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Systematic Review: Antifungal Efficacy of *Morinda Citrifolia* Against *Candida Albicans*

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Abstract

Candidiasis, caused by *Candida species*, remains a significant global health concern, with oral and vulvovaginal forms prevalent among immunocompromised populations and women, respectively. Increasing antifungal resistance, particularly among *non-albicans* species, underscores the need for novel therapeutic options. This systematic literature review assesses the antifungal efficacy of *Morinda citrifolia* (noni fruit) against *Candida albicans*. Utilizing the PRISMA protocol, we analyzed studies from Scopus, ScienceDirect, PubMed, and Google Scholar databases published between 2014 and 2024. Seven in vitro studies were included, examining the inhibitory effects of *M. citrifolia* extracts on *C. albicans*. Findings indicate that *M. citrifolia* exhibits significant antifungal properties, attributed to its bioactive compounds such as scopoletin and flavonoids, which disrupt fungal cell membranes. The effectiveness of *M. citrifolia* is concentration-dependent, with higher concentrations yielding greater antifungal activity. Given the increasing resistance to conventional antifungals, in vitro studies suggest that *M. citrifolia* may be a promising alternative, warranting further *in vivo* and clinical investigations to substantiate its therapeutic potential.

Keywords: Candidiasis, *Morinda citrifolia*, Antifungal

1. Introduction

An opportunistic infection of the *Candida species* can lead to candidiasis, which can affect various body parts, such as vagina, oral cavity, and invasive, each with distinct epidemiological characteristics and risk factors. Untreated cases can result in a systemic infection that can lead to sepsis.[1] Oral candidiasis often attacks people with impaired immune systems, such as 82% of HIV patients and other blood cancers.[2–5]

Vulvovaginal candidiasis is a common condition that affects many women around the world. *Candida* infections are to blame for about one out of every three cases. Diabetes and hormonal birth control are two other things that can raise your risk.[6,7] It can be hard to treat this condition when it keeps coming back, which can happen for years at a time.[7] The epidemiology of this condition shows a move toward species other than *C. albicans*. This includes *Candida glabrata*, which is known to be resistant to antifungal drugs.[8,9] Patients who are having medical procedures or have underlying conditions can get invasive candidiasis, which is a very serious infection.[10] It affects babies and kids and causes a lot of sickness. Invasive candidiasis can be hard to treat because new species are coming up and fungi are becoming resistant to antifungal drugs.[11,12]

According to the Infectious Diseases Society of America (IDSA), treatment of candidiasis is based on its location and severity. In mild or topical cases, the therapy used is the nystatin or miconazole group. While in systemic cases, echinocandin and fluconazole can be used.[13–15] In cases of newborn candidiasis, amphotericin B can be used because of its minimal side effects.[16,17]

The development of antifungals, especially from natural ingredients, continues to increase due to the increasing cases of resistance to existing antifungals such as the

azole group. Various antifungals are developed from natural ingredients derived from plants or honey, which come from materials that are easier to find and cheaper. The development of this new antifungal aims to see its effectiveness in inhibiting the development of pathogenic fungi.[18–20]

Morinda citrifolia of the *Rubiaceae* family[21] is a plant that grows up to six meters tall and originates from Southeast Asia.[22,23] This fruit is commonly used as an alternative medicine by the community.[23,24] Various components of the plant, such as fruit, flowers, and leaves, are rich in natural compounds that can act as therapeutic agents. The secondary metabolites of *M. citrifolia* consist of various compounds, such as terpenoids, anthraquinones, flavonoids, and saponins.[25–27] These substances are known to contribute to its antimicrobial, anti-inflammatory, antifungal, and antioxidant properties. The ripeness of the fruit can increase its antioxidant capacity.[28] The high levels of ascorbic acid in noni fruit make it an antioxidant and anti-inflammatory. Also, when it comes into contact with Iridoid substances like deacetylasperside acid, it can start to work against fungi and bacteria.[29]

The antifungal properties of *M. citrifolia* come mostly from its bioactive compounds, such as eugenol and scopoletin, which have been shown to have strong effects against *C. albicans*. Researchers have looked into how well *M. citrifolia* works as an antifungal by comparing it to other antifungals. They have done this by looking at different extracts and fractions, as well as the concentration and exposure time.[23,29,30] Based on this, this systematic literature review study aims to determine the effectiveness of *M. citrifolia* as an antifungal in inhibiting the development of *Candida albicans* from various in vitro studies that have been conducted.

2. Method

A systematic literature review study was conducted according to the PRISMA protocol. Papers published from databases such as Scopus, ScienceDirect, PubMed, and Google Scholar were included based on inclusion criteria from 2014 to 2024. We searched and collected data using search terms using Boolean Operators with keywords, "*Morinda citrifolia* OR noni's fruit'' AND "antifungal OR natural antifungal" AND "*Candida albicans*". The inclusion criteria of this study were (1) the population was *Candida albicans* strain; (2) the intervention carried out was the use of *M. citrifolia* fruit or leave extract; (3) the comparison was made between *M. citrifolia* extract and positive control (standard conventional antifungal); (4) the results were in the form of the effectiveness of *M. citrifolia* extract in inhibiting the growth of *Candida albicans*; and (5) the study design chosen was an in vitro study. We excluded articles with the following criteria: (1) duplication; (2) published not in English; (3) irrelevant titles and/or abstracts, reviews, comments, dissertations not published in journals, incomplete data, and poor availability; (4) the desired outcome was not reported; and (5) outside the specified time frame, namely 2014-2024. The following details were extracted from each study: (1) first author; (2) year of publication; (3) country of study; (4) origin of *M. citrifolia*; (5) parameters used; and (6) outcomes. Quality assessment was conducted by two authors assigned to evaluate all articles found in this database. They then checked the titles, abstracts, and full texts of the articles before excluding unrelated studies. Disagreements between the selected papers were resolved through discussion and interaction with a third author.

3. Result

Literature search was conducted using four electronic databases, namely Scopus, Science Direct, PubMed, and Google Scholar.

The search results yielded 468 citations. After removing duplicates, screening based on title, abstract and full text and assessment based on eligibility criteria, 7 papers were obtained to be analyzed in a systematic review. The complete descriptive literature search has been outlined in the PRISMA flowchart in Figure 1.

In vitro research on the potential of *Morinda citrifolia* in inhibiting *C. albicans* was conducted mostly in 2020. Most of these studies were conducted in Indonesia, Brazil and India, with 2 studies each. Of the 7 studies conducted, the part of *M. citrifolia* that was most widely used as research material was the fruit, with various concentrations.

The measurement method used in some of these studies, the majority (5 studies) used the method of measuring the diameter of the inhibition zone. However, there were two studies that used cell viability and fungal cell death as well as minimum inhibitory concentration (MIC).

There are various strains of *C. albicans* used in the study such as ATCC, MTCC, cEC and UFPEDA. The *M. citrifolia* extract solvents used in some of these studies varied from ethanol, methanol, n-hexane, acetone and distilled water. But the most widely used are ethanol and methanol.

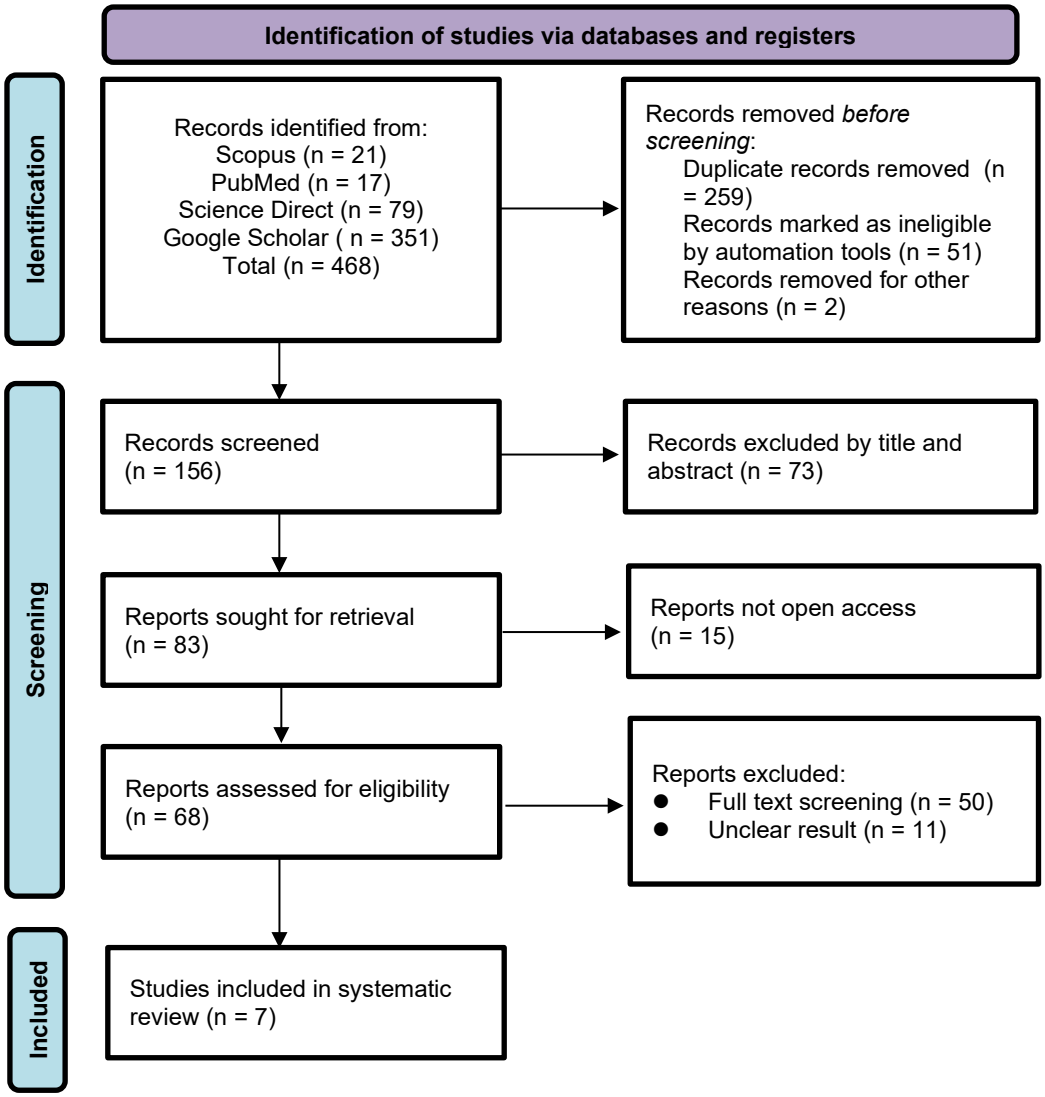

Figure 1. PRISMA flowchart of the study the selection process of included studies

Table 1. Characteristics study included in the study

Author, Year	Country	Population	Study Design	Intervention	Comparison	Outcome
Barani K, et al.[31] 2014	India	*C. albicans*, MTCC 3958	In vitro	Fruit extract of *Morinda citrifolia* at concentrations 100 µg/ml, 250 µg/ml, 500 µg/ml, 1000 µg/ml	Amphotericin B	At a concentration of 1000 µg/ml, the extract produced an inhibition zone of 16.6 ± 0.3 mm, which is close to the effectiveness of amphotericin B (20.6 ± 0.6 mm) used as a positive control. At a concentration of 500 µg/ml, the inhibition zone produced was 13.6 ± 0.3 mm. At a concentration of 250 µg/ml, the inhibition zone produced was 8.6 ± 0.3 mm. At a concentration of 100 µg/ml, the inhibition zone produced was 8.3 ± 0.3 mm.
Simatupang OC, et al.[32] 2017	Indonesia	*C. albicans*	In vitro	Leaves ethanol extract of *Morinda citrifolia*	Ketoconazole	The inhibition zone diameter of *M. citrifolia* leaf extract was 16.0 mm and the inhibition zone diameter of ketoconazole was 19.5 mm
Singh M, et al.[30] 2019	India	*C. albicans*, ATCC 29212 and ATCC 90028	In vitro	Fruit extract of *Morinda citrifolia* at concentrations of 100%, 3%, 1,5%, 0,75%.	Chlorhexidine	*Morinda citrifolia* juice in its different concentrations, when tested against *C. albicans*, showed larger zones of inhibitions at higher concentrations (100%) (2 mm). There was no inhibition for (0.75%, 1.5%). At concentration (3%) (2mm), some antimicrobial effectiveness was seen. Chlorhexidine (17-26 mm)
Hardani R, et al.[33] 2020	Indonesia	*C. albicans*	In vitro	*M. citrifolia* fruit extract with various solvents	Nystatin	The diameter of the inhibition zone obtained was extract with distilled water solvent of 3.4 cm, extract with acetone solvent of 6.72 cm, extract with n-hexane solvent of 6.08 cm, while the positive control of nystatin was 4 cm

Miguel CB, et al.[23] 2022	Brazil	*C. albicans*, cEC 1291	In vitro	*M. citrifolia* fruit extract	Amphotericin B	Noni extract significantly reduced the viability of THP-1 cells (human monocyte cells), with a decrease of about 25% after 12 hours and reaching 67% after 36 hours. Noni extract showed strong fungicidal activity, with 99.99% C. albicans cell death achieved after 6.82 hours of exposure at certain concentrations (1:1 and 1:2)
Colmenares SMM, et al.[29] 2024	Peru	*C. albicans*	In vitro	*Morinda citrifolia* methanolic extract from each fruit part	Chlorhexidine	*Morinda citrifolia* pulp methanolic extract presented the lower antifungal activity with average inhibition halos of 11.56 mm, whereas its seed methanolic extract presented higher antifungal activity with average inhibition halos of 15.94 mm, the highest average antifungal activity was obtained by 0.12% chlorhexidine (control) with 22.13 mm
Holanda L, et al.[34] 2020	Brazil	*C. albicans*, UFPEDA 1007	In vitro	*M. citrifolia* fruit methanolic extract	Metronidazole and fluconazole	MICs values of *M. citrifolia* ranging from 39 µg.mL−1. MICs values for the esterified essential oil of *M. citrifolia* 1250 µg.mL−1. Metronidazol (10 µg.mL−1) and Fluconazol (50 µg.mL−1)

From the 7 studies, it was found that the diameter of the inhibition zone of *M. citrifolia* extract ranged from 2 mm to 67,2 mm. With a MIC value of 39 µg.mL−1. This means that *M. citrifolia* extract has a smaller inhibition zone diameter when compared to amphotericin B, ketoconazole and chlorhexidine. However, it is larger than nystatin. Likewise with MIC, *M. citrifolia* extract has a higher MIC than metronidazole but lower than fluconazole.

The characteristics of the studies and the results information have been summarized in Table 1. Our analysis includes 9 studies to determine the inhibitory power of *M. citrifolia* against *C. albicans* in vitro, with an average of the most studies conducted in Indonesia, which is one of the countries in Southeast Asia, namely 4 studies in the period 2014-2024.

4. Discussion

Candida albicans is an important component of our normal flora, but under certain conditions *Candida* can become pathogenic. *Candida* is known to trigger various infections, including systemic and life-

threatening cases. The ability of *Candida* to evolve in various forms, including yeast, hyphae, and pseudohyphae, contributes to its adaptability and pathogenicity in various environments.[35–37] Antifungal susceptibility testing is crucial for effective treatment, as different *Candida species* exhibit varying levels of resistance to antifungal agents. *C. albicans* generally remains susceptible to azoles and echinocandins, but resistance patterns are evolving, complicating treatment strategies. This has prompted the development of new antifungal agents from natural sources[38,39]

In the study of the characteristics of several studies above, it was found that research on *M. citrifolia* was mostly conducted in Indonesia. This is in accordance with the theory that *M. citrifolia* is most commonly found in Southeast Asia.[22,23] Based on several studies above, it was found that *M. citrifolia* has been proven to have strong inhibitory power against *Candida albicans*. The strength of the inhibitory power depends on the concentration of *M. citrifolia* extract given. The higher the concentration of the extract used, the higher the inhibition power. This can be seen in a study conducted by Singh et al., where no inhibition zone was formed at low concentrations.[30] The diverse array of compounds found in *M. citriifolia* makes it suitable for diverse applications. These include terpenoids, phenolics, flavonoids, anthraquinone and terpenoids, among others.[22,24] Its bioactive constituents contribute to its usual utilization in a wide range of therapeutic applications, such as antioxidant, antimicrobial, anti-inflammatory, and antifungal.[40–42]

The inhibition zone demonstrates the *M. citrifolia* extract's ability to suppress *C. albicans*. The presence of secondary metabolite chemicals from the extract is what causes the inhibition zone to form around the well. Based on the diameter of the inhibition zone, Davis and Stout have classified inhibitory activity into four categories: (1) very strong (>20 mm), (2) strong (11-20 mm), (3) moderate (5-10 mm), and (4) weak (<5 mm).[32] Because acetone solvent is semi-polar, it may draw in both polar and non-polar antifungal substances, including flavonoids, alkaloids, saponins, phenolics, and terpenoids. This is why using it to extract noni fruit produces positive results.[33]

M. citrifolia totally stopped *C. albicans* cells from growing for eight hours in the Miguel et al. investigation, but after six hours, the cells resumed their regular linear development. This might be because the cells resumed their normal development after the initial effect, and ½ MIC represents a sub-lethal dose. Therefore, a second dose after six hours could be required if the extract is to be utilized in clinical applications in order to fully prevent the development of *C. albicans*.[23]

One of the phenol coumarin compounds contained in *M. citrifolia* extract that is known to have antifungal effects against *C. albicans* is scopoletin (6-Methoxy-7-hydroxycoumarin), by damaging the fungal cell membrane and reducing protein aggregation.[23,29,42–45] In addition to being antifungal, this scopoletin compound also has anti-inflammatory and antioxidant properties which can certainly help reduce clinical symptoms in candidiasis therapy.[24,46,47]

In addition to scopoletin, *M. citrifolia* contains secondary metabolites of flavonoids which are known to be antifungal. This is in accordance with research conducted by Medrano-Colmenares et al. which stated that the methanol extract of *M. citrifolia* has a strong effect on *C. albicans.*[29,48,49] According to Singh et al. the contact time with the fungus and its concentration of *M. citrifolia* extract affect its effectiveness as an antifungal.[30] In addition, it is also known that the growing location and age of the *M. citrifolia* plant

affect the content of its secondary metabolites, especially flavonoids.[28]

Other secondary metabolites in the plant, like terpenoids and anthraquinones, might also make its antifungal effects stronger. The combined effect of these chemicals might make it work better.[50] Because of this, *M. citrifolia* is a great candidate for making antifungal drugs. The way the extraction is done can have a big effect on the compound's activity and yield. This shows how important it is to make processes better so that *M. citrifolia* can be used to fight fungi.[51]

5. Conclusion

Based on the results of a systematic literature review of several in vitro studies, *M. citrifolia* has shown an inhibitory effect on the growth of *Candida albicans*, which is influenced by concentration, contact time and solvent used. These findings highlight the strong potential of *M. citrifolia* as an alternative antifungal candidate for cases of resistance or allergy to existing antifungal agents. Therefore, in the future, more comprehensive studies are needed, including in vivo studies and clinical trials to evaluate the effectiveness of *M. citrifolia* as an antifungal agent.

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