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IN VITRO ANTIMICROBIAL ACTIVITY OF CAPPARIS SPINOSA EXTRACT AGAINST ORAL PATHOGENS FROM PEDIATRIC DENTAL CARIES

Layla Fadhil Azeez Albedhiani[1], Sura Hameed Mohammed[2]*, Safaa Abbas Abd AL-Kahdum Hussain[3], Rahaf Esam Naji[4], Yasmeen Imad Kadhum[2], Dhay Haider M. Hassan[5], Hany Akeel Al-Hussaniy[6]

[1]Department of Community and Preventive Dentistry, College of Dentistry, Ahl al-Bayt University, Iraq.
[2]*Department of Basic Sciences, College of Dentistry, Ahl al-Bayt University, Iraq.
[3]Department of Biology, College of Science, University of Babylon, Iraq.
[4]Department of Orthodontics, College of Dentistry, Ahl al-Bayt University, Iraq.
[5] Department of Pedodontics and Preventive Dentistry, College of Dentistry, Ahl al-Bayt University, Iraq.
[6]Department of Pharmacology, College of Pharmacy, Al-Nisour University, Baghdad, Iraq.

***Corresponding Author: Sura Hameed Mohammed**

Department of Basic Sciences, College of Dentistry, Ahl al-Bayt University, Iraq.

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ABSTRACT

Aim: Dental caries is one of the most common oral diseases of children worldwide. Natural agents with antimicrobial activity are increasingly being explored in light of the rise in resistance of oral microorganisms to conventional antibiotics. **Objective**: This study aimed to isolate and phenotypically identify oral pathogens associated with dental caries in children and to evaluate the *in vitro* antifungal effect of the aqueous extract of *Capparis spinosa* leaves. **Materials and Methods:** Thirty clinical specimens were obtained from children aged 7-9 years, including 20 samples with dental caries and 10 from healthy children as controls. The samples were incubated for 24–48 hours after being cultured on Sabouraud Dextrose Agar (SDA) and Brain Heart Infusion (BHI). Data were analyzed using one-way ANOVA by LSD test at a $p \leq 0.05$ probability level**. Results**: The isolated fungi, comprising *Candida albicans* (60%), *C. parapsilosis* (30%), and *C. krusei* (10%), *Streptococcus mutans* (70%), and *Lactobacillus* spp. (30%), were isolated from carious samples. The aqueous extract of *C. spinosa* demonstrated a strong effect against *C. albicans* but a weak antibacterial effect against *Streptococcus mutans*. **Conclusion**: Findings of this *in vitro* study indicate that *Capparis spinosa* extract showed antifungal activity against specific fungal species associated with pediatric dental caries. Further *in vivo* investigations are recommended to explore its incorporation into pediatric oral care formulations.

KEYWORDS: Dental caries, plant extract, Antifungal activity, pediatric dentistry, oral microbiology.

INTRODUCTION

Oral health is crucial for overall health and plays a vital role in protecting against systemic diseases. According to the World Health Organization, poor oral health is one of the most significant global health burdens of non-communicable diseases, particularly in low- and middle-income countries.[16] Untreated tooth decay affects billions of people worldwide and is considered one of the most prevalent diseases globally.[8,13] The State of Global Oral Health Report (2022) also indicates that early childhood caries is among the most common chronic diseases worldwide, and higher sugar consumption is associated with more cavity formation.[10]

The oral microbiome is a diverse and complex ecosystem that inhabits more than 750 species of microorganisms, many of which are associated with dental and oral diseases.[15,17,10] Imbalances in oral organisms are responsible for the development of dental and periodontal diseases, as indicated by next-generation sequencing.[12,5,30] *Streptococcus mutans* and various *Lactobacillus species*, both lactic acid–producing bacteria, convert dietary sugars into lactic acid, leading

to enamel demineralisation and cavity formation.[11,17,10] According to the latest scientific research, the main bacteria causing tooth decay, acid-producing bacteria in the oral biofilm, are *Streptococcus mutans*, but other bacteria, such as *Streptococcus sobrinus* and *Lactobacillus spp.*, also contribute to cavities.[22]

In addition to bacterial species, fungi are increasingly recognized as risk factors for early childhood caries, specifically *Candida albicans*, which is more prevalent among immunocompromised children or those with reduced salivary flow.[7] *Candida albicans* can co-aggregate with *S. mutans*, leading to greater biofilm and acid production.[18,7,5] Other non-*Candida albicans* species, including *Candida tropicalis*, *Candida parapsilosis*, and *Candida krusei* and similar strains, have been associated with various microbial infections in tooth decay.[19]

Extracts of *Capparis spinosa* exhibit antifungal activity against *C. albicans* and antibacterial effects against *S. mutans.*[2 ,20,28,23] Studies have also shown that certain botanicals can effectively inhibit oral biofilm formation by interfering with bacterial adhesion.[27]. Other studies have shown that *C. spinosa* contains bioactive phytochemicals, including flavonoids, phenols, alkaloids, and glucosinolates, which contribute to its antimicrobial and antioxidant activities.[6,14,23,25,29]

Despite growing interest in plant-derived antiprotozoal agents, studies on the use of plant extracts as antifungal agents against oral candidiasis remain limited.

Research investigating the antifungal activity of *Capparis spinosa* against *Candida albicans* remains limited and, in some cases, contradictory.[20] Some studies suggest that the effectiveness of the extraction method and phytochemical concentration vary.[6,25] The present study aims to isolate and identify bacterial and fungal species associated with dental caries in children. Additionally, it evaluates the antimicrobial properties of aqueous *Capparis spinosa* extract against *S. mutans* and *C. albicans.*

MATERIALS AND METHODS

1. Study Design and Setting

This study was conducted in the Microbiology Laboratory of the College of Science at the University of Babylon, Iraq, from January 2022 to March 2023.

2. Sample Collection and Microbial Isolation

Oral swabs and clinical samples were collected from children aged 7 to 9 years. A total of 30 specimens (20 of decayed teeth and 10 from healthy children as controls) were collected. To collect samples from subjects with active dental caries, sterile cotton swabs were used; the samples were then firmly smeared onto the decayed teeth and placed in their respective transport gel for transport to the laboratory. Through the streaking method, smear samples were cultured on Brain Heart Agar and Sabouraud Dextrose Agar (SDA). To examine morphological characteristics of isolates derived from children presenting with dental caries on morphological traits (the colony's colour, shape, and texture and posterior colour of the colony), all plates were incubated at 37 °C for 24–48 hours under aseptic conditions. To ensure purity of growth, the isolates were routinely re-cultured on SDA media.[1]

3. Preparation of Plant Extracts

Plant *Capparis spinosa* leaf samples were taken from diverse locations in Babylon Governorate/Iraq. After cleaning, they were left to dry at room temperature. They were ground electrically into a fine powder after drying.[6,9] The aqueous extract of caper was prepared according to Amed *et al.* (1998), who used 20 g of dried caper powder, which was added to 500 mL of sterile distilled water in a 1000 mL beaker. The mixture was well mixed and allowed to sit for 24 hours to extract the active compounds. It was then centrifuged at 3000 rpm for 10 minutes. The supernatant, which was clear water, was collected and evaporated in an oven at 45 °C for 24 hours. The precipitate was then skimmed and collected. This process was repeated 2-3 times to obtain a sufficient quantity of extract. A 5% extract concentration was prepared by dissolving 0.5 g in 10 ml of distilled water. The extract was sterilized by microfiltration through a 0.45 µm membrane filter before its use in antimicrobial testing, and its efficacy against *Candida albicans* and *Streptococcus mutans* was tested.[3]

4. Culture Media and Identification of Microorganisms

Culture media were prepared following standard protocols.

SDA liquid (65 g/L), blood agar (40 g/L + 3–5% human blood), and BHI (52 g/L) were prepared and sterilized with pressurized steam at 121°C for 15 minutes. They were then poured into sterile Petri dishes and stored at 4°C until use.[20,25] CHROMagar Candida (47.7 g/L) was heated until dissolved, then cooled to approximately 45°C. It was not sterilized by pressurized steam and was poured into sterile dishes for virtual identification based on colony colour.[3]

Culturing Technique

Oral swab samples were collected from decayed teeth by pressing the swab firmly on the decay and preserved in the gel itself before being transported to the laboratory. The samples were cultured on Sabouraud Dextrose Agar (SDA) and Brain Heart Agar using the streaking method. The plates were incubated at 37 **°C** for 24–48 hours to assess the isolate's growth based on morphological characteristics, including colony colour, shape, and texture, as well as the background colour. The isolates were periodically and sterilely re-cultured on SDA agar to maintain their purity.[1]

Identification of Candida spp. on CHROM agar

A small piece of each Candida fungal colony was taken and subcultured on Chromagar medium, then incubated at 37 °C for 24 hours. We identified the species based on the colonies' coloration observed in the medium: Apple green = *C. albicans*, White to purple = *C. parapsilosis*, Pink = *C. krusei*.[20]

Identification of *Streptococcus mutans* on Blood agar.

A small portion of the *Streptococcus mutans* colony was inoculated onto blood agar and incubated at 37 °**C** for 24 hours. Bacterial identification was based on hemolysis on blood agar.[22]

5. Antimicrobial Activity Assay

The 5% aqueous extract of *Capparis spinosa* was evaluated for its antimicrobial activity using the well-diffusion method in accordance with standard antimicrobial susceptibility testing protocols.[3,4] Six-mm-diameter wells were cut into Sabouraud Dextrose Agar (SDA) plates for Candida spp. and into Brain Heart Infusion (BHI) Agar plates for *Streptococcus mutans*, as commonly used for antimicrobial susceptibility testing of oral bacteria.[4] Then, 100 µL of the 5% aqueous plant extract was added to each well. Fluconazole for *Candida spp*. and Nystatin were the positive controls, while sterile distilled water served as the negative control. All plates were incubated at 37 °C for 24-48 hours. The diameter of the inhibition zone was measured in mm using a sterile ruler.[27,3] All antimicrobial experiments were performed in triplicate (n = 3) to ensure reproducibility.

6. Statistical Analysis

The mean ± standard deviation (SD) was calculated. Data were analyzed by means of one-way ANOVA, while the differences among means were determined through the least significant difference (LSD) test at $p \leq 0.05$.[3]

RESULTS

1. Isolation and Identification of *Candida* spp. of Teeth Biofilms

Twenty strains were isolated using the agar plate technique, and an additional 10 samples were collected from a control group of healthy children using the same method. The distribution of case by age among infected and healthy children is presented in Table 1. Isolations were identified based on colony characteristics and classified at the species level based on colour reactions on Chromagar Candida medium. Figure 1: The number of colonies on SDA plates was as follows: *C. albicans* (12), *C. parapsilosis* (6), and *C. krusei* (2), as shown in Figure 1.

Table 1: Distribution of cases by age among infected and healthy children.

Age -years	Infected Control	Healthy Control	Percentage of Total
7	10	3	43%
8	10	7	57%
Total	**20**	**10**	**100%**

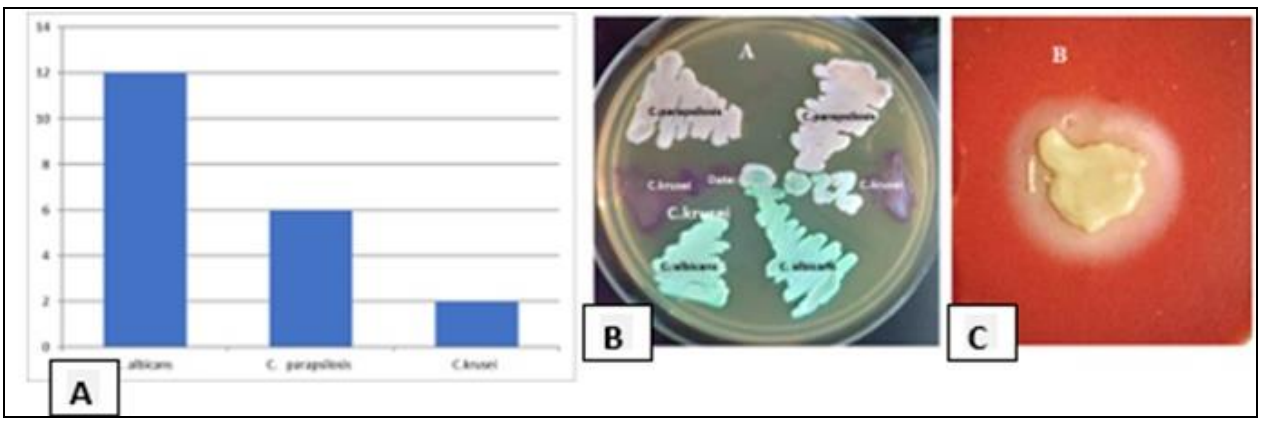

Figure 1: Identification and distribution of Candida in carious lesions of children.

A: Bar chart shows the sharing of *Candida albicans*, *C. parapsilosis*, and *C. krusei* was taken from oral cavity samples, with *Candida albicans* representing the highest frequency. **B:** The colour of the colonies on the Chromagar Candida plate indicates *C. albicans:* white-to-pink colonies; *C. parapsilosis* appears pinkish; *C. krusei*. **C:** Hemolytic activity of *C. albicans* grown on blood-enriched Sabouraud Dextrose Agar exhibited clear hemolytic zones, while non-albicans species showed no hemolysis.

2. Hemolytic Activity of Candida Species

Culturing isolates on blood agar evaluated hemolytic activity. The results showed that *C. albicans* had distinct hemolytic zones, while *C. parapsilosis* and *C. krusei* did not show hemolytic activity Figure 1.

3. Isolation and Identification of Bacterial Species *Streptococcus mutans* and *Lactobacillus* spp

Samples collected from infected and healthy children were cultured on Blood agar and Brain Heart Infusion (BHI). The colonies detected on BHI plates included

Streptococcus mutans (14) and *Lactobacillus spp*. *Streptococcus mutans* isolates showed clear alpha-hemolysis on blood agar, as illustrated in Figure 2.

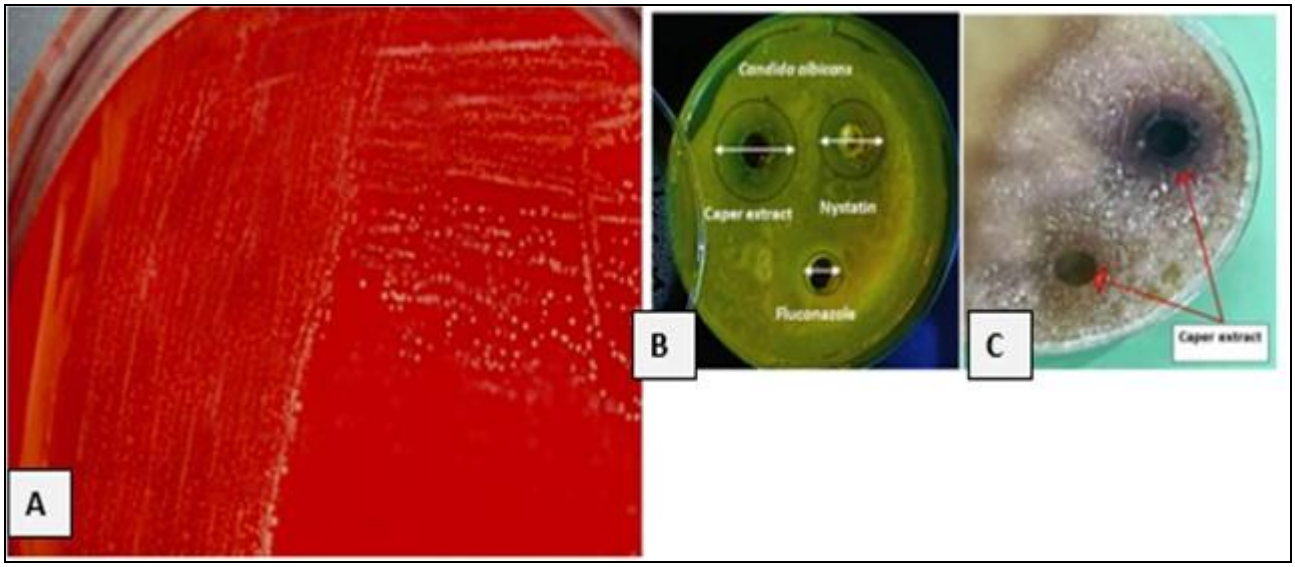

Figure 2: Antimicrobial property of *Capparis spinosa* extract against oral pathogens.

A: This is a blood agar plate showing the growth and hemolytic pattern of *Streptococcus mutans*. **B:** A wide diffusion test is used to measure the effects of a 5% aqueous *Capparis spinosa* extract against standard antifungal agents (Nystatin and Fluconazole) and *Candida albicans*, and to compare them. **C:** More antimicrobial assay plates representing inhibition zones due to caper extract against the established biofilm of the fungus Candida, suggesting antifungal activity and weak antibacterial effect.

4. Antimicrobial Activity of *Capparis spinosa* Extract

5% aqueous extract of *Capparis spinosa* showed apparent antimicrobial activity against the isolated microorganisms. The extract showed measurable inhibition zones on *Candida albicans*, while *C. parapsilosis and C. krusei* showed weak or no inhibition. The extract also showed a limited antibacterial effect against *Streptococcus mutans.*

The antimicrobial assay was performed in triplicate against *Candida albicans, Candida parapsilosis, Candida krusei*, and *Streptococcus mutans*. The average inhibition zones of *Capparis spinosa* extract compared with standard antifungal agents are summarized in Table 2.

C. albicans exhibites the largest inhibition zone, measuring 17 mm ± 1.2 mm when treated with 5 % caper plant extract. The inhibition patterns of *Candida spp.* are illustrated in Figure 2.

Table 2: Inhibition zones (mm ± SD) of Capparis spinosa aqueous extract compared with standard antifungal agents.

Isolates	Capparis Extract (5%)	Nystatin (100,000 U/mL)	Fluconazole
Candida albicans	17.0 ± 1.2 mm	13.0 ± 1.0 mm	12.0 ± 0.9 mm
Candida parapsilosis	14.0 ± 0.8 mm	11.0 ± 0.7 mm	10.0 ± 1.0 mm
Candida krusei	13.0 ± 1.1 mm	10.0 ± 0.9 mm	9.0 ± 1.0 mm
Streptococcus mutans	9.0 ± 0.6 mm	–	–

Values are expressed as mean ± standard deviation (n = 3)

DISCUSSION

The study showed that the 5% aqueous extract of *Capparis spinosa* exhibited strong antifungal activity against *Candida albicans* but weak or limited antibacterial activity against *Streptococcus mutans.*[3, 20 28] The inhibition zones recorded for *C. albicans* were the largest among all tested microorganisms. The extract appears to be more effective against fungal isolates than against bacterial species.[24,25,21]

The ability of *Streptococcus mutans* to form biofilms on tooth surfaces, together with its acidogenic and aciduric properties, is associated with enamel demineralization.[17,22] Due to its ability to withstand high acidity levels, this bacterium can survive in carious lesions.[11,17] *Candida albicans'* ability to produce hemolysin enables it to obtain iron from red blood cells

and to support infection.[7,20] These virulence factors can explain the microbial behaviour observed in this study.

The inhibition of *C. albicans* observed in this study is consistent with previous reports.[7,20,23] In earlier studies, the antimicrobial potential of *Capparis spinosa* was attributed to its phytochemicals, such as flavonoids, alkaloids, and glucosinolates, which disrupt microbial membranes and intracellular enzymes.[6,23,24,25,29] This study reported inhibition zones consistent with other studies, demonstrating the extract's effectiveness against *Candida species.*[3,20,23] The antimicrobial effect of *C. spinosa is* likely due to the bioactive compounds in the extract, which destabilize the cell walls of fungi and bacteria. These active compounds include phenolic compounds and flavonoids.[6,23,29] These compounds can inhibit essential metabolic pathways in microorganisms.[24,25] The much higher level of inhibition shown by the fungal isolates indicates that the extract interacts more readily with the fungal cell membrane than with bacterial cells.[24,25] Co-aggregation between *S. mutans* and *C. albicans* plays a significant role in mixed oral biofilms.[11,17] In this study, *C. albicans* showed greater inhibition than *S. mutans*, indicating that caper extract is more effective against fungal components of the biofilm community.[22] This may mean that the extract interacts more readily with fungal cell membranes than with bacterial cell membranes.[24,25,27]

Earlier studies showed that aqueous extracts of *Capparis spinosa* produced inhibition zones as large or

larger than those from other plant parts.[6,3,23] The current findings provide further evidence for the antimicrobial activity of aqueous preparations against important oral pathogens.[3,20,28]

Limitations: This study has several limitations. All experiments were done *in vitro*, which does not reflect the oral environment, including saliva, pH changes, and complex mixed-species biofilms.[26] Microbial identification also relied on phenotypic and microscopic characteristics that may lack taxonomic specificity. The study did not characterize the extract's chemical profile, so the precise concentrations of active metabolites are unknown.

Evaluation of *Capparis spinosa* extract under actual oral conditions should be conducted through a clinical trial to assess safety and efficacy.

It is recommended to use HPLC, GC–MS, or spectrophotometric assays to quantify the active compounds responsible for antimicrobial activity. Creating standardised medicine formulations or mixing the extract with other biocompatible materials can improve its efficiency as a pediatric oral medicine.

CONCLUSIONS

1. The aqueous extract of *Capparis spinosa* has shown vigorous antifungal activity against various *Candida species* isolated from children with dental caries.
2. The antibacterial activity against *Streptococcus mutans* was limited, suggesting the extract's potential as a natural antimicrobial agent for dental applications.
3. With further validation, *C. spinosa* may help improve children's oral health

Availability of data and materials

The datasets generated and analyzed during the current study are included within the article.

Mandatory Statements

Patient's/Guardian's Consent

We obtained verbal informed consent from parents and guardians of all children.

Ethical Clearance

This study did not involve any invasive investigations; it only used oral swabs, which have a very low risk, so approval from the Institutional Ethics Committee, University of Babylon, Iraq (UB-SCI-MICRO/2022/014, 15 December 2022) was waived.

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This study did not get money from governments, businesses, or not-for-profit groups.

Conflict of Interest: The authors declare no conflicts of interest.

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