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Antibacterial Activity of Crude Herbal Mixture (Oak bark, Miswak , Cinnamon , Mint, Clove , Common Camomile and Glycerin oil) on Oral Pathogenic Bacteria

Karama T. Al-Taei 1, Sadad S. Al-Ani 2 , Khadija K. Al-Dulaimi 3
and Mohammed M . Al-Dulaimi 4

1,2,3 Assistant Lecturer Department of Basic Sciences, College of Dentistry.

4 University of Anbar, B .Sharia Islamic

Abstract

Objective: To investigate the antibacterial activity of the crude herbal mixture on some oral pathogenic bacteria.

Material and Methods : Out of seventy clinical samples (17 gingival swab, 7 saliva , and 46 dental caries swabs) , fifty four isolates were identified as (18.5%)10 isolates *Streptococcus mutans* ,(11.1%) 6 isolates *Staphylococcus aureus* ,(42.6%) 23 isolates *Staphylococcus epidermidis*,(9.3%) 5isolates *Micrococcus* and(18.5%) 10 isolates *Lactobacillus* .

The crude herbal mixture composed of (10% *Quercus aegilops* L. (ground of oak bark) , 20% *Salvadora persica* L.(ground of miswak) , 20% *Cinnamomum zeilanicum* (ground of Cinnamon bark) , 10% *Mentha spicata* L. (leaves of mint) , 5% *Syzygium aromaticum* (dried flower buds of clove) ,30% glycerin oil and 5% *Matricaria chamomilla* L. (flowers of camomile) .

The crude herbal mixture was evaluated against *Streptococcus mutans*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus* and *Lactobacillus* isolated from seventy oral infection samples . Antimicrobial activity was determined by the agar diffusion method and the zones of growth inhibition were measured.

Results: The crude herbal mixture was found to possess strong antibacterial activity against range of studied bacteria which was isolated Conclusion : The crude herbal mixture can be used as tooth paste component .

Keyword : antibacterial .herbal mixture. oral bacteria .

Introduction

Dental caries and periodontal diseases are among the most important global oral health problems ,which increase resistance by bacteria to antibiotics, adverse effects of some antibacterial agents are currently used in dentistry and financial considerations in developing countries.

There is a need for alternative prevention and treatment options that are safe, effective and economic ⁽¹⁾.

Oral cavity has numerous types of bacteria, most of them are considered as normal flora and opportunistic pathogen like *Staphylococcus*,*Streptococcus*, *Actinomyces* and *Lactobacillus* ⁽²⁾.

The link between oral diseases and the activities of microbial species that form part of the microflora of the oral cavity is well established ⁽³⁾. Subsequent aspiration of respiratory pathogens shed from oral biofilms into the lower airway increases the risk of developing a lung infection. In addition, patients may aspirate inflammatory products from inflamed periodontal tissues into the lower airway, contributing to lung insult ⁽⁴⁾.

The global need for alternative prevention and treatment options and products for oral diseases that are safe, effective and economic comes from the rise in disease incidence which increases resistance by pathogenic bacteria to currently used antibiotics and chemotherapeutics ⁽⁵⁾. In this study we used crude herbal mixture as antibacterial activity against these pathogens. Herbal mixture is composed of many plants, ground of oak bark, miswak(ground of chewing sticks), ground of Cinnamon, ground leaves of peppermint, ground dried flower buds of clove, ground flowers of Chamomile and glycerin oil. The herbs are mixed as soft paste.

Quercus aegilops L.(oak bark) is a powerful astringent that has been used for centuries to control diarrhea, internal and external bleeding, nosebleed and hemorrhoids. Oak contains tannins which are antibacterial, anti-viral and anti-inflammatory ⁽⁶⁾.

Salvadora persica(miswak) (chewing sticks) is used for centuries as a natural tooth brush. Its fibrous branches have been promoted by the World Health Organization for oral hygiene use ⁽⁷⁾. Many studies have demonstrated the antibacterial, anti-caries, anti-periodontic,

and anti-fungal properties of aqueous extracts of various chewing sticks ^{(8),(9)}.

Cinnamomum zeylanicum (Cinnamon) the oil of Cinnamon initially causes a considerable decrease in the metabolic activity and in the replication capacity of *Ps. aeruginosa* and *Staph. aureus* and caused losing of membrane ⁽¹⁰⁾.

Matricaria chamomilla (Camomile) is considered to be an antiseptic, antibiotic, disinfectant antibacterial, antifungal and antiviral ⁽¹¹⁾. Compounds in the essential oil of chamomile are effective against *Staphylococcus* and *Candida* ⁽¹²⁾.

Mentha spicata L. (mint) contains major compounds of α -terpinene isomenthone trans carveol and piperitininone oxide. The essential oils have good or excellent antimicrobial activities against all microorganisms tested ⁽¹³⁾.

Glycerin is used in medical and pharmaceutical it is found in cough syrups, elixirs and expectorants, toothpaste, mouthwashes and skin care products because it attracts water and helps skin look better ⁽¹⁴⁾.

Syzygium aromaticum L., The Turkish clove oil is found to be better antagonistic agent by inhibiting both bacteria & fungi. The oil is found to be very effective against *Staphylococcus epidermidis* & *Staphylococcus aureus* ⁽¹⁵⁾. Aqueous and methanol extracts of clove are shown to affect the cariogenic properties of *Streptococcus mutans* as exhibited by the ability of the extracts to inhibit adhesion of the bacteria to glass, and the production of glucosyltransferase ⁽¹⁶⁾. The aim of this study is to investigate the antibacterial activity of the crude herbal mixture on some oral pathogenic bacteria.

Materials and Methods

Collection of samples

Seventy oral specimens (17 gingival swab, 7 saliva, and 46 dental caries swab) are taken from (50) secondary school students in Ramadi city (26 with dental caries and 24 caries free) and (20) adult patients with dental caries from both sexes in clinics of Dentistry College.

Specimens are cultured on blood agar, chocolate agar and MacConky agar and incubated aerobically and anaerobically at 37 °C for 24 hrs. Specimens are cultured also on blood agar 5% CO₂. Bacteria isolates are diagnosed according to their morphological and cultural criteria⁽¹⁷⁾.

crude herbal mixture Preparation of

We mixed 10% ground of oak bark, 20% ground of miswak, 20% ground of Cinnamon bark, 10% ground of mint dry leaves, 5% ground dried flower buds of clove tree, 5% ground of dried flowers of camomile and 30% glycerin oil. The mixture of crude herbal were they already prepared in present study prepared as a tooth paste preparation. All herbal plants were obtained from herbalist lawful from Ministry of Health / Center of Medical Herbs (NO.175-2000/1/12) table (1). Herbs were approved by the National Herbal Of Iraq State Board for Seed Testing and Certification. Ministry of Agriculture, Iraq /Baghdad.

Table -1-The percentage of parts of plants that used in crude herbal mixture

Plant name (common)	Scientific name	Parts used of plant	Percentage of parts used of plant
Oak	<i>Qurecus aegilops L.</i>	bark ground of	10%
Miswak(tooth-brush)	<i>Salvadora persica</i>	stem of ground	20%
Cinnamon	<i>Cinnamomum zeilanicum</i>	bark ground of	20%
Mint	<i>Mentha spicata L.</i>	ground of dried leaves	10%
Clove tree	<i>Syzygium aromaticum L.</i>	ground of dried flower buds	5%
Common Camomile	<i>Matricaria chamomilla L.</i>	dried flowers	5%
Cotton	<i>Gossypium herbaceum L.</i>	Oil of cottonseed (glycerin)	30%

Bacterial isolates

Bacterial standardization

Fifty four isolates of, *Staphylococcus aureus* *Staphylococcus epidermidis*,

Streptococcus mutans, *Micrococcus Spp.* and *Lactobacillus Spp.*, are obtained from oral specimens (17 gingival swab(9 males and 8 females),

7 saliva(males) , and 46 dental caries swab(26 students 18 males and 8 females) and (20 patients 7 males and 13 females) .Bacterial strains were grown overnight on nutrient broth at 37 °C and diluted with sterile saline to achieve an approximate density of 1×10^8 colony forming units (cfu) per ml (18) .

Determination of antibacterial activity:

The antibacterial activity of the crude herbal mixture is determined by using agar well diffusion method following published procedure (19) (20) .

Nutrient agar is inoculated with the given microorganisms by spreading the bacterial inoculums on the media The seeded agar plates are then left for about 15 min. Wells (8 mm diameter) are punched in the agar and filled with volume of plant mixture. The plates are incubated at 37 °C for 18 hrs. Control wells containing distilled water (negative control) or standard,

antibiotic solution (positive control) ciprofloxacin (5 µg/ml) are also run parallel in the same plate (21) .

The resultant clear zones around the well are measured in mm. The antibacterial activity of plant mixture is indicated by clear zones of growth which inhibit it. Zones of inhibition are measured and recorded in millimeter diameter according to the methods of Kirby-Bauer (18) (22) .

Results

Forty one (58.5%) of males and twenty nine (41.4%) of females oral specimen are taken from (50) secondary school students(26 dental caries and 24 caries free) and (20) adult patients from both sexes in clinics of Dentistry College . The ages of the students (males) are between 15-18 and females are 12-17 (table 2) while the ages of patients (males) between 25-60 and females are 40-45 (table3).

Table (2) Oral specimens types of students

Type of sample	Age of males	Mean of age	% of sample number from total samples	Age of females	Mean of age	% of sample number from total samples
Gingival swab	15-18	16.5	12.8	12-17	14.5	11.4
Saliva	15-17	16	10	-	-	-
Caries swab	15-18	16.5	25.7	12-15	13.5	11.4

Table (3) Oral specimens types of patients

Type of sample	Age of males	Mean of age	% of sample number from total samples	Age of females	Mean of age	% of sample number from total samples
Caries swab	25-60	42.5	10	40-45	42.5	18.6

The isolation of bacteria results are referred that *Staphylococcus epidermidis* is the highest percentage of isolation and followed by *Streptococcus mutans* and

Lactobacillus Spp. The *Micrococcus Spp.* is the lowest percentage of isolation table 4.

Table -4- The percentages of bacterial isolates that isolated from oral specimen

Isolated bacteria	Number of isolates	%
<i>Staphylococcus. Epidermidis</i>	23	42.6
<i>Streptococcus mutans</i>	10	18.5
<i>Lactobacillus Spp.</i>	10	18.5
<i>Staphylococcus aureus</i>	6	11.1
<i>Micrococcus Spp.</i>	5	9.3
Total	54	100

The antibacterial activity of crude herbal mixture are indicated by clear zones of growth which inhibit it . The inhibition zoon are measured and recorded in millimeter diameter (table 5) .

Table -5-Effect the crude herbal mixture against fifty four isolates of bacteria on nutrient agar plates after 18hrs incubation (inhibition zoon in millimeter)

bacteria Tested	Inhibition zone diameter(mm) mean	± SD
<i>Streptococcus mutans</i>	33.8	± 4.4
<i>Staphylococcus aureus</i>	28.7	± 1.2
<i>Staphylococcus epidermidis</i>	38.6	± 2.7
<i>Micrococcus Spp.</i>	33.3	± 1.4
<i>Lactobacillus Spp.</i>	40.5	± 0.8

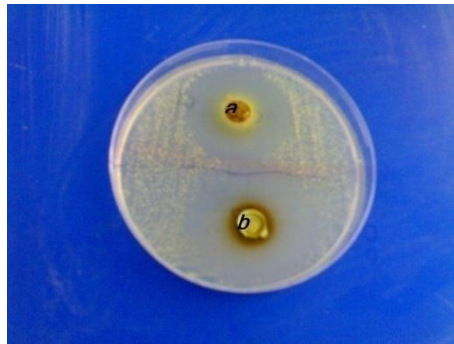


Figure (1)-antibacterial activity of crude herbal mixture on a- *Staphylococcus aureus* and b- *Staphylococcus epidermidis* on nutrient agar plate after incubation 18hrs at 37°C

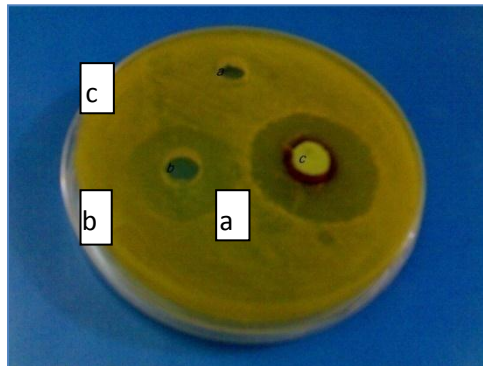


Figure (2)-antibacterial activity of crude herbal mixture on *Streptococcus mutans* a-negative control (distilled water) b-positive control (5 µg/ ml ciprofloxacin) c-crude herbal mixture on nutrient agar plate after incubation 18hrs at 37°C .

Discussion

Early studies have clearly established that a number of substances is potential to be utilized in the dental industry, giving their activity against cariogenic bacteria and those bacteria associated with periodontal diseases.

The previous studies proved that the impact of the bark of oak, Chamomile, cinnamon, cloves miswak and glycerin is effective on bacteria, fungi and viruses, especially the bacteria isolated from the mouth and tooth decay .

So the objective of this research is to mix these parts used of plants in certain percentages and in terms of importance to give the most effective result . We mix glycerin oil by the largest percentages 30% because this article enforce the gums also have a therapeutic effect ⁽²³⁾ . We also added ground of miswak 20% and that because of its tremendous impact on the content of bacteria in the mouth and remove plaque accumulated on the teeth ⁽²⁴⁾ .

Twenty percentage ground of Cinnamon were added in order to give off the smell, fragrant natural mouth and has effective anti-bacteria⁽¹⁰⁾. The proportion of added dried leaves of mint is 10%.

The article points to studies in which a special extract of leaves which have the effect of anti-bacterial pathogens, especially bacteria, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Serratia marcesens*, *Streptococcus* and *Staphylococcus aureus*⁽²⁵⁾. Ground of Oak bark is mixed by 10%, to get the active ingredient against resistant bacteria to antibiotic such as *Staphylococcus aureus*. Also it is used to stop bleeding⁽²⁶⁾ and the proportion of dried flowers of Chamomile is 5% as recommended by the NCCAM, 2009 because they cause deodorizing and bleaching teeth, killing germs, stopping necrosis and bleeding and finally add ground of dried flower buds of clove 5%, the material is used widely in dental fillings and it is active against bacteria and fungi⁽²⁷⁾.

Fifty-four isolated bacteria were isolated from oral samples which are divided into 17 swab gums, 7 saliva samples and 46 swab of Dental caries as indicated in table (2) and (3). The types of bacteria isolated showed that the percentage of isolated bacteria *Staphylococcus epidermidis* had the highest percentage which was 42.6%. *Staphylococcus epidermidis* is responsible for 50 to 70% of infections⁽²⁰⁾ while *Micrococcus* is the lowest 9.3% table (4).

The effectiveness of the crude herbal mixture against the bacteria isolated was tested, the test result indicates antibacterial effect against bacteria. The antibacterial activity of crude herbal mixture was indicated by clear zones of growth which is inhibited. Zones of inhibition were measured in millimeter diameter according

to table (5). The result of inhibition zone mean was between 28.7 mm to 40.5 mm.

The zone of inhibition assay results demonstrated that the 5 µg of crude herbal mixture was able to produce same effect as that of 5µg of ciprofloxacin. The results from the present study are very encouraging and indicate that these herbs should be studied more extensively to explore their potential in the treatment of infection.

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