Original Article

Punica granatum as a salutiferous superfruit in the treatment of oral candidiasis – An *in-vitro* study

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Abstract

Context: The rise in the incidence of clinical resistance to antifungal therapy and failure to respond in recent years underscores the need for a time-honored approach to treat the disease using natural drugs instead of synthetic drugs, which have lesser adverse effects and good patient response. *Punica granatum* (pomegranate) is a fruit that has admirable medicinal value.

Aims: The study aimed to evaluate the *in vitro* antifungal efficacy of *P. granatum* peel extract against oral *Candida* compared with clotrimazole.

Settings and Design: The study design involves an *in-vitro* study.

Subjects and Methods: Saliva from candidiasis patients was inoculated and cultured on 60 separate Sabouraud dextrose agar plates and incubated at 37°C for 48 h from which *Candida* species were collected. Agar well-diffusion method was followed. Different concentrations of *P. granatum* peel extracts, ethanol solvent (control) and standard clotrimazole were added into the wells and allowed to diffuse at room temperature for 2 h. The plates were incubated at 37°C for 48 h. The antifungal potential of test compounds was determined based on the mean diameter of the zone of inhibition around the well in millimeters.

Statistical Analysis Used: Statistical analysis was performed using IBM software SPSS version 20 at one-way ANOVA.

Results: Antifungal efficacies of *P. granatum* peel extract and clotrimazole were statistically significant, and there was an increase in inhibitory efficacy with an increase in concentration. Minimum inhibitory concentration of *P. granatum* peel extract approximated with that of clotrimazole.

Conclusions: The results of this study indicate that *P. granatum* peel extract can be used as an effective natural substitute for synthetic antifungal agents.

Keywords: Candidiasis, clotrimazole, minimum inhibitory concentration, *Punica granatum*

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INTRODUCTION

"Nature itself is the best physician" stated Hippocrates. One such gift from nature is *Punica granatum* (pomegranate) with innate therapeutic properties, and the peel extract contains active antifungal compounds such as punicalagin, castacalagin, granatin, catechin, gallocatechin, kaempferol and quercetin.[1-3]

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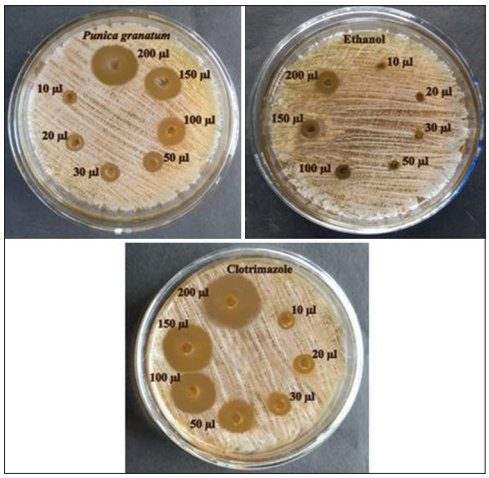

Figure 1: Zones of inhibition exhibited by *Punica granatum*, clotrimazole and ethanol at different concentrations

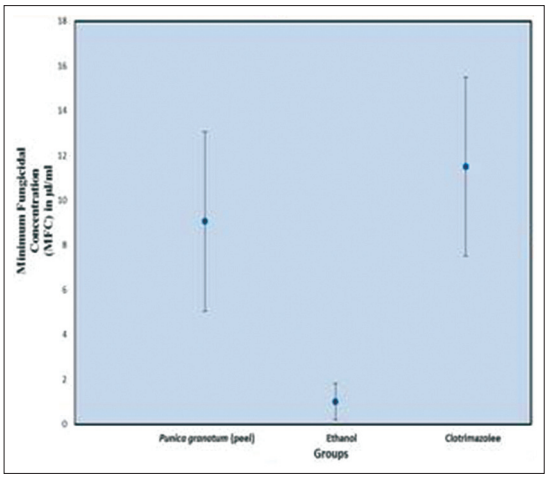

Figure 2: Antifungal efficacy of *Punica granatum*, ethanol and clotrimazole against *Candida* species

Oral candidiasis is the most prevalent opportunistic infection affecting the oral mucosa caused by *Candida albicans*.[4] The development of resistance to antifungal agents underscores the need to use natural drugs instead of synthetic drugs, which have lesser adverse effects.[1] This study aims to evaluate the *in vitro* antifungal efficacy of *P. granatum* peel extract against oral *Candida* compared with clotrimazole.

SUBJECTS AND METHODS

An *in-vitro* study was conducted in the department of oral medicine and radiology. A total of 60 patients who were diagnosed and confirmed by clinical and mycological examination as suffering from oral candidiasis were enrolled in the present study.

Determination of antimicrobial activity

The antimicrobial activity was performed by well diffusion method.[4,5]

Sabouraud dextrose agar

Sabouraud dextrose agar is recommended for the isolation of *C. albicans*.

Composition of media

Ingredients	Gm/L
Dextrose (Glucose)	40.000
Mycological peptone	10.000
Agar	15.000
Final pH (at 25°C)	5.6 ± 0.2

Preparation of the medium

Suspend 65.0 g in 1000 ml distilled water. Heat to boiling point to dissolve the medium completely. Sterilize the medium using autoclave at 15 lbs pressure (121°C) for 15 min. Cool to 45°C–50°C. Mix well and pour into sterile Petri plates.

Preparation of the extract

About 10 g of sample powder was soaked in 99.9% ethyl alcohol for 4 days and filtered using Whatman filter paper. The obtained filtrate was subjected to rotary evaporator at a temperature of 70°C and 120 rpm, and crude extract was obtained. The weight of crude extract was calculated by measuring the difference between the weights of beaker before and after the collection of extract. The final material (crude extract) was dissolved in ethanol and made into different concentrations of 10, 20, 30, 50, 100, 150 and 200 µl. Ethanol group was considered negative control in this study to investigate the presence of any antifungal property for ethanol because the crude extract was dissolved in ethanol. The final crude extract was subjected to antifungal susceptibility test.

The minimum inhibitory concentration (MIC) value is defined as the lowest concentration of plant extract in the medium that inhibited visible growth of the test fungal strains.[6,7] The minimum fungicidal concentration (MFC) is defined as the concentration required to give 50% inhibition of hyphal growth.[6]

Preparation of sample solutions for the experiment

The samples were weighed (10 mg/10 ml) and dissolved in sterile distilled water to prepare appropriate dilution to get required concentrations of about 10 µl (10 µg), 20 µl (20 µg), 30 µl (30 µg), 50 µl (50 µg), 100 µl (100 µg), 150 µl (150 µg) and 200 µl (200 µg). These solutions were refrigerated till they were used for the experiment.

Microorganism

The saliva samples were collected from Diamond Dental Clinic, Thanjavur. Saliva was collected by spitting method

in graduated container from patients diagnosed as having candidiasis. The collected saliva was inoculated and cultured on 60 separate Sabouraud dextrose agar (SDA) plates. These plates were incubated at 37°C for 48 h from which *Candida* species were collected.

Antimicrobial assay

With the help of agar well diffusion method, antifungal property was evaluated. SDA plates were swabbed (sterile cotton swabs) with 8 h old broth culture of fungi. Wells (6 mm diameter and about 1 cm apart) were made in the plates using sterile cork borer. Different concentrations of plant extracts, ethanol solvent and standard clotrimazole were added using micropipette into the wells and allowed to diffuse at room temperature for 2 h. Later, these plates were incubated at a temperature of 37°C for a period of 48 h. The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the well in millimeters. The zones of inhibition of the tested microorganisms by the samples were measured using a millimeter scale.

Statistical analysis

Data were analyzed using IBM software SPSS version 20 at one-way ANOVA with a significance level 0.05.

RESULTS

The results were obtained based on the zones of inhibition produced by *P. granatum* extract, ethanol and clotrimazole in the sixty samples. The widths of the zones of inhibition in each group have shown increase with increase in their concentrations, as tabulated in Table 1. The width of zones of inhibition of *P. granatum* approximated with that of clotrimazole at concentrations of 30 µl, 50 µl, 100 µl, 150 µl and 200 µl as depicted in Figures 1 and 2. This proves that *P. granatum* has almost equal antifungal efficacy as clotrimazole.

DISCUSSION

The Indian subcontinent is well known for its richness in the presence of numerous plants with medicinal properties.[1] The reasons behind the need for the discovery of natural products with therapeutic properties are the adverse effects caused by synthetic drugs and the increasing resistance of microorganisms to the antimicrobials prescribed currently.[3]

Table 1: Zones of inhibition of *Punica granatum*, ethanol and clotrimazole group at different concentrations

Concentration	*Punica granatum* (peel)	Ethanol	Clotrimazole
10 µl	1.32±0.39	0	2.87±0.25
20 µl	2.62±0.47	0	3.60±0.59
30 µl	4.62±0.47	0	5.50±0.77
50 µl	5.85±0.31	0	10.17±0.56
100 µl	10.50±0.43	1.02±0.05	15.10±1.31
150 µl	16.42±0.95	2.22±0.23	19.20±0.54
200 µl	22.12±0.78	3.85±0.36	24.55±0.79
P	0.000	0.000	0.000

$P<0.05$ is significant

P. granatum is an antique, asomatous and novel fruit, which has therapeutic properties with the ability to heal various ailments of the human body.[1] Plants have a natural capacity to synthesize phenols and tannins which provide defensive action against microorganisms.[1,8] *P. granatum* peel extract has high reserves of tannins and phenols as compared to the different parts of *P. granatum* plant in a study performed by Pai *et al*.[1] Scalbert (1991) proposed different mechanisms to explain the antimicrobial activity of tannin. These include (i) inhibition of extracellular microbial enzymes, (ii) deprivation of substrates and metal ions required for microbial growth and (iii) direct action on microbial metabolism through the inhibition of oxidative phosphorylation.[7] Shafighi *et al*. in an *in vitro* study proved that the MIC of peel extract is greater than that of flower, stem and leaf.[1]

As observed by the scanning electron microscopy, the peel extracts showed morphological alterations along with cell aggregation and growth inhibition in the yeasts used for the trial. The appearance of the control sample was regular and homogeneous, with a normal budding profile at the cell and hyphae, whereas the treated cells presented an irregular cell wall, with viscous material on the surface along with the rupture of the hyphae and desquamation.[7]

In the present study, the extract of *P. granatum* peel has the effect on *C. albicans* in all concentrations. This finding is in agreement with Duraipandian's study.[3] *P. granatum* has proved to be effective against *C. albicans*, which is in accordance with the study conducted by Endo EH. It is also in accordance with the studies conducted by Vasconcelos *et al*. and Duraipandiyan *et al*. Vasconcelos *et al*. through a clinical trial showed that *P. granatum* peel extract was effective as a topical antifungal drug against *C. albicans* for treating two cases of denture stomatitis patients. According to Vasconcelos *et al*., in their *in-vitro* study, antimicrobial efficacy of *P. granatum* extract gel was proven to be higher than miconazole.[1]

The MIC and MFC exhibited by *P. granatum* in this study were at concentrations of 30 µl/ml and 20 µl/ml, respectively, as tabulated in Table 2, which is in close correlation with the results obtained by Madugula *et al*. in their study where the MIC and MFC were found to be 20 µl/ml and 10 µl/ml, respectively.[1]

Table 2: Determination of minimum inhibitory concentration and minimum fungicidal concentration for *Candida* species

	MIC (µl/ml)	MFC (µl/ml)
Punica granatum (peel)	30	20
Ethanol	10	10
Clotrimazole	20	10

MIC: Minimum inhibitory concentration, MFC: Minimum fungicidal concentration

In the present study, the *P. granatum* peel extract had MIC against *C. albicans* at all concentrations and the antifungal efficacy of *P. granatum* peel extract approximated with that of clotrimazole. *P. granatum* peel extract can be used as an antifungal agent in clinical trials. This study is limited as it is an *in-vitro* study. Further *in-vivo* studies should be carried out to confirm the results of this study.

CONCLUSIONS

Our results indicate that *P. granatum* peel extract is an effective antifungal agent against *C. albicans* and its antifungal efficacy approximates that of standard clotrimazole. The discovery of a natural antifungal agent with beneficial qualities of biosafety and efficacy will be a great boon in the therapy of fungal infections. Apart from providing treatment to drug-resistant patients, cost-effectiveness also plays a major role in the replacement of synthetic drugs with natural ones, especially in developing countries.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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