Antifungal activity of *Morinda citrifolia* fruit extract against *Candida albicans*

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Objective. The objective of the study was to investigate the antifungal activity of *Morinda citrifolia* fruit extract on *Candida albicans*.
Materials and methods. Juice extract from *M. citrifolia* fruit was lyophilized and used in antifungal testing. Antifungal activity of *M. citrifolia* fruit extract against *C. albicans* was tested in vitro at various concentrations and for different contact times. The inhibitory effect of *M. citrifolia* extract on *C. albicans* was determined by cultures and an applied broth dilution test.
Results. Using cultures, growth of *C. albicans* was not detected with 50 mg/mL of extract at 30-minute contact time or with 60 mg/mL of extract at 15-minute contact time. By the broth dilution test, the minimum fungicidal concentration of extract against *C. albicans* was 40 mg/mL at 90-minute contact time or with 50 mg/mL at 15-minute contact time.
Conclusion. *M. citrifolia* fruit extract had an antifungal effect on *C. albicans* and the inhibitory effect varied with concentration and contact time. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:394-398)**

Morinda citrifolia is commonly known as "Indian mulberry," "cheese fruit," or "noni." It is called "Yor" in Thailand and can be grown in all parts of the country. *M. citrifolia* was first discovered as a medicinal plant in Southeast Asia and the subcontinent and is widely used for this purpose. The *M. citrifolia* plant is reported to have a broad range of therapeutic effects, including antimicrobial, anti-inflammatory, anticancer, analgesic, and hypotensive effects and immune enhancement.[1-10] All of the parts of *M. citrifolia* (root, bark, leaf, bud, and fruit) have been used to treat a wide range of health problems.[11] *M. citrifolia* fruit is oval in shape and will turn from a greenish hue to a yellowish-white color when it ripens (Fig. 1). It has a bitter taste and a pungent smell. The fruit contains polysaccharides, scopoletin, proxeronine, vitamins, and minerals.[11-13] Southeast Asians and Pacific Islanders consume the fruit as food, either raw or cooked. The juice of this fruit is popular as a medicinal drink and is used to treat different kinds of illnesses such as muscle pain, arthritis, diabetes, hypertension, cardiovascular disease, menstrual disorders, gastrointestinal disturbances, and cancers.[6,14] It is also used as an immune stimulant and as an antibacterial, antiviral, antiparasitic, and antifungal agent.[6,10,11,15]

Previous studies have shown the *M. citrifolia* plant has antimicrobial activities. Its beneficial antimicrobial effects may result from components such as phenolic compounds including acubin, L-asperuloside, alizarin, scopoletin, and other anthraquinones.[16] Recently, Murray et al.[17] tested *M. citrifolia* juice as an endodontic irrigant. These authors showed an antimicrobial effect against *Escherichia faecalis* and also demonstrated *M. citrifolia* juice was more effective than chlorhexidine gluconate and saline for the removal of the smear layer. Bushnell et al.[1] and Locher et al.[3] reported antimicrobial properties of *M. citrifolia* fruit extract against several species of bacteria, viruses, and fungi. However, there are very few documented reports regarding the antifungal activity of *M. citrifolia* on *Candida albicans*. In an in vitro study, the fruit extract of *M. citrifolia* was shown to interfere with the serum-induced morphological conversion of *C. albicans* from cellular yeast to the filamentous form,[18] therefore it might have a potential therapeutic value against candida infections.

Candida is the predominant commensal fungus inhabiting the human oral cavity. *C. albicans* is one of the many oral *Candida* species and is responsible for most oral candidal infections.[19,20] The prevalence of *C. albicans* has been reported to vary from 3% to 78% in healthy, dentate subjects.[21] The prevalence and density

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Fig. 1. The ripe fruits *of M. citrifolia*.

of *C. albicans* was greater in healthy denture wearers and in patients with denture stomatitis as compared with healthy dentate subjects.[21-23] More than 90% of the *Candida* found in 11 cases of candidal leukoplakia were identified as *C. albicans*.[21] Systemic and local factors which reduce host resistance will promote the transition of *Candida* from commensalism to parasitic forms. The aforementioned factors include malnutrition, endocrinologic disorders, medications, malignancies, immunopathies, xerostomia, trauma, and poor denture hygiene.[24-26] Medications associated with the emergence of clinical candidiasis are anticholinergic agents, antibiotics, corticosteroids, and immunosuppressives.[24,26] Oral candidiasis is commonly reported in denture wearers, immunocompromised patients, and patients being treated with radiotherapy and chemotherapy.[24-26] Also, candidiasis sometimes occurs following the treatment of oral lesions with topical steroids.[27,28] Treatment with antifungal agents is costly and systemic administration sometimes causes liver and kidney toxicity[29] and long-term therapy may result in resistance. Plants that are traditionally used in the treatment of fungal infections could be a good source of a new, safe, and inexpensive antifungal drug. In spite of the broad use of *M. citrifolia* as a traditional medicine, there is limited scientific support for its various uses including the treatment of fungal infection. Therefore, the aim of this study was to investigate the antifungal activity of *M. citrifolia* fruit extract against *C. albicans* in vitro.

MATERIALS AND METHODS

Preparation of *M. citrifolia* fruit extract

Ripe fruits of *M. citrifolia* were cleaned, rinsed with distilled water, and air-dried. The fruits were collected in a sterile glass jar for 2 to 3 days to allow the juice to seep out. The juice was centrifuged twice at 1000 g for 15 minutes. The supernatant was lyophilized to dryness and the powder was kept in the refrigerator at 4°C and analyzed within 3 months. Stock solution of extract was prepared by adding 20 mL of 0.85% sterile saline solution into 1200 mg of dry powder, resulting in a concentration of 60 mg/mL. This solution was sterilized by filtration through a 0.45-μm millipore-filter. Serial dilutions of extract were prepared and final concentrations of 60, 50, 40, 30, 20, and 10 mg/mL were obtained for further testing.

Antifungal property of *M. citrifolia* fruit extract on *C. albicans*

The antifungal activity of *M. citrifolia* extract against *C. albicans* was tested in vitro at various concentrations (10, 20, 30, 40, 50, and 60 mg/mL) and for different contact times (15, 30, 45, 60, 75, and 90 minutes). A pure culture of *C. albicans* (strain ATCC 9028) was used in the experiment and prepared as follows: *C. albicans* from stock was incubated in Sabouraud dextrose broth (SDB) for 24 hours at 37°C and then yeast cells were grown on a Sabouraud dextrose agar (SDA) dish for 48 hours at 37°C. An inoculum suspension was prepared by selecting 5 colonies of *C. albicans* and suspending them in 5 mL of SDB. After incubation for 24 hours at 37°C, the suspension was vibrated and the cell density was adjusted with a spectrophotometer by adding sufficient sterile saline to increase the transmittance to that produced by a 0.5 McFarland standard at a 530-nm wavelength. The inoculum suspension was diluted until an optical density of 0.11 was obtained, resulting in a suspension of 10^5 cells/mL of yeast cells. The inoculum size of 50 μL was verified by the enumeration of colony forming units (CFUs) obtained by subculture on SDA. The resulting standardized sample of 50 μL of *C. albicans* was inoculated into 1.5 mL of fruit extract at various concentrations (10, 20, 30, 40, 50, and 60 mg/mL), and incubated at room temperature for the designated contact times (15, 30, 45, 60, 75, and 90 minutes). For the *Candida* cultures, 50 μL from undiluted and diluted (1:10) inoculum suspension was spread on each SDA dish. After incubation at 37°C for 24 hours, each agar plate was evaluated for candida growth and the number of CFUs was counted. Fruit extract-free agar plates were included as positive controls in each set of experiments. For each concentration and each contact time, the experiment was done in triplicate. The number of CFUs on each plate was compared with the number of CFUs on the positive control plate. The criteria for an inhibitory effect were set as follows: no inhibition was noted if the number of CFUs on the test plate was not lower than the number

Table I. The inhibitory effect of *M. citrifolia* fruit extract on *C. albicans* at various concentrations and for different contact times

	Concentrations of fruit extract, mg/mL					
Contact times, min	*10*	*20*	*30*	*40*	*50*	*60*
15	33800 (12458)	37000 (5724)	28400 (8335)	14667 (12709)	67 (67)	0
30	28067 (6901)	33933 (8456)	29067 (11685)	3933 (2198)	0	0
45	35533 (3142)	33600 (7621)	20133 (8642)	400 (115)	0	0
60	26733 (6896)	29600 (5974)	18733 (8002)	133 (67)	0	0
75	25133 (6181)	26800 (4772)	15733 (7681)	533 (291)	0	0
90	22467 (4339)	25800 (6512)	17867 (9395)	67 (67)	0	0

Data expressed as a mean value (standard error) in colony-forming units (CFUs).
For positive control plates, the candida count was 21475 (687) CFUs.

of CFUs on the positive control plate. Partial inhibition was recorded if the number of CFUs on the test plate was lower than the number of CFUs on the positive control plate. Complete inhibition was documented if no CFU of *C. albicans* was detected on the test plate.

The minimum fungicidal concentration (MFC) of *M. citrifolia* fruit extract was determined by an applied broth dilution test. After exposure to fruit extract at the designated concentrations and contact times, 100 μL of the resulting suspension was transferred into 900 μL of SDB and incubated at 37°C for 24 hours. The turbidity of the broth was estimated visually and the MFC was defined as the lowest concentration of fruit extract that showed no growth.

Table II. The results of the applied broth dilution tests of *M. citrifolia* fruit extract on candida growth at various concentrations and for different contact times

	Concentrations of fruit extract, mg/mL					
Contact times, min	*10*	*20*	*30*	*40*	*50*	*60*
15	+	+	+	+	–	–
30	+	+	+	+	–	–
45	+	+	+	+	–	–
60	+	+	+	+	–	–
75	+	+	+	+	–	–
90	+	+	+	–	–	–

+, positive culture; –, negative culture.

RESULTS

Table I demonstrates the inhibitory effect of *M. citrifolia* fruit extract on *C. albicans* at various concentrations and for different contact times. *M. citrifolia* fruit extract at concentrations of 10 and 20 mg/mL showed no inhibitory effect on candida growth at all contact times. For the *M. citrifolia* fruit extract at a concentration of 30 mg/mL, no inhibitory effect was detected at contact times less than 45 minutes. At all higher concentrations, *M. citrifolia* fruit extract demonstrated an inhibitory effect. *M. citrifolia* fruit extract at a concentration of 40 mg/mL showed a partial inhibitory effect on candida growth at all contact times. *M. citrifolia* fruit extract at a concentration of 50 mg/mL partially inhibited candida growth at 15-minute contact time and completely inhibited candida growth at contact times of 30 minutes and over. *M. citrifolia* fruit extract at a concentration of 60 mg/mL showed a complete inhibitory effect on candida growth at all contact times.

Table II shows the results of the applied broth dilution tests of *M. citrifolia* fruit extract on candida growth at various concentrations and for different contact times. Turbidity (positive culture) was observed in the test tubes containing fruit extract at concentrations of 10, 20, and 30 mg/mL at all contact times. For the concentration of 40 mg/mL, turbidity was also observed in all test tubes at contact times of 15, 30, 45, 60, and 75 minutes, but no turbidity (negative culture) was observed in the test tubes at 90-minute contact time. Therefore 40 mg/mL of extract is defined as the MFC at 90-minute contact time. At concentrations of 50 and 60 mg/mL, no turbidity was seen in any of the test tubes for all contact times. Therefore 50 mg/mL of fruit extract was defined as the MFC at 15-minute contact time.

DISCUSSION

Previous reports have claimed that fruit extract of *M. citrifolia* exerts an antifungal effect on candida infections. However, scientific studies on the antifungal properties of *M. citrifolia* are scarce. Oral candidiasis is common in denture wearers, the elderly, and in HIV-infected patients. Secondary candidiasis also occurs in patients with long-term use of broad-spectrum antibiotics, corticosteroids, and immunosuppressants. In these cases, antifungal agents are often prescribed. Our goal was to find an herbal plant that may serve as an antifungal agent. *M. citrifolia* was chosen for testing because it is a readily available plant in Southeast Asia and it is well

known for its medicinal properties in this region. The ripe fruit of *M. citrifolia* was used because it has been reported that the antimicrobial effect is greater when it ripens.[1,16] *C. albicans* was selected as the organism to be tested because it is the most common species in oral candida infections. The results of this preliminary in vitro study showing the inhibitory effect of *M. citrifolia* fruit extract against *C. albicans* suggest that it may be an effective agent when applied to a clinical situation. We have also varied both the concentration and contact time in our tests to obtain the lowest concentration of *M. citrifolia* fruit extract and shortest contact time that demonstrated an inhibitory effect.

Our findings are in agreement with those of Banerjee et al.[18] who showed that *M. citrifolia* fruit extract had anticandidal activity in vitro. Recently, Jayaraman et al.[30] performed an in vitro study to test the antifungal activity of *M. citrifolia* fruit extract. They found the highest percentage of inhibition against *C. albicans* was very low (8%) and concluded *M. citrifolia* fruit extract showed no significant activity against *C. albicans*. Relative to our findings, this discrepancy may be explained by differences in the materials and methods used. In the contradictory Jayaraman et al.[30] study, dried fruits were used and extracted using 3 solvents (methanol, ethyl acetate, and hexane).[30] The active compounds that exert anticandidal activity may not have been effectively dissolved in the solvents used. Also, the concentration of extract used (1 mg/mL) was much lower than the concentrations used in our study. Our data showed the minimum concentration of extract had to be at least 30 mg/mL and above 45-minute contact time to show partial inhibition.

The results of this study confirm that *M. citrifolia* fruit extract exerts an antifungal effect on *C. albicans* and the inhibitory effect varies with the concentration and contact time. The higher the concentration of fruit extract and the longer the contact time, the higher is the inhibitory effect. The findings as reported in the present study indicate a strong potential therapeutic value of *M. citrifolia* fruit extract against in vivo candida infections. Oral therapeutic products derived from *M. citrifolia* fruit extract should be safe for use in clinical applications; this is supported by reports indicating that in spite of wide consumption of *M. citrifolia* fruit in several countries for centuries, no serious side effects have occurred.[6,11] Also, our pilot study showed no toxic effect of *M. citrifolia* fruit extract on fibroblastic cells in tissue culture (Jainkittivong and Swasdison, 2008, unpublished data).

M. citrifolia fruit extract may be used to prevent and/or to treat oral candida infections. In clinical applications, *M. citrifolia* fruit extract may be used as a mouthwash or as a topical medication. It may also be useful as disinfectant for dentures. However, the toxicity of *M. citrifolia* fruit extract on the oral mucosa should be investigated if a topical application is to be formulated. The results of our study indicate that doses above 40 mg/mL demonstrate candicidal activity and may be tested in clinical applications. However, the contact times showing antifungal properties in our experiments may not be practical in some clinical situations and more advantageous in other applications such as under dentures and for soaking prostheses. Shorter contact times may be achieved by increasing the concentrations of the extract. However, the effects of our tested concentrations on the oral mucosa and higher concentrations require further clinical study.

In our studies, some limitations should be mentioned: although the *M. citrifolia* fruit extract exhibited antifungal activity, it should be noted the present testing was limited to only one strain of *C. albicans.* Studies to evaluate the applicability of *M. citrifolia* fruit extract on other strains as well as on clinical isolates of *C. albicans* need further verification. *M. citrifolia* is a natural plant, thus the concentration of active compounds in each fruit may not be consistent at all times. Also, the fruit extract used in the present study was a crude extract, thus the active compounds that exert antifungal properties are still unknown. Future research is warranted to identify the active compounds that inhibit candida growth as well as appropriate dosages and formulations for clinical use.

CONCLUSION

M. citrifolia fruit extract has an antifungal effect on *C. albicans* and the inhibitory effect varies with concentration and contact time. Doses above 40 mg/mL might be useful for clinical applications. The results of this study indicate the potential application of *M. citrifolia* fruit extract as an antifungal agent.

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