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Determination of Inhibition Activity for Arak and Cloves Against Aerobic Bacterial Isolates from Patients of Dental Caries and Gingivitis

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ABSTRACT

One hundred and nine samples were collected from General Hospital and some outpatient dental clinics in Al-Shirqat city through the period from 10/10/2018 to 20/3/2019 to determine the bacterial species causing dental caries and gingivitis by culturing on culture media, it was diagnosed phenotypically and biochemically as well as modern methods using Vitek-2 compact system technique. The species isolated as were: *Staphylococcus epidermidis* (51.37%), *Streptococcus mutans* (19.26%), *Staphylococcus aureus* (12.84%), *Lactobacillus acidophilus* (7.33%), *Escherichia coli* (5.50%), *Streptococcus pneumoniae* (3.66%). The sensitivity of isolates was tested for fourteen antibiotics. The plant extracts were more effective than antibiotics, especially at 100 mg / ml. Not all isolates were affected by 10 mg / ml.

Introduction

Dental caries has been the focus of attention for researchers and dental professionals, because it poses very serious difficulties for specialists, in addition to causing more severe pain than other infectious diseases, as well as it remains the main factor responsible for the loss of most teeth at all ages no other etiology [1]. The oral cavity is endemic in a plethora of diverse pathogens that cause various diseases of the mouth [2], where recent estimates show that the oral cavity contains more than 700 different bacterial species, many of which are unknown [3].

The global problem of oral disease persists despite progress and improvement in the treatment of oral problems in several countries and the World Health Organization has recommended that a lack of attention to oral health can have a significant impact on public health and that there are several diseases in the mouth related to chronic diseases. Tooth decay is known as a local breakage of hard and sensitive tooth tissues due to acidic products resulting from bacterial fermentation of carbohydrates in food, a chronic disease that progresses slowly in most people, resulting from an environmental imbalance between dental minerals and the biofilm of the mouth called "plaque" which is distinguished by microbial activity resulting in fluctuations in the plate pH due to bacterial production of acids as well as the buffer action of saliva and the synthesis of the surrounding tooth. As a result, the tooth surface is in a dynamic equilibrium with the surrounding environment [4]. Caries lead to gingivitis is the lightest type of mouth infections can be treated with daily cleaning of teeth and the use of medical floss and regular cleaning by the dentist in addition to the use of appropriate medicines and thus reduce the infection, in this disease is not losing tissue or alveolar bone supporting the tooth so it is necessary to treat gingivitis as soon as possible to prevent advanced periodontal disease, periodontitis [5].

Periodontitis is a highly prevalent chronic inflammatory disease that results in soft tissue pockets between the gums and teeth, loss of connective tissue and eventual bone destruction, a leading cause of tooth loss in adults [6]. Many patients are increasingly using medicinal plants and herbs for health purposes, so scientific scrutiny of their therapeutic potential, biologic properties and safety of use will encourage their proper use. Recent references have indicated that plants produce many secondary metabolites manufactured biologically and derived from primary metabolites and are a source of many drugs and the effects of medicinal plants in preventing and treating many diseases are largely

attributed to their antioxidants, substances that prevent ,eliminate or delay oxidative damage of the target cell, so these antioxidants may control the level of free radicals to repel oxidative damage [7].

Most bacterial pathogens became resistant to antibiotics and this led to the failure to treat bacterial infections with antibiotics, which necessitated the use of medical alternatives to treat these infections [8].

Materials and Methods

Samples collection:

One hundred and nine samples were collected from General Hospital and some outpatient dental clinics in Al-Shirqat city through the period from 10/10/2018 to 20/3/2019 to determine the bacterial species causing dental caries and gingivitis.

Three culture media was used in current study which MacConkey agar, blood agar and mannitol salt agar.

Identification

Bacterial isolates were identified based on morphology of colony, gram stain and some of biochemical tests used according to [9], single colony of the bacterial culture was transferred by the loop carrier to a clean glass slide and after flame fixation was stained with Gram stain and examined by an optical microscope under the oil lens to determine the shape and size of the cells and their interaction with the Gram stain and how they combine. Biochemical tests such as catalase, oxidase, indole, methyl red, Voges proskauer, citrate utilization, coagulase and motion testing were also used in the identification process. The identification was confirmed using the Vitek-2 compact system technique.

Antibiotics susceptibility test:

1- Bacterial suspension was prepared by transferring a number of colonies by sterile loop from a 24-hour new culture to test tubes containing (5) ml of normal saline with good shaking to homogenize the solution (Macfarland tube).

2- Bacterial sample spreading with a sterile cotton swab on the Muller Hinton agar. Media were left at room temperature for (5-10) minutes until the implant was absorbed and dried, and then placed antibiotic discs by sterile forceps with equal dimensions (5 to 6) discs per dish, then incubated at 37° C for 24 hours.

3- The results were read by determine the inhibition zones around the antibiotic discs in millimeters by a ruler and compared with the indices of the antimicrobial inhibition zone diameters [10,11].

Plant Samples Preparation:

Plant samples Arak (*Salvadora persica*) and Cloves (*Dianthus caryophyllus*) get up from local markets in Shirqat city, it was cleaned and placed in containers at laboratory temperature to ensure they are not exposed to direct sunlight, and don't continue by moving and ensure don't rot until they dry completely, then the grinder was used to grind the plant samples to obtain the fine powder and then put again in sterile and airtight plastic containers, then stored at the temperature of the laboratory refrigerator to be used in preparing the extracts.

Preparation of aqueous extracts:

Plant extracts were prepared by mixing 40grams of the plant with 160 ml of distilled water, in a ratio of 4 :1w / v in the blender then stir the mixture by magnetic stirrer for at least one hour to lyse the plant cell wall, the mixture left in the refrigerator for 24 hours then filtered through several layers of gauze and filtered again by a Buchner's funnel using the Whatman (No.1) filter paper with discharge by the vaccum. In order to remove the residue of the fibers and the un-crushed parts, the raw aqueous extract was prepared and cooled under pickled pressure by Edwards's lyophilizer. After drying, the samples were placed in glass bottles with airtight lids in moisture-free conditions. These aquatic extracts preserved by freezing for use in the study [12].

Preparation of alcoholic extracts

The alcoholic extracts were prepared by mixing of 20 gm of plant in 200 ml of ethyl alcohol with 95% concentration in water bath, the mixture is shaken Stirrer well, kept in refrigerator for 24 hours, then filtered through several layers of gauze filter. In order to get rid of alcohol, the mixture is placed in the rotary evaporator, which is based on the evaporation work under the pressure of the mixture and the temperature does not exceed 40^0C, then after the evaporation of the solvent (alcohol), the extract is cooled in the freeze-dried dehydration apparatus, and finally the samples are then preserved by freezing until use in the study [13].

Results and Discussion

Sixty-six (60.55%) samples from male and forty-three (39.44%) from female was collected, as shown in Table (1). The results showed that 103 (94.49%) samples were positive growth and 6 (5.50%) samples were negative growth, the reason for the emergence of negative bacterial cultures is that some bacteria need special growth requirements or because a person was used antibiotics [14].

Table 1: Distribution of Samples according to gender

Gender	Samples	
	Number	Percentage %
Female	43	39.44
Male	66	60.55
Total	109	100

Six different bacterial species were isolated in this study. The highest isolation rate was attributed to *Staph. epidermidis* by 53 (51.37%), *Strep. Mutans* 20 (19.26%), *Staph. aureus* 13 (12.84%), *Lactobacills acidophillus* 7 (7.33%), *E. coli* 6 (5.50%) and *Strep. pneumoniae* 4 (3.66%).

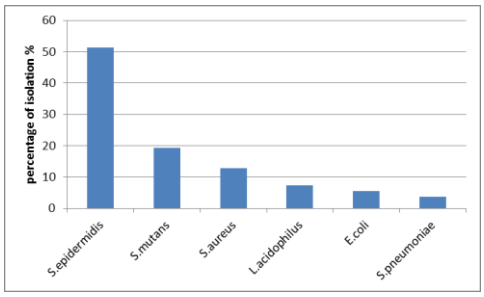

Fig. 1: percentage of bacterial spp. isolates from oral cavity

Antibiotic susceptibility test

The results showed that the vast majority of these isolates were resistant to all antibiotics used in the study as in table (2), due to many factors that affected the test result such as pH and the chemical content in the antibiotic disk and its suitability and storage location [15].

Table 2: Resistance of bacterial isolates to the antibiotics used

Antibiotics	Bacterial species					
	E.coli	***S.pneumoniae***	***L.acidophilus***	***S.mutans***	***S.aureus***	***S.epidermidis***
Ciprofloxacin	R	R	R	I	R	R
Sulfamethoxazole	R	R	R	I	R	R
Amikacin	R	R	R	R	R	R
Cefotaxime	R	R	R	R	R	R
Gentamycin	R	R	R	R	R	R
Streptomycin	R	R	R	I	R	R
Amoxicillin	R	R	R	R	R	R
Ampicillin	R	R	R	R	R	R
Erythromycin	R	R	R	R	R	R
Meropenem	R	R	R	R	R	R
Cefixime	R	R	R	R	R	R
Nitrofurantoin	R	R	I	R	R	R
Azithromycin	R	R	R	R	R	R
Imipenem	R	R	R	R	R	R

R: Resistant S: Sensitive I: Intermediate

Effectiveness of plant extracts against bacterial isolates:

The results of the present study indicated that these plant extracts of *Salvadora persica* and *Dianthus caryophyllus* (alcoholic and aqueous) contain active antibacterial substances. These antimicrobial agents are more effective with increasing concentrations and bacterial species. The concentrations of extracts were used in this study was (10, 30, 50, 70 and 100) mg/L as shown in table (3).

Effect of clove extract on bacterial species:

The results in table (3) showed the effect of Clove (*Dianthus caryophyllus*) extracts alcoholic and aqueous (hot and cold) on bacterial species isolated from people with dental caries and gingivitis. It is observed that the inhibition zone increases with gradient with concentration, and the most effective concentration was 100 mg / L of alcoholic extract which recorded the largest inhibition zone against *E. coli* with an average inhibition zone of 24.4 mm followed by the same concentration against *Staph. aureus* with an average inhibition zone of 20.8 mm and the same concentration of the same extract against *E.coli* with an average diameter of 20.75 mm inhibition zone and then the same concentration of cold aqueous extract against the same bacteria with an average diameter of 20 mm inhibition zone then the same concentration of the same extract against *Staph. aureus* bacteria with an average inhibition zone of 19.4 mm.

The results of the present study also found that the concentration of 10 mg / L was ineffective and had no effect on all bacterial isolates and cold water clove extract at different concentrations did not effect on streptococcal bacteria. The cold and hot water clove extract and their different concentrations also did not effect on lactobacillus acidophilus.

Table 3: Effect of clove extracts on isolated bacterial species

Bacterial isolates	Average diameter of Inhibition zone at mm (Hot water extract)					Average diameter of Inhibition zone at mm (cold water Extract)					Average diameter of Inhibition zone at mm (alcoholic extract)				
	Concentrations of plant extracts mg/l														
	10	30	50	70	100	10	30	50	70	100	10	30	50	70	100
Staph. epidermidis	-	-	8.3	12	16.3	-	-	7.2	11.8	16	-	-	10.7	12.9	16.2
Strep. mutans	-	-	-	-	-	-	-	-	-	-	-	-	-	9.1	12
Staph. aureus	-	-	9.6	13.8	18.2	-	7.4	11	15.2	19.4	-	-	11.6	16.6	20.8
L. acidophillus	-	-	-	-	-	-	-	-	-	-	-	-	-	9.3	13.3
E. coli	-	-	-	11	15.7	-	-	10	15	20	-	8.5	15.3	20.8	24.4
Strep. pneumoniae	-	7.2	12	13.6	15.3	-	-	10	13	16.5	-	7.4	11.4	13.6	15.6

(-) no effect

Effect of Arak *(Salvadora persica)* extracts on bacterial isolates:

The results of the present study showed the inhibitory activity of alcoholic and aqueous Arak extracts in concentrations (10, 30, 50, 70 and 100) mg / ml against isolated bacteria. It is noted from Table (4) that the alcoholic and aqueous (hot and cold) extract of Arak at a concentration of 10 mg / ml has no effect on *S.epidermidis*. The average of diameters of the inhibition zones were graded according to the concentration used.

The concentration 100 mg/ml for aqueous extract was recorded with the average diameter of the inhibition zone 16.6 mm followed by the same concentration of alcoholic extract with the average diameter of the inhibition zone 15.3 mm and then the same concentration of cold water extract with the average diameter of the inhibition zone 15 mm. The concentration of 70 mg/ml was not less than the concentration of 100 mg / ml. The average inhibition diameter was 12.66 mm for the three extracts followed by the concentration of 50 mg/ml with an average inhibition diameter of 8.6 mm. And the lowest average inhibition diameter against these bacteria was 30 mg / mL which was not effect against isolates and the results were consistent with [16,17].

While for *S. mutans* bacteria, that they are affected by the extracts since only alcohol concentration 100 mg /ml had an effect on these bacteria with different inhibition diameter 8.6 mm. Hot and cold aqueous extracts of different concentrations had no effect on these bacteria. This finding differs with the results of the study in [17,18,19].

The extracts used had a clear inhibitory activity on *S. aureus* and with the exception at a concentration of 10 mg / ml for all extracts. Alcohol extract of 100 mg / ml was most effective among other concentrations and extracts with an average inhibition diameter of 17.4 mm followed by the same concentration of hot aqueous extract with an average inhibition diameter of 16.2 mm and then the same concentration of cold water with an average inhibition diameter of 15 mm and then the concentration of 70 mg / ml of three extracts was it has an inhibitory activity with an average inhibition diameter of 12.3 mm. A concentration of 50 mg / ml with an average inhibition diameter of 8.6 mm. This finding was consistent with [20] .

As for *Lactobacillus acidophilus*, it was not different from *S. mutans* in terms of its effect on the extracts, as the aqueous extracts had no effect on these bacteria, while the inhibition was limited to alcoholic extract with concentrations (70 and 100) mg / ml was 9.3 mm, 12.2 mm, respectively. This result differed with [21,22].

With respect to *E. coli*, the aqueous extract has the greatest inhibitory effect compared with the extracts used against isolated bacterial species with the exception of concentration 10 mg / ml for all extracts and 30 mg/ml for alcoholic extract as these concentrations have no effect. The concentration of 100mg / ml of hot aqueous extract inhibited these bacteria with the highest average inhibition area of 17 mm followed by the same concentration of cold aqueous extract with an average inhibition diameter of 15.6 mm and then the concentration of 70 mg / ml of hot aqueous extract with an average inhibition diameter of 14.6 mm and then the concentration 100 mg / ml for alcohol extract with an average inhibition diameter of 14.5 mm. The concentration of 30 mg / ml of alcoholic extract had no inhibitory activity against these bacteria, while the concentration of 50 mg / ml had an inhibitory activity of 8.2 mm. The concentration of 70 mg / ml of the three extracts had an inhibition effect with an average inhibition diameter of 12.23 mm. This result differed with [23].

As for *S. pneumoniae*, the concentration of 10 mg/ ml and 30 mg/ml of the extracts of the alcohol extract did not affect them. The highest concentration of inhibitory effect is 100 mg / ml for hot aqueous extract, alcoholic extract and then cold aqueous with diameters of inhibition zones 15.2 mm, 13.5 mm and 13 mm, respectively. The average 10.8 mm is the concentration inhibition of 70 mg / ml while 8.2 mm is the average concentration inhibition of 50 mg/ml. The concentration of 30 mg / ml of cold and hot aqueous extract had an inhibitory effect on these bacteria where the average diameters of the inhibition zones were 9.6 **mm.**

Table 4: effect of Arak extracts against bacterial isolates

Bacterial isolates	Average diameters of Inhibition zone at mm (hot water arak)					Average diameters of Inhibition zone at mm (cold water arak)					Average diameters of Inhibition zone at mm (alcoholic arak)				
	Concentrations of extracts mg/ml														
	10	30	50	70	100	10	30	50	70	100	10	30	50	70	100
Staph. epidermidis	-	-	8.2	13	16.6	-	-	8.5	13	15	-	-	9.1	12	15.3
Strep. mutans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8.6
Staph. aureus	-	-	8.4	12.2	16.2	-	9.6	10.6	12.8	15	-	-	-	12	17.4
Lacto. acidophilus	-	-	-	-	-	-	-	-	-	-	-	-	-	9.3	12.2
E. coli	-	-	10	14.6	17	-	-	8.6	12.6	15.6	-	-	-	9.5	14.5
Strep. pneumoniae	-	-	10.2	12.2	15.2	-	-	10.6	11	13	-	-	-	9.3	13.5

Comparison between the plant extracts and antibiotics activity:

In Table (5), the results showed that the extracts of plants used are clearly superior to other antibiotics used against *S. epidermidis* and that Ciprofloxacin, Sulfamethoxazole and Amikacin are relatively close to the effectiveness of these extracts with averages of inhibition diameters of 8 mm, 9 mm and 7mm respectively.

The mean efficacy of Clove and Arak extract concentrations at 100 and 70 mg / ml was 16.6, 12 and 15.6, 12.7 respectively. While these antibiotics were less effective against *S. aureus* compared to the efficacy of the extracts, while the efficacy of Amoxicillin, Ampicillin and Erythromycin were 7.5, 7 and 8 mm respectively, while the efficacy of the extracts were 19.5, 15 mm and 16, 12 mm respectively.

As for *S. mutans*, the antibiotic activity was stronger compared to the extracts for example: Amikacin was the strongest antagonist with an average inhibition diameter of 13 mm followed by Ciprofloxacin with an average diameter inhibition of 12.5 mm and then Sulfamethoxazole with an average inhibition diameter of 12 mm while the efficacy of the extracts was not effect against the isolates. Likewise, *Lactobacillus acidophillus* was the most potent antibiotic. Imipenem had the highest average inhibition diameter of 9.5 mm. In case of *S. pneumoniae*, the effectiveness of the extracts is stronger compared to antibiotics in which the sulfamethoxazole is the strongest compared with other antibiotics with an average inhibition diameter of 7 mm. The effectiveness of the extracts was 16, 13.5 mm and 14,11 mm respectively.

E. coli is not different from other species as the extracts are more effective compared to the antibiotics and Sulfamethoxazole is the strongest among the rest antibiotics against these bacteria with an average inhibition diameter of 7 mm, while the effectiveness of the extracts was 20, 15.5 mm and 16, 12 mm respectively.

Table 5: Comparison between the effectiveness of extracts and the antibiotics used

Bacterial isolates	*S. epidermidis*	*S. aureus*	*S. mutans*	*L.acidophilus*	*S. pneumoniae*	*E. coli*
Antibiotics	Average of inhibition zone diameters for antibiotics and extracts					
Ciprofloxacin	8	4.5	12.5	7	5	6
Sulfamethoxazole	9	4.5	12	7.5	7	7
Amikacin	7	4.5	13	7	-	6
Cefotaxime	4	-	10	8.5	-	-
Gentamycin	4	-	8.5	9.5	-	-
Streptomycin	4	-	11	8	-	-
Amoxicillin	-	7.5	4.5	-	-	-
Ampicillin	-	7	4	-	-	-
Erythromycin	-	8	5	-	-	-
Meropenem	-	-	6	6	-	-
Cefixime	-	-	5	4.5	-	-
Nitrfurantoin	-	-	7	9	4.5	-
Azithromycin	-	-	7	9.5	-	-
Imipenem	-	-	7	9.5	-	-
C.,70 ,100 clove	12 , 16.5	15,19.5	-	-	13.5 , 16	15.5 , 20
C.,70 , 100 arak	,15.612.7	12 ,16	-	-	11 ,14	12 ,16

(−)no effect

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تحديد الفعالية التثبيطية لمستخلصات الاراك والقرنفل تجاه العزلات البكتيرية الهوائية المعزولة من المصابين بالتهابات الاسنان واللثة

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الملخص

تضمن البحث جمع 109 عينة من المراجعين لمستشفى الشرقاط العام وبعض عيادات اطباء الاسنان الخارجية في قضاء الشرقاط خلال المدة من 2018/10/10 ولغاية 2019/3/20 لتحديد العزلات المسببة لتسوس الاسنان واختبار فعالية بعض المضادات الحيوية الشائعة ونوعين من المستخلصات النباتية (الاراك والقرنفل) تجاه هذه العزلات. تم تشخيص العزلات الجرثومية بعد زرعها على الاوساط الزرعية وشخصت مظهريا وبالاعتماد على نتائج الاختبارات الكيموحيوية بالاضافة الى تأكيد التشخيص اعتمادا على الطرق الحديثة بتقنية vitek 2 compact system .

وقد أظهرت نتائج التشخيص الانواع التالية ونسبها المئوية: *Streptococcus mutans* (19.26 %), *Staphylococcus epidermidis* (51.37%), *Staphylococcus aureus* (12.84%), *Lactobacillus acidophilus* (7.33%), *Escherichia coli* (5.50%), *Streptococcus pneumoniae* (3.66%).

تبين من نتائج البحث ان المستخلصات المائية والكحولية كانت اكثر فعالية من المضادات الحيوية وخاصة التركيز 100 ملغم/مل في حين ان التركيز 10 ملغم/مل لم يكن فعالا تجاه جميع العزلات.