Alqalam Journal of Medical and Applied Sciences. 2026;9(9):2931-2935
https://doi.org/10.54361/ajmas.269939

Original article

Antifungal Efficacy of Pomegranate Peel Extract Against *Candida albicans* in Patients Wearing Removable Orthodontic Appliances in Tripoli, Libya

Aesha Alabyad[1], Fawzia Shawesh[2], Rema Hwas[3]*, Khaled Aburass[4]

[1]*Department of Dental Technology, Faculty of Medical Technology, Sabratha University, Libya.*
[2]*Department of Medical Laboratories, Faculty of Medical Technology, University of Zawia, Libya.*
[3]*Department of Orthodontics, Faculty of Dentistry, University of Zawia, Libya*
[4]*Libyan Center for Medical Research, Zawia,Libya.*
Email. *r.hawas@zu.edu.ly*

Abstract
Orthodontic treatment with removable appliances may increase *Candida albicans* colonization, a key opportunistic pathogen in the oral cavity. Herbal extracts, including pomegranate peel, exhibit promising antifungal properties and may offer natural therapeutic alternatives. This study aims to determine the association between removable orthodontic appliances and *Candida albicans* oral infections, and to evaluate the antifungal potential of pomegranate peel extract (PPE). This study was conducted in Tripoli, Libya (May 2023 – June 2025), enrolling 100 patients (50 orthodontic appliance users as the study group; 50 non-users as controls). Oral swabs were cultured on Sabouraud Dextrose Agar (SDA). Positive isolates were confirmed using the germ tube test and growth at 45°C. PPE was prepared by alcoholic extraction and tested via well-diffusion and microdilution methods. Antifungal susceptibility to fluconazole, nystatin, and miconazole (Daktarin) was assessed by disk diffusion. Culture positivity was 70% in the control group and 72% in the orthodontic group, with no statistically significant difference between groups (Chi-square test, p = 0.828). PPE inhibition zones ranged from 9 mm (12.5 mg/mL) to 22 mm (150 mg/mL); the MIC was 12.5 mg/mL. Fluconazole resistance was 84.5%, miconazole resistance 73.2%, and nystatin resistance only 12.7%. Removable orthodontic appliances may promote *C. albicans* colonization by altering the oral environment. PPE effectively inhibits yeast growth in vitro, suggesting potential as a complementary antifungal agent. High fluconazole resistance highlights the urgent need for alternative treatments in Libya.
Keywords. *Candida albicans*, Removable Orthodontic Appliances, Pomegranate Peel Extract, Oral Candidiasis.

Introduction

Candida species are ubiquitous opportunistic fungi that colonize the oral cavity, gut, and genital tract of over 70% of healthy individuals. Among them, *Candida albicans* is responsible for approximately 70% of fungal infections worldwide and is considered the most pathogenic member of the genus [1,2]. It is also the leading cause of healthcare-associated fungal infections, particularly threatening immunocompromised patients [3]. Oral candidiasis is one of the most prevalent fungal infections in the oral cavity, with colonization rates ranging from 20%–75% in the general population and up to 95% in HIV patients [4]. Predisposing factors include poor oral hygiene, use of dental prostheses and orthodontic appliances, xerostomia, antibiotic therapy, high-carbohydrate diets, smoking, and systemic immunosuppressive conditions [2, 5].

Orthodontic appliances —both fixed and removable — have been shown to alter the oral microenvironment, promoting microbial adhesion and biofilm formation. They increase plaque retention, modify salivary pH and flow, and cause frictional mucosal trauma that facilitates *Candida* overgrowth [5,6]. Removable appliances, made of acrylic materials that serve as effective substrates for yeast adhesion, pose a particularly underexplored risk in this regard [7].

The rise in antifungal resistance, especially to azole drugs such as fluconazole, has become a serious public health concern. This has intensified interest in plant-derived alternatives with documented antimicrobial activity [8]. Pomegranate (*Punica granatum* L.) peel is rich in bioactive polyphenols — including punicalagins, ellagic acid, gallic acid, and flavonoids — which exhibit potent antifungal properties through disruption of cell membranes, ergosterol reduction, and inhibition of biofilm formation [9,10].

In Libya, data on the epidemiology and antifungal susceptibility of oral *Candida* infections in orthodontic patients remain scarce. The present study was designed to address this gap by: (1) determining the prevalence of *C. albicans* oral infections in patients using removable orthodontic appliances, and (2) evaluating the in vitro antifungal efficacy of pomegranate peel extract against clinical isolates.

Methods

Study Design and Setting

A cross-sectional study was conducted from May 2023 to June 2025 at two dental centers in Tripoli, Libya: The Health Services Administration in Janzour and the Dolphin Dental Clinic for Dentistry and Oral Surgery. Ethical approval was obtained from the Libyan Academy, and all data were collected anonymously with confidentiality maintained.

Study Population and Sampling

A convenience sample of 100 participants was recruited and divided into two equal groups: (1) study group — 50 patients using removable orthodontic appliances; and (2) control group — 50 individuals not undergoing orthodontic treatment.

Received: *14-07-2026 - **Accepted:** 13-09-2026 - **Published:** 20-09-2026*

Participants included 28 males and 72 females, with ages ranging from 5 to 45 years. All participants were in generally good health. Exclusion criteria included age below 5 years.
Data were collected via a structured questionnaire covering demographics, oral hygiene practices, dietary habits, medical history, medication use, and clinical oral symptoms.

Specimen Collection and Microbiological Analysis
Sterile oral swabs (COPAN Diagnostics) were rotated on the tongue surface and around dental caries or appliance surfaces, then transported to the National Animal Health Center microbiology laboratory in Tripoli. Swabs were inoculated onto Sabouraud Dextrose Agar (SDA) supplemented with 2% chloramphenicol and incubated at 37°C for 48 hours. Presumptive *Candida* colonies (cream-colored, smooth, raised) were identified by Gram staining (Gram-positive budding yeast cells and pseudohyphae), the germ tube test (incubation in human serum at 37°C for 2.5–3 hours), and growth assessment at 45°C to differentiate *C. albicans* from *C. dubliniensis*.

Preparation of Pomegranate Peel Extract (PPE)
Fresh pomegranate fruits purchased from local markets in Tripoli were peeled, washed, and dried at room temperature (25°C). Peels were ground into powder. A stock solution was prepared by mixing 100 g of powder with 500 ml of 95% ethanol for 72 hours, followed by the extract mixture was filtered into small tubes and subjected to centrifugation at 300 rpm for 5 minutes.and rotary evaporation (50°C, 120 rpm, 4 hours). The extract was stored in a sterile dark container at 4°C. Working concentrations of 12.5, 25, 50, 100, and 150 mg/mL were prepared in 70% ethanol and filter-sterilized through 0.22 µm membranes.

Antifungal Activity Testing
Well Diffusion Method: Mueller-Hinton agar plates were inoculated with a 0.5 McFarland-adjusted *C. albicans* suspension. Wells of 6 mm diameter were bored, and 50 µl of each PPE concentration was added. Fluconazole (150 mg) served as the positive control; 70% ethanol as the negative control. Inhibition zone diameters (IZDs) were measured after 48 hours at 37°C.
Minimum Inhibitory Concentration (MIC): Serial two-fold dilutions (3.12–150 mg/mL) in SDA medium were dispensed in 96-well microtiter plates. Each well received 50 µl of yeast suspension (1.5×10^6 cells/ml). Plates were incubated at 37°C for 48 hours. The MIC was defined as the lowest concentration with no visible turbidity.
Antifungal Susceptibility Testing: The Kirby-Bauer disk diffusion method on Mueller-Hinton agar was performed following CLSI M44A guidelines, Pre-prepared paper discs containing different antifungals, namely fluconazole (150 mg), Daktarin (40 g), and Nystatin (100 U), were placed on the agar surface. The plates were further incubated at 37°C for 24 hours. Isolates were classified as susceptible, intermediate, or resistant based on manufacturer interpretive criteria.

Statistical Analysis
Data were analyzed using SPSS v25. Categorical variables were reported as frequencies and percentages; chi-square tests assessed within-group distributions. Independent-samples t-tests and one-way ANOVA compared the two groups on continuous variables (e.g., age). Chi-square tests (or Fisher's exact test where expected cell counts were <5) were used to compare categorical and binary variables between the control and study groups (e.g., culture positivity, oral fissures). Spearman's rank correlation assessed the association between gender distributions across the two groups. Statistical significance was set at $p < 0.05$.

Results

Demographic Characteristics
The study enrolled 100 participants (50 cases, 50 controls). In both groups, females predominated: 66.0% of controls and 78.0% of cases were female (chi-square, $p = 0.024$ and $p < 0.001$ respectively). Spearman's correlation confirmed a significant association between gender distributions across the two groups ($rs = 0.740$, $p < 0.001$), yet an independent-samples t-test revealed no significant between-group difference in gender ($p = 0.185$), confirming that the groups were balanced for sex.
The age distribution differed between groups. Cases were predominantly aged 17–28 years (68%), while controls were more evenly distributed across age ranges (5–10 years: 24%; >45 years: 18%). Despite this distributional difference, one-way ANOVA identified no statistically significant between-group difference in mean age ($F = 1.010$, $p = 0.317$), indicating adequate comparability.

Table 1. Demographic characteristics of study participants

Variable	Controls (n=50)	Cases (n=50)	p-value (between groups)
Female, n (%)	33 (66.0%)	39 (78.0%)	0.185
Male, n (%)	17 (34.0%)	11 (22.0%)	
Age 17–28 yrs, n (%)	7 (14.0%)	34 (68.0%)	0.317 (ANOVA)
Age >45 yrs, n (%)	9 (18.0%)	0 (0.0%)	

Received: 14-07-2026 - **Accepted:** 13-09-2026 - **Published:** 20-09-2026

Oral Hygiene and Appliance Maintenance Practices

All participants in both groups reported performing daily oral hygiene. Dental floss use was uncommon, with 84% of controls and 88% of cases reporting non-use (p = 0.569 between groups). Similarly, only 8% of participants in each group cleaned their appliance with a dedicated disinfectant (p = 1.000 between groups). These uniformly low hygiene-enhancement practices underscore an important modifiable risk factor in this population.

Table 2. oral hygiene and appliance maintenance practices

Appliance Maintenance Practices	Controls n (%)	Cases n (%)	p-value
Dental floss use	8 (16%)	6 (12%)	0.569
Appliance disinfection	4 (8%)	4 (8%)	1.000

Oral Symptoms and Clinical Risk Factors

White tongue coating was reported by approximately 58–60% of participants in both groups, with no significant between-group difference (p = 0.841). However, a statistically significant difference was observed for the presence of oral fissures (tongue and lip commissures): 66% of controls versus 46% of cases reported fissures (Chi-square test, p = 0.044).
Chronic disease prevalence was significantly higher in the case group (12% vs. 0%; p = 0.013). Vitamin deficiencies were also significantly more prevalent among cases (70%) compared to controls (44%; p = 0.008). Iron deficiency affected approximately 46–50% of participants in both groups without significant between-group difference (p = 0.693). Gingivitis, dry mouth, psychological stress, diet high in sugars, smoking, and unprescribed antibiotic use did not differ significantly between groups (all p > 0.05).

Table 3. Selected clinical risk factors by group

Risk Factor	Controls n (%)	Cases n (%)	p-value
White tongue coating	29 (58%)	30 (60%)	0.841
Oral fissures	33 (66%)	23 (46%)	0.044*
Chronic diseases	0 (0%)	6 (12%)	0.013*
Vitamin deficiency	22 (44%)	35 (70%)	0.008*
Iron deficiency	23 (46%)	25 (50%)	0.693
Gingivitis	31 (62%)	33 (66%)	0.861
High-sugar diet	34 (68%)	29 (58%)	0.305
Smoking	7 (14%)	3 (6%)	0.186
Psychological stress	35 (70%)	31 (62%)	0.404

** Statistically significant (p < 0.05)*

Prevalence of Candida albicans

Culture analysis revealed *C. albicans* in 70.0% of controls and 72.0% of cases (p = 0.828, between groups), confirming that both groups exhibited comparable baseline colonization rates. These findings validate the study design and suggest that appliance use alone does not confer a statistically distinct colonization advantage under the current study conditions.

Table 4. Candida albicans culture results

Result	Controls n (%)	Cases n (%)	p-value
Positive	35 (70.0%)	36 (72.0%)	0.828
Negative	15 (30.0%)	14 (28.0%)	

Antifungal Susceptibility of C. albicans Isolates

Disk-diffusion susceptibility testing of all *C. albicans* isolates against three standard antifungals revealed a high rate of resistance to fluconazole (84.5%) and miconazole/daktarin (73.2%). Nystatin retained the highest efficacy, with 87.3% of isolates classified as susceptible and a resistance rate of only 12.7%.

Table 5. Antifungal susceptibility profile of C. albicans isolates (n = 71)

Antifungal Agent	Resistant n (%)	Susceptible n (%)
Nystatin	9 (12.7%)	62 (87.3%)
Fluconazole	60 (84.5%)	11 (15.5%)
Miconazole (Daktarin)	52 (73.2%)	19 (26.8%)

Antifungal Activity of Pomegranate Peel Alcoholic Extract

The alcoholic extract of pomegranate peel demonstrated concentration-dependent antifungal activity against all *C. albicans* isolates tested. Inhibition zone diameters (IZD) ranged from 9 mm at 12.5 mg/mL to 22 mm at 150 mg/mL. The positive control (fluconazole 150 mg disc) yielded a mean IZD of 0.5 mm, reflecting the high resistance rate observed, while

Received: 14-07-2026 - **Accepted:** 13-09-2026 - **Published:** 20-09-2026

the negative control (70% ethanol) produced a mean IZD of 4.33 mm. All tested *C. albicans* isolates were sensitive to the pomegranate peel extract.

Table 6. Inhibition zone diameters of pomegranate peel alcoholic extract against C. albicans (well-diffusion method)

Concentration (mg/mL)	Mean IZD (mm)
150	22.00
100	19.33
50	14.50
25	13.90
12.5	9.00
Fluconazole 150 mg (control +)	0.50
70% Ethanol (control −)	4.33

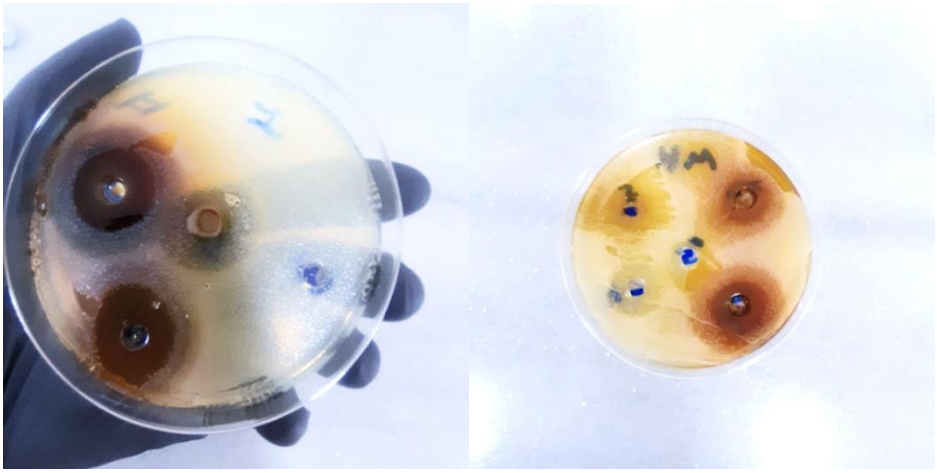

Figure 1. Inhibition zone diameters of Alcoholic extract

The MIC of the pomegranate peel extract was determined to be 12.5 mg/mL by broth microdilution, representing the lowest concentration that completely inhibited visible *C. albicans* growth.

Discussion

This study investigated the relationship between removable orthodontic appliances and oral *C. albicans* colonization, alongside the antifungal potential of pomegranate peel extract. The high overall prevalence of *C. albicans* positivity (70–72%) in both groups is consistent with published reports from the region and comparable populations [11, 12]. The relatively high baseline colonization in the control group may reflect the generally low dental floss usage (16%) and limited appliance disinfection practices observed in both groups — factors well established as risk factors for oral candidiasis [2].

While a marginal increase in colonization was observed in the orthodontic group (72% vs 70%), this difference was not statistically significant (Chi-square test, $p = 0.828$). This finding aligns with some earlier studies suggesting that the impact of removable appliances is more pronounced in the context of fixed appliances or with specific oral hygiene deficiencies [5, 7]. The high vitamin deficiency rate in the orthodontic group (70% vs 44%) is noteworthy, as nutritional deficiencies are recognized predisposing factors for candidiasis [2]. *C. albicans* was identified based on characteristic colony morphology, Gram stain appearance, positive germ tube formation, and growth at 45°C. These conventional methods remain reliable and cost-effective for routine identification and are consistent with established diagnostic protocols [13].

The in vitro antifungal activity of PPE was clearly demonstrated, with inhibition zones increasing proportionally with concentration and a MIC of 12.5 mg/mL. These results align with previous studies reporting significant anti-*Candida* activity of pomegranate extracts [4,9,14]. The antifungal mechanism is attributed primarily to punicalagins, ellagic acid, and gallic acid, which disrupt the fungal cell membrane, reduce ergosterol content, and precipitate cytoplasmic proteins [10]. The alarming fluconazole resistance rate of 84.5% observed in this study surpasses previously reported resistance levels in Libya and is consistent with the global trend of increasing azole resistance among *C. albicans* isolates [15]. This underscores the critical importance of continuous antifungal surveillance and the exploration of alternative therapeutic agents. Nystatin retained superior efficacy (87.3% sensitivity), affirming its continued value as a first-line topical antifungal in resource-limited settings.

This study has several limitations. It relied on self-reported questionnaire data for many risk factors, which may introduce reporting bias. The cross-sectional design limits causal inference. Molecular confirmation of *C. albicans* identity was not performed. Future studies should incorporate PCR-based species confirmation, quantitative oral yeast counts, longitudinal follow-up, and in vivo evaluation of PPE formulations.

Received: *14-07-2026 -* ***Accepted:*** *13-09-2026 -* ***Published:*** *20-09-2026*

Conclusion

This study found high rates of oral *C. albicans* colonization in both orthodontic and non-orthodontic patients in Tripoli, with no statistically significant difference between groups. Removable orthodontic appliances may nonetheless contribute to the conditions favoring *Candida* growth through changes in the oral microenvironment. Pomegranate peel extract demonstrated significant dose-dependent inhibitory activity against *C. albicans* in vitro, with a MIC of 12.5 mg/mL, representing a promising natural antifungal candidate. The high prevalence of fluconazole resistance (84.5%) in this cohort demands urgent attention from health authorities and highlights the need for alternative treatments and enhanced antifungal stewardship in Libya.

Clinical recommendations

1. Clinicians should provide routine oral hygiene counseling to all orthodontic patients and encourage regular monitoring for oral candidiasis symptoms.
2. Given the high fluconazole resistance rates, nystatin should be prioritized as the first-line topical antifungal for oral candidiasis management in this region.
3. National surveillance programs for antifungal resistance patterns in Libya should be established or expanded.

Conflict of interest

The authors declare no conflict of interest.

References

1. Wang H, Xu Z, Gao L, Hao B. A fungal phylogeny based on 82 complete genomes using the composition vector method. *BMC Evol Biol*. 2009;9:195.
2. Talapko J, Juzbašić M, Matijević T, Pustijanac E, Bekić S, Kotris I, Škrlec I. *Candida albicans*—the virulence factors and clinical manifestations of infection. *J Fungi (Basel)*. 2021;7(2):79.
3. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. *Sci Transl Med*. 2012;4(165):165rv13.
4. Bassiri-Jahromi S, Pourshafie MR, Mirabzade Ardakani E, Ehsani AH, Doostkam A, Katirae F, Mostafavi E. In vivo comparative evaluation of the pomegranate (*Punica granatum*) peel extract as an alternative agent to nystatin against oral candidiasis. *Iran J Med Sci*. 2018;43(3):296-304.
5. Freitas AO, Marquezan M, Nojima MdC, Alviano DS, Maia LC. The influence of orthodontic fixed appliances on the oral microbiota: a systematic review. *Dental Press J Orthod*. 2014;19(2):46-55.
6. Topaloglu-Ak A, Ertugrul F, Eden E, Ates M, Bulut H. Effect of orthodontic appliances on oral microbiota--6 month follow-up. *J Clin Pediatr Dent*. 2011;35(4):433-436.
7. Khanpayeh E, Jafari AA, Tabatabaei Z. Comparison of salivary *Candida* profile in patients with fixed and removable orthodontic appliances therapy. *Iran J Microbiol*. 2014;6(4):263-268.
8. Wise R, Hart T, Cars O, Streulens M, Helmuth R, Huovinen P, Sprenger M. Antimicrobial resistance. Is a major threat to public health. *BMJ*. 1998;317(7159):609-610.
9. Endo EH, Cortez DA, Ueda-Nakamura T, Nakamura CV, Dias Filho BP. Potent antifungal activity of extracts and pure compound isolated from pomegranate peels and synergism with fluconazole against *Candida albicans*. *Res Microbiol*. 2010;161(7):534-540.
10. Bar-Ya'akov I, Tian L, Amir R, Holland D. Primary metabolites, anthocyanins, and hydrolyzable tannins in the pomegranate fruit. *Front Plant Sci*. 2019;10:620.
11. Altayyar IA, Alsanosi AS, Osman NA. Prevalence of vaginal candidiasis among pregnant women attending different gynecological clinic at South Libya. *Eur J Exp Biol*. 2016;6(3):25-29.
12. Mustafa MHM, Abrantes PDS, Africa CWJ. *Candida* species carriage in diabetic patients in Misrata, Libya. *S Afr J Infect Dis*. 2017;32(4):ID832.
13. Mastammanavar D, Hunasgi S, Koneru A, Vanishree M, Surekha R, Vardendra M. Techniques used for isolation and identification of *Candida* from the oral cavity. *Ann Essences Dent*. 2014;6(1):44-48.
14. Anibal PC, Peixoto IT, Foglio MA, Höfling JF. Antifungal activity of the ethanolic extracts of *Punica granatum* L. and evaluation of the morphological and structural modifications of its compounds upon the cells of *Candida* spp. *Braz J Microbiol*. 2013;44(3):839-848.
15. Ellabib MS, Agaj M, Khalifa Z, Kavanagh K. Yeasts of the genus *Candida* are the dominant cause of onychomycosis in Libyan women but not men: results of a 2-year surveillance study. *Br J Dermatol*. 2002;146(6):1038-1041.

Received: *14-07-2026 -* ***Accepted:*** *13-09-2026 -* ***Published:*** *20-09-2026*