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EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF HERBAL MOUTHWASHES ON MULTIDRUG-RESISTANT GRAM-POSITIVE BACTERIA ISOLATED FROM PATIENTS WITH DENTAL CARIES

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ABSTRACT

Dental caries is a widespread oral health issue that can progress to serious complications if untreated. Multidrug-resistant (MDR) Gram-positive bacteria poses great challenge in dental infections. Herbal mouthwashes, containing natural bioactive compounds are been used against these resistant strains. This study evaluates the antibacterial activity of three herbal mouthwashes against multidrug-resistant Gram-positive bacteria isolated from dental caries patients. Bacterial isolates were collected from 50 patients with active dental caries at the University of Port Harcourt Teaching Hospital, Nigeria. Standard microbiological methods, including Gram staining and biochemical tests, were used for bacterial identification. Thirteen multidrug-resistant bacteria were isolated. The antimicrobial susceptibility of herbal mouthwashes was assessed using agar well diffusion and Kirby-Bauer disc diffusion methods. Three herbal formulations X, Y and Z were tested against the resistant bacterial strains. The bacterial isolates used include *Staphylococcus aureus* and *Bacillus subtilis*. The percentage occurrence of *Staphylococcus aureus* was (83%) while *Bacillus subtilis* was (17%). The results showed positive control had inhibition zone diameter (IZD) of 14mm (25%), Herbal Mouthwash X, Y, Z and normal saline (negative control) had IZD of 13mm (23.2%), 14mm (25%), 15mm (27%) and 0mm (0%) respectively. Among the tested mouthwashes, Herbal Mouthwash Z, containing *Thymus vulgaris* and *Sesamum indicum*, demonstrated the highest antimicrobial efficacy, showing statistically significant inhibition zones against the resistant isolates. However, Herbal Mouthwashes X and Y exhibited moderate activity. The findings showed that Herbal Mouthwashes gave promising antimicrobial activity and could be further investigated for broader clinical applications.

Keywords: Dental Caries, Herbal Mouthwashes, Multi-Drug-Resistant, Gram-positive bacteria, *Thymus vulgaris*.

1 INTRODUCTION

Dental caries is a dynamic process characterized by the localized breakdown and dissolution of dental hard tissues due to acidic byproducts produced during the bacterial fermentation of carbohydrates [Longbottom, *et al.*, 2009]. According

to the ecological plaque hypothesis, microbes play a crucial role in the development of dental caries [Marsh and Bradshaw, 1997]. While caries is a multifactorial condition, key causative agents include bacteria like *Streptococcus mutans* and *Lactobacillus* [Hahn, *et al.*, 1997].

Dental caries is widespread and arises from the interaction between dietary components and specific causative microbes [Longbottom, *et al.*, 2009]. Dental plaque acts as a reservoir, creating a stable environment conducive to destructive processes [Marsh and Bradshaw, 1997]. Over time, dental plaque can develop into a cariogenic biofilm as bacteria colonize the surfaces of the substrate [Marsh and Bradshaw, 1997].

Dental caries, commonly referred to as tooth decay, is a biofilm-mediated, multifactorial, and dynamic condition caused by sugar intake. It leads to alternating phases of demineralization and remineralization of dental hard tissues and affects individuals across all age groups [Pitts, *et al.*, 2017; Zou, *et al.*, 2018].

Bacteria like *Streptococcus* and *Lactobacillus* species are recognized as primary culprits in dental caries [Wade, 2013; Matsumoto-Nakano, 2018; Ahirwar, *et al.*, 2019]. For example, *Streptococcus mutans* and *S. salivarius* are gram-positive, facultative anaerobes closely linked to tooth decay, while *S. mitis* is a mesophilic, alpha-hemolytic pathogen. These bacteria are significant contributors to oral infections and systemic issues like infective endocarditis, highlighting their public health relevance [Monk, *et al.*, 2024; Yu, *et al.*, 2024].

Caries is often considered a disease of childhood and adolescence. However, limited research addresses its prevalence among adults in Nigeria, who are the primary users of mouthwashes [Zou, *et al.*, 2018; Alsadat, *et al.*, 2018]. Despite the availability of various mouthwash products in Nigeria, there is little data on their efficacy against local strains of oral pathogens [Iyun, *et al.*, 2014; Uguru, *et al.*, 2020; Oluremi, *et al.*, 2010]. This study aimed to evaluate the antimicrobial activity of three herbal mouthwashes against multi-drug-resistant Gram-positive bacteria isolated from patients with dental caries.

2 METHODOLOGY

3 MATERIALS

These includes the chemicals, reagents, culture media, equipment and other recommended consumables used in the course of the research.

3.1 Reagents

Hydrogen peroxide (for catalase test), Kovac's reagent (for indole test), Oxidase reagent, Crystal violet dye, Lugol's iodine, Safranin red.

3.2 Culture media

The following culture media were used;

Nutrient agar, Nutrient broth, Mueller Hinton agar, Mannitol salt agar, MacConkey agar

4 METHODS

4.1 Study Design

The study is a prospective study involving consenting adults aged 18 years and above with active dental caries who attended the Oral Diagnosis Dental Clinic at the University of Port Harcourt Teaching Hospital, Obio Akpor, Rivers State, Nigeria. The study was conducted on *Staphylococcus* and *Bacillus* isolates.

4.2 Participant Recruitment

Adults aged 18 and above who voluntarily participated were included in the study after being assessed and confirmed by a consultant dentist to have dental caries. Recruitment took place among individuals receiving care at the Oral Diagnosis Dental Clinic at the University of Port Harcourt Teaching Hospital, Obio Akpor, Rivers State, Nigeria.

4.3 Collection of Mouthwashes

The mouthwashes were randomly procured from nearby shops and open markets within the Port Harcourt metropolis. Also, the manufacturing date, expiry date, NAFDAC Registration numbers and active ingredients were documented as shown in Table 1.

4.4 Details of Mouthwashes

Table 1: mouthwashes used in the study

Mouthwashes	Code	Composition	Manufactured Date	Expiry Date	NAFDAC REG. NO.
Positive control	1	Thymol 12,78mg, Alcohol (95%v/v), 4, 54ml, sodium benzoate and benzoic acid.	06/2023	05/2025	04-9471
Herbal Mouthwash X	2	<i>Zingiber officinale</i> 35%, <i>Allium sativum</i> 35%, <i>Potash alum</i> 20%, Menthol 5%	2024	2029	A7-2646L
Herbal Mouthwash Y	3	<i>Aloe Vera</i> and 3 other dental herbs	09/2023	09/2026	Nil
Herbal Mouthwash Z	4	<i>Thymus vulgaris</i> 65%, <i>Sesamum indicum</i> 35%, water qs	03/2024	03/2027	A7-101523L
Negative control	5	0.9% sodium chloride	08/2022	07/2025	A11-0235

4.5 Ethics Approval

The study was approved by the Ethics and Research Committee (ERC) at the University of Port Harcourt Teaching Hospital. Protocol number: (UPTH/ADM/90/5.11/VOL.XI/17011). All patients provided informed consent.

4.6 Collection of Dental Caries Samples

Using a sterile swab, fifty carious dental caries samples were collected under the supervision of the dentist and transferred into an already prepared nutrient broth medium (McCartney bottle) for preservation. It was transported to the Pharmaceutical Microbiology Laboratory, Department of Pharmaceutical Microbiology and Biotechnology, University of Port Harcourt. This was done within one hour thirty minutes.

It was stored in an incubator at 37°C for 24 hours.

4.7 In-Vitro Evaluation of the Antimicrobial Activity

The test organisms used were isolated from the caries of dental patients early in the morning and were taken to the microbiology laboratory for culturing.

4.8 Culturing of Dental Caries Samples

Culturing and isolation were done using the streak plate technique as in [Cheesebrough, 1984; Cruickshank, *et al.*, 1986].

4.8.1 Identification of Test Organisms

This was properly carried out to identify the true nature of the organisms through the following:

4.8.1.1 Gram Staining

Gram staining is a method used to classify bacterial cells into Gram-positive and Gram-negative categories based on their cell wall's ability to retain a specific dye.

4.8.2 Procedure

- A wet sample from various bacterial colonies was spread on a clean glass slide, heat-fixed, air-dried, and sequentially stained with different dyes. The process involved:
- The smear was flooded with 1% Crystal Violet for one minute, followed by rinsing with water.
- Lugol's iodine was applied for one minute, then rinsing with water.
- A 95% alcohol was used as a decolorizer for thirty seconds, immediately rinsed with water.
- The smear was flooded with 1% Safranin red for one minute, rinsing with water, and air drying.
- The stained smear was observed under an oil immersion microscope. Gram-positive bacteria retained the primary Crystal Violet stain, appearing purple, while Gram-negative bacteria were decolorized and took up the Safranin stain, appearing red.

4.8.2.1 Biochemical and Qualitative Identification of Test Organisms

This process utilizes bacteria's ability to ferment sugars, glycosides, and polyhydric alcohols to differentiate members of the Enterobacteriaceae family.

4.8.2.1.1 Catalase Test

The catalase test distinguishes catalase-producing organisms, such as *Staphylococcus* species, from non-catalase producers, like *Streptococcus* species. A drop of 3% hydrogen peroxide was placed on a clean slide, and a colony of the test organism was mixed into the drop. The production of gas bubbles indicated a positive result (catalase-positive), while no bubbles indicated a negative result.

4.8.2.1.2 Indole Test

This test is key for identifying enterobacteria that can produce indole, such as *E. coli*, *Proteus vulgaris*, *Proteus rettgeri*, *Morganella morganii*, and *Providencia* species. The test organism was cultured in a tryptophan-containing medium. The presence of indole was detected using Kovac's reagent, which reacts with indole to form a red-colored compound, indicating a positive result.

4.8.2.1.3 Oxidase Test

The oxidase test aids in identifying species such as *Pseudomonas*, *Neisseria*, *Vibrio*, *Brucella*, and *Pasteurella*, which produce the enzyme cytochrome oxidase. A piece of filter paper was moistened with a freshly prepared 1% aqueous solution of tetramethyl-p-phenylenediamine hydrochloride. A small sample of the test isolate was smeared onto the moistened filter paper using a sterile, grease-free glass slide that had been flamed and cooled. The development of a purple color within seconds along the smear indicated a positive result, while the absence of color signified a negative result.

4.8.2.1.4 Citrate Test

This test determines whether an organism can use citrate as its sole source of carbon and energy. Citrate-positive organisms, such as *Klebsiella pneumoniae*, cause the medium to change color from green to blue, while citrate-negative organisms, such as *Escherichia coli*, do not produce a color change. Using a sterile wire loop, bacterial colonies were streaked onto a citrate agar slant in a test tube and incubated overnight at 37°C.

4.8.2.1.5 Urease Test

The following is the procedure for preparing urea agar base with urea salt to carry out the urease test:

4.8.2.1.6 Preparation of the Medium Base:

The required amount of urea agar base powder was weighed and dissolved in distilled water, (typically about 15g in 195ml of distilled water). The mixture was heated gently while stirring until completely dissolved.

- **Sterilize the Base:** The solution was sterilized in an autoclave at 121°C for 15 minutes to eliminate contaminants.
- **Prepare Urea Solution:** A sterile 40% urea solution was prepared separately. 2g of urea salt was weighed and dissolved in 5ml of distilled water. This step was done aseptically, as urea is heat-sensitive and would degrade if autoclaved.
- **Cool the Medium:** The sterilized urea agar base medium was allowed to cool to approximately 50°C to prevent denaturation of the urea during the mixing process.
- **Mix Urea with Base:** The sterile urea solution was aseptically added to the cooled agar base and thoroughly mixed to ensure even distribution.
- **Dispense the Medium:** The prepared medium was poured into sterile test tubes and allowed to solidify. They were typically slanted to create a larger surface area for inoculation.
- **Perform the Urease Test:** Test organisms were inoculated onto the prepared urea agar medium and incubated at the appropriate temperature (e.g., 35–37°C). Color changes in the medium were observed to determine urease activity.

4.8.2.1.7 Antimicrobial Susceptibility Testing

This test was carried out to determine the sensitivity of the test organisms against the antimicrobial agent using the agar well diffusion method and Kirby-bauer disc diffusion method.

4.8.2.1.8 Agar Well Diffusion Method

Pure isolates were transferred using a sterile loop into a tube containing 0.1% peptone water. The density of each microbial suspension was adjusted to that of 0.5 McFarland standard and used as inoculums. Wells of 6mm in diameter were bored into the seeded Muller Hinton agar plates where the different mouthwashes were dispensed. The plates were allowed to stand for one hour for diffusion of the mouthwashes to take place and after diffusion; the plates were incubated at 37°C for 24 hours. The antimicrobial activity, indicated by an inhibition zone surrounding the well containing the mouthwashes was recorded. The zones of inhibition were measured using an accurately calibrated measuring scale.

4.8.2.1.9 Kirby-Bauer Disc Diffusion Method

The Kirby-Bauer Disc Diffusion Method is the most commonly used method for antimicrobial susceptibility testing with commercial antibiotic discs. Here is an overview of the procedure:

4.8.3 Procedure

4.8.3.1 Preparation of Inoculum

A bacterial suspension was prepared by diluting the test organism in sterile broth to match the turbidity of a 0.5 McFarland standard.

4.8.3.2 Inoculation of Agar Plate

- A sterile swab was dipped into the bacterial suspension, and excess liquid was removed by pressing against the tube wall.
- The swab was then streaked evenly over the surface of the Mueller Hinton Agar (MHA) plate to create a uniform lawn of bacteria.

4.8.3.3 Placement of Antibiotic Discs

With a sterile forcep, the antibiotic discs were placed on the agar surface. Adequate spacing was ensured to prevent overlapping zones of inhibition.

4.8.3.4 Incubation:

- The plates were incubated at 35–37°C for 24 hours.
- The diameter of the zones of inhibition (in millimeters) around each disc was measured and recorded using a calibrated meter rule.

5 RESULTS

5.1 Biochemical Test Result for Oral Isolates

Table 2: Providing details about the biochemical test result for oral isolates.

Isolate Code	Catalase	Oxidase	Citrate	Urease	Tsiaz Butt	Tsiaz Slope	Tsiaz Gas	Tsiaz H ₂ S	Gram Staining	Indole	Organisms Isolated
Isolate 3	+	-	-	-	Red	Red	-	-	+Cocci	-	<i>Staphylococcus aureus</i>
Isolate 4	+	-	+	-	Yellow	Yellow	-	-	+Rod	+	<i>Bacillus Subtilis</i>
Isolate 7	+	-	-	-	Red	Red	-	+	+Cocci	-	<i>Staphylococcus aureus</i>
Isolate 8	+	+	+	-	Yellow	Yellow	+	+	+Rod	+	<i>Bacillus Subtilis</i>
Isolate 13	+	-	-	-	Yellow	Yellow	+	-	+Cocci	-	<i>Staphylococcus aureus</i>

Isolate 24	+	-	-	-	Yellow	Yellow	-	+	+Cocci	-	<i>Staphylococcus aureus</i>
Isolate 32	+	-	-	-	Red	Red	-	-	+Cocci	-	<i>staphylococcus aureus</i>
Isolate 44	+	-	-	-	Yellow	Yellow	+	-	+Cocci	-	<i>Staphylococcus aureus</i>
Isolate 34	+	-	-	-	Red	Red	-	-	+Cocci	-	<i>Staphylococcus aureus</i>
Isolate 38	+	-	-	-	Red	Red	+	-	+Cocci	-	<i>Staphylococcus aureus</i>
Isolate 39	+	-	-	-	Red	Red	-	-	+Cocci	-	<i>Staphylococcus aureus</i>
Isolate 43	+	-	-	-	Red	Red	-	-	+Cocci	-	<i>Staphylococcus aureus</i>
Isolate 49	+	-	-	-	Red	Red	-	-	+Cocci	-	<i>Staphylococcus aureus</i>

From the table 2 above the percentage of count of the organisms isolated are *Staphylococcus aureus*: 84.6% and *Bacillus subtilis*: 15.4%

5.1.1 Interpretation

The table summarizes the biochemical test results for various microbial isolates, showcasing their enzymatic activities, Triple Sugar Iron Agar (TSIA) reactions, gram staining, indole production, and identified organisms. The isolates primarily include *Staphylococcus aureus* and *Bacillus subtilis*, with their distinct biochemical profiles aiding identification. *Staphylococcus aureus* isolates were observed in Isolates 3, 7, 13, 24, 32, 44, 34, 38, 39, 43, and 49. These were consistently positive for catalase, gram-positive cocci, and showed negative reactions for oxidase, citrase, urease, and indole. TSIA reactions were predominantly red for both butt and slope, indicating no fermentation, with no gas or H₂S production. *Bacillus subtilis* isolates were identified in Isolates 4 and 8. These showed positive reactions for catalase, citrase, oxidase (Isolate 8), and indole (Isolate 4 and 8), along with yellow TSIA butt and slope, suggesting glucose fermentation. The percentage distribution of the isolates is demonstrated in fig.1 below. They were gram-positive rods with variable gas production. This biochemical differentiation highlights the utility of enzymatic and TSIA reactions in distinguishing between bacterial species in a laboratory setting.

5.2 Antimicrobial Susceptibility Test Using Kirby-Bauer Disc Diffusion Method

Table 3: Antimicrobial susceptibility test using Kirby-Bauer Disc diffusion method

SAMPLE CODE	GN mm	CTX Mm	CRO mm	ZEM mm	LBC Mm	CIP mm	IMP mm	CXM mm	OFX mm	ERY mm	AUG mm	AZN mm
Isolate 3	9	0	26	0	35	24	0	0	20	0	16	0
Isolate 4	0	0	0	0	25	20	0	0	0	0	0	0
Isolate 7	0	0	0	0	0	0	0	0	0	0	0	0
Isolate 8	0	0	0	0	26	24	0	0	11	0	0	0
Isolate 13	0	0	0	0	26	16	0	0	11	0	0	0
Isolate 24	0	0	0	0	25	24	0	0	14	0	0	0
Isolate 32	11	0	0	0	27	25	0	0	14	0	0	0
Isolate 34	0	0	14	0	19	0	0	0	0	0	0	0
Isolate 38	0	0	0	9	15	10	0	12	0	0	0	0
Isolate 39	23	24	22	0	23	22	0	0	0	17	0	25
Isolate 43	0	0	0	0	19	12	0	0	0	16	0	21

Isolate 44	0	0	0	0	22	16	0	0	0	0	0	0
Isolate 49	13	0	0	0	14	10	0	0	0	0	0	0

Where; 0= No Inhibition Zone Diameter, GN= Gentamicin, CTX= Cefotaxime, CRO= Ceftriaxone, ZEM= Cefepime, LBC= Lincomycin, CIP= Ciprofloxacin, OFX= Ofloxacin, ERY=Erythromycin, AUG= Amoxicillin-Clavulanic acid (AUGMENTIN), AZN= Azithromycin, CXM= Cefuroxime, IMP= Imipenem.

From table 5 above, the most effective agents in inhibiting microbial Isolates were LBC (Lincomycin) and CIP (Ciprofloxacin), each inhibiting 12 and 11 Isolates respectively. Ofloxacin (OFX) followed, with 6 Isolates inhibited. Other antibiotics like Ceftriaxone (CRO), Azithromycin (AZN), and Gentamicin (GN) were effective against 4 Isolates. Less frequently, Erythromycin (ERY), Cefuroxime (CXM), and Cefepime (ZEM, CTX, AUG) were able to inhibit only 2 to 1

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Table 4: The number of antimicrobial agents resistant against the isolated Isolates.

Isolate Code	Number of Antimicrobial agent Resistant against	Outcome	Organism Isolated
Isolate 3	6	Multiresistant	<i>Staphylococcus aureus</i>
Isolate 4	10	Multiresistant	<i>Bacillus Subtilis</i>
Isolate 7	8	Multiresistant	<i>Staphylococcus aureus</i>
Isolate 8	12	Multiresistant	<i>Bacillus Subtilis</i>
Isolate 13	9	Multiresistant	<i>Staphylococcus aureus</i>
Isolate 24	9	Multiresistant	<i>Staphylococcus aureus</i>
Isolate 32	8	Multiresistant	<i>Staphylococcus aureus</i>
Isolate 44	10	Multiresistant	<i>Staphylococcus aureus</i>
Isolate 34	7	Multiresistant	<i>Staphylococcus aureus</i>
Isolate 38	3	Multiresistant	<i>Staphylococcus aureus</i>
Isolate 39	8	Multiresistant	<i>Staphylococcus aureus</i>
Isolate 43	10	Multiresistant	<i>Staphylococcus aureus</i>
Isolate 49	8	Multiresistant	<i>Staphylococcus aureus</i>

From the table 3, for *Staphylococcus aureus* the average number of drugs resistant to it is 8, while for *Bacillus Subtilis*, the average number of drugs resistant to it is 11

5.2.1 Interpretation

The above table shows that all the isolated organisms from the Isolates are resistant to equal to or more than 3 Antimicrobial agents of different classes, thus all strains in the collected Isolates are Mult-resistant to antimicrobial agents.

5.3 Antimicrobial Susceptibility Test with Mouthwash Using Well Agar Diffusion Method

Table 5: The antimicrobial susceptibility test with mouthwash using Well Agar Diffusion method

S/No	SAMPLE CODE	POSITIVE CONTROL	HERBAL MOUTHWASH (Majestic) X	HERBAL MOUTHWASH (Opat) Y	HERBAL MOUTHWASH Z (Jalin)	NORMAL SALINE
		Inhibition zone diameter (mm)				
1	Isolate 3	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
2	Isolate 4	8.5±8.5	12.5±0.5	0.0±0.0	17.0±1.0	0.0±0.0
3	Isolate 7	12.5±1.5	0.0±0.0	0.0±0.0	13.0±1.0	0.0±0.0
4	Isolate 8	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
5	Isolate 13	0.0±0.0	0.0±0.0	0.0±0.0	16.5±2.5	0.0±0.0
6	Isolate 24	14±2.0	12.0±1.0	13.0±3.0	14.0±4.0	0.0±0.0
7	Isolate 32	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
8	Isolate 34	8.0±8.0	6.5±6.5	13.0±1.0	14.5±0.5	0.0±0.0
9	Isolate 38	7.0±7.0	12.5±0.5	13.0±1.0	0.0±0.0	0.0±0.0
10	Isolate 39	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
11	Isolate 43	13.0±1.0	6.5±6.5	7.0±7.0	7.5±7.5	0.0±0.0
12	Isolate 44	0.0±0.0	6.0±6.0	9.5±9.5	0.0±0.0	0.0±0.0
13	Isolate 49	12.5±0.5	7.5±7.5	8.0±8.0	7.5±7.5	0.0±0.0

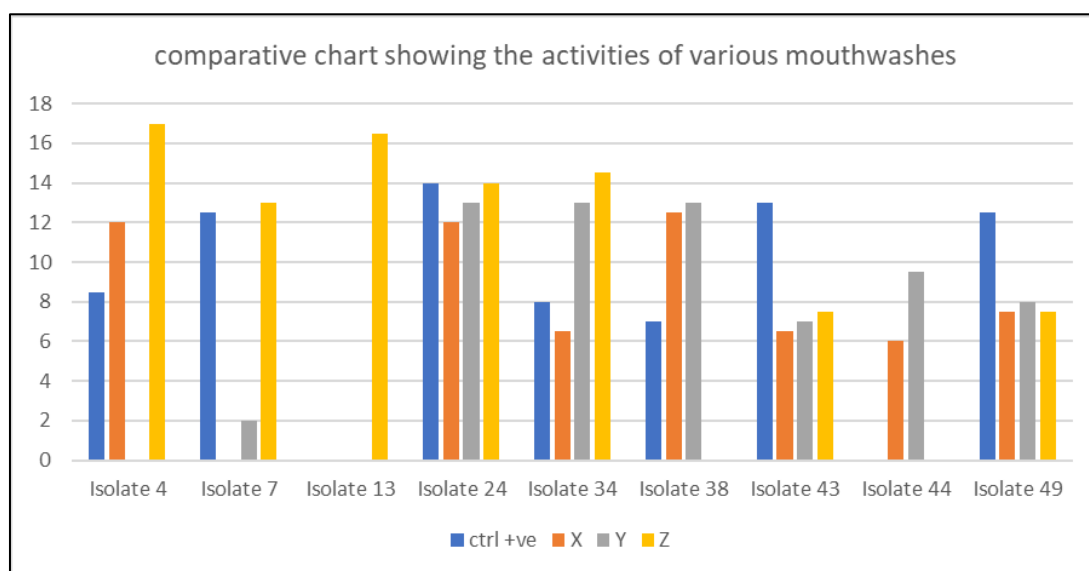


Figure 1: Comparative Chart Showing the Mean Zone of Inhibition of Various Herbal Mouthwashes with the positive control

6 DISCUSSION

This study was carried out to test or evaluate the efficacy of herbal mouthwashes containing *Zingiber officinale*, Thymol oil, *Allium sativum*, Potash alum, Menthol, *Thymus vulgaris*, *Sesamum indicum*, *Aloe vera* etc. which are notably incorporated into Herbal Mouthwashes X, Y and Z on most selected oral pathogens (dental caries). Although a synthetic product was also tested and it served as the positive control while normal saline served as the negative control.

The combination of various herbs in a single preparation provides dual advantage in obtaining a purely herbal mouthwash with enhanced antimicrobial activity along with the

application of various excipient properties like flavoring, sweetening and preservative agents.

Table 1 outlines the composition of mouthwashes X, Y, Z, positive control, and Negative control.

Mouthwashes with herbal components (e.g., *Zingiber officinale*, *Thymus vulgaris*, *Sesamum indicum*) are well-documented for antimicrobial activity. For example, studies show *Thymus vulgaris* essential oil exhibits strong antimicrobial effects due to thymol and carvacrol components, which disrupt bacterial membranes (Salehi et al., 2018). Similarly, *Zingiber officinale* (ginger) reduces biofilm formation by *Streptococcus mutans* (Abdel-Aziz et al., 2015).

Table 4 showed the oral isolates. Identification of isolates showed predominance of *Staphylococcus aureus* (11 isolates) and *Bacillus subtilis* (2 isolates). *Staphylococcus aureus* is known to contribute to dental infections and biofilm formation. Studies confirm its ability to develop multidrug resistance through biofilm-mediated tolerance mechanisms (Khairnar et al., 2015). The identification of *Bacillus subtilis* suggests its opportunistic involvement, which aligns with research showing its potential pathogenicity in compromised oral cavities (Loesche, 1986).

Table 3 showed that all isolates demonstrated multidrug resistance, with up to 12 antibiotics resisted by some strains. For *Staphylococcus aureus*, the average number of drugs it is resistant to is 8, while for *Bacillus Subtilis*, the average number of drugs it is resistant to is 11. The high resistance underscores the global concern of antimicrobial resistance (AMR) in oral pathogens. According to the World Health Organization (WHO), such MDR bacteria significantly complicate treatment options, emphasizing the need for alternative therapies like herbal mouthwashes (WHO, 2021).

From table 5. the antimicrobial activity of the positive control mouthwash, which is a commercially available synthetic product containing thymol, alcohol, and sodium benzoate. Zones of inhibition were recorded around the wells, indicating the efficacy of the synthetic mouthwash against resistant *Staphylococcus aureus* and *Bacillus subtilis* isolates.

Thymol as an Active Ingredient: Thymol is a major compound in synthetic mouthwashes due to its antimicrobial activity. It disrupts bacterial membranes, leading to cell lysis [Mikulski et al., 2020].

Alcohol and Sodium Benzoate: Alcohol enhances the antimicrobial properties of the mouthwash by denaturing proteins in bacterial cells [Cunha et al., 2017]. Sodium benzoate acts as a preservative and exhibits mild antimicrobial effects by lowering the pH to levels unsuitable for bacterial survival [Farrell et al., 2006]. Synthetic mouthwashes generally provide consistent and reliable antimicrobial activity, which aligns with the zones of inhibition seen in table 4

For the antimicrobial activity of Herbal Mouthwash X containing *Zingiber officinale* (ginger), *Allium sativum* (garlic), Potash alum, and menthol. Zones of inhibition were moderate, indicating some effectiveness of the mouthwash against resistant *S. aureus* and *Bacillus subtilis* isolates.

***Zingiber officinale* (Ginger):** Ginger contains gingerol and shogaol, compounds with potent antimicrobial activity against oral pathogens like *S. mutans* and *P. gingivalis* [Abdel-Aziz et al., 2015]. Studies have shown that ginger extracts reduce bacterial adhesion and biofilm formation, which is crucial in managing dental caries [Kalemba and Kunicka, 2003].

***Allium sativum* (Garlic):** Garlic contains allicin, a sulfur compound with broad-spectrum antimicrobial activity [Bakri and Douglas, 2005]. Garlic extracts have demonstrated inhibitory effects against multidrug-resistant bacteria, including *S. aureus* [Khairnar et al., 2015].

Potash alum and Menthol: Potash alum acts as an astringent and antimicrobial agent, helping to reduce bacterial load [Singh and Gupta, 2013]. Menthol provides a cooling effect while contributing to the inhibition of bacterial growth. The moderate activity observed in Figure 4 aligns with findings that herbal mouthwashes containing ginger and garlic show efficacy against oral pathogens. [Kalemba and Kunicka, 2003].

Herbal Mouthwash Y, containing Aloe vera and other dental herbs, exhibited variable antimicrobial activity with zones of inhibition observed for some isolates. The efficacy was moderate compared to the synthetic mouthwash and Herbal Mouthwash Z.

Aloe vera: *Aloe vera* contains anthraquinones and saponins, which exhibit antimicrobial and anti-inflammatory properties [Saini et al., 2011]. Research indicates that *Aloe vera*-based mouthwashes are effective against *S. mutans* and other Gram-positive bacteria due to their inhibitory effects on biofilm formation [Jesumirhewe and Ariyo, 2023]. Herbal formulations combining multiple plant extracts have shown synergistic antimicrobial effects [Malik et al., 2014]. *Aloe vera*, combined with other bioactive dental herbs, can help reduce plaque and gingivitis while targeting cariogenic bacteria. The results in Figure 5 align with literature showing that *Aloe vera*-based mouthwashes are effective [Saini et al., 2011].

Herbal Mouthwash Z, containing *Thymus vulgaris* (thyme) and *Sesamum indicum* (sesame oil), demonstrated the highest antimicrobial efficacy among the herbal formulations. Significant zones of inhibition were observed, especially against *S. aureus* isolates.

Thymus vulgaris (Thyme): Thyme oil, rich in thymol and carvacrol, exhibits strong antimicrobial activity by disrupting bacterial membranes [Salehi et al., 2018]. Studies have confirmed thyme's effectiveness against oral pathogens like *S. mutans* and *F. nucleatum*, reducing periodontal disease risks [Mikulski et al., 2020].

Sesamum indicum (Sesame Oil): Sesame oil is traditionally used in oil pulling and contains bioactive compounds like sesamin and linoleic acid, which exhibit antimicrobial properties. [Asokan et al., 2008]. Sesame oil reduces bacterial load and inhibits plaque formation, making it effective in oral health management. The combination of thyme and sesame oil enhances antimicrobial activity through synergistic mechanisms, which explains the larger zones of inhibition in Figure 6 [Kalemba and Kunicka, 2003].

Herbal Mouthwash Z's superior efficacy aligns with literature highlighting the antimicrobial potential of thymol-rich formulations [Salehi et al., 2018].

In all, herbal Mouthwash Z showed the largest inhibition zones across multiple isolates. The efficacy observed in Herbal Mouthwash Z can be attributed to bioactive components like thymol (thymol essential oil) and linoleic acid (sesame oil), which disrupt bacterial membranes and metabolic pathways (Mikulski et al., 2020).

On the antimicrobial susceptibility test in table 5, results showed variable inhibition zones for antibiotics like Gentamicin, Ciprofloxacin, and Lincomycin. Multidrug-resistant isolates like *Staphylococcus aureus* often retain sensitivity to certain antibiotics (e.g., ciprofloxacin), but over-reliance on synthetic antibiotics has promoted resistance. This aligns with research advocating herbal alternatives to combat AMR (Thomas et al., 2015).

7 CONCLUSION

The study highlights the antimicrobial effectiveness of herbal mouthwashes against multidrug-resistant Gram-positive bacteria isolated from patients with dental caries. Among the tested mouthwashes, Herbal Mouthwash Z, containing *Thymus vulgaris* and *Sesamum indicum*, demonstrated the highest antimicrobial efficacy, followed by the mouthwashes X and Y when compared to the positive control. In all the mouthwashes could be used effectively in the management of some dental caries

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

Authors declare no conflict of interest.

Statement of ethical approval

The study was approved by the Ethics and Research Committee (ERC) at the University of Port Harcourt Teaching Hospital. Protocol number: (UPTH/ADM/90/5.11/VOL.XI/17011).

Statement of informed consent

All the individuals who participated in the study gave their informed consent.

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