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Biological Activity of Active Compounds Extracts Tannins, Alkaloids, Glycosides and Saponins from *Cuscuta lehmanniana*

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ABSTRACT
The present study aimed to extract the active content of saponins, glyclosides, alkaloids and tannins from the Cuscuta lehmanniana (Growing on Populus Sp) And test their biological activity against some negative and positive bacterial species (Staphylococcus epidermidis Staphylococcus aureus gram positive and Escherichia coli, shigella, Klebsiella pneumonia, Salmonella Paratyphi gram negative) And pathogenic fungus is Trichophyton rubrum, The results showed the activities of the studied extracts against the tested bacterial species, that this activity was higher in tannin extract compared to the tested extracts with an Averaged inhibition zoon (AIZ) 13.97 mm , It was also found that the concentration of 100 mg / ml is the most effective in inhibition of bacteria on that the most inhibitory activity recorded by tannin extract with an (AIZ 23.33 mm) against S.epidermidis. The results also showed that the treatment of positive control (Nystatin) for the fungus is more effective in inhibiting T. rubrum with a growth diameter of 12 mm , and that the concentration of 5 mg / ml of saponins and alkaloids extract is most effective in inhibiting the fungus with an average growth of 33.33 mm and 30 mm, respectively.
Key words: active content, Cuscuta lehmanniana, tannin extract, activities, saponins
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INTRODUCTION

Cuscuta lehmanniana, which is classified as a Convolvulaceae (Yang *et al*, 2016), is a helioparasitic flowering plant that depends entirely on other green plants for water and food, and is characterized by having a stem. color pink or pale brown with spots of pink spots, cylindrical, leaf-free, wrapped in a spiral open around the host plant and connected to the host plant by specialized members known as Haustoria penetrate into the tissues of the host to absorb water and food from it and intrude on many cultivated and wild plants This causes significant damage to the host plant (Fahmy, 2008 and Alwan , 2009). The Cuscuta genus is spread in various regions of the world as it is widespread in the subtropical and temperate regions (Yanghan, 1994). The seriousness of this species lies in the parasitism of its species on a wide range of plant families as well as its ability to produce seeds in large numbers that are resistant to inappropriate conditions as well as their ability to grow rapidly (Nickrent & Musselman,2004). Cuscuta species has been used from ancient times for various purposes (Adnan *et al*, 2020). The genus Cuscuta is also used as a purgative in the treatment of liver disorder, cough, itching, constipation, flutance, body pain, jaundice, gout, rheumatism, antiviral, urination disorders muscles pain, impotence, premature ejaculation, sperm leakage, lower back pain, sore knees, ringing in the ear, leucorrhea, dry eyes, It exhibits anti-inflammatory, antibacterial, anticonvulsant, antiseptic, analgesic, anesthetic, antipyretic, bodycardia, antisteroidogenic, anti-oxidant, hemodynamic, antiplasmodic, nematicide, anti-androgenic, dermatigenic, hypocholestrolemic, antiandrogenic, hemolytic, diuretic, immunostimulant, antiarthritic, antiasthma and anticancer activities, antimicrobial (Lalchand *et al* , 2017 , Verma & Yadav, 2018, Zhang *et al* , 2019 and Alam & Sharma 2020). *C. lemannianais* used in the treatment of ulcers and its flavonoids extract is effective in treating diabetes (Sharifzadeh *et al* 2003 , Farhadi *et al* 2011) . A study (Al-Smail, 2011) indicated that the aqueous and alcoholic extracts of *C.lehmanniana* parts are effective against *Staphylococcus aureus, Protius vulgaria* and Fungai *Alternaria alternata* and *Fusarium solani*. .It turns out from the periodical review available scientific scarcity of studies on *C.lehmanniana* in Iraq and in many countries of the world The current study aimed, for the first time in Iraq, to separate the active compounds (tannins, alkaloids, glycosides and saponins) and test their anti-microbial activity.

Collection of plant samples

Samples were collected for dodder plant *Cuscuta lehmanniana* from the Populus Sp plant in July / June2018/2019 during the flowering phase of. Al-Shirqat district / Salahuddin Governorate. These samples included the entire flowering plant, after which these samples were washed with distilled water and dried at room temperature (to prevent oxidation Photovoltaic) and then ground with an electric grinder and kept in sealed plastic boxes until use.

Extraction of Tannins

Prepare the aqueous extract by boiling 0.5 g of the sample in 50 ml of distilled water for 30 minutes. The extract was filtered and presented to the centrifuge (2000 rpm) for 20 minutes. Transfer the supernatant to a 100 mL volumetric flask, add 20 mL of 4% lead acetate solution and complete the volume with distilled water. The extract was filtered and dried in an electric oven at a temperature of 60 ° C until completely dry. (Ahmad& Nazil,1989)

Extraction of Crude Alkaloids

100g grams of plant powder was extracted with 500ml of

70% ethanol
using soxhlet apparatus at (40) ºC for 24hrs , then filtered through filter paper (Whattman No . 1) . Ammonium hydroxide (1%) was added to make pH = 9 , then the solution was transferred to a rotary evaporator at (40) ºC and, transferred in a separating funnel , 10 ml of chloroform was added to the residue . The chloroform layer was collected ; this step was repeated for three times . The collected chloroform layers were mixed and evaporated in a rotary evaporator representing the total alkaloids (Harborne,1973).

Extraction of Glaycosides
10 g of the sample was added to 100 ml 80% ethyl alcohol and left the mixture for 24 hours in the refrigerator (7˚C) and filtered to obtain the ethanol extract .Concentrate the extract to a third of its volume with a rotary evaporator and add 50 ml of ether and 5 ml of lead acetate solution 3.0 molar to the separation funnel with shaking and pull the aqueous layer , Repeat the process three times and dry the drawn water layer at a temperature of 30 ° C until completely dry . (Ukida *et al* ,2006)

Extract of saponins
10 g of the sample was added to 50 ml 20% ethyl alcohol heat after the solution on a water bath for a period of 3 hours with continuous stirring and at a temperature of 55 ° C. Then the remaining extract was filtered and treated again with 100 ml of aqueous ethanol 20% preheat the resulting solution on a water bath at a temperature of 90 ° C. Until the final solution volume is approximately 40 ml , then the resulting solution was transferred to a separating funnel to which 20 mL diethyl ether was added with continuous shaking. The aqueous layer was separated while the ether layer was neglected(Harborne,1973).

Preparation of test microorganisms
In this study, two types of Gram-positive bacteria (*Staphylococcus epidermidis Staphylococcus aureus*) and four types of Gram-negative bacteria (*Escherichia coli, shigella, Klebsiella pneumonia, Salmonella Paratyphi*) were used, obtained from diagnostic laboratories at the Department of Science / College of Education Al-Shirqat / Tikrit University has been directly activated before use and the fungus *Trichophyton rubrum* was obtained from the laboratories of the Faculty of Science / Tikrit University

Detection of Tannins
10 g of vegetable samples were boiled in 50 ml of distilled water, then the solution was filtered and left to cool. A few drops of lead acetate solution of 1% were added to it, as the presence of tannins was inferred by the appearance of a gelatinous precipitate.(Shihata,1951)

Detection of Alkaloids
The method (Fahmy, 1933) was followed by boiling 10 g of vegetable powder with 50 ml of distilled water acidified with 4% hydrochloric acid, then the solution was filtered after cooling and a 0.5 ml of filtrate was tested in a test tube with each of the following reagents: Dragenderov Orange , Mayer is white(Mitscher,1972)

Detection of glycosides
Mix two equal parts of the Falken reagent with aqueous plant extracts, then leave the mixture in a boiling water bath for 10 minutes, and the test positive is indicated by the appearance of a red precipitate which is evidence of the presence of sugars. (Fahmy,1933)

Detection of Saponins
The aqueous solution of plant samples was strongly shaken in a test tube, and the presence of saponin is inferred by the appearance of a thick foam that remains for a long time , (1 -3) ml of mercury chloride solution was added to (5) ml of the plant extract.The appearance of a white precipitate indicated the positive of the detection(Ukida *et al* , 2006).

Antimicrobial activity study
For the purpose of using extracts in inhibition experiments, a method was adopted
Mitscher *et al* (1972) in preparing the stock solution and sterilize it 5 g of dry vegetable extract powder was taken and dissolved in 10Ml of sterile distilled water, so we have a storage solution of 500 mg / ml. The solution was sterilized by filtering to get rid of microbial contaminants in it and obtaining it On a sterile storage solution, and this solution was used as a source for preparing the dilution 5,10,25,75 and 100 mg / ml.

Preparation of cultures for testing plant extracts antibacterial
The bacterial suspension was prepared from a 24-hour colony using a normal saline solution compared to a MacFarland solution of 10^8 x 1.5 cells / ml (Baron *et al*, 1994).
The agar diffusion method by drilling (well) was used according to Egorove (1985) to note the sensitivity of microorganisms to the extracts of plants studied at concentrations 5 ,10,25,50,75 and 100 mg / ml. Bacterial isolates were cultures in a Petri dish containing container on the Mueller Hinton Agar medium by spreading 0.2 ml of bacterial suspension prepared on the dishes using an L-shaped glass rod sterilized with flame and then left the plates at the laboratory temperature for half An hour for impregnation to take place. After drying the surface of the acar layer, holes were made (cork borer) by a sterile cork borer with a diameter of (10) mm and by 3 holes per plate. Dishes at a temperature of (37 ± 2 ° C) for a period of (24) hours. Diameter measurements of the inhibition region were taken, if they were found in millimeters, according to the repeated rate. two control treatments were prepared
1- In the previous method, with the exception of making a pit instead of 3 pits, take (0.5 microliter / pit) of sterile distilled water and place it in the pit designated for it and with three dishes (three replications per treatment) (control negative)
2- Place a Tetracycline antibacterial tablet (30 mg) in the center of the dish, with three replications per treatment (positive control of bacteria).

Preparing cultures to test plant extracts antifungal

Fungal isolates were grown in container petri dishes on the center of Potato Dextrose Agar impregnated with different concentrations of plant extracts(Fungal isolates with a diameter of 5 mm were taken from the side of a pre-prepared petri dish by a cork borer (the colony's lifespan does not exceed more than two weeks) by three replicates each concentration the dishes were incubated in the incubator at a temperature 25 ± 2 ° C. for ten days, as readings were taken every three days and by repetition by measuring the average diameter of the fungi growth in millimeters and how it was affected by the concentrations of plant extracts (Jethinlakhosh &Lathika ,2012) .two control treatments were prepared:

1- In the previous method, except for not adding the extract to the culture medium as a negative control

2- In the previous method, except for the addition of nystatin in the vegetable extract, to the culture medium, as a positive control

RESULTS AND DISCUSSION

The results of the current study showed the effective of isolated compounds extracts and their concentrations (5, 10, 25, 50, 75, 100) against the studied microorganisms. However, there is a marked variation between the extracts.

Antibacterial active

Table (1) shows that tannin extract with a concentration of 100 mg / ml was more effective in inhibiting the studied bacterial species compared to control (Tetra and DW) as Averaged inhibition zoon (AIZ) reached 23.33 mm against *S.epidermidis* bacteria, and AIZ reached 22 mm against *S.aureus*, AIZ reached 17.66 mm against *E. coli*, AIZ reached 21 mm against *Shigella* sp, AIZ reached 18 mm against *Klebsiella pneumonia*, and 16.66 mm against *Salmonella Paratyphi* . Tannins are a group of polycyclic phenolic compounds found in almost all parts of the plant, and their anti-bacterial activity is due to their ability to inhibit the adhesins proteins, enzymes, and some of the transporting proteins present within the cell membrane (Engels *et al* ,2011) . The tannins are divided into two groups: biodegradable tannins and tannins Intensive, both of which have antagonistic activity toward microorganisms, and some of their members have a high ability to inhibit enzymes Peroxidase in prokaryotes (Okuda,2005). attributed Scalbert(1991)

Increase the inhibitory efficacy of tannins by increasing the concentration that tannins can interfere with The function of the cell membrane, and may affect the effective of some enzymes when the concentrations are high . And Al-Smail (2017) pointed out the effective of tannin extracts for Geranium and Erodium genus against bacteria *S. aureus*, *Streptococcus lactis*, *E. coli* and *Proteus mirabilis*.

(Table 2) shows the alkaloid extracts the concentrations of 50, 75 and 100 mg / ml were most effective in inhibition of *S. aureus*, *Shigella sp* and *Klebsiella pneumoniae* as the AIZ was 10, 10 and 10 mm, respectively, while the concentration was 100 mg / Ml is most effective in inhibition of *S.epidermidis* and *Salmonella Paratyphi* as it reached AIZ 10 and 8 mm, respectively, and in *E. coli* concentrations 75 and 100 mg / ml are the most effective against bacteria as AIZ reached 6 and 6 mm, respectively, compared to Controlled (tetra and D.W).The effect of alkaloids antimicrobial may be attributed to The interact with metabolic reactions , or Plasma membrane destruction, or due to Changes in the enzymes that is vital to growth and reproductions(Abdul-Rahman,1995) . Bacterial membrane disruption. some types of alkaloid , acts through a detergent-like mechanism of action against Gram-negative bacteria , leading to the disruption of their outer membranes , and it depolarizes Gram-positive bacterial membranes (Alhanout *et al*, 2010). Respiratory inhibition and enzyme inhibition in bacteria. (Arai *et al*, 2014).

And (Table 3) showed in the extract of glycosides concentrations 50, 75 and 100 mg / ml were most effective in inhibition of *S.epidermidis* and *S.aureus* as it reached AIZ 10, 10 and 10 mm, respectively, while the positive control treatment (Tetra) It is most effective in inhibition of *E. coli* and *Klebsiella pneumonia* as it reached AIZ 6 and 9.33 mm, respectively, and the concentration was 100 mg / ml is most effective in inhibition of *Shigella* sp and *Salmonella Paratyphi* as it reached AIZ 9 and 4.66 mm, respectively.

The effective of the antimicrobial glycosides is due to the non-diabetic component in their composition (Peach and Tracy, 1998) , and the non-saccharide part differs from glycoside to another, which may give the glycosides high efficiency and a wider spectrum of effect. (Cowan, 1999) indicated that the mechanism of action of biologically active plant substances includes interference with the functions of the cell membrane, especially the binding with proteins, adhesins and membranes of membranes, and inhibition of the effectiveness of some enzymes

It is noted from (Table 4) , That in saponins extract , the positive control treatment (Tetra) was the most effective in inhibition of *S.epidermidis* and *Klebsiella pneumonia*, as AIZ was 6 and 9.33 mm, respectively, while concentrations 50, 75 and 100 mg / ml were the most It is effective in inhibition of *S. aureus* as it reached AIZ 10, 10 and 10 mm, respectively, and the concentration was 100 mg / ml was most effective in inhibition of *E. coli* and *Shigella* sp as it reached AIZ 10 and 9 mm, respectively, and the concentrations were 50 and 75 And 100 mg / ml is most effective in inhibition of *Salmonella Paratyphi* as AIZ was 6.66, 7 and 7.33 mm, respectively. Saponins is one of the diverse groups of plant sources compounds with valuable medial values and bioactivities , possess detergent-like properties and might increase the permeability of bacterial cell membranes ; this activity might facilitate antibiotic flow through the bacterial cell wall (Jacob *et al* , 1999) . The saponin interaction with cholesterol in the cell membrane accounts for many of its biological effects . The saponin extracts contain a mixture of saponins that can possibly interact with the cholesterol molecules in the cell membrane and consequently disturb the cell membrane organization and the ability of membrane receptors utilized by pathogens to function properly (Johnson,2013).The results of a study (Khanna and Kannabiran 2008) indicated that plant

saponins can be used as therapeutic agent to control common microbial diseases .

From Table 5, it was found that there was no significant difference between the tested plant extracts and the positive control treatment Tetra (regardless of the type of extract and concentration) in its effectiveness against the tested bacterial species, that the tannins extract is the most effective in inhibiting the tested bacteria with an average of 13.97 mm.

Antifungal active

The results showed that the treatment of positive control (Nystatin) is the most effective in inhibition of *Trichophyton rubrum* with an average diameter of 12 mm compared to the concentrations of the studied extracts and negative control, and showed no significant differences between the concentrations of the studied extracts and the negative control treatment except for the concentration of 5 mg / ml in saponins and Alkaloids extract has shown active in inhibiting fungi with an average growth diameter of 33.33 mm and 30 mm respectively compared to negative control. (table, 6)

CONCLUSIONS

Extracts of the active components isolated from *C.lehmanniana* and their concentrations were active antimicrobial in varying degrees, while the tannins showed the highest inhibiting activity against the studied bacterial species, and the alkaloid extract and saponins extract at a concentration of 5 mg / ml showed inhibitory activity against the fungui *T. rubrum* and did not show any other concentrations of the extracts active in inhibition of fungi . The results of the current research have shown the biological importance of some of the active compounds of *C.lehmanniana*, which requires further studies on the quantitative and qualitative assessment of the active compounds of the *C.lehmanniana*, and the species of *Cuscuta* genus

Table 1: The effects of crude Tannins compounds extracted from Cuscuta lehmanniana on tested bacteria.

Bacteria / Concentration	Average Inhibition zone mm					
	S.epidermidis	*S.aureus*	*E.coli*	*Shigella* sp	*Klebsiella pneumoniae*	*Salmonella Paratyphi*
5	10 C	10.66 D	11.33 B	10 E	10.66 C	11.66 B
10	10.66 C	11.33 D	11 B	10.33 E	10.66 C	10.66 B
25	11.66 C	14.33 CD	11.66 B	11.33 DE	11.66 C	8 BC
50	16.66 B	17.66 BC	12.66 B	14.33 C	15 B	12.66 B
75	16.66 B	19.33 AB	17 A	16 B	15.33 B	13.66 AB
100	23.33 A	22 A	17.66 A	21 A	18 A	16.66 A
Tetracycline	6 D	7 E	6 C	7 F	9.33 C	0 D
Distilled water	0 E	0 F	0 D	0 G	0 D	0 D

* The numbers represent an average of three iterations

- Similar capital letters in one column mean that there are no significant differences between them at the probability level (0.05).

Table 2: The effects of crude Alkaloids compounds extracted from Cuscuta lehmanniana on tested bacteria.

Bacteria / Concentration	Average Inhibition zone mm					
	S.epidermidis	*S.aureus*	*E.coli*	*Shigella* sp	*Klebsiella pneumoniae*	*Salmonella Paratyphi*
5	3 E	2 C	3 C	2 E	5.33 D	0 D
10	4 D	6.33 B	4.66 B	3 D	7.66 C	0 D
25	6 B	6.66 B	6.66 A	5 C	9 B	2.66 C
50	6 B	10 A	5 AB	10 A	10 A	5.33 B
75	5 G	10 A	5.66 A	10 A	10 A	5.66 B
100	10 A	10 A	6 A	10 A	10 A	8 A
Tetracycline	6 B	7 B	6 A	7 B	9.33 B	0 D
Distilled water	0 F	0 D	0 D	0 F	0 E	0 D

* The numbers represent an average of three iterations

- Similar capital letters in one column mean that there are no significant differences between them at the probability level (0.05).

Table 3: The effects of crude Glycosides compounds extracted from Cuscuta lehmanniana on tested bacteria.

Bacteria / Concentration	Average Inhibition zone mm					
	S.epidermidis	*S.aureus*	*E.coli*	*Shigella* sp	*Klebsiella pneumoniae*	*Salmonella Paratyphi*
5	3.66 C	2 C	0 C	0 D	0 D	0 B

10	5 C	6.33 B	0 C	0 D	0 D	0 B
25	9 A	6.66 B	0 C	5.33 B	5 C	0 B
50	10 A	10 A	0 C	4.66 BC	7 B	0 B
75	10 A	10 A	5 B	7 B	7 B	0 B
100	10 A	10 A	5 B	9 A	7.33 B	4.66 A
tetracycline	6 BC	7 B	6 A	7 B	9.33 A	0 B
Distilled water	0 E	0 D	0 C	0 D	0 D	0 B

* The numbers represent an average of three iterations

- Similar capital letters in one column mean that there are no significant differences between them at the probability level (0.05).

Table 4: The effects of crude Saponins compounds extracted from Cuscuta lehmanniana on tested bacteria .

Bacteria / Concentration	Average Inhibition zone mm					
	S.epidermidis	*S.aureus*	*E.coli*	*Shigella* sp	*Klebsiella pneumoniae*	*Salmonella Paratyphi*
5	0 E	0 E	0 E	2.66 E	0 E	0 C
10	2 D	5.66 D	2 D	4.66 D	0 E	0 C
25	4 C	7.66 B	7 B	8 B	0 E	2.66 B
50	B5	10 A	7 B	8 B	2.33 D	6.66 A
75	B5	10 A	5.66 C	8 B	4.33 C	7 A
100	B5	10 A	10 A	9 A	6.33 B	7.33 A
Tetracycline	A 6	7 BC	6 C	7 C	9.33 A	0 C
Distilled water	0 E	0 E	0 E	0 F	0 E	0 C

* The numbers represent an average of three iterations

- Similar capital letters in one column mean that there are no significant differences between them at the probability level (0.05).

Table 5: The effect of types of plant extracts on laboratory bacteria

Bacteria	Average Extraction mm				Average
	Tannins	alkaloids	Glycosides	Saponines	
S.epidermidis	14.82 a	5.66 bc	7.94 b	3.5 c	7.98 A
S.aureus	15.88 a	7.49 b	7.49 b	7.22 b	9.52 A
E.coli	13.55 a	6.16 b	1.66 b	5.27 b	6.41 A
Shigella	13.83 a	6.66 b	4.33 b	6.72 b	7.88 A
Klebsiella pneumonia	13.55 a	8.66 b	4.38 c	2.66 d	7.31 A
Salmonella Paratyphi	12.21 a	3.60 b	0.77 bc	3.94 b	5.13 A
Tetracycline	5.88 a	5.88 a	5.88 a	5.88 a	5.88 A
Average	13.97 a	6.37 b	4.42 bc	4.88 b	

- Similar small letters in a row mean that there are no significant differences between them at the probability level (0.05).

- Similar capital letters in one column mean that there are no significant differences between them at the probability level (0.05)

Table 6: Activity of tannins, alkaloids, glycosides and saponins extract from *cuscuta lehmanniana* against *Trichophyton rubrum* fungi

Extraction / Concentration	Average Inhibition zone mm			
	Tannins	alkaloids	Glycosides	Saponines
5	50 CD	30 C	55 A	33.33 C
10	56.66 BC	48.33 B	51.66 A	60 A
25	65 A	51.66 B	48.33 A	55 AB
50	55.66 BC	50 B	56 A	43.33 B
75	61.66 A	50 B	66 A	45 B
100	46.66 D	54.33 B	62.33 A	43.33 B
Control 1	12 E	12 D	12 B	12 D
Control 2	61.66 A	61 A	61.66 A	61.66 A
Average extraction	55.94 b	47.39 ab	56.55 b	41.66 a

* The numbers represent an average of three iterations

- Similar capital letters in one column mean that there are no significant differences between them at the probability level (0.05).

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