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RESEARCH ARTICLE

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Bio-efficacy of *Allium sativum* L., *Syzygium aromaticum* L. and Selected Commercial Toothpastes Against Microbial Isolates from Recurrent Aphthous Stomatitis (RAS)

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ABSTRACT

Mouth ulcers, also called Recurrent Aphthous Stomatitis (RAS) posse a significant global health burden, impacting quality of life through pain, discomfort, and difficulties in eating or speaking. African medicinal plants are highly sought after natural resources that can be used in the treatment of RAS. This study evaluated the bio-efficacy of *Allium sativum* Linn. (Garlic) / *Syzygium aromaticum* Linn. (Clove) extracts and selected toothpastes against microbial isolates from Recurrent Aphthous Stomatitis (RAS). Samples were obtained from six individuals with mouth ulcer sources (MU 1 - MU 6). Isolation, characterization, and identification of microbes were performed using conventional methods. Antimicrobial activity of toothpastes and garlic/clove ethanol extract was assessed using agar well diffusion. Microbial loads, antimicrobial susceptibility, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) minimum fungicidal concentration (MFC), and time-kill kinetics were determined using standard methods. A total of 10 bacteria (*Bacillus cereus*, *Bacillus licheniformis*, *Corynebacterium matruchotii*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus reuteri*, *Lactobacillus brevis*, and *Streptococcus salivarius*) and 4 fungi (*Candida tropicalis*, *Geotrichium candidum*, *Pichia fermentans* and *Saccharomycopsis fibuligera*) were identified. Bacterial counts averaged 1.4×10^5 CFU/mL and fungal counts averaged 1.7×10^5 SFU/ml. Toothpaste (Colgate) showed inhibition zones of up to 37 mm and MIC of 3.125% against *K. pneumoniae* while garlic achieved 31 mm and 0% MIC against *S. salivarius*. Clove also exhibited 27 mm zones and 12.5-50% MIC. Time-kill assays revealed >90% reduction within 8-24 hours for garlic against most isolates, outperforming toothpastes (~70-75% kill by 24-32 hours). In conclusion, the activities of garlic and clove extracts surpass commercial toothpastes in combating mouth ulcer pathogens, supporting their use as natural alternatives amid antimicrobial resistance.

Highlights

- Garlic and clove extracts showed strong antimicrobial activity against RAS isolates.
- Garlic achieved >90% microbial reduction within 8 – 24 h.
- Natural extracts matched commercial toothpastes against oral pathogens.

1. Introduction

Recurrent Aphthous Stomatitis (RAS), also known as canker sores or mouth ulcers are painful lesions of the oral mucosa characterized by eroded epithelium, often presenting as shallow, round sores with a whitish or yellowish center and erythematous borders. They are the most common oral mucosal conditions affecting the global population,

with RAS being the most prevalent. Mouth ulcers vary in size, frequency, and duration, with minor ulcers healing within 7-14 days, while major or herpiform ulcers may persist longer and cause scarring (Sánchez-Bernal *et al.*, 2020).

Microorganisms, particularly *Streptococcus* spp. and *Candida* spp. have been associated with mouth ulcer development and exacerbation. Dysbiosis or microbial imbalance can allow opportunistic pathogens to proliferate, contributing to inflammation and tissue damage (Sanz *et al.*, 2020). *Streptococcus salivarius*, a common oral commensal can become pathogenic in dysbiotic conditions, promoting inflammation and biofilm formation that worsen ulcerative lesions (Taraji *et al.*, 2021). Similarly, *Candida albicans*, a common oral fungus, is a key contributor to ulcerative conditions like oral candidiasis, where its overgrowth leads to erythematous or pseudomembranous lesions, particularly in immunocompromised individuals or those with disrupted microbiome due to antibiotics or poor hygiene (Vila *et al.*, 2020). These microorganisms amplify ulcer severity through toxin production, immune evasion, and synergistic interactions within complex microbial communities, highlighting their critical role in ulcer pathogenesis.



Fig 1: Recurrent Aphthous Stomatitis (Vila *et al.*, 2020).

Toothpastes are a cornerstone of oral hygiene, primarily designed to prevent dental caries and periodontal diseases, but their role in treating mouth ulcers is limited and varies depending on their formulation. Toothpastes containing antimicrobial agents, such as fluoride, triclosan, or herbal extracts, can indirectly alleviate mouth ulcers by reducing microbial load and inflammation in oral cavity, which may prevent secondary infections in ulcerative lesions (Walsh *et al.*, 2020). Their antimicrobial activity targets oral pathogens, including bacteria such as *Streptococcus salivarius* and fungi like *Candida albicans*, which are implicated in ulcer pathogenesis (Vila *et al.*, 2020). The effectiveness of toothpastes in inhibiting these microbes depends on their active components, formulation and delivery mechanisms, making them relevant for managing mouth ulcers. Herbal toothpastes, incorporating ingredients like aloe vera, chamomile, clove, have shown mild anti-inflammatory and soothing effects, which may provide symptomatic relief for mouth ulcers, though evidence for direct healing is limited (Varghese *et al.*, 2022).

Allium sativum is among the oldest of all cultivated medicinal plants (Hayat *et al.*, 2022). It has been used as a spice, food and folklore medicine for many years, and has been widely researched as a medicinal plant. *Allium sativum* is a species in the onion genus, Allium. Its close relatives include the onion, shallot and leek (van der Meer *et al.*, 2020). *Allium sativum* is native to Central Asia and North-eastern Iran. The early Egyptians were reported to have used garlic to treat diarrhoea and its medical power was described on the walls of ancient temples. In Africa, particularly in Nigeria, it is used to treat certain

conditions such as abdominal discomfort, diarrhoea and respiratory tract infections (Adeleye *et al.*, 2021).



Fig 2: *Allium sativum* (Bhatwalkar *et al.*, 2021)

Allium sativum is one of the most important bulb vegetables, which is used as a spice and flavouring agent for foods as reported by (Enebeli-Ekwutoziam *et al.*, 2021). It is widely used around the world for its pungent flavour as a seasoning or condiment. The pungency, lachrymatory effects, and spicy aroma of garlic are due to the presence of organosulfur compounds such as allicin and diallyldisulfide. *Allium sativum* adds to the taste of foods as well as helps to make them digestible. *Allium sativum* as a spice is utilized in both fresh and dehydrated states in the food industry. It is dehydrated into different products such as flakes, slices, and powders as described by Singh (2020). *Syzygium aromaticum* are a highly valued spice with a rich history in traditional medicine, particularly noted for their antimicrobial and anti-inflammatory properties (Osuntokun *et al.*, 2024). Extensively studied for their therapeutic applications, cloves have been used across cultures to alleviate toothache and respiratory issues (Singh, 2020). Their significance extends to being one of the oldest cultivated spices, with ongoing research into their phytochemical benefits (Chaudhary *et al.*, 2022).



Fig 3: *Syzygium aromaticum* (Osuntokun *et al.*, 2024).

Syzygium aromaticum are one of the most important spices, widely used as a flavouring agent and medicinal ingredient for foods as reported by (Sharma *et al.*, 2022). They are extensively utilized around the world for their warm, aromatic flavour as a seasoning or condiment in culinary dishes such as teas, baked goods, and preserved foods. The pungency, aromatic effects, and spicy aroma of cloves are due to the presence of active compounds such as eugenol and caryophyllene. Cloves are utilized in both fresh and dehydrated forms in the food

industry, often processed into whole buds, ground powder, and essential oils as described by Patel and Khan (2023).

Oral diseases represent a growing global health challenge, often linked to microbial imbalances in the oral cavity and compounded by the rise of antibiotic-resistant oral isolates, necessitating comparative evaluations of commercial toothpastes versus natural extracts from medicinal plants like *A. sativum* and *S. aromaticum* for their antimicrobial efficacy (Kumar *et al.*, 2021). Despite promising *in-vitro* evidence showing that garlic exhibit strong antibacterial activity against key oral pathogens such as *Porphyromonas gingivalis*, which are implicated in ulcer inflammations, there is lack of comparative studies integrating toothpastes with plant-based formulations to address biofilm formation and ulcer potential (Gabriel *et al.*, 2022). This gap is further highlighted by a research on clove oil, which demonstrates superior inhibition zones against dental plaque bacteria compared to aqueous extracts, yet limited data exists on their comparative effects with modern toothpastes in managing mouth ulcer amid increasing resistance (Gupta, 2021). Addressing this gap with clear comparisons could improve oral health prevention by using these plants to manage ulcers.

2. Materials and Methods

Study area

This study was conducted within Akungba-Akoko, Ondo state. All analyses were conducted at the Department of Microbiology laboratory, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria, 7.4823° N, 5.7552° E.

Collection of Recurrent Aphthous Stomatitis (RAS) Samples

The test organisms used in this study were RAS isolates obtained from the oral cavities of six (6) individuals (AAUA-HREC Approval No: 210323ERC16) using sterile swab stick. The volunteers were students of Adekunle Ajasin University, Ondo State, Nigeria. Samples were collected by gently rubbing sterile cotton swabs over the ulcer site. The swabs were then transported to the Microbiology Laboratory, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

Pure culture of the test organisms

Fresh Nutrient Agar (NA) and Potato Dextrose Agar (PDA) (Oxoid, UK) were prepared according to the manufacturer's instruction, homogenized, autoclaved at 121°C for 15 minutes. Fungal colonies were obtained by streaking on freshly prepared PDA while bacterial colonies were obtained by streaking on NA. The plates were inverted and incubated aerobically at 37°C for 24 hours (bacteria) and at 28°C for 3-5 days (fungi). Isolates were sub-cultured to obtain pure isolates, which were aseptically inoculated onto labeled slants, incubated until visible growth were observed, and preserved in the refrigerator afterwards.

Identification of bacterial isolates from mouth ulcer samples

The combination of cultural, morphological, and biochemical characteristics were employed in the identification of the bacteria isolates with reference to the Bergey's manual of systematic or determinative bacteriology. The cultural identification that were carried out on the bacterial isolates upon growth include the appearance of colonies on agar media such as; shape, color, margin, elevation, edge, texture, and transparency. Gram staining reaction and Biochemical tests

were carried out on bacterial isolates. Biochemical tests included; coagulase, catalase, indole, methyl red, motility, oxidase, sugar fermentation (sugar used included; glucose, sucrose, lactose, galactose, mannitol and arabinose), Voges-Proskauer, citrate utilization, and urease tests.

Identification of fungal isolates from the mouth ulcer samples

Fragment of fungal colony was placed on a slide, and stained with lactophenol cotton blue stain, covered with a cover slip, and then examined under the microscope using x40 objective lens. The fungal morphology was examined by observing the colony features, including the colour, shape, size, spore-bearing structure and hyphae (Kak and Kishor, 2025).

Procurement of toothpastes and medicinal plants

Six different brands of toothpastes were purchased from different merchants shops in two local markets (Okusa and Ibaka market) in Akungba-Akoko, Ondo state. These toothpastes included: Close-up (tingly red), Oral-B, Dabur herbal (Red denitrifice), Colgate maximum protection, Xtreme fluoride (Activated charcoal) and Pepsodent (triple action). Their batch numbers, registration numbers, manufacture and expiry dates as well as the presence of the manufacturer's seal were documented.

Collection, authentication and preparation of plant samples

Garlic (*Allium sativum*) and clove (*Syzygium aromaticum*) were purchased from the same Akungba-Akoko markets. The plants were authenticated by Prof. Obembe of the Department of Plant Science and Biotechnology Herbarium (PSBH), Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. The garlic bulbs were washed, peeled and sliced into small chips (dried). The clove buds were also washed. These samples were then dried to reduce moisture content. The dried samples were gathered together and powdered using a sterile blender and stored in clean containers. The powdered samples were extracted using ethanol as solvent. Twenty grams of each sample were soaked with 100 ml of 95% ethanol for 72 hours with constant agitation to permit solvent penetration and full extraction of the active ingredients. After 72 hours, the homogenates were filtered using Whatman No1 filter paper to separate the liquid extracts from the solid residues. The resulting extracts were collected in a round bottom flask, filtered and concentrated using a rotary evaporator at 25°C and then oven dried. The crude extracts were stored aseptically in airtight containers and kept in the refrigerator at 4°C for further screening (Osuntokun, 2021).

Standardization of the plants' extracts

Plants' extracts were reconstituted by adding 1g of each extract to 2.5 ml of dimethyl sulphoxide (DMSO) and 100ml of sterile distilled water, to make a stock solution of 100 mg/ml. Two-fold serial dilution of the stock extracts were made by drawing 20 ml from the stock extracts (100 mg/ml) into 20 ml of sterile distilled water in sterile bottles to obtain concentrations of 50, 25, 12.5, 6.25, 3.125 mg/ml (Osuntokun, 2021).

Preparation of toothpaste solution

The different toothpaste solutions were made by mixing the calculated amount of toothpaste (2 g) in measured volume (2 ml) of sterile pyrogen-free distilled water to give 1:1 dilution (50%); they were further diluted in sterile water and four different dilutions of 1:2 (25%),

1:4 (12.5%), 1:8 (6.25%) and 1:16 (3.125%) were made. These dilutions were used for the antimicrobial assay.

Determination of antibacterial/antifungal activity of toothpaste and garlic/clove ethanol extracts against microbial isolates using agar well diffusion method

Antibacterial activities of the extracts were determined by the agar well diffusion method as described by several authors (Ahmed *et al.*, 2021). The bacterial isolates were standardized to 0.5 McFarland standards (10^6 CFU/mL). A twenty (20 ml) of sterilized Mueller-Hinton agar was aseptically poured into sterile Petri dishes and allowed to set, after which the standardized inoculum were seeded on the plates. This was allowed to stand for ten minutes. With the aid of sterile cork borer, four wells were bored on the solidified agar culture. One millilitre each of the plant extract samples was then added into two of the wells and appropriately labelled while antibiotics drug (Ampicillin) was introduced into the third (positive control) and sterile water into the fourth (negative control). The same procedure was repeated using concentrated toothpaste slurries. The plates were allowed to stand on the laboratory bench for 15 minutes to allow diffusion of the solution into the medium before incubating in an incubator at 37°C for 24 hours. The plates were examined for clear zones of inhibition around wells. These were measured in millimetres using transparent meter ruler. The same method was used to access the antifungal activity using fungal isolates and antifungal drug (Fluconazole) as a positive control (Osuntokun, 2021).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of Toothpastes and Garlic/Clove Ethanol Extracts against Test Organisms

The MIC of the extract was determined using macro-broth dilution technique as modified by (Huanfang *et al.*, 2024)). Different concentrations of the extracts were prepared at 50, 25, 12.5, 6.25 and 3.125 % in test tubes containing Mueller-Hinton broth for bacteria and potatoes dextrose broth for fungi. This was followed by the addition of 0.1 ml of the standardised inoculum (1×10^6 CFU/mL) into each test tube. A set of test tubes containing only Mueller-Hinton broth (bacteria) or potatoes dextrose broth (fungi) were used as negative controls, another set of test tubes containing antibacterial or antifungal agent in Mueller-Hinton or potatoes dextrose broth plus each test organism were used as positive controls. All the test tubes were then incubated at 37°C for 24 hours. Growth in each tube was checked by using a spectrophotometer. Growth inhibition was indicated by no and low turbidity while growth was indicated by high turbidity. The concentration at which there was no/least growth as indicated by clear broth was taken as the MIC. The MBC/MFC was determined by culturing 1 ml from each tube that showed no growth during MIC determination on Mueller-Hinton agar for bacteria and PDA for fungi, followed by incubation at 37°C for 24 hours. The least concentration with no growth was taken as the MBC/MFC.

Time-kill Kinetics Assay of the Extracts (*A. sativum* and *S. aromaticum*) and Toothpaste

A time-kill kinetics assay was conducted to evaluate the antimicrobial activity of the selected toothpaste, clove extract and garlic extract against fourteen (14) microbial isolates (bacterial and fungal species). This test was designed to evaluate the growth rate of the

isolates and determine their kill time on exposure to the clove/garlic extracts and toothpaste. A total of 48 test tubes, each containing 9 ml of nutrient broth or potato dextrose broth, were divided into three sets of 14 test tubes, each corresponding to one test substance and labelled A, B and C. Then, the isolates were inoculated into the test tubes in each set and were labelled with the correct isolate codes. Six (6) additional test tubes containing nutrient broth only served as controls. These test tubes were incubated at appropriate temperatures (bacteria at 37°C for 24 hours) and fungi (28°C for 2 days). Prior to measurement, the spectrophotometer was calibrated to a wave length of 620nm and allowed to warm up for 30 minutes. The initial optical density was recorded at the zero-hour mark for all the three sets, serving as the baseline control. Subsequent readings were taken every 8 hour for a total of five readings. At the fifth reading, corresponding to the 32-hour mark in set A, the toothpaste was introduced to evaluate the kill rate of the isolates. In set B and C, to assess the killing rate, clove and garlic extract were applied at the same 32-hour mark (Etah, 2025).

3. Results

Table 1 details the cultural characteristics of the bacterial isolates analyzed from mouth ulcer, including shape, texture, opacity, color, elevation and margin. The isolates exhibited the following colours; grayish-white, white and creamy, with varying elevations such as raised and convex. Some were brittle, mucoid and dry while others were slimy and moist; some were round and irregular in shape. It was also observed that the isolates had lobate, entire (smooth) or wavy margin. Notably, the opacity of all isolates is predominantly opaque. Table 2 shows the Gram's reaction and biochemical characteristics confirmation of the isolates which indicated that some were Gram-positive while some were Gram-negative; all were rod-shaped organisms, with the exception of isolate M5A10⁵, which is coccus in shape. The isolates were either positive or negative for catalase, motility, indole, urease, citrate, oxidase, coagulase, methyl red, Voges-Proskauer, gas, lactose, sucrose, galactose, arabinose, mannitol and glucose test. Table 2 shows the mouth ulcer bacterial isolates identified using Bergey's Manual of Determinative Bacteriology. The identified organisms were: *C. matruchotii*, *L. casei*, *S. salivarius*, *E. cloacae*, *L. acidophilus*, *L. reuteri*, *K. pneumoniae*, *Bacillus cereus*, *L. brevis* and *B. licheniformis*.

Table 3 details the morphological and cultural characteristics of the fungal isolates analyzed from mouth ulcer, including appearance of colony on PDA, nature of hyphae, spore bearing structures and spore producing structures (as shown in table 4). The isolates appeared on PDA as; creamy, white, butyrous, cottony, powdery, wrinkled and smooth. Some have septate hyphae that are colorless while others have pseudohyphae. Most possess ascospores as spore bearing structures while some have blastoconidia and conidiophores as spore bearing structures. Table 5 shows the microscopic characteristics of the fungal isolates. The isolates were stained with lactophenol cotton blue and examined under a microscope with objective lens of x40. Table 6 shows the mouth ulcer fungal isolates identified upon staining with lactophenol cotton blue stain and examining under a microscope. The probable organisms confirmed from the fungal staining test are: *Pichia fermentans*, *Geotrichium candidum*, *Saccharomycopsis fibuligera* and *Candida tropicalis*.

Table 1: Cultural characteristics of recurrent aphthous stomatitis (mouth ulcer) bacterial isolatesKey: M1A10⁵, M6A10³, M5A10⁵, M2A10³, M6B10³, M6A10⁵, M3A10³, M3B10⁵, M2B10⁵ and M5A10³ represent the isolate code.

| Isolate Code | Shape | Texture | Opacity | Colour | Elevation | Margin |
|--------------------|-----------|---------|---------|---------------|-----------|-----------------|
| M1A10 ⁵ | Irregular | Brittle | Opaque | Grayish-white | Raised | Lobate |
| M6A10 ³ | Round | Moist | Opaque | Creamy | Convex | Entire (smooth) |
| M5A10 ⁵ | Round | Moist | Opaque | White | Convex | Entire (smooth) |
| M2A10 ³ | Round | Slimy | Opaque | Creamy | Convex | Entire (smooth) |
| M6B10 ³ | Round | Moist | Opaque | Creamy | Convex | Entire (smooth) |
| M6A10 ⁵ | Round | Moist | Opaque | White | Convex | Entire (smooth) |
| M3A10 ³ | Round | Mucoid | Opaque | Creamy | Convex | Entire (smooth) |
| M3B10 ⁵ | Irregular | Dry | Opaque | White | Raised | Undulate (wavy) |
| M2B10 ⁵ | Round | Moist | Opaque | Creamy | Convex | Entire (smooth) |
| M5A10 ³ | Irregular | Dry | Opaque | White | Raised | Undulate (wavy) |

Table 2: Microscopic and biochemical characteristics of recurrent aphthous stomatitis (mouth ulcer) bacterial isolates

| Isolate code | Gram's reaction | Citrate | Motility | Coagulase | Urease | Oxidase | Catalase | Indole | Methylred | Voges Proskauer | Arabinose | Mannitol | Galactose | Glucose | Lactose | Sucrose | Probable organism |
|--------------------|-----------------|---------|----------|-----------|--------|---------|----------|--------|-----------|-----------------|-----------|----------|-----------|---------|---------|---------|------------------------------------|
| M1A10 ⁵ | +rod | + | - | - | + | - | + | - | - | - | +G | +G | +G | +G | +G | +G | <i>Corynebacterium matruchotii</i> |
| M6A10 ³ | +rod | - | - | - | - | - | - | - | - | + | +G | +G | +G | +G | +G | +G | <i>Lactobacillus casei</i> |
| M5A10 ⁵ | +cocci | - | - | - | - | - | - | - | - | + | -g | +G | +G | +G | +G | +G | <i>Streptococcus salivarius</i> |
| M2A10 ³ | -rod | + | + | - | + | - | - | + | - | + | +G | +G | +G | +G | +G | +G | <i>Enterobacter cloacae</i> |
| M6B10 ³ | +rod | - | - | - | - | - | - | - | - | + | +G | +g | +G | +G | +G | +g | <i>Lactobacillus acidophilus</i> |
| M6A10 ⁵ | +rod | - | - | - | - | - | - | - | - | - | +G | +G | +G | +G | +G | +G | <i>Lactobacillus reuteri</i> |
| M3A10 ³ | -rod | + | - | - | + | - | + | - | - | + | +G | +G | +G | +G | +g | +G | <i>Klebsiella pneumonia</i> |
| M3B10 ⁵ | +rod | + | + | - | - | - | + | - | - | + | +g | +G | +g | +g | +G | +G | <i>Bacillus cereus</i> |
| M2B10 ⁵ | +rod | + | + | - | - | - | - | - | - | + | +g | +g | +G | +g | +G | +g | <i>Lactobacillus brevis</i> |
| M5A10 ³ | +rod | + | + | - | + | - | + | - | - | + | +g | +g | +g | +g | +g | +g | <i>Bacillus licheniformis</i> |

Key: M1A10⁵, M6A10³, M5A10⁵, M2A10³, M6B10³, M6A10⁵, M3A10³, M3B10⁵, M2B10⁵ and M5A10³ represent the isolate code.

+G represents positive for acid and gas; +g represents positive for acid and negative for gas; -g represents negative for both acid and gas.

Figure 4 shows the colony forming units (CFU/mL) of the bacteria enumerated from mouth ulcer samples at a dilution of 10³. The results indicated that the CFU/mL values ranged from 9.0 x 10⁴ CFU/mL (MU 4) to 2.0 x 10⁵ CFU/mL (MU 1). MU 2 showed a bacterial count of 1.0 x 10⁵ CFU/mL, followed by MU 3 (1.3 x 10⁵ CFU/mL), MU 5 (1.3 x 10⁵ CFU/mL), and MU 6 (1.4 x 10⁵ CFU/mL). MU 1 recorded the highest microbial load while sample MU 4 had the lowest count. Also, the results revealed that among the toothpastes tested, Colgate (COL)

was the most effective overall with inhibition zones ranging from 0-37 mm. It was highly effective against eight isolates (*K. pneumoniae*, 37 mm, *L. casei*, 33 mm), moderately effective against 1 (*B. cereus*, 25 mm) and ineffective against 1 (*C. matruchotii*, 0 mm). Xtreme charcoal, Dabur herbal (DAB), Pepsodent (PEP), and Oral-B (OB) all demonstrated significant inhibition against these isolates, with zones ranging from 15-30, 12-30, 15-30, and 15-26 mm, respectively. Close up (tingly red) had comparatively lower activity with inhibition zone

ranging from 9-29 mm. *Allium sativum* and *S. aromaticum* ethanol extracts exhibited similar antibacterial activity against these isolates with inhibition zones ranging from 12-31mm and 14-29 mm, respectively. Negative control wells (without any active agent) showed no inhibition and positive control wells (with ampicillin) showed the highest zone of inhibition at 20 mm against *L. acidophilus* and the lowest zone of inhibition (8 mm) for *B. cereus* (Figure 5).

Figure 6 represents the MIC values (in percentage, %) of six different toothpastes and *A. sativum* /*S. aromaticum* ethanol extracts inhibited the growth of various mouth ulcer bacterial isolates. Lower MIC values indicate higher antibacterial activity. *Klebsiella pneumoniae* showed the lowest MIC (3.125%) with Colgate. *Corynebacterium matruchotii* and *E. cloacae* showed variable antibacterial activity with some extracts (e.g., Colgate at 0% and Garlic at 50%) while *Lactobacillus* spp., generally had MICs between 6.25 and 25%. Garlic and Clove extracts (GAR and CLV) exhibited consistent inhibition (12.5% to 50%) across the isolates with notable effectiveness against *S. salivarius* and *Bacillus cereus*. Toothpaste brands like Colgate and Pepsodent showed varying potency with Colgate being particularly effective against *K. pneumoniae* (3.125%) and less effective against *C. matruchotii* (0%).

Figure 7 represents the MBC values (in percentage %) of six different toothpastes and garlic/clove ethanol extracts required to kill various mouth ulcer bacterial isolates. Lower MBC values indicate higher bactericidal activity. Based on this table, *Lactobacillus reuteri* showed the lowest MBC (6.25%) with Colgate and Pepsodent. Similarly, *C. matruchotii* and *S. salivarius* showed variable effectiveness with both Colgate and Close up at 0%. *Lactobacillus* spp., revealed MBC values between 6.25% and 100% with Colgate and Pepsodent showing lower values (6.25%) for some isolates. Garlic (GAR) and Clove (CLV) extracts exhibited higher MBC values (up to 100%) against *L. reuteri* and *K. pneumoniae*. Toothpaste brands like Colgate and Pepsodent show varying potency with Colgate being particularly effective against *L. reuteri*, *B. licheniformis* (6.25%) while Oral-B and Close-up showed higher MBC values (up to 100%) against *L. casei* and *L. brevis*.

Figure 8 showed the time-kill kinetics assay of *A. sativum* at 50% against mouth ulcer bacterial isolates. The reductions ranged from approximately 66% for *E. cloacae* (0.192 to 0.065) to 96% for *L. casei* (0.303 to 0.012). *Allium sativum* showed strong bacteriostatic activity for all isolates. For *L. casei*, *S. salivarius* and *L. brevis*, it demonstrated significant bactericidal potential, achieved $\geq 90\%$ reduction in viable biomass. The strongest antimicrobial effect was observed post-24 hours. Figure 9 showed the time-kill kinetics assay of *S. aromaticum* at 50% against mouth ulcer bacterial isolates. The reductions ranged from approximately 79% for *L. brevis* (0.259 to 0.055) to 95% for *B. cereus* (0.432 to 0.022). Clove showed strong bacteriostatic activity for all isolates, effectively inhibited proliferation. Potential bactericidal effects ($\geq 90\%$ reduction) were noted for *L. acidophilus*, *L. casei*, *Bacillus cereus* and *L. brevis*. The strongest antimicrobial effect was observed at 32 hours. The reductions ranged from approximately 84% for *L. reuteri* (0.215 to 0.030) to 98% for *S. salivarius* (0.301 to 0.007). Colgate exhibited strong bacteriostatic activity across all isolates. Potential bactericidal effects ($\geq 90\%$ reduction) are noted for *L. casei*, *S. salivarius*, *E. cloacae*, *B. licheniformis* and *L. brevis* (Figure 10).

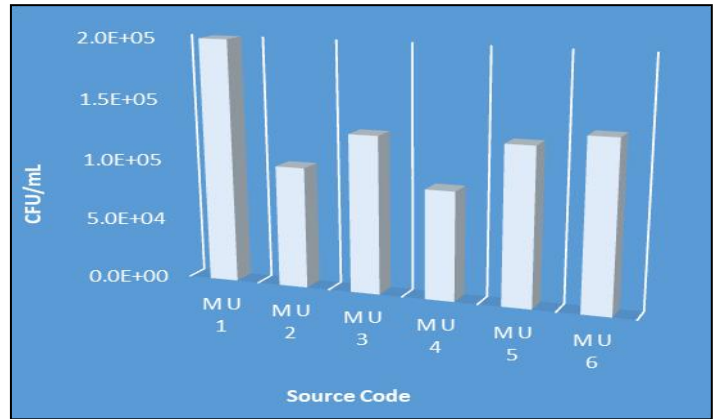


Fig 4: Colony counts of bacterial isolates on nutrient agar from mouth ulcer. Key: MU = mouth ulcer

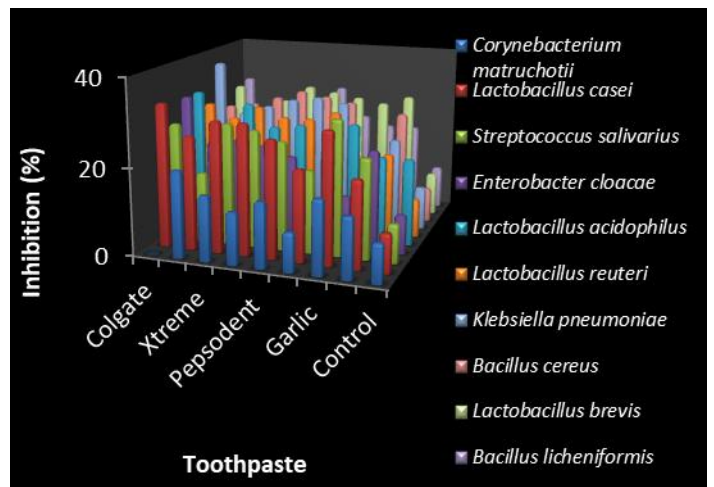


Fig 5: Synergistic antibacterial activity of selected toothpastes and *A. sativum* / *S. aromaticum*, ethanol extracts against bacterial isolates from mouth ulcer. Key: Zone of inhibition (mm).

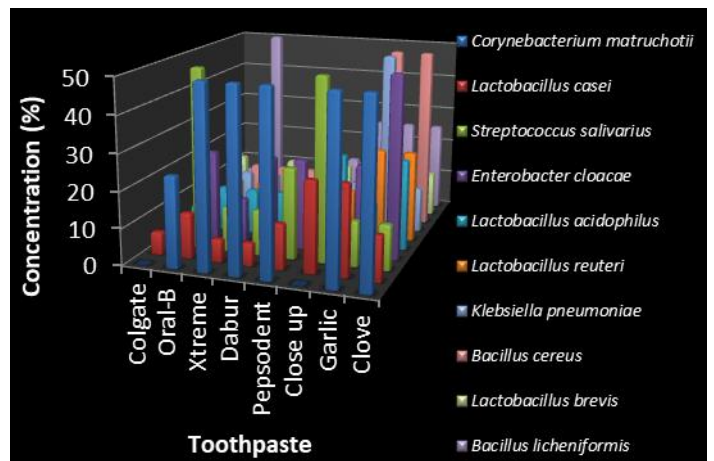


Fig 6: Minimum Inhibitory Concentration (MIC) of selected toothpastes & *A. sativum*/*S. aromaticum*, ethanol extracts against mouth ulcer bacterial isolates

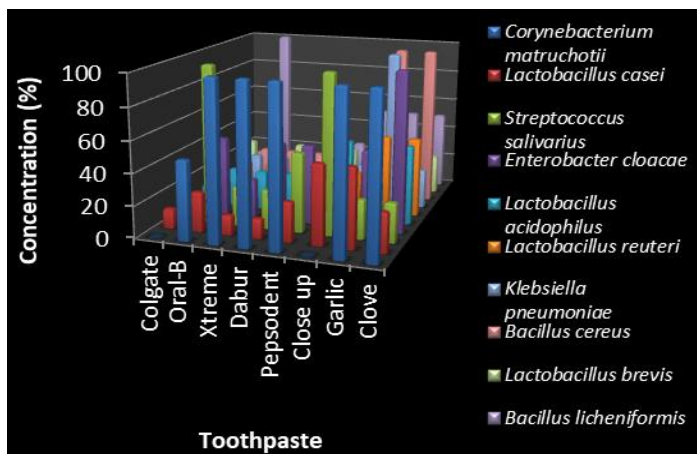


Fig 7: Minimum Bactericidal Concentration (MBC) of selected toothpastes & garlic/clove ethanol extracts against mouth ulcer bacterial isolates

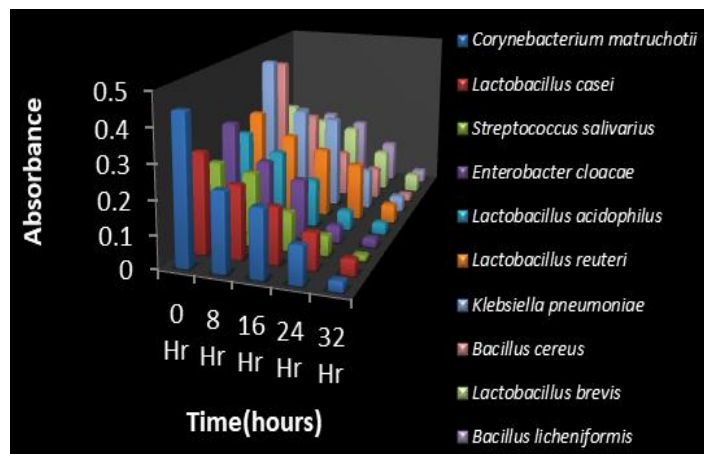


Fig 9: Time-kill kinetics assay of *S. aromaticum* at 50% against mouth ulcer bacterial isolates

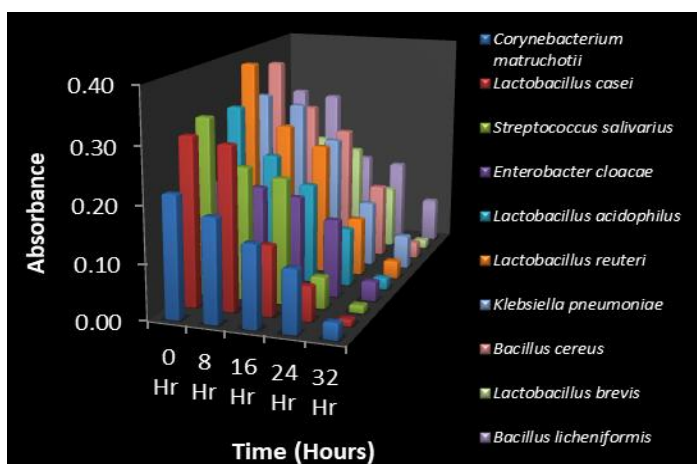


Fig 8: Time-kill kinetics assay of *A. sativum* at 50% against mouth ulcer bacterial isolates

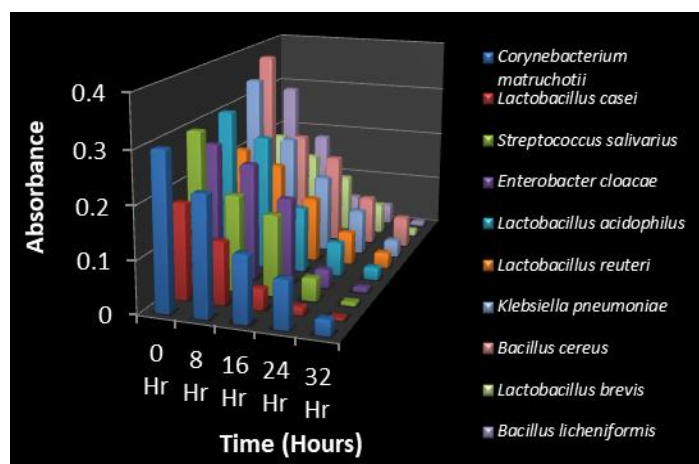


Fig 10: Time-kill kinetics assay of toothpaste at 50% against mouth ulcer bacterial isolates

Table 3: Morphological characteristics of recurrent aphthous stomatitis (mouth ulcer) fungal isolates

| Isolate code | Appearance of colony on PDA | Nature of hyphae | Spore bearing structure and spore producing structure | Micrograph | Probable organism |
|--------------------|-----------------------------|-------------------|--|------------|------------------------------------|
| M5A10 ⁵ | Creamy, butyrous, wrinkled | Pseudohyphae | Evanescent (short-lived) asci bearing 2-4 hat-shaped ascospores; may arise from conjugated or unconjugated cells | | <i>Pichia fermentans</i> |
| M2B10 ³ | White, cottony | Septate (hyaline) | Simple conidiophores bearing unicellular, rectangular or barrel-shaped conidia formed by fragmentation of hyphae (arthroconidia) | | <i>Geotrichium candidum</i> |
| M3D10 ³ | White, powdery | Septate (hyaline) | Forms evanescent asci (spore-bearing) containing 2-4 oval ascospores (spore-producing); spores may adhere to the ends or sides of hyphae | | <i>Saccharomycopsis fibuligera</i> |
| M2C10 ⁵ | Creamy, smooth | Pseudohyphae | Forms blastoconidia; produced by budding from pseudohyphae or yeast cells | | <i>Candida tropicalis</i> |

Figure 11 represents the colony forming units (SFU/ml) of the fungi enumerated from mouth ulcer samples at a dilution of 10^{-3} . The results indicate that the SFU/ml values ranged from 8.0×10^4 SFU/ml (MU 3) to 2.9×10^5 SFU/ml (MU 5). MU 1 had the fungal load of 1.0×10^5 SFU/ml, followed by MU 2 (9.0×10^4), MU 4 (2.7×10^5), and MU 6 (2.4×10^5) SFU/ml, showing a moderate variation in microbial concentration among the samples. MU 5 recorded the highest load while MU 3 had the lowest count. The results revealed that among the toothpastes tested, Colgate (COL) was the most effective with inhibition zones ranging from 25-31 mm. It was highly effective against two isolates against *P. fermentans* (31 mm) against *S. fibuligera* (28 mm) while moderately effective against two against *C. tropicalis* and *G. candidum* (25mm). Xtreme charcoal (XC), Dabur herbal (DAB), Oral-B (OB), Pepsodent (PEP) and Close-up (CLP) all demonstrated significant inhibition against the isolates with zones ranging from 25-27, 23-30, 24-26, 21-27, and 22-26 mm, respectively. Among the extracts, clove ethanol extracts exhibited greater antifungal activity against the isolates with inhibition zones ranging from 23-30 mm; garlic ethanol extract had a comparatively lower antifungal activity, with inhibition zones ranging from 20-25 mm. Negative control wells (without any active agent) showed no inhibition and the positive control wells (with fluconazole) showed the highest zone of inhibition at 33 mm against *G. candidum* and the lowest zone of inhibition (20 mm) was recorded for *C. tropicalis* (Figure 12).

Figure 13 presents the MIC values (in percentage %) of *A. sativum* / *S. aromaticum* ethanol extracts required to inhibit the growth of various mouth ulcer fungal isolates. *Pichia fermentans* showed the lowest MIC (6.25%) with Colgate. *G. candidum* and *S. fibuligera* generally revealed MICs between 12.5% and 25% with Colgate and Garlic showing consistent inhibition at 12.5%. *Candida tropicalis* exhibited uniform sensitivity across most extracts with MICs consistently at 12.5%, except for Dabur herbal (25%). Garlic and Clove extracts (GAR and CLV) demonstrated effective inhibition (12.5%) across all tested isolates, while toothpaste brands like Colgate and Pepsodent showed varying potency, with Colgate being particularly effective against *P. fermentans* (6.25%).

All isolates showed a consistent MFC of 25% across most extracts, indicating uniform fungicidal activity. It was observed in this table that *C. tropicalis* and *P. fermentans* have an MFC of 25% against all toothpastes and extracts. *Saccharomycopsis fibuligera* and *G. candidum* exhibited higher MFC values (50%) with Oral-B and Close-up. Garlic (GAR) and Clove extracts maintained an MFC of 25% across all isolates, showing consistent fungicidal activity. Toothpaste brands like Colgate, Pepsodent, Dabur herbal, and Xtreme charcoal demonstrate

uniform effectiveness at 25% MFC, while Oral-B and Close-up showed reduced potency (50%) against *S. fibuligera* and *G. candidum* (Figure 14). The reductions range from approximately 75% for *C. tropicalis* (0.240 to 0.060) to 95% for *S. fibuligera* (0.321 to 0.016) with *P. fermentans* at 92% (0.201 to 0.017) and *G. candidum* at 90% (0.325 to 0.032). Garlic showed strong fungistatic to potentially fungicidal effects across all isolates (Figure 15). The reductions range from approximately 66% for *G. candidum* (0.281 to 0.095) to 84% for *C. tropicalis* (0.215 to 0.035) with *P. fermentans* at 79% (0.241 to 0.050) and *S. fibuligera* at 83% (0.439 to 0.076). Clove demonstrated strong fungistatic to

potentially fungicidal effects across all isolates (Figure 16). Also, the reductions range from approximately 88% for *C. tropicalis* (0.244 to 0.030) to 95% for *S. fibuligera* (0.253 to 0.012), with *P. fermentans* at 91% (0.540 to 0.050) and *G. candidum* at 89% (0.325 to 0.035) were observed. Colgate exhibited strong fungistatic to potentially fungicidal effects across all isolates.

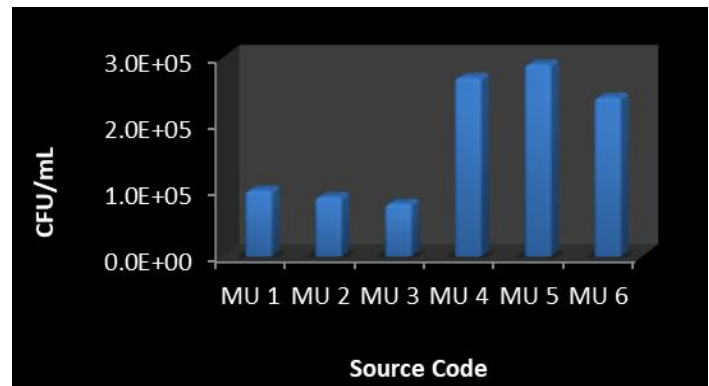


Fig 11: Colony counts (SFU/ml) on potato dextrose agar from mouth ulcer fungal isolates

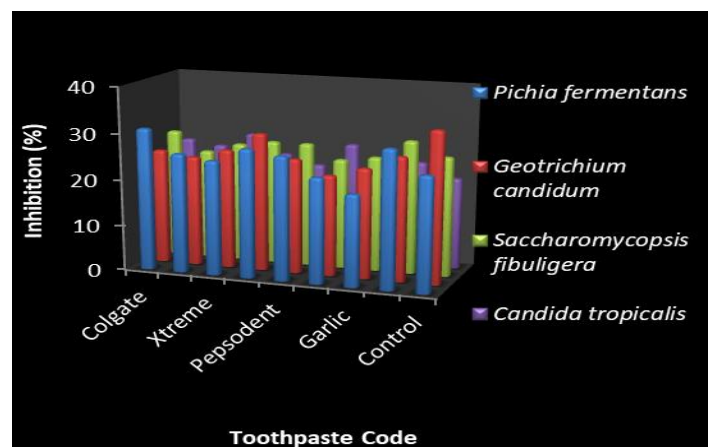


Fig 12: Antifungal activity of selected toothpastes and *A. sativum* / *S. aromaticum* ethanol extracts against mouth ulcer fungal isolates (Zone of Inhibition mm).

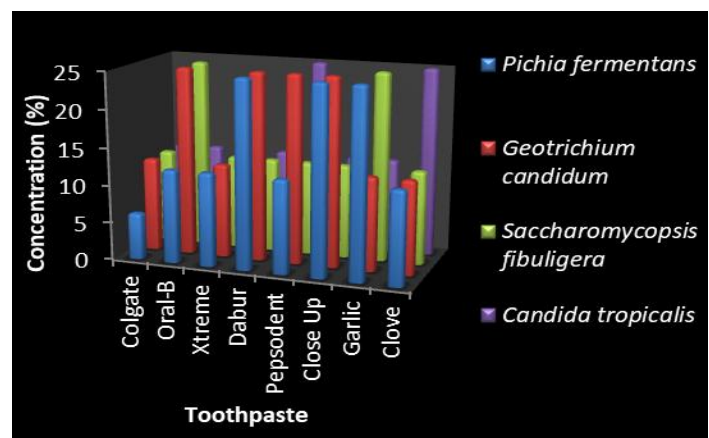


Fig 13: Minimum Inhibitory Concentration (MIC) of selected toothpastes / *A. sativum* / *S. aromaticum* ethanol extracts against mouth ulcer fungal isolates

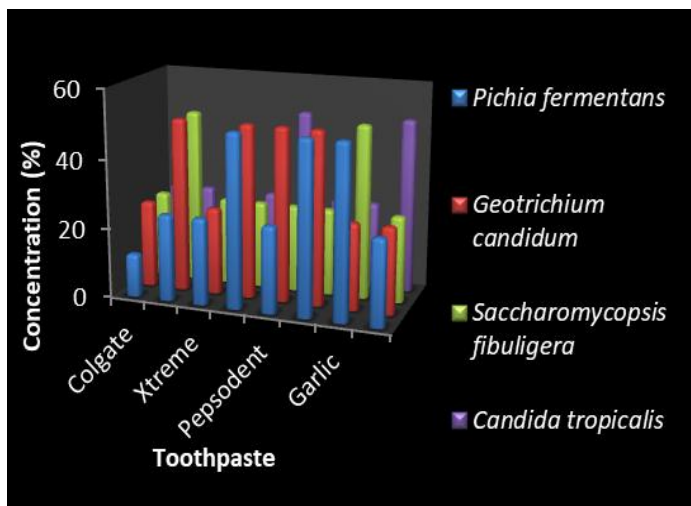


Fig 14: Minimum Fungicidal Concentration (MFC) of selected toothpastes and *A. sativum*/*S. aromaticum* ethanol extracts against mouth ulcer fungal isolates

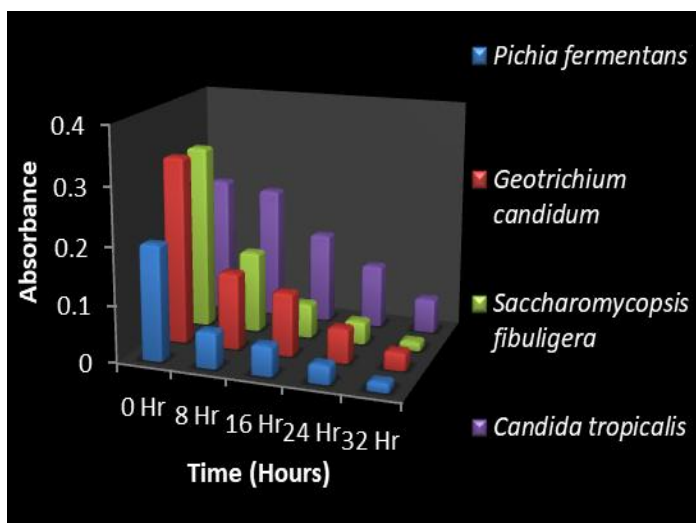


Fig 15: Time-kill kinetics assay of *A. sativum* at 50% against mouth ulcer fungal isolates

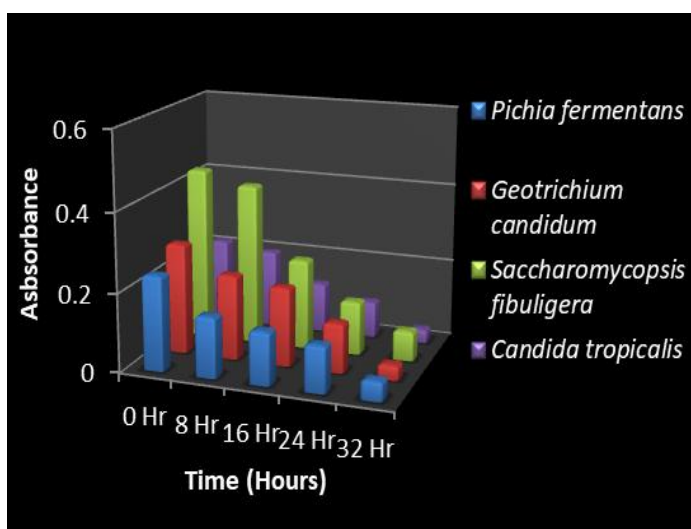


Fig 16: Time-kill kinetics assay of *S. aromaticum* at 50% against mouth ulcer fungal isolates

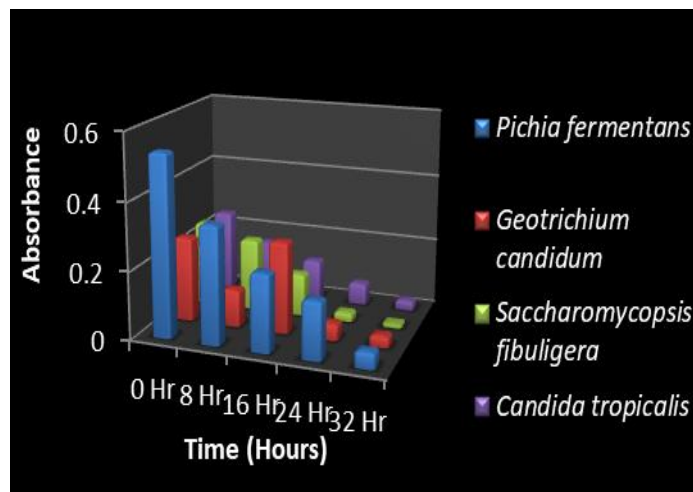


Fig 17: Time-kill kinetics assay of toothpaste at 50% against mouth ulcer fungal isolates

4. Discussion

Bacterial and fungal counts from mouth ulcer samples (MU 1 - MU 6) on NA and PDA revealed substantial microbial loads, highlighting the potential for pathogen proliferation in ulcer environments. Bacteria counts varied from 9.0×10^4 CFU/mL (MU 4) to 2.0×10^5 CFU/mL (MU 1), with an average of approximately 1.3×10^5 CFU/mL. Higher bacterial counts in MU 1 and MU 6 (2.0×10^5 and 1.4×10^5 CFU/mL, respectively) indicated differences in ulcer aetiology or microbial virulence. Fungal counts were notable, ranging from 8.0×10^4 CFU/mL (MU 3) to 2.9×10^5 CFU/mL (MU 5), averaging 1.7×10^5 CFU/mL. Higher bacterial counts in MU 5 and MU 6 (2.9×10^5 and 2.4×10^5 CFU/mL, respectively) suggested yeast dominance, possibly exacerbated by pH shifts. These observations are consistent with recent research carried out by Etebarian *et al.* (2023) who documented comparable bacterial densities ($10^4 - 10^6$ CFU/mL) in oral ulcers, emphasizing *Lactobacillus* spp. as opportunistic contributors to acidic microenvironments that exacerbate lesions.

The isolates obtained in this study included bacteria such as *C. matruchotii*, *Lactobacillus* spp., *S. salivarius*, *E. cloacae*, *K. pneumoniae*, *Bacillus* spp., and fungi like *P. fermentans*, *Geotrichium candidum*, *S. fibuligera* and *C. tropicalis*. This showed that opportunistic pathogens are implicated in mouth ulceration. These microbes exploit compromised oral mucosa, promoting inflammation and hindering healing. This is similar to the findings of Yang *et al.* (2023) who through a meta-analysis of case-control studies identified elevated abundances of *S. salivarius*, *L. casei* and other Enterobacteriaceae genera (including *E. cloacae*-like species) in active ulcer lesions compared to healthy mucosa, mirroring the opportunistic roles to acidogenic biofilm formation and immune dysregulation. Similarly, Min *et al.* (2023) reported dysbiotic overgrowth of opportunistic fungi such as *C. tropicalis* in RAS lesions, which exploit mucosal breaches to promote inflammation and delay epithelial repair, corresponding to the hindering mechanisms observed in these isolates.

Antibacterial efficacy assessed via zones of inhibition showed that toothpastes such as Colgate and Dabur herbal displayed robust antibacterial activity, with zones of inhibitions reaching 37 mm against *K. pneumoniae* (susceptible category: 28-40 mm). Colgate consistently

produced larger zones reaching 33 mm against *L. casei*, attributable to its fluoride and triclosan formulations that compromise bacterial cell walls. Plant extracts exhibited comparable performance; garlic (GAR) yielded 31 mm against *S. salivarius* and clove achieved 29 mm against *Lactobacillus brevis*. These results aligned with the report of Enebeli-Ekwutoziam *et al.* (2021) who reported garlic's allicin inhibiting *Streptococcus* spp. with zones of 25-30 mm through biofilm disruption via organosulfur compounds. Clove's eugenol demonstrated intermediate efficacy (11-25 mm) in several isolates, corresponding to findings of Hayat *et al.* (2022) on its role in combating multi-drug resistant oral pathogens.

Further quantification through MIC and MBC revealed low thresholds for effective inhibition. For instance, Colgate had MIC of 3.125% against *K. pneumoniae* with MBC often twice the MIC. This indicated bactericidal potential. Garlic displayed exceptional potency with 0% MIC against *S. salivarius*. This is likely due to synergistic sulfur-based mechanisms, as evidenced in Sunil *et al.* (2025) where aged garlic extracts achieved MICs below 6.25% against oral biofilms. Clove MIC ranged from 12.5-50%, effective yet generally higher than those of toothpastes like Oral-B (6.25-50%).

In antifungal evaluations, toothpastes like Darbur herbal revealed zones up to 30 mm against *G. candidum* while clove matched this with 30 mm against *P. fermentans*. The MIC and MFC data confirmed Colgate low MICs (12.5% against most fungi) with 25-50% MFC of garlic denoting fungicidal activity. These outcomes resonates with recent research carried out by (30) in which garlic extract showed zones of 4.89 mm against *C. tropicalis*, significantly smaller than the 30 mm evaluated in this study. This may be due to pure vs. ethanol extracts, highlighting this study's method enhanced inhibition. For clove, Didehdar *et al.* (2022) observed inhibition at 12.5-25% MIC against *Candida* spp. via eugenol-mediated membrane disruption, matching the MIC range in this study but with the MIC indicating stronger fungicidal effects against *G. candidum* and *S. fibuligera*.

Time-kill kinetics assays for bacteria, garlic extract exhibited >90% reduction by 8-24 hours across strains, clove exhibited approximately 85-95% reduction by 16 hours, and Colgate toothpaste exhibited approximately 70% reduction by 24 hours with residual viability. Fungi showed similar patterns: garlic exhibited >95% reduction by 16 hours, clove exhibited approximately 80% reduction by 24 hours. Colgate exhibited approximately 75% reduction by 32 hours. No re-growth indicated bactericidal/fungicidal action with garlic/clove outperforming toothpaste in speed. Comparatively, garlic and clove extracts often equalled or exceeded commercial toothpastes, especially garlic against bacterial isolates. The elevated inhibition zones for garlic in this study (up to 30 mm) surpass those in Osuntokun (2015), Torbati *et al.* (2021), Osuntokun *et al.* (2024), and Osuntokun *et al.* (2024), with the value of 18-24 mm. This may be due to optimized ethanol extraction method which enhances bioactive availability.

5. Conclusion

In conclusion, this study showed that toothpastes and extracts from *A. sativum* and *S. aromaticum* effectively inhibited microorganisms associated with mouth ulcers and oral infection when incorporated into toothpaste. Pathogens like *Corynebacterium matruchotii*, *Lactobacillus* spp., and *Candida tropicalis* were prevalent, indicating dysbiosis in

ulcer environments that promotes inflammation and delay healing. Antimicrobial assays showed robust efficacy with zones of inhibition up to 37 mm (Colgate against *Klebsiella pneumoniae*), low MIC values (0% for garlic against *Streptococcus salivarius*), and rapid time-kill reductions (>90% within 8-24 hours for garlic). Extracts (garlic and clove) often matched or outperformed commercial toothpastes, attributed to allicin and eugenol, respectively. This study recommended incorporating *A. sativum* and *S. aromaticum* extracts into toothpaste as natural antimicrobial agents to effectively manage mouth ulcers, leveraging their proven efficacy against pathogens.

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There is no funding obtained for this research work.

Competing Interest

There is no competing interest for this research work.

Data Availability Statement

Available upon request.

Ethics Approval

AAUA Human Research Ethics Committee granted approval for the conduct of this research (Approval No: 210323ERC16).

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