International Journal of Design & Nature and Ecodynamics
Vol. 20, No. 5, May, 2025, pp. 1177-1183
Journal homepage: http://iieta.org/journals/ijdne

Synergistic Antifungal Effects of Alcoholic Pomegranate Extract and Chlorhexidine Against Oral *Candida albicans*

Fadia Abd Almuhsin Al-khayat[1*], Eslah Shakir Rajab[2], Dhuha H. Almarsomy[3], Muhammad Shuaib[4]

[1] Department of Basic Sciences, College of Dentistry, University of Baghdad, Baghdad 10001, Iraq
[2] Department of Microbiology, College of Science, Al-Kharkh University of Science, Baghdad 10001, Iraq
[3] Department of Conservative and Aesthetic Dentistry, Ahl Al Bayet College of Dentistry, University of Ahl Al Bayet, Karbala 56001, Iraq
[4] Arid Zone Small Ruminants Research Institute, Kohat 26010, Pakistan

Corresponding Author Email: fadia.khayat@codental.uobaghdad.edu.iq

https://doi.org/10.18280/ijdne.200522

Received: 21 April 2025
Revised: 17 May 2025
Accepted: 23 May 2025
Available online: 31 May 2025

Keywords:
Candida albicans, chlorhexidine, pomegranate extract, antifungal effect, synergistic effect

ABSTRACT

Compared to synthetic chemicals, which often pose risks to human health, natural plant-based antimicrobials are gaining increasing attention due to their lower toxicity and biocompatibility. Among these, pomegranate extract has emerged as a promising plant-derived antimicrobial agent for controlling oral pathogens. The aim of this work was to study the effect of pomegranate alcoholic extract and analyze its synergistic effects with chlorhexidine on oral *Candida albicans* isolates from the mouths of diabetic patients. Ten isolates of *Candida albicans* were evaluated for the antifungal effect of pomegranate alcoholic extract at different concentrations (10, 25, 50, 75, and 100%), compared to chlorhexidine at 0.22%. The well diffusion and tube dilution methods were used to determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC), and to evaluate the synergistic effect of pomegranate alcoholic extract combined with chlorhexidine as an anti-*Candida* agent. The results indicate that the 100% concentration recorded the highest antifungal activity compared to the other tested concentrations, except for the 10% which did not record any effect. The minimum inhibitory concentration (MIC) and minimum fungicide concentration (MFC) were 12.5% and 25%, respectively, for the pomegranate alcoholic extract. There were also highly statistically significant differences (p<0.001) in the synergistic effect between chlorhexidine and the lowest lethal concentration of alcoholic pomegranate extract, where the inhibition zone was recorded at 2.8 cm compared to what was recorded by both chlorhexidine 2 cm and alcoholic pomegranate extract 1.7 cm. Effective treatment of oral *Candida albicans* could help prevent conditions such as oral candidiasis and other systemic infections, especially in people with compromised immunity or chronic diseases. This synergistic formula offers a natural alternative to traditional antifungals, with a reported 40% increase in effectiveness, reducing reliance on synthetic agents.

1. INTRODUCTION

Candida albicans, a dimorphic fungus, serves as a prominent pathogen in both immunocompetent and immunocompromised individuals, often leading to oral candidiasis. This opportunistic yeast is a significant contributor to mucosal infections, particularly in individuals with altered microbial flora or diminished immunity, such as diabetic patients [1]. Treatment of invasive fungal infections is challenging due to the limited availability of antifungals, their relative toxicity, drug interactions, and the challenges of drug resistance. These challenges have led to the need for alternative antimicrobial strategies, prompting research into natural products with synergistic properties [2].

Medicinal herbs have long been used worldwide to treat a variety of diseases. Their use in healing practices dates back to ancient times [3]. Pomegranate (*Punica granatum*) is known for its rich phytochemical composition, including ellagitannins, which disrupt cell wall integrity and membrane function, and flavonoids, which interfere with fungal metabolism and induce oxidative stress [4, 5]. The pomegranate extracts possess significant antifungal effects against various pathogens, including *C. albicans*, through mechanisms that may include disruption of cell membrane integrity and inhibition of biofilm formation [6].

Chlorhexidine (CHX), a broad-spectrum antiseptic, is widely employed in oral hygiene to manage microbial populations in the oral cavity [7]. Its effectiveness against yeast infections is well-documented, making it a standard adjunctive treatment for oral candidiasis. However, concerns regarding its cytotoxicity and the potential for resistance have prompted the exploration of complementary therapies that enhance efficacy while minimizing side effects.

Synergistic effects are defined as the phenomenon in which

two or more agents, when used together, produce a total effect greater than the sum of their individual effects. This can be described as a "trigger" effect, where the system integrates its functional components to produce a result that exceeds the contribution of each component alone. When combined with synthetic drugs, plant-derived compounds offer a promising therapeutic avenue by enhancing efficacy and reducing adverse effects. From the perspective of pharmacology of medicinal plants, bioactive compounds have been proven to act as a 'synergist' in drug combinations in increasing the absorption, bioavailability, and simultaneously decreasing the toxicity [8, 9].

Natural products have gained increasing attention in disease treatment due to the limited availability of new drug candidates [10], and both plant extracts and plant medicines as herbal therapeutics are well known in history for containing numerous bioactive compounds such as flavonoids, alkaloids, and terpenoids which have broad clinical activities including anti-inflammatory, antioxidant, and antimicrobial activities [11]. Improvement in treatment outcomes is possible through the action or metabolism of drugs using phytochemical constituents, as these compounds are capable of influencing drug metabolism and action. Some of the considered studies in this area demonstrated the inhibition effect of a few plant extracts on drug-metabolizing enzymes, which might increase the bioavailability of the drugs when used together [12]. On the other hand, some extracts may have other supplementary mechanisms of action which permit the use of less potent doses of the more potent synthetic drugs without losing their intended effect [13].

This study aims to investigate the inhibitory effects of pomegranate alcoholic extract at various concentrations on the growth of *Candida albicans*, the causative agent of oral candidiasis. Additionally, the study evaluates the synergistic effect of the pomegranate alcoholic extract, at its lowest lethal concentration, in combination with chlorhexidine. This will provide insights into alternative antifungal strategies and enhance the effectiveness of current treatments against Candida infections.

2. MATERIAL AND METHODS

2.1 Isolates of *Candida albicans*

The known isolates of *C. albicans* were sourced from the microbiology lab of the College of Dentistry, University of Baghdad. Out of the ten isolates, all were obtained from the mouths of diabetic patients who had been attending the hospital. Activation was carried out by growing on Sabouraud dextrose agar (SDA) (HIMEDIA, India) and was placed in an incubator at 37 degrees Celsius for a duration of one day [14].

The identification of *C. albicans* was through the colony morphology, microscopical examination with Gram stain, and Germ tube formation. To verify these results, biochemical identification was performed through the Vitek 2 test [15].

2.2 Preparation of plant material

After harvesting, the parts of the pomegranate fruit were dried and milled to increase surface area; 300 g was obtained for extraction that took place in the Biotechnology Research Center, Al-Nahrain University, through the cold maceration technique by submerging the ground plant material in ethanol. The plant material was put in a sterile glass container with two liters of 70% ethanol and placed in a ratio of 1:5 (1 part plant material to 5 parts ethanol) by weight/ volume. The container was tightly capped and stored in a dark, cool place at 25℃ for three days and was rocked or gently stirred several times, as this helps extract more of the beneficial compounds from the plant material. First, a gauze filter was applied, and afterwards, the solution was placed in a rotary evaporator (Heidolph, Germany) at 45℃ to finish extracting the dried extract. The extract was stored in a dark, dry container at 4℃ until called to use it. The yield of the extract was calculated with the following equation (%) yield of extract = [weight (g) of dried extract/weight (g) of dried plant sample] × 100 [16, 17].

2.3 Preparation of the stock solution and concentrations

A stock solution of plant extract was prepared by dissolving 10g of extract in 100 mL of sterile distilled water. Then, different concentrations were made: 10, 25, 50, 75, and 100%, each was prepared with sterile distilled water, and all were syringe filtered using 0.22 μm pore size filters [18].

2.4 Antifungal testing of pomegranate and chlorhexidine zone of inhibition test

The agar plate of Muller-Hinton (HIMEDIA, India) was inoculated by spreading a *C. albicans* inoculum of 0.5 McFarland (1.5×10^8 CFU/ mL) over the entire agar surface. Then, a hole with a diameter of 6 mm was punched aseptically with a sterile cork borer, and the extract solution at different concentration 10, 25, 50, 75, and 100% were introduced into the well, then plates were left at room temperature for 3 to 5 min to allow adsorption of excess moisture. after that, agar plates were incubated at 37℃ for 24 hr. The antifungal agent diffuses in the agar medium and inhibits the growth of the microbial strain tested [19].

2.5 Determination of minimal inhibitory concentration (MIC)

Using the broth dilution method, the extract was serially diluted, and eight tubes were obtained, each having a volume of 1 ml of Mueller-Hinton broth (MHB) (HIMEDIA, India). One of these tubes served as a control and contained only MHB with *C. albicans*. To the first tube, extract was added to obtain a concentration of 200 mg/ml. One milliliter of solution from the first tube was transferred into the second tube, and this process was repeated until the 8th tube. In the end, one ml was pipetted out from the eighth tube. This process made the concentration halves in the tubes, and the following concentrations were obtained: 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56 mg/mL. Each of the dilutions developed was supplemented with a standard inoculum (50 μL) of 0.5 McFarland (1.5×10^8 CFU/ mL) activated *C. albicans* suspension. These dilutions were kept for 24 hours at a temperature of 37℃. The concentration that exhibited no visible growth was recorded as MIC.

2.6 Determination of minimum fungicide concentration (MFC)

The MFC was evaluated from a negative tube (presented no visible growth after microplate incubation). For this, an aliquot of 10 μL from the corresponding tubes was inoculated on

Mueller-Hinton Agar (MHA) plates. After incubation at 37°C for 24 hr, the colony development was followed in each inoculation place. The MFC value was noted for the concentration that yielded no microbial colonies. The ratio MFC/MIC is used to classify antimicrobials; the ratio lower than 4 corresponds to bactericidal activity, and the ratio higher than 4 corresponds to bacteriostatic activity [20].

2.7 Evaluation of the synergistic effect of pomegranate with chlorhexidine

There is currently no standardized methodology for measuring synergistic interactions between plant and chemical antibacterial. Researchers have used various methods. In this study, we used the combination index to analyze potential synergistic effects between pomegranate alcoholic extract and chlorhexidine.

2.8 Statistical analysis

Statistical analysis will be conducted using IBM SPSS Statistics 24, while Microsoft Excel 10 will be used for figure preparation. Descriptive statistics, including mean, standard deviation (SD), standard error (SE), and mean difference, will be calculated to summarize the dataset. Inferential statistical tests will be performed to determine significant differences between groups. A one-way analysis of variance (ANOVA) will be used to compare group means, followed by an F-test to assess overall model significance. If ANOVA results indicate a significant difference ($p < 0.05$), a post hoc Least Significant Difference (LSD) test will be applied to identify specific group differences. The results will be interpreted based on the p-value, with $p < 0.05$ considered statistically significant. Tables and figures will be generated using Microsoft Excel 10 to illustrate key findings clearly [21].

3. RESULTS

3.1 Identification of *Candida albicans*

Colonies were examined and diagnosed in reference to their morphological characteristics on Sabouraud Dextrose Agar (SDA) as selective media, *C. albicans* appear as creamy, smooth, and pasty convex colonies, and the microscopic examination showed that *C. albican*s in gram stained smears, appears as gram positive budding yeast cells (Figure 1).

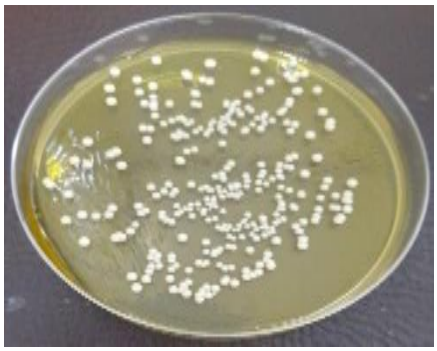

Figure 1. Colonies of *C. albicans* on SDA

The microscopic examination showed that *C. albican*s in Gram-stained smears appears as gram-positive budding yeast cells (Figure 2).

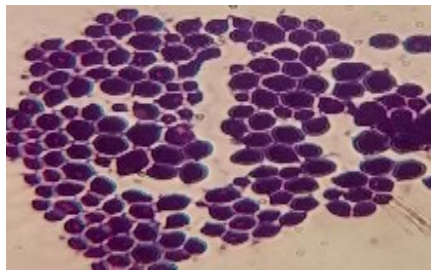

Figure 2. *C. albicans* under magnification power 1000×

For germ tube formation, the germ tube appears as an appendage that is half the width and three to four times the length of the yeast cell from which it arises (Figure 3).

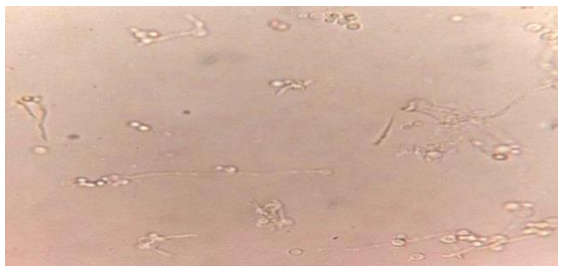

Figure 3. *Candida albicans* germ tube magnification power 400×

Vitek 2 system, which showed the presence of *C. albicans* with 98% excellent identification with bionumber 6542546075325771.

3.2 Yield of alcoholic pomegranate extraction

The percentage yield of ethanolic extract of 300g dried pomegranate was 76.56g (25.522% w/w).

3.3 Antifungal sensitivity for alcoholic pomegranate extract and chlorhexidine

3.3.1 Zone of Inhibition test

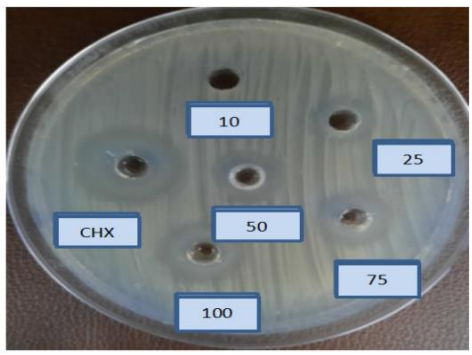

Figure 4. Zone of inhibition of the extract against *C. albicans* of alcoholic pomegranate extract in addition to CHX 0.22%

The inhibition zone method is used to evaluate the effectiveness of antimicrobial agents against specific microorganisms by measuring the diameter of the clear zone around wells in an agar plate inoculated with the microorganism. A larger zone indicates greater susceptibility of the microorganism to the antibiotic, while a smaller or

absent zone indicates resistance. The results showed the highest effect of the alcoholic extract of pomegranate at a concentration of 100%, with a mean of 1.75, while each of 25,50, and 75% showed a lesser effect. No effect was recorded at the concentration of 10%, comparable to the effect of chlorhexidine, which recorded a mean of 2.11 (Figure 4 and Table 1).

Table 1. Descriptive of groups

Statistic	10%	25%	50%	75%	100%	Chlorhexidin
Mean	N.I	1.29	1.48	1.53	1.75	2.11
SD	N.I	0.073786	0.091894	0.105935	0.108012	0.07378648

3.4 Determination of minimal inhibitory concentration (MIC)

The bioactive compounds from alcoholic pomegranate extract were used to determine their MICs against the *C. albicans* isolates. The concentrations of purified bioactive compounds were used from 200, 100, 50, 25, 12.5, 6.25, 3.12, and 1.56% of Mueller-Hinton broth. Figure 5 summarizes these results. *C. albicans* showed inhibition of growth with an MIC of 12.5%.

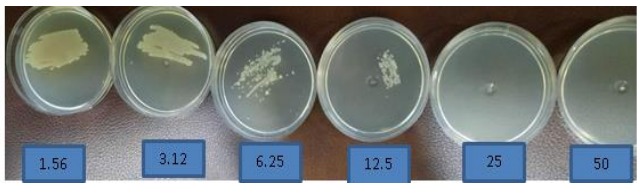

Figure 5. MIC and MFC for alcoholic pomegranate extract against *C. albicans*

3.5 Determination of minimum fungicide concentration (MFC)

Using the procedure described above to determine the MFC, the results indicate that the concentration of alcoholic pomegranate extract was 25% (Figure 5).

3.6 Synergistic effect of alcoholic pomegranate with chlorhexidine

The results indicate that the combination of chlorhexidine 0.22% and alcoholic pomegranate extract at the MFC 25% produced a larger zone of inhibition compared to each agent alone, suggesting a synergistic effect between the two. Increasing the diameter of the inhibition zone from 1.7 cm (pomegranate extract) and 2.0 cm chlorhexidine to 2.8 cm with the combination treatment demonstrates enhanced antifungal activity, potentially due to the synergistic interaction between the two substances (Figure 6). Highly significant differences were recorded at $p<0.001$ (Table 2 and Table 3).

To calculate the percentage increase in effectiveness due to the synergistic effect between chlorhexidine and pomegranate extract, the following equation can be used [19]:

Increase in effectiveness (%) = (Synergistic inhibition zone - max Individual inhibition zone) /max Individual inhibition zone ×100

$$((2.8-2)/2)\times 100=(0.8/2)\times 100=0.4\times 100=40\%$$

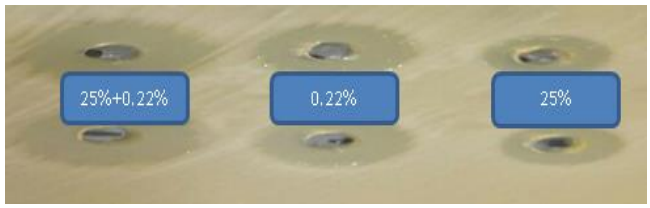

Figure 6. Comparison of the individual and synergistic effects of pomegranate alcoholic extract and chlorhexidine

Table 2. Descriptive of groups

	N	Mean	SD	SE	ANOVA F-test	P-value
25% Pomegranate	10	1.50	0.0816	0.0258	446.538	$p<0.001$ HS
CHX 0.22%	10	2.00	0.1054	0.0333		
Pomegranate Extract + CHX	10	2.80	0.1054	0.0333		

Table 3. Least significant difference

G1	G2	MD	SD	Sig.
25% Pomegranate	CHX 0.22%	-.50000	0.04389	0.000
	Pomegranate Extract + Chlorhexidine	-1.30000	0.04389	0.000
CHX 0.22%	Pomegranate Extract + Chlorhexidine	-.80000	0.04389	0.000

4. DISCUSSION

The results of this study, which indicate that pomegranate alcoholic extract has an effect on the growth of *Candida albicans* at high concentrations, while the absence of an inhibition zone observed with 10% pomegranate extract may be attributed to insufficient concentrations of antifungal compounds or the extract might not diffuse well through the agar medium [22], these results show some promise in treating fungal infections. This may stem from the fact that the phytochemicals in pomegranate, which include antioxidants and anti-inflammatory compounds, may help inhibit the growth of Candida, further enhancing its use as a natural antifungal.

The results are consistent with what Endo et al. [23] reported about the potential to inhibit the growth of fungi such as Candida, as pomegranate contains bioactive compounds ranging from ellagitannins to flavonoids. On the other hand, Malkawi et al. [24] reported that the antioxidant nature of pomegranate may be important for modulating the immune response and enhancing the body's defense mechanisms against fungal infections.

The results also indicated that the synergistic effect of chlorhexidine and pomegranate alcoholic extract in inhibiting the growth of *Candida albicans* was greater than that of either extract alone. Chlorhexidine, a well-known antiseptic, exhibits antimicrobial activity, inhibiting the growth of a variety of microorganisms, including *Candida albicans* [25]. Additionally, pomegranate extract, which contains polyphenols, exhibits antifungal activity that complements the effect of chlorhexidine [26].

The combined effect of these agents results in greater inhibition of *Candida albicans* growth than the sum of their individual effects. This is particularly important in clinical cases where *Candida albicans* infections do not respond to treatment. This is because combining chlorhexidine with pomegranate extract improves membrane permeability and increases fungal cell lysis, enhancing treatment efficacy [27]. Furthermore, the antioxidant effect of pomegranate, particularly its ability to control Candida biofilm formation, can enhance overall antifungal activity when used in combination with chlorhexidine [28]. Thus, this synergistic strategy not only highlights the role of herbal adjuvants in modern antifungal therapy but also calls for further research aimed at improving treatment strategies targeting *Candida albicans* infection.

The antimicrobial activity of pomegranate extract is primarily attributed to its high content of polyphenolic compounds, such as ellagic acid, tannins, and flavonoids. These compounds are believed to disrupt the cell wall and membrane of *Candida albicans*, leading to its death. Pomegranate also possesses antioxidant properties, which may help inhibit Candida growth by neutralizing reactive oxygen species and limiting its ability to proliferate [22].

Chlorhexidine works by disrupting the integrity of the microbial cell membrane. Chlorhexidine binds to negatively charged components of the cell membrane, leading to the leakage of intracellular components and, ultimately, cell death. Chlorhexidine is particularly effective against oral pathogens due to its ability to remain active in the oral cavity for extended periods [29, 30].

4.1 Synergistic antioxidant effects

Combining the antioxidants found in pomegranates with the antimicrobial properties of chlorhexidine may produce a synergistic effect, enhancing protection against oxidative stress and microbial infection.

In 2020, Bescos et al. [31] addressed the clinical implications. Therefore, combining pomegranate extract with chlorhexidine could be a promising alternative or adjunctive treatment for oral *Candida albicans* infections, particularly in patients with oral candidiasis or at risk due to conditions such as diabetes or immunocompromised individuals. Pomegranate extract could also provide a less toxic alternative to synthetic antifungals. Furthermore, the synergistic effect of these two agents could allow for the use of lower concentrations of each, potentially reducing common side effects associated with higher doses of chlorhexidine, such as mucosal irritation or taste disturbance.

The results of this study are consistent with what was suggested by Fernandes et al. [32], in that the combination of pomegranate and chlorhexidine to combat Candida fungi may be a good approach, as the side effects of prolonged use of chlorhexidine can be mitigated by the synergistic effect of pomegranate.

The synergistic antimicrobial effect of pomegranate extract with chlorhexidine may result from complementary mechanisms, including enhanced membrane disruption, improved biofilm penetration, multi-target cellular damage, and reduced potential for resistance development. These effects are attributed to the action of polyphenolic compounds like punicalagin and ellagic acid in combination with the cationic activity of chlorhexidine [33-35]. The observed synergistic effect between chlorhexidine and pomegranate extract, as evidenced by the increased zone of inhibition, highlights the potential benefits of combining natural and synthetic antimicrobials. However, the reliability of these results can be influenced by several experimental factors, including diffusion variability, agar composition, and incubation conditions. Additionally, a limited sample size reduces the statistical power of the findings and increases the risk of random error. To strengthen the validity of such results, future studies should employ standardized methodologies, larger sample sizes, and appropriate statistical analyses. These measures will ensure more accurate interpretation of synergistic interactions and support their potential application in clinical or pharmaceutical settings [36].

This study helps to demonstrate the importance of combining natural extracts with traditional therapies as modern approaches in the treatment of fungal infections of the oral cavity.

5. CONCLUSIONS

The results indicate that the alcoholic extract of pomegranate exhibits potent antifungal activity against oral candida, suggesting its potential as a natural antifungal agent. Furthermore, the synergistic interaction between the alcoholic extract of pomegranate and chlorhexidine demonstrated antifungal activity against oral candida, exceeding the effects of either agent alone, with a 40% increase in effectiveness.

This study demonstrates potential for developing more effective therapeutic strategies against oral candida infections, especially with increasing resistance to conventional antifungal treatments. Further research could shed light on the mechanisms underlying this synergy and explore the potential for combining these agents into therapeutic formulations for practical applications.

ACKNOWLEDGMENT

The authors thank the College of Dentistry-University of Baghdad, College of Science - Al-Kharkh University of Science, Ahl Al Bayet College of Dentistry, University of Ahl Al Bayet, Iraq, and Arid Zone Small Ruminants Research Institute, Ghulam Banda, Kohat, Pakistan, for their help in completing this work.

REFERENCES

[1] Talapko, J., Juzbašić, M., Matijević, T., Pustijanac, E., Bekić, S., Kotris, I., Škrlec, I. (2021). Candida albicans—The virulence factors and clinical manifestations of infection. Journal of Fungi, 7(2): 79. https://doi.org/10.3390/jof7020079

[2] Busquets, M.A., Fernández-Pradas, J.M., Serra, P., Estelrich, J. (2020). Superparamagnetic nanoparticles with efficient near-infrared photothermal effect at the second biological window. Molecules, 25(22): 5315. https://doi.org/10.3390/molecules25225315

[3] Pathak, A.K. (2012). Anti-Candida activity of aqueous extracts of some herbals. Indian Journal of Fundamental and Applied Life Sciences, 2(2): 1-6.

[4] Lavaee, F., Motaghi, D., Jassbi, A.R., Jafarian, H., Ghasemi, F., Badiee, P. (2018). Antifungal effect of the

bark and root extracts of Punica granatum on oral Candida isolates. Current Medical Mycology, 4(4): 20-24. https://doi.org/10.18502/cmm.4.4.382

[5] Bakkiyaraj, D., Nandhini J.R., Malathy, B., Pandian, S.K. (2013). The anti-biofilm potential of pomegranate (Punica granatum L.) extract against human bacterial and fungal pathogens. Biofoulin, 29(8): 929-937. https://doi.org/10.1080/08927014.2013.820825

[6] Celiksoy, V., Moses, R.L., Sloan, A.J., Moseley, R., Heard, C.M. (2022). Synergistic activity of pomegranate rind extract and Zn (II) against *Candida albicans* under planktonic and biofilm conditions, and a mechanistic insight based upon intracellular ROS induction. Scientific Reports, 12: 19560. https://doi.org/10.1038/s41598-022-21995-7

[7] Vitkov, L., Hermann, A., Krautgartner, W.D., Herrmann, M., Fuchs, K., Klappacher, M., Hannig, M. (2005). Chlorhexidine-induced ultrastructural alterations in oral biofilm. Microscopy Research and Technique, 68(2): 85-89. https://doi.org/10.1002/jemt.20238

[8] Mundy, L., Pendry, B., Rahman, M. (2016). Antimicrobial resistance and synergy in herbal medicine. Journal of Herbal Medicine, 6(2): 53-58. https://doi.org/10.1016/j.hermed.2016.03.001

[9] Rossato, L., Camargo dos Santos, M., Vitale, R.G., de Hoog, S., Ishida, K. (2021). Alternative treatment of fungal infections: Synergy with non-antifungal agents. Mycoses, 64(3): 232-244. https://doi.org/10.1111/myc.13203

[10] Newman, D.J., Cragg, G.M. (2016). Natural products as sources of new drugs from 1981 to 2014. Journal of Natural Products, 79(3): 629-661. https://doi.org/10.1021/acs.jnatprod.5b01055

[11] Afzal, I., Habiba, U., Yasmeen, H. (2023). Review on therapeutic potential of phytochemicals from medicinal plants. Journal of Bioresource Management, 10(4): 62-75.

[12] Thiengsusuk, A., Boonprasert, K., Na-Bangchang, K. (2020). A systematic review of drug metabolism studies of plants with anticancer properties: Approaches applied and limitations. European Journal of Drug Metabolism and Pharmacokinetics, 45: 173-225. https://doi.org/10.1007/s13318-019-00582-8

[13] Bonincontro, G., Scuderi, S.A., Marino, A., Simonetti, G. (2023). Synergistic effect of plant compounds in combination with conventional antimicrobials against biofilm of *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Candida* spp. Pharmaceuticals, 16(11): 1531. https://doi.org/10.3390/ph16111531

[14] Mallick, D.C., Kaushik, N., Goyal, L., Mallick, L., Singh, P. (2025). A comprehensive review of candidemia and invasive Candidiasis in adults: Focus on the emerging multidrug-resistant fungus *Candida auris*. Diseases, 13(4): 93. https://doi.org/10.3390/diseases13040093

[15] Lass-Flörl, C., Kanj, S.S., Govender, N.P., Thompson III, G.R., Ostrosky-Zeichner, L., Govrins, M.A. (2024). Invasive candidiasis. Nature Reviews Disease Primers, 10(1): 20. https://doi.org/10.1038/s41572-024-00503-3

[16] Sultana, B., Anwar, F., Ashraf, M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules, 14(6): 2167-2180. https://doi.org/10.3390/molecules14062167

[17] Shafiq, N.E., Mahdee, A.F., Mohammed Hasan, Z.Y. (2024). Leaf extracts of Moringa oleifera cultivated in Baghdad: Characterization and antimicrobial potential against endodontic pathogens. The Scientific World Journal, 2024(1): 6658164. https://doi.org/10.1155/2024/6658164

[18] Phuyal, N., Jha, P. K., Raturi, P.P., Rajbhandary, S. (2020). Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of Zanthoxylum armatum DC. The Scientific World Journal, 2020(1): 8780704. https://doi.org/10.1155/2020/8780704

[19] Balouiri, M., Sadiki, M., Ibnsouda, S.K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, 6(2): 71-79. https://doi.org/10.1016/j.jpha.2015.11.005

[20] De Castro, R.D., de Souza, T.M.P.A., Bezerra, L.M.D., Ferreira, G.L.S., de Brito Costa, E.M.M., Cavalcanti, A.L. (2015). Antifungal activity and mode of action of thymol and its synergism with nystatin against Candida species involved with infections in the oral cavity: An in vitro study. BMC Complementary and Alternative Medicine, 15: 417. https://doi.org/10.1186/s12906-015-0947-2

[21] Taylor, T.R. (1967). Principles of Medical Computing. Oxford: Blackwell Scientific Publications.

[22] Al-Zoreky, N.S. (2009). Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peels. International Journal of Food Microbiology, 134(3): 244-248. https://doi.org/10.1016/j.ijfoodmicro.2009.07.002

[23] Endo, E.H., Cortez, D.A.G., Ueda-Nakamura, T., Nakamura, C.V., Dias Filho, B.P. (2010). Potent antifungal activity of extracts and pure compound isolated from pomegranate peels and synergism with fluconazole against Candida albicans. Research in Microbiology, 161(7): 534-540. https://doi.org/10.1016/j.resmic.2010.05.002

[24] Malkawi, R., Jarwan, B., Waleed, R., Younis, R. (2024). Pharmaceutical prospects of pomegranate antioxidants in combating microbial infections. International Journal of Food Properties, 27(1): 768-782. https://doi.org/10.1080/10942912.2024.2360985

[25] Brookes, Z.L., Belfield, L.A., Ashworth, A., Casas-Agustench, P., Raja, M., Pollard, A.J., Bescos, R. (2021). Effects of chlorhexidine mouthwash on the oral microbiome. Journal of Dentistry, 113: 103768. https://doi.org/10.1016/j.jdent.2021.103768

[26] Derakhshan, Z., Ferrante, M., Tadi, M., Ansari, F., Heydari, A., Hosseini, M.S., Conti, G.O., Sadrabad, E.K. (2018). Antioxidant activity and total phenolic content of ethanolic extract of pomegranate peels, juice and seeds. Food and Chemical Toxicology, 114: 108-111. https://doi.org/10.1016/j.fct.2018.02.023

[27] Tavazo Zadeh, E., Aref, P., Askarizadeh, N., Emadi, F. (2023). In vitro antimicrobial effect of punica granatum extract versus Chlorhexidine on Streptococcus sobrinus, Streptococcus sanguinis, and Candida albicans. Journal of Research in Dental and Maxillofacial Sciences, 8(1): 18-27.

[28] Dahham, S.S., Ali, M.N., Tabassum, H., Khan, M. (2010). Studies on antibacterial and antifungal activity of pomegranate (*Punica granatum* L.). American-Eurasian Journal of Agricultural & Environmental Sciences, 9(3): 273-281.

[29] Wiegand, I., Hilpert, K., Hancock, R.E. (2008). Agar and broth dilution methods to determine the minimal

inhibitory concentration (MIC) of antimicrobial substances. Nature Protocols, 3(2): 163-175. https://doi.org/10.1038/nprot.2007.521

[30] Barasch, A., Safford, M.M., Dapkute-Marcus, I., Fine, D.H. (2004). Efficacy of chlorhexidine gluconate rinse for treatment and prevention of oral candidiasis in HIV-infected children: A pilot study. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 97(2): 204-207. https://doi.org/10.1016/j.tripleo.2003.09.005

[31] Bescos, R., Ashworth, A., Cutler, C., Brookes, Z.L., et al. (2020). Effects of chlorhexidine mouthwash on the oral microbiome. Scientific Reports, 10(1): 5254. https://doi.org/10.1038/s41598-020-61912-4

[32] Fernandes, G.L., Vieira, A.P.M., Danelon, M., Emerenciano, N.G., et al. (2022). Pomegranate extract potentiates the Anti-Demineralizing, Anti-biofilm, and anti-inflammatory actions of non-alcoholic Mouthwash when Associated with Sodium-Fluoride Trimetaphosphate. Antibiotics, 11(11): 1477. https://doi.org/10.3390/antibiotics11111477

[33] Bassiri-Jahromi, S., Katiraee, F., Hajimahmoodi, M., Mostafavi, E., Talebi, M., Pourshafie, M.R. (2015). *In vitro* antifungal activity of various Persian cultivars of *Punica granatum* L. extracts against *Candida* species. Jundishapur Journal of Natural Pharmaceutical Products, 10(3): e19754. https://doi.org/10.17795/jjnpp-19754

[34] Braga, L.C., Leite, A.A., Xavier, K.G., Takahashi, J.A., Bemquerer, M.P., Chartone-Souza, E., Nascimento, A.M. (2005). Synergic interaction between pomegranate extract and antibiotics against Staphylococcus aureus. Canadian Journal of Microbiology, 51(7): 541-547. https://doi.org/10.1139/w05-022

[35] Gallas, J.A., Pelozo, L.L., Corona, S.A.M., Shen, Y., Haapasalo, M., Sousa-Neto, M.D., Souza-Gabriel, A.E. (2024). Effect of pomegranate solution alone or combined with chlorhexidine against oral multispecies biofilm. International Endodontic Journal, 57(12): 1819-1828. https://doi.org/10.1111/iej.14135

[36] Maphetu, N., Unuofin, J.O., Masuku, N.P., Olisah, C., Lebelo, S.L. (2022). Medicinal uses, pharmacological activities, phytochemistry, and the molecular mechanisms of *Punica granatum* L. (pomegranate) plant extracts: A review. Biomedicine & Pharmacotherapy, 153: 113256. https://doi.org/10.1016/j.biopha.2022.113256

NOMENCLATURE

℃	Degrees Celsius
CFU	Cell forming unit
CHX	Chlorhexidin
Cm	Centimeter
g	Gram
hr	Hour
MD	Mean Difference
mg	milligram
MHA	Mueller-Hinton Agar
MHB	Mueller-Hinton broth
MIC	Minimum Inhibitory Concentration
MFC	Minimum Fungicide Concentration
ml	Milliliter
NI	No Inhibition zone
SDA	Sabouraud Dextrose Agar
SD	Stander deviation
Sig	Significant
SE	Standard Error
w/w	Weight/Weight

Greek symbols

µL	Microliter