



**ANTIFUNGAL ACTIVITY OF KARANJA (*PONGAMIA GLABRA*) ON MEDICALLY IMPORTANT CLINICAL ISOLATES OF CANDIDA FUNGI**

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**ABSTRACT**

In the present study antifungal activity of *Karanja* (*Pongamia glabra*) was evaluated against clinical isolates of *Candida albicans* and *C. tropicalis*. The aqueous, ethanol, methanol and chloroform extracts of the dried *karanja* were evaluated for their antifungal activity using standard procedures. Methanol extract showed moderate inhibitory action against *C. albicans*, while methanol and chloroform extract showed significant effect against *C. tropicalis*. Results of the present study suggest that the *karanja* extracts have immense potential to be developed as alternative antifungal agents especially in a situation where available drugs are not effective following development of multidrug resistance in fungi of clinical importance.

**Keywords:** *Karanja, Candida albicans, C. tropicalis*

**INTRODUCTION:** Fungal infestations are emerging as major health hazard in recent times. These infections are usually treated by topical antifungal agents, which include nystatin, miconazole, fluconazole, itraconazole and amphotericin B. Opportunistic fungal infections, mainly resulting from *Candida*, *Cryptococcus*, and *Aspergillus* spp. are life-threatening in immunocompromised patients (with AIDS, cancer, or organ transplant)<sup>1</sup>. However, the management of these infections faces a number of problems including; limited number of effective antifungal agents, toxicity of the available antifungal agents, resistance of fungus to commonly used antifungal drugs, relapse of infections and the high cost of treatment. The difficulties associated with the management of fungal infections necessitate the discovery of new antifungal agents. In Ayurvedic textual references several *dravyas* including *Karanja*<sup>2</sup> has been mentioned as having antimicrobial activity. *Karanja* (*Pongamia glabra*) is a

valuable remedy for a vast range of diseases. According to ayurvedic literature the bark skin juice is a keen stimulant for digestive system and is beneficial in anorexia, piles, worm infestations and flatulence and liver diseases. However, the antifungal and antibacterial activity of *Karanja* was attributed to Pongarotene, a new rotenoid and karanjin, a known flavonol<sup>3</sup>. The present study reports the anticandidal effect of *karanja* on clinically important isolates of candida.

**MATERIALS AND METHODS:**

**Collection and identification of plant material:** Based on the textual references in Ayurveda and the available recent literatures *Karanja* was considered for its antifungal activity in the present study. The authenticity of the drug was identified and confirmed using morphological and anatomical features by Professor N.P.Kaur, Department of Botany, College of Basic Sciences, Punjab Agriculture University, Ludhiana, Punjab. A voucher specimen was deposited at the Herbarium

of the Babe Ke Ayurvedic College and Hospital, Daudhar, Moga, Punjab.

#### **Preliminary phytochemical analysis:**

Preliminary phytochemical analysis like, tests for tannins, alkaloids, saponins, cardiac glycosides, anthroquinone glycosides, steroids, resins and volatile oils was done for qualitative assessment of phytoconstituents as per the standard protocols mentioned in elsewhere<sup>3</sup>.

**Extraction of drug from the plant material:** Extraction of drug from the plant material was done as per the standard procedures published elsewhere. The aqueous extraction of the *karanja* dried powder was done by soxhlet extraction while ethanol, methanol and chloroform extraction was done by cold percolation on magnetic stirrer for 24 hrs. The extracts were first filtered through double layer of muslin cloth and then with Whatman filter paper No.1. The filtrate was air dried under low heat of 50<sup>0</sup> C once entire solvent was evaporated the powder was weighed to calculate the yield and the powder was re-dissolved to appropriate concentration. The extract diluted to predetermined concentration was stored at -20<sup>0</sup> C till further use.

**Fungal strains:** Standard fungal strains *Candida albicans* and *Candida tropicalis* were procured from Institute of Microbial Technology (IMTEC), Chandigarh and Department of Microbiology, Christian Medical College, Ludhiana, while clinical isolation of fungal strains was done from urinary and genital infections at Department of Medical Microbiology, Christian Medical College (CMC), Ludhiana, Punjab. The isolates were identified and characterized based on colony morphology and staining characters as per the standard protocols mentioned in

Berge's Manual of Systematic Bacteriology<sup>4</sup>.

**Testing the antifungal activity of *karanja* extracts:** Antifungal activity of the plant extracts was done against *C. albicans* and *C. tropicalis* following the standard disc diffusion method or dilution method mentioned elsewhere. Sabouraud dextrose agar (SDA) and Sabouraud dextrose broth, were applied for growing and diluting the microorganisms suspensions. Fungal isolates were aseptically inoculated on Petri dishes containing autoclaved, cooled and settled SDA medium. The Petri dishes were incubated at 31<sup>0</sup>C for 48 h to observe white round colonies against yellow background. These were aseptically subcultured on SDA slants. The yeast colonies from SDA slants were suspended in sterilized 0.9% sodium chloride solution (normal saline), which was compared with McFarland solution. One ml of yeast suspension in normal saline was added to 74 ml of sterile medium, to give concentration of 2x10<sup>7</sup> cells/ml.

Antifungal activity of the different extracts of *karanja* was determined by a viable colony count. Nine hundred microliter of a suspension of 10<sup>8</sup> fungi/ml was added to 100  $\mu$ l of plant extract and incubated at 37<sup>0</sup>C in shaker incubator for 60 min. Similarly, positive fungal control was kept in each experiment in which isolates were diluted in DMSO. Following incubation serial tenfold dilution was made in a broth (10<sup>1</sup> through 10<sup>4</sup>) and suspension from the last dilution was spread on to SDA plates and incubated. Following incubation number of colonies in each plate was counted colony forming unit (CFU) per plate. The effectiveness of the plant at killing fungal isolates was expressed as percentage inhibition of colony growth

(i.e. percentage of fungi killed) compared to the control.

**RESULTS AND DISCUSSION:** Results of the qualitative phytochemical analysis for tannins, alkaloids, saponins, cardiac glycosides, steroids: turpenoids and flavonoids confirmed the authenticity of the drugs collected for the present study all the results were in accordance with the previously published standard observations.

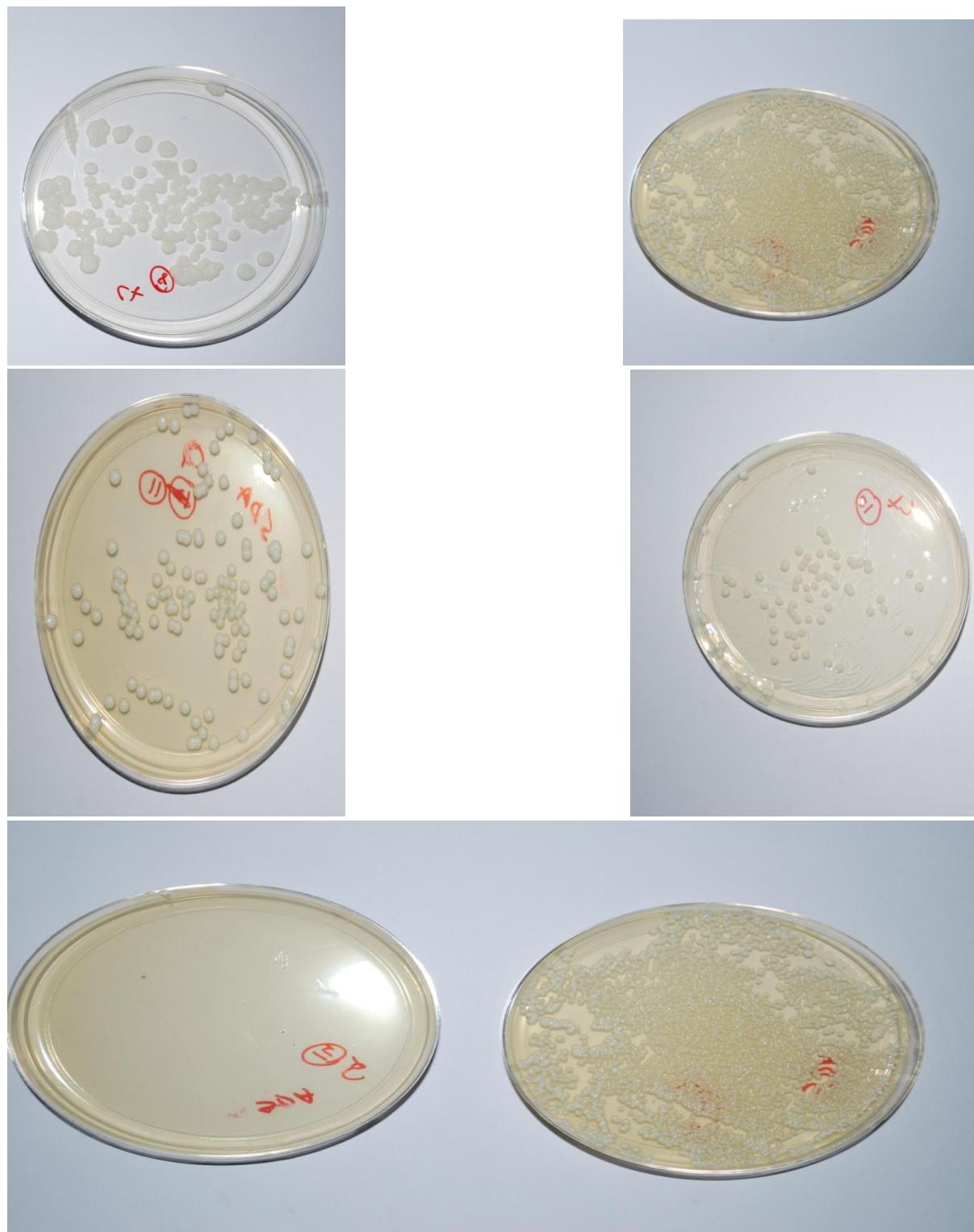
Aqueous and ethanol extract of *karanja* showed no inhibition on both fungal species of isolates *C.albicans* and *C.tropicalis* tested. However, methanol extract showed moderate inhibition of *C.albicans* while showing significant inhibition of *C.tropicalis* fungal isolates. Chloroform extract showed no inhibition of *C.albicans* whereas highly significant inhibition was observed on *C.tropicalis* fungal isolates. Results are presented in Table 1.

**Table: 1 Antifungal activity (percentage inhibition) of different crude extracts of Karanja (*Pongamia glabra*) against clinical isolates of candida spp.**

Sl.No.	Crude extracts	<i>Candida albicans</i>	<i>Candida tropicalis</i>
1.	Aqueous	0%	0%
2.	Ethanol	0%	0%
3.	Methanol	50%	75%
4.	Chloroform	0%	90%
5.	Fungi control	0%	0%
6.	DMSO control	0%	0%

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. A number of medicinal plants, traditionally used for over 1000 years named *rasayana* are present in herbal preparations of Indian traditional health care systems. In the

present study *karanja* (*Pongamia glabra*) was subjected to evaluation for its antifungal activity. Their authenticity was established, phytochemical analysis was performed and crude extracts of drug was prepared with different solvents using standard established extraction protocols. Their activity against clinical isolates of *C.albicans* and *C.tropicalis* was evaluated.



- a. Methanol extract of *Karanja* showing 50% inhibition of *C. albicans*.
- b. Ethanol or water extract showing no inhibition.
- c. Methanol extract of *Karanja* showing 75% inhibition of *Candida tropicalis*

- d. Chloroform extract of *Karanja* showing 90% inhibition of *Candida tropicalis*
- e. Negative control showing 0% inhibition

Candida species are now recognized as a significant cause of hospital-acquired infection. *Candida albicans* is the

organism most often associated with serious fungal infection and it is showing increased resistance to traditional antifungal agents. Recently, non-*C. albicans* species, such as *Candida tropicalis*, *Candida glabrata*, *Candida guilliermondii*, *Candida krusei*, and *Candida parapsilosis*, have also shown dramatic increases in fungal infections and antifungal resistance. The development of resistance in known fungal pathogens and emergence of new fungal pathogens intrinsically resistant to the currently available drugs demonstrate the urgent importance of identifying novel antifungal agents.

In the present study methanol extract showed moderate inhibitory action against *C. albicans* while methanol and chloroform extract showed significant effect against *C. tropicalis*. Earlier studies have revealed that ethanol extract of the drug contains isoliquiritigenin, maackiain, pterocarpin, medicarpin and homopterocarpin<sup>5</sup>. Earlier Simin and others<sup>6</sup> had identified bioactive compounds pongarotene, rotenoid and karanjin from the seeds of *Pongamia pinnata*. Antifungal activity of pure compounds as well as of the methanol and ethyl acetate crude extracts was also reported. Results of the present study suggest that the *karanja* extracts have immense potential to be developed as alternative antifungal agent especially in a situation where available drugs are not effective following development of multidrug resistance in fungi of clinical importance.

#### REFERENCES:

1. Liu, W. D., and C. H. Lian. 2003. The early stage diagnosis of deep-seated fungal infections. Chin. J. Lab. Med. 26:583–584.

2. Caraka Samhita; Commentary by Chakrapani Datta, Edited by Vaidya Yadavaji Trikamaji Acharya and Naryana Rama Acharya, 5<sup>th</sup> Edition 2001, Chaukambha Surabharati Prakashan, Varanasi (C.S.Vi. Ch 7)
3. Trease GE, Evans WC (1983). Textbook of Pharmacognosy. 12th edn. Balliere Tindall and Company Publisher, London. pp. 343-383.
4. Bergey, D.H., Krieg, N.R. and Holt, J.G. 1984. Bergey's manual of systematic bacteriology. Baltimore, MD : Williams & Wilkins, ©1984-©1989.
5. Sangwan, S., Rao, D.V. and Sharma, R.A. A Review on *Pongamia Pinnata* (L.) Pierre: A Great Versatile Leguminous Plant. Nature and Science 2010;8(11):130-139.
6. Simin K., Ali Z, Khaliq-Uz-Zaman SM and Ahmad VU (2002). Structure and biological activity of a new rotenoid from *Pongamia pinnata*. Natural Product Letters, 16: 351–7.

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