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Antimicrobial Effects of *Azadirachta indica* and *Vachellia nilotica* Resin Extracts on *Streptococcus mutans* and *Candida albicans* isolated from Dental Plaque

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ABSTRACT

This study evaluated the antimicrobial efficacy of ethanolic and aqueous extracts of *Azadirachta indica* and *Vachellia nilotica* against *Streptococcus mutans* and *Candida albicans*, key pathogens involved in dental caries and oral candidiasis. Sixty dental caries patient samples were cultured, with 44 positives for *S. mutans* and 5 for *C. albicans*. Both plants' ethanolic extracts demonstrated significantly greater antimicrobial activity compared to their aqueous counterparts, as evidenced by larger zones of inhibition and lower minimum inhibitory concentrations (MIC) and minimum bactericidal/fungicidal concentrations (MBC/MFC). For *S. mutans*, the MIC and MBC of ethanolic extracts ranged from 0.4 to 0.8 µg/ml and 0.8 to 1.6 µg/ml, respectively, whereas aqueous extracts showed higher MIC/MBC values. Similarly, against *C. albicans*, ethanolic extracts exhibited MIC and MFC values between 0.4 and 0.8 µg/ml, outperforming aqueous extracts which required concentrations up to 6.4 µg/ml. These findings indicate that bioactive compounds such as nimbidin and azadirachtin, more effectively extracted in ethanol, contribute to the potent antimicrobial effects. This study supports the traditional use of *A. indica* and *V. nilotica* in oral health and highlights their potential as natural alternatives for managing dental infections.

Introduction

The human oral cavity harbors a diverse microbiota, comprising approximately 700–1,000 bacterial species, which are closely linked to oral diseases such as dental caries and periodontal disorders (Mahdi, Shaker, & Alamiery, 2024). *Streptococcus mutans* and *Candida albicans* are key pathogens involved in dental plaque formation and cariogenesis (Moussa, Ahmad, Mansour, Siqueira, & Microbiology, 2022). Dental caries is an infectious disease characterized by enamel demineralization caused by acids produced from carbohydrate fermentation by oral bacteria (Inchingolo et al., 2022). Risk factors include high sugar consumption, poor oral hygiene, and socioeconomic status (Issrani et al., 2023). The dental biofilm protects bacteria and facilitates acid production, disrupting the balance between demineralization and remineralization (Mosaddad et al., 2019). Globally, dental caries affects over 90% of school-age children and adults, posing a significant public health burden (Palombo & Medicine, 2011). Medicinal plants such as *Azadirachta indica* and *Vachellia nilotica* have demonstrated antimicrobial activity against oral pathogens, presenting potential alternatives for dental disease management (Byakod & Health, 2023).

Dental caries is a multifactorial disease primarily driven by the dynamic interplay between fermentable carbohydrates and oral microbial communities, notably *Streptococcus mutans* and *Candida albicans* (Zhu et al., 2023). The formation of dental biofilms, complex microbial matrices adhering to tooth surfaces, is crucial in caries development and presents challenges for effective therapeutic targeting (Neelakantan et al., 2025). Conventional treatments, including fillings, crowns, root canals, and extractions, address damage but do not fully prevent recurrence or biofilm persistence (Mandurino et al., 2023). Furthermore, increasing antibiotic resistance among oral pathogens limits the efficacy of standard antimicrobials (Brooks, Narvekar, McDonald, & Mullany, 2022). Traditional medicinal plants such as *Azadirachta indica* (neem) and *Vachellia nilotica* (babul) have demonstrated promising antibacterial and anti-biofilm properties against *S. mutans* and *C. albicans* (Pradhan, Devkota, & Ali, 2024). Despite their widespread use in regional dental care, the effects of these mastics on clinical isolates remain underexplored. This study aims to evaluate their antimicrobial impact against dental plaque pathogens, potentially offering cost-effective alternatives to conventional treatments.

Despite extensive research on *Streptococcus mutans* and *Candida albicans* as primary contributors to dental caries, current antimicrobial treatments face significant limitations, including rising antibiotic resistance and limited efficacy in disrupting biofilms. While *Azadirachta indica* (neem) and *Vachellia nilotica* (babul) are traditionally used in regional dental care for their antimicrobial properties, scientific validation of their effects on clinical isolates of these cariogenic pathogens remains insufficient. Moreover, the specific mechanisms by which these plant mastics influence biofilm formation and microbial viability have not been thoroughly investigated in clinical settings. This lack of detailed, evidence-based evaluation constitutes a critical gap that limits the integration of these natural products into mainstream dental therapeutics. For this study following objectives: To evaluate the antimicrobial efficacy of *Azadirachta indica* and *Vachellia nilotica* mastics against clinical isolates of *Streptococcus mutans* and *Candida albicans*. To assess the impact of these plant extracts on the biofilm formation ability of *S. mutans* and *C. albicans*. To compare the antimicrobial potential of these natural mastics with conventional antibiotic treatments. To explore the potential of these plant-based products as alternative or adjunctive agents in preventing and managing dental caries.

Material and Methods

The present study was conducted in Kohat, Pakistan, involving patients diagnosed with dental caries at the District Headquarters Hospital. A total of 60 dental swab samples were aseptically collected from patients presenting with dental caries lesions. Ethical approval was obtained from the Kohat University of Science and

Technology (KUST) Ethical Committee, and informed consent was obtained from all participants after explaining the study objectives. The collected dental swabs were immediately placed into sterile tubes containing 1 mL of Brain Heart Infusion (BHI) broth to preserve microbial viability during transport to the microbiology laboratory at KUST. For isolation of *Streptococcus mutans*, the samples were incubated aerobically in BHI broth at 37°C for 24 hours. Subsequently, aliquots were streaked onto Tryptone Yeast Cysteine Sucrose Bacitracin (TYCSB) agar plates and incubated at 37°C for an additional 24 hours, following established protocols for selective isolation (Momeni, 2016). To isolate *Candida albicans*, samples were inoculated on Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol and incubated aerobically at 37°C for 24 to 48 hours (Orwa, 2020).

Identification of *S. mutans* was performed based on colony morphology on TYCSB media, Gram staining, and biochemical tests. Colonies were characterized by their size, shape, margins, and color, with Gram staining confirming their Gram-positive cocci nature (Forbes et al., 2007). *C. albicans* colonies were identified by their distinct morphology on SDA, with confirmation by the germ tube test, where yeast cells were incubated in serum at 37°C for 90 minutes to observe hyphal germ tube formation (Orwa, 2020). Gram staining for *S. mutans* involved heat-fixing smears on glass slides followed by sequential application of crystal violet, Gram's iodine, ethanol decolorization, and safranin counterstaining, as described by Cappuccino and Sherman (2014). Lacto-phenol cotton blue staining was used for *C. albicans*, which stains fungal cell walls blue, enabling clear visualization under the microscope (Chaitra, 2014).

Biochemical tests for *S. mutans* included the catalase test, where bacterial colonies were mixed with 3% hydrogen peroxide to observe oxygen bubble formation, distinguishing catalase-positive from catalase-negative species (Mutua, 2017). The oxidase test involved applying a 1% oxidase reagent to bacterial cultures to detect cytochrome oxidase activity (Shafi et al., 2022). The bile solubility test distinguished *S. pneumoniae* by incubating bacterial suspensions with bile salts and observing tube clearing, as described earlier (Keith, 2011). Haemolysis was assessed by streaking bacteria on blood agar plates containing 5% sheep blood and incubating at 37°C for 24 hours to observe hemolytic patterns (Mutua, 2017). Bacitracin sensitivity was tested by placing bacitracin discs on inoculated Mueller-Hinton agar plates and measuring zones of inhibition after 24 hours incubation ((Carvalhaes, Klauer, Rhomberg, Pfaller, & Castanheira, 2022). For *C. albicans*, the germ tube test was performed by incubating yeast suspensions in pre-warmed serum at 37°C for 1 to 2 hours and examining under a microscope for germ tube formation (Odds, 1988). Antibiotic susceptibility of *S. mutans* isolates was assessed using the disc diffusion method on Mueller-Hinton agar plates in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (Carvalhaes et al., 2022). Antibiotics tested included azithromycin, chloramphenicol, clindamycin, ofloxacin, tetracycline, bacitracin, and nystatin for fungal isolates. Plates were incubated at 35°C for 20–24 hours, and inhibition zones were measured to determine sensitivity profiles.

Plant extracts were prepared from *Azadirachta indica* leaves collected from Shimla Pahari and *Vachellia nilotica* from KDA, Kohat. Plant materials were washed, shade-dried for 1–2 weeks, and ground into fine powder at the Botany Department of KUST. Ethanolic and aqueous extracts were prepared by soaking 50 g of powdered plant material in 100 mL of solvent, followed by continuous shaking for one week at room temperature, as described by (Musyimi, Opande, Chesire, Sikuku, & Buyela, 2017). The organic and inorganic phases of the extracts were separated using solvent extraction. After soaking, extracts were filtered and centrifuged at 4000 rpm for 15 minutes at room temperature. The supernatant containing dissolved bioactive compounds was concentrated using a rotary evaporator in the Department of Chemistry at KUST.

The antimicrobial activity of these plant extracts against *S. mutans* and *C. albicans* was evaluated using the agar well diffusion method. Microbial suspensions standardized to McFarland 0.5 were spread on Mueller-Hinton agar for *S. mutans* and SDA for *C. albicans*. Wells (6 mm diameter) were cut in agar plates, and 100 µL of plant extracts were added to each well. Distilled water and ethanol served as negative controls, while tetracycline, chloramphenicol, azithromycin, clindamycin, ofloxacin, and nystatin were used as positive controls. Plates were incubated at 37°C for 24 hours, and zones of inhibition were measured in triplicate. Minimum inhibitory concentrations (MIC) of plant extracts were determined by preparing serial dilutions ranging from 0.2 to 10 µg/mL in sterile distilled water and ethanol. Agar well diffusion assays were conducted, and the lowest concentration producing a visible zone of inhibition after 24 hours incubation at 37°C was recorded as the MIC ((Balouiri, Sadiki, & Ibnsouda, 2016)). Minimum bactericidal concentrations (MBC) were determined by sub-culturing aliquots from MIC assays showing no growth onto fresh agar plates and incubating for 24 hours at 37°C. The lowest concentration that completely inhibited microbial growth on subculture was noted as the MBC (Pankey & Sabath, 2004).

Result

A total of 60 dental plaque samples were collected from patients with dental caries at the District Headquarters Hospital (KDA) in Kohat. After initial incubation in Brain Heart Infusion broth at 37°C for 18–24 hours, samples were inoculated onto selective media: TYCSB agar for *Streptococcus mutans* and Sabouraud Dextrose Agar (SDA) for *Candida albicans*. Plates were incubated overnight at 37°C with 5% CO_2. Growth was observed in 49 samples (81.7%), of which 44 (89.8%) were positive for *S. mutans* and 5 (10.2%) for *C. albicans* (Figure 1).

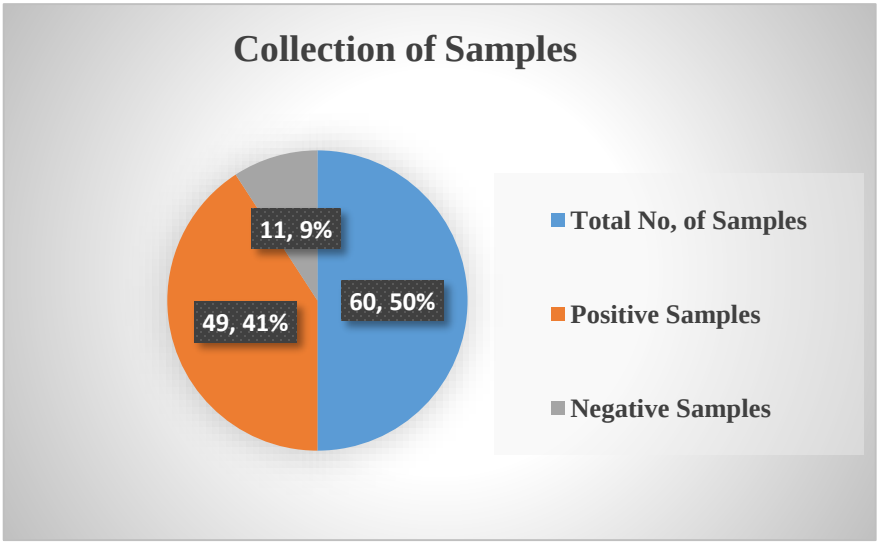


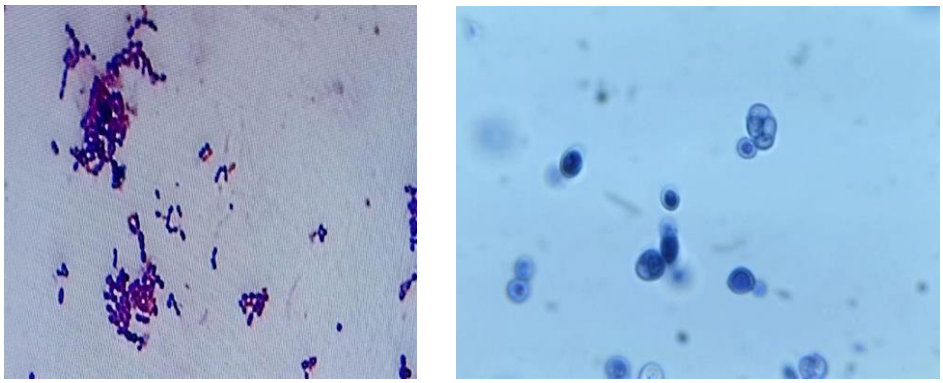
Figure 1: Prevalence of *S. mutans* and *C. albicans* among dental caries samples (n=60).

Streptococcus mutans colonies appeared as small, translucent colonies with clear zones on TYCSB agar after 24 hours, while *C. albicans* formed white, smooth, yeast-like colonies on SDA after 48 hours (Figure 2). Microscopic examination revealed Gram-positive cocci arranged in chains for *S. mutans* (Figure 3a), and polymorphic, oval yeast cells for *C. albicans* stained with lacto-phenol cotton blue (Figure 3b). Biochemical tests confirmed *S. mutans* identification: all isolates were catalase and oxidase negative, bile insoluble, and exhibited alpha hemolysis on blood agar (greenish discoloration). Bacitracin sensitivity testing showed

resistance with zones of inhibition between 7–8 mm (Carvalhaes et al., 2022) (Figures 4a–4e). The germ tube test confirmed *C. albicans* through the presence of characteristic hyphal extensions (Figure 4).

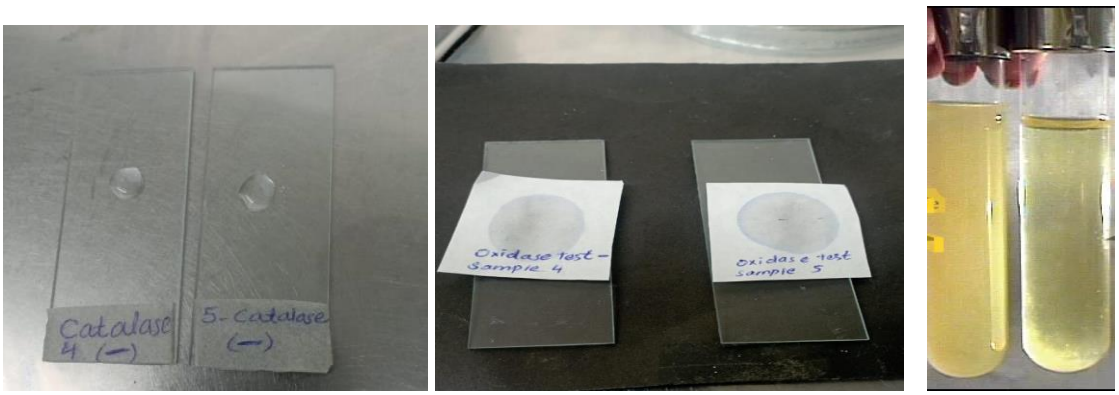
A B

Figure 2: Colony morphology on selective media: (a) *S. mutans* on TYCSB agar; (b) *C. albicans* on SDA.

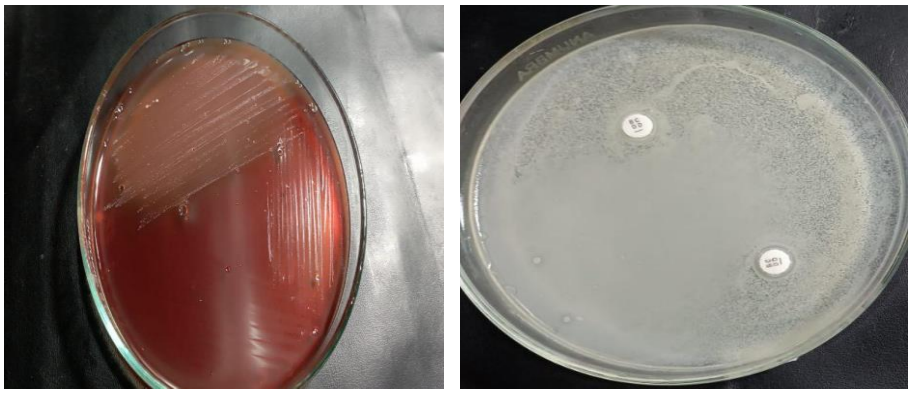


A B

Figure 3: Microscopic images: (a) Gram stain of *S. mutans* showing Gram-positive cocci in chains; (b) Lacto-phenol cotton blue staining of *C. albicans*.



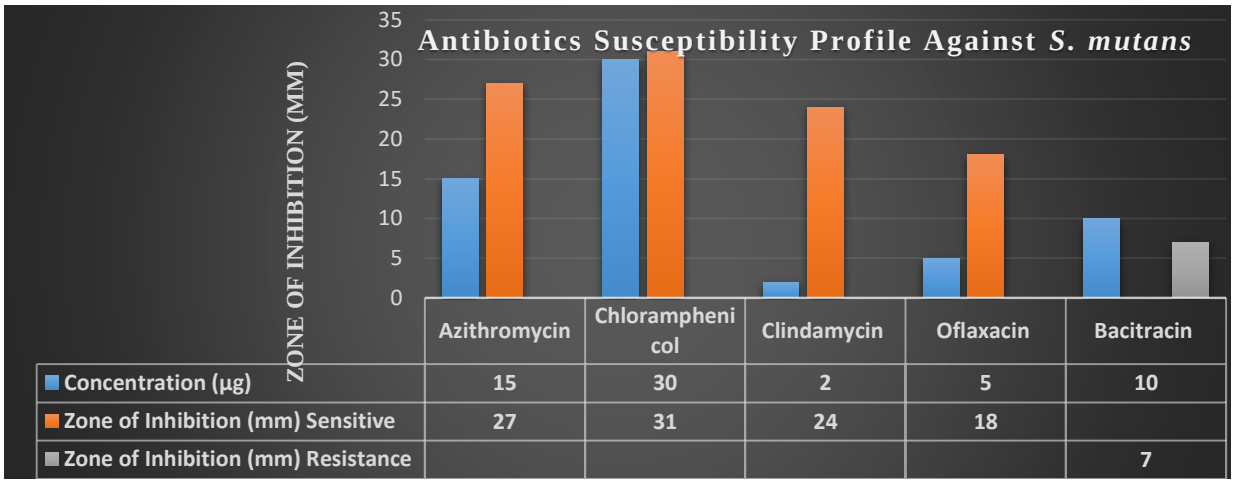
A **b** **c**

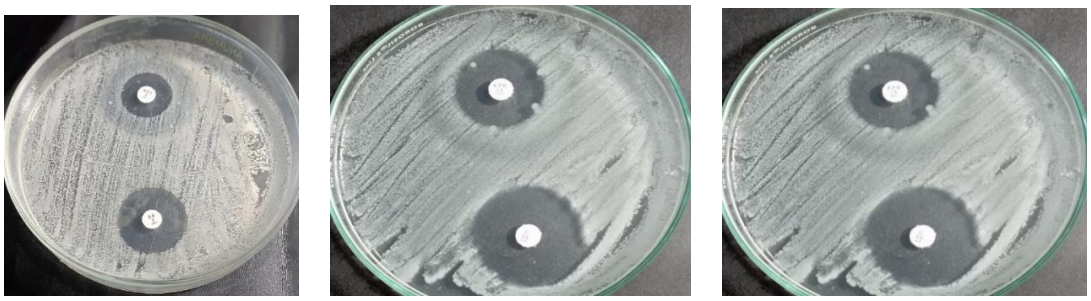


D **e**

Figure 4: Biochemical tests of *S. mutans*: (a) Catalase negative; (b) Oxidase negative; (c) Bile solubility negative; (d) Alpha hemolysis on blood agar; (e) Bacitracin resistance; (f) Germ tube test confirming *C. albicans*.

Antibiotic susceptibility testing of *S. mutans* isolates showed susceptibility to ofloxacin (16–18 mm), chloramphenicol (23–31 mm), clindamycin (19–24 mm), azithromycin (25–27 mm), and tetracycline (25–27 mm). Resistance was noted against bacitracin (6–7 mm) (Figure 5). Phytochemical screening revealed that *Azadirachta indica* contains active compounds such as nimbin, nimbidin, and azadirachtin with antibacterial and antifungal properties, primarily soluble in organic solvents. Similarly, *Vachellia nilotica* contains flavonoids, saponins, terpenoids, and alkaloids, contributing to its antimicrobial activity. Consequently, ethanolic extracts exhibited superior antimicrobial effects compared to aqueous extracts (Figure 6).





Oflaxacin & Clindamycin **Azithromycin & Chloramphenicol** **Bacitracin**

Figure 5: Antibiotic susceptibility profiles of *S. mutans* isolates showing zones of inhibition for tested antibiotics.

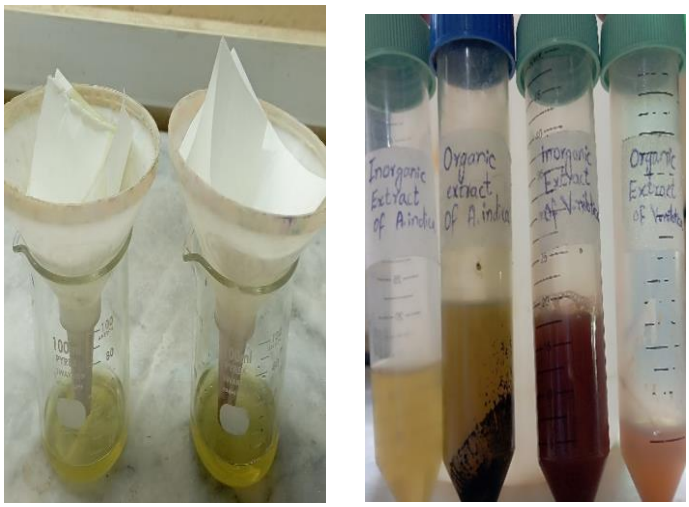
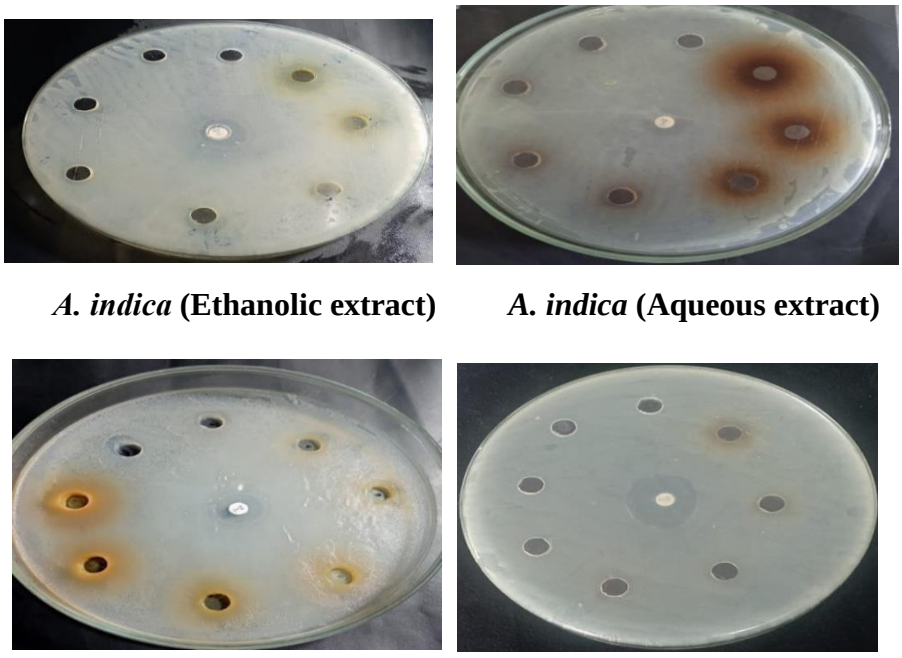


Figure 6: Separation of organic and aqueous phases of plant extracts and their antimicrobial activities.

The antimicrobial activity of plant extracts was assessed against *S. mutans* and *C. albicans*, with zones of inhibition summarized in Table 1. The ethanolic extract of *A. indica* produced zones of 20 mm and 19 mm against *S. mutans* and *C. albicans*, respectively, while the aqueous extract showed 16 mm and 15 mm zones. The ethanolic extract of *V. nilotica* demonstrated zones of 18 mm and 22 mm, compared to 15 mm and 14 mm for its aqueous extract.

Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) further confirmed the greater efficacy of ethanolic extracts. MIC and MBC values for the ethanolic extracts of *A. indica* and *V. nilotica* were 0.4 µg/mL and 0.8 µg/mL, respectively, whereas aqueous extracts showed higher MIC and MBC values of 1.6 µg/mL and 3.2 µg/mL (Figure 7).



***A. indica* (Ethanolic extract)** ***A. indica* (Aqueous extract)**

***V. nilotica* (ethanolic extract)** ***V. nilotica* (Aqueous extract)**

Figure 7: MIC and MBC determination for ethanolic and aqueous extracts of *A. indica* and *V. nilotica* against *S. mutans*.

Table 1. Antimicrobial Activity of Plant Extracts Against *S. mutans* and *C. albicans* (Zone of Inhibition in mm)

Organism	Extract Type	*V. nilotica*	*A. indica*	Positive Control	Negative Control
S. mutans	Ethanolic	18	20	24	0
	Aqueous	15	16	24	0
C. albicans	Ethanolic	22	19	24	0
	Aqueous	14	15	24	0

MIC and MBC of Plant Extracts Against *Streptococcus mutans*

The antimicrobial efficacy of ethanolic and aqueous extracts of *Azadirachta indica* and *Vachellia nilotica* was evaluated against *S. mutans* by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). As shown in **Table 3**, the ethanolic extract of *A. indica* exhibited inhibitory activity starting at 0.4 µg/ml with a mean inhibition zone of 11.5 ± 0.5 mm, increasing progressively to 19.6 ± 0.5 mm at 10 µg/ml. The aqueous extract of *A. indica* showed antibacterial activity beginning at a higher concentration (1.6 µg/ml) with zones of inhibition ranging from 10.3 ± 0.5 mm to 15.3 ± 0.5 mm.

Similarly, the ethanolic extract of *V. nilotica* demonstrated inhibitory activity from 0.4 µg/ml (8.3 ± 0.5 mm) to 18.3 ± 0.5 mm at 10 µg/ml, while its aqueous extract showed zones ranging from 9.3 ± 0.5 mm to 15.5 ± 0.5 mm over the same concentration range. Statistically significant increases in inhibition zones ($p < 0.01$) were observed at concentrations ≥ 3.2 µg/ml for all extracts tested. The ethanolic extracts generally showed superior antimicrobial activity compared to the aqueous extracts, indicating higher potency against *S. mutans*.

Table 2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Azadirachta indica* and *Vachellia nilotica* extracts against *Streptococcus mutans*.

Concentration (µg/ml)	*A. indica* (E) Mean Zone ± SD (mm)	P-Value	*A. indica* (A) Mean Zone ± SD (mm)	*V. nilotica* (E) Mean Zone ± SD (mm)	P-Value	*V. nilotica* (A) Mean Zone ± SD (mm)
0.2	–	–	–	–	–	–
0.4	11.5 ± 0.5	0.91	–	8.3 ± 0.5	0.88	–
0.8	13.1 ± 0.2	0.93	–	11.6 ± 0.5	0.94	–
1.6	14.1 ± 0.2	0.83	10.3 ± 0.5	13.1 ± 0.2	0.82	9.3 ± 0.5
3.2	16.6 ± 0.5	0.005	11.8 ± 0.2	14.1 ± 0.2	0.005	11.3 ± 0.5
6.4	18.1 ± 0.5	0.006	13.6 ± 0.5	15.1 ± 0.2	0.007	13.3 ± 0.5
10	19.6 ± 0.5	0.008	15.3 ± 0.5	18.3 ± 0.5	0.009	15.5 ± 0.5

(E = ethanolic extract, A = aqueous extract; SD = standard deviation; "–" indicates no activity or not tested)

MIC and MFC of Plant Extracts Against *Candida albicans*

The antifungal activity of the same plant extracts was evaluated against *C. albicans*, and the results are summarized in Table 4. The ethanolic extract of *A. indica* exhibited zones of inhibition ranging from 8.0 ± 0.2 mm at 0.4 µg/ml to 18.0 ± 0.2 mm at 10 µg/ml, with MIC and minimum fungicidal concentration (MFC) determined at 0.4 µg/ml and 0.8 µg/ml, respectively. The aqueous extract of *A. indica* demonstrated slightly lower activity, with inhibition zones between 6.0 ± 0.2 mm and 15.1 ± 0.4 mm, and MIC and MFC values of 1.6 µg/ml and 3.2 µg/ml, respectively.

For *V. nilotica*, the ethanolic extract inhibited *C. albicans* growth with zones ranging from 6.0 ± 0.2 mm to 22.1 ± 0.3 mm and MIC/MFC values of 0.4 µg/ml and 0.8 µg/ml, respectively. The aqueous extract showed inhibition zones between 8.0 ± 0.2 mm and 14.0 ± 0.3 mm with MIC and MFC values at 3.2 µg/ml and 6.4 µg/ml, respectively. Statistical analysis revealed borderline significance ($p = 0.05$ to 0.09) at higher concentrations, indicating consistent dose-dependent antifungal activity. These findings suggest that ethanolic extracts of both plants have greater antifungal potency against *C. albicans* compared to their aqueous counterparts.

Table 3. Antifungal activity of ethanolic and aqueous extracts of *Azadirachta indica* and *Vachellia nilotica* against *Candida albicans*.

Concentration (µg/ml)	*A. indica* (E) Mean Zone ± SD (mm)	P-Value	*A. indica* (A) Mean Zone ± SD (mm)	*V. nilotica* (E) Mean Zone ± SD (mm)	P-Value	*V. nilotica* (A) Mean Zone ± SD (mm)
0.2	–	–	–	–	–	–
0.4	8.0 ± 0.2	0.88	–	6.0 ± 0.2	0.94	–
0.8	10.0 ± 0.2	0.95	–	9.0 ± 0.2	0.96	–
1.6	11.9 ± 0.2	0.94	–	10.9 ± 0.2	0.96	–
3.2	13.1 ± 0.2	0.05	11.9 ± 0.2	12.0 ± 0.4	0.06	8.0 ± 0.2
6.4	15.0 ± 0.2	0.07	13.8 ± 0.7	20.1 ± 0.3	0.08	13.0 ± 0.3
10	18.0 ± 0.2	0.06	15.1 ± 0.4	22.1 ± 0.3	0.09	14.0 ± 0.3

Discussion

The present study investigated the antimicrobial efficacy of ethanolic and aqueous extracts of *Azadirachta indica* and *Vachellia nilotica* against *Streptococcus mutans* and *Candida albicans*, common oral pathogens implicated in dental caries and candidiasis. The results demonstrated that ethanolic extracts of both plants exhibited significantly greater antimicrobial activity compared to aqueous extracts, consistent with previous findings that organic solvents better extract bioactive phytochemicals such as flavonoids, alkaloids, and terpenoids (Issrani et al., 2023; Kumar, Sreedharan, Kashyap, Singh, & Ramchiary, 2022). The MIC and MBC values for ethanolic extracts of *A. indica* and *V. nilotica* against *S. mutans* ranged between 0.4 to 0.8 µg/ml and 0.8 to 1.6 µg/ml, respectively, indicating potent bactericidal effects. This aligns with prior studies where neem extracts showed strong antibacterial properties, attributed mainly to compounds like nimbidin and azadirachtin, which interfere with bacterial metabolism and biofilm formation (Subapriya & Nagini, 2005; Raut et al., 2016). The aqueous extracts, in contrast, showed higher MIC and MBC values, suggesting lower efficacy likely due to limited solubility of active compounds in water (Cowan, 1999).

Similarly, the antifungal activity against *C. albicans* was more pronounced in ethanolic extracts, with MIC and MFC values between 0.4 and 0.8 µg/ml, compared to aqueous extracts which required higher concentrations (1.6 to 6.4 µg/ml). This supports previous reports where neem and acacia extracts inhibited *C. albicans* growth, potentially through disruption of fungal cell membranes and inhibition of germ tube formation (Barua, Basavanna, Varghese, & JCDR, 2017). The superior antimicrobial activity of ethanolic extracts underscores the importance of solvent choice in phytochemical extraction, affecting the yield and potency of bioactive compounds (Savickiene & Raudone, 2024). These findings support the traditional use of *A. indica* and *V. nilotica* in oral health management and suggest their potential development into natural therapeutic agents against dental pathogens. Future studies should focus on isolation of specific active constituents and in vivo efficacy to validate these promising in vitro results.

Conclusion

The present study demonstrates that ethanolic extracts of *Azadirachta indica* and *Vachellia nilotica* exhibit strong antimicrobial activity against *Streptococcus mutans* and *Candida albicans*, outperforming their aqueous extracts in both efficacy and potency. The lower MIC and MBC/MFC values of ethanolic extracts suggest higher concentrations of bioactive compounds responsible for inhibiting and killing these oral pathogens. These findings validate the traditional medicinal use of these plants in oral health care and highlight their promising potential as natural, effective agents for preventing and managing dental caries and oral fungal infections. Further research and clinical evaluation are recommended to develop plant-based therapeutic formulations.

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For correction of English artificial intelligence has been used

References

1. Balouiri, M., Sadiki, M., & Ibnsouda, S. K. J. J. o. p. a. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *6*(2), 71-79.
2. Barua, D. R., Basavanna, J. M., Varghese, R. K. J. J. o. c., & JCDR, d. r. (2017). Efficacy of neem extract and three antimicrobial agents incorporated into tissue conditioner in inhibiting the growth of *C. albicans* and *S. mutans*. *11*(5), ZC97.

3. Brooks, L., Narvekar, U., McDonald, A., & Mullany, P. J. M. O. M. (2022). Prevalence of antibiotic resistance genes in the oral cavity and mobile genetic elements that disseminate antimicrobial resistance: A systematic review. *37*(4), 133-153.
4. Byakod, A. S. J. I. J. o. C. M., & Health, P. (2023). Phytochemical screening of Acacia nilotica extract: an in vitro study. *10*(4), 1525.
5. Carvalhaes, C. G., Klauer, A. L., Rhomberg, P. R., Pfaller, M. A., & Castanheira, M. J. J. o. C. M. (2022). Evaluation of rezafungin provisional CLSI clinical breakpoints and epidemiological cutoff values tested against a worldwide collection of contemporaneous invasive fungal isolates (2019 to 2020). *60*(4), e02449-02421.
6. Chaitra, P. (2014). *Diagnosis of Onychomycosis: a Comparative Study of Potassium Hydroxide-Based Direct Microscopy and Fungal Culture with Histopathology Using Periodic-Acid Schiff.* Rajiv Gandhi University of Health Sciences (India),
7. Cowan, M. M. J. C. m. r. (1999). Plant products as antimicrobial agents. *12*(4), 564-582.
8. Inchingolo, A. D., Malcangi, G., Semjonova, A., Inchingolo, A. M., Patano, A., Coloccia, G., . . . Ciocia, A. M. J. C. (2022). Oralbiotica/oralbiotics: the impact of oral microbiota on dental health and demineralization: a systematic review of the literature. *9*(7), 1014.
9. Issrani, R., Reddy, J., Bader, A. K., Albalawi, R. F. H., Alserhani, E. D. M., Alruwaili, D. S. R., . . . Rao, K. J. D. (2023). Exploring an association between body mass index and Oral Health—a scoping review. *13*(5), 902.
10. Keith, E. R. (2011). *Laboratory and Epidemiological Characteristics of Streptococcus pseudopneumoniae.* University of Otago,
11. Kumar, A., Sreedharan, S., Kashyap, A. K., Singh, P., & Ramchiary, N. J. H. (2022). A review on bioactive phytochemicals and ethnopharmacological potential of purslane (Portulaca oleracea L.). *8*(1).
12. Mahdi, A. S., Shaker, L. M., & Alamiery, A. J. J. o. O. (2024). Recent advances in organic solar cells: materials, design, and performance. *53*(2), 1403-1419.
13. Mandurino, M., Di Domenico, G. L., Baldani, S., Collivasone, G., Gherlone, E. F., Cantatore, G., & Paolone, G. J. B. (2023). Dental Restorations. In (Vol. 10, pp. 820): MDPI.
14. Momeni, S. S. (2016). *Molecular analysis of Streptococcus mutans genetic diversity for the epidemiological study of oral disease.* The University of Alabama at Birmingham,
15. Mosaddad, S. A., Tahmasebi, E., Yazdanian, A., Rezvani, M. B., Seifalian, A., Yazdanian, M., . . . Diseases, I. (2019). Oral microbial biofilms: an update. *38*, 2005-2019.
16. Moussa, D. G., Ahmad, P., Mansour, T. A., Siqueira, W. L. J. F. i. C., & Microbiology, I. (2022). Current state and challenges of the global outcomes of dental caries research in the meta-omics era. *12*, 887907.
17. Musyimi, D. M., Opande, G. T., Chesire, J., Sikuku, P. A., & Buyela, D. J. I.-J. o. B. S. (2017). Antimicrobial potential and screening of phytochemical compounds of Lantana camara Linn. *3*(6), 18-35.
18. Mutua, J. M. (2017). *Antimicrobial Susceptibility Profiles Of Bacteria From The Respiratory Tract Of Camels In Samburu, Nakuru And Isiolo Counties, Kenya.* University of Nairobi,
19. Neelakantan, P., Kalimuthu, S., Pudipeddi, A., Krom, B. P., Ramage, G., Butcher, M., & Brown, J. L. (2025). The Oral Mucosa-Fungi Interactome. In *Oral Biofilms in Health and Disease* (pp. 189-231): Springer.
20. Orwa, P. (2020). *Diversity and Biological Control Potential of Haloalkaliphilic Fungi from Lake Magadi, Kenya.* University of Embu,
21. Palombo, E. A. J. E. B. C., & Medicine, A. (2011). Traditional medicinal plant extracts and natural products with activity against oral bacteria: potential application in the prevention and treatment of oral diseases. *2011*(1), 680354.

22. Pankey, G. A., & Sabath, L. J. C. i. d. (2004). Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. *38*(6), 864-870.
23. Pradhan, Y., Devkota, H. P., & Ali, A. J. B. B. R. A. (2024). Potential Role of Medicinal Plants and their Phytochemicals Against Plaque Forming Oral Microbiota. *21*(2), 371-389.
24. Savickiene, N., & Raudone, L. J. P. (2024). Trends in Plants Phytochemistry and Bioactivity Analysis. In (Vol. 13, pp. 3173): MDPI.
25. Shafi, S., Aziz, M. A., Padder, S., Mir, A. H., Bhat, M. A., Dar, Z. M., . . . Azad, A. (2022). Phenotypic characterization of root-associated Rhizomicroflora of (Phaseolus vulgaris) French Yellow Bean under temperate ecologies.
26. Zhu, Y., Wang, Y., Zhang, S., Li, J., Li, X., Ying, Y., . . . Wang, Q. J. F. i. m. (2023). Association of polymicrobial interactions with dental caries development and prevention. *14*, 1162380.