In vitro antifungal activity of methanolic extract of various parts of *Punica granatum* L.

Mahsa Shafighi, Leila Amjad, Mahboobeh Madani

Abstract— Candida species are now fourth common organisms isolated from hospitalized patients. It is important to increase effective therapy. In the past decade numerous reports of treatment failures are reported. In the other hand, plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases including epidemics. The aim of this study was to investigate the antifungal effect of methanolic extract of pomegranate peel, flower, leaf and stem against *Candida albicans* NCPF 3153 by well diffusion, also MIC snd MFC were determined. *Punica granatum* L. was collected from Isfahan, Iran. All part of plants were dried and pwodred seperatly. The dried powders were extracted by Soxhlet apparatus for 8 hours. Results showed that maximum inhibition zones of antifungal effect were obtained in 200 µl concentration by flower extract. This provides a complementary preventive value for this plant and supports its gaining popularity as an antifungal source.

Index Terms— *Candida albicans*, Flower, Leaf, Peel, *Punica granatum*, Stem.

1 Introduction

PUNICA granatum L. is a small tree originating from Persia and from there it spread into Asia, North Africa, Mediterranean Europe and USA. Pomegranate is one of the oldest Holley edible fruits [1], [2], [3], [4], [5]. The peel, flower, leaf, stem, bark, and seed of this plant employed in folk medicine for treatment of different diseases such as skin diseases and wound healing, fever, diarrhea and microbial infection, ulcers, helminthiasis, acidosis, dysentery, haemorrhage and respiratory pathologies [6]-[8]. Different parts of pomegranate are rich sources of polyphenol compounds, flavonoids, tannins, alkaloids and organic acids [9], [10], [11]. Candida species are harmless saprophyte yeast, a normal component of the human biota in the gastrointestinal tract, oral and vaginal mucosae [5], [6], [12]. In addition, Candida species can cause a lot of systemic infections in patients with immune deficiency such as human immunodeficiency virus (HIV), anticancer therapy, organ transplantation, abdominal surgery, catheters, diabetes and the use of broad- spectrum antibiotics [6], [12].

For controlling Candida species, the most popular way is using limited synthetic drugs and fungicides. They are limited due to the eukaryotic nature of fungal cells, which are similar to host cells. On the other hand, structures of synthetic drugs create resistant strains [3], [6]. Hence, researchers focus on natural compounds such as essential oils and extracts for biological control of pathogens. Therefore, the aim of present study was to assess antifungal properties of crude extract isolated from pomegranate peel, flower, leaf and stem against *Candida albicans* (NCPF 3153).

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2 Materials and Methods

Fresh samples of *P. granatum* peel, flower, leaf, and stem were collected and identification in June and September 2011, from the research Institute of Isfahan Forests and Rangelands, Isfahan, Iran. The samples were separated, washed thoroughly with tap water and then, they were air- dried in a low light at room temperature for seven days. The samples were thereafter homogenized in an electric grinder to fine powder separately. They stored in airtight bottles.

30 gram of dried powder of each part was extracted with 100% Methanol (MERCK) using Soxhlet apparatus for 8 hours [5], [6], [8], [9].

The extracts were dried over night and freed of solvent under reduce pressure, using a rotary vacuum evaporator. The dried extracts were weighted and then, they were solved in dimethyl sulfoxide (MERCK). All extracts were frigid at -20 °c in dark and airtight bottles, until each experiment [6], [7], [9].

The yeast strain *Candida albicans* (NCPF 3153) were used in this study. It obtained from Institute of Scientific and Industrial Researches, Tehran, Iran. At the first, it has been maintained at 4 °c on Sabouraud Dextrose Agar (SDA) plates and subcultured at 25 °c in Sabouraud Dextrose Broth (SDB) before each experiment to ensure viability and purity.

Petri dishes contained SDA, have been used for agar well diffusion assay. Well (6 mm) have been prepared in the SDA plates. In agar well diffusion, 10, 50, 100, and 200 µl of each extract have been inoculated in each well, separately. Then 100 µl of 10^6 CFU/ml yeast suspension was spread uniformly onto the SDA plate using cotton swabs.

Fluconazole and dimethyl sulfoxide used as positive and negative control, respectively. The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) values were studied. The experiments were performed four times to minimize errors.

Data were analyzed using SPSS version 12.0. ANOVA test was used to evaluate significance of parameters. P-value of < 0.05 was considered statistically significant.

3 Results

Antifungal activity of the methanolic extracts of *Punica granatum* peel, flower, leaf and stem were determined against *Candida albicans* were shown in Table 1.

Maximum inhibition zones of flower, peel, stem and leaf extracts against *C. albicans* were obtained in 200 µl concentration (Fig. 1) respectively.

It should be noted that, the flower, leaf, and stem extracts had no any antifungal activity in 10 µl concentration. It is show that there is direct relationship between concentration and inhibition zone.

The MIC and MFC of these fractions reported in Table 2 and Fig. 2. Meanwhile, there are significant changes of antifungal effects between effective fungi and control sample (P-Value < 0.05). The inhibitory effects of the extracts were compared with standard antifungal, fluconazole.

TABLE 1
INHIBITION ZONE'S OF PEEL, FLOWER, LEAF, AND STEM EXTRACTIONS ON *CANDIDA ALBICANS* NCPF 3153 (X±SD)

Concentration (µl)	INHIBITION ZONES (mm)			
	Peel	Flower	Leaf	Stem
10	11.50 ± 1.29	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
50	18.25 ± 0.50	20.50 ± 2.08	19.25 ± 0.95	19.25 ± 3.86
100	25.00 ± 4.08	22.75 ± 4.34	20.25 ± 3.40	21.50 ± 4.65
200	26.50 ± 6.45	27.50 ± 2.38	21.75 ± 1.89	25.75 ± 2.21

The test results reported as mean of 4 times.

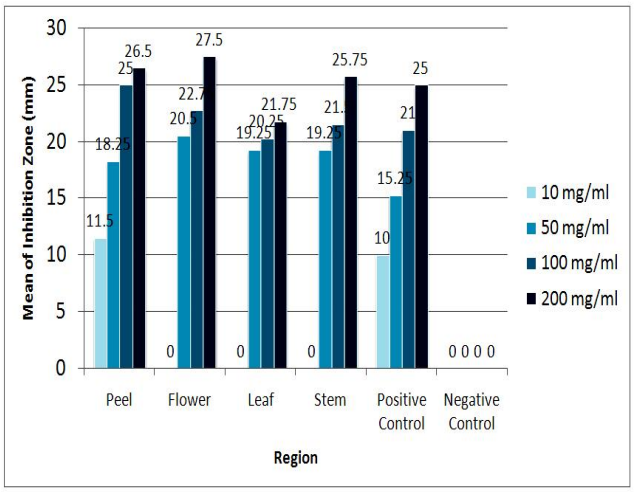

Fig. 1. Means of inhibition zone's of different parts of *Punica granatum* exrtact's on *Candida albicans* NCPF 3153.

TABLE 2
DETERMINATION OF MIC AND MFC FOR *CANDIDA ALBICANS* NCPF 3153

Region	Minimum Inhibition Concentration (mg/ml)	Minimum Fungicidal Concentration (mg/ml)
Peel	250	125
Flower	62.5	31.25
Leaf	15.62	7.81
Stem	125	62.5

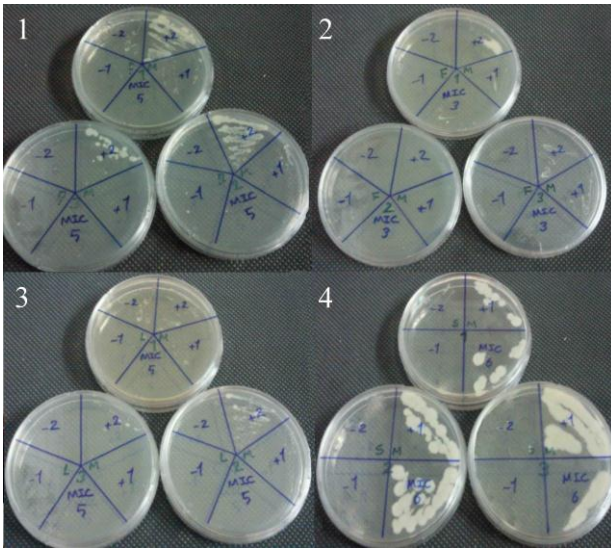

Fig. 2. Different region MFC's in *Candida albicans* NCPF 3153

1. Peel, 2. Flower, 3. Leaf, 4. Stem

4 Discussion

Candida species are now the fourth most common organism recovered from the blood of hospitalized patients. Notwithstanding the increasing need for effective therapy, the range of antifungal agents available is limited, and some of the most effective agents are also toxic. In addition, although azoles have been used successfully for the treatment of Candida infections, numerous reports of treatment failures are now appearing in the literature [6], [12].

Plants have a highly ability to synthesize aromatic products, most of which are phenols. These are secondary metabolites and in many cases, these products serve as plant defense mechanisms against predation by microorganisms, insects, and so forth [13]. Generally, herbal products cause to changes in microorganism's cell, such as cytoplasm granulation, cytoplasmic membrane disruption, inactivation or inhibition of enzyme activity within cell and outside cell, and cell wall col-

lapse [14], [15], [16]. An antimicrobial activity of pomegranate extract is related to attendance of antibiotic compounds [3], [17]. Major antibiotic components in the peel extract from *P. granatum* are flavonoids (such as Quercetin, Rutin, Naringenin, Luteolin, Pelargonidin, Prodelphinidin, Kaempferol, and Flavan), and tannins (including Methyl gallate, Methyl Ellagic acid, and Peduncalagin). Antifungal properties in the flowers methanolic extracts are refer to some flavonoids like Punica flavones, and tannins such as Pomegranatate, gallic acid, and ellagic acid. Also methanol extracted flavonoid (Apigenin, and Luteoline) and tannins (Brevifolin, Corilagin, Gallic acid, and Ellagic acid) from the pomegranate's leaf. The stem extract have plenty of tannin compounds such as Ellagitannin, Punicalagin, Punicalin, and Punicatonic, as well as numerous piperidine alkaloids [6], [11], [17]. Specific mechanism of action of tannins against Candida is unclear. However, it has been suggested that they may act on the cell membrane, because these compounds can precipitate proteins [6]. Some mechanisms of antimicrobial action of phenolic compounds are Substrate deprivation, Membrane disruption, Adhesin binding, Complex with cell wall, Enzyme inactivation, and Interaction with eukaryotic DNA (antiviral activity). Main mechanisms of antimicrobial action of tannins are protein binding, adhesion binding, enzyme disruption, and metal- ion complex; also alkaloids have intercalation into cell wall and/or DNA [17]. It is logical that, synergistic interactions of these compounds increase the antifungal activity of pomegranate.

On the other hand, level of compound isolated from plants depends on polarity of these compounds and solvent. Methanol is a polar solvent. In hence, polar compounds such as phenoles, tannins and flavonoids are more effectively extracted by polar solvents [18]. Therefore, high antifungal potential of methanol extractions are due to polarity solvent.

5 Conclusion

Consequently, methanol solvent extracted maximum rate of phenol and tannins compounds. The present study demonstrated that *Punica granatum* peel, flower, leaf and stem extracts show high antifungal property. It is expected that this ability is referring to high amounts of phenol and tannins. This provides a complementary preventive value for this plant and supports its gaining popularity as an antifungal source.

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