RESEARCH ARTICLE

Evaluation of the Biological Efficacy and Physicochemical Evaluation of Toothpaste Prepared from Few Types of Plant Extracts against Some Pathogenic Bacteria Isolated from the Mouth

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ABSTRACT

The demand for a herbal-based products such as toothpaste is high these days. Consumers believed that herbal-based toothpaste are safe, effective and less toxic because few and safe chemicals is used as compared to synthetically produced toothpaste. Therefore, this study was amied to formulate and evaluate new polyherbal toothpaste containing herbal extracts available to treat periodontal problems. The polyherbal toothpaste was formulated using three herbal extracts namely lemongrass (*Cymbopogon citratus* L.) marjoram (*Qriganum majorana* L.) and salivia (*Salvia officinalis* L.*)* and tested against *Streptococcus mitis-orul Streptococcus pyogen*, *Streptococcus mutan*s, *Enterococcus faecalis Enterococcus faecium, Gemella morbilloe, Staphylococcus aureus, Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Escherichia coli, Klebsiella pneumonia ssp*, *Acinetobacter baumannii* complex*, Pseudomonas aeruginosa, with different* concentrations varying from 100, 50, 25, 12.5, and 6.25 mg/mL. The significant inhibition has seen against *Streptococcus mutans* (27 mm) and there is no inhibition for both *Acinetobacter baumannii* complex *and P. aeruginosa.* The formulated toothpaste was also evaluated with the standard physiochemical parameters along with the antimicrobial activity. it opens a window for future study to enhance the ability of the toothpaste and to prove the efficacy and safety of the formulated toothpaste. Then a comparison was made with commercial toothpastes for three selected types.

Keywords: Antibacterial activity, Comparative evaluation with commercial toothpaste, *Cymbopogon citratus* L., Herbal toothpaste, Oral bacteria, *Qriganum majorana* L., Salvia officinalis L.

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INTRODUCTION

Dental care addresses problems such as bad breath, tooth decay, periodontal, tooth sensitivity, etc., lemongrass, majorana, and saliva from medicinal plants. Herbal used all over the world and does not cause any side effects as used as an anti-inflammatory and antimicrobial as well as used as disinfectant, suppressor and treatment of dentistry in this formula uses calcium carbonate used as adhesives and sealants, sodium lauryl sulphate as a cleaner, glycerin as a sweetener and preserves and attracts moisture, acacia glue as a thickening agent, water for dilution purposes, sugar as a sweetener, and preservatives as a shield For the growth of microorganisms as the use of toothpaste, toothbrush and mouthwash containing antimicrobial agents is commonly used as products that improve oral hygiene dating back to ancient times and continue to date toothpaste is considered an indispensable factor in the effective home care system as it is a gel or paste used with a toothbrush to clean and maintain teeth in order to promote oral health.[1] Given the huge number of brands of many toothpastes on the market, the effectiveness of these toothpastes in controlling the number of bacteria should be analyzed scientifically as the high popularity of herbal and natural products has led dental professionals to assess the effectiveness of these products and make evidence-based suggestions to their patients to make a better choice [2]

MATERIALS AND METHODS

Botanical Plant Collection

Get the leaves of the lemongrass plant, majorana, and salvia plant, from local markets in Diyala governorate. It was purified and broken into small pieces, then washed with distilled water twice, and then left to dry in the room and in the presence of an air stream, in which it dried completely, Then it was crushed

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and kept in a bottle in a vial at a temperature of 25°C for use in the process of preparing plant extracts.

Clinical Specimen Collection

62 clinical samples were collected from different pathological injuries of patients from specialized dental centers in the governorates of Baghdad and Diyala. The collection cases included gingivitis, tooth decay and inflamed roots in both sexes and of different ages. The collected samples were distributed as follows: 8 clinical samples from the Martyr Dr. Waseem Khudhair Abbas specialized Dental Center in Al-Hussainiya City, Baghdad, Al-Rusafa, 5 clinical samples from the specialized Dental Center in Al-Shaab City, and 18 clinical samples from the first specialized dental center in Baqubah City, Diyala Governorate and 31 clinical samples from the second specialized dental center in the new city of Baquba, Diyala governorate. The samples were collected under the supervision of the specialized doctor through sterilized cotton swaps, and then the swabs were transferred to the scientific laboratories, Baquba Teaching Hospital in Diyala governorate for the purpose of isolation and diagnosis of bacteria using the Vitek 2 device.

Preparation of Extracts

Preparation of alcoholic and crude hot water extracts for plants under study adopted a method[3] with some modifications. The powder was packed in soxhlet apparatus and continuous extraction process was done for about 6 hours at 50°C with methanol. After the extraction process, the product was collected, shade dried for 10 days, and the extract was powdered. The standard toothpaste base was formulated. Powdered extracts were incorporated in the base in various concentrations as given in Table 1. The formulations were filled in tubes, and the chemical components were weighed and crushed in the mortar as indicated in Table 1. Then glycerin and soaked *acacia* were added to the above mixture, then this solution was added by drop to a blender containing herbal ingredients and crushed well until a paste consistency is formed, as shown in Figure 1.[4]

Figure 1: Prepared toothpaste mixture.

Antimicrobial Evaluation of Toothpaste

Three conventional toothpastes and the formulated toothpaste were selected, conducting prepared toothpaste tests and evaluation criteria. The inhibitory effect on bacterial isolates was studied as follows:

- The bacterial suspension was prepared by taking a newly grown colony grown on the solid nutrient media and placing it in the physiological saline solution. The tube was shaken by the media mixer and the turbidity was adjusted as it became equal to the 0.5 McFarland solution, which is equivalent to 1.5×10^8 cells/mL.
- Transfer 100 mL of the bacterial suspension prepared in the previous step to the solid nutrient medium and spread it on all plates using a sterile cotton swab and leave for 10–15 minutes to dry.
- Six holes were made in the dishes using a sterile cork borer with a diameter of 6 mm, filled with each of the prepared and commercial toothpastes, as well as the negative control group represented by the chemical components of the prepared pastes without adding plant extracts and separately in each hole.
- The petri dish were placed in the incubator for 24 hours at a temperature of 37°C
- Then, the diameters of the inhibition zone were measured, which was the non-growth zone surrounding the hole.[5]

Physicochemical Evaluation of Manufactured Toothpaste

Appearance

The prepared toothpaste and the marketed toothpaste were exposed to detect any change in the appearance of the toothpaste when kept for a long period of time, which is three months starting from the date of preparing the formula From 1-7 to 1-10/2021.[6]

pH

pH of formulated herbal toothpaste was determined by using pH meter. One gram of toothpaste was placed in 100 mL of beaker. Allow the 10 mL of distilled water and stir vigorously until a mixture is formed.[7]

Foam

The foaming ability of formulated toothpaste was evaluated by taking a small amount of formulation with water in a measuring cylinder; initial volume was noted and then shaken for 10 times. The final volume of foam was noted. The foam was calculated for the prepared toothpaste through the following equation

Foam height = L1 - L2

L1 = Volume in milliliters of foam with water L2 = Volume in milliliters of water only.[8]

Moisture and Volatile Matter

Determination of moisture and volatile matter 5 g of formulation placed in a porcelain dish in it. Dry the sample in an oven at 101°C. Calculation moisture percentage = (After drying weight - real sample weight)/(real sample weight) x 100.[9]

Table 1: shows the quantities of plant extracts and the chemical substance.

No.	*Ingredients*	*Quantity used (%)*	*Importance*
1	Lemon grass	1-gm	anti-bacterial
2	Marjoram	1-gm	anti-bacterial
3	Salvia	1-gm	anti-bacterial
4	Calcium carbonate	2.5 gm	abrasive
5	Glycerine	625 mL	moisturizer
6	Sodium lauryl sulphate	0.125 gm	foaming agent
7	*Acacia* gum	2 gm	binder
8	Sodium chloride	2 gm	preservative
9	Sodium saccharin	0.025 gm	local factor
10	Para hydroxide benzoic acid	0.125	detergent and foaming agent
11	Distilled water	10 mL	

Cleaning

In this method, one egg was used (eggshells contain a high amount of calcium and tooth enamel), which is the most approximate ratio to test the cleaning ability of toothpaste. One eggshell was used for each toothpaste tested. By heating 200 mL of water until boiling in a beaker. Then add 15 mL and 20 drops of vinegar and red food coloring, respectively. Submerge a boiled egg in the food coloring solution for 5 minutes until it stains red, then draw a line along the eggshell dividing it in half. And using a toothbrush after moistening it with distilled water to brush one side of the egg for 10 strokes (every motion was a complete back and forth motion.) The ability of water to remove any color was noted. Then rinsed the toothbrush with water, dried the water, and put a pea-sized amount of compound toothpaste on the toothbrush to brush the opposite side of the egg for 10 strokes. The egg was rinsed and checked for color removal. The procedure was repeated for each toothpaste tested.[3]

Stability

The test was carried out for the formulations prepared at room temperature studied for 7 days at 40°C for 30 days. The formulations were kept both at room and elevated temperature and observed on 0, 30, determined various parameters specified.[10]

Spreadability

In this method, the sliding and pulling characteristics were determined. A prepared paste of 2 g of prepared toothpaste was placed on a slide. The prepared paste, was placed between this slide and other glass slides for 5 minutes like a sandwich to expel the air, and to provide a uniform layer of paste between the slides. The excess was removed from the dough from the edges was pulled out of the upper slice by means of a thread weighing 80 g, and the time required to pull the first slice was recorded, as the short period of withdrawal indicated a better spreading ability. The following equation was used to calculate the diffusion capacity

$S = M \times L / T$

S = Spreadability M= Weight in the pan ((tied to the upper slide) L = length moved by the glass slide T = Time(sec) taken to separate the upper slide from the ground slide.[10]

Scraping

A small amount of prepared toothpaste was placed on a clean from a glass microscope slide, and a drop of distilled water was added and rubbed with a clean cotton swab with back and forth movements for 30 times. Then the slide was carefully rinsed and dried with a clean and soft cotton swab. Then the slide was examined under the microscope. The number of scratches was determined and rated on a scale from 0 to 5 (no scratches).[4]

Homogeneity

Marketed as a uniform distribution in the cream confirmed by visual appearance and tactile production laboratory scale.[11]

Viscosity

This test was done using a viscometer, 25 g of the prepared toothpaste formula was placed in a glass beaker with a volume of 50 mL, then the device was set at 100 cycles per minute for a period of 5 minutes and at a temperature of 37°C, then the viscosity value was read.[9]

Statistical Analysis

Use the statistical program statistical analysis system SAS (2012). In data analysis to study the effect of different treatments on the studied traits, according to a completely random design (CRD), the significant differences between the means were compared with the least significant difference test (least significant difference - LSD). The readings were considered significant at the significance level $p \leq 0.05$.

RESULTS AND DISCUSSION

Classification of Plants under Study

The plant under study was classified based on plant classification by the teacher Dr. Arij Abdul Sattar Al-Rawi of the College of Education Ibn al-Haytham, University of Baghdad, Plant classification specialty as shown in Table 2 and Figure 2. Form 2 shows the parts of the dried plant studied and the parts used.

Determination of Microbial Content

The results of the inhibitory effect of the manufactured toothpastes were shown and compared with the commercial

Table 2: The plants under study with the parts used.

No.	*Local name*	*Scientific name*	*Family*	*The part of the plant used*
1	Lemongrass	*Cymbopogon citratus* L.	Gramineae	papers
2	Marjoram	*Qriganum majorana* L.	Lamiaceae	whole plant
3	Salvia	*Salvia officinalis* L.	Lamiaceae	whole plant

toothpaste selected for the purpose of comparison and evaluation of their antimicrobial activities, alcoholic plant extracts and similar to commercial pastes, with the largest inhibition diameter of 27 mm. the microorganisms tested in this study are closely related to oral diseases *S. mutans* is one of the most common bacteria found in dental biofilms *P aeruginosa*, *E. faecalis* is well known for its roles in gum disease and inflammation *E. coli*, *S. pyogines* and other types of oral microorganisms were also evaluated, as shown in the table to compare the antimicrobial activity Figure 3.[12] The results showed differences between the tested microorganisms, where the average diameter of the largest inhibition was in *S. mutans* and *S. mittis* isolates, with a diameter of inhibiting 26 and 27, respectively for the alcoholic toothpaste formula, while the aqueous solvent toothpaste formula did not affect the pathological isolates *A. baumannii* complex and *P. aeruginosa*. The results differed with the findings[13] due to the change in concentrations of other extracts or technique of preparing toothpaste formula. After conducting a statistical analysis of the obtained results, it was noted that there were significant differences under the level * ($p \leq 0.05$). The paste formulations prepared from aqueous solvent showed greater ability to inhibit the studied pathogenic bacterial isolates (Table 3).

Figure 2: The study of parts of the dried plants and the parts used.

Physicochemical Tests

The study revealed that the formulations formulated in the laboratory had a nearly constant pH homogeneous appearance and spreadability. The formulated formulations also showed a good cleaning ability and a relative density similar to that of the marketed formulations. Several studies that evaluate the relationship between the abrasive potential of toothpaste

Table 3: The rate of measuring the diameters of the inhibition zones for the formulations of formulated pastes and the commercial pastes selected for comparison.

Plant extracts						*Isolates*
LSD	*Herbal clove toothpaste*	*Dabur herbal basil*	*Lacalut aktiv medical toothpaste*	*Toothpaste formula watery*	*Alcoholic toothpaste formula*	
3.69 *	26	22	22	24	27	*Streptococcus mitis-orul*
2.84 NS	23	24	24	25	26	*Streptococcus pyogen*
2.76 NS	26	25	25	27	27	*Streptococcus mutans*
4.34 *	18	27	27	18	22	*Enterococcus faecalis*
2.68 NS	23	22	23	22	23	*Enterococcus faecium*
4.77 *	16	19	19	16	22	*Staphylococcus aureus*
3.81 *	16	10	12	16	18	*Staphylococcus epidermidis*
3.67 *	22	12	12	21	18	*Staphylococcus haemolyticus*
4.94 *	22	9	9	23	24	*Escherichia coli*
4.72 *	14	11	111101 10	12	22	*Gemella morbilloe*
4.66 *	10	9	8	11	23	*Klebsiella pneumonia ssp*
5.01 *	6	9	8	0	19	*Acinetobacter baumannii complex*
4.78 *	4	0	4	0	22	*Pseudomonas aruginosa*
----	4.97 *	5.26 *	5.08 *	5.71 *	4.87 *	LSD

* ($p \leq 0.05$).

Table 4: The results of re-testing the physicochemical composition of the prepared pastes from for the time period from 1/7 to 1/8/2021

S.no	*Parameters*	*Alcoholic toothpaste formula*	*Toothpaste formula watery*	*Daber herbal basil*	*herbal clove toothpaste*	*Lacalut aktiv medical toothpaste*
1	Appearance	Dark green and herbal scent	Dark brown and smell herbal	light green and a special smell	brouwn and the smell of cloves	White and a special smell
2	pH	8.26	7	8	5	5
3	Foamability	10.5	10.3	10	10	10
4	Cleaning	Good	Middle	Good	Good	Good
5	Diffusion (cm/sec)	3.7	3.7	3.6	3.6	3
6	Stability	Good	Good	Good	Good	Good
7	Homogenecity	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous
8	Viscosity	1000–0007 CPS	1000–4000 CPS	700–1000 CPS	700–1000 CPS	700–1000 CPS

Table 5: The results of re-testing the physicochemical composition of the prepared pastes for the period from 1/8 to 1/9/2021

S.no	*Parameters*	*Alcoholic toothpaste formula*	*Toothpaste formula watery*	*Daber herbal basil*	*herbal clove toothpaste*	*Lacalut aktiv medical toothpaste*
1	Appearance	Dark green and herbal scent	Dark brown and smell herbal	light green and a special smell	brown and the smell of cloves	White and a special smell
2	pH	8.26	7	8	5	5
3	Foamability	10.5	10.3	10	10	10
4	Cleaning	Good	Middle	Good	Good	Good
5	Diffusion (cm/sec)	3.7	3.7	3.6	3.6	3
6	Stability	Good	Good	Good	Good	Good
7	Homogenecity	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous
8	Viscosity	1000–0007 CPS	1000–4000 CPS	700–1000 CPS	700–1000 CPS	700–1000 CPS

Table 6: The results of re-testing the physicochemical composition of the prepared pastes for the period from 1/9 to 1/10/2021

S.no	*Parameters*	*Alcoholic toothpaste formula*	*Toothpaste formula watery*	*Dabyr herbal basil*	*herbal clove toothpaste*	*Lacalut aktiv medical toothpaste*
1	Appearance	Dark green and herbal scent	Dark brown and smell herbal	light green and a special smell	brouwn and the smell of cloves	White and a special smell
2	pH	8.26	7	8	5	5
3	Foamability	10.5	10.3	10	10	10
4	Cleaning	Good	Middle	Good	Good	Good
5	Diffusion (cm/sec)	3.7	3.7	3.6	3.6	3
6	Stability	Good	Good	Good	Good	Good
7	homogenecity	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous
8	Viscosity	1000–5000 CPS	1000–3000 CPS	700–1000 CPS	700–1000 CPS	700–1000 CPS

and the alteration on enamel have revealed that the decrease in abrasive potential of toothpaste, the less enamel gets worn out.[14,15] However, the higher the abrasive potential the better the stain removal. In the present study, the watery and alcoholic toothpaste formula is the only toothpaste containing calcium carbonate in its composition. Toothpaste with this composition are less abrasive.[15] Hydrated silica is abrasive in lacalut aktiv medical toothpaste, Dabur herbal basil and herbal clove toothpaste. Reports from previous studies have shown that silica exhibit strong abrasive properties that can increase roughness.[16] All toothpaste showed the ability to clean the enamel irrespective of the absence or presence of detergent in their composition. The stability test is a close approximation of the shelf life of the products. All products remain effective for months. Usually, a sample stored at room temperature, 40°C, refrigerator temperature 6°C, for 12 weeks is equivalent to one stored at room temperature for a 3 months. as the results obtained from the current study are presented in table below and for the time period from 1/7 2021 to 1/8 2021, *i.e.,* for a period of 30 days from the date of the first formulation with continuing the examination for a period of 3 months, i.e., until 10/1/2021, as shown later in Tables 4-6.

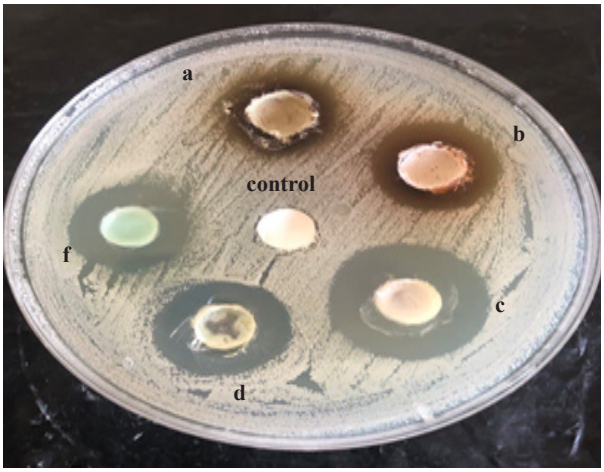

Figure 3: The effect of manufactured toothpaste on bacterial isolates *S.mutans.*
F = commercial toothpaste A = Toothpaste formula with aqueous extract B = The composition of the toothpaste with alcoholic extract C = Clove toothpaste D = basil toothpaste

CONCLUSION

The extract of marjoram, cinnamon oil, lemongrass, and salvia was used for the formulation of toothpaste. Marjoram and lemongrass plays a vital role in herbal medicinal toothpaste. The evaluation parameter of the toothpaste suggested that the formulated toothpaste and marketed during stability study. The study shows that, the formulation of toothpaste containing herbal extract can be possible, which is used as a good toothpaste.

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