

Anticandidal Activity of Ornamental *Punica granatum* Linn. Flowers

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Abstract

Punica granatum Linn. is a shrub belonging to the family Punicaceae. It is often a cross pollinated food crop and has large forms of cultivars in various regions of the world. Double flower – *Punica granatum* is an ornamental type where the flowers have numerous petals and look like a rose flower and have a significant ornamental value. In Unani system of medicine, these flowers are called as “Gulnar” and are used as one of the ingredient in many formulations. Though the flower has significant therapeutic use since antiquity, many fundamental aspects including pharmacological and microbiological activities are lacking. Hence, in the present study, the anticandidal potency of the flowers of *Punica granatum* was studied against the clinical strains of *Candida albicans* collected from various laboratories and hospitals responsible for causing oral thrush or candidiasis in human. The alcoholic extract of the flowers found to have good anticandidal effect and had a significant control against most of the strains tested. The MIC dosage level was also determined.

Keywords: Anticandidal activity, *Punica granatum* L., Candidiasis

Introduction

The use of natural products with therapeutic properties is as ancient as human civilization. For a long time, mineral, plant and animal products were the main sources of drugs (De Pasquale, 1984). In recent years, throughout the world, there has been a growing interest in alternative therapies and the therapeutic use of natural products especially those derived from plants (Goldfrank *et al.*, 1982; Vulto and Smet, 1988). In India, traditional medicine is mainly based on three major systems of medicines namely, Ayurveda, Siddha and Unani. In almost all the system, the medicinal plant plays a major role and constitutes the backbone. Among various plants of commercial value, pomegranate (*Punica granatum*) is known worldwide for its delicious taste and health promoting properties. The plant *Punica granatum* is a member of the Punicaceae that has a bushy appearance, tends to develop multiple trunks, and grows upto a height of 12 to 16 feet (Sharrif and Kashani, 2012).

Varietal wealth of *Punica granatum*

In different pomegranate growing areas of the world a good number of varieties have been identified by growers and breeders and cultivated either as commercial crop for the production of pomegranates or as ornamental crop (Jaliop, 2010).

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Double flowers of *Punica granatum*

Double flower bearing *Punica granatum* is an ornamental type and finds a significant use in horticulture for its beautiful flowers. The plant is considered to be native of Iran, Arabia, Afghanistan and Baluchistan and cultivated all over India. It grows in the hill slopes of Jammu & Kashmir and Himachal Pradesh between 900m and 1800m. The plant mostly flowers in April - June, but may occur even at other seasons also (Dina Nath Tiwari *et al.*, 2001). Double flower as the name implies are large attractive and look like rose flowers, wherein numerous stamens are modified into petals. Due to these modifications, some of these cultivars do not set fruits (abortive). As per the literature survey the commercial samples of the Unani drug used under the name Gulnar have been identified as the flowers of the horticulture form of *Punica granatum* (which does not produce fruits) (Tiwari *et al.*, 2001).

Medicinal uses of *Punica granatum* double flowers

Gulnar - the abortive double flowers of *Punica granatum*, in Unani system of medicine is prevalently used in various forms like decoction, powder, syrup, infusion, nasal drop, gargle and pessery for treating various ailments (Nazamuddin *et al.*, 2013). These flowers have different pharmacological activities and being used as astringent, haemostatic, antihelmintic, stomachic, desiccant and cicatrizant. It is also used as one of the ingredients in many formulations prescribed by the Unani physicians for treating various ailments.

Oral thrush or Candidiasis

Oral thrush or candidiasis is the fungal infection caused by the yeast *Candida albicans*. The organism is usually present as commensals in all human skin and mucous membranes. Infection occurs when there is overgrowth of the organisms with signs and symptoms like; (i) creamy white lesions with a cottage cheese-like appearance on tongue, inner cheeks, on the roof of mouth, gums, tonsils; (ii) redness or soreness that may be severe to cause difficulty while eating or swallowing; (iii) slight bleeding if the lesions are rubbed or scraped; (iv) cracking and redness at the corners of mouth; (v) loss of taste etc. The infection occurs mainly to those who have either diabetes, glandular (endocrine) disorders, genetic disorders such as down syndrome, leukemia, lymphoma, immunodeficiency such as HIV/AIDS or those who have underwent a course of oral antibiotics especially inhaled steroids for certain lung condition, chemotherapy etc (Mariappan *et al.*, 2011).

With this view, the present study was designed to determine the anticandidal activity of ethanol extract of ornamental flowers of *P.granatum* against the strains of *Candida albicans* responsible for oral thrush.

Materials and Methods

Collection of study material

Double flowers of *P. granatum* were collected from Herbal Garden of Regional Research Institute of Unani Medicine, Royapuram, Chennai, Tamil Nadu, during the month of April to June 2012 and were authenticated by the Botanist, Plant Anatomy Research Centre, Chennai, India (Figure-1).

Preparation of ethanol extract of flowers

Fresh abortive double flowers of ornamental type of *Punica granatum* were collected, shade dried at room temperature and coarsely powdered (Sieve No.10). Ten grams of the powder was extracted with ethanol using Soxhlet apparatus for 6 hrs. The extract was filtered using sterile Whatman filter paper No.2, dried using rotary vacuum filter at 40°C.

Collection of microorganism

Swab samples (taken from the mouth along with the history of the patients) were obtained from various hospitals and laboratories, Chennai.

Confirmation for *Candida albicans* was performed in the Department of Microbiology, RRIUM, Chennai by performing the following tests (Myers and Koshis, 1982)

- Smear test
- Germ tube test
- Culture test

Five confirmed strains of *Candida albicans* coded as CAI, CAII, CAIII, CAIV, CAV and *Candida albicans* ATCC 10231 were stored in the SDA plates for further analysis of anticandidal study.

Anticandidal activity

The in-vitro anticandidal activity was performed using the cup plate method (Anonymous, 1996). The required number of Muller Hinton agar plates were prepared and swabbed with different isolates of log phase cultures of above mentioned *Candida albicans* cultures coded CAI, CAII, CAIII, CAIV, CAV and *Candida albicans* ATCC 10231. The plates were allowed to stand for few minutes. Required numbers of 6 mm diameter wells were made over the plates at an equidistant position. Wells were loaded with 70µl of the drug at the concentration of 100 mg/ml. Dimethylsulphoxide (DMSO) was used as the solvent. Separate

control disc was also included using the solvent. The ampicillin (10 mcg) dissolved in sterile DMSO was used as standard for comparison. All the plates were kept at 37°C for 18-24 hrs. The zone of inhibition was measured using the calipers.

Minimum inhibitory concentration (MIC)

The MIC, the lowest concentration of the extract required to inhibit the microorganism was determined by the agar dilution method (Myers and Koshis, 1982). Different concentrations of alcohol flower extract ranging from 100 ìg/ìl, 50 ìg/ìl, 25 ìg/ìl, 12.5ìg/ìl, 6.25ìg/ìl and 3.125 ìg/ìl were used in the study. The lowest concentration of the drug (MIC) that completely inhibits the growth was determined after overnight incubation at 37°C for 18 to 24 hrs. The zone of inhibition was measured using the calipers (Table-1; Figure-2).

Table 1: MIC value

S.No.	Culture tested	MIC µg/µl	Std (Amp) mcg/ml
1.	<i>Candida albicans</i> CAI	3.125	Intermittently sensitive
2.	<i>Candida albicans</i> CAII	6.25	
3.	<i>Candida albicans</i> CAIII	6.25	
4.	<i>Candida albicans</i> CAIV	3.125	
5.	<i>Candida albicans</i> CAV	3.125	
6.	<i>Candida albicans</i> ATCC 10231	12.5	



Figure 1: Double flower of *Punica grantum* Linn.

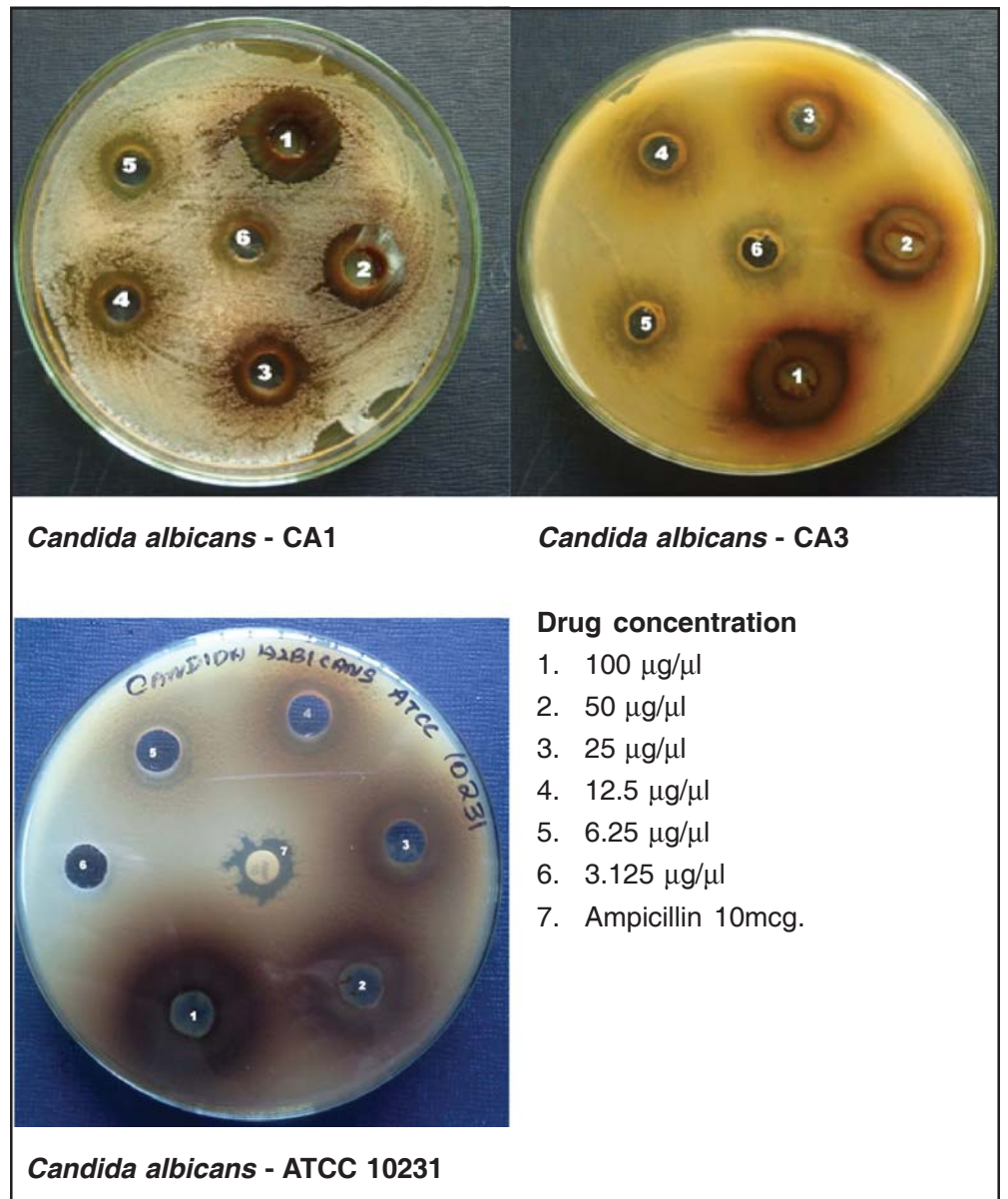


Figure 2: Plates showing Anticandidal activity

Results and Discussion

Oral hygiene and topical antifungal gels are usually adequate for the uncomplicated oral candidiasis. Modern drugs presently used in the treatment of *Candida albicans* are Gentian violet an aniline dye, but because of their staining of oral mucosa is replaced by other drugs like nystatin, Amphotericin B, Clotrimazole, Ketoconazole and Fluconazole which has its own potential side effects. In this situation, as per our research findings, the alcohol extract of the flowers of ornamental *Punica granatum* finds to possess good controlling capacity against the tested strains of *candida albicans*. Hence the study can be concluded as, the double flowers of

Punica granatum could be given as an alternative medicine either separately or in combination with modern medicine in the treatment of patients having oral thrush.

Acknowledgement

The authors are extremely thankful to Director General, CCRUM, New Delhi, for his valuable guidance, encouragement and providing necessary research facilities.

References

- Anonymous, 1996. Microbiology assays and Tests. Indian Pharmacopoeia, pp.100-106.
- De Pasquale, A., 1984. Pharmacognosy: the oldest modern science. *Journal of Ethnopharmacology* 11: 1-16.
- Dina Nath Tewari, Kaushal Kumar, Arti Tripathi, 2001. Anar, Ocean Books, 1st Edt., pp. 29-43.
- Goldfrank, L., 1982. The Pernicious Panacea: Herbal Medicine, Hospital Physician, 10: 64-86.
- Jalikip S. H., 2010. Pomegranate breeding. *Fruit, vegetable and cereal science and Biotechnology* 4 (2spl): 26-34.
- Mariappan Premanathan, Fathi Abdullah Amaar Shakurfow, Ahmadali Ismail, Mohammed Ayad Berfad, Adel Tawfik Ebrahim and Moussa Milad Awaj, 2011. *International Journal of Medicine and Medical Sciences* 3 (3): 83-86.
- Myers and Koshis, 1982. Manual of diagnostic Procedures in Medical Microbiology and Immunology/Serology.1st ed., Christian Medical College and Hospital, Vellore, pp. 74-87.
- Nazamuddin, Wadul Abdul, NajeebJahan, TanwirAlam M., Nafis M Iqbal, Asim Mohammed Khan, 2013. Gulnar (Flowers of *Punica granatum*Linn. Precious medicinal herbs of Unani medicine – An Overview). *Int J. Cur. Res. Rev.* 5 (20): 16-21.
- Sharrif Moghaddasi Mohammed and Hamed Haddad Kashani., 2012. Chemical composition of the plant *Punica granatum* L. (Pomegrante) and its effect on heart and cancer. *Journal of Medicinal Plants Research* 6 (40): 5306-5310.
- Vulto, A.G., Smet, P.A.G.M., 1988. Meyler's Side Effects of Drugs, In: Dukes, M.M.G. 11thEdt. Elsevier, Amsterdam, pp. 999-1005.

