ORIGINAL RESEARCH

Anti-fungal activity of Morinda citrifolia (noni) extracts against Candida albicans: An in vitro study

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ABSTRACT

Aim: The aim of this study was to investigate the anti-fungal activity of Morinda citrifolia fruit extract on Candida albicans.

Materials and Methods: Juice extract from M. citrifolia fruit was lyophilized and used in anti-fungal testing. Anti-fungal activity of M. citrifolia fruit extract against C. albicans was tested in vitro at various concentrations. The inhibitory effect of M. citrifolia extract on C. albicans was determined by agar culture and applied broth dilution test.

Results: M. citrifolia extract at 1000 µg/ml concentration effectively inhibited the growth of C. albicans (16.6 ± 0.3) compared with the positive control - amphotericin B (20.6 ± 0.6). It was found to be a dose-dependent reaction.

Conclusion: M. citrifolia fruit extract had an anti-fungal effect on C. albicans and the inhibitory effect varied with concentration.

Key words: Anti-fungal, Candida albicans, Morinda citrifolia

Candidiasis is a fungal infection caused by genus Candida involving almost all parts of the body tissue which ranges from mild, localized lesions to severe, systemic ones. Candida albicans is the species most frequently important in causing oral infections. The most common infection of the oropharynx is oral candidiasis.[1] It is one of the most prevalent opportunistic infections affecting the oral mucosa. It is clinically presented in various forms such as angular chelitis, denture stomatitis, hyperplastic (candidial leukoplakia), chronic atrophic (erythematous), median rhomboid glossitis, endocrine-candidiasis syndrome, inflammatory papillary hyperplasia, muco-cutaneous, and pseudomembranous.[2,3] The increasing prevalence of acquired immune deficiency syndrome and other immune-compromised patient groups in the society, the increased usage of broad-spectrum antibiotics, and corticosteroids, commonly occurring endocrine disorders such as diabetes mellitus, and severe nutritional deficiencies have resulted in the revival of oral candidiasis as a relatively common illness in patients.[4] Due to the recently seen increased resistance to anti-fungal agents by these fungi and the adverse effects of the anti-fungal agents currently used in dentistry, there is a need for a safe and efficient alternative option for the prevention and treatment of these infections. Several anti-fungal agents are commercially available in the market, but these agents can alter oral micro-flora and have side-effects such as vomiting, diarrhea and staining of teeth.[5] Hence, the search for alternative natural products with less or no side-effects continues and phytochemicals isolated from plants used as traditional medicines are considered as better alternatives. The WHO in 1998 has estimated that 80% of the people in developing countries use traditional medicine exclusively.[5] Morinda citrifolia commonly known as “Indian mulberry or “noni” was first discovered as a medicinal plant in Southeast Asia, and is now prevalent almost all parts of the world invariably. It is reported to have a broad range of therapeutic effects, including anti-inflammatory, anti-microbial, anticarcinogenic, analgesic, hypotensive effects, and also proved to show immune enhancement in humans.[6-8] The juice of this fruit is used popularly as a medicinal drink and is used for the treatment of different kinds of diseases such as diabetes, hypertension, arthritis, muscle pain, cardiovascular disease, gastrointestinal disturbances, menstrual disorder, and also cancer.[8] It is also used as an immune enhancer and as an antiviral, anti-fungal, antibacterial, and antiparasitic agent. A previous study shows that the beneficial anti-microbial effects of M. citrifolia may result from phenolic compounds abundantly present in the fruit which includes L-asperuloside, acubin, scopoletin,
alizarin, and other anthraquinones. However, there are very few documented reports regarding the anti-fungal activity of M. citrifolia on C. albicans. In an in vitro study, an aqueous extract of the M. citrifolia fruit was shown to interfere with the serum induced morphological conversion of C. albicans from cellular yeast to a filamentous form; therefore, it might have significant therapeutic effects against all the Candida infections. With this background this study was conducted to assess the in vitro effect of M. citrifolia extract on C. albicans.

MATERIALS AND METHODS

Plant material

Source, collection and preparation of extract

Ripe fruits of M. citrifolia were collected from Madurai, a city in the southern part of Tamil Nadu, India. The fruits were cleaned in distilled water and juice was collected. The juice was centrifuged and later lyophilized to dryness to obtain a powder extract. Serial dilutions of extract were prepared (solvent - water) and final concentrations of 1000, 500, 250, 100 µg/ml were obtained for further testing.

Preparation of inoculum

The microbial strain used for this study was procured from the Institute of Microbial Technology, Chandigarh (C. albicans, MTCC 3958). Stock cultures were maintained at 4°C on the slant of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of nutrient broth for fungi that were incubated for 24 h at 37°C. The assay was performed by disc diffusion method.

Disk diffusion method

Anti-fungal activity of the given sample was determined by disk diffusion method on Muller Hinton agar (MHA) medium. The MHA medium is poured in to the petri plate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moisture with the bacterial suspension. The disks were placed on MHA plate with the help of sterile forceps and different concentration (1000 µg, 500 µg, 250 µg and 100 µg) of each samples were loaded on disks. Blank disc impregnated with solvent dimethyl sulfoxide followed by drying off was used as a negative control and amphotericin B (10 µg/disc) used as a positive control. The plates were incubated for 24 h, at 37°C. Then the microbial growth was determined by measuring the diameter of zone of inhibition.

Data were analyzed using independent t-test. The level of significance was set at 0.05.

RESULTS

M. citrifolia extract at 1000 µg/ml concentration effectively inhibited the growth of C. albicans (16.6±0.3) compared with the positive control - amphotericin B (20.6±0.6) [Figure 1]. M. citrifolia showed maximum inhibition at 1000 µg/ml, followed by 500 µg/ml (13.6±0.3), 250 µg/ml (8.6±0.3), and 100 µg/ml (8.3±0.3). The inhibition pattern was seen to increase with the increase in concentration of the extract [Table 1]. Thus, it was the dose-dependent reaction. An independent t-test was done to compare the action of M. citrifolia extract and amphotericin B against C. albicans. The P value was found to be >0.05, it is concluded that there is no significant difference in the mean value between M. citrifolia extracts and amphotericin B against C. albicans [Table 2].

DISCUSSION

The incidence of C. albicans in the oral cavity has been reported to be 45% in neonates, 45-65% in healthy children, 30-45% in healthy adults, 50-65% in people wearing removable dentures, 65-88% in people living in acute and long-term care facilities, 90% in patients with acute leukemia who are undergoing chemotherapy, and 95% in patients with HIV infection.[10]

Our aim was to find a herbal anti-fungal agent, which would effectively replace the commercially available agents. M. citrifolia was chosen because of its well-known

Table 1: Anti-fungal activity M. citrifolia fruit extract

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>1000 µg</th>
<th>500 µg</th>
<th>250 µg</th>
<th>100 µg</th>
<th>DMSO</th>
<th>Amphotericin B 100 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>16.6±0.3</td>
<td>13.6±0.3</td>
<td>8.6±0.3</td>
<td>8.3±0.3</td>
<td>-</td>
<td>20.6±0.6</td>
</tr>
</tbody>
</table>

C. albicans=Candida albicans, M. citrifolia=Morinda citrifolia, DMSO=Dimethyl sulfoxide
Morinda citrifolia extracts against candida albicans

Table 2: Comparison of anti-fungal activity of amphotericin B and M. citrifolia extracts on C. albicans (independent t test)

<table>
<thead>
<tr>
<th></th>
<th>t</th>
<th>df</th>
<th>Significant (2-tailed)</th>
</tr>
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<tbody>
<tr>
<td>Amphotericin B 100 µg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equal variances assumed</td>
<td>2.439</td>
<td>1</td>
<td>0.64*</td>
</tr>
<tr>
<td>Equal variances not assumed</td>
<td>2.94</td>
<td></td>
<td>0.75*</td>
</tr>
<tr>
<td>M. citrifolia extract 1000 µg</td>
<td>1.98</td>
<td>1</td>
<td>0.52*</td>
</tr>
<tr>
<td>Equal variances assumed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equal variances not assumed</td>
<td>2.3</td>
<td></td>
<td>0.61*</td>
</tr>
</tbody>
</table>

P>0.05. M. citrifolia=Morinda citrifolia, C. albicans=Candida albicans

anti-microbial and therapeutic properties. It is said to be an underutilized, miracle plant found growing naturally in all types of lands right from the sea coast to interior, even without proper care and management and is now being cultivated by the farmers as a crop in different parts of India. [11]

Previous studies have claimed that the M. citrifolia fruit extract shows anti-fungal effects on Candida infections. [9,11] However, scientific studies on the anti-fungal properties of M. citrifolia are scarce.

The major micro nutrients identified in the M. citrifolia plant are phenolic compounds, alkaloids and organic acids. Of these phenolic compounds, the most commonly reported are anthraquinones (damnacanthal, morindone, morindin, etc.), aucubin, asperuloside and scopoletin. The main organic acids are caproic and caprylic acids and the mainly reported alkaloid is xeronine. [11] The results of a previous study done by Banerjee et al. indicate that noni contains water soluble components that interfere with the conversion of C. albicans morphologically and thus may have potential therapeutic value against candidiasis. [9]

Results obtained from our study were similar to other studies, which showed that M. citrifolia fruit extract had an effect on C. albicans in vitro. [9,12] except for one study done by Jayaraman et al. where the results were contradictory. [13] This discrepancy may be explained by the differences in the methodology. In his study, dried fruits were used whereas in our study, ripe fruits were used. It is reported that the anti-microbial property of M. citrifolia increases as the fruit ripens. [13]

The results of our study indicate that M. citrifolia extract shows anti-fungal effects on C. albicans and the inhibitory effect varies with concentration. The inhibitory effect has shown to increase as the concentration of the extract increased. A double-blinded controlled trial was conducted to test the toxicity of fruit and the results proved that up to 750 ml of the fruit juice taken per day was shown to have no toxic effects and was well-tolerated by the humans [14] and reports also show widespread consumption of the fruit in several parts of the world for over several centuries without any known side-effects. [15] Therefore, oral therapeutic anti-fungal preparations can be formulated from higher concentrations of M. citrifolia fruit extracts to treat oral candidiasis, which can occur in patients with long term use of antibiotics, in immune-compromised hosts and in patients undergoing chemotherapy. [16]

As with other studies, this study also has its limitations as it is an in vitro study. Further in vivo studies have to be conducted to check the safety, tolerance, and cost-effectiveness of the extract. Other important factor that needs to be analyzed is that, M. citrifolia is a natural plant and the active ingredients may not always be stable and the exact mechanism of action against Candida is also not explained. Thus, further studies should confirm the action of the active ingredients present in M. citrifolia fruit in the inhibition of candidal growth and also the dosages and formulations as an oral anti-fungal agent.

REFERENCES