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

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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. values at 5% significant level.

Results showed that *A. circinalis* was the most sensitive to alkaloid extract and the diameterinhibitionzone was 40 mm in 20 mg/ml concentration, while this alga was less sensitive to phenol extract anthe inhibition zone was 17 mm. also the results showed that alkaloids extract was most active against all algae usedin this study followed by terpens extracts, while the phenols extracts had lower antialgal activity.

Keywords: algae, leaves extracts , *Tinospora cordifolia* , phytochemical.

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

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الخلاصة

توضح الدراسة الحالية تأثير المستخلصات التربينية والقلويدية والفينولية لأوراق نبات *Tinospora cordifolia* على الطحالب. حيث اختبرت فعالية هذه المستخلصات على ثلاثة أنواع من الطحالب هي: *Anabaena circinalis* و*Scenedesmu squadricauda* و*Mougeotia scalaris* بطريقة الحفر والانتشار في الاكار لتحديد الفعالية التثبيطية لتلك المستخلصات حيث استخدمت ثلاثة تراكيز هي: 5و 10و 20 ملغم/مل. أُستعمل التصميم العشوائي الكامل في تنفيذ التجربة وبثلاث مكررات . بينت النتائج ان طحلب *A. circinalis* كان الأكثر حساسية للمستخلصات القلويدية لأوراق نبات القنقينة وكان قطر منطقة التثبيط يعادل 40 ملم عند استخدام التركيز 20ملغم/مل. بينما كان الطحلب نفسه اقل حساسية للمستخلص الفينولي اذ كان قطر منطقة التثبيط يعادل 17ملم عند استخدام التركيز 20ملغم/مل. و اوضحت الدراسة ان المستخلص القلويدي هو الاكثر تاثيراً على الطحالب تلاه المستخلص التربيني بينما كان المستخلص الفينولي هو الأقل تاثيراً على الطحالب المستخدمة في هذه الدراسة.

Introduction:

The development of extensive algal blooms is a worldwide problem. Cyanobacteria produce secondary metabolites, which are toxic to a variety of aquatic and terrestrial organisms, including human. Some species also produce blooms that may block filters used in drinking water supply systems [1]. Many problems, such as, dermatitis diseases, fish toxicity and amenity of water…..etc. were considered due to the presence of algal blooms in general and cyanobacterial species in particular

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in the environment [2]. However, research to find ways of controling their growth were encouraged such as the use of algicidal substances and mechanical cleaning for stable tanks and filtration to control algae in 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 as a tonic, vitalizer and as a remedy for diabetes and other metabolic disorders [3]. 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 [4].

Tinospora cordifolia Willd. belongs to 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 [5]

Evidence hints that Tinospora may have anti-cancer [6], immune stimulating [7], anti-diabetic [8], cholesterol-lowering [9] and liver-protective actions[10]. *T. cordifolia* has also shown some promising speed in healing the diabetic foot ulcers [11]. Due to the wide use of plant extracts, methods were suggested to use plant extracts in control of algal blooms [12-16].

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

Materials and Methods:

Algal samples : Water samples were taken from the canal inside the University of Baghdad during 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 [17] were used to isolate algae and incubated at 26±1°C with illumination tensity about 200µE/m^2/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 and 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 µE/m²/s and 26± 2 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 compound microscope, this method was repeated till a uni algal culture had been gained [18]. 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 [12,13] 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 [12,14]. Obtained algal isolates were identified with help of algal classification references [19,20].

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

Preparation of plant extracts:

1- Terpens:

Terpenswere extracted 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 [21].

2- Alkaloids:

Extraction refers to the process of obtaining an 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_2So_4 until pH becomes between 1-2. The resulted solution extracted with chloroform 3 times then alkaloids were precipitated by the addition of concentrated NH_4OH 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 [21].

3- Phenols:

The process of extraction done 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 throught 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 [22].

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

The isolated compounds indicators:

1- Acetic anhydride reagent

This test used for the detection of terpens, According to [23], 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_2SO_4. 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 $HgCl_2$ in 60 ml H_2O, stock solution (2) was prepared by dissolving 5g KI in 10 ml H_2O, then combined with stock (1) and (2) and diluted with H_2O 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 [24].

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 [21].

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: $C_1V_1=C_2V_2$ 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 $\mu E/m^2/S$ and 26±2°C for 2-3 days until the plates turn into greenish color [13,14].

B- Control of algae:

Algicidal effects of plant extracts were detected by using the agar- well diffusion method according to [13,14] 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. 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- [25] and Means were compared according to L.S.D. at 95 significant level.

Results and Discussion

Isolation and Characterization of algae:

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

1- *M. scalaris*

Vegetative cells 34µ 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 [20].

2- *A. 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, heterocystissubspherical, 8- 10 µ broad, spores cylindrical, sometimes curved, ends rounded, 16- 18 µ broad up to 34 µ long, ordinarily away from the heterocyst epispore smooth and colourless[19].

3- *S. 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, andswampy habitats [20].

Table 1- The isolated algae in this study and their classification:

Algae	Division	Class	Order	Family
A. circinalis	Cyanophyta	Cyanophyceae	Nostocales	Nostocaceae
S. quadricauda	Chlorophyta	Chlorophyceae	Chlorococcales	Chlorococcaceae
M. scalaris	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 *A. 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 Figure-1.

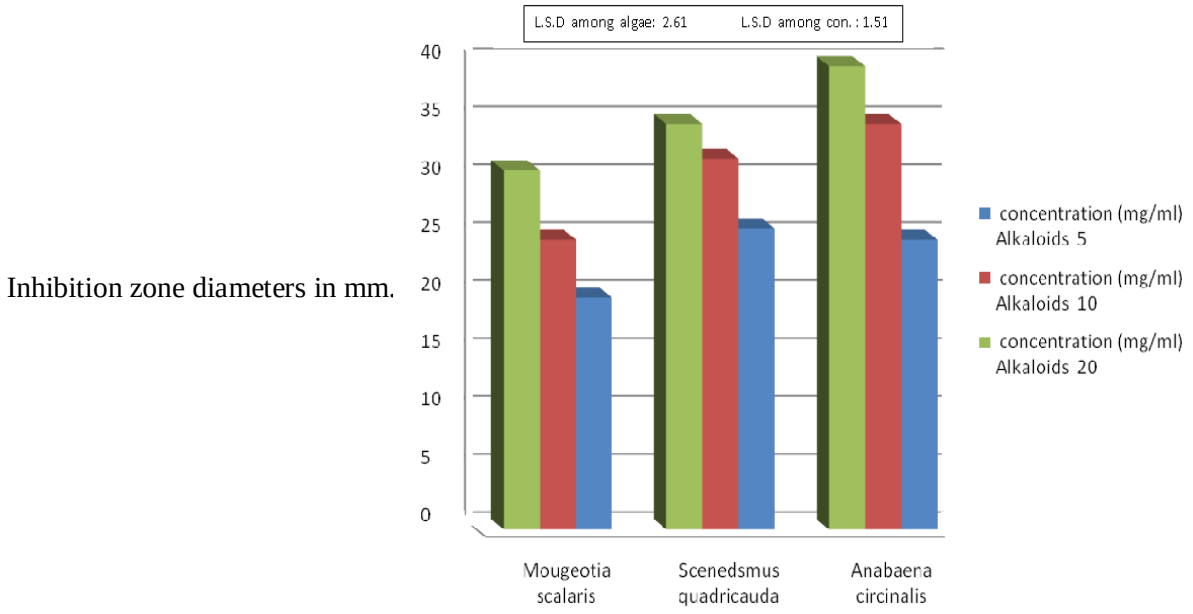

Figure 1- Evaluation of inhibitory effects of Alkaloids leaves extracts against algae .

Also the results in Figure-2 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.

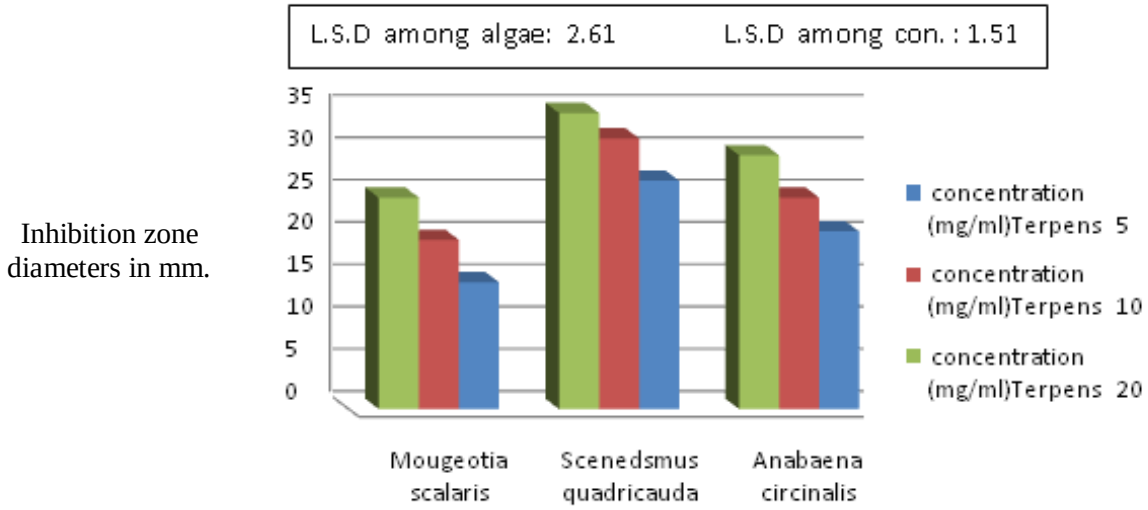

Figure 2- Evaluation of inhibitory effects of Terpens leaves extracts against algae

In Figure-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.

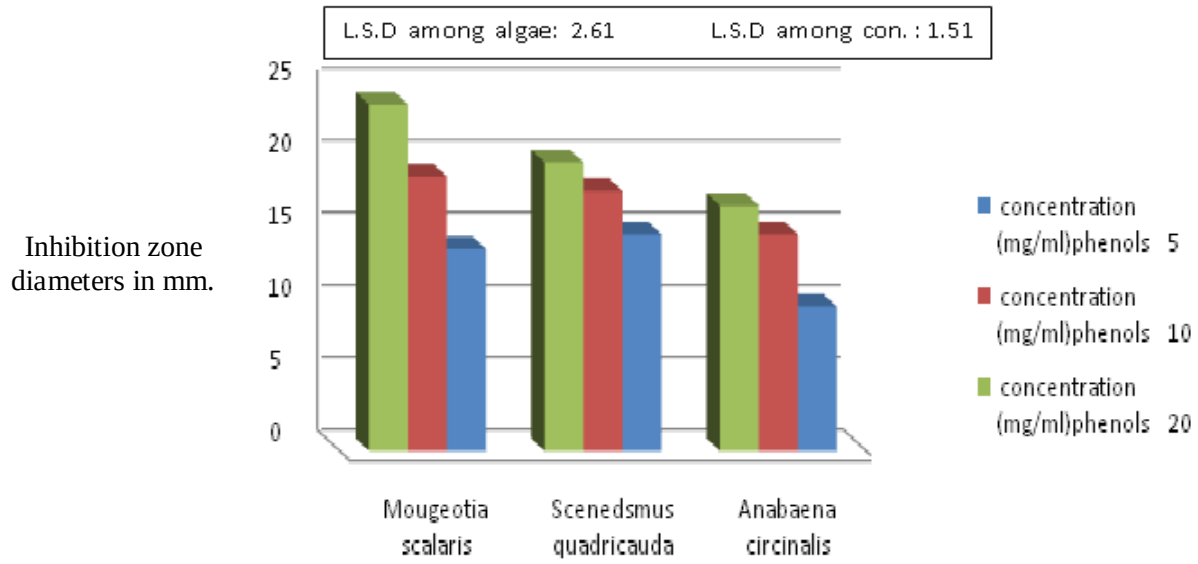

Figure 3- Evaluation of inhibitory effects of phenols leaves extracts against algae

Moreover in Figure-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.

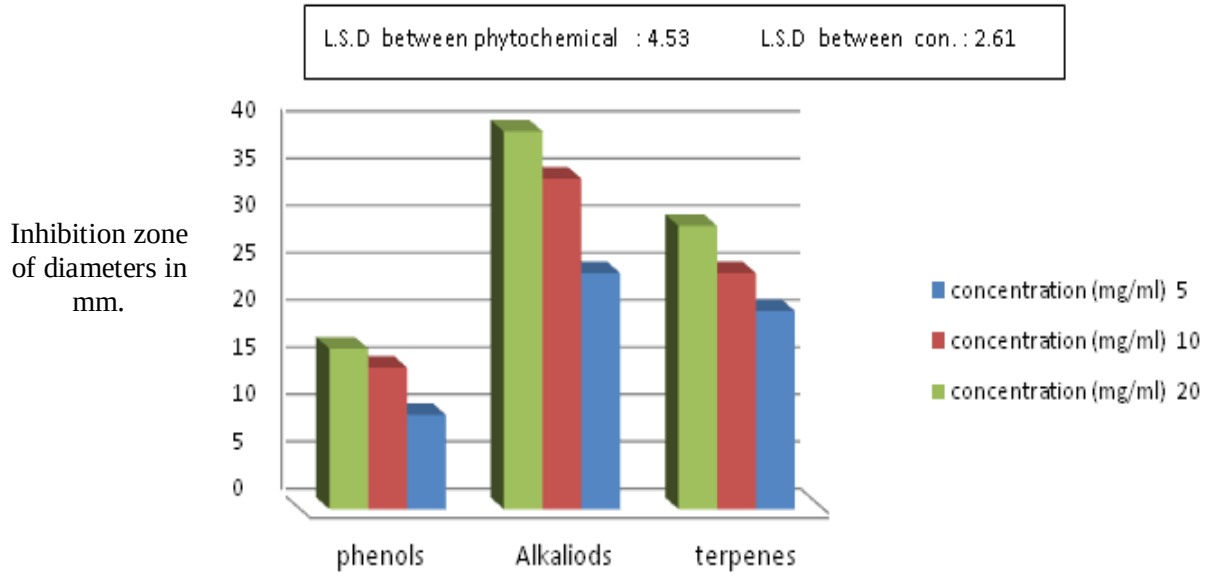

Figure 4- Inhibition zones diameters in mm. caused by leaves extracts against algae *A. circinalis* .

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

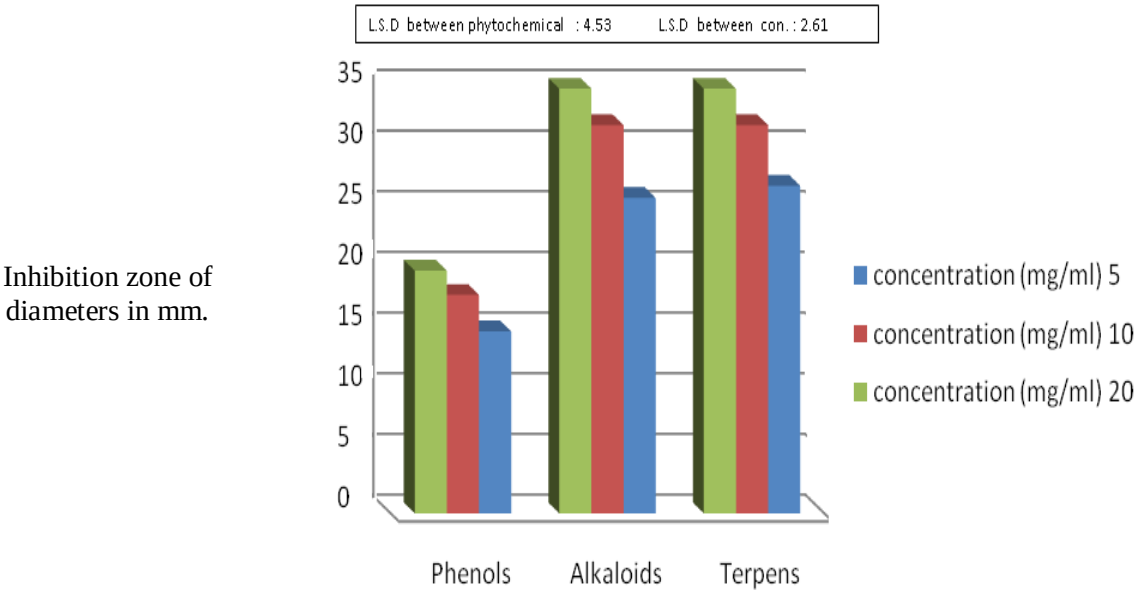

Figure 5- Inhibition zones diameters in mm. caused by leaves extracts against algae *S. quadricauda* .

But in Figure-6 almostterpens 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 duo to alkaloids.

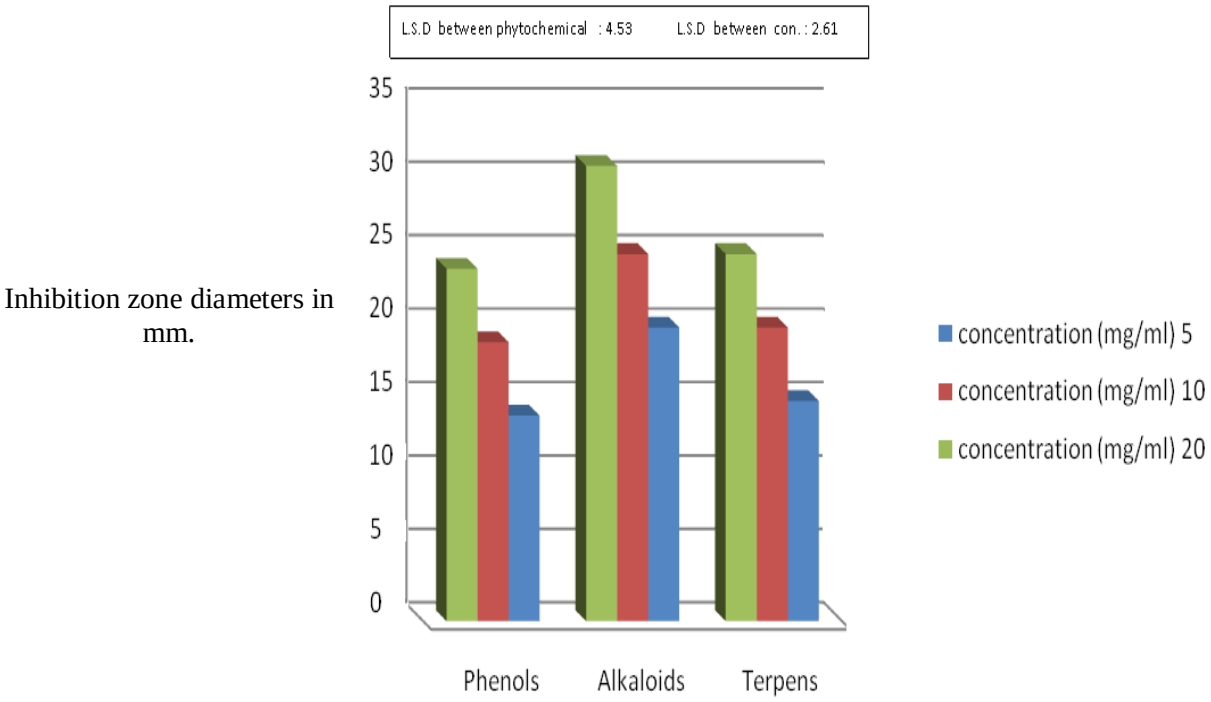

Figure 6- Inhibition zones diameters mm. caused by leaves extracts against *M. scalaris* .

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) [26]. 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 .furanolactonediterpenes, furanolactoneclerodane, diterpenes, furanoidditerpenes, tinosporaside, ecdysteronemakisterone and several glucosides isolated as poly acetate[26]. 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 use 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.

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