

Research Article



ANTIFUNGAL ACTIVITY OF CRUDE AQUEOUS AND METHANOLIC AMCHUR (*MANGIFERA INDICA*) EXTRACTS AGAINST *CANDIDA* SPECIES

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ABSTRACT

The *in-vitro* antifungal activity of crude aqueous and methanolic extracts of amchur (dried pulp of unripe *Mangifera indica*) was evaluated against four *Candida* spp. which are reported to be present in diabetic foot ulcers, by agar-well diffusion method. The crude extracts exhibited potential antifungal activity. The extracts were most effective against *Candida glabrata* (45-47 mm). The MIC values for crude extract ranged between 0.3 and 0.6 mg/ml. The phytochemical analysis of methanolic extract of amchur revealed the presence of tannins and terpenoids. This study depicts that dried pulp of unripe *Mangifera indica* (amchur) possesses very good antifungal activity. Therefore, amchur would be an interesting topic for further study and possibly for reducing fungal load of diabetic foot ulcers.

Keywords: Antifungal activity, Amchur (*Mangifera indica*), *Candida*, Minimum inhibitory concentration, Phytochemical Analysis.

INTRODUCTION

A diabetic foot ulcer is a complication related to diabetes. It is estimated that 15% of all diabetic individuals are affected from diabetic foot ulcers during their lifetime. It is now appreciated that 15-20% of patients with foot ulcers go on to need amputation due to infections, gangrene and subsequent necrosis¹. It was reported in a study conducted by Chellan *et al.*² that mixed fungal and bacterial infections were seen in 21.4% of patients with diabetic foot ulcers, while 58.3% had only bacterial infection and 5.8% had only fungal infection. *Candida* spp. is the most frequent agent in fungal infections³. Clinicians and surgeons treating diabetic foot ulcers suspects only bacterial infections and treat them with antibacterial agents, either due to lack of literature support or due to the assumption there would not be any fungal infections in the diabetic foot ulcers². Resistance among *Candida* spp. to orally administered azole-derivatives has been observed⁴. Conventional medicine is gradually losing its popularity due to concerned raised by resistance to commercial antibiotics. New antimicrobial agents are needed to treat infections caused by drug resistant microorganisms⁵. Natural antimicrobial compounds in spices have been found to possess antimicrobial activity⁶. In order to use spices to reduce fungal load of diabetic ulcers, it is essential that antifungal effects of crude extracts of spices be investigated.

Amchur (dried pulp of unripe *Mangifera indica*) is a common condiment used in Indian cookery. It is used as a souring agent and flavour enhancer. *Mangifera indica* is a commonly used herb in ayurvedic medicine⁷. Mangoes are good source of antioxidants in human diet⁸. Mango pulp extract is reported to be effective in combating oxidative stress and thus helps in wound healing⁹. Amchur is also reported to have antidiabetic activity¹⁰.

Very limited literature is available on antimicrobial activity of amchur extract. Therefore, in the present study aqueous and methanolic extracts of amchur were examined for antifungal activities against four *Candida* spp. which are reported to be frequently involved in diabetic foot ulcer infections¹¹. Phytochemical screening of amchur extract was also subsequently performed.

MATERIALS AND METHODS

Procurement and maintenance of test pathogens

The *Candida* species were procured from Microbial Type Culture collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, which included *C. albicans* (MTCC 3017), *C. glabrata* (MTCC 3019), *C. parapsilosis* (MTCC 1965) and *C. tropicalis* (184). The slants of *Candida* agar (HiMedia, Mumbai) were made to preserve the cultures. All the slants were kept at 4°C in the refrigerator for future studies.

Collection of plant material

Unripe mangoes were collected from local market in Kurukshetra (Haryana, India).

Preparation of plant extract

The pulp of unripe mango (*Mangifera indica*) was dried and powdered in Mixer Grinder to obtain fine dry powder called amchur. The powder was weighed using electronic weighing balance. The herbal extract was prepared at the rate of 25gms/100ml of solvent (Distilled water and Methanol) in a 250mL Erlenmeyer flasks. The flasks were closed with cotton plug and aluminum foil. The spice powder was soaked in solvent for 48h at room temperature with intermittent shaking. The mixture was centrifuged at 7000 rpm for 10min and finally filtered through filter paper. The pellet was discarded and the



supernatant was collected and heat sterilized at 120°C for 30 min. This served as a mother extract. These were stored in the refrigerator at 4°C for testing antimicrobial sensitivity¹².

Antimicrobial Activity Assay

Antifungal activity of aqueous and methanolic extracts was determined by agar-well diffusion method on candida agar medium. Microbial inoculum was spread on the Candida agar plates with a sterile swab and wells were made in the plates using cork borer (8 mm). 100 µl of the extract was placed in the wells made in inoculated plates, the treatments also included 100 µl of DMSO, which served as negative control. All the plates were incubated for 72 h at 27°C and zone of inhibition if any around the wells was measured in mm (millimeter). An antibiotic disc of ketoconazole was used as positive control¹³.

Determination of MIC

A twofold serial dilution of each extract was prepared by first reconstituting the powder in dimethylsulphoxide (DMSO) to achieve a decreasing concentration range of 10mg/ml to 0.01mg/ml. The powder was prepared by concentrating the mother extract in water bath at 60°C until semisolid substance was obtained. This was dried inside the oven under a controlled temperature (45°C) to obtain solid powder¹⁴. A 100 µl volume of each dilution was introduced into wells (duplicates) in the candida agar plates already swabbed with 100µl of microbial inoculum of the test microbial strain. All test plates were incubated

aerobically at 27°C for 72 hrs and observed for the inhibition zones. The lowest concentration of each extract showing a clear zone of inhibition (>8mm), considered as the MIC, was recorded for each test organism¹⁵.

Phytochemical screening of Amchur methanolic extract

The method of Treas and Evans¹⁶ was applied for phytochemical analysis. Methanolic amchur extract was evaluated for the presence of saponins, tannins, steroids, flavanoids, terpenoids and cardiac glycosides.

RESULTS AND DISCUSSION

The antifungal activity of amchur extract against *Candida* has been investigated for the first time in the present study. The data in the present study revealed very promising antimicrobial potential of both the extracts of amchur. Methanolic extract of amchur exhibited maximum activity against *Candida glabrata* exhibiting zone of growth inhibition of 47mm (Table 1). The minimum activity of methanol extract was found against *Candida parapsilosis* with zone of growth inhibition being 35mm. Aqueous extract exhibited no antifungal activity against *Candida tropicalis* while methanolic extract produced 40 mm zone of growth inhibition against the same microorganism. Therefore, in this study methanolic extract was found to exhibit antimicrobial potential as compared to aqueous extract. MIC values for tested pathogens ranged from 0.3 mg/ml to 0.6 mg/ml. *Candida albicans* was found to be most resistant and showed resistance at MIC value of 0.3 mg/ml, at which growth of all other tested pathogens was inhibited.

Table 1: Antifungal activity of aqueous and methanolic amchur extract

Test pathogens	Amchur (aqueous)	Amchur (Methanolic)	Positive control	Negative control
	Zone of growth inhibition (mm)			
<i>Candida albicans</i>	33	45	NA	NA
<i>Candida glabrata</i>	45	47	NA	NA
<i>Candida parapsilosis</i>	42	35	NA	NA
<i>Candida tropicalis</i>	NA	40	34	NA

NA- No Activity

Ketoconazole taken as positive control failed to inhibit growth of *Candida albicans*, *Candida glabrata* and *Candida parapsilosis* while methanolic extract of amchur produced zones of inhibition of 45 mm, 47 mm and 35 mm respectively. The phytochemical screening revealed the presence of tannins and terpenoids in crude methanolic extract of amchur (Table 2). Our results are in agreement with Gupta *et al.*¹⁷ The antifungal activity of amchur may be due to presence of tannins and terpenoids.

It may be concluded that amchur extract may be used as an inexpensive source for reducing microbial infection in diabetic foot wounds by inhibiting antibiotic resistant *Candida* species which are a threat to human health. Spices can serve as an important source for the development of inexpensive, safe and effective medicines. Toxicology studies of amchur extracts,

purification of phytoactive components and *in vivo* trials are required to be carried out so that it can be used as a potential source for the development of phytomedicine for reducing fungal load of diabetic foot ulcers.

Table 2: Phytochemical analysis of methanolic amchur extract

Phytochemical compounds	Results
Tannins	+
Saponins	-
Flavanoid	-
Steroid	-
Terpenoid	+
Cardiac glycosides	-

+ Positive; - Negative



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REFERENCES

1. Pecoraro RE, Reiber GE, Burgess EM. Pathways to diabetic limb amputation. Basis for prevention. *Diabetes Care*. 13, 1990, 13-21.
2. Chellan G, Shivaprakash S, Ramaiyar SK, Varma AK, Varma N, Sukumaran MT, Vasukutty JR, Bal A, Kumar H. Spectrum and Prevalence of Fungi Infecting Deep Tissues of Lower-Limb Wounds in Patients with Type 2 Diabetes. *J. Clin. Microbiol.* 48, 2010, 2097–2102.
3. Celik AD, Yulugkural Z, Kuloglu F, Akata F. *Candida glabrata*: Etiology agent of soft tissue abscess in a diabetic patient. *Ind. Jour. Path. Microbiol.* 53, 2010, 590-591.
4. Odds FC Resistance of yeasts to azole-derivative antifungals. *J. Antimicrob. Chemother.* 31, 1993, 63-471.
5. Zink DL. The impact of consumer demands and trends on food processing. *Emerg. Infect. Dis.* 3, 1997, 446-467.
6. Shelef LA. Antimicrobial Effects of Spices. *J. Food Safet.* 6, 1983, 29-44
7. Shah KA, Patel MB, Patel RJ, Parmar PK. *Mangifera Indica* (Mango). *Phcog Rev.* 4, 2010, 42-48
8. Rocha Ribeiro SM, Queiroz JH, Lopes Ribeiro ME, Campos FM, Pinheiro Santana HM (2007) Antioxidant in mango (*Mangifera indica* L.) pulp. *Plant Foods Hum. Nutr.* 62, 2007, 7-13.
9. Prasad S, Kalra N, Shukla Y. Hepatoprotective effects of lupeol and mango pulp extract of carcinogen induced alteration in Swiss albino mice. *Mol. Nutr Food Res.* 51, 2007, 352-359
10. Agte VV, Mengale SS, Bhonde RR. Potential of Indian spices for their Antidiabetic and Nutraceutical Value. In: Singh V K, Govil J N, Sharma RK (ed) *Phytopharmacology and therapeutic values*. Studium Press Llc, India, 2008, pp. 71-80.
11. Missoni EM, Kalenic S, Vukelic M, De Syo D, Belicza M, Kern J, Babic VV. Role of yeasts in diabetic foot ulcer infection. *Acta Med. Croatica.* 12, 2006, 42-48.
12. Satish S, Raghavendra MP, Raveesha KA. Evaluation of the antibacterial potential of some plants against human pathogenic bacteria. *Adv. Biol. Res.* 2, 2008, 44-48.
13. Ahmad I, Beg AJ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multidrug resistant human pathogens. *J. Ethnopharmacol.* 74, 2001, 113-123
14. Elgayyar M, Draughon FA, Golden DA, Mount JR. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. *J. Food Protect.* 64, 2001, 1019-1024.
15. Rajasekaran C, Meignanam E, Vijayakumar V, Kalaivani T, Ramya S, Premkumar N, Siva R, Jayakumararaj R Investigations on antibacterial activity of leaf extracts of *Azadirachta indica* A. Juss (Meliaceae): a traditional medicinal plant of India. *Ethnobotanical Leaflets* 12, 2008, 1213-1217
16. Treas GE, Evans MC. Textbook of Pharmacognosy 13th ed. Bailliere Tindal London, 1989, pp. 683-684
17. Gupta C, Garg AP, Gupta S. Antimicrobial and Phytochemical Studies of Fresh Ripe Pulp and Dried Unripe Pulp of *Mangifera indica* (Amchur). *Middle-East J. Sci. Res.* 5, 2010, 75-80.

