RESEARCH ARTICLE

Preparation and Evaluation of the Effectiveness of Mouthwash Prepared from Few Types of Plant Extracts against Some Types of Pathogenic Bacteria Isolated from Mouth

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ABSTRACT

Problems associated with drug resistance and the toxicity of some medicinal compounds currently in use are a major problem that requires searching for new compounds to overcome these problems. In the current study, two types of mouthwash were prepared, the first representing the mixture of aqueous extracts of *Melissa officinalis* L., *Pimpinella anisum* L. and *Silybum marianum* L. plants and the second represented the mixture of their methanolic extracts. The inhibitory activity of the two prepared types of mouthwash was tested in comparison with the effectiveness of commercial products available in the Iraqi markets, namely oral B and alpha zac against some types of pathogenic bacteria isolated from the mouth of patients attending to some specialized dental centers in the city of Baghdad, where three of them were negative for gram stain represented by (*Enterobacter cloacae ssp dissolvens, Kluyvera intermedia* and *Serratia marcescen)*. The four gram-positive represented by *Enterococcus faecium, Streptococcus mutans, Streptococcus parasanguinis* and *Staphylococcus aureus*, using the diffusion agar method and tube method to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The methanolic and aqueous extracts showed inhibitory activity against most types of bacteria included in this study, as the inhibitory values ranged between 10 to 24 mm. The aqueous's lowest inhibitory dilution (MIC) was 28% at a concentration of 6.25 µg/mL, and the lowest bactericidal dilution (MBC) was 14% at a concentration of 12.5 µg/mL. These results are close to the inhibitory properties of the methanolic extract, as no significant differences were observed in both MIC and MBC values between both lotions. The prepared aqueous and methanolic extracts lotion showed distinctive stability in terms of pH, density, color, smell and homogeneity over a period of three months from the date of preparation under different storage conditions.

Keywords: Antimicrobial activity, *Melissa officinalis* L., Mouthwash, Pathogenic bacteria, *Pimpinella anisum* L., *Silybum marianum* L .

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INTRODUCTION

Bad health habits are one of the main problems that affect public health, while oral diseases are one of the most important diseases associated with these practices. Oral health is often a reflection of the health of the individual[1] as the oral cavity is an environment suitable for the growth of germs moist, constant temperature and a pH close to neutral, in addition to the nature of the composition of oral tissues, which makes it a source of different types of bacteria. A number of gram-negative and gram-positive bacteria have been recorded, which includes more than 500 species endemic the oral cavity.[2] The presence of many of them in the mouth has been classified naturally and is called oral flora.[3] At the same time, some of them are harmful and cause tooth decay and gum disease. They are either gram-negative such as *Pseudomonas spp, Enterobactar spp* and *Actinomycetes* or gram-positiv, such as, *Streptococcus mutans, Streptococcus viridians, Staphylococcus aureus, Porphyromonas gangivalis and Lactobacillus acidophilus.* So maintaining oral hygiene greatly contributes to reducing the harmful microbial content and thus controlling diseases that can affect the mouth, tooth decay and gum disease.[4] Many types of pathogenic bacteria that infect the mouth, such as *S. mutans, S. aureus* and *P. spp* possess a number of virulence factors such as adhesion factors on the surface of the microbe, which is the first stage of colonization of host tissues and it possesses the enzyme hemolysin, and it also produces many

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enzymes such as proteases, nucleases, gelatinase and lipases, as well as its production of biofilm. Due to the emergence of bacterial strains with multiple resistance to antibiotics, long treatment period and its high cost, so recently, there has been a call to return to nature for safe health and to search for natural therapeutic alternatives. Therefore, many plant extracts have been used nowadays as a treatment for diseases, especially in the treatment of bacterial infections, to reduce errors caused by the excessive use of synthetic, medicinal drugs.[5]

Medical mouthwash is one of the current effective solutions to treat gingivitis, toothache and, plaque because it contains effective chemical compounds such as thymol, hexedene, fluoride, calcium and sodium benzoate, which works to obtain the refreshing smell of mouthwash, as well as sweeteners such as sorbitol, sodium saccharin, and xylitol, which is used as a bacterial inhibitor, but the presence of ethanol in these mouthwashes is most likely associated with oral cancer.[6] In recent years, the tendency towards the use of medicinal herbs has appeared imposed by several factors, including that many of them are considered as natural food, in addition to the fact that plants are free from side effects caused by chemical and industrial materials.[7] Either by chewing the leaves of herbs, using their water soak, or applying its essential oil to the affected tooth. Among these herbs are eucalyptus, whose oil contains the active substance (Cineol), peppermint (Menthol) and cloves (Eugenol) and (Carvacrol) in licorice and cardamom.[8] This study aims to prepare and evaluate the effectiveness of a mouthwash prepared from the aqueous and methanolic extracts of *Melissa officinalis* L., *Pimpinella anisum* L. and *Silybum marianum* L. against a number of pathogenic bacteria isolated from the mouth (*Enterobacter cloacae ssp., Kluyvera intermedia occus, Serratia maroceens, Enterobacter cloacae ssp., Faecium, S. mutans, Streptococcus parasanguinis*) isolated from the mouth and comparing its efficacy with that of a number of oral mouthwash available in the Iraqi markets.

MATERIALS AND METHODS

Clinical Specimen Collection

Clinical samples (30) were collected from the mouths of patients attending the specialized dental centers in Baghdad governorate from different parts of the mouth, including (gingiva, roots, canines, premolars, centrals and molars). The collected samples were distributed as follows:

- clinical samples (19) from the specialized dental health center in Sheikh Omar.
- clinical samples (11) from the specialized dental center in Al-Maghrib Street.

Clinical samples were collected by the specialist doctor using sterilized cotton swaps. Then swabs were transferred in a sterile container to the postgraduate laboratory, Department of Science, College of Education, Al-Mustansiriyah University, where they were classified with the Vitek 2 system.

Plants used in the Study

Three types of plants (melissa, galangal and anise) that grow in the Iraqi environment were collected for use in experiments. Prof. Dr. Areej Abdul-Sattar Farman classified them in the College of Education for Pure Sciences, Ibn Al-Haytham College, University of Baghdad.

Melissa Officinalis L.

Dried *Melissa* leaves were obtained from the local markets in Wasit Governorate, Iraq. The leaves were washed with distilled water, dried in the shade for seven days, ground to a fine powder, and kept in a bottle at 25°C until use.

Pimpinella anisum L.

Dried anise seeds were obtained from the local markets in Wasit Governorate, Iraq. The seeds were purified, ground into powder, and kept in a bottle at 25°C until use.

Sillybum marianum L.

The leaves and stems of the *Silybum marianum* L. were obtained from farms in Al-Aziziyah, Wasit governorate in Iraq. The collection process took from 27/2/2021 to 3/08/2021, washed, cut into small pieces and left to dry in the shade; this process was carried out away from sunlight and took two weeks. Then kept in a bottle at 25°C until use.

Preparation of Aqueous and Methanolic Extracts of *Melissa*, *Anisum* and *Sillybum*

The 10 gm of crushed *M. officinalis* L. leaves, *Silybum marianum* L .leaves and stems and *Pimpinella anisum* L. seeds were placed separately in the soxhlet extractor and the active substances were extracted using 300 mL of distilled water or methanol for 12 hours. The extracts were filtered using whatman no. 1 filter paper, this procedure was repeated twice,

Table 1: Ingredients for mouthwash using aqueous and methanolic extracts of *M. officinalis* L., *P. anisum* L. and *S. marianum* L.

Ingredient	*Function*	*Formulation*	
		Methanolic Mouthwash	*Aqueous Mouthwash*
Aqueous (*M.officinalis* L.)	Active drug	-	1 g
Aqueous *(S. marianum* L.)	Active drug	-	1 g
Aqueous (*P.anisum* L)	Active drug	-	1 g
Methanolic (*M. officinalis* L.)	Active drug	1 g	-
Methanolic (*S. marianum* L.)	Active drug	1 g	-
Methanolic *(P. anisum* L.)	Active drug	1 g	-
Deionized water	Base	10 mL	10 mL
Glycerol	Surfactant	0.65 µL	0.65 µL
Sodium benzoate	Preservative	0.01 g	0.01 g
Honey	Sweetener	0.01 g	0.01 g
Tween-20	Surfactant	0.35 µL	0.35 µL

Table 2: Types and number of bacteria isolated from clinical samples and the media on which they were grown

No.	*Kind of Bactria*	*Culture medium*	*Bactria No.*
1	*Strptococcus parasanguinis*	De-Man Rogosa agar (MRS)	5
2	*Strptococcus mutans*	Mitis Salivarius Agar (M-S Agar)	5
3	*Enterococcus faecium*	Bile esculin agar	5
4	*Enterobacter cloacae ssp dissolvens*	Eosin-methylene blue agar (EMB)	4
5	*Kluyvera intermedia*	MacConkey agar	4
6	*Staphylococcus aureus*	salt agar Mannitol	5
7	*Serratia marcescens*	Cetrimide agar	2

after that the extracts were poured into glass dishes, dried and kept in glass bottles in the refrigerator at 4°C until use.[9]

Preparation of Mouthwash from Aqueous or Methanolic Extracts of *Melissa*, *Anisum* and *Silybum*

Mouthwash was prepared using a mixture of aqueous or methanolic extracts of *Melissa*, *Anisum* and *Silybum* plants, based on reports[10] as shown in Table 1.

Study the Inhibitory Effect of Mouthwashs (methanolic , aqueous, alpha zac, Oral B) using Diffusion Agar.

The inhibition effect of aqueous and methanolic mouthwash against bacterial isolates in comparison to Alpha zac, Oral B was studied according to the following method. The bacterial suspension was prepared by taking a smear from the bacterial colony to be tested, which is a newly grown colony on the solid nutrient medium its age ranges between 18 to 24 hours, and placed in the physiological saline solution.Then, shake the tube with a vortex mixer and adjust the concentration to 0.5 McFarland solution, equivalent to 1.5 x 10^8 cells/mL. The 100 µL of the prepared bacterial suspension was transferred to nutrient agar, spread on all plates using a sterile cotton swab, and left for 10–15 minutes to dry. After that, five holes were made in the dishes using a sterile 6 mm diameter cork drill, then 100 µL of each of aqueous, methanolic, Alpha zac, Oral B mouthwashes and negative control sample were placed separately in each hole, then the dishes were incubated in the incubator for 24 hours at of 37°C, and the diameters of the inhibition zone were measured, which represented the non-growth zone surrounding the hole, as mentioned by.[11]

Study of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) for Methanolic, Aqueous, Alpha zac and Oral B Mouthwash using the Tubes Method

The MIC and MBC of the mouthwash prepared using the tubes method were calculated by dissolving the prepared mouthwash (methanolic and aqueous) and the commercial Alpha zac and Oral B that were purchased from a pharmacy in Baghdad, with different concentrations in 2 mL of nutrient broth , with mixing, to prepare mouthwash with the following half diluted concentrations (100, 50, 25, 12.5, 6.25, 3.125) µ/mL.

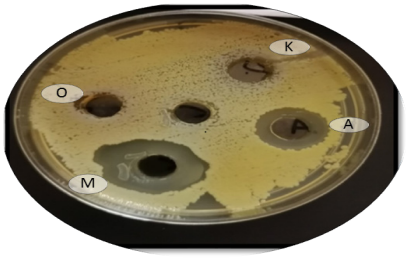

Figure 1: The inhibition of alcoholic, aqueous, Alpha zac and Oral B mouthwashes on Muller Hinton agar medium against *Staphylococcus aureus* bacteria (A = Alpha zac, O = Oral-B, K = methanolic mouthwash, M = aqueous wash**)**

The bacterial suspension was prepared by taking a smear from the newly grown bacterial isolate and diluting it with physiological saline, then it was compared with McFarland's solution to give an approximate number of cells equal 1.5 x 10^8 cells/mL. A quantity of 10 µL of the bacterial suspension was placed in each tube, then the tubes were exposed to the vortex mixer to ensure the distribution of bacteria in the suspensions. And then it was placed in the incubator for 24 to 48 hours at 37°C in aerobic and anaerobic conditions. After the end of the incubation period, each dilution of the tubes was grown on the solid nutrient medium by adding 10 mL of the mixture to the dish, and then the dish was incubated for 24 hours at 37°C in aerobic and anaerobic conditions, as the solid nutrient medium was used instead of Muller Hinton medium. The results were read, considering MIC was the area in which the last growth occurred on the medium, while the area that preceded it was considered to be the MBC area, as mentioned in.[12]

Evaluation of prepared Methanolic and Aqueous Mouthwash by Physical Factors (color, odor, relative density, homogeneity, pH)

Relative Density of the Mouthwash

This test was carried out in a density apparatus, which is a short-necked glass container of 5 mL volume with a glass cover containing a capillary tube through which the liquid exceeding the volume of the vial passes. This bottle is used to calculate the density of liquids by weighing it first with the cap when it is empty (m1), and secondly with distilled water (m2), then weighing it with mouthwash inside. By weighing the liquid inside it from the difference between the weight of the bottle filled and the weight of the empty bottle, we calculate the result by dividing the mass by the volume of the bottle, we get the density in units of g/cm^3.[13]

$$\mathbf{d} = \frac{m1-m2}{\mathbf{v}}$$

$$\mathbf{d} = \frac{m}{\mathbf{v}}$$

m = the mass of the liquid in grams, (v) represents the volume of the liquid (cm^3) and (d) represents the density of the liquid in units (g/cm^3)

Table 3: The inhibitory effect of each mouthwash (alcoholic, aqueous, Alpha zac, Oral B) using diffusion agar method.

Type of Bactria	*Bactria Name*	*Isolate NO.*	*Methanol*	*aqueous*	*Control*	*Alpha Zac*	*Oral-B*	*LSD*
			inhibition zone diameter mm					
gram-positive	*Streptococcus parasanguinis*	6	24	22	19	12	0	3.71*
		7	15	17	0	0	0	2.55*
		10	0	9	0	0	0	2.16*
		29	0	0	0	0	0	NS
		30	11	13	0	0	0	2.26*
	Stapylococcus aureus	2	17	18	0	16	12	2.63*
		5	18	18	0	12	10	2.58*
		17	12	0	0	13	18	2.66*
		18	20	20	0	10	10	3.09*
		29	22	0	0	11	0	3.15*
	streptococcus mutans	4	15	14	0	13	12	2.75*
		9	22	16	0	15	11	2.91*
		30	20	20	0	16	10	3.20*
	Enterococcus Faecium	1	0	0	0	0	0	NS
		6	13	12	10	0	0	2.16*
		26	11	11	0	0	11	2.08*
		27	0	0	0	0	0	NS
		28	15	14	11	13	10	2.77*
		29	17	18	12	0	11	2.96*
		30	8	0	0	0	0	2.16*
gram-negative	*Kluyvera intermedia*	9	15	22	0	15	13	4.09*
		13	20	24	0	15	14	4.12*
		16	14	15	0	13	10	2.65*
		18	12	15	0	10	11	2.41*
	Enterobacter cloacae ssp dissolvens	1	22	24	0	12	9	3.66*
		9	15	0	0	0	0	2.52*
		13	20	18	0	12	0	2.96*
		16	15	20	0	14	0	2.81*
		19	22	18	0	10	10	3.28*
	Serratia marceseens	13	13	0	0	0	11	2.63*

($p \leq 0.05$)* NS= Nonspecific

Table 4: Study of MIC and MBC of methanolic, aqueous, Alpha zac ad Oral-B for *Enterobacter Cloacae ssp dissolvens***.**

	S. marianum L. alcohol extract of aerobic negative bacteria												
Effect ratio		Enterobacter Cloacae ssp dissolvens						*Enterobacter Cloacae ssp dissolvens*					
Mg/mL	LSD value	3.125	6.25	12.5	25	50	100	3.125	6.25	12.5	25	50	100
MIC%	7.61 *	0	0	50	0	0	0	0	100	0	0	0	0
MBC%	8.60 *	0	0	50	100	100	100	0	0	100	100	100	100
Effect ratio%	*Kluyvera intermedia*							*Kluyvera intermedia*					
MIC%	7.35 *	0	20	80	0	0	0	0	80	80	0	0	0
MBC%	9.05 *	0	0	20	100	100	100	0	0	20	100	100	100
Effect ratio%	Serratia marcesens							*Serratia marcesens*					
MIC%	7.51 *	0	100	0	0	0	0	0	100	0	0	0	0
MBC%			0	100	100	100	100	0	0	100	100	100	100

Table 5: Study of MIC and MBC of methanolic, aqueous Alpha zac ad Oral-B for, *Enterococcus faecium*, *Streptococcus mutans*, *Streptococcus parason guinis*

	Methnolic mouthwash , positive anaerobic bacteria												
Effect ratio		*Enterococcus faecium*						*Enterococcus faecium*					
mg/mL	LSD value	3.125	6.25	12.5	25	50	100	3.125	6.25	12.5	25	50	100
MIC%	7.50 *	0	0	100	0	0	0	0	28	71	0	0	0
MBC%	8.33 *	0	0	0	100	100	100	0	0	28	100	100	100
Effect ratio%		Streptococcus mutans						Streptococcus mutans					
MIC%	7.21 *	0	33	66	0	0	0	0	33	66	0	0	0
MBC%	8.05 *	0	0	33	100	100	100	0	0	33	100	100	100
Effect ratio%		*Streptococcus parason guinis*						*Streptococcus parason guinis*					
MIC%	7.01 *	0	28	71	0	0	0	0	28	57	42	0	0
MBC%	9.44	0	0	28	100	100	100	0	0	14	71	100	100

Table 6: Study of MIC and MBC of methanolic, aqueous, Alpha zac ad Oral-B for *Enterobacter Cloacae ssp dissolvens*, *Kluyvera intermedia*, *Serratia marcesens*

	Oral-B mouthwash is aerobic negative												
Effect ratio		*Enterobacter Cloacae ssp dissolvens*						*Enterobacter Cloacae ssp dissolvens*					
mg/mL	LSD value	3.125	6.25	12.5	25	50	100	3.125	6.25	12.5	25	50	100
MIC%	7.50 *	0	0	100	0	0	0	0	100	0	0	0	0
MBC%	8.02 *	0	0	0	100	100	100	0	0	100	100	100	100
Effect ratio%		*Kluyvera intermedia*						*Kluyvera intermedia*					
MIC%	9.33 *	0	0	100	0	0	0	0	80	20	0	0	0
MBC%	8.61 *	0	0	0	100	100	100	0	0	80	100	100	100
Effect ratio%		*Serratia marcesens*						*Serratia marcesens*					
MIC%	7.50 *	0	0	100	0	0	0	0	100	0	0	0	0
MBC%	8.02 *	0	0	0	100	100	100	0	0	100	100	100	100

Table 7: Study of MIC and MBC of methanolic, aqueous, Alpha zac ad Oral-B for *Enterococcus faecium*, *Streptococcus mutans*, *Streptococcus parason guini*

	Oral-B mouthwash anaerobic positive												
Effect ratio		*Enterococcus faecium*						*Enterococcus faecium*					
mg/mL	LSD value	3.125	6.25	12.5	25	50	100	3.125	6.25	12.5	25	50	100
MIC%	7.50 *	0	0	100	0	0	0	0	100	0	0	0	0
MBC%	8.02 *	0	0	0	100	100	100	0	0	100	100	100	100
Effect ratio%		*Streptococcus mutans*						*Streptococcus mutans*					
MIC%	7.50 *	0	0	100	0	0	0	0	100	0	0	0	0
MBC%	8.02 *	0	0	0	100	100	100	0	0	100	100	100	100
Effect ratio%		*Streptococcus parason guinis*						*Streptococcus parason guinis*					
MIC%	7.50 *	0	0	100	0	0	0	0	100	0	0	0	0
MBC%	8.02 *	0	0	0	100	100	100	0	0	100	100	100	100

Table 8: Study of MIC and MBC of methanolic, aqueous, Alpha zac ad Oral-B for *Staphylococcus aureus*.

	Oral-B mouthwash aerobic positive												
Effect ratio		*Staphylococcus aureus*						*Staphylococcus aureus*					
mg/mL	LSD value	3.125	6.25	12.5	25	50	100	3.125	6.25	12.5	25	50	100
MIC%	7.61 *	0	42	57	0	0	0	0	57	42	0	0	0
MBC%	9.46 *	0	0	42	100	100	100	0	0	57	100	100	100

Table 9: Observable pH values of alcoholic, aqueous, and commercial (Alphazac and Oral –B) mouthwash within three months.

pH							
4-9-2021			4-8-2021			4-7-2021	Date
40	25	4	40	25	4	4	Temperature (°C)
4.14	4.2	4.3	4.22	4.29	4.3	5	methanolic
5.0	5.17	5.2	5.12	5.2	5.2	6	aqueous
6.3	6.4	6.4	6.4	6.5	6.3	6.8	Alphazac
5.4	5.5	5.6	5.7	5.7	5.6	10.9	Oral B

Table 10: The physical characteristics of the methanolic and aqueous, Oral B and Alpha zac mouthwash within three consecutive months.

Macroscopic characteristics of the prepared and commercial mouthwash at 4°, 25 and 40°C

4-7-2021				4-8-2021				4-9-2021					
Color	Odor	sedimentation	homogeneity	Color	Odor	sedimentation	homogeneity	color	odor	sedimentation	homogeneity	mouthwash	mouthwash
Dark nutty	Acceptable	Non	homogeneous	Dark nutty	Acceptable	Non	homogeneous	Dark nutty	acceptable	Non	homogeneous	methanolic	
Dark nutty	Acceptable	Non	homogeneous	Black	Acceptable	Non	homogeneous	black	acceptable	Non	homogeneous	aqueous	
Green	Mint	Non	homogeneous	Green	Mint	Non	homogeneous	green	acceptable	Non	homogeneous	Alphazac	
light blue	Mint	Non	homogeneous	light blue	Acceptable	Non	homogeneous	light blue	acceptable	Non	homogeneous	Oral B	

Table 11: Density test for methanolic and aqueous lotion, Alpha zac and Oral B. within three consecutive months.

Third month 4-7-2021	*Second month 4-7-2021*	*First month 4-7-2021*	*Inside the apparatus*
15.0	15.0	15.0	Distilled water
15.6	15.7	15.6	Methanolic
15.7	15.7	15.7	Aqueous
15.1	15.1	15.1	Alpha-zac
15.1	15.0	15.0	Oral B

The temperature at the time of measurement was between 30-35

Macroscopic characteristics of the mouthwash

A - The macroscopic characteristics of each mouthwash were read for a period of three consecutive months. These characteristics are color, odor, phase separation, and homogeneity (1)

B - Macroscopic characteristics of the mouthwash

C - pH of the mouthwash

The pH values for each mouthwash were recorded over a period of three months using a pH meter. (1.)

Statistical Analysis

The statistical program (Statistical Analysis System -SAS 2012) was used in data analysis to study the effect of different treatments on the studied traits according to a complete random design (CRD), and the significant differences between the means were compared with the least significant difference-LSD test.

RESULTS AND DISCUSSION

Collection and Diagnosis of Pathogenic Bacteria in the Mouth Bacteria in the mouth

Clinical specimen collection

The 30 clinical samples were collected from patients attending specialized dental centers in Baghdad governorate from different parts in the mouth of female and male, children and adults. Collecting samples continued from 12/28/2020 until 15/2/2021 from the Specialized Dental Health Center in Al-Maghrib Street and the Specialized Dental Health Center in the Sheikh Omar area. The clinical samples showed a diversity in the presence of bacteria types, as there were aerobic, anaerobic, pathogenic and non-pathogenic bacteria. Plaque samples were collected from deep pockets around the tooth and outside of the tooth by cotton swabs from patients diagnosed with gingivitis by a specialist doctor. After the swab was taken, it was placed in a special bag to store the samples. The 5 to 7 samples were collected at each time, depending on the number of patients who visited the Dental Health Center. Then they were transferred to the postgraduate laboratory at the College of Basic Education, Al-Mustansiriyah University. The results of the diagnosis with the Vitek 2 system showed that there are seven types of bacteria: (*E. cloacae ssp, E. dissolvens, S. marcescens, E. faecium, K. intermedia., S. aureus, S. mutans, S. parasanguinis*) shown in Table 2.

Studying the Inhibitory Effect of each of the Lye (alcoholic, aqueous, Alpha zac, Oral B) using Diffusion Agar Method.

The effect of aqueous, methanolic, Oral-B and Alpha zac mouthwashes on bacterial isolates was studied. The results were as follows for *E. cloacae ssp* E. *dissolvens* (gram-negative), highest concentration of the inhibition zone was 22 mm for methanolic mouthwash and the lowest concentration was 10 mm for Oral B. As for the bacteria *Kluyvera intermedia* (gram-negative), the highest inhibition was 24 mm for aqueous mouthwash , and the lowest was 9 mm for Oral B. As for *Serratia marceseens* (gram-negative), the highest inhibition was 13 mm for methanolic mouthwash and the lowest inhibition for 11 mm for Oral B. As for *E. Faecium* (gram-positive), the highest inhibition was 18 mm for aqueous mouthwash, and the lowest inhibition was 8 mm for methanolic mouthwash, while *S. aureus* (gram-positive) had the highest inhibition of 22 mm for methanolic mouthwash and the lowest inhibition was 10 mm for Oral B. As for *S. parasanguinis* (gram-positive), the highest inhibition was 24 mm for methanolic mouthwash and the lowest inhibition was 12 mm for Alpha zac, while *S. mutans* (gram-positive) had the highest inhibition of 22 mm for methanolic mouthwash and the lowest inhibition was 10 mm for Oral B . The discrepancy that occurred in inhibition was due to the differences in resistance and virulence factors for each type of bacteria studied (Table 3).

Study of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) for Methanolic, Aqueous, Alpha zac and Oral B mouthwash

The results in Table 4 show that the lowest inhibitory dilution (MIC) of the aqueous mouthwash was 28% at a concentration of 6.25 µg/mL, and the lowest bactericidal dilution (MBC) was 14% at a concentration of 12.5 µg/mL. It was also found that the lowest inhibitory dilution (MIC) of methanolic mouthwash was 14% at a concentration 12.5 µg/mL , and the lowest bactericidal dilution was 14% at a concentration 25 µg/mL, meaning there were no significant differences between methanolic and water mouthwash and this was attributed to the effectiveness of the plants from which the mouthwash was prepared.

As for Alpha zac, it was found that MIC was 20% at concentration 12.5 µg/mL, and MBC was 57% at concentration 12.5 µg/mL. As for Oral-B, it was found that MIC was 42% at concentration 6.25 µg/mL and MBC was 42% at concentration 12.5 µg/mL (Table 5-8).

Physical Properties of Methanolic, Aqueous, Alpha zac and Oral -B mouthwash

The pH was read for the four alcoholic, aqueous, Oral B and Alpha zac mouthwash for three consecutive months, and the results were as shown in the following (Table 9). The physical characteristics of the methanolic and aqueous, Oral B and Alpha zac mouthwash within three consecutive months (Table 10). The Density test for methanolic and aqueous lotion, Alpha zac and Oral B. within three consecutive months (Table 11).

CONCLUSIONS

Some types of oral bacteria have shown a high resistance to the commercial mouthwash available in the local markets in Iraq, with a distinct sensitivity, 100%, to the mouthwash prepared from a mixture of extracts from *M. officinalis* L., *P. anisum* L., *S. marianum* L. including antibiotic-resistant bacteria, which gives the possibility of using it in the treatment of oral diseases caused by these harmful bacteria. Note that some types of oral studied bacteria have the ability to form biofilm in varying degrees.

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