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 necrosis. It was reported in a study conducted by Chellan el al. 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 infections. 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 that there would not be any fungal infections in the diabetic foot ulcers. Resistance among Candida spp. to orally administered azole-derivatives has been observed. 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 microorganisms. Natural antimicrobial compounds in spices have been found to possess antimicrobial activity. 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 medicine. Mangoes are good source of antioxidants in human diet. Mango pulp extract is reported to be effective in combating oxidative stress and thus helps in wound healing. Amchur is also reported to have antidiabetic activity.

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 infections. 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
superficial 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 sensitivity.

**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 control.

**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 powder. 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 organism.

**Phytochemical screening of Amchur methanolic extract**

The method of Treas and Evans 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>
<thead>
<tr>
<th>Test pathogens</th>
<th>Amchur (aqueous)</th>
<th>Amchur (Methanolic)</th>
<th>Positive control</th>
<th>Negative control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>33</td>
<td>45</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>45</td>
<td>47</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td>42</td>
<td>35</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>NA</td>
<td>40</td>
<td>34</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA: No Activity

<table>
<thead>
<tr>
<th>Phytochemical compounds</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Flavanoid</td>
<td>-</td>
</tr>
<tr>
<td>Steroid</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
</tr>
</tbody>
</table>

+ Positive; - Negative

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. 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,
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REFERENCES


