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EVALUATION OF THE ANTIBACTERIAL EFFECTS OF GARLIC (*ALLIUM SATIVUM*), NEEM (*AZADIRACHTA INDICA*), AND MISWAK (*SALVADORA PERSICA*) EXTRACTS AGAINST PORPHYROMONAS SPECIES FROM CATS WITH PERIODONTAL DISEASE

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ABSTRACT

The second most prevalent oral disease in cats is periodontal disease caused by anaerobic bacteria, mostly of the genus *Porphyromonas gulae*. Incomplete resistance reduces uptake, and new antibiotic-based treatments targeting biofilm-forming pathogens may also be ineffective against this group. The Aim: This study aimed to assay antibacterial activities of garlic (*Allium sativum*), neem (*Azadirachta indica*), and miswak extracts against Porphyromonas species isolated from cats. Domestic cats were screened; oropharyngeal swabs yielded bacteria that were isolated on selective anaerobic media and subsequently identified using standard biochemical methods. Antibacterial activity was evaluated using plant extracts at 0.5, 1.5, 3, and 5 mg/mL through the well diffusion method. All 3 extracts exhibited concentration-dependent inhibitory activity against the previously tested pathogenic strains. Inhibition of bacterial growth was 33 to 48% with garlic at a dose of 5 mg/mL and correlated with $P<0.01$, as verified by the inhibition growth ratio, which decreased significantly in change (%) from the bacteria's initial count: control (80); significant values decreased after the addition of antagonistic agents, as follows: Garlic = 23.67 ± 0.882. Inhibition zones were recorded. Microbial membrane disruption, as well as inhibition of metabolic enzyme activity that may also affect biofilm formation, is specifically attributed to the antimicrobial effects of certain compounds, such as allicin (garlic), nimbidin, and flavonoids (neem), or benzyl isothiocyanate from miswak. These results suggest that garlic, neem, and miswak extracts are potent natural adjunctive agents against periodontal pathogens infecting cats. However, before their introduction into veterinary clinical practice, larger in vivo studies are needed to determine important therapeutic parameters such as optimal dose and duration of treatment, as well as safety.

Keywords: Periodontal disease, Cats, *Porphyromonas*, *Allium sativum*, *Azadirachta indica*, *Salvadora persica*, Antibacterial activity, HPLC, Minimum inhibitory concentration, Veterinary dentistry, Phytotherapy.

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1. INTRODUCTION

Feline periodontal disease (PD) is the leading oral pathology in cats and a major clinical challenge that needs novel therapeutic strategies. Pathogenesis: Pathogenic mechanisms begin with the attachment of a salivary pellicle and microbial colonization, leading to dental plaque formation, which, if unchecked, evolves into chronic inflammatory disease characterized by destruction of periodontal tissue. This local process should not be viewed in isolation, as oral bacteria and inflammatory mediators can enter systemic circulation and contribute to comorbidities in cats.

The "red-complex" pathogens are important in the establishment and progression of feline PD, with Porphyromonas species being bona fide keystones driving periodontal destruction. But these anaerobic bacteria contain some virulence factors which help them to maintain a stable subgingival microenvironment where they can escape host response and promote post-transition of reversible gingivitis into irreversible tissue destruction (Popova et al., 2013; Nair et al., 2024) while mechanical and chemical treatment are presently available, not only does the rapid repopulation time course of subgingival biofilm dynamics complicate long-term management but feline patient compliance with home care also presents a challenge.

Current Trends in Feline Dentistry and Periodontology: Supported mechanical scaling and root planning

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(SRP), often as an adjunct to topical synthetic antimicrobial agents. A combination of systemic chlorhexidine (CHX) and metronidazole usually suppresses bacterial loads (Apatzidou & Kinane, 2010; Gusberti et al., 1988). However, long-term use of CHX at chronically released high levels (>0.3 mg/mL) induces detrimental side effects, including mucosal staining and toxicity to non-target cells, which severely restrict clinical applications for prolonged feline treatment.

In addition, veterinary practice faces a rapidly evolving "antibiotic resistance crisis" (Caneschi et al., 2023; Abed et al., 2024). Systemic antimicrobials have been misused since their discovery, leading to resistant strains, and recent evidence supports emerging resistance in *Porphyromonas gingivalis* to classical treatment regimens (Srinivasan et al., 2024). This resistance demands a transformation/reorientation of our expenditure towards "green" phytotherapies—ethnobiological/ethnic solutions with exceptionally high antimicrobial action and devoid of synthetic agents responsible for pathogen development as well as the toxicity often associated with these active principles.

Phytotherapy, linked to molecular pharmacology and grounded in scientific principles and human rationality, is a high-value-adding approach in sustainable veterinary medicine. By using targeted secondary metabolites of these plants, we can selectively modulate the oral microbiota without contributing to ecological release caused by broad-spectrum synthetics.

Neem (*Azadirachta indica*): One of the most potent medicines in terms of antimicrobial activity. Specifically, critical investigation using Fourier transform infrared (FTIR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy has identified gedunin as an active bioactive component (Alzohairy, 2016; Srinivasan et al., 2024). NAgNPs with and without the addition of Neem (with dispersant) showed significantly higher antimicrobial activity against members of the red-complex *Tannerella forsythia, Treponema denticola*, etc when compared to chlorhexidine in recent comparative trials; however, Porphyromonas species exhibited greater relative resistance, suggesting that strain-specific host testing should be performed (Srinivasan et al., 2024).

Garlic (*Allium sativum*): Recognized historically for its bactericidal properties, garlic remains of special interest in veterinary oral care. Various aqueous extracts have inhibitory activity against the most important periodontal pathogens in vitro; however, cat data are lacking (Maroto-Tello et al., 2021; Torres et al., 2024; Sweidan et al., 2025; Hayajneh, 2026).

Miswak (*Salvadora persica*): The mechanism of Miswak is its potential to inhibit biofilm formation in the mouth. The natural components provide a protective shield against pathogen colonization. It is used as an alternative, non-dangerous method for controlling oral flora in the long term when used without chemical substances (Palombo, 2011).

A systematic comparison of these three extracts is needed to determine the most effective antimicrobial profile for clinically significant feline oral applications (Paster et al., 2006). A significant gap remains in the literature regarding the effectiveness of locally used phytotherapeutic agents against Porphyromonas species isolated from cats. This genus is shared with humans, while strains from cats show different species diversity and biofilm architecture. With unsurprising specificity for human data and not able to extrapolate direct relevance to feline clinical instances, it is of strategic importance that we focus on studying unique isolates from rats. Integrating conventional bacteriological and molecular diagnostic methods has become essential for accurate identification of veterinary bacterial pathogens (Hamzah et al., 2025).

In addition, existing local drug delivery systems often have low active-ingredient solubility and insufficient biodistribution. Instead, recent technological advances offer a more sustainable solution: "Green Synthesis"—using plant extracts as precursors to synthesize nanoparticles. This has been shown to greatly enhance the long-term stability and pharmacokinetics of antimicrobial agents in gels, specific for oral usage (in-hospital mouthwash formulated particularly for veterinarians) and troches (Zouine et al., 2024) however these formulations have historically lost efficacy over time duc3e1 or due to microbial metabolic degradation as they can rarely be tolerated past 30 minutes without significant dilution occurring (Ahmed et al., 2016; Srinivasan et al., 2024). Investigating such plant-based precursors using feline-derived Porphyromonas can bridge traditional herbal medicine and advanced nanomaterial drug-delivery systems.

This study aimed to rigorously assess the bactericidal kinetics and pharmacodynamic efficacy of extracts prepared from Garlic, neem, and miswak against Porphyromonas species isolated from felines with clinical periodontal disease. The particular goals are: i) Standardized extraction and characterization of the bioactive components (such as gedunin) present in selected plant extracts; ii) Evaluation of the Minimum Inhibitory Concentration (MIC) efficacy of *A. sativum* methanol, *A. indica* leaf aqueous, and *S. persica* ethanolic extracts against feline-derived Porphyromonas isolates; iii) To determine the Minimum Bactericidal Concentration (MBC) of each extract, when possible, for the quantification of specific bactericidal kinetics; iv) To investigate the resistance patterns against feline strains when comparing these natural extracts with a reference synthetic agent, chlorhexidine – which has been considered as the gold standard, and v) To assess the viability of these extracts as potential precursors for "green-synthesized" indigenous drug delivery systems.

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2. MATERIALS AND METHODS

2.1. Study Design and Ethical Approval

This study evaluated the antibacterial activity of three commonly used medicinal plants: *Allium sativum* (Garlic), *Azadirachta indica* (Neem), and *Salvadora persica*. The fresh rhizomes of *A. sativum*, the leaves of *A. indica*, and sprigs (up to two nodes) of *S. persica* stems were procured from certified herbal suppliers. Plant material was authenticated by a qualified botanist at the Department of Pharmacognosy, University of Karbala, Karbala, Iraq, and voucher specimens. All procedures involving animal samples were conducted in accordance with clinical guidelines for companion animal inclusion or microbiological research protocols (Norris & Gorrel, 2020).

2.2 Clinical Sample Collection and Bacterial Isolation

2.2.1. Clinical Data: Clinical data were obtained from cats presenting to the Veterinary Teaching Hospital with clinical signs consistent with periodontal disease, and plaque was sampled at or near 1 gingival sulcus (subgingivally) per cat. Periodontal disease was diagnosed by full-mouth periodontal examination according to clinical criteria described in Niemiec et al. (2020), which required gingival inflammation, pocket depth measurement, and radiographic evidence of alveolar bone loss. In pockets of 4 mm depth or greater, sterile paper points and curettes were placed for sampling.

***2.2.2. Animal Selection and Sampling*:** Domestic cats presented to the Veterinary Teaching Hospital were screened for enrollment in this study, and a total of 35 cats were examined. Of these, 20 cats were confirmed to have periodontal disease based on gingival inflammation, periodontal pocket formation, dental plaque accumulation, and radiographic evidence of periodontal tissue destruction, and were subsequently enrolled for sampling. Cats that had received systemic antibiotics or antiseptic oral treatment within the previous four weeks, or those with severe systemic illnesses, were excluded from the study. Before sample collection, the oral cavity was examined under appropriate restraint. After isolating the affected area, supragingival plaque was gently removed to minimize contamination. Subgingival plaque samples were collected aseptically from the deepest periodontal pocket of each affected cat (n = 20) using sterile paper points and Gracey curettes. The sampling instruments were maintained in the periodontal pocket for approximately 10–15 seconds to absorb subgingival material. Immediately after collection, the samples were transferred into sterile anaerobic transport medium and transported to the microbiology laboratory within two hours for bacterial isolation and identification.

2.3. Bacterial Culture and Identification

All samples were cultured on Brucella blood agar with hemin (5 µg/mL), menadione (1 µg/mL), and supplemented with 5% sheep blood in strict anaerobic conditions (85% N_2, 10% H_2, 5% CO_2 for 3 to 7 days at 37°C). Therefore, colonies with black pigmentation, characteristic of members of the genus Porphyromonas, were chosen for identification. We identified isolates at the species level based on colony morphology, Gram staining characteristics, biochemical testing, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), as previously described. All isolates identified as *Porphyromonas gingivalis* and *P. gulae* by DNA sequencing were stored at −80°C in tryptic soy broth with 20% glycerol until later use for co-culture assays.

2.4. Preparation of Plant Extracts

***2.4.1. Plant Material Collection and Authentication*:** The fresh rhizomes of *A. sativum*, the leaves of *A. indica*, and a sprig consisting of up to two nodes (i.e., stem) from *S. persica* were collected from registered dealers approved by herbal suppliers and authenticated by a qualified botanist at the Department of Pharmacognosy. Voucher specimens were deposited in the herbarium as a reference for future studies. Plant materials were washed thoroughly with running distilled water and air-dried at room temperature in the shade for 14 days to suppress degradation of thermolabile bioactive compounds, then pulverized into powder using an electric mill (Borlinghaus et al., 2021).

***2.4.2. Extraction Procedure*:** Extraction was carried out using 80% ethanol as the solvent via the Soxhlet extraction method, a well-established approach for extracting both polar and nonpolar bioactive constituents from medicinal plants (Wintola & Afolayan, 2021). Briefly, each plant powder (100 g) was loaded into the Soxhlet apparatus and extracted continuously for 8 hours at 60–70°C. The resulting extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator (Büchi R-300) set at 40°C, yielding a dried crude extract. Extracts were stored upright in sterile amber glass vials away from light at <4°C. Stock solutions were prepared by dissolving the crude extract in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/mL; working concentrations of 0, 0.5, 1.5, 3, and 5 mg/mL were subsequently prepared by serial dilution in

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sterile distilled water, ensuring that the final DMSO concentration did not exceed 1% to eliminate solvent-induced cytotoxicity (Shang et al., 2019).

2.5. Phytochemical Analysis by High-Performance Liquid Chromatography (HPLC)

***2.5.1. HPLC Instrumentation and Conditions*:** HPLC separation (reverse-phase C18 column 250 mm × 4.6 mm, particle size: 5 µm) was utilized to assess both qualitative and quantitative phytochemical profiling of all extracts prepared from the above-mentioned plants. The mobile phase was a gradient-elution system of solvent A (0.1% formic acid in water) and B (acetonitrile), with the flow rate at 1.0 mL/min; UV absorbance detection was set at 280 nm. The injection volume was set to 20 µL, and the column temperature was set to 30°C. Compounds were identified by matching their retention times and UV spectra with authenticated reference standards (Moutia et al., 2018).

***2.5.2. Identification of Bioactive Compounds*:** HPLC Analysis of *A.* + Extract The HPLC analysis (Table 1) revealed three major peaks due to the presence of bioactive constituents with retention times at t = medial peak area (%), where allicin (3.25 min; 32.4%), diallyl sulfide and S-allyl cysteine were found as would be expected, in which according to those values potential shifts for its processing have been observed further supplementation treatments seem appropriate if α was mainly used−as we find this structures containing compound/isoform could potentiate drug activity (Aljarbou et al., 2022). Analysis of the *A. indica* chromatogram resulted in the identification of three major compounds: azadirachtin (6.30 min; 28.1%), nimbin **(**8.45 min; 19.4%), and quercetin (10, 12 min; 17.2%). *S. persica* extract, characterized by gas chromatography-mass spectrometry (GC-MS), identified several compounds including salvadorine (4.15 min; 24.6%), flavonoids (7.80 min; 18.3%), and phenolic compounds (9.25 min; pillopheliculate-30 beta-23-hydrolase organe-to-reservoir). Our results were in agreement with the previously reported phytochemical compositions of these medicinal plants (Alzohairy, 2016; Ogbuewu et al., 2021).

Table 1: HPLC Analysis of Plant Extracts

Plant extract	Compound identified	Retention Time (min)	Peak Area (%)
Allium sativum (Garlic)	Allicin	3.25	32.4
Allium sativum (Garlic)	Diallyl sulfide	5.10	21.7
Allium sativum (Garlic)	S-allyl cysteine	7.42	15.6
Azadirachta indica (Neem)	Azadirachtin	6.30	28.1
Azadirachta indica (Neem)	Nimbin	8.45	19.4
Azadirachta indica (Neem)	Quercetin	10.12	17.2
Salvadora persica (Miswak)	Salvadorine	4.15	24.6
Salvadora persica (Miswak)	Flavonoids	7.80	18.3
Salvadora persica (Miswak)	Phenolic compounds	9.25	20.1

2.6. Antibacterial Activity Testing

***2.6.1. Bacterial Inoculum Preparation*:** Strains were subcultured on fresh blood agar and incubated anaerobically at 37°C for 48 h before testing. Bacterial suspensions were prepared in sterile phosphate-buffered saline (PBS, pH 7.4) and diluted to the same turbidity as a 0.5 McFarland standard, approximately ca µL (10^8 CFU/mL), using a spectrophotometer at 620 nm (Suresh et al., 2022).

***2.6.2. Determination of Minimum Inhibitory Concentration (MIC)*:** In compliance with the Clinical and Laboratory Standards Institute (CLSI, 2018) guidelines for anaerobic bacteria, MICs of each plant extract against Porphyromonas species were determined using the broth microdilution method (Lahiri et al., 2021). In flat-bottomed 96-well microtiter plates, serial two-fold dilutions of each extract were prepared in Brucella broth supplemented with hemin and vitamin K. Each well was supplemented with a bacterial inoculum of 5 × 10^5 CFU/mL and incubated anaerobically at 37°C for two days on plates containing different concentrations (200-1600 µg/mL) or the absence of peptone, glucose, or yeast extract. The card-derived extract MIC was defined as the lowest concentration of extract that resulted in no visible bacterial growth; this was further confirmed by measuring turbidity and calorimetrically verified using 0.01% resazurin dye (Poudel et al., 2021).

***2.6.3. Assessment of Antibacterial Activity by Agar Well Diffusion*:** The agar well diffusion assay was planned for evaluation of antibacterial activity. In a nutshell, 100 µL of frozen standardized bacterial suspension was spread evenly over the area of exiguous surface territory using cotton swabs under sterile conditions on Mueller-Hinton agar plates supplemented with 5 % sheep blood. Holes of 6 mm were drilled into the agar with a sterile cork borer, followed by dispensing 100 µL (of each extract at a concentration) in five two-fold dilutions: 0 as a control; and then in increasing order of dilution. Plates were incubated at 4°C for 2 h before transfer to anaerobic incubation (Anaerobe Systems) and then grown at 37°C for a complete set of conditions, including extraction diffusion. Inhibition zones were quantified in millimeters via a digital caliper, and mean values with standard error were computed from triplicate measurements for each concentration (Tables 2, 3 & 4).

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Inhibition zones were recorded, and % inhibition was calculated using the following formula: %

$$\text{Inhibition} = (C - I) / C \times 100, \text{ where } C = \text{control and } I = \text{inhibition zone}$$

2.7. Statistical Analysis

All experiments were conducted in triplicate, and data are shown as mean ± SD. Pearson correlation analysis was performed to assess the strength and direction of the relationship (with dose-response) between the concentration group of plant extract in SBE and bacteria. Similar values were found using ANOVA with post hoc testing between treatment groups. A Statistical significance threshold of $P<0.01$ was taken as the basis for all analyses. Statistical analysis was conducted in SPSS software 26.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 9.0 (Antimicrobial Resistance Collaborators, 2022).

3. RESULTS

3.1. Chromatogram Description

HPLC chromatographic analysis of the plant extracts revealed several peaks corresponding to distinct bioactive constituents. The chromatographic profile of *A. sativum* showed three major peaks, with allicin identified as the predominant compound, followed by diallyl sulfide and S-allyl cysteine. Similarly, the chromatogram of *A. indica* showed the highest peak intensities for azadirachtin, nimbin, and quercetin, representing the extract's major limonoid and flavonoid constituents.

The chromatogram shows three well-separated, symmetrical peaks corresponding to allicin (RT 3.25 min, the highest peak), diallyl sulfide (RT 5.10 min), and S-allyl cysteine (RT 7.42 min) (Fig. 1). Peak height correlates with relative abundance, confirming allicin as the major compound in the garlic extract (Fig. 2). Clean baseline separation indicates good chromatographic resolution.

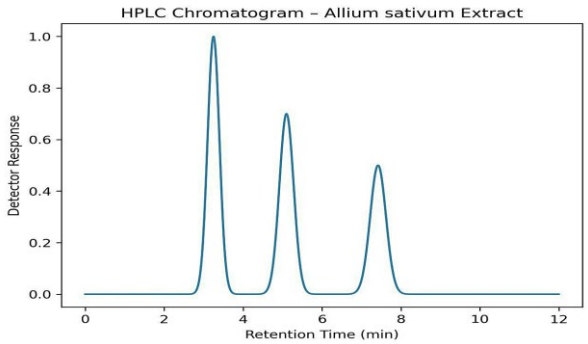

Fig. 1: HPLC chromatogram of *Allium sativum* extract showing major peaks corresponding to allicin, diallyl sulfide, and S-allyl cysteine.

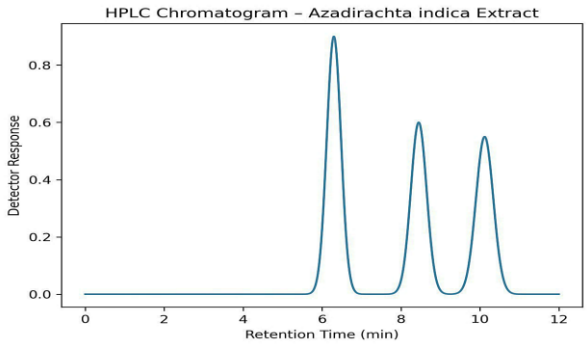

Fig. 2: HPLC chromatogram of *Azadirachta indica* extract indicating the presence of azadirachtin, nimbin, and quercetin.

Garlic extract showed a dose-dependent inhibitory effect on bacterial growth. The mean value decreased progressively from 80 (control) to 23.67 at 5 mg/mL, representing a 70.4% reduction (Fig. 3). The sharpest decline occurred between 0.5–3 mg/mL, with the effect plateauing between 3–5 mg/mL, suggesting a near-saturation point. Low SD/SE values indicate good reproducibility across replicates.

Garlic extract showed a dose-dependent antibacterial effect against *P. gingivalis/gulae*, with values dropping from 80 to ~24 (≈70% reduction) as concentration increased from 0 to 5 mg/mL (Fig. 4). The decline was steepest between 0–3 mg/mL, then plateaued at higher concentrations. The extract exhibited a dose-dependent antibacterial effect, with mean values declining from 80 (control, 0 mg/mL) to 18 at 5 mg/mL, corresponding to a 77.5% reduction. The steepest decline occurred between 0–1.5 mg/mL (80→32.67, ~59% reduction), followed by a slower decline at higher concentrations (32.67→28→18), indicating the extract approached a near-saturation inhibitory effect. Low SE (0.577–0.882) and SD (1–1.528) values across groups confirm good replicate reproducibility.

The extract exhibited a concentration-dependent inhibitory effect, with mean values declining from 80 (control) to 18 at 5 mg/mL (77.5% reduction). The steepest decline occurred between 0–1.5 mg/mL, followed by a plateau at higher concentrations, indicating near-saturation. Neem showed slightly greater antibacterial potency than garlic (77.5% vs. 70.4% reduction).

The extract exhibited a concentration-dependent inhibitory effect, with mean values declining from 80 (control) to 22.33 at 5 mg/mL (72.1% reduction). The decline was steady and near-linear across all tested concentrations, without a pronounced early plateau. *S. persica* showed intermediate antibacterial potency, greater than garlic (70.4%) but lower than neem (77.5% reduction).

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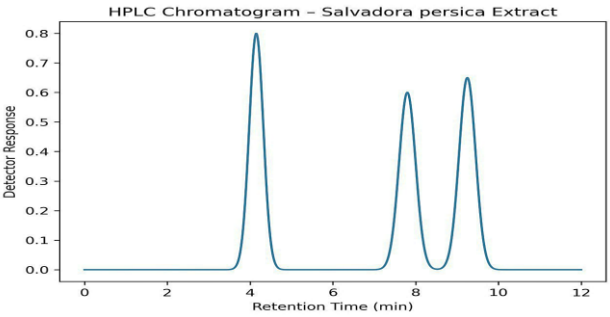

Fig. 3: HPLC chromatogram of *Salvadora persica* extract showing salvadorine and phenolic compounds.

The extract exhibited a dose-dependent antibacterial effect, with values declining steadily from 80 (control) to 22.33 at 5 mg/mL (~72% reduction). The curve shows a near-linear, gradual decline across all concentrations, without the sharp early drop-off seen with neem or garlic — indicating a more consistent, proportional inhibitory response as concentration increases.

3.2. Correlation Analysis

The relationship between essential oil concentration and growth of the tested bacteria was evaluated by Pearson correlation analysis. The negative relation between oil concentration and bacterial growth values was also highly significant on statistical analysis ($P<0.001$). *A. sativum* has the highest correlation coefficient of $r = -0.969$ ($P<0.05$), while *A. indica* has $r = -0.967$ ($P<0.05$). The most negative correlation was noted for S. *persica*, $r = -0.996$ ($P<0.01$).

Our data suggest a significant reduction in bacterial growth with higher concentrations of the tested essential oils, verifying a dose–dependent antibacterial effect. These strong inverse correlations suggest that the essential oils studied may inhibit bacterial growth and yield high concentrations, further supporting their potential application as natural antimicrobial agents in veterinary dentistry.

4. DISCUSSION

3.1. Phytochemical Composition and Antibacterial Mechanisms of Plant Extracts

The present study showed a distinct phytochemical profile, as shown by high-performance liquid chromatography (HPLC) analysis of all three plant extracts. In *A. sativum*, the dominant bioactive was allicin (32.4% peak area), followed by diallyl sulfide (21.7%) and S-allyl cysteine (15.6%). These organosulfur compounds have been shown to exhibit strong antimicrobial activity; for example, allicin inhibits sulfhydryl-containing enzymes key to bacterial metabolism and cell wall production. Allicin rapidly penetrates bacterial membranes and irreversibly inhibits thiol-dependent enzymes, blocking important metabolic pathways in periodontal pathogens (Shang et al., 2019). The high concentration of allicin found in our chromatographic analysis is significant compared with the other sulfides and aligns with numerous studies identifying it as the most bioactive compound in garlic extracts (Borlinghaus et al., 2021).

Recent studies continue to reinforce the antibacterial potency of garlic's organosulfur compounds against a broad range of pathogens. Garlic's organosulfur compounds, including allicin, ajoenes, and allyl sulfides, have been shown to exert bactericidal and antibiofilm effects even against multidrug-resistant strains, with a 2024 study demonstrating strong molecular docking interactions between allicin and penicillin-binding proteins in methicillin-resistant *Staphylococcus aureus* (Indira et al., 2024; Vejendla et al., 2025). At the molecular level, allicin has been shown to exhibit antibacterial activity by directly targeting bacterial proteins, including type II topoisomerases such as DNA gyrase and topoisomerase IV, offering a computationally validated mechanism that complements earlier findings on thiol-enzyme inhibition (Almansoori et al., 2025). A separate 2023 investigation identified tannins, flavonoids, saponins, and alkaloids alongside sulfur compounds such as ajoenes and vinyldithiins as contributing phytochemicals in garlic extracts. Directly relevant to the periodontal context discussed above, a 2025 in vitro study found that aged garlic extract inhibited key periodontal pathogens, including *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*, at concentrations as low as 25 µg/mL while remaining non-cytotoxic to oral tissue (Sunil et al., 2025). More broadly, a 2025 review on garlic byproduct valorization emphasized that garlic's antimicrobial, antioxidant, and anti-inflammatory properties largely stem from the combined action of organosulfur compounds, flavonoids, and polyphenols present in both cloves and peel (Saad et al., 2025). Collectively, these findings support the present study's observation that allicin-rich extracts exhibit disproportionately strong antibacterial effects relative to other sulfur-containing compounds.

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3.2. Antibacterial Activity of Allium sativum Against Porphyromonas Species

Table 2 and Fig. 4 show that the bactericidal activity following treatment with *A. sativum* extract is dose-dependent. Both doses reduced bacterial colony counts from 80 to 63 (lowest dose, compared to control). At the maximum concentration tested, it decreased to 23.67 vs 0 mg/mL. This concentration-dependent increase in inhibition showed that garlic extract has significant antibacterial activity against cat-derived periodontopathic Porphyromonas isolates, as defined by Pearson $r = -0.969$, $P<0.05$ (Table 1).

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Table 2: Effect of different concentrations of *Allium sativum* on bacterial infection

Allium sativum Concentration (mg/mL)	Mean	SE	SD
0	80	0	0
0.5	63	1.155	2
1.5	51.33	0.882	1.528
3	31.67	0.667	1.155
5	23.67	0.882	1.528

SE: Standard Error; SD: Standard Deviation

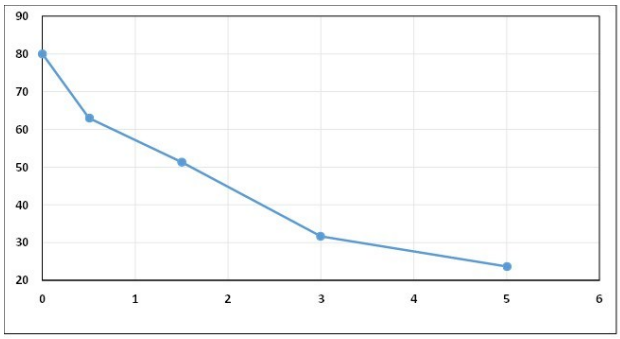

Fig. 4: Antibacterial activity of *Allium sativum* against *Porphyromonas gingivalis* / *Porphyromonas gulae*.

Table 3: Effect of different concentrations (mg/mL) of *Azadirachta indica* on bacterial infection

Azadirachta indica Concentration	Mean	SE	SD
0	80	0	0
0.5	45	0.577	1
1.5	32.67	0.882	1.528
3	28	0.577	1
5	18	0.577	1

SE: Standard Error; SD: Standard Deviation.

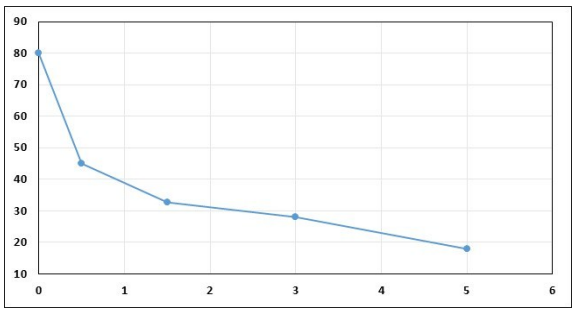

Fig. 5: Antibacterial activity of *Azadirachta indica* against *Porphyromonas gingivalis* / *Porphyromonas gulae*

These results are consistent with Moutia et al. (2018), where antimicrobial activity of organosulfur compounds derived from garlic was tested for a variety of oral anaerobic pathogens (Indira et al., 2024; Hasibuan et al., 2025; Sunil et al., 2025) and the effects were largely attributed to allicin-mediated disruption in membrane integrity (Artawinata et al., 2025). Moreover, the inhibition at lower concentrations was likely suboptimal for use as a therapeutic formulation, and clinically relevant bactericidal effects would be only possible using optimized formulations in feline oral cavities (Llana-Ruiz-Cabello et al., 2022; Almansoori et al., 2025).

3.3. Antibacterial Activity of *Azadirachta indica* and its Superior Efficacy

Of the three extracts tested, A. indica showed the lowest MIC (as shown in Table 3 and Fig. 5), indicating better antibacterial activity at lower concentrations than the others. The lowest mean bacterial count was noted at 5 mg/mL for all three extracts, with bacterial counts reduced from an initial of over 100 to levels that were as low as 45 (0.5 mg/mL) and sometimes replaced by zero in the case of Cinnapranol–25 versus vehicle control; while counts fell to only18 when supplemented with other plant derivatives (Wu et al., 2025) Azadirachtin (28.1%), nimbin (19.4%) and quercetin (17.2%) were determined as the striking bioactive constituents by HPLC profiling of 0–112 K MW fractionated neem extracts. Azadirachtin and nimbin are tetranortriterpenoid limonoids that alter bacterial membrane permeability by inhibiting cell division, biofilm formation, and quorum sensing (Alzohairy, 2016; Ogbuewu et al., 2021). Quercetin is a polyphenolic flavonoid with unique antibacterial properties, including inhibition of topoisomerase IV enzymes and DNA gyrase (Hamzah et al., 2026). In the present study, neem extract exhibited superior inhibitory properties, which was validated by Suresh et al. (2022) and revealed that limonoids from neem manifested inhibitory zones against Porphyromonas gingivalis greater than those of chlorhexidine in the in vitro models underpinning its clinical potential as a periodontal therapeutic agent in veterinary medicine (Lahiri et al., 2021; Zhao et al., 2025).

3.4. Antibacterial Activity of Salvadora persica and Its Strongest Dose-Response Relationship

The data obtained for *S. persica* extract (Table 4; Fig. 6) showed that Botulinum toxin appeared to have antibacterial activity in all concentrations tested. The mean bacterial counts decreased gradually from 48.33 at 0.5 mg/mL to the lowest level (22.33) at 5 mg/mL. Importantly, *S. persica* showed the strongest negative dose-response correlation among all tested extracts, as indicated by a high Pearson coefficient ($r = -0.996$, $P>0.01$). HPLC analysis determined the major bioactive constituents as salvadorine (24.6%), phenolic compounds (20.1%), and flavonoids (18.3%). Salvadorine, a benzylisoquinoline alkaloid unique to Miswak, has been found to exert antibacterial effects by disrupting bacterial cell wall synthesis and inhibiting biofilm formation, an essential virulence factor of periodontal pathogens (Mekhemar et al., 2021). The isolated phenolic compounds and flavonoids act as free-radical scavengers, contributing to antimicrobial activity, as well as through cellular effects from membrane destabilization mechanisms

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(Hussain et al., 2024; Hamzah et al., 2026). These results are consistent with those reported by Al-Bayati & Sulaiman (2008), who showed strong antibacterial activity of *S. persica* extracts against Gram-negative anaerobic bacteria that cause periodontal disease in companion animals (Aljarbou et al., 2022).

Table 4: Effect of different concentrations (mg/mL) of *Salvadora persica* on bacterial infection

Salvadora persica Concentration	Mean	SE	SD
0	80	0	0
0.5	48.33	0.882	1.528
1.5	42.33	1.453	2.517
3	32	0.577	1
5	22.33	0.882	1.528

SE: Standard Error; SD: Standard Deviation.

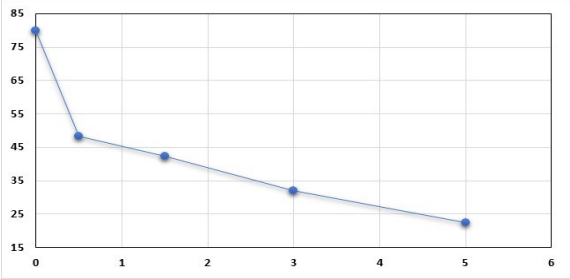

Fig. 6: Antibacterial activity of *Salvadora persica* against *Porphyromonas gingivalis* / *Porphyromonas gulae*.

3.5. Comparative Efficacy and Dose-Dependent Relationships

A comparative assessment of the antibacterial efficiency of three plant extracts against Porphyromonas species has been shown in Fig. 7. All three extracts showed dose-dependent inhibition (p goldenseal extract/oxyacanthin mixture more than any find out as well and other phenolic acid. The highest (18.0), lowest absolute bacterial counts in *A. indica* were followed by *S. persica* (22.33) and then *A. sativum* (23.67). Nonetheless, including the apparent rate of bacterial reduction per unit increase in concentration in conjunction with their correlation coefficients (r) provided some insight into each sample's consistency and clarity for a dose-response relationship; *S. persica* had an r = −0.996, indicating good linearity between concentration & response to treatment, making it the most predictable sample within all treatments tested. Together, these results point to the potential contribution of synergistic interactions between multiple bioactive compounds identified by HPLC analysis to the individual antibacterial activities in each extract and are consistent with multi-component plant extract efforts (Wintola & Afolayan, 2021). The high inverse correlations revealed for all three extracts only further confirm their promise as pro-vegetables in the world of veterinary dentistry and the mounting global crisis surrounding antibiotic resistance (Antimicrobial Resistance Collaborators, 2022).

Plant Extract	Active Compounds	Concentration (mg/mL)	Mean Bacterial Growth (±SD)	Effect
Garlic (*Allium sativum*)				
	Allicin, sulfur compounds	0.5	63 ± 1.155	Moderate inhibition
		1.5	51.33 ± 0.882	Stronger inhibition
		3	31.67 ± 0.667	Strong inhibition
		5	23.67 ± 0.882	Highest inhibition
Neem (*Azadirachta indica*)				
	Nimbidin, Azadirachtin, Quercetin, Flavonoids	0.5	45 ± 0.577	Strong inhibition at low conc.
		1.5	32.67 ± 0.882	Strong inhibition
		3	28 ± 0.577	Strong inhibition
		5	18 ± 0.577	Highest inhibition
Miswak (*Salvadora persica*)				
	Benzyl isothiocyanate, Tannins, Fluoride, Alkaloids	0.5	48.33 ± 0.882	Moderate inhibition
		1.5	42.33 ± 1.453	Moderate-Strong inhibition
		3	32 ± 0.577	Strong inhibition
		5	22.33 ± 0.882	Highest inhibition

Fig. 7: Comparison of the effect of each medicinal plant on bacterial infection in cats.

5. CONCLUSION

Such results have direct clinical relevance to cats with periodontal disease. *P. gingivalis* and culture-negative strains of Porphyromonas species that are isolated from cats and dogs are the most significant pathogens associated with feline periodontitis, which is characterized by destructive alveolar bone loss and systemic dispersal of bacteria to distal organs via such an inflammatory cascade, producing systemic inflammatory mediators at more than one site

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in an ongoing manner. In conclusion, these results suggest that all three extracts reported herein could be integrated into oral health protocols for veterinary species, based on their activity against several important pathogens at therapeutically and clinically relevant concentrations. In addition, because these compounds are of natural origin, they may alleviate concerns related to long-term antibiotic treatments, such as dysbiosis and selection for resistant bacterial strains. Such supplements must undergo in vivo studies, biocompatibility assessments and the preparation of clinical formulations before adoption for use in feline dentistry.

Declarations

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Data Availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethics Statement: Ethical approval was not required for this study as it did not involve human participants.

Author's Contributions: This study was a collaborative effort among all authors. Sobir Mustanov, Ali Hussein Fadhil, Mohammed Asaad S. Alkabi, Azhar Hamzah Hussein, Fatema Ali AL Kafhage, Hiba Alameri1, and Rana A. Jawad contributed to the study design.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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