



## تقييم فعالية مستخلصات نبات القنقينة على نمو بعض الطحالب *Tinospora cordifolia* Willd.

### Evaluation of *Tinospora cordifolia* Willd. Extracts Against Algal Growth

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#### الملخص

بهدف دراسة تأثير المستخلصات التربينية والقلويدية والفينولية لأوراق نبات *Tinospora cordifolia* في الطحالب، تم اختبار فعالية هذه المستخلصات على ثلاثة أنواع من الطحالب هي: *Mougeotia scalaris* و *Scenedsmus quadricauda* و *Anabaena circinalis* بطريقة الحفر والانتشار في الأغار لتحديد الفعالية التثبيطية لتلك المستخلصات. استُخدمت ثلاثة تركيزات هي: 5، 10 و 20 مغ/مل، ووضعت التجربة وفق التصميم العشوائي الكامل في تطبيق التجربة وبثلاثة مكررات. بينت النتائج أن طحلب *A. circinalis* كان الأكثر حساسية للمستخلصات القلويدية لأوراق نبات القنقينة، وبلغ قطر منطقة التثبيط نحو 40 ملم عند استخدام التركيز 20 مغ/مل، بينما كان الطحلب نفسه أقل حساسية للمستخلص الفينولي، فبلغ قطر منطقة التثبيط 17 ملم عند استخدام التركيز 20 مغ/مل. كما أوضحت النتائج أن المستخلص القلويدي كان الأكثر تأثيراً في الطحالب، تلاه المستخلص التربيني، بينما كان المستخلص الفينولي الأقل تأثيراً في الطحالب المستخدمة في هذه الدراسة.

**الكلمات المفتاحية :** طحالب ، مستخلصات ورقية ، *Tinospora cordifolia*

#### Abstract

The present study has been conducted to evaluate the antialgal activities of *Tinospora cordifolia* leaves extracts, these extracts included Terpens, Alkaloids, and Phenols of that plant against 3 algal isolates: *Anabaena circinalis*, *Scenedsmus quadricauda* and *Mougeotia scalaris*. The agar well diffusion method was used to evaluate the inhibitory actions of these extracts with 3 concentrations: 5, 10, and 20 mg/ml. The experiments were conducted and analyzed as factorial experiments with three replications using a completely Randomized Design. Means were compared according to L.S.D. value at 5% significant level.

The results showed that *Anabaena circinalis* was the most sensitive to alkaloid extract and the diameter of inhibition zone was 40 mm in concentration of 20 mg/ml., while this alga was less sensitive to phenol extract and the inhibition zone was 17 mm. The results also showed that alkaloids extract was the most active against all algae used in this study followed by terpens extracts, while the phenols extracts has the lowest antialgal activity.

**Key words:** Algae, Leaves extracts, *Tinospora cordifolia*.

## Introduction

The development of extensive cyanobacterial and algal blooms is a worldwide problem. Cyanobacteria produce secondary metabolites, which are toxic to a variety of aquatic and terrestrial organisms, including humans. Some species also produce create filamentous blooms that may block filters used in drinking water supply systems ( Schrader et al.,2002).

Many of problems, such as, dermatitis diseases, fish toxicity, amenity of water....etc. were considered due to the presence of algal blooms in general and cyanobacterial species in particular in the environment (Lund,1972). However, researches to find ways of controlling their growth were encouraged such as the use of algicidal substances and mechanical cleaning for stable tanks and filtration to control algae which is known as Gulancha in English, Guduchi in Sanskrit, and Giloya in Hindi. It is a large, glabrous, deciduous climbing succulent shrub, commonly found in hedges. It has been known for long in Ayurvedic literature as a tonic, vitalizer and as a remedy for diabetes and other metabolic disorders (Edwards,1972). There were some of organic chemical compounds act as algicides which were more effective from the non-organic salts such as copper sulphate, potassium permanganate, chlorine but these compounds were toxic and very expensive (Kameswararao et al., 2003).

*Tinospora cordifolia* (Willd.) belongs to the Menispermaceae family. It has been known for long in Ayurvedic literature as a tonic, vitalizer and as a remedy for diabetes and other metabolic disorders (Singh et al.,2005).

Evidence hints that *Tinospora* may have anti-cancer (Singh et al.,2005), immune stimulating (Rawal et al., 2004), anti-diabetic (Rathi et al.,2002), cholesterol-lowering (Bishayi et al.,2002) and liver-protective (Jawad,2007) actions.

*T. cordifolia* has also shown some promising speed in healing the diabetic foot ulcers (Purandare and Supe,2007). Due to the wide use of plant extracts, methods were suggested to use plant extracts in control of algal blooms (Jawad,1982).

The aim of this study was to evaluate the algicidal activity of different concentration of Guduchi in controlling the growth of isolated algae.

## Material and methods

Algal samples: Water samples were taken from the canal around the University of Baghdad in 15 November 2011 and a series of dilutions were made after hand shaking. The dilution series ranged from  $10^{-1}$  to  $10^{-5}$ . Solid and liquid modified CHU-10 medium were used to isolate algae and incubated at  $26\pm1^{\circ}\text{C}$  with illumination tensity about  $200 \mu\text{E}/\text{m}^2/\text{Sec}$ . for two weeks in cooled illuminated incubator.

### Isolation and purification of algae

Uni-green, blue-green algae and diatoms were obtained by using the following methods:

**a** - Chu-10 nutrient solution solidified by 2% agar-agar and sterilized with autoclave, after sterilization Chu-10 with  $45-50^{\circ}\text{C}$  was poured in petri-dishes which left to solidify. Then the surface of each plate was inoculated with 1 ml of water sample, the inoculum distributed with a sterile spreader or streaking by using a sterile loop. The inoculated plates were kept in a cooled illuminated incubator with light intensity about  $200 \mu\text{E}/\text{m}^2/\text{s}$  and  $26\pm2^{\circ}\text{C}$  for 7- 10 days. Aggregated colonies were observed on the surface of plates. Part from these colonies was stroke on another plates. Each subculture was examined by using a compound microscope, this method was repeated till a uni algal culture or cultures had been gained (Stein, 1973).

A small part of unialgal culture was transferred which was microscopically confirmed as uni algal culture into Chu-10 nutrient solution within a 250 ml sterile flask and incubated for 2-3 weeks under the growth conditions which explained by Jawad (1982) to get appropriate growth. In order to sustain the viability of the uni algal growth, these cultures should be renewed every two weeks by sub culturing into another Chu-10 nutrient solution.

**b** - Serial dilutions from the collected samples were prepared starting with 1ml of sample inoculated into 9 ml of Chu-10 nutrient solution. This procedure was repeated with examining of each dilution with a compound microscope until one species of algae was obtained. After the target dilution was microscopically examined several time and confirmed as unialgal culture (2 ml) was transferred into (20 ml) of fresh Chu-10 enhancement solution then incubated

under suitable conditions for algal growth which described previously till the culture turn into greenish color (Jawad, 1982). Obtained algal isolates were identified with help of classical algal classification references (Desikachary, 1959; Prescott, 1973).

The plant samples include leaves of *T. cordifoloid* were collected and cleaned prior to dryness at room temperature and then ground down to powder form.

### **Preparation of plant extracts**

#### **1- Terpens**

Extraction refers to the process of obtaining Terpens constituents by using 15 g. of dried materials extracted in a soxhlet for 8 hrs. with chloroform. The solvent was removed by rotary evaporator at 40°C, then the extract kept in refrigerator until used (Harborne, 1984).

#### **2- Alkaloids**

Extraction refers to the process of obtaining Alkaloids constituents by using 100 g. of dried materials were homogenized with 350 ml of (4:1) ethanol:distilled water, then filtered through muslin then through filter paper in Bouknner funnel. The concentrated volume was acidified by drops of 2% H<sub>2</sub>SO<sub>4</sub> until pH becomes between 1-2. The resulted solution extracted with chloroform 3 times then alkaloids were precipitated by the addition of concentrated NH<sub>4</sub>OH drops and the pH become 9-10. Then extracted with chloroform-methanol (3:1) twice and with chloroform once.

The lower layer was dried and the residue contains weak alkaloids, the upper layer was dried and the residue was extracted with methanol (Harborne, 1984).

#### **3- Phenols**

Extraction refers to the process of obtaining Phenols constituents by using 10 g of plant powder mixed with 400ml of 2% acetic acid then put in reflex condenser in water bath 70°C for 8 hrs., then filtered through muslin cloth and mixed with equal volume of N- propanol, then put it in separated funnel and saturated with sodium chloride. After a period, it separated into 2 layers, the lower layer is neglected, and the upper layer was collected and put it in oven at 40°C to evaporate the solvent, and kept in refrigerator until use (Ribereu-Gayon, 1972 ).

The antialgal activity of the isolated compounds (terpens, alkaloids, and phenols) extracted from leaves of *T. cordifolia* was determined by using different concentrations (5, 10, and 20) mg/ml.

### **The isolated compounds indicators**

#### **1- Acetic anhydride reagent**

This test was used for the detection of terpens. According to ( Al-Bid, 1985), 1 ml of the extract was added to 1-2 drops of chloroform then 1 drop of anhydried acetic acid, then 1 drop of concentrated H<sub>2</sub>SO<sub>4</sub>. The appearance of brown colour indicated the presence of terpens.

#### **2- Mayer reagent**

This reagent was used for the detection of alkaloids. The stock solution (1) was prepared by dissolving 13.5 g HgCl<sub>2</sub> in 60 ml H<sub>2</sub>O, stock solution (2) was prepared by dissolving 5g KI in 10 ml H<sub>2</sub>O, then combined with stock (1) and (2) and diluted with H<sub>2</sub>O up to 100 ml, then 1-2 ml of Mayer reagent were added to 5 ml of aqueous, or alcohol extract. A creamy or white precipitate indicated the presence of alkaloids ( Jones and kinghorn , 2006).

#### **3- Ferric chloride and Potassium ferric cyanide reagent**

It was used for the detection of general phenols. It prepared by taking 2 equal volumes of aqueous solution of ferric chloride 1% and potassium ferric cyanide 1%. blue-green color appeared indicating that the test is positive (Harborne , 1984).

### **Preparation of concentration**

Stock solution were prepared by mixing 2 g from the dried extract with 20 ml of ethylen glycol. Then the concentrations (5, 10 and 20)mg / ml were prepared by mixing known volume from the stock solution with ethylen glycol using the following equation: C<sub>1</sub>V<sub>1</sub>=C<sub>2</sub>V<sub>2</sub> to prepare these three concentrations.

Control treatment is ethylen glycol which used to prepare the extracts.

## 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:

### A- Preparation Lawns of algae

- 1- Bright green culture of alga was selected.
- 2- CHU-10 medium was prepared in flat bottom flask, agar- agar 2% was added for solidification.
- 3- Agar- agar was dissolved by using water bath with 100°C, the media was sterilized using the autoclave.
- 4- The media cooled up to 35-40°C.
- 5- 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.
- 6- petri dishes were incubated in reverse position within a cooled illuminated incubator with 200 µE/m<sup>2</sup>/S and 26±2°C for 2-3 days until the plates turn into greenish color (Jawad,1982).

### B- Control of algae

Algicidal effects of plant extracts were detected by using the agar- well diffusion method according to (Jawad,1982) as follows:

Certain numbers of wells were prepared in the plates contained the lawns of tested algae with the help of sterile cork borer (6 mm in diameter), the tested concentration of plant extracts were inculated into the well. Controls 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 incubator for 24 hrs.. Inhibition zones were determined by measuring their diameters. Three replicates were made and the mean values were recorded.

### Statistical analysis

The experiments were conducted and analyzed as factorial experiments with three replications using a completely Randomized Design, Data were analyzed by using statistical analysis system- SAS (2001) and Means were compared according to L.S.D. at 5% significant level.

## Results and Discussion

### Isolation and Characterization of algae

The algal isolates included two species of green algae (*S. quadricauda* and *M. scalaris*) and one species of blue-green algae (*A. circinalis*). These isolates and their classification are shown in Table (1).

#### 1- *Moigeotia scalaris*

Vegetative cells 34 µ in diameter, 40-180 µ long . 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 (Prescott,1973).

#### 2- *Anabaena circinalis*

Thallus frothy, floating, trichome mostly circinate, seldom straight, mostly without a sheath, 8-14 µ broad, cells barrel-shaped or spherical, somewhat shorter than broad, with gas- vacuoles, heterocystis subspherical, 8- 10 µ broad, spores cylindrical, sometimes curved, ends rounded, 16- 18 µ broad up to 34 µ long, ordinarily a way from the heterocyst episore smooth and colourless (Desikachary,1959).

#### 3- *Scenedsmus quadricauda*

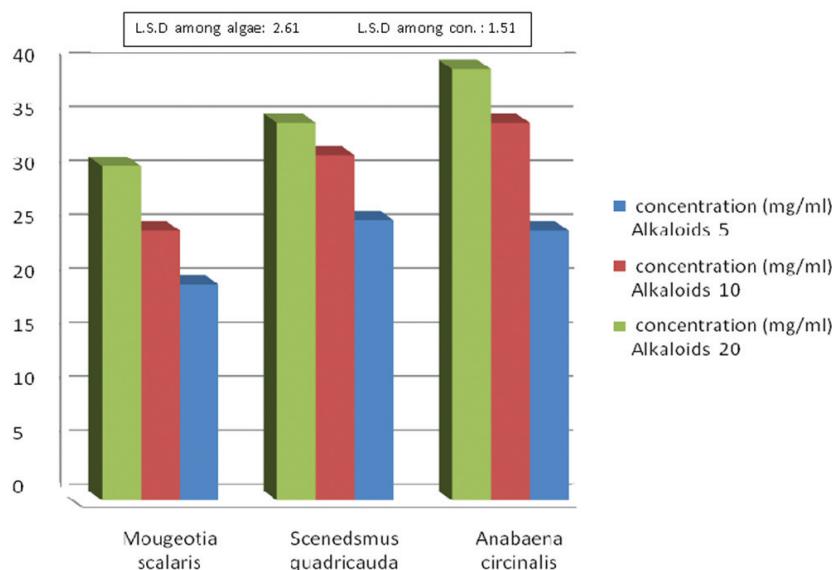
Colony composed of 4-8 ovate cells with broadly rounded apices cells 5-8 µ in diameter, 10-18 µ- long (Ribereu-Gayon,1972), spines relatively short, often strongly refluxed. Rare, but found in the plankton of great variety of lakes, ponds, and swampy habitats (Prescott,1973).

**Table 1. The isolated algae in this study and their classification.**

Algae	Division	Class	Order	Family
<i>A. circinalis</i>	Cyanophyta	Cyanophyceae	Nostocales	Nostocaceae
<i>S. quadricauda</i>	Chlorophyta	Chlorophyceae	Chlorococcales	Chlorococcaceae
<i>M. scalaris</i>	Chlorophyta	Chlorophyceae	Zygnematales	Zygnemataceae

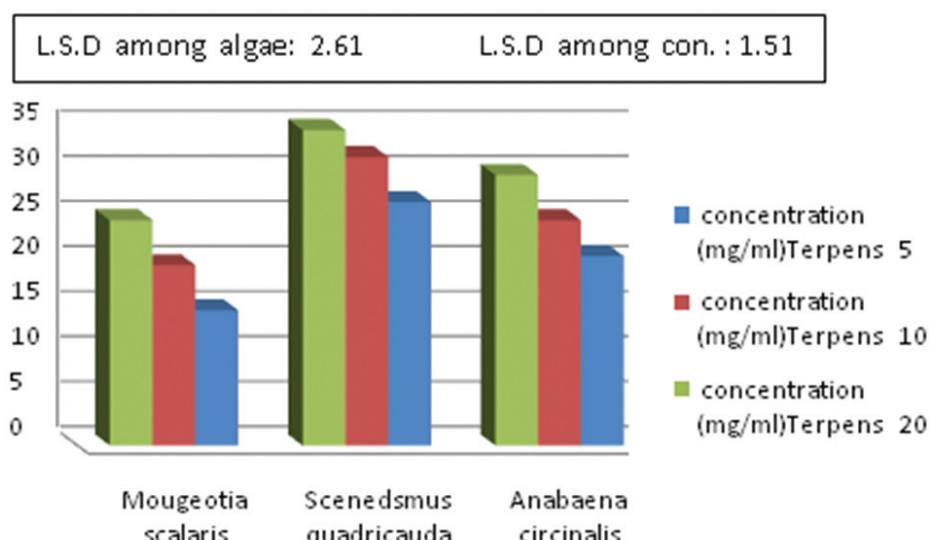
### Evaluation of inhibitory effects of leaves extracts against algae

Results showed that the highest value of inhibitory action caused by alkaloid extracts in concentration 20 mg/ml against *Anabaena circinalis* and the inhibition zone equal 40 mm in diameter, while the lower value of inhibitory action (10 mm in diameter) caused by phenolic extracts in concentration 20 mg/ml against *M. scalaris* as showed in fig. 1.



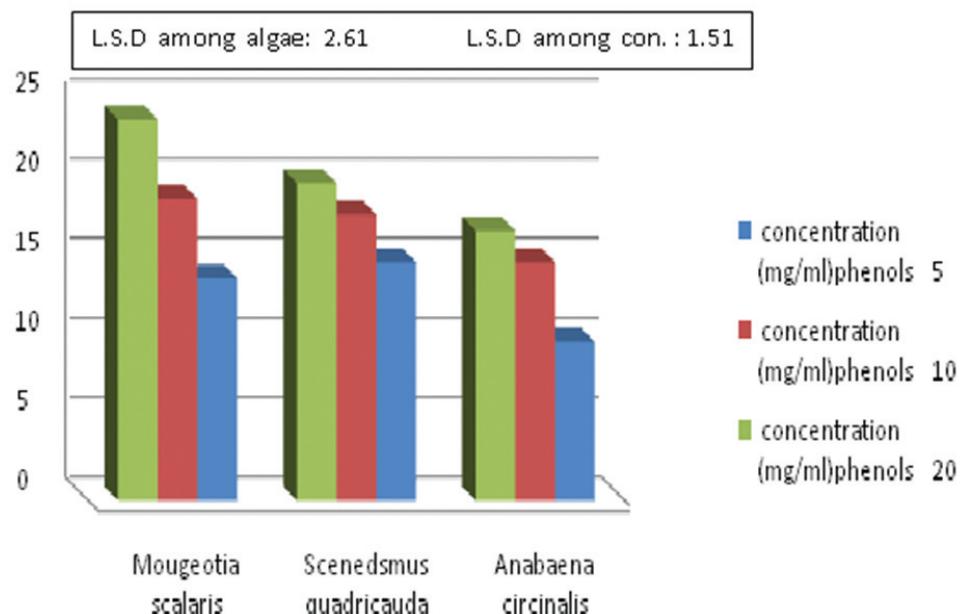
**Figure 1 . Evaluation of inhibitory effects of Alkaloids leaves extracts against algae .**

Also the results in Fig2. showed that terpen extracts have the highest value of inhibition zones (35 mm. in diameter) in concentration of 20 mg/ml against *S. quadricauda*, and the lower value (15 mm in diameter) against *M. scalaris* in concentration of 5 mg/ml.



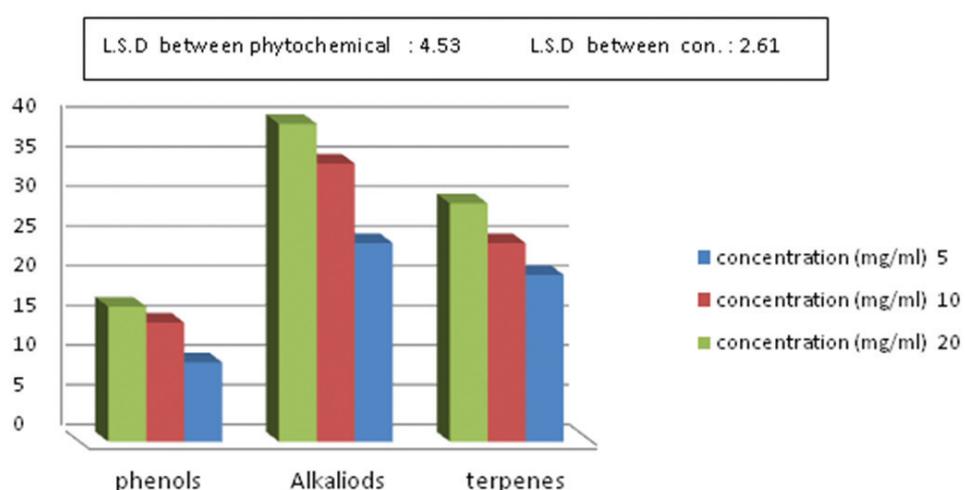
**Figure 2. Evaluation of inhibitory effects of Terpens leaves extracts against algae .**

In Fig .3 the results showed the highest value of inhibition zones caused by phenols extracts (24 mm in diameter) against *M. scalaris* in concentration of 20 mg/ml, and the lowest value of inhibition zones (7 mm in diameter) caused against *A. circinalis* in concentration of 5 mg/ml.



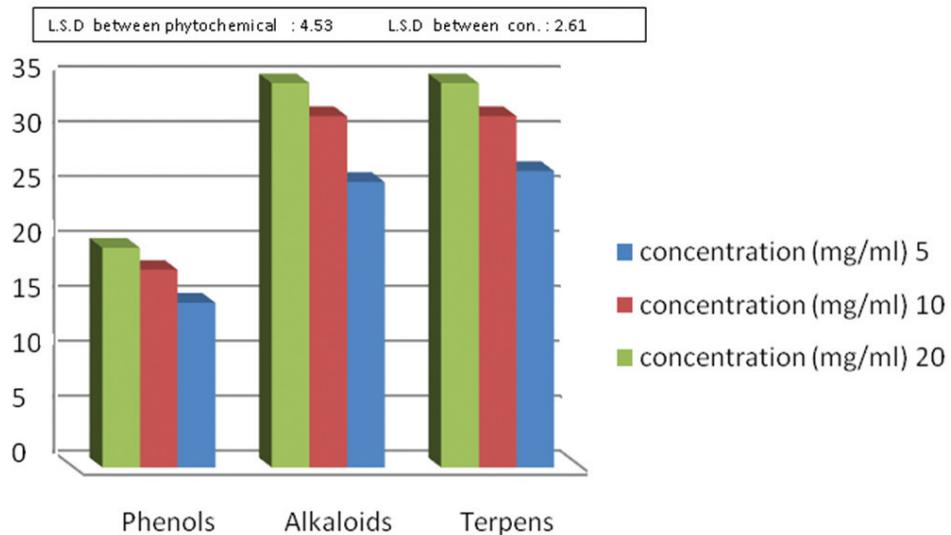
**Figure 3. Evaluation of inhibitory effects of phenols leaves extracts against algae**

Moreover in Fig . 4 the results recorded that *A. circinalis* was the most sensitive to alkaloid extract and the diameter of inhibition zone was 40 mm in concentration of 20 mg/ml., while this alga was less sensitive to phenol extract and the inhibition zone was 17 mm.



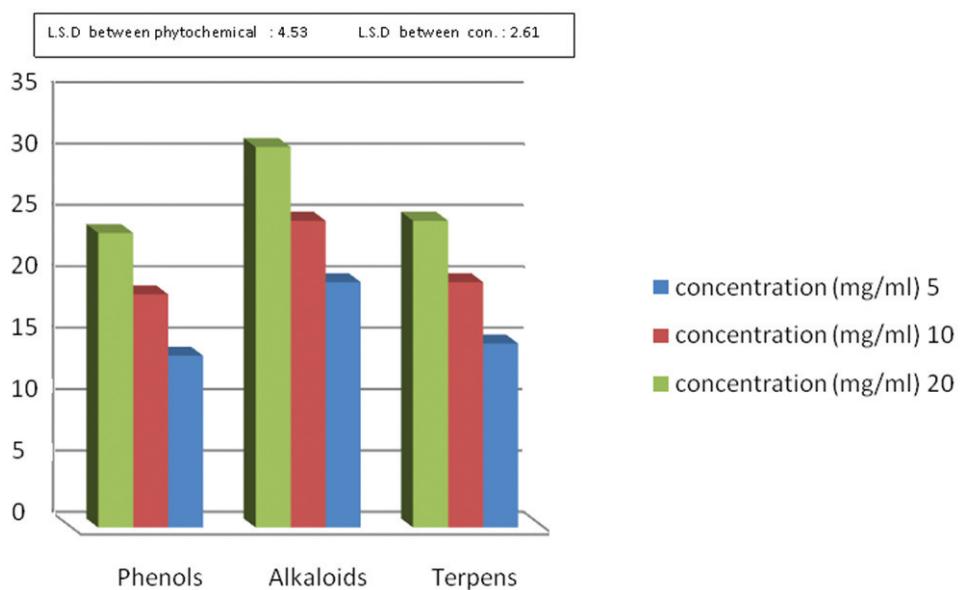
**Figure 4. Inhibition zones diameters in mm. caused by leaves extracts against algae *A. circinalis*.**

Another result was the terpens and alkaloids have a same effective on *S. quadricauda* as shows in Fig (5) .



**Figure 5. Inhibition zones diameters in mm. caused by leaves extracts against algae *S. quadricauda*.**

But in fig. 6 almost terpens and phenols have the same effect against *M. scalaris* and that because no significant differences between them. Based on these results the higher activity was due to alkaloids.



**Figure 6. Inhibition zones diameters mm. caused by leaves extracts against *M. scalaris*.**

The tested extract gave positive results for different phytochemical constituents like alkaloids, terpens and phenols. These results indicated that alkaloid extracts were more effective as antialgal effects against these 3 algae followed by Terpens extracts, while the phenols extracts were less effective. Plants *T. cordifolia* extracts contain a number of biologically active compounds, including alkaloids (of which more than thirty have been previously identified)(Sinha et al., 2004)(Daniel et al., 2007). However, the terpens were effective against algae followed by phenolic compounds, and may also could explain due to attributed to the terpens extract contains like, fuinosporide .furanolactone diterpenes, furanolactone clerodane, diterpenes, furanoid diterpenes, tinosporaside, ecdysterone makisterone and several glucosides isolated as poly acetate (Sinha et al., 2004). All the plant extracts in this study showed high inhibitory effects against the selected algae especially at high concentrations which cause complete lysing to the algal cell walls which confirmed microscopically, thus, intracellular toxins could be release and become a threat to the environment, so this study is applicable for water treatment which could be used for industrial uses.

There are probably also other bioactive compounds that play an important role in toxicity of the examined extracts and there may also occur synergistic effects between several components.

## Conclusion

Algae were generally more sensitive to all extracts, especially at high concentrations which cause significant biological activity with complete lysing to the algal cell walls, and the presence or absences of different chemical constituents in extracts were responsible for different biological activities. The results obtained in the present study supports that the plants contain biological active compounds with effective in resisting the growth of the algae. It is difficult to imagine that plant extracts will replace industrial algicides used in reservoirs or dams, but they could be an alternative method for small lakes, ponds, or aquaria. This approach could provide environmentally safe alternatives to chemical compounds used in water management for the control of noxious algae.

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