

Journal of Pharmaceutical and Biological Research

ISSN: 2347-8330 | <https://doi.org/10.30904/j.jpbr.2026.4938>

Bacterial Biofilm Formation on Infant Pacifiers and Its Impact on Feeding Habits: Evaluation of the Antimicrobial Activity of Selected Plant Extracts

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Received: 17-01-2026 | Revised: 05-02-2026 | Accepted: 27-02-2026 | Published: 23-03-2026

ABSTRACT

Pacifiers are widely used as soothing devices for infants, but their frequent exposure to saliva and environmental contaminants makes them potential reservoirs for microbial colonization. The present study aimed to investigate bacterial contamination and biofilm formation on infant pacifiers and to evaluate the antimicrobial activity of selected plant extracts. Pacifiers were collected from infants aged 6 months to 2 years, and bacterial isolates were identified using morphological and biochemical methods. The antimicrobial activity of *Prosopis juliflora* and *Allium cepa* extracts was tested using the agar well diffusion method. Results revealed significant bacterial contamination, including *Staphylococcus spp.*, *Streptococcus spp.*, *Escherichia coli*, *Klebsiella spp.*, *Pseudomonas spp.*, and *Bacillus spp.* The plant extracts showed dose-dependent antibacterial activity. The findings highlight the importance of proper hygiene practices and suggest that plant-based antimicrobials can be effective alternatives for controlling microbial contamination on pacifiers.

Keywords: Pacifiers, Biofilm, Infant health, *Prosopis juliflora*, *Allium cepa*, Antimicrobial activity

Introduction

Pacifiers are widely used infant care devices designed to satisfy the non-nutritive sucking reflex, an innate behavior that develops early in life and plays a significant role in soothing infants. Their use has been associated with calming effects, reduced crying, and improved sleep patterns (Schmid *et al.*, 2018). Globally, the prevalence of pacifier use varies considerably, ranging from 25% to 84%, influenced by cultural norms, parental beliefs, and caregiving practices (Ponti, 2003; Buccini, Pérez-Escamilla, & Venancio, 2017). Despite their benefits, prolonged pacifier use has raised concerns due to its potential negative impacts on infant health, particularly in relation to breastfeeding duration and dental development. Studies have shown that extended pacifier use may interfere with breastfeeding practices and contribute to dental malocclusions such as anterior open bite and posterior crossbite (Buccini *et al.*, 2017; World Health Organization [WHO], 2018).

In addition to these developmental concerns, microbial contamination of pacifiers has emerged as a significant public health issue. Pacifiers are frequently exposed to infant saliva, environmental contaminants, and improper handling, creating favorable conditions for microbial colonization. The repeated use of pacifiers without adequate sterilization facilitates the accumulation of microorganisms and promotes the formation of biofilms on their surfaces (Comina *et al.*, 2006). Biofilms are complex, structured communities of microorganisms embedded in a self-produced extracellular matrix, which enhances their survival and resistance to antimicrobial agents (Costerton, Stewart, & Greenberg, 1999). Several pathogenic microorganisms have been identified on contaminated pacifiers,

including *Streptococcus mutans*, *Staphylococcus aureus*, and *Candida albicans*, all of which are associated with oral and systemic infections in infants (Mattos-Graner *et al.*, 2001). Infants are particularly vulnerable to such infections due to their immature immune systems, making the presence of these microorganisms a matter of clinical concern. The persistence of biofilms on pacifier surfaces further complicates disinfection efforts, as biofilm-associated microbes exhibit increased resistance to conventional cleaning methods.

In recent years, there has been growing interest in the use of plant-derived antimicrobial agents as safer and eco-friendly alternatives to synthetic chemicals. Medicinal plants have long been recognized for their therapeutic properties, largely attributed to their rich content of bioactive compounds such as flavonoids, phenolics, alkaloids, and sulfur-containing compounds (Cowan, 1999). Among these, *Prosopis juliflora* and *Allium cepa* have confirmed notable antimicrobial activity against a wide range of microorganisms. *Prosopis juliflora*, a member of the Fabaceae family, is known for its diverse phytochemical profile and adaptability to harsh environmental conditions, while *Allium cepa* (onion) contains sulfur compounds and flavonoids that exhibit potent antimicrobial and antioxidant properties (Dilfy *et al.*, 2020). Therefore, the present study aims to evaluate microbial contamination and biofilm formation on infant pacifiers and to assess the antimicrobial efficacy of selected plant extracts. This investigation seeks to contribute to the development of safer, natural alternatives for controlling microbial contamination and improving infant health outcomes.

Materials and Methods

Study Area and Sample Collection

Pacifiers were collected from infants aged 6 months to 2 years in Puduvalasai Village with parental consent. Samples were transported under sterile conditions and processed within 2 hours to prevent changes in microbial load (Comina *et al.*, 2006).

Sample Processing

Pacifiers were immersed in sterile physiological saline (0.85% NaCl), vortexed to dislodge surface-adhered microorganisms. The resulting suspension was used for microbiological analysis (Moshrefi, 2002).

Serial Dilution

Serial dilution from 10^{-1} to 10^{-7} was performed to obtain countable colonies using standard microbiological procedures (Harborne, 1984).

Isolation of Bacteria: Samples were cultured on selective and differential media: Nutrient Agar, Blood Agar, MacConkey Agar, Mannitol Salt Agar. Plates were incubated at 37°C for 24-48hours (Cappuccino & Sherman, 2014).

Enumeration of Bacteria

Colony-forming units (CFU/mL) were calculated from plates containing 30–300 colonies using standard plate count methods (Prescott *et al.*, 2005).

Identification of Bacteria

Bacterial isolates were identified using: Gram staining, Morphological analysis, Biochemical tests (Catalase, Oxidase, Indole, MR-VP, Citrate, Urease, TSI). Identification was performed based on Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994).

Preparation of Plant Extracts

Methanolic extracts of *Prosopis juliflora* leaves and *Allium cepa* peels were prepared using cold maceration techniques (Harborne, 1984; Orhan *et al.*, 2007).

Antimicrobial Assay: Antimicrobial activity was evaluated using the agar well diffusion method at concentrations of 1 µg, 10 µg, 25 µg, and 50 µg. Kanamycin was used as a positive control (Bauer *et al.*, 1966).

Questionnaire Survey

A structured questionnaire was administered to 20 families to assess pacifier usage, hygiene practices, and awareness of associated health risks (Nelson-Filho *et al.*, 2015).

Results and Discussion

The survey findings indicated that pacifier use was universal among the studied population, with 100% of families reporting usage. This highlights the widespread acceptance of pacifiers as a soothing aid for infants. Notably, approximately 50% of caregivers reported prolonged use exceeding six months, a practice that has been associated with an increased risk of dental malocclusion, including anterior open bite and posterior crossbite (Warren & Bishara, 2002). Although a high proportion of families (90%) reported cleaning pacifiers by boiling, only 5% were aware of the potential health risks associated with improper pacifier hygiene (Table 1). This discrepancy suggests a significant gap between hygienic practices and awareness, consistent with previous findings emphasizing limited parental knowledge regarding microbial contamination (Nelson-Filho *et al.*, 2015).

Table 1: Awareness and Practices Regarding Pacifier Use (n = 20 families).

Question	Yes (n)	Percentage (%)
Do you use a pacifier for your baby?	20	100%
Using pacifier for more than 6 months	10	50%
Use pacifier to calm the baby	9	45%
Pacifier affects breastfeeding	5	25%
Clean pacifier 1–2 times per day	10	50%
Use boiled water for sanitizing	18	90%
Store pacifier in a clean box	14	70%
Aware of pacifier-related health risks	1	5%
Gap between baby teeth observed	5	25%
Use pacifier during sleep	11	55%

Microbiological analysis revealed a substantial bacterial load on pacifiers (Figure 1), ranging from 1.2×10^4 to 4.6×10^6 CFU/mL, indicating heavy microbial contamination. These findings are in agreement with earlier studies that identified pacifiers as reservoirs of microorganisms due to repeated exposure to saliva and environmental contaminants (Comina *et al.*, 2006). The bacterial isolates identified in the present study (Figure 2) included *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., and *Bacillus* spp. These microorganisms are commonly associated with oral infections, gastrointestinal disturbances, and environmental contamination, suggesting potential health risks for infants (Prescott, Harley, & Klein, 2005).



Figure 1: Microbiological analysis of on pacifiers

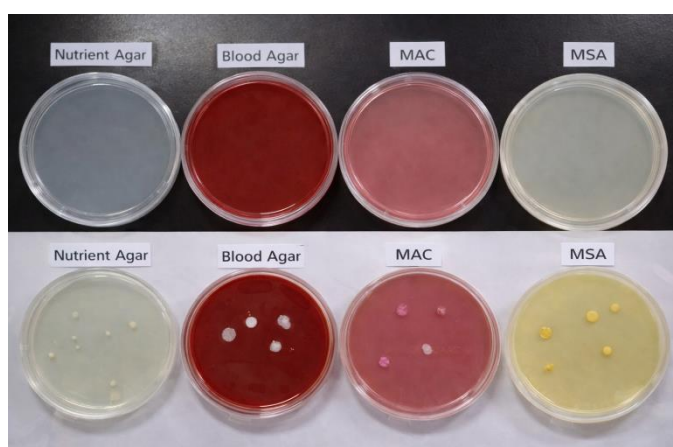


Figure 2: The bacterial isolates identified in the present study.

The detection of a diverse range of microbial species indicates a high likelihood of biofilm formation on pacifier surfaces. Biofilms are structured microbial communities enclosed within

a self-produced extracellular matrix that enhances bacterial adhesion and survival. This mode of growth significantly increases resistance to antimicrobial agents and conventional cleaning methods, making infections more persistent and difficult to control (Costerton, Stewart, & Greenberg, 1999). The presence of biofilm-forming bacteria on pacifiers is particularly concerning given the immature immune system of infants, which makes them more susceptible to infections. The antimicrobial activity of the selected plant extracts confirmed a clear dose-dependent effect against the tested bacterial isolates. An increase in extract concentration resulted in larger zones of inhibition, indicating enhanced antibacterial efficacy. Although the standard antibiotic kanamycin exhibited higher inhibitory activity, the plant extracts showed considerable antimicrobial effects, suggesting their potential as natural alternatives. These findings support earlier reports that plant-derived compounds such as flavonoids, phenolics, and sulfur-containing compounds possess strong antimicrobial properties (Cowan, 1999).

Overall, the results emphasize that while pacifier use is widespread and often accompanied by basic cleaning practices, microbial contamination remains a significant concern. The presence of pathogenic and biofilm-forming bacteria underscores the need for improved hygiene awareness among caregivers. Furthermore, the promising antimicrobial activity of plant extracts highlights their potential application as eco-friendly agents for controlling microbial contamination on infant care products.

Conclusion

The present study demonstrates that pacifier use is highly prevalent among infants, reflecting its importance as a soothing tool in early childhood care. However, the findings highlight significant concerns regarding prolonged usage and inadequate awareness of associated health risks. A considerable proportion of caregivers reported extended pacifier use beyond six months, which may contribute to adverse effects such as dental malocclusion and interference with normal oral development. Microbiological analysis confirmed that pacifiers serve as potential reservoirs of diverse microorganisms, with high bacterial loads observed across samples. The isolation of pathogenic bacteria such as *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., and *Bacillus* spp. indicates a substantial risk of infection in infants. The presence of these organisms further suggests the formation of biofilms, which enhance microbial persistence and resistance to conventional cleaning methods. Despite the majority of caregivers adopting boiling as a cleaning method, the persistence of microbial contamination suggests that current hygiene practices may not be consistently effective or properly implemented. Additionally, the low level of awareness regarding potential health risks underscores the need for improved education among caregivers. The study also highlights the promising antimicrobial potential of plant extracts such as *Prosopis juliflora* and *Allium cepa*. The observed dose-dependent antibacterial activity against tested pathogens suggests that these natural agents could serve as effective and eco-friendly alternatives to synthetic antimicrobial agents. Although their efficacy was slightly lower than that of standard antibiotics, their safety, availability, and reduced risk of

resistance make them valuable candidates for further research and application.

Acknowledgment

The authors would like to express their sincere gratitude to Management and Principal, Syed Ammal Arts and Science College, Ramanathapuram, and the Department of Zoology for providing the necessary facilities and support to carry out this research work successfully.

Conflict of Interests

The authors declare no conflict of interest

Ethics Approval

Not applicable

Funding: This study received no specific funding from public, commercial, or not for profit funding agencies.

AI Tool Declaration

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

Data Availability

Data will be available on request

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