

Effect of Tannin Fruit Extracts on Some Bacterial Species

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Abstract :

Background: The results of the chemical detection showed that the active groups in the fruit extracts of the tannin plant contained glycosides, saponins, flavonoids, tannins, terpene and phenols.

Aim: To evaluate Tannins inhibition activity against *Escherichia coli* and *Klebsiella pneumoniae* .

Materials and methods: A number of aqueous, alcoholic and acetone fruit extracts of Tannins were prepared, and their effect on inhibition of *E.coli* and *K.pneumonia* was tested. Four concentrations were used [10, 20, 30, 40 (mg / ml)].

Results: The current study aims to determine the effect of the aqueous and alcoholic tannins plant on some bacterial species The results showed that the effect of aqueous and alcoholic

extracts against the species *E.coli* and *K. pneumoniae* The effectiveness of the biological agent increased with increasing concentration used, as it was found that the aqueous extract was highly effective, as the average diameter of inhibition reached 22.0mm for *K. pneumoniae* and was equal to the alcoholic extract for bacteria. The concentration was 40 mg/ml compared to the antibiotic in which the average diameter of inhibition was 5.14 mm. While the effect of the alcoholic extract was very high compared to the aqueous extract and the antibiotic, as it reached 24.0 and 27.6mm at concentrations of 30 and 40 mg/ml, while the diameter of inhibition for *E.coli* reached the average diameter of inhibition for bacteria. For the same bacteria, 17.3 and 20 mm at concentrations of 30 and 40 mg/ml for the aqueous extract.

Conclusion: Tannin extracts show antibacterial activity against *Escherichia coli* and *Klebsiella pneumoniae*.

Keywords: Tannin fruit , *E.coli* , *K. pneumonia*, Glycosides.

Introduction :

Plant extracts have been utilized as supplements in animal feeding due to their antibacterial activities. These extracts have shown potential to inhibit the pathogenic effects of various unwanted intestinal bacteria, thereby improving animal health [1,2]. Consequently, numerous studies have investigated the phytochemical composition, biological activities, and antimicrobial properties of these plant extracts [3].

Tannins act directly against cellular organelles and within the cell membranes of various microorganisms ,inhibiting their growth [4] .Recent studies indicate that tannins present great potential in binding proteins and adhesions, interfering directly in the substrate availability necessary for bacterial metabolism and growth [5].

The percentage of tannins present in the plants, however, varies. While they are present in significant proportions in some plants, many others have too little of them.[6] Tannins are usually found in large quantities in the bark of trees where they act as a barrier for microorganisms and protect the tree. Apart from tanning, tannins are also used in dyeing, photography, refining beer and wine as well as an astringent in medicines[7,8].

Antibiotics provide the main basis for treating infection with germs (bacterial). Since the discovery of these antibiotics and their use as chemotherapeutic agents, there has been a belief among scientists that this will lead to the final eradication of infectious diseases, but

the excessive use of antibiotics has become a “major” factor in the emergence of and the spread of strains of microorganisms that are resistant to a number of antibiotics [9].

In recent years, there has been interest in medicinal plants and the healing secrets they contain, and many countries, most notably the United States, have given great interest to specialized plants and herbs that are exclusively natural sources of medicines and skilled treatments for diseases [10,11] They have also made diligent efforts and continuous attempts on both sides to investigate the stimulating effect of many of medicinal herbs and place a variety of diverse things "short for germs and then mastered as a treatment that donates the germ, this is a continuous continuation in separating the wonderful efforts and learning about their activity [12]. Thus this study was conducted to evaluate its inhibition activity against on some bacterial species .

Materials and Methods :-

Preparation of Plant Extracts

Three types of solvents were used, distilled water, 95% alcohol. Forty gram of plant form was mixed with 160 ml of distilled water, and the mixture was stirred by a shaker. Leave the mixture in the refrigerator to soak for 24 hours. It was then filtered through several layers of gauze and filtered again using filter papers (Whatman No1) to get rid of the unpulverized plant parts and remaining fibers. Then the extract was placed in the oven at a temperature of 40 °C until all the water evaporated and the extract remained in the base of beaker.[13] Then the extracts, after drying, were placed in glass vials with tightly closed lids and kept in the freezer until use.

Preparation of Alcoholic Extract

The alcoholic extract was prepared in the same way as in the preparation of the previous aqueous extract, except for replacing the distilled water with ethyl alcohol at a concentration of 95% [13].

Concentrations used in the study:

Concentrations were used (10,20,30,40) in mg/ml.

Sterilization of extracts and preparation of dilutions:

Prepare a stocking solution by taking 1 gm of dry plant extract powder and dissolving it in 10 ml of sterile distilled water. The concentration of the storage solution became 100 mg / ml. Sterilize the solution by filtration using sterile filter papers (Whatman No 1) to get rid of contaminants and obtain a sterile storage solution. And used as a source for the preparation of

fears.[14]. The method of diffusion in the pits was performed as described before [15], as follows:

- 1- Pour (25) ml of the Mueller Hinton agar into each plate.
- 2- Inoculated the feeding acres by spreading (0.1) ml by means of a sterile spreader from the bacteria culture containing (1.5 x 10⁸) cells/ml, by comparing it with the standard stable turbidity solution, then the plates were left to dry at room temperature.
- 3- A hole with a diameter of (5) mm was made in the culture medium with a sterile cork (borer)
- 4- An amount of (0.2) ml of graduated concentrations prepared for plant extracts was added using a micropipette. A positive control hole was made by adding anti-nystatin 30 mg / ml.
- 5- Three replications were made for each plate, then the plates were incubated at a temperature of (37) C for a period of (24) hours, in a manner that determined the effectiveness of each concentration of the extract by measuring the inhibition zone.

Statistical analysis

The results of the study were analyzed statistically according to the Duncan polynomial test at a probability level of 5%, and this was implemented using the Excel program in the electronic calculator

Result and Discussion :-

Effect of Tannins fruit extract against bacterial species

The effect of Tannins fruit extract against bacterial species Effect of aqueous extract of tannins fruits against bacterial species. The results showed that the effect of the aqueous extract depends on the concentration of the extract as well as on the types of bacteria, as in the Table (1).

The aqueous extract showed higher inhibitory activity compared to the antibiotic on *K. pneumoniae* bacteria. The average diameter of inhibition for these bacteria was 9.5 and 12.5 mm, respectively, at concentrations of 30 and 40 mg/ml, while the extract showed good inhibitory activity compared to the antibiotic on *E. coli* bacteria. The average diameter of inhibition was (11.16 and 14.5) mm, respectively, at concentrations of 10 and 20 mg/ml, and increased to (17.33 and 20) mm, respectively, at concentrations of 30 and 40 mg/ml.

Table.1. Effect of aqueous extract of Tannins fruits against isolated bacterial species

Bacterial species	Concentrations used (mg/ml)				Positive control	Negative control
	10	20	30	40		
<i>E.coli</i>	11.6E	14.5CD	17.3CB	20.0BA	00.0 F	22.5A
<i>K.pneumonia</i>	9.1ED	12.6 ED	20.3BA	22.0A	00.0 F	14.5CD

There were a significant differences according to Duncan's test [$P < 0.05$] in the effect of the aqueous extract of tannin fruits between the tested bacterial isolates.

These results were consistent with others [16] in Nigeria, that the aqueous extract of the plants *Alstoniaboonei* and *Morindalucida* gave a high inhibitory activity against two types of gram-positive bacteria and five types of gram-negative bacteria, which included *Staph.aureus*, *B. subtilis*, and *K. pneumoniae*, *E.coli*, *Ps.aeruginosa*, and *Flavobacterium*. Also the present study finding agree with other study [17] in Brazil which reported the aqueous extract of 16 Amazon forest plants shows inhibitory activity against gram-positive species, but did not show inhibitory activity against gram-negative species, which included *E.coli*, *Ps.aeruginosa*, and *E. faecalis*. Additionally, this study findings agree with other [18] in India, who indicated that the aqueous extract of the *Caesalpiniamapulsherrima* plant showed high inhibitory activity against bacterial species that included *Staph.aureus*, *B. subtilis*, *E.coli*, *K. pneumoniae*, and *E. aerogenes*. Also our findings agreed with other studies [19, 20] that indicated that the aqueous extract of turmeric rhizomes has good inhibition of the growth of *E.coli*, *Staph.aureus*, and *K. pneumonia* bacteria.

The reason for the efficiency of the aqueous extract may be due to the nature of the active compounds present in it, especially phenols, flavones, glycosides, and soaps [21, 22] It is known that these compounds have the ability to dissolve in water when used in extraction.[23,24]

The effect of the alcoholic extract of the Tannins fruit against bacterial species.

The alcoholic extract of Tannins fruit showed very high effectiveness against both *E. Coli* and *K.pneumonia* bacteria. Comparing with radiation. As for chloramphene, as in Table (2) the infection rate in *E.coli* bacteria reached (11.5 and 14.0) mm, respectively, at concentrations of 10 and 20 mg/ml.

As for the *K. Pneumonia* bacteria, the average hair diameter reached (12.6 and 17.3) ml on the toilet at concentrations of 10 and 20 mg/ml, while it reached (19.33 and 22.0) ml on the toilet at concentrations of 30 and 40 mm/ml .

Table (2) Effect of alcoholic extract of Tannins fruits against isolated bacterial species

Bacterial species	Concentrations used (mg/ml)				Positive control	Negative control
	10	20	30	40		
<i>E.coli</i>	11.5E	19.0CB	24.0 BA	27.6A	00.0F	22.5B
<i>K.pneumonia</i>	12.6DE	17.3CD	19.8CB	22.0B	00.0F	14.5D

There were a significant differences according to Duncan's test [$P < 0.05$] in the effect of the alcoholic extract of tannin fruits between the tested bacterial isolates.

These results were consistent with others finding [25] that the alcoholic extract of tannins has a high inhibitory activity against a number of Gram-negative and positive bacteria. They also proved that the alcoholic extract of tannins has a very high effectiveness in inhibiting the production of enzymes and toxins in bacterial species and fungi as a result of inhibiting the process of protein synthesis and deposition. These results are consistent with what reported by others [26], who indicated that the *Holarrenapubescens* extract was effective against both Gram-positive and Gram-negative species. In addition, the present study findings agrees with Kelmanson et al [27], they found that the alcoholic extract of *Thymus vulgaris*, black pepper *Capsicum annum*, and nettle *Urticadioico* showed high inhibitory activity on Gram-positive and Gram-negative bacteria species. Our findings agreed with a Khan and Khan (2002) study conducted in India, who found that the alcoholic extracts of the *Clerodendruminerme* plant showed good inhibition against 15 out of 18 gram-positive and gram-negative bacteria isolates.

The present study findings also agreed with that of Pepeljnjak [29] in Croatia as they found that the dried leaf extract of *Pelargonium radula* showed high inhibitory activity against Gram-positive and Gram-negative bacteria species, which included *Ps.aeruginosa*, *E.coli*, *K.pneumonia*, *E. faecalis*, and *B. pumilus*. Nwaogu eta al [30] in a study conducted in Nigeria, shows that the alcoholic extract of the leaves of the *Landolphiaowariensis* plant showed good inhibitory activity against bacteria, *Klebsiella spp.*, *E.coli*, *Proteus sp.*

Previous studies indicated that green tea has an inhibitory effect on the growth of Gram-negative and Gram-positive bacteria, as its alcoholic extract inhibits the growth of *Klebsiella species*, *Shigella species* *E.coli* bacteria [31,32].

From the above, we note that the alcoholic extract of the fruits of the tannin plant had a higher inhibitory effect than the aqueous extract, and this result is supported by many studies, as the compounds that have antimicrobial activity are mostly poorly soluble in water and well soluble in ethanol, such as phenolic compounds and aldehydes and flavonoids [33,34] , the active substances are more concentrated in the alcoholic extract than in the aqueous extract [35].

Conclusion

It was concluded from the study that the higher the concentration used, the greater the diameter of inhibition against bacterial species. The alcoholic extract of the fruits of the tannin plant comes in first place in inhibiting *staph. Aureus* bacteria, and the aqueous extract comes second in antibacterial activity. While the aqueous extract of the fruits of the tannin plant comes in first rank in inhibiting *E.coli* bacterial, and the alcoholic extract comes in second place .

ETHICAL APPROVAL

The research protocol was approved by the Ethical Committee of Kirkuk Education Authority.

HUMAN AND ANIMAL RIGHTS

The study not included human and animal.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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