The antimicrobial activity of leaves and callus extracts of *Thevetia peruviana In vitro*

الفعالية المايكروبية لمستخلصات اوراق وكالس نبات الدفلة الصفراء *Thevetia peruviana* خارج الجسم الحي

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Abstract

The antimicrobial activity for *Thevietia peruviana* was evaluated by measuring inhibition zone diameter in agar using well diffusion assay. The aim of the present study was to evaluate the antimicrobial potential of *Thevietia peruviana* leaf extract as compared with callus extract against some bacterial strains and fungi. The results showed that the addition of 2,4-D at the concentration of 9 mg/l, and 0.1 mg/l of kinetin led to obtain callus weight reached 800 mg. It was noticed that the reduction of 2,4-D concentration up to 6 mg/l resulted in compact and green pieces of callus. The optimal weight and friable callus was obtained at 9 mg/l. Among the susceptible bacteria are the gram negative *pseudomonas aeruginosa* seemed to be sensitive against all concentration of *Thevietia peruviana* leaf and callus extracts, While *Escherichia coli* showed resistance with all concentrations of extracts. It was noted that the extracts were more active against gram positive *staphylococcus aureus*, as compared with other bacterial species. Results of this study revealed that callus extract of *Thevetia peruviana* possess higher activity in comparison with leaf extract against gram positive bacteria (*Staphylococcus aureus, Bacillus cereus*) and gram negative (*Pseudomonas aeruginosa*). Finally all the bioextracts were well stable at room temperature during the period of the study and did not show any reduction of activity against the bacterial strains used in this study experiments.

المستخلص

قيمت الفعالية المايكروبية لنبات الدفلة الصفراء *Thevetia peruviana* وذلك بقياس اقطار مناطق تثبيط على الاكار باستعمال كشف الانتشار بالحفر . وكان هدف الدراسة الحالية لتقييم الجهد المايكروبي لمستخلص اوراق نبات الدفلة الصفراء *Thevetia peruviana* بالمقارنة بمستخلص الكالس ضد بعض السلاسل البكتيرية والفطريات. اظهرت النتائج ان اضافة الـ 2,4-D عند التركيز 9 ملغم/ لتر، و0.1 ملغم/ لتر من الكاينتين ادى الى الحصول وزن للكالس وصل الى 800 ملغم. لوحظ ان خفض تركيز الـ 2,4-D الى 6 ملغم/ لتر نتج عنه قطع كالس خضراء متماسكة. الوزن الاعلى والكالس الهش تم الحصول عليه عند التركيز 9 ملغم/ لتر.

Key words: callus extracts, *Thevetia peruviana,* callus

من بين البكتريا الحساسة كانت السالبة لصبغة كرام ***Pseudomonas aeruginosa*** والتي ابدت حساسية تجاه جميع التراكيز المستخدمة من مستخلصات الاوراق والكالس لنبات الدفلة الصفراء. بينما اظهرت البكتريا ***E. Coli*** مقاومة لجميع تراكيز المستخلصات، وقد لوحظ بان المستخلصات كانت اكثر فعالية تجاه البكتريا الموجبة لصبغة كرام ***Staphylococcus aureus*** مقارنة ببقية انواع البكتريا . بينت نتائج هذه الدراسة ان مستخلص كالس نبات الدفلة الصفراء يمتلك فعالية اعلى مقارنة بمستخلص الاوراق ضد البكتريا الموجبة لصبغة كرام (***Staphylococcus aureus, Bacillus cereus***) والسالبة للصبغة (***Pseudomonas aeruginosa***). واخيراً فان جميع المستخلصات الحيوية كانت ثابتة عند درجة حرارة الغرفة اثناء فترة البحث ولم تظهر اي نقص في الفعالية تجاه السلالات البكتيرية التي استخدمت في هذه الدراسة.

Introduction

Thevetia peruviana Schum. (Family-Apocynaceae), commonly known as yellow oleander, it is a small ornamental shrub, which grows up to a height of about 3 to 4 meters. *Thevetia peruviana* is a plant probably native to Central and South America but now frequently grown throughout the tropical and sub-tropical region of the world. It is an evergreen small shrub that bears yellow, trumpet like flowers and its fruit is deep red/black in color encasing a large seed that bears some resemblance to Chinese "lucky nut" [1,2].

It contains a milky sap containing a compound- thevetin that is used as a heart stimulant but in its natural form, it is extremely poisonous, as all part of the plants, especially the seeds. It has immense medicinal properties. This plant is particularly known for its ability to produce cardiac glycosides; flavonol glycoside from leaves. *Thevetia peruviana* has inhibitory effect against HIV-1 reverse transcriptase and HIV-1 Integrase [3]. It has also been regarded as a potential source of biologically active compounds, namely insecticides, rodenticides, and bactericides [4]. *Thevetia peruviana* plant extracts have also been reported to have antifungal properties against *Cladosporium cucumerinum* [5]. The presence of unsaturated linoleic acid in Yellow oleander oil, which has drying properties [6,7], makes Yellow oleander oil suitable for making a surface coating such as paint. Many studies have been conducted using different levels and combinations of plant growth regulators to callus induction. The MS (Murashige and Skoog) medium supplemented with 2,4-D and Kinetin combination given highest callus induction [8, 9]. Evaluation of natural products to access new effective antimicrobial agent is one of the scientific strategies to combat drug-resistant pathogens with this perspective leaf and callus extracts of *T. peruviana* which had documented uses in traditional medicine, were investigated for antimicrobial activity against fungus and bacterial strains. *T. peruviana* plant species contain glycosides, whose toxicity against snails, slugs, bacteria, and insects has been documented [10].

Materials and Methods

Plant materials

Yellow oleander *Thevetia peruviana* plants were purchased from Nurseries in Baghdad on March/2012. The leaves were washed three times with tap water and subjected to surface sterilization using 50% alcohol for 15 minutes then washed with sterilized distilled water.

Bacteria and Fungi Strains

Bacterial and fungal strains were obtained from College of Science laboratory/ Baghdad University. These strains were used previously for student training.

Callus induction

Leaf explants were cultured vertically on MS medium [8] supplemented with 9 mg/l of 2,4-D and 0.1 mg/l of kinetin to get out most favoring growth of callus. [11, 12]. The medium was fortified with 3% (w/v) sucrose and PH was adjusted to 6 before being solidified with 0.8 % (w/v) agar. The culture vessels containing the media were autoclaved at 15 lb/inch and 121C° for 20 min. Cultures were maintained at 25 ± 2C°, photoperiod of 16/8 hours (light/ dark) for 30 days. Observations were made for callus induction percent after one month of callus growth.

Preparation of the extracts

Ten g of callus and 100g of *Thevetia peruviana* leaves were extracted separately with 95% alcohol at 60-80C° in a soxhlet apparatus. The extract was collected in a container and concentrated to dryness in an evaporator apparatus under reduced pressure and controlled temperature 40-60C° until it used in antimicrobial assay.

Microorganisms

Bacterial isolates of *klebsiella pneumoniee, Pseudomonas aeruginos, Salmonella typhi, Escherichia Coli, Bacillus Cereus, Staphyloccus aureus* and *Candida albicans* were obtained as slants from microbiology laboratory, College of Science. The bacterial and fungal cultures were maintained on nutrient agar and sabourand dextrose agar medium respectively, and were stored at 4C°of callus for determining antimicrobial activity [13, 14].

Antimicrobial activity

In vitro antibacterial and antifungal activities were assayed by using agar well diffusion method. The pure cultures of different pathogens were grown overnight in sterile nutrient broth for 24 hours.

The 0.1 ml of the culture was seeded on 25ml and incubated at 37 of solidified nutrient agar plate and sabouraud dextrose agar plates for bacterial and fungal cultures. The wells were bored with 6 mm borer in seeded agar and then the particular concentrations (2 mg, 4 mg, 6 mg and 8 mg /0.2ml / well) were used, the plates were then kept at 10C° for 30 min. After normalized to room temperature the plates were incubated at 37°/ 24 hr. Later, the zone of inhibition was measured and recorded [15].

Results

Callus induction

The results showed that the addition of 2,4-D at the concentration of 9 mg/l, and 0.1 mg/l of kinetin led to obtain callus weight reached 800 mg. It was noticed that the reduction of 2,4-D concentration to 6 mg/l or 4 mg/l resulted in compact and green pieces of callus.

The optimal weight of callus was obtained at 9 mg/l due to several literatures [8,9, 12].

Leaf extract

Table (1) showed that the higher inhibition zone 9mm were recorded for the fungus *Condida albicans* at the concentration 200 mg/ml, while it was recorded 8 mm for the lower concentration 75 mg/ml. The gram positive bacteria *Bacillus cereus* showed 7 mm as inhibition zone at the concentrations 150,175,200mg/ml of the extract while

Staphylococcus aureus recorded 12 mm as the higher inhibition zone at the concentration 150 mg/ml of the extract. The gram negative bacteria *Pseudomonadas aeruginose* recorded the higher inhibition zone 13mm at the concentration of 200 mg/ml followed by *Klebseilla Pneumonia*e which recorded 12 mm at the concentration of 150 mg/ml. *E.coli* appeared to be resistance against all concentrations of leaf extract. The lowest inhibition zone diameter 7 mm was recorded for *Salmonella typhi* at the concentration of 50 mg/ml.

Table (1): Inhibition zone diameter (mm) for the fungi and bacteria species as affected by leaf extract of *Thevetia peruviana*

Microorganism	Inhibition zone diameter (mm) Concentrations (mg/ml)						
	200	175	150	125	100	75	50
Candida albicans	9	8	-	-	-	-	-
Bacillus cereus	7	7	7	-	-	-	-
Staphylococcus aureus	10	10	12	10	9	7	6
Klebsiella pneumonia	_	_	12	10	9	7	6
Escherchia coli	-	-	-	-	-	-	-
Salmonella typhi	9	8	8	10	9	8	7
Pseudomonas aeruginosa	13	9	8	_	_	_	_

Callus extract

Table (2) showed that the higher inhibition zone 7mm were recorded for the fungus *Candida albicans* at the concentration 200 mg/ml, while it was recorded 6 mm for the lower concentration 125 mg/ml. The gram positive bacteria *Bacillus cereus* showed 12 mm as inhibition zone at the concentration 175 mg/ml of the extract, while *Staphylococcus aureus* recorded 15 mm as the higher inhibition zone diameter at the concentration of 200 mg/ml. The gram negative bacteria *Pseudamonas aeruginosa* recorded the highest inhibition zone 13mm at the concentration of 200mg/ml followed by *Klebseilla Pneumonia*e which recorded 9 mm at the concentration of 175 mg/ml. *E.coli* appeared to be resistance for all concentrations of callus extract. The lowest inhibition zone diameter 7 mm was recorded for *Salmonella typhi* at the concentrations of 175, 200 mg/ml.

Table (2): Inhibition zone diameter (mm) for the fungi and bacteria species as affected by callus extract of *Thevetia peruviana*

Microorganism	Inhibition zone diameter (mm) Concentrations(g/ml)						
	200	175	150	125	100	75	50
Candida albicans	7	-	-	6	-	-	-
Bacillus cereus	8	12	10	9	8	9	9
Staphylococcus aureus	15	10	9	10	8	10	10
Klebsiella pneumonia	8	9	7	7	7	6	6
Escherchia coli	-	-	-	-	-	-	-
Salmonella typhi	7	7	6	6	6	6	-
Pseudomonas aeruginosa	13	10	9	8	8	9	9

Discussion

The results imply that the extracts leaf and callus of *Thevetia peruviana* plant exhibited more or less pronounced antibacterial and antifungal potencies affecting gram positive, gram negative bacteria and fungi used in this study.

Leaf extract

Results of this study on *Thevetia peruviana* showed that *Condida albicans* fungus was affected by leaf extract at the higher concentration 200 mg/ml, while low concentrations did not show any effect. These results are in agreement with Monkillkin et.al, which proved that fungus was affected by oil from the seed of *Thevetia peruviana* at the higher concentrations [16].

Gram positive bacteria *Staphylococcus aureus* showed resistance to the lower concentrations. *Bacillus cereus* also showed lower inhibition zone diameter at the lower concentration 50-125 mg/ml. The results were in agreed with [17] Who found that the lower concentration of leaf extract of *Nerium Oleander* didn't show any significant effects against gram positive bacteria *Corynebacterium ulerans* and *Bacillus subtilis*. Gram negative bacteria *Pseudomonas aeruginosa* showed the highest inhibition zone diameter 13 mm as compared with other bacterial species, followed by *Klebsiella pneumoniae* which was recorded 12 mm. Other bacteria species showed less sensitivity.

Callus extract

Data revealed that *Candida albicans* fungus was affected by callus extract at the higher concentration 200 mg/ml, while low concentration did not show any effect. These results were agreed with [18] who proved that funguses were affected by oil from the callus extract of *Thevetia peruviana* at the higher concentration.

Gram positive bacteria *Staphylococcus aldreus* showed resistance to the lower concentrations. *Bacillus cereus* also showed lower inhibition zone diameter at the lower concentration. These results were agreed with [19] who found that the lower concentrations of callus extract obtained from *Thevetia peruviana* did not show any significant effects against *Streptococcus lactic* and other bacterial species. Gram negative bacteria *Pseudomonas aeruginosa* showed the highest inhibition zone diameter 13mm as compared with other bacterial species, followed by *Klebsiella pneumoniae*, which was recorded 9 mm as inhibition zone diameter. Other bacterial species showed less sensitivity. These results were agreed with [20] they found that a significant effect of callus extract of *Thevetia peruviana* shown against *Salmonella typhi* and *Salmonella paratyphi.*

From these results it was clear that callus extract of *Thevetia peruviana* showed higher activity in comparison with leaf extract against gram positive bacteria *Staphylococcus aureus, Bacillus cereus* and gram negative *Pseudomonas aeruginosa*. This effect may be due to flavonol glycosides which have antimicrobial activity against bacteria and fungi as found by [4, 5]. *E. coli* showed resistance at all concentrations used from callus and leaf extraxts. This resistance may be due to their cell wall structure that contains peptioglycan and polysaccarides, these substances give rigidity for cell wall and reduce the effect the antimicrobial agents (extracts). The ethanol extract of *Thevetia peruviana* and *Nerium indicum* showed a wide range of inhibition against *Bacillus subtilus, Bacillus pumilus,*

Staphylococcus aureus, *Escherichia coli*, and the fungus *Aspergillus nigar*, these results are in harmony with the results of the present study which indicate the antimicrobial activity of ethanol extracts of leaf and callus of *Thevetia peruviana* [21].

Conclusion and Recommendations:

It was concluded that *Thevetia peruviana* callus extract is more effective than leaf extract especially against *Staphylococcus aureus* and *Psudomonas aeruginosa*, and both extracts in this study have antimicrobial activity against fungi and bacteria, and can used as a source of natural drugs for treatment of some diseases that caused by bacteria depending on these results and results from other literatures. It was recommended to use the extracts of this plant in manufacturing some useful drugs in medicine. Other studies must be done in this field to develop plant products, for using as natural antimicrobial agents.

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