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PHARMAKEFTIKI, 37, 2S, 2025 | 376-378 RESEARCH

https://doi.org/10.60988/p.v37i2S.234

Antifungal effects of silver nanoparticles produced by *Cinnamomum verum* bark extract against fungi isolated from toothbrushes

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KEY WORDS:
toothbrush; green synthesis; antifungal activity; silver nanoparticles; *Cinnamomum verum*

ARTICLE INFO:
Received: December 28, 2024
Revised: February 28, 2025
Accepted: March 01, 2025
Available online: October 10, 2025

ABSTRACT

The findings revealed that 50 toothbrush samples used by individuals aged 20–50 years were contaminated with fungi. The infection rate was higher among females (40%), and fungal contamination increased when toothbrushes were used for more than three months. The most frequently isolated fungal species were *Aspergillus niger* (45%) and *Penicillium citrinum* (18%), while *Fusarium oxysporum* was the least common (2%). Silver nanoparticles (Ag NPs) were synthesized *via* a green method using *Cinnamomum verum* bark extract. The Ag NPs inhibited the growth of *A. niger*, *A. flavus*, *A. terreus*, and *P. citrinum* by 44.44%, 75.11%, 84.83%, and 98.66%, respectively, at a concentration of 200 µg/mL. Complete fungal inhibition was observed at 500 µg/mL, with statistically significant differences (p<0.05) compared to the untreated control.

1. Introduction

The toothbrush remains the most effective tool for cleaning the surfaces of the teeth and tongue. It also serves as a conduit between the external environment and the body's digestive and respiratory tracts, thereby creating a favourable setting for microbial colonization[1]. Although findings remain limited and subject to debate, studies investigating the antibacterial activity of nanoparticles (NPs) have demonstrated their potential to mitigate harmful microbial effects[2].

Silver NPs (Ag NPs) exhibit notable antibacterial properties even at low concentrations[3]. In the present study, *Cinnamomum verum* bark

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extract was employed for the green synthesis of Ag NPs, owing to its broad biological potential. Antibacterial activity was evaluated using both the disc diffusion and broth microdilution methods, with results corroborated by fluorescence microscopy observations[4].

2. Methodology

This study was conducted on 50 toothbrush samples collected from students and staff at the University of Kufa (Iraq), aged between 20 and 50 years, representing both sexes. All samples were submitted to the mycology laboratory during the period of September to October 2024. Toothbrush heads were sectioned and transferred using sterile forceps onto culture dishes containing Sabouraud dextrose agar (SDA) for fungal cultivation. The inoculated plates were then incubated for three days[5].

3. Results and Discussion

Fifty toothbrush samples from individuals of varying ages and genders were processed in the mycology laboratory. The toothbrush heads were dissected and cultured on SDA, followed by a three-day incubation period[5]. Twelve SDA flasks were divided into four experimental groups, each treated with Ag NPs at varying concentrations. The distribution of fungal contamination by age revealed the lowest infection rate in the 20–30-year group (28%) and the highest in the 41–50-year group (40%). Females exhibited a higher infection rate (76%) compared to males (24%), with a 2:1 female-to-male ratio, particularly pronounced in the 41–50-year group. This trend may be attributed to age-related hormonal changes and diminished immune function.

Fungal and bacterial contamination was more prevalent in toothbrushes used for longer than three months. Accordingly, it is recommended that toothbrushes be replaced every three to four months so as to preserve their efficacy in dental plaque removal[6].

The most frequently isolated fungal species were *Aspergillus niger* (45%) and *Penicillium citrinum* (18%), while *Fusarium oxysporum* was the least common (2%). These findings are consistent with previous reports[7].

Transmission electron microscopy analysis of the synthesized Ag NPs revealed an average particle diameter of 18–20 nm, confirming monodispersity. The spherical morphology and minimal agglomeration of the particles suggest that *C. verum* bark extract was highly effective as both a reducing and stabilizing agent.

The Ag NPs synthesized from *C. verum* demonstrated *in vitro* antifungal activity against *A. niger*, *A. flavus*, *A. terreus*, and *P. citrinum*, with inhibition rates of 33.5%, 11.1%, 44.4%, and 66.7%, respectively, at a concentration of 100 μg/mL. Inhibition increased with higher concentrations, culminating in complete fungal growth suppression at 500 μg/mL, consistent with prior findings[8].

Studies have shown that Ag NPs synthesized from biological sources such as plants, algae, and bacteria possess antimicrobial, antioxidant, and anticancer properties. The formation of Ag NPs begins when the biological extract is mixed with silver nitrate, resulting in a colour change from light to dark brown, which confirms NP synthesis[7]. In green synthesis, the extract functions as both a reducing and stabilizing agent, with 60°C identified as the optimal reaction temperature. This method yields stable, spherical Ag NPs[9].

4. Conclusion

Fungal contamination was observed in toothbrushes used by both adult males and females, with a higher infection rate among women. The most prevalent fungal species were *Aspergillus niger* and *Penicillium citrinum*, while *Fusarium oxysporum* was the least frequently detected. The environmentally friendly synthesis of Ag NPs using *C. verum* bark extract proved effective.

Acknowledgements

None.

Conflicts of interest

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None exist.

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HOW TO CITE:
Sahib R.A., Obaid R.F., Ali H.A. Antifungal effects of silver nanoparticles produced by *Cinnamomum verum* bark extract against fungi isolated from toothbrushes. *Pharmakeftiki* 37(2s), 376-378, 2025. https://doi.org/10.60988/p.v37i2S.234