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Effect of Fungal GrowthInhibition from Pomegranate Flower and Peel Extracts

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Abstract

Candida species are now one of the most common organisms isolated from hospitalized patients. The range of antifungal agents available is limited, and some of the most effective agents are also toxic. 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 petroleumether, ethyl acetate and n-butanol fractions isolated from pomegranate pericarp and flower against Candida albicans (ATCC 3153). The maximum inhibition zone of Candida albicans was obtained by peel's n-butanol fraction, 35 mm. Petroleumether fractions had no any antifungal activities.

Keywords: Punica granatum L., peel, flower, petroleumether, ethyl acetate, n-butanol

1. Introduction

The *Punica granatum L.*, is one of the oldest known edible fruits. It is native to Persia and from there it spreads into Asia, North Africa and Mediterranean Europe, including Turkey [1, 2]. According to Qur'an, Bible, Torah and Babylonian Talmud, pomegranate is a gif and heavenly fruit of God.

The extracts of traditional herbs have been shown to exert biological activity in vitro and in vivo. *Punica granatum* is employed in man medicine for the treatment of various diseases such as skin diseases, and wound healing, ulcers, fever, diarrhoea, and microbial infection. In the recently years, the *Punica granatum* has been the subject of much scientific research which have showed its antimicrobial, antioxidant and anti-cancer effects [3, 4].

The different types of phytochemical that have been showed identified from pomegranate pericarp (peel, rind) and pomegranate flower. Pomegranate pericarp's constituents are Luteolin, kaempferol, EA glycosides, EA, Pedunculagin, punicalin, phenolic punicalagins, Gallic acid and other fatty acids, catechin, EGCG, quercetin, rutin and other flavonols, flavones, flavonones, anthocyanidins. Pomegranate flower's constituents are Polyphones, punicalagins punicalin, EA, Gallic acid, ursolic acid, triterpenoids, including maslinic and Asiatic acid [1, 5- 7].

Candida species are harmless saprophyte yeasts, a normal component of the human biota in the gastrointestinal tract and oral and vaginal mucosae. These yeasts can cause superficial infections

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such as thrush and vaginitis; however, if the immune defences of the host become compromised, they can cause severe systemic infections. Risk factors for patients include infection by the human immunodeficiency virus (HIV), anticancer therapy, organ transplantation, abdominal surgery, catheters, diabetes and the use of broad-spectrum antibiotics [4, 11].

The aim of this study was to investigate the antifungal effect of some fractions isolated from pomegranate pericarp and flower against *Candida albicans.*

2. Materials and Methods

Samples of *P. Granatum* flowers and peels were collected and identification in June and September 2011 respectively from the Agricultural Research Centre in Isfahan, Iran.

The flowers and peels were air-dried in a low light at room temperature for 1 weak. The material was thereafter ground in an electric grinder to produce a powder separately.

Peels and flowers powder extracted with 100% petroleumether (F1) at room temperature for 24 hours respectively, the extracts were filtered. Then 20 gram of remains powders were extracted with 75% Ethanol by Soxhlet extraction for 8 hours.

The residues were dried over night and then evaporated by using a rotary evaporator. The dried extracts were suspended in distilled water (F2) and partition with ethyl acetate (F3) followed by n-butanol (F4). All fractions were frigid at -20°C until each experiment.

The yeast strain *C. albicans* (ATCC 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 sub cultured at 25°C in Sabouraud dextrose broth (SDB) before each experiment to ensure viability and purity.

Petri dishes contained 20 ml of SDA have been used for well-diffusion assay. Wells have been prepared in the SDA plates. In agar well diffusion, 10, 50, 100 and 200 μl of each fractions have been inoculated to each well separately. Then 100 ml of 10^6 CFU/ml yeast suspension was spread uniformly onto the agar plate using cotton swabs. Diameters (in mm) of growth inhibition zones were measured after incubation at 25°C for 24- 48 hours.

3. Results and 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 [4]. In view of the lack of new classes of drugs or different molecular targets, drug combinations might be considered a viable strategy for therapy, considering the multiplicity of fungal targets against which current agents are effective.

The antimicrobial and antifungal effects of alcoholic extracts of different parts of pomegranate tree were previously studied [1, 8-11]. Nevertheless, few studies have showed the antifungal effect of different pomegranate fractions. This study showed the antifungal activity of 4 pomegran-

ate fractions. Antifungal effects of peel and flower fractions by well-diffusion assay method have been shown in Figure 1 and Figure 2 respectively.

The maximum inhibition zones of peel fractions against C. albicans ATCC 3153 were obtained in 200 µl concentrations by n- butanol fraction, water fraction and ethyl acetate fraction respectively.

C. albicans ATCC 3153 only was sensitive to flower n- butanol fraction. In addition C. Albicans ATCC 3153 was resistant to peel and flower Petroleumether fractions.

The fractions had no any antifungal activity in 10 µl, and there was direct relationship between concentration and inhibition zone.

Also, n- butanol, ethyl acetate, water and petroleumether had no any antifungal activities. In the other words, the extracted compounds of peel and flower were effective [4].

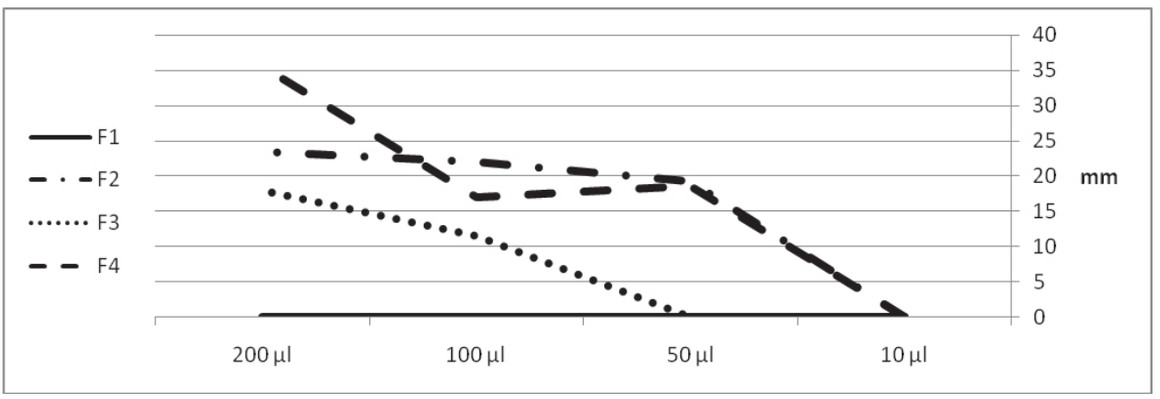

Figure 1. The inhibitory zons (mm) of peel's fractions in 4 concentrations against *C. albicans (*ATCC 3153). F1: Petroleumether Fraction, F2: Water Fraction, F3: Ethyl acetate Fraction, F4: n-butanol Frcation.

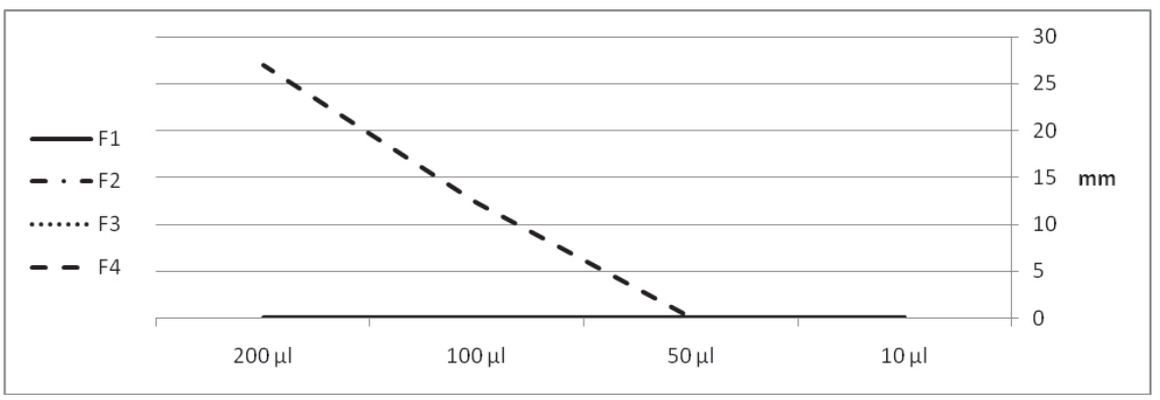

Figure 2. The inhibitory zones (mm) of flower's fractions in 4 concentrations against C. albicans (ATCC 3153). F1: Petroleumether Fraction, F2: Water Fraction, F3: Ethyl acetate Fraction, F4: n-butanol Frcation.

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