The antimicrobial activity of leaves and callus extracts of

*Thevetia peruviana* In vitro

*Thevetia peruviana* الفعالية المايكروبية لمستخلصات أوراق وكاتالس نبات الدفلة الصفراء خارج الجسم الحي

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Abstract

The antimicrobial activity for *Thevetia 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 *Thevetia 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 *Thevetia 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.

Key words: callus extracts, *Thevetia peruviana*, callus
from the bacteria sensitivity test, the pathogenic bacteria Pseudomonas aeruginosa. All preparations were tested against E. Coli. The results revealed that there were more significant differences found in the pathogenic bacteria Pseudomonas aeruginosa compared to Staphylococcus aureus, which was found in the analysis and the results were of statistical significance. Therefore, it can be concluded that all parts of the plant, especially the seeds, are toxic. This plant has immense medicinal properties. This 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 121°C for 20 min. Cultures were maintained at 25 ± 2°C, 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 60-80°C 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 40-60°C until it used in antimicrobial assay.

Microorganisms
Bacterial isolates of klebsiella pneumoniae, Pseudomonas aeruginos, Salmonella typhi, Escherichia Coli, Bacillus Cereus, Staphylococcus 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 4°C 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 10°C for 30 min. After normalized to room temperature the plates were incubated at 37°C / 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 Pseudomonadus aeruginose recorded the higher inhibition zone 13 mm at the concentration of 200 mg/ml followed by Klebsiella Pneumoniae 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

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<td>Escherichia coli</td>
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<td>Salmonella typhi</td>
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<td>Pseudomonas aeruginosa</td>
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Callus extract

Table (2) showed that the higher inhibition zone 7 mm 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 Pseudomonas aeruginosa recorded the highest inhibition zone 13 mm at the concentration of 200 mg/ml followed by Klebsiella Pneumoniae 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

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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 ulcerans* 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 *Condida 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 alderus* 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 polysaccharides, 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 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.

**References**


