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# The Effect of Aqueous and Alcoholic Extract of Ziziphus Spina-christi on some Bacterial Isolates Causing Gingivitis

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## Abstract

Bacterial gingivitis is a most common and mild form of periodontal disease that causes bleeding, redness and swelling (inflammation) of gingiva, around the cervical area of teeth. Untreated gingivitis can progress to periodontal disease, periodontitis, by spreading to supporting tissue of teeth involving bone and periodontal ligament, a much more serious condition that can lead to tooth loss. The study was conducted to investigate the bacteria causing gingivitis with an attempt to investigate the bacterial inhibitory effect of seder leaves extract. Samples consist from (230) gingivitis swab were collected from the patients which are admitted to dental clinics in Tikrit city , among the period extended from March 2016 to February 2017 .Cultural, microscopical and biochemical tests were performed to identify the bacteria causing gingivitis .The results showed 165positive heavy culture of causative bacteria as follows :Gram positive ; Streptococcus mutans (% 18.78 ) , Streptococcus pyogens (% 8.48 ), Streptococcus salivarius (% 6.06 ) , Streptococcus angiosus (% 4.84 ) ,Streptococcus pneumoniae (%3.03 ), Staphylococcus aureus (% 16.96 ), Staphylococcus epidermidis (% 9.09 ) ,while gram negative included Pseudomonas aeruginosa (% 13.93),Escherichia coli (% 9.69 ) ,Klebsiella .spp (%6.66) , and finallyPseudomonas monilli (%2.42).The present study showed that the aqueousand alcoholic extract of Ziziphus spina-christi leaves had inhibitory activity agiants the bacterial species . At the concentration of (200 mg/ml) of seder leaves extract showed the highest effect on bacteria . All the types of bacteria that isolated from the patients with gingivitis had revealed disproportionately sensitivity against antibiotics : Amikacin , ampicillin , tetracycline , gentamicin , chlirompheniсol , nalidixic acid , amoxicillin , trimethoprim .The mixture of aqueous extract of seder leaf and antibiotic Gentamicin , Amikacin and amoxicillin revealed high inhibitory synergetic effect against aginst resistant bacteria.

## Introduction:

Gingivitis is the most common form of periodontal disease that causes inflammation of the gingiva by the bacterial biofilms and the plaques on the surface of the teeth. The dental plaque is the main cause of periodontal disease. It is necessary to remove the alkaline accumulations continuously as they are considered collection of the bacteria causing the infection. Larg number of studies had been conducted on the effect of microorganisms on teeth and gingiva. These studies had shown that Streptococcus. Speciesare mostly responsible for inflammation and dental carries, as well as the emplication of other types of bacteria. Some studies had also found that cranberry and sugary foods help to bind the bacterium to the tooth and the gingiva wall [(1)]. The use of antibiotics such as ampicllin, clindamymcin, tetracycline, kanamycin, trimethaprim and erythromycin have shown an importance in the treatment of bacterial infections, including gingivitis for many years. However, there have been many problems in usingthem continuously because of the emergence of resistant strains, bacterialpossess two types of resistance; natural resistance and acquired resistance. Gram-negative bacteria have a natural resistance to a large number of high-effective and effective antibodies in gram-positive bacteria. This resistance is due to the presence of the multi-sugar layer lipopolysachrrides in the cell wall of gram negative bacteria that inhibit the permeability of inhibitory concentrations of antibodies into the bacterial cell [(2)]. The problem is the acquired resistance, which has been increasing rapidly in recent years [(3)] Plant extracts in treatment, characterized by their effectiveness and accessibility as well as being inexpensive and had no polluting environment [(4).] Ziziphus spina-christi is an evergreen tree or plant native to northern and tropical Africa, Southernand Western Asia. It is native to the regions Levant, East Africa and some tropical countries. It was called Seder (associated with the Lote-trees of the Quran). The most important natural plants in our world today is called nativly the Seder plant. It is used in many therapeutic uses. It is used in treatment of headaches, fever, stomach ache, purifying the intestines and skin, strengthening and using its leaves in the treatment of painful joints, malaria and immunodeficiency Syndrome as well as its use in the treatment of gastric pain [(5)]. Seder also has a high efficacy against the growth of fungi and bacteria. It has an inhibitory effect on the growth of many positive and negative bacteria. Also, the extract of Seder leaves has a high efficiency against the growth of positive bacteria [(6)]. For the purpose of using extracts of this plant in the medical field and the possibility of using them in the treatment of many diseases caused by these ,this study aimed at the inhibitory effect of seder leafs extract on bacterial isolates isolated from gingivitis.

## Materials and Methods:

1- Collection and examination of samples

A total of 230 swabs were collected using sterile cotton swabs after the diagnosis was made by the specialized physician from our specialized dental clinics in Tikrit city of those patients attending the center, within a period extended from March 2016 to February 2017. The swabs were transferred to the laboratory of the Faculty of Dentistry for culturing on MacConkey and blood agars. Then the dishes were incubated at 37 ° C for 24 hrs. Developing colonies were diagnosed based on the macroscopical and microscopical characteristics of the colonies and also the biochemical characteristics mentioned in [(7)].

2- Collection of plant samples:

The leaves of the Seder plant were collected from the public parks in Tikrit city, after that cleaning the leaves from the dust by the tap water was peformed. The leaves were placed in the shade to dry and then crushed to be powdered. The powder

was placed in clean dry nylon bags in the refrigerator for the antimicrobial purposes

.

3- Preparation of the cold water extract of the seder plant:

About 20 g of dry powder were taken and mixed with 400 ml of distilled water by using the electric mixer and left for 24 hr. at room temperature. After filtering with several layers of medical gauze the mixture was centrifuged at 3000 rpm / (10 min) and then filtered by useing of 0.1 Whatman no. and dried in over at temperature of 40°C then refrigerated until use [8] .

4- Preparation of the alcoholic extract of the seder plant:

In this extraction Ladd and others method (1978) was followed by taking 20 g of dry matter powder and then extracting the substance by utilizing Saxholate byusing 400 mL of ethyl alcohol (95%) for 24 hours. After drying the extract in Electric oven at a temperature of 40 ° C.

5- Preparation of reagents for secondary chemical compounds
The following reagents such as Alkaloides reagents, Mayers reagent, Phenoles reagents, Leadacetate reagents, and Terpenoid Reagents were prepared in order to detect the chemical substances in cold water and ethyl alcohol extracts of Seder [9].

6- Preparation of different concentrations of plant extracts:

For the preparation of stock solution of water extract (2) g of the powder was taken and dissolved in (10) ml sterile distilled water (as in previous section), so we have a storage stock solution at a concentration of (200) mg / ml. This solution was used as a source of concentration (200, 100) mg / mL. While in alcohol extract 2 gm was taken and dissolvedin 3 mL ethyl alcohol and completed to 10 mL with distilled water. The solution concentration was 200 mg / ml. The preparation of 50, 100 and 200 mg/ml was performed.

**Preparation of bacterial suspension:**
Nutrient broth was prepared according to the instructions of the company (Himedia India) and distributed in test tubes of 5 ml per tube and then inoculated with 3 bacterial colonies growing on the nutrient agar medium at 24 hours. Then incubated for 18 hrs. At 37 C.By McFarland the concentration kept in diluted suspension at (1.5 × 10 8) cell / ml, and saved at 4 C until use [10].

**Bacterial Inhibitory effect of plant extract:**
Agar diffusion method was followed by preparing wells [11]. The method includes by cutting wells equal dimensions in Muller Hinton agar dishes according to the instructions of the company (Himedia / India) with 6 mm in diameter. About 0.2 ml of each concentration of the water and alcohol extract was added to 3 wells and the fourth one was left for controle by placing 0.2 ml of distilled water. Then the dishes incubated at 37 ° C for 24 hours and the results were read by measuring the inhibitory effect by the ruler [12].

**Testing the sensitivity of bacteria to antibiotics:**
According to the, the standard method of Bauer and his group (1966) was followed by utilizing the different standard antibiotic discs (Kirby-Bauer method) [13]. Eight antibiotics were used for this purpose. Meanwhile, the prepared bacteria was inoculated and distributed in the plates and by a sterile forceps antibiotic discs were placed and gently pressed on the agar. The plates were incubated overnight at 37 C resistant and sensitive bacteria were identified by measuring the inhibition zone of bacterial growth.

## Results:

In the course of the present study 11 species of bacteria causing gingivitis were isolated , belonging to two genera of gram positive bacteria (67.26 %), as well as types of gram negative bacteria (32.72

%).A Gram positive Streptococci recorded the highest frequently isolated genera with a high rate of more than (40 %) of bacteria with five types as follows ; Streptococcus mutans (18.78 %), Streptococcus pyogens (8.48 %)Streptococcus salivarius (6.06 %), Streptococcus angiosus (4.48 %) Streptococcus pneumoniae (3.03 %), all Followed by two types of Staphylococcus sp , Staphylococcus aureus (16.96 %), Staphylococcus epidermidis (9.09 %), While, The gram-negative types were Pseudomonas (Ps) aeruginosa with the highest rate of 13.93 %, E.coli (9.69 %) followed by Klebsiella sp (6.66 %) and the lowest proportion were Ps .monilli which scored 3.70 %. Table (1) Frequency of Bacterial species isolated from patients with gingivitis. Table (2) shows that the effect of the plant extract varies according to the concentrations used. The effect of the concentration started with 50 mg / ml. The most significant effect was the concentration of 200 mg / ml on E. coli and Streptococcus mutans, followed by the concentration of 100 mg / ml on different bacteria. The least effect achieved with 50 mg/ml in both water and alcoholic extract. The results showed in Table (3) indicate that the gram-positive bacterial isolates showing a difference in their resistance pattern to the tested antibiotics under study. The highest resistance was expressed by gram-positive bacteria to the ampicillin while the highest sensitivity was toward amikacin antibiotic.

On the other hand, Gram-negative bacteria was the highest resistance to tetracycline, while the least resistance to amikacin based on the measurement of inhibition zones The study showed that non-effective concentration (50 mg/ml) of gentamicin and amikacin in combination with the waterextract of Seder leaves, in its, had an effect on bacteria causing gingivitis and showed the highest inhibition diameter recording12 mm and even 13 mm inhibition zone against moderately resistant isolates.which is higher than the diameters indicated in the sensitivity test by using sole antibiotic. Synergistic effect between antibiotic and plant extract was remarkably obviousespecially with gentamicin amikacin, and amoxicillin Table (4).

## Discussion

The present results demonstrated that Streptococcus species are common isolated bacteria because they play an important role in dental carries and they are called cariogenic bacteria also playing the pivotal role opportunistic infections caused by the other group of gram-positive bacteria represented by Staphylococci because of their presence [(14)]. The reason for their spread in the mouth is because they have fast and easy resistance mechanisms through plasmids through conjugation, transitions, surface antigens, lytic and hydrolitic enzyme that helps to penetrate body tissues as well as in cluster bacteria [(15)]. The Gram-negative bacteria were less than gram-positive bacteria. According to several studies, including Waltimo and his group (1997). Most gram-negative bacteria came from respiratory infections or gastrointestinal tract, which appeared in the mouth. The study was conducted by [(16-19)]. The results showed that gram positive isolates gave a significant difference in their susceptibility to antimicrobial agents, which may be due to the production of beta-lactase enzymes or other mechanisms of antibiotic resistance. The genes that have been chromosomally encoded on plasmids [(20)], or bacterial resistance may be attributed to one of these three [(21)]. The cause of the resistance may be due to increased random use of antibiotics, resulting in resistance caused by bacteria due to the use of sub-therapeutic doses or the arbitrary usage of antibiotic, resulting in the formation of mutant isolates [(22, 23)]. The results of the preliminary chemical detection revealed that the seder plant contains many active ingredients, the most important of which are quinoids, including spinanina, jujube, which is responsible for antiviral activity (Al-Abed, 2008). It also contains flavonoids of various types including anti-oxidant classics, phenols, sapphones, pectin, fat and tannic acid Zidic acid acid, tannins, and turbines are consistent with the study conducted. As for the nature of the extracts, it was characterized by viscous strength, dark green color and aromatic aroma. The appearance of green

color is attributed to the color of chlorophyll and zantin [24]. The aromatic aroma of seder can be attributed to the seder containing volatile oils [25]. It also contains mucus, vegetable gel and glue. The results showed that all types of bacteria were sensitive to the water and alcohol extract of the plant and through Table (1,2,3,4) through the apparent contrast of the concentrations used to influence the growth of isolated bacteria until the maximum effect at the concentration of 200 mg /ml. It was noticed that the increase in concentration had an effect on the increase of inhibitory effect in the growth of these bacteria. The highest inhibitory effect was observed at the concentration of 200 mg / ml of the extract of the bacteria in Stretococcsus mutans (14 mm) and the lowest effect was at 100 mg /ml of water extract in bacteria Klebsiella sp. As it reached (8) mm. The effect of the extract on the permeability of the cell membrane and the work of the bacterial cell can be increased. The effectiveness of the seder extracts is attributed to the presence of phenols that have inhibitory effect on positive and negative chromosomes. The results showed that the tested bacteria were sensitive to the water and alcoholic extractof seder leaves and the effect may be larger or similar when compared to the sensitivity of antibiotics. This can be explained either because these bacteria never faced these extracts before and therefore could not resist them or on the basis that the extracted materials have a chemical affinity to interact with Cell components or the presence of special receptors on the bacterial cell wall and appropriate vectors that move their molecules into the cell to stop the action of helper enzymes and other effective biologic molecules) [26]. The combination of gentamicin and the water extract of cloves showed a synergistic inhibition against the test bacteria especialy against the oral Streptococci.

## Recommendations

The study recommends the extraction of volatile oils and their chemical data to identify the effective compound, as well as a subsequent study to test the effectiveness of these oils against other types of bacteria that affect humans and thus use as an alternative to antibiotics.

Table (1): Frequency of Bacterial species isolated from patients with gingivitis.

| | **Percentage (%)** | **No. of Isolates** | **Gram Positive bacteria** |
|---|---|---|---|
| **1** | 18.78% | 31 | *Streptococcus mutans* |
| **2** | 8.48% | 14 | *Streptococcus pyogenes* |
| **3** | 6.06 | 10 | *Streptococcus salivarius* |
| **4** | 4.84 | 8 | *Streptococcus angiosus* |
| **5** | 3.03 | 5 | *Streptococcus pneumonia* |
| **6** | 16.96 | 28 | *Staphylococcus aureus* |
| **7** | 9.09 | 15 | *Staphylococcus epidermidis* |
| **8** | 67.26 | 111 | Total No. |
| | **Percentage** | **No. of Isolates** | **Gram negative bacteria** |
| **9** | 13.93 | 23 | *Pseudomonas aeroginosa* |
| **10** | 9.69 | 16 | *Escherichia coli* |
| **11** | 6.66 | 11 | *Klebsiella sp* |
| **12** | 2.42 | 4 | *Pseudomonas monilli* |
| **13** | 32.72 | 54 | Total No. |

Table (2): The effect of the plant extract varies according to the concentrations used.

| Diameter of inhibition zone | | | | | | | Extract Conc. | |
|---|---|---|---|---|---|---|---|---|
| 2% | | 100% | | 50% | | 25% | | |
| 12 | 13* | 8** | 9* | 1** | 1* | _ | *Streptococcus mutans* | 1 |
| 14 | 14 | 10 | 9 | 3 | 2 | _ | *Streptococcus pyogenes* | 2 |
| 12 | 14 | 7 | 9 | 1 | 1 | _ | *Streptococcus salivarius* | 3 |
| 13 | 15 | 10 | 7 | 1 | 1 | _ | *Streptococcus angiosus* | 4 |
| 10 | 11 | 7 | 8 | 4 | 2 | _ | *Streptococcus pneumoniae* | 5 |
| 9 | 11 | 5 | 8 | _ | 1 | _ | *Staphylococcus aureus* | 6 |
| 12 | 13 | 5 | 7 | 3 | 1 | _ | *Staphylococcus epidermidis* | 7 |
| 11 | 10 | 6 | 6 | 3 | 2 | - | *Escherichia coli* | 8 |
| 10 | 10 | 7 | 8 | 2 | 1 | _ | *Klebsiella sp* | 9 |
| 9 | 11 | 6 | 8 | 2 | 1 | _ | *Pseudomonas aeroginosa* | 10 |
| 11 | 10 | 6 | 7 | 3 | 1 | _ | *Pseudomonas monilli* | 11 |

*The inhibition zone measured by mm with alcoholic extract.

** The inhibition zone measured by mm with water extract.

Table (3): Antibiotic susceptibility testing of the bacterial isolates isolated from gingivitis.

| Ps monilli (4) (2)S (2)R | Ps aerogi nosa (22) (14)S (9)R | Klebsiell a (11) (10)S (1)R | E. col i ) 16 ( )S (9 )R (7 | Staph Epider midis (15) (14)S (1)R | Strep Aure us (28) 19)S ( (9)R | Strep Pneum onia (5) (4)S (1)R | Strep Angio sus (8) (7)S (1)R | Strep Saliva rius (10) S(9) (1)R | Stre p pyo gen es (14) S (10) R (2) | Str pm uta ns No. 31) ( )S (17 )R (14 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 23 | 25 | 22 | 24 | 22 | 24 | 26 | 23 | 21 | 22 | 22 | **Amikacin** |
| - | - | - | - | 8 | 9 | 9 | 7 | - | 9 | - | **Ampicillin** |
| 22 | 23 | 25 | 20 | 22 | 23 | 12 | 23 | 21 | 20 | 18 | **Nalidixic acid** |
| 21 | 22 | 21 | 21 | 23 | 22 | 25 | 21 | 22 | 20 | 13 | **gentamicin** |
| 19 | 1 | 17 | 15 | 10 | 12 | 13 | 17 | 18 | 13 | 12 | **Amoxicillin** |
| 22 | 21 | 19 | 18 | 15 | 13 | 19 | 15 | 13 | 20 | 18 | **Chloromphenicol** |
| 11 | 9 | 11 | 10 | 18 | 15 | 13 | 15 | 10 | 12 | 13 | **Tetracyclin** |
| 13 | 22 | 21 | 20 | 15 | 18 | 22 | 17 | 21 | 19 | 22 | **Trimethoprim** |

No : number of isolates S ; Sensitive Isolates R: Resistant isolates

Table (4): The effect of sub inhibitory concentration of seder leaf water extract in combination with antibiotics against moderately resistant bacteria.

| **Ps aeroginosa*** | **E.coli *** | **Strep Aureus *** | **Strep Pneumonia *** | **Strep pyogenes*** | **Strp mutans *** | Resistant Isolates / Antibiotic type ** |
|---|---|---|---|---|---|---|
| 2 | 5 | | 11 | 11 | 7 | Ampicillin** |
| 8 | 11 | 9 | 8 | 9 | 12 | Gentamicin** |
| 5 | 6 | 6 | 12 | 10 | 13 | Amoxicillin** |
| | 7 | | 5 | 4 | 12 | Tetracyclin** |
| 7 | 14 | 11 | 9 | 9 | 12 | Amikacin** |

*Moderatly resistant isolates

**Antibiotics in combination with non-effective 50 mg/ml of seder leaves water extract.

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