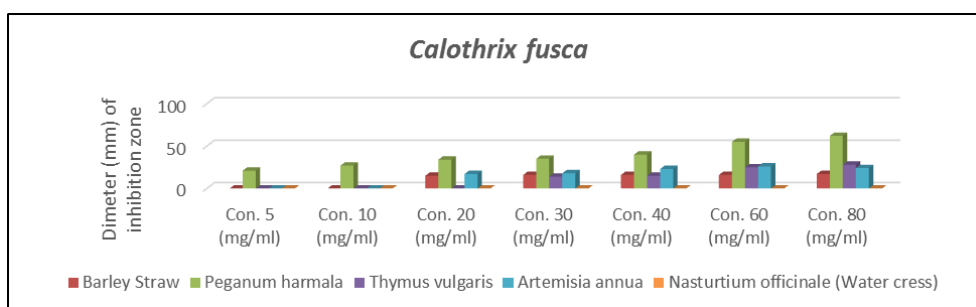


**Figure 4-3: Diameters (in millimeter) of inhibition zone caused by crude extracted from selected plants against *Chlorella vulgaris***

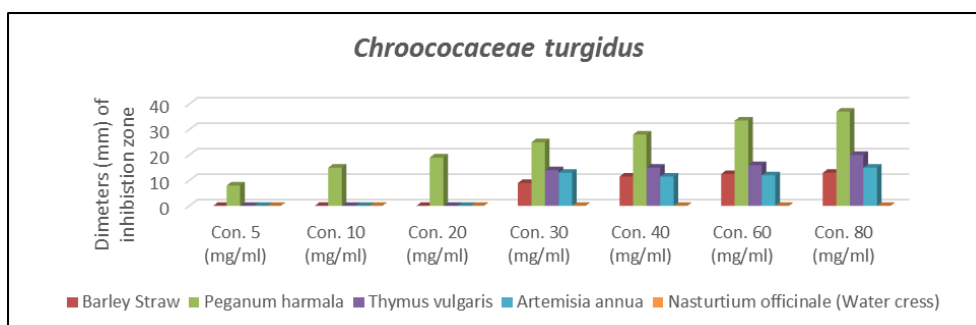


**Figure 4-4: Diameters (in millimeter) of inhibition zone caused by crude extracted from selected plants against *Desmodemus itascaensis***

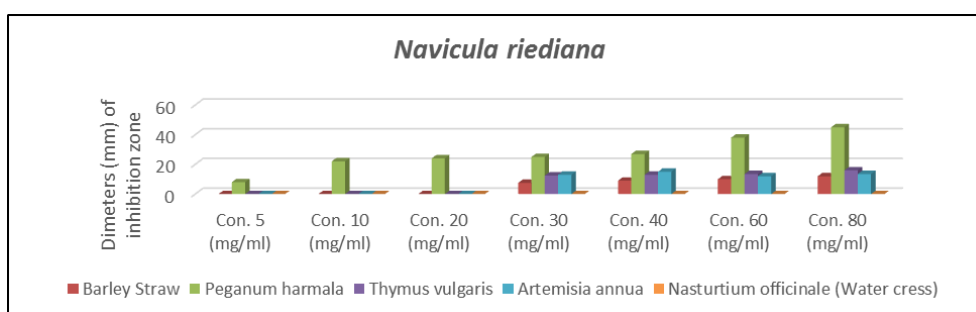




**Figure 4-5: Diameters (in millimeter) of inhibition zone caused by crude extracted from selected plants against *Calothrix fusca***



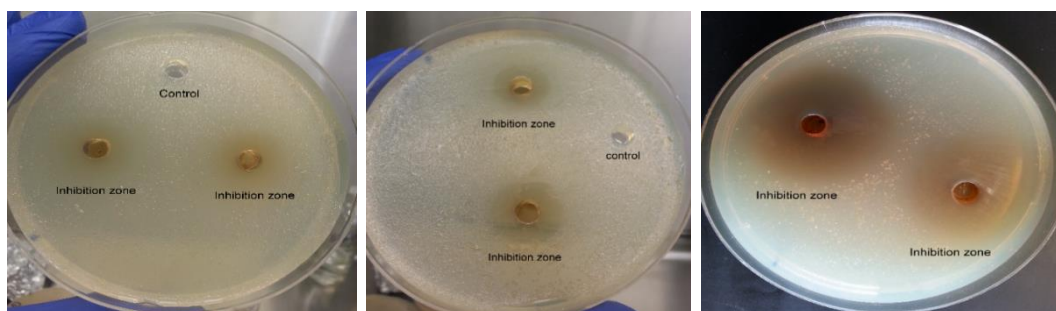
**Figure 4-6: Diameters (in millimeter) of inhibition zone caused by crude extracted from selected plants against *Chroococcus turgidus***



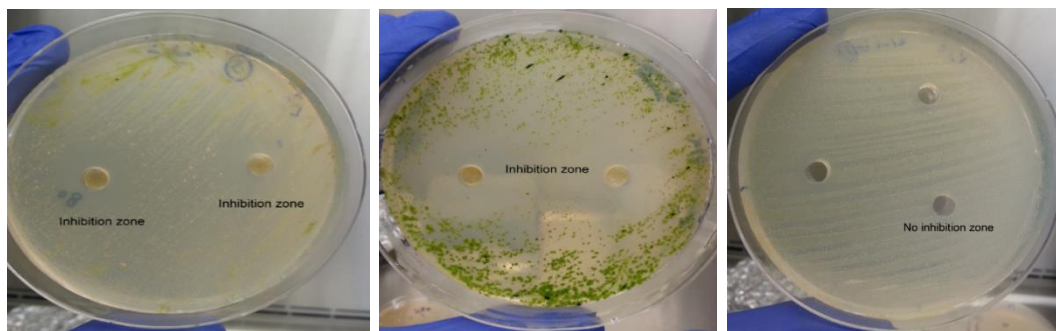
**Figure 4-7: Diameters (in millimeter) of inhibition zone caused by crude extracted from selected plants against *Navicula riediana***

Inhibition zones diameters differed according to the algal types and types and concentration of plant's extracts (Pictures 4-3 show the inhibition zone of isolated algae by plant extracts). *Calothrix fusca* has highest value of inhibition zone diameter that means *Calothrix fusca* (blue-green algae) was more sensitive to treatment by plant extracts than other types of

algae used in this study for example *navicula riediana* (diatom), this may be due to the structure of the cell walls since the cell walls of diatoms are thick and contain large amounts of silica, therefore, these algae (diatom) have more resistance to treatment by plant extracts than other algae.



**Pictures 4-3 Models for Inhibition zones caused by crude plant extracts against isolated algae**



**Continued Pictures 4-3 Models for Inhibition zones caused by crude plant extracts against isolated algae**

[18] described the effect of barley straw as being algistatic (preventing new growth of algae) rather than algicidal (killing already existed algae). The inhibitory effect of barley straw might be due to chemical compounds such as oxidized phenolics and hydrogen peroxide, which occur during the decomposition process. It appears that good aeration, neutral to alkaline pH, and open sunlight water are essential for optimum algal control by barley straw [19]. The study by [20] showed that barley straw liquor inhibited the growth of three algal species, had no effect on other five algal species, and enhanced the growth of another four algal species instead.

Both laboratory and field experiments have shown that barley straw releases phenolic substances that both before and after decomposing, suppress algal growth [19] and [21].

The results of the current study showed inhibition of all types of algae isolated at concentrations of 30, 40, 60 and 80 mg/ml with different diameters of inhibition zone depending on the type of algae, and no inhibition occurs when using low concentrations (5, 10, and 20 mg/ml) of the extract of barley straw. These results coincided with studies that showed the effectiveness of inhibitory barley Straw against the types of algae as in the studies mentioned above. This ability to inhibit algae may be because the Barley straw decomposing releases substances that inhibit algal growth such as 2, 6-Dimethoxy-4-(2-propenyl) phenol and octanoic acid [19]. In a previous study, also found that the flavonolignans salcolin A and salcolin B in barley straw inhibited cyanobacteria growth and induced an increase on intracellular ROS (Reactive Oxygen Species) levels and esterase activity suppression [22] and [23]. However, Barley straw is antialgal under conditions that may promote oxidation of phenolic hydroxyl groups to quinones; tannins are antialgal under similar conditions [21].

When using *Peganum harmala* extract, the results showed high inhibition of isolated algae and it's the highest of all inhibitions which happen with the others plant extracts in all concentrations (As shown in the Table 4-2), It is considered the first inhibitor of

algae and this is may be due to the fact that seeds of *Peganum harmala* contained the highest levels of alkaloids. The high amount of alkaloids in seeds might explain the significant inhibitory activities of their ethanol extracts. Among the total alkaloids of harmine and harmaline, harmaline exhibited the most potent inhibitory effect on growth of the tested algae, as in a study by [24].

Also, the antialgal activity of extract from thyme showed the ability of inhibition for all isolated algae by varying inhibition diameters for different concentrations. The reasons for the inhibition of algae by using thyme extract may be because of the major components containing thyme extract such as p-cymene (It is a constituent of a number of essential oils),  $\gamma$ -terpinene (classified as terpenes), thymol (classified as phenolic compound) and others compounds, these compound act as antimicrobial as mentioned in [25]. These compounds may play a role in the inhibition of algae.

The extract of *Artemisia annua* showed positive results in the inhibition of all isolated algae in concentrations 20 (in *Calothrix fusca* only), 30, 40, 60 and 80, as it is shown in Figures (4-1 to 4-7) and Table 4-2. The main reason for this inhibition that the extract of *Artemisia annua* contains compounds work as anti-algae, this result is according to a study [26] which include isolate and identify an anti-algal compound from extracts of *Artemisia annua* and study its mode of action on *Microcystis aeruginosa*, and it also the anti-algal compound was isolated from the extracts using column chromatography and activity-guided fractionation methods. Artemisinin with strong anti-algal activity was identified by gas chromatography-mass spectrometry and 1- H Nuclear Magnetic Resonance.

The extract of *Nasturtium officinale* (watercress) showed negative results in the inhibition of all isolated algae in all concentrations, this is because it doesn't contain inhibitory compounds. Watercress is a good source of vitamins A and C, along with niacin, ascorbic acid, thiamine, riboflavin, and iron [27].

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## عزل وتنقية وتشخيص سبعة أنواع من الطحالب في ثلاث مشاريع لمياه الشرب ينبوعية المصدر في محافظة السليمانية والسيطرة على نمو الطحالب المعزولة منها باستخدام بعض المستخلصات النباتية

بشار طارق اسماعيل الشنداح<sup>1</sup>، رياض عباس عبد الجبار<sup>1</sup>، تريفه كمال جلال فرخة<sup>2</sup>

<sup>1</sup>قسم علوم الحياة، كلية العلوم، جامعة تكريت، تكريت، العراق

<sup>2</sup>قسم علوم الحياة، كلية العلوم، جامعة السليمانية، السليمانية، العراق

### الملخص

في هذه الدراسة تم اختيار ثلاث مشاريع لمياه الشرب ينبوعية المصدر ضمن مواقع مختلفة من محافظة السليمانية – اقليم كردستان العراق. جمعت العينات لمدة ثمانية اشهر اعتبارا من شهر ايار 2015 وحتى نهاية شهر كانون الاول 2015. اختيرت خمس محطات للدراسة، المحطات الثلاثة الاولى تقع في مشروع بيستان سور لمياه الشرب في قضاء شارزور، وتقع المحطة الرابعة في مشروع سراي سبحان اغا لمياه الشرب في قضاء سيد صادق، وتقع المحطة الخامسة في مشروع خورمال لمياه الشرب في ناحية خورمال ضمن قضاء حلبجة.

شملت الدراسة عزل وتنقية وتشخيص سبعة أنواع من الطحالب باستخدام الوسطين Chu-10 وBBM، وكذلك تقييم كفاءة المستخلصات النباتية لتعمل كمثبطات طحلبية تم تجربتها في المختبر على سبعة أنواع من الطحالب المعزولة عن طريق استخدام Agar-Well Diffusion Method.

تم عزل وتنقية وتشخيص سبعة أنواع من الطحالب وهي:

*Ulothrix zonata*, *Desmodesmus lunatus*, *Desmodesmus itascaensis*, *Chlorella vulgaris*, *Calothrix fusca*, *Chroococcus turgidus* and *Navicula riediana*.

هذه الانواع السبعة المعزولة تضمنت نوعان من الطحالب الخضراء المزرقة واربعة انواع من الطحالب الخضراء و نوع واحد من الديتومات تم اقتراح 5 انواع من المستخلصات النباتية لاستخدامها كمثبطات نمو للطحالب وهذه النباتات تنتمي لعوائل نباتية مختلفة وهي نبات الشعير (*Barley*) *H. vulgare* (تبن الشعير)، ونبات الحرمل *Peganum harmala*، ونبات الشيح (*Artemisia annua*)، ونبات الزعتر (*Thymus vulgaris*)، ونبات كرفس الماء (*Nasturtium officinale* Watercress). اما فيما يتعلق بتقييم فعالية المستخلصات النباتية الخام (كمثبطات) لنمو الطحالب تم استخدام طريقة Agar-Well Diffusion، اظهرت النتائج ان اكبر قطر للتثبيط حصل ضد النوع الطحلي *Calothrix fusca* بطول قطر 62 ملم عند استخدام مستخلص نبات الحرمل عند التركيز 80 ملغم/مل، واقل قطر تثبيط 7 ملم فقد سجل ضد النوع الطحلي *Ulothrix zonata* عند استخدام مستخلص نبات الشيح عند التركيز 30 ملغم/مل، اما نبات كرفس الماء فلم يظهر اي فعالية ضد اي نوع من الطحالب المعزولة وفي جميع التراكيز المستخدمة. بشكل عام قطر التثبيط يختلف تبعا للنوع الطحلي ونوع وتركيز المستخلص المستخدم.