

ANTIBACTERIAL ACTIVITY & ACUTE TOXICITY TEST (LD50) FOR ASPARAGUS OFFICINALIS, & APIUM GRAVEOLENS

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ABSTRACT

Medicinal plants have been used in traditional medicine for the treatment of infectious diseases. In the present study, the crude extract of seed of *Asparagus officinalis*, & *Apium graveolens* were studied -in vitro- for their antibacterial activity, & in vivo for their acute toxicity. Antibacterial sensitivity test disks were prepared from the aqueous extract of the test plants in concentrations 5, 10, 15%. LD50 were tested using 60 mice, which received oral doses of 250, 500, 1000, & 10000-mg/kg b.w. The results showed that *Asparagus officinalis*, & *Apium graveolens* had a zone of inhibition in concentration of 5%, which is dose-related effect, *Asparagus officinalis* was slightly toxic, while the seed of *Apium graveolens* was practically safe. The results support the traditional used of test plants. It can be concluded that both *Asparagus officinalis* & *Apium graveolens* can be used treatment of bacterial infection safely.

INTRODUCTION

The interest in the folk medicine is increasing at the present time, because many patients believed that such products are effective and less harmful. This trend stimulates our interest in the scientific investigations of medicinal plants that are used in Iraqi folk medicine for the treatment of bacterial diseases, to determinate the anti bacterial activity, and the numerous value of LD50, because there is no information concerning the toxicity of both. Among the list of medicinal plants *Asparagus officinalis*, & *Apium graveolens* were used (1,2).

Acute toxicity in animals: is the adverse effect occurred within a short time of oral administration of a single dose of a substance or multiple doses given within 24 hrs. In term of human exposure refers to life threading crises such as accidental over doses, suicide attempts. The numeric value of LD50 has been to classify, and compare acute toxicity among chemicals, but its only one of many reference points. (2)

MATERIALS & METHODS

Preparations of the test samples:

In Iraqi, these drugs are generally prepared from the plant materials by boiling in water on an open fire. In the current study the extracts were prepared by such method by adding 10 grams of dried plants to 100ml of boiling water. Then boiled for 3 min later, the resulted solutions were obtained. The solutions were dried using reduce- pressure. The solid materials were obtained, and weighted, then different concentrations were prepared (5%, 10%, & 15%) for the preparing the antibacterial disks, Then the solutions exposed to ultra violate radiation for 6 hours. (3)

Preparations of medicinal plants sensitivity disks:

Disks were prepared from No.1 Whatman filter paper; sterilized by dry heat for 60-min; the solutions were also prepared in concentrations of 5%, 10%, and 15%. Using micropipette, 0.01ml of each concentration were added for each disk. The disks kept in screw-capped bottles, and kept at 4°C (4).

Sensitivity test:

E. Coli was first cultured, identified in Basrah military hospital. Then *E. coli* tested against commercially available antibiotic disks: Ampicilline, Gentamicine, Methoprim, Naledexic acid, Nitrofurantin, and Refampicin. The sensitivity tests were done according to Kirby - Baurer method. All samples showed that Refampicin had a wider zone of inhibition, so Refampicin used as a control antibiotic disk. The second stage was using of medicinal plant disks with refampicin disks to test the anti bacterial activity and to compare such effect with effective drug, each concentration was tested for at least 3 times. (5, 6).

Preparation of animals for acute toxicity test:

Mice (n=60) of both sexes were used, the mean body weight for each group was 22-25 g. Mice divided into groups consisting of three males and three females, fasted for 24 hrs, using mesh to prevent coprophagy, only free-access drinking water was allowed. For each test plant 5 groups were used, one is control received only bidistilled water, the others received a single dose of 250, 500, 1000, & 10000 mg/kg b.w. of aqueous extract of the plants. (7)

RESULTS

1-Antibacterial effects:

Both plants showed a zone of inhibition to the culture of *E. coli* (15-20mm), as in figure 1&2. This inhibition effect is a-dose dependent, till reach to 25-30mm-with concentration 15%. In comparison with control antibiotic, there is a slight difference (inhibition zone of refampicin=4mm).

LD50 test

The results showed that no death had been occurred with all groups that received treatment as well as in control group. Only *Asparagus officinalis* in a dose of 10000 mg/kg b.w. induced death in 2 mice (33.3%).

Signs of toxicity:

Death occurred within 2 days. The animals showed depression, loss of appetite with signs of spasm. Recovery of survival animals occurred within 5-6 days.

P.M findings:

Congestion had been observed in the liver, kidneys, & lungs, with signs of bronchitis.

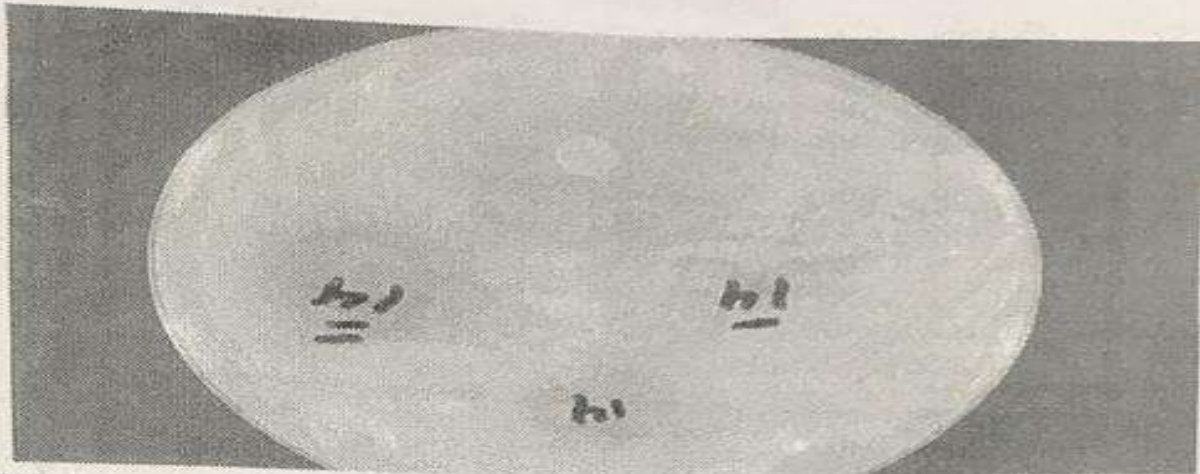


Fig.1: Showed the inhibition zone produced by using the *Asparagus officinalis*. 14⁻ indicate concentration 5%, 14^{''} indicate concentration 10%, 14^{'''} indicate concentration 15%,

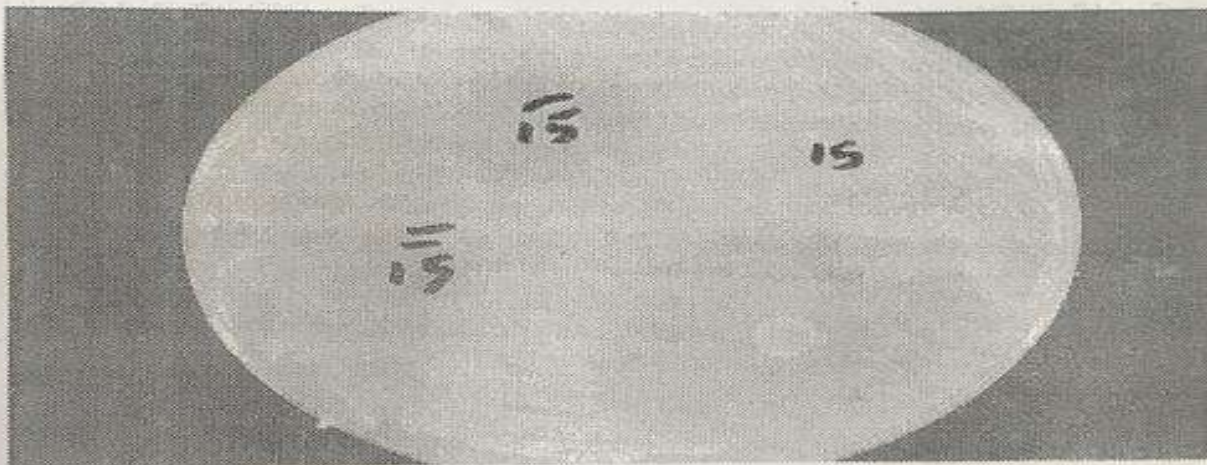


Fig.2: Showed the inhibition zone produced by using the seed of *Apium graveolens*. 15⁻ Indicate concentration 5%, 15^{''} indicate concentration 10%, 15^{'''} indicate concentration 15%,

DISCUSSION

In the folk medicine, the aqueous extracts should be taken orally with out any limitation as much as the patient could endure for a period of at least 15 days, and reach to 6 months. These applications suggest that there would be no toxic effects with the administration of those extracts. To the best of our knowledge, there is no very well documented information about the antibacterial, acute toxicity, the lower lethal dose, and chronic toxicity, which is an important data to start further studies in man. In the present study, the results indicate that *E.coli* sensitive to *Asparagus officinalis*, and to less extent *Apium graveolens*, also showed that *Asparagus officinalis* slightly toxic, while *Apium graveolens* was practically non toxic as in table one (8), according to approximate toxicity in everyday values.

LD50	Correlation to ingestion by a 150 pound adult man	Toxicity
0-50mg/kg	0-1tea spoon	Extreme
50-500mg/kg	1 Oz to 1 pt	Moderate
5-15 g/kg	1 pt-1qt	Slight
Over 15g/kg	More than 1qt	Practically non toxic

J.W.Trevaan first used the LD50 in 1927 (9), to determine the safety dose of Digitalis and insulin. In fact the safe dosage was later found using careful clinical trials. The median lethal dose test, dose not accurately measure the human health hazards, and is a very crude & imprecise, because it can not tell us about the non-lethal damage, and the results can be affected by the age, sex of the animals, their housing and nutritional conditions, temperture, time of the day and year. Most users, of a substance will need to judgment to assessed the reavelance to human toxicity data derived from animals including LD50 figures under European law. The Food & Drug administration (FDA) requires tests on animals that each ingredient in a cosmetic products be adequately substantiated for safety prior to marketing (10). It can be concluded that both *Asparagus officinalis* & *Apium graveolens* can be used treatment of bacterial infection safely.

دراسة الفاعلية المضادة للبكتيرية والسمية الحادة لنباتات الهليون وبذور الكرفس

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الخلاصة

تم استخدام النباتات الطبية في الطب الشعبي لعلاج الأمراض المعدية. وفي هذه الدراسة تم استخدام المستخلص المائي لنباتات الهليون (الاهليلج الأسود) وبذور الكرفس لدراسة فاعليتها -خارج الجسم- المضادة للبكتيرية، ودراسة السمية الحادة في داخل الجسم. وقد تم تحضير أقراص اختبار الحساسية من المستخلص المائي لنباتات المختبرة بتركيز (5، 10، 15%)، وتم استخدام 60 فاراً مختبرياً لدراسة نصف الجرعة القاتلة وذلك بإعطائها فمويًا 250، 500، 1000، 10000 ملغم/كغم من وزن الجسم. أشارت النتائج إلى وجود مناطق تثبيط بتركيز 5%، وهذا التثبيط معتمد على التركيز، وأن نبات الهليون ذو سمية خفيفة بينما بذور الكرفس عملياً غير سامة. وهذه النتائج تدعم الاستخدام الشعبي لهذه النباتات. ومن هذا يمكن الاستنتاج بأن النباتات المستخدمة في الدراسة يمكن استخدامها في علاج الإصابات البكتيرية بأمان.

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