Original Article

Evaluation of antifungal efficacy of albedo extract of *Punica granatum* on *Candida albicans* – An *in vitro* study

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Abstract

Introduction: The study aims to investigate the antifungal efficacy of albedo extract of Punica grantum on candida albicans by evaluating the inhibitory capacity of alcoholic albedo extracts by disc diffusion method and by comparing the antifungal efficacy of alcoholic extract of albedo with clotrimazole and ethanol.

Methods: Using a conventional disc diffusion method, the effectiveness of Punica Granatum albedo extract against Candida albicans was assessed and evaluated depending on the presence or absence of inhibition zones, as well as the average diameter of inhibition zones. Albedo extract of Punica granatum serial dilutions were prepared ranging from 1%, 5%, 10%, 15%, 20% and 25% and its antifungal efficacy was tested against Candida albicans in comparison with clotrimazole and ethanol.

Results: When compared to clotrimazole, the albedo extract of punica granatum showed significant anticandidal activity. The mean zone of inhibition of extract was recorded at 27.6 mm whereas clotrimazole was 21.6 mm and no zone of inhibition was recorded for ethanol. Statistically significant p value 0.015 was recorded within the different dilutions of albedo extract of Punica grantum which is less than 0.05.

Conclusion: The present investigation found that Punica Granatum albedo extract had greater potent antifungal activity when compared to clotrimazole and ethanol.

Keywords: Antifungal Efficacy, *Candida albicans*, *Punica Granatum*

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INTRODUCTION

Oral cavity harbours wide diversities of microbial organisms, constituting the second-largest habitat of the cell densities of microbes in humans after gut.[1] Oral microbial flora contains many bacteria, protozoa, fungi and viruses. Fungi are ubiquitous, and they colonise various surfaces of skin and oral mucous membranes. Oral Candida infections are regarded as opportunistic, earning it the epithet "disease of the diseased."[2]

Oral candidiasis is the most common fungal infection affecting the oral cavity caused by *Candida albicans*. *C. albicans*

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is an asexual, thermal fungus which is dimorphic. About 70% of candidiasis is caused by *C. albicans*.[3,4] *C. albicans* apart from causing candidiasis also has a contributing role in the pathogenesis of various diseases in the oral cavity like dental caries, periodontitis and oral cancer. *C. albicans* sometimes enter into the bloodstream and cause candidemia which is a lethal condition in immunocompromised individuals.[5]

Many drugs have been developed to target this fungus, among which fluconazole and amphotericin B are generally used.[6] Traditional herbal remedies made from natural plant materials are safe and efficient medicinal agents. They serve as the main source of treatments for the illnesses. Due to its strong astringency property, pomegranate (*Punica granatum*) has been extensively used in traditional medicine.[7] To date, many studies have been conducted evaluating the efficacy of barks, roots, juice, oil and peel extract of *P. granatum* against fungal organisms.[8-14] The literature study revealed that there has been no research on the effectiveness of *P. granatum* albedo extract against candidal organisms.

MATERIALS AND METHODS

Sixty patients were selected and saliva sample was collected from them in GSL Dental College, Rajahmundry, after obtaining written informed consent. Patients who were using antifungal medication either topically or systemically were excluded. Ethical committee clearance has been approved by the Gsl Dental College, institutional ethics committee.

Methodology

Fungicidal activity was assessed by the agar disc diffusion method by measuring zone of inhibition method.

Preparation of crude extract

Fresh samples of mesocarp (fleshy middle layer) of pomegranate fruit were collected. The samples were separated and shade-dried in a low light at room temperature for 7 days. One hundred grams of the sample were placed and weighed in Soxhlet apparatus for 8 hours at a temperature of 60 degrees centigrade with 99.9% ethanol of 200 ml, periodically agitated, and the residue collected was subjected to rotary vacuum evaporator at 40- 60°C and this was repeated for 3-4 times to obtain the *P. granatum* extract.

Preparation of sabouraud's dextrose agar (SDA) and sabouraud's dextrose broth medium

According to manufacturer instructions, 6.5 gms of agar medium was added to 100 ml of distilled water, 3 gms of broth medium was added to 100 ml of distilled water respectively, and both were sterilised in autoclave for 15 minutes at 15 lbs pressure. After the sterilisation process, SDA is poured into a sterile petri dish of 2 mm thickness, placed in the laminator at a temperature of 37°C for 1-2 hours and allowed to solidify. The broth was poured into sterile test tubes of 2 mm thickness.

Collection of the sample

A sterilised wooden toothpick was used to collect supragingival dental plaque samples from the tooth surfaces. It was placed in a sterile container containing the 0.2-millilitre saline solution right after the sample was taken.

Isolation of *Candida albicans*

The specimen collected in the sterile container was inoculated onto the SDA plate using a preheated inoculating loop. The sample-streaked agar plate has been incubated at 37 degrees centigrade for 24-48 hours. After incubation, the specimen was transferred to the test tubes containing Sabouraud's dextrose broth for the preparation of culture inoculum and was placed in the incubator for 4-5 hours. After the incubation period, the broth is spread uniformly over the SDA plate.

Disc diffusion for crude extracts

Sterile Whatman paper no. 1 filter paper was used to prepare discs with a diameter of 5.0 mm. Ten microlitres of the extract dilutions and ethanol were incorporated into the Whatman filter paper. Crude extracts at serial dilutions were incorporated into the sterile filter paper discs and placed on the SDA plate inoculated with culture inoculum of Sabouraud's dextrose broth. Serial dilutions of 1%, 5%, 10%, 15%, 20% and 25% were tested. These inoculated plates were incubated for 24-48 hours at 37-degree centigrade; zones of inhibition around the discs indicated the presence of anticandidal activity. Mean diameter of zones of inhibition was measured.

Disc diffusion for clotrimazole

Standard antifungal drug clotrimazole was taken and stored in the refrigerator. Discs impregnated with standard drug of clotrimazole were placed in petri dish, as positive control or reference standard drug for comparing the sensitivity of tested fungi of *C. albicans*, with the tested active compound of *P. granatum*.

Measurement of fungicidal activity

The fungicidal activity was determined by comparing the mean diameter of inhibition zones at each concentration to the mean diameter of inhibition zones of standard

antifungal drugs (using an antibiotic zone scale). The inhibitory concentrations in diffusion (ICD) approach was used to determine the minimum inhibition concentrations (MIC) (Guerin-Faublee *et al*., 1996).[15] The MIC value for that strain was determined by noting the lowest concentration that inhibited growth.

Statistical analysis

The Excel data obtained was uploaded into a computer database for statistical analysis, and SPSS software was used for analysis. To assess inhibitory activity between the three groups, a one-way analysis of variance (ANOVA) and two-pair T-test were used, with a *P*-value lower than 0.05 considered statistically significant.

RESULTS

The present study includes three groups:

Group 1 – Crude ethanolic albedo extract of *P. granatum*.

Group 2 – A standard antifungal drug, clotrimazole

Group 3 – Ethanol

The antifungal activity of *P. granatum* albedo extract was measured by preparing stock solutions of extract in ethanol followed by the preparation of serial dilutions ranging from 1%, 5%, 10%, 15%, 20% and 25% which were tested against *C. albicans*. There was a steady decrease in the diameter of zone of inhibition with increasing dilutions.

Zone of inhibition

Graph representing mean of diameter of inhibition zones and its comparison among groups was recorded in Graph 1. It can be inferred from the data below that the mean zone of inhibition was greater for crude *P. grantum* albedo extracts when compared with the clotrimazole standard drug, and no zone of inhibition was recorded around ethanol, negative control Preethi M *et al*.[16]

Mean, standard deviation, test of significance values of zones of inhibition (in mm) among different groups have been tabulated in Table 1. The one-way ANOVA test and two-pair T-test revealed a statistically significant difference in *P. granatum* ($P = 0.015$), clotrimazole ($P = 0.000$) and ethanol concentrations ($P = 0.000$).

Table 1: Significance of P Value

Group	*n*	Mean zone of inhibition in mm	Standard deviation	*P*
Punica granatum albedo extract	60	27.60	1.76	0.015*
Clotrimazole	60	21.83	1.53	0.00
Ethanol	60	0.00	0.00	0.00

*$P<0.05$

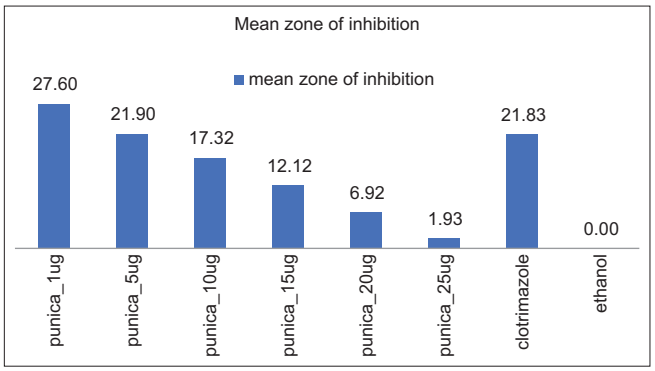
DISCUSSION

Traditional medicine has a significant history, a collection of practices based on theories, beliefs and experiences from several cultures and time periods, which are often inexplicable. Medicinal herbs have long been used to treat a variety of diseases all around the world.[17] They have been used for healing since the beginning of time.

The early nineteenth century led to a change in the understanding and application of medicinal herbs. The isolation of glycosides, followed by the discovery, substantiation and separation of alkaloids from poppy, ipecacuanha, strychnos, quinine, pomegranate and other plants, marked the beginning of scientific pharmacy. Pomegranate is one of the first domesticated fruits known to man. It belongs to the *Punica* L. genus, Punicaceae family.[18]

Pomegranate extracts have been found to exhibit antimicrobial activity against a variety of oral bacteria. *F. nucleatum*, *P. gingivalis*, *P. intermedia*, *S. mutans* and *A. actinomycetomomitans*.[19] The primary and secondary coloniser bacteria of dental plaque were said to be resistant to them. Pomegranate extract's role in antifungal activity was previously not well documented. *P. granatum*'s efficacy against pathogens found in the oral cavity, which harbours a variety of organisms like bacteria and fungi such as *Candida* sp., should be investigated further.[20]

Oral infections brought on by *Candida* species are referred to as oral candidiasis (OC).[21] The intensity and spread of the fungus are inversely connected to the host's immunity, and it can manifest at any age.[22] One



Graph 1: Comparison between mean zone of inhibition of punica, ethanol and clotrimazole

option for treatment is the local application of antifungal medications. Chemotherapeutic medications such as nystatin, amphotericin B and azoles are effective against these infections.[23] At the moment, azoles are the most widely used local treatment for candidal infections.

Since present antifungal medicines for the treatment of illnesses like candidiasis and other Candida-related infections have negative side effects, new antifungal therapies derived from natural ingredients are desperately required. Natural product interest has grown over time, and medicinal plants have been recognised as sources of bioactive chemicals. Natural compounds like *P. granatum* were identified and subjected to extensive testing to determine their modes of action and target locations.

Therefore, this study was aimed at assessing the efficacy of crude ethanolic albedo extracts of *P. granatum* against *C. albicans* compared to a phenolic compound ethanol and the fungicidal activity of an azole derivative, clotrimazole.

In the present study, 60 saliva samples were collected from patients, which were validated by clinical and mycological examination and cultured on SDA medium and incubated at 37°C for 24-48 hours, similar to the study by Preethi M *et al*.[16]

A wide range of phenols can be dissolved by ethanol, which can be used to extract plant components, among other extraction techniques like methanol, ethanol and water. Moreover, ethanol is an effective way to evaporate water and ethanolic combinations were safe for human consumption. In the current study, ethanolic pomegranate extracts were used to study antifungal efficacy.

P. granatum albedo extract was considered as Group I, clotrimazole as a positive control as Group II, Ethanol as a negative control (Group III), and ethanol exhibited zero or minimal antifungal activity similar to the study conducted by Preethi Madugula in which zero antifungal activity was noted against *C. albicans* and Jayan *et al*.[24] where minimum/zero antifungal activity was recorded against *Candida glabrata*.

Clotrimazole was used as positive control group, and inhibition zones were compared using national committee for clinical laboratory standards (NCCLS) zone interpretation criteria for clotrimazole. In the study conducted by Preethi M *et al*.,[16] the zone of inhibition for various concentrations of clotrimazole in comparison to the various concentrations of *P. granatum* peel extract was recorded, and the mean zone of inhibition of clotrimazole was noted at 100 µ l as 22.24 mm. In the current study, the zone of inhibition of clotrimazole in the standard concentration of 10 mcg was recorded at 21.83 mm.

In contrast to the study conducted by Jayan *et al*. to determine the anticandidal properties of *C. sativum*, *M. piperita*, and *P. granatum* extracts against fluconazole-resistant *C. glabrata* by determining the zone of inhibition and the extracts' minimum inhibitory concentration against the organisms where zone of inhibition of fluconazole of 250 mg was recorded, the zone of inhibition of clotrimazole in the standard concentration of 10 mcg in the This variation in the inhibitory zone may be due to the variations in the medications and the organisms to whom they were delivered.

In the present study, the mean zone of inhibition of *P. granatum* albedo against *C. albicans* ranged from 1.9 mm to 27 mm, almost in comparison with the study conducted by Jayan *et al*.,[24] where mean zone of inhibitions of *P. granatum* against *Candida glabrata* were recorded from 1.9 mm to 21 mm. The difference in zones of inhibition could be due to differences in the extract used and the organisms against which it was tested.

The dilutions were prepared in concentrations of 1%, 5%, 10%, 15%, 20% and 25% according to the study by Mehta V.V *et al*.,[25] where pomegranate peel, lotus leaf, guava leaf and coffee extracts were diluted in the concentrations of 1%, 5%, 10%, 15% and 20%, and efficacy testing was performed using the disc diffusion method against *Streptococcus mutans*, *Streptococcus mitis*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *C. albicans*. The zones of inhibition were recorded at 1% concentration as 1.15 mm, 5% as 0.82 mm, 10% as 0.00 mm, 15% as 0.00 mm and 20% as 0.00 mm, whereas in the current study the zones for 1% dilution was recorded as 27.6 mm, 5% as 21.9 mm, 10% as 17.32 mm, 15% as 12.12 mm, 20% as 6.92 mm and 25% as 1.93 mm.

The current study's findings indicate that *P. granatum* albedo extract has antifungal properties comparable to the other part extracts of *P. granatum*. Albedo extract exhibited a greater zone of inhibition when compared to the standard antifungal drug clotrimazole. Albedo extracts explored in this study make a viable supplement and/or alternative to synthetic medications. Cost-effectiveness and no side effects offer more advantages to albedo extract compared to synthetic drugs. The inhibitory effect of extract varies with different concentrations against *C. albicans*. The compound's inhibitory impact was proportionate to its dilutions. As a result, these dilutions

can be used to develop topical antifungal treatments to treat oral candidiasis. Additional *in vivo* long-term studies must be conducted to validate the findings of the current study.

CONCLUSION

The crude ethanolic albedo extracts of *P. granatum* have shown significant potency and efficacy against the tested *C. albicans*. Thus, considering the benefits of natural derivatives, such as less resistance, low cost, and minimal side effects compared to azoles, this herbal extract could be used against *C. albicans* as an alternative to standard azoles. Further clinical trials are required to utilise the compounds derived from this herbal extract against infections caused by *C. albicans*.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Deo PN, Deshmukh R. Oral microbiome: Unveiling the fundamental. J Oral Maxillofac Pathol 2019;23:122-8.
2. Avila M, Ojcius DM, Yilmaz O. The oral microbiota: Living with a permanent guest. DNA Cell Biol 2009;28:405-11.
3. Murray PR, Rosenthal KS, Pfaller MA. Textbook of Medical Microbiology, Opportunistic Mycoses. 5th ed. Elsevier; 2005. p. 80-6.
4. Rao PK. Oral candidiasis: A review. Schol J Med 2012;2:110-4.
5. Cheng M-F, Yang Y-L, Yao T-J, Lin C-Y, Liu J-S, Tang R-B, *et al*. Risk factors for fatal candidemia caused by Candida albicans and non-albicans Candida species. BMC Infect Dis 2005;5:22.
6. Lavaee F, Motaghi D, Jassbi AR, Jafarian H, Ghasemi F, Badiee P. Antifungal effect of the bark and root extracts of Punica granatum on oral Candida isolates. Curr Med Mycol 2018;4:20-4.
7. Bassiri-Jahromi S. Punica granatum (Pomegranate) activity in health promotion and cancer prevention. Oncol Rev 2018;12:345.
8. Endo EH, Garcia 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;161:534–40.
9. Abdollahzadeh SH, Mashouf RY, Mortazavi H, Moghaddam MH, Roozbahani N, Vahedi M. Antibacterial and antifungal activities of Punica granatum peel extracts against oral pathogens. J Dent (Tehran) 2011;8:1-6.
10. 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;44:839–48.
11. Shafighi M, Amjad L, Madani M. *In vitro* antifungal activity of methanolic extract of various parts of Punica granatum L. Int J Sci Eng Res 2012;3:2229–5518.
12. Al-Zoreky NS. Antimicrobial activity of pomegranate (Punica granatum L) fruit peels. Int J Food Microbiol 2009;134:244–8.
13. Heber D, Schulman RN, Seeram NP. Pomegranates: Ancient Roots to Modern Medicine. Florida: CRC Press; 2006.
14. Dixon DM, Walsh TJ. Antifungal agents. In: Baron S, editor. Medical Microbiology. 4th ed. Galveston (TX): University of Texas Medical Branch at Galveston; 1996.
15. Faubee VG, Muller MLD, Vigneulle M, Flandrosis JP. Application of Disc Diffusion technique to antimicrobial testing of Vibrio Anguillarum and Aeromonas Salmonicida Clinical Isolates. Veterinary Microbiology 1996:51:1-2.
16. Preethi M, Reddy S, Koneru J, Rao AS, Sruthi R, Dalli DT. "rhetoric to reality" - efficacy of Punica granatum peel extract on oral candidiasis: An *in vitro* study. J Clin Diagn Res 2017;11:ZC114–7.
17. Pathak AK. Anti-Candida activity of aqueous extracts of some herbals. J Fundame Appl Life Sci 2012;2:1-6.
18. Petrovska BB. Historical review of medicinal plants' usage. Pharmacogn Rev 2012;6:1–5.
19. Gościniak A, Paczkowska-Walendowska M, Skotnicka A, Ruchała MA, Cielecka-Piontek J. Can plant materials be valuable in the treatment of periodontal diseases? Practical review. Pharmaceutics 2021;13:2185.
20. Celiskoy V, Heard CM. Antimicrobial Potential of Pomegrante Extracts. Intechopen; 2021. p. 11.
21. Akula ST, Nagaraja A, Ravikanth M, Raj Kumar N G, Kalyan Y, Divya D. Antifungal efficacy of lauric acid and caprylic acid – Derivatives of virgin coconut oil against Candida albicans. Biomed Biotechnol Res J 2021;5:229-34.
22. Taylor M, Raja A. Oral Candidiasis. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2021.
23. Whaley SG, Berkow EL, Rybak JM, Nishimoto AT, Barker KS, Rogers PD. Azole antifungal resistance in candida albicans and emerging non-albicans candida species. Front Microbiol 2017;7:2173.
24. Jayan L, Priyadharsini N, Ramya R, Rajkumar K. Evaluation of antifungal activity of mint, pomegranate and coriander on fluconazole-resistant Candida glabrata. J Oral Maxillofac Pathol 2020;24:517-22.
25. Mehta VV, Rajesh G, Rao A, Shenoy R, B H MP. Antimicrobial efficacy of Punica granatum mesocarp, nelumbo nucifera leaf, psidium guajava leaf and coffea canephora extract on common oral pathogens: An in-vitro study. J Clin Diagn Res 2014;8:ZC65-8.