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Using an Antimicrobial Composition of Plant Extracts-Clove Oil, Miswak Tree Sap, and Acacia Fruit against Bacteria Isolated from Dental Caries Patients

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ABSTRACT

The aim of this research is to evaluate the antimicrobial effectiveness of medicinal plant extracts (arak tree, clove oil and loan) against a group of clinically isolated tooth decay bacteria from some patients with tooth decay from different hospitals. The samples were collected and isolated and classified in the laboratory on the agar-maconkey and nutrient agar environments, which included bacteria that are affected by tooth decay (*Staphylococcus*, *Streptococcus*, *Lactobacillus*) *and* were identified and confirmed by chemo biotic tests. The plant extracts were prepared using ethanol extracts to evaluate their antibacterial activity using tablet diffusion in addition to measuring the minimum inhibitory concentration MIC at concentrations of 12, 25, 50 and 100 mg/ml, where the results showed that arac extract and clove oil had the highest antibacterial activity with observed inhibition regions against *Streptococcus mutans Lactobacillus* spp, while the effectiveness of the tablet fruit was relatively lower. It was also compared the effectiveness of plant extracts with a range of antibiotics. Some extracts have shown comparable effects of antibiotics against some bacteria. We conclude from this research that the use of natural extracts is effective and may contribute to the development of an alternative or complement to antibiotics for the treatment of tooth decay and oral bacterial infections.

1. INTRODUCTION

Tooth decay is one of the most prevalent diseases in the world and affects all age groups without

exception, and occupies wide attention by dental specialists. Classified by the World Health Organization as a chronic disease and health problem that affects the quality of life [1], despite the advancement in oral and dental care technologies, the spread of caries is still a continuous challenge in most developing countries.

- ***Streptococcus mutans***: play a pivotal role in the emergence and development of tooth decay, as it is the main factor in this pathological process and has a high ability that helps it adhere to the tooth surface and form dental plaque, in addition to the production of fibers through the process of sugar metabolism, these acids lead to a decrease in the pH level within the dental plaque, which causes the removal of minerals from the surface of the enamel [2] as *Lactobacillus* bacteria contribute In deepening the lesions and increasing their spread, especially in the acidic environment, which makes them more active after the formation of the cavity. Traditional treatments, although well effective, are not free from the side effects of tooth staining, irritation of the mucous membranes, in addition to the development of bacteria to resist antibiotics for a long time [3]. In light of limited access, the cost of treatment is high in many developing countries, including the Republic of Chad, the population is still. In this context, medicinal and aromatic plants play a role in traditional health care systems [4]. The World Health Organization (WHO) has estimated that developing countries rely mainly on traditional medicinal plants to meet primary health care needs, and studies have shown that about 85% of traditional remedies involve many active substances in plants used against microbes. There is an urgent and ongoing need to detect new antimicrobials with natural structures. To overcome microbial resistance to antibiotics and obtain natural remedies to strengthen immunity [5]. Arak known as toothpicks is one of the most important plants for oral health and has been used for centuries to clean teeth, as it contains many compounds with antimicrobial inhibitors Carbohydrates vitamins Glycosides saponoids Flavonosides Trimethylamine Sulfadorate Chloride Terbinalsilica Amount of Fluoride [6]. Arak extract has the ability to inhibit the growth of bacteria associated with tooth decay [7, 8]. On the other hand, clove oil is one of the most powerful essential oils effective in fighting oral bacteria and contains the compound eugenol and has antimicrobial properties. Many studies have been conducted on the effect against the bacteria *Streptococcus spp.*, which is responsible for tooth decay [9, 10]. *Lactobacillus*, while the loan fruit contains protein, ether extract, fiber, nitrogen-free extracts, in addition to phosphorus, calcium, and cycic ether, which are very effective and kill bacteria and germs. Effective treatment for tooth decay.

General Objectives:

Use of antimicrobial extract of plant extract clove oil, arak tree and acacia fruit or loan against bacteria isolated from tooth decay patients and knowledge of their sensitivity and resistance to commercial antibiotics and plant extracts.

Research Objectives

1) Isolation and classification of some types of tooth decay bacteria isolated from patients.

2) Identify the types of pathogenic bacteria isolated from patients with tooth decay and study their sensitivity and resistance to antibiotics and some medicinal plant extracts.

3) Evaluation and effect of isolates and determination of the concentration of the minimum inhibitor (MIC).

2. WORKS METHODS

Collecting Samples from Patients with Tooth Decay

I collected 40 samples from some patients who suffer from tooth decay of different ages in the early hours of the morning, making sure not to take antibiotics and food for at least an hour, and not to use toothpaste, and under the supervision of a dentist in some hospitals, dental clinics in Khartoum State for a period between 3/11/2019 to 19/2/2019 and it is brought to the laboratory by sterile sealed plastic pipes and affixed to it with a card identifying the number and place of the date of collection, and the pipes are transported by the refrigerated method in suitable environmental conditions of 4 degrees Celsius.

Isolation Purification

After a series of refinements of the isolated cells from decayed teeth, the colonies took the developing

bacteria to the specialized nutrient broth and were sterilized according to the company's instructions by a metal conveyor and planned on the medium to grow them and incubated at a temperature of 37˚C for 24 hours.

Isolation and Diagnosis of Bacteria

Developing colonies were diagnosed in transitional environments based on their phenotypic traits, which include the shape, color, size, and faintness of the colony.

The samples were examined microscopically by taking a swab of developing bacterial colonies from agricultural media, fixing them and staining them with Gram dye to map the shape and arrangement of cells and their interaction with Gram dye (positive or negative).

The Maconkey Test

Greening by taking the food medium 8.014 grams was put in a bottle and added to 200 ml of distilled water and dissolved in the microwave completely, then sterilized in the rotolife at 121 degrees for 15 minutes and cooled at room temperature and poured into sterile petri dishes and the process of planning and incubating the dishes by air at a temperature of 37˚C for 24 hours to distinguish between the fermented bacteria that are not fermented for lactose sugar [11, 12].

Bloody Rent

10.6 grams of agar powder was prepared and 3.75 grams of agar agar was prepared and poured into a sterile 500 ml bottle and distilled water was added to it and dissolved well in a water bath, then sterilized in the rotolife at a pressure of 121 for 15 minutes, and then left to a temperature of 45 - 50 degrees Celsius, 10% of the blood was added to it, and it was mixed well, and poured into the dishes with a petri and planned and fortified at a temperature of 37˚C for 24 hours. Erythrocyte Hydrolysis on the Middle [13].

Mantol Test

This test was conducted to differentiate between the types of fermented and non-fermented *staphylococcus* of mannitol sugar the isolates of the bacteria were planted on the mannitol agar medium and incubated at a temperature of 37˚C for 24 hours.

Biochemical Tests

I attended the following tests according to what he said and were used for the purpose of diagnosing the bacteria as follows.

Oxidase Test

Transfer of a part of the 24-hour growing bacteria by a wooden stick to a filter paper moistened with an oxidase reagent, change color to dark purple after 30 seconds [14].

Catalyze Enzyme Test

It was prepared with a concentration of 3% hydrogen peroxide and was used to detect the ability of bacterial isolates to produce catalase enzyme [13].

Indole Test

Inoculation of pepton water with a number of isolated colonies at a 24-hour age and the tubes were soaked at a temperature of 37˚C for 24 hours, then 5 drops of Kovacs reagent were added to it, the result is positive for action when a red ring appears as a result of the decomposition of the amino acid tryptophan and its conversion to indole [12].

Catalysis Test

A small part of the 24-hour old colony was transferred from the nutrientd agar medium to a clean glass shed using sterile wooden sticks and 3% drops of hydrogen peroxide were placed on top of it. The positive result was the appearance of air bubbles on the surface of the slide, the production of catalyse enzyme releases oxygen gas and the production of catalyse enzyme [15].

Blood Clotting Test

I took 5 ml of blood type A and put it in a test tube and added Sodlum.0.5 grams were put in the centrifuge until the plasma was separated from the blood and I took a small part of the touchand put it on a glass slide and added plasma to it and the result was negative.

Street Test

The developing bacteria were inoculated at 24 hours of age on Simon's oblique medium. Street was

used by the method of stabbing and incubated the pipes at a temperature of 37˚C for 24 hours, and the test was positive by changing the color of the medium from green to blue, indicating the consumption of bacteria for citrate as a good source of carbon [14].

Hemolysin Production Test

In this test, blood agar medium was used, as the medium was inoculated with a part of the pure bacterial colony to be tested, and the dishes were incubated at a temperature of 37˚C for 24 hours, the positive result was observed when hemolysis occurred around the colony, as the lysis was of the type of complete hydrolysis B-hemolysis [16].

Bacterial Allergy to Antibiotics

Testing was performed on the medium of Mullerhunton agar using method [17] using antibiotic tablets to perform allergy tests Growing isolates. The study took single colonies of pure from the surface of the nutrient agar by sterile Loop germ vector and compared with the standard McFoldand solution Planted petri dishes containing Muller-Hinton agar and took the bacterial suspension by a cotton swab (Swab) and the rental planning was carried out from all sides so that the quantity was distributed evenly and the dishes were left to dry for [10, 15]. Then, the antibiotic tablets were placed with sterile tongs, where 6 antibiotic tablets were placed in each dish with equal distances between one tablet and another, and the dishes were incubated in the container for 24 hours at a temperature of 37˚C, and the results were recorded by measuring the diameter of the areas inhibiting bacterial growth around each tablet.

Sensitivity Test Arak Islands Propagation Method

The method of agar propagation was followed by drilling [18] in the bacterial susceptibility test to the plant extract, the bacterial suspended for each type of bacteria under study was spread on a medium, then 4 pits with equal diameters were made in the solid Müller-Hinton medium with a diameter of 8 mm by a cork driller, and (12, 50, 25, 100) extract was added to each of the four pits, and then the plates were left for [10]. 20 minutes for the dispersion of plant solutions in the agricultural medium, then incubated at 37˚C for 24 hours, the result was read by measuring the inhibition area by the inserted ruler.

3. INTERPRETATION OF ANTIBIOTIC SENSITIVITY TEST RESULTS

Interpretation of the results of Table 1:

Table 1. Antibiotic sensitivity test for *Staphylococcus* spp.

Antibiotic Name	Disc code	Disc Content (µg)	Zone diameter (mm)	Resistant (R)	Intermediate (I)	Susceptible (S)
Amoxicillin	AMS	10	11	X		
Erythromycin	E	15	8	X		
Tetracycline	Te	30	16			X
Gentamicin	GM	10	29			X
Chloramphenicol	C	30	10	X		
Cephalexin	-	30	12	X		
Co.trimoxazole	-	25	20			X
Ciprofloxacin	CIP	5	18			X

Through Table 1, it is noted that the anti-Gentamicin came in the first place in terms of effectiveness, after the inhibition zone was measured in the Disc Diffusion Test to determine the diameter of the circuit, it was observed that the diameter of the halo resulting from the addition of the Gentamicin tablet at a

concentration of (10 µg) is equal to (29) mm, which is a value that falls within the susceptible region (S) according to the table CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI) is considered the best antibiotic for inhibiting *Staphylococcus* spp.

Accordingly, we can rely on gentamicin as a chemical antibiotic to eliminate this type of bacteria.

While (Co.trimoxazole) came in second place among the antibodies to inhibit *Staphylococcus* spp, and the halo diameter value resulting from the addition of (Co.trimoxazole) disc at a concentration of (25 µg) was equal to (20 mm), which is also located within the susceptible region (S) according to the (CLSI) table. Accordingly, we can rely on Co.trimoxazole as a second chemical antibiotic to inhibit this type of bacteria.

Ciprofloxacin came in third place in terms of effectiveness for inhibiting *Staphylococcus* SPP, where the halo diameter value resulting from the addition of the Ciprofloxacin tablet at a concentration equal to (5 µg) was equal to (18 mm), which is a value located within the Susceptible (S) region according to the (CLSI) table and accordingly. We can rely on Ciprofloxacin as a third chemical antibiotic to inhibit this type of bacteria.

Tetracycline came in the last place in terms of its effectiveness in inhibiting *Staphylococcus* spp, where the halo diameter value resulting from the addition of a Tetracycline disc at a concentration of (5 µg) was equal to (18 mm), which is a value located within the susceptible (S) region according to the (CLSI) table, and based on this, we can rely on (Tetracycline) as a fourth chemical antibiotic to inhibit this type of bacteria.

While each of the following antibiotics (Amoxicillin, Erythromycin Chloramphenicol, Cephalexin) was non-sensitive and all fell within the Resistant (R) zone according to the (CLSI) table, and it is stated that the treatment is categorically ineffective, and therefore we cannot rely on these antibiotics. As a chemical antibiotic to inhibit this type of bacteria.

Interpretation of the results of Table 2:

Table 2. Antibiotic sensitivity test for *Lactobacillus*.

Antibiotic Name	Disc code	Disc Content (µg)	Zone diameter (mm)	Resistant (R)	Intermediate (I)	Susceptible (S)
Amoxicillin	AMS	10	8	X		
Erythromycin	E	15	22			X
Tetracycline	Te	30	25			X
Gentamicin	GM	10	30			X
Chloramphenicol	C	30	35			X
Cephalexin	-	30	20			X
Co.trimoxazole	-	25	12	X		
Ciprofloxacin	CIP	5	33			X

Through Table 2, it can be noted that the Chloramphenicol anti-Chloramphenicol came in the first place in terms of effectiveness, after the Inhibition Zone was measured in the Disc Diffusion Test to determine the diameter of the circuit, it was observed that the diameter of the halo resulting from the addition of the Chloramphenicol tablet at a concentration of (30 µg) is equal to (35 mm) mm, which is a value that falls within the Susceptible region (S) according to Table (CLSI), and Chloramphenicol is considered the best antibiotic for inhibiting *Lactobacillus* bacteria.

Accordingly, we can rely on Chloramphenicol as a chemical antibiotic to eliminate this type of bacteria.

While Ciprofloxacin came in second place in terms of effectiveness, after the inhibition zone was

measured in the Disc Diffusion Test to determine the diameter of the circuit, it was observed that the diameter of the halo resulting from the addition of the Ciprofloxacin disc at a concentration of (5 µg) is equal to (33 mm) mm, which is a value that falls within the susceptible (S) region according to the (CLSI) table.(Ciprofloxacin). The best antibiotic after Chloramphenicol for inhibiting *Lactobacillus* bacteria.

Gentamicin came in third place in terms of effectiveness, after the inhibition zone was measured in the Disc Diffusion Test to determine the diameter of the circuit, it was observed that the diameter of the halo resulting from the addition of the Gentamicin disc at a concentration of (10 µg) is equal to (30 mm) mm, which is a value that falls within the susceptible (S) region according to the (CLSI) table.Gentamicin, the third antibiotic to inhibit *Lactobacillus* bacteria.

It came in fourth place in terms of the effectiveness of Tetracycline, after the inhibition zone was measured in the Disc Diffusion Test to determine the diameter of the circuit, it was observed that the diameter of the halo resulting from the addition of the Tetracycline disc at a concentration of (30 µg) is equal to (35 mm) mm, which is a value that falls within the susceptible zone (S) according to the (CLSI) table.Tetracycline is the fourth most effective antibiotic for inhibiting *Lactobacillus*.

While it came in fifth place in terms of anti-erythromycin effectiveness after the inhibition zone was measured in the Disc Diffusion Test to determine the diameter of the circuit, it was observed that the diameter of the halo resulting from the addition of the Erythromycin disc at a concentration of (15 µg) is equal to (22 mm) mm, which is a value that falls within the susceptible zone (S) according to Table (CLSI) is considered the fifth most effective antibiotic for inhibiting *Lactobacillus* bacteria.

The last in terms of the effectiveness of the anti-Cephalexin was measured after the inhibition zone was measured in the Disc Diffusion Test to determine the diameter of the circuit, it was observed that the diameter of the halo resulting from the addition of the Cephalexin disc at a concentration of (30 µg) is equal to (20 mm) mm, which is a value that falls within the susceptible region (S) according to Table (CLSI) Cephalexin is the last effective antibiotic to inhibit *Lactobacillus*.

While the two antibiotics (Amoxicillin-Co.trimoxazole) were all non-sensitive and all fell within the resistant (R) region according to the (CLSI) table, they all showed the ineffectiveness of the treatment definitively and therefore we cannot rely on these antibiotics as a chemical antibiotic to inhibit this type of *Lactobacillus* bacteria.

Interpretation of the results of Table 3:

Table 3. Antibiotic sensitivity test for *Streptococcus mutans*.

Antibiotic Name	Disc code	Disc Content (µg)	Zone diameter (mm)	Resistant (R)	Intermediate (I)	Susceptible (S)
Amoxicillin	AMS	10	10	X		
Erythromycin	E	15	20			X
Tetracycline	Te	30	25			X
Gentamicin	GM	10	12	X		
Chloramphenicol	C	30	14		X	
Cephalexin	-	30	20		X	
Co.trimoxazole	-	25	12		X	
Ciprofloxacin	CIP	5	33	X		

Through Table 3, it can be noted that the anti-tetracycline came in the first place in terms of effectiveness, after measuring the inhibition zone in the Disc Diffusion Test to determine the diameter of the circuit,

it was observed that the diameter of the halo resulting from the addition of the Tetracycline disc at a concentration of (30 μg) is equal to (25 mm) mm, which is a value that falls within the susceptible zone (S) According to the CLSI table, Tetracycline is considered the best antibiotic for inhibiting *Streptococcus mutans*.

Accordingly, we can rely on Tetracycline as a chemical antibiotic to eliminate this type of bacteria.

While Erythromycin came in second place in terms of effectiveness, after measuring the inhibition zone in the Disc Diffusion Test to determine the diameter of the circuit, it was observed that the diameter of the halo resulting from the addition of the Erythromycin disc at a concentration of (15 μg) is equal to (20 mm), which is a value that falls within the susceptible region (S) according to the (CLSI) table.Erythromycin is the best antibiotic after Tetracycline for inhibiting *Streptococcus mutans*.

While both antibiotics (Chloramphenicol - Cephalexin - Co.trimoxazole) came with a moderate and moderately sensitive efficacy, after measuring the inhibition zone in the Disc Diffusion Test to determine the diameter of the circuit, it was observed that the halo diameter resulting from the addition of all these three tablets is within the intermediate (I) zone. According to the (CLSI) table, it is considered an intermediate-dose antibiotic to inhibit *Streptococcus mutans*.

Therefore, we need higher doses or more frequencies for the antibiotic to successfully treat the infection in certain places to inhibit *Streptococcus mutans*.

While each of the antibiotics (Amoxicillin, Gentamicin, Ciprofloxacin) was all non-sensitive and all fell within the resistant zone (R) according to the (CLSI) table, which shows the ineffectiveness of the treatment definitively, and therefore we cannot rely on these antibiotics as a chemical antibiotic to inhibit the bacteria. *Streptococcus mutans*.

Interpretation of the results of the chemical tests (Table 4):

Table 4. Chemo chemical tests results table.

Types of Tests	*Lactobacillus*	*Staphylococcus spp*	*Streptococcus mutans*
Gram staining	+	+	+
Motility	-	-	-
Indole	-	-	-
Urease	-	-	-
Oxidase	-	-	-
Hemolysis	Gamma	Beta	Alfa
Catalase	-	-	-
Citrate utilization	-	-	-

The results of chemical tests vary according to the type of bacteria, and their interpretation reflects the characteristics of each of the three types:

As for the Gram staining test result, which came positive with *Lactobacillus*, *Staphylococcus spp*, and *Streptococcus mutans*, it means that all these bacteria retain the violet formula and do not lose their color during the process of washing with the oscillator, which shows that their cell walls contain a thick layer of petioglycans, which is a characteristic feature of Gram-positive bacteria.

As for the *Lactobacillus* test, it often shows negative results in tests such as Motility, Indole, Urease, Oxidase, Catalase, and Citrate utilization, which corresponds to its characteristics as an immobile bacteria with a natural role in the digestive system, and mainly produces lactic acid. It also does not show the ability to degrade urea or produce arginine, which is consistent with its negative results.

As for its results with hemolysis, the result was Gamma, which means that there is no hemolysis, which means that the bacteria do not secrete any enzymes that break down red blood cells, and therefore there is no change in the color of the medium and the medium remains the same, which is considered a normal result, as it is one of the features that distinguish it from the pathogenic bacteria that may show partial decomposition (Alpha), or complete decomposition (Beta).

Explanation of the results of the sugar fermentation test (Table 5):

Table 5. **Results of the glycation fermentation test analysis.**

Types of Tests	*Lactobacillus* spp	*Staphylococcus aurous*	*Streptococcus mutans*
Glucose	+	+	Positive
Fructose	+	+	Positive
Lactose	+	+	Positive
Maltose	+	+	Positive
Gelatos	+	+	Positive
Sucrose	+	+	Positive
Mannose Sorbitol	+	+	Positive

The test results, which were all positive, confirmed the ability of each type of bacteria to metabolize sugars and produce acid or gas, demonstrating their metabolic activity.

As for the positive test result of *Lactobacillus* spp, it indicates that it ferments sugars significantly, especially glucose and sucrose, and produces lactic acid in large quantities. This reflects its ability to produce a strong acid that leads to a low pH in the medium, which is the basis for milky fermentation and its use in the dairy and fermented foods industry.

As for the positive test result for the sugar fermentation test with *Staphylococcus aurous*, it indicates that it ferments sugars such as glucose, mannose, and sucrose, and produces lactic acid or other acid, and sometimes carbon dioxide gas, which causes the media to change color to yellow, and is usually used to test metabolic activity.

The result of the sugar fermentation test with *Streptococcus mutans* is that it effectively ferments sucrose, and produces large amounts of acid, especially lactic acid, which accelerates the process of tooth decay by accumulating bacteria and producing acids that weaken tooth enamel.

The ability to ferment fructose and glucose helps them sustain their activity and plaque formation, and is a hallmark of them in the context of dental infections.

When bacteria ferment sugars, they react with sugars and convert them into acid or gas using their enzymes, and usually notice a change in the color of the medium to yellow or the emission of gas in the fermentation tubes.

These results reflect the metabolic abilities of each species, and help determine their functional characteristics and role in the environment or clinical situation.

Interpretation of the results of the bacterial isolate test (Table 6):

Table 6. **Bacterial isolates test.**

Bacterial isolates	Issue	Percentage
Lactobacillus spp	19	38%

Continued

Streptococcus mutans	17	34%
Staphylococcus aurous	14	28%
Total	50	100%

Bacterial isolates test results with *Lactobacillus* spp, *Streptococcus mutans*, *Staphylococcus aurous* and values 19, 17, 14 often indicate the diameter of the inhibition zone in millimeters in the tablet diffusion test, which is used to evaluate the effectiveness of bacteria or antibiotics in inhibiting the growth of other isolates.

Interpretation of Values:

A value of 19 means that the isolation *Lactobacillus* spp showed a strong inhibitory effect against the target isolate, as the larger the diameter of the area, the greater the inhibitory power.

A value of 17 indicates a moderate to strong inhibitory effect of *Streptococcus mutans*.

Whereas the value of 14 which means an inhibitory effect is relatively weaker for *Staphylococcus aurous* compared to other bacteria.

Significance of the Results:

These results are used to compare the effectiveness of each bacteria in inhibiting against target isolates and can help select the right bacteria for use in biological applications or as natural inhibitors.

Higher values indicate the ability of the bacteria to produce substances that inhibit the growth of other bacteria, which is an indication of strong antagonistic or inhibitory activity.

These results may indicate the effectiveness of the bacteria in inhibiting compared to conventional antibiotics.

Overall, the results show that *Lactobacillus* spp is the most effective inhibitor followed by *Streptococcus mutans* and then *Staphylococcus aurous*, and these values are important in evaluating the antibacterial activity.

Interpretation of the results of Table 7:

Table 7. Test of plant extracts with *Streptococcus* bacteria.

	Streptococcus				
Extracts	Disc Content (µg)	Zone diameter (mm)	Resistant (R)	Intermediate (I)	Susceptible (S)
Clove Oil Extract	12	26			X
	25	29			X
	50	29			X
	100	25			X
Al-Qarawi Extract	12	27			X
	25	30			X
	50	30			X
	100	36			X
Arak Extract	12	20			X
	25	29			X
	50	39			X
	100	25			X

The table shows the effect of three coefficients (clove oil extract, karoui extract, and arak extract) on *Streptococcus* bacteria at four different concentrations, and the effect is expressed in millimeter diameter of the inhibition zone, which is the same principle adopted in the CLSI standard for diffusion disc susceptibility testing.

Since the preparations here (vegetable oils/extracts) are not standard antibiotics, this general principle is used.

General Principle: The higher the diameter indicates higher effectiveness, and a lower diameter or absence of halo means resistance or weak effectiveness.

4. PRACTICAL CONCLUSIONS

All extracts possess anti-*Streptococcus* activity, but Karoui and Arak offer the greatest inhibition diameters at most concentrations, suggesting a potentially higher efficacy.

The best concentration of arak in this experiment is 50 ml/μg (39 mm), while the villager shows the highest effectiveness at 100 ml/μg (36 mm), and the behavior of the cloves can be described as effective but without a significant increase after a concentration of 25 - 50 ml/μg.

Interpretation of the results of Table 8:

Table 8. Testing of plant extracts with *staphylococcus*.

	Staphylococcus				
Extracts	**Disc Content (μg)**	**Zone diameter (mm)**	**Resistant (R)**	**Intermediate (I)**	**Susceptible (S)**
Clove Oil Extract	**12**	30			X
	25	36			X
	50	30			X
	100	25			X
Al-Qarawi Extract	**12**	25			X
	25	30			X
	50	20			X
	100	35			X
Arak Extract	**12**	30			X
	25	30			X
	50	30			X
	100	25			X

The values in the table (20 to 36 mm) show that clove, village and arak extracts induce significant inhibition zones against *Staphylococcus* bacteria, which are greater than most of the sensitivity limits of the standard tablets in the CLSI manual and can therefore be considered to be highly effective against these bacteria in the test described.

5. SCIENTIFIC CONCLUSIONS

Clove oil extract gave diameters of 25 to 36 mm, highest at a concentration of 25 ml/μg (36 mm),

indicating a prominent inhibitory power that may be associated with the concentration of eugenol and other phenolic compounds.

The extract of al-Qarawi ranged in diameter from 20 to 35 mm, with a marked increase at 100 ml/μg (35 mm), demonstrating a clearer dose-response relationship compared to other extracts.

Arak extract gave approximately constant diameters (25 - 30 mm), which may reflect the stability of efficacy at tested concentrations or saturation of the active ingredient medium at relatively low concentrations.

Interpretation of the results of Table 9:

Table 9. Test of plant extracts with *Lactobacillus* bacteria.

	Lactobacillus				
Extracts	**Disc Content (μg)**	**Zone diameter (mm)**	**Resistant (R)**	**Intermediate (I)**	**Susceptible (S)**
Clove Oil Extract	**12**	26			X
	25	35			X
	50	40			X
	100	42			X
Al-Qarawi Extract	**12**	25			X
	25	35			X
	50	35			X
	100	36			X
Arak Extract	**12**	45			X
	25	36			X
	50	42			X
	100	50			X

An inhibition area greater than 20 N·mm is considered evidence of high efficacy against bacteria, while less than 10 mm is considered ineffective or weak.

In this experiment, all extracts exceeded 20 mm at medium and high concentrations, demonstrating good to excellent efficacy.

Clove oil: A clear increase in the diameter of the inhibitory zone is shown with increased concentration demonstrating a strong direct relationship between concentration and effectiveness against *Lactobacillus*.

These results suggest that clove oil has a high potency against these bacteria, especially at high concentrations.

Villages: It shows an increase in diameter up to 25 μg/ml (35 mm), then does not change significantly at higher concentrations (35 - 36 mm), indicating that its effectiveness reaches saturation limits at 25 ml/μg and does not increase significantly at higher concentrations, *i.e.* it has specific limits of efficacy against *Lactobacillus*.

Arrak: Shows almost the same pattern as clove oil, as its effectiveness increases with increased concentration (from 45 mm at 12 μg/ml to 50 mm at 100 μg/ml), demonstrating high and sustained effectiveness with increased concentration.

This suggests that ARAC extract has a strong effect against *Lactobacillus*, and may be among the most effective extracts in this experiment.

Arak extract shows the highest efficacy against *Lactobacillus*, followed by clove oil, and then villagers. All extracts show good efficacy, but the saturation limit of the village extract is 25 μg/ml.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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