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Effectiveness of aqueous and alcoholic extract of "Annona Squamosa" plant against some types of Gram positive and negative bacteria.

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ABSTRACT

The attention to plants and medicinal herbs has increased. It use as key sources for the production of medical drugs or as a source of effective materials that go into the composition of the drug. In order to determine the medical significance of *Annona Squamosa* plant and its anti-microbial activity against some types of Gram positive and negative bacteria by means measure the diameter of the inhibition zone of it by aqueous and alcoholic extract of each parts of fruit, where make water extract and methanol extract from pulp, shell and seed of the "apple honey". These bacteria were isolated from clinical samples of people whom Fallen asleep in Al-Hilla teaching hospital, Babylon during a period from October 2015 to January 2016. In the present study; the diagnosis of bacterial strains where after its culturing on suitable media and then the diagnosis was performed by using Vitek 2 system to identify species level of isolates strains. These strains were included; *Aeromonas hydrophila*, *Klebsiella pneumoniae*, *Escherichia coli*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella paratyphi*. In order to Detection antibacterial activity of aqueous and alcoholic extract of *Annona Squamosa*. The methanolic extracts of *Annona squamosa* have higher antimicrobial activity than aqueous extract seed and fruit of present plant, while the shell of the extract have no activity on studied bacteria. Also higher diameters of inhibition zone was to alcoholic extract against *Escherichia coli* were (26) mm at the concentration was (50) mg \ ml, While less diameters of inhibition zone was in *Klebsiella pneumoniae* 10 mm in the concentration (50) mg \ ml in of aqueous extract of the seed. From the result we concluded the *A. squamosa* extracts has beneficial effect on pathogenic bacteria. The methanol and water extracts of seed have better activity than other extracts that can be used to prevent enteric diseases.

Keywords: *Annona squamosa*, Antimicrobial activity, Agar disc diffusion method, ethanol, aqueous extract.

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INTRODUCTION

In recent years, the attention to plants and medicinal herbs has increased. It uses as key sources for the production of medical drugs or as a source of effective materials that go into the composition of the drug. It is also used as a raw material for the production of certain chemical compounds in the production of some pharmaceutical substances (1). In this research, both plant extract (aqueous and alcoholic extract) of *Annona squamosa* was studied as antimicrobial agents against some pathogenic bacteria. It has several names which are Guanabana fruit or fruit of a tree Graveola, cream in Egypt, Almstafl in Oman and other names such as Alcherimoya and Asersph (2). Every *A. squamosa* part possesses medicinal property (3). The root acts as a drastic purgative, while the leaves infusion is considered efficacious in children prolapsus ani, as well as crushed leaves are sniffed to overcome fainting spells and hysteria, in addition to its benefit to recover of ulcer and wounds. Roots are employed internally in depression of spirits and spinal diseases (4). Fruits are good tonic, used as expectorant, increases muscular strength. It's also for cooling, lessens burning sensation and tendency to biliousness; sedative to heart and relieves vomiting. Seed yields oil and resin which acts as detergent for hair wash after gram flour (5). The previous phytochemical investigations made on the *A. squamosa* plant have proved that they possess a wide variety of compounds like acetogenins, alkaloids, protein, amino acid, carbohydrate, glycosides, phytosterols, tannins and phenolic compounds (6). This may explain its diversity of curing diseases safely and well tolerated remedies compared to the conventional medicines. In this research, we experiment extract of this fruit (pulp and shell) as well as seed parts on some diseases bacteria which *E. coli*, *S. pneumoniae*, *K. pneumoniae* and *A. hydrophila*, *S. aureus*, *P. aeruginosa*, *S. paratyphi*. **Aim:** In order to determine the medical significance and antimicrobial activity of *Annona squamosa* against some types of Gram positive and negative bacteria.

MATERIALS AND METHODS

Preparation of plant sample:

A fresh sample of *A. squamosa* plant was collected from Al Bayaa area /Baghdad /Iraq and prepared with both type of extracts (Aqueous & Methanol extracts) according to (7).

Collection of Bacterial strains and diagnosis:

Different seven clinical microbial isolates Gram positive, Gram negative (*E. coli*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Aeromonas hydrophila*, *S. aureus*, *P. aeruginosa* and *S. paratyphi*) were isolated from different clinical samples. During period from January to Jun, 2016 and identified by using conventional biochemical tests and Vitek 2 system (Biomeraux, France), and cultivated in pure culture, at microbiological laboratory/college of Medicine / Babylon University.

Determination of antimicrobial activity of *A. squamosa*:

Measuring of inhibition zone was done according to (8) in which discs of 6 mm in diameter was prepared then sterilized by autoclaving and dried. The discs were then impregnated with extract of the plants. To assessment the antibacterial activities of plant extracts by disc diffusion method. The antimicrobial susceptibility test was done according to the standard method by Kirby-Bauer (9).

To inoculate Mueller Hinton agar plates evenly; a bacterial suspension adjusted to 0.5 McFarland standard (1.5×10^8 CFU/ml) was used by sterile swab. The discs impregnated with four concentrations of plant extracts (and were placed individually on the Mueller Hinton agar surface. The plate was then incubated at 37°C for 18 hours in inverted position to look for a circular area of clearing around the plant extract impregnated discs (zones of inhibition). The diameter of the inhibition zone of the disc was measured to the nearest millimeter and The experiment was carried out in triplicates to get rid of any error (10).

RESULT AND DISCUSSION

The results of antibacterial activity of seed, pulp and shell of *A. squamosa* against bacterial isolates with different pattern of inhibition zone (Figure 1, 2).

The aqueous extract and methanolic extracts of *Annonasquamosa* were studied; the result showed that the antimicrobial activity of methanolic extracts more than aqueous extract, due to the low concentrations of active substances which dissolved in water extracts compare with methanol extracts. Active substances were soluble in organic solvents and therefore, not present in water extracts(11). The seed of *Annonasquamosa* showed higher activity for all tested bacteria compared to the pulp and shell. On the other hand, the phytochemical studies of the extracts conclude that the methanol and water extracts of seed had good results contributed to alkaloids, oils, tannins, phenols and flavonoids (12) which are well known as an active chemical substance for antimicrobial activity that can be used to prevent enteric diseases. Moreover, the elevated of inhibition zone was revealed by the methanolic extract of *Annonasquamosa* seed against *E.coli* was 26mm/50µl and lowest for *K. pneumoniae* 10mm/50µl. Furthermore, these results were accordance with (13). Regarding, The fruit pulp water extract exhibit no any effect on all tested bacteria while the methanol extract of it showed a good anti-microbial effect since highest zone of inhibition was 18 mm/50 µl, while the lowest diameter of inhibition 11mm/50 µl. This result contributed to the fruit contains; flavanoids, saponins, tannins, carbohydrates, amino acids, terpenoids, and glycosides (14). The potential effect of fruit extract may be due to flavanoid in the fruit. The antimicrobial activity of fruit of *Annonasquamosa* due to the tannins present in its fruits. The active components of plant extract have antimicrobial activities against fungi, yeast and bacterial isolates without and side effect for human. The useful of plant extract as a treatment to reduce the bacterial resistance for antibiotics.

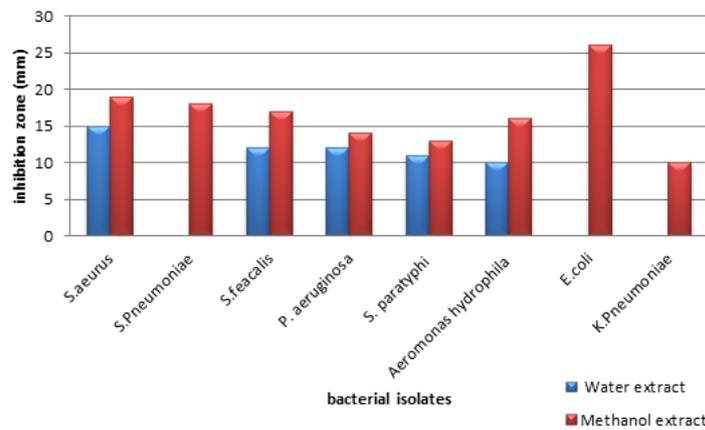


Figure 1: antibacterial activity of seed of *A. Squamosa* selection by agar disc diffusion against tested bacterial isolates

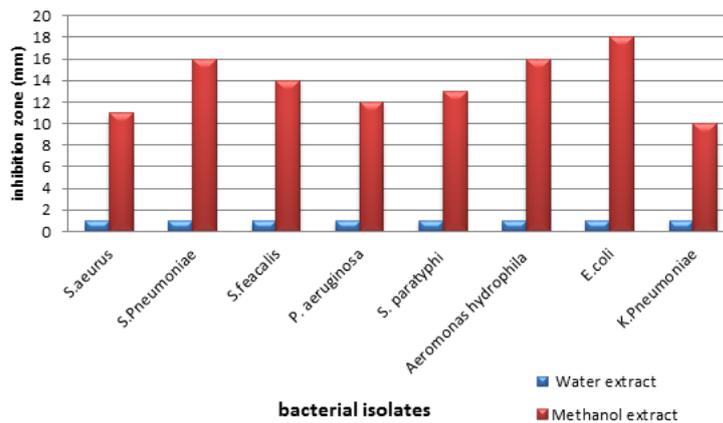


Figure 2: antibacterial activity of pulp of *A. Squamosa* selection by agar disc diffusion against tested bacterial isolates

CONCLUSION

From the above experiment, it was concluded that *A. squamosa* plant has beneficial effect on tested pathogens bacteria. The methanol extracts more effective than other extract, and can be used to prevent enteric diseases. The active components of plant extract have antimicrobial activities against bacterial

isolates without and side effect for human. The useful of plant extract as a treatment to reduce the bacterial resistance for antibiotics.

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