RESEARCH PAPER

Evaluation of the Antifungal Activity of Methanolic Leaf Extracts of Morinda tinctoria Against Candida albicans

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ABSTRACT

Background

Morinda tinctoria commonly known as Indian mulberry, belongs to the family Rubiaceae grows widely and distributed throughout Southeast Asia. It is a small tree with immense medicinal properties. There is a greater demand for fruit extract of morinda species in treatment for different kinds of illness such as arthritis, cancer, gastric ulcer and other heart disease. It shows Anti Convulsant, analgesic, anti-inflammatory, antioxidant activity and cytoprotective effect.

Aim and Objective

The aim of this study is to investigate the anti-fungal activity of Morinda tinctoria Methanol leaf extract on Candida albicans.

Materials and Methods

Fresh leaves of Morinda tinctoria were collected, shade-dried, powdered, and extracted using methanol. The crude extract was fractionated, and the antifungal activity of the fractions was assessed against Candida albicans using the agar well diffusion method. The most active fraction was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis for the identification of bioactive constituents.

Results

All fractions exhibited varying degrees of antifungal activity against Candida albicans. Among them, Fraction 3 demonstrated the highest zone of inhibition, indicating superior antifungal efficacy. GC-MS analysis of the active fraction revealed the presence of several phytochemicals, with Methyl 3-bromo-1-adamantaneacetate identified as a major compound that may contribute to the observed anticandidal activity.

Conclusion

The methanolic leaf extract of Morinda tinctoria, particularly Fraction 3, exhibited significant antifungal activity against Candida albicans. These findings suggest that Morinda tinctoria is a promising source of natural antifungal compounds and may have potential applications in the prevention and management of oral fungal infections and caries-associated microbial biofilms. Further studies are required to isolate the active compounds and evaluate their clinical applicability.

Keywords: Morinda tinctoria, Candida albicans, antifungal activity, methanolic leaf extract, phytochemicals, oral candidiasis, dental caries.

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INTRODUCTION:

Morinda tinctoria Roxb., commonly known as Aal or Indian mulberry, is a medicinal plant belonging to the

family Rubiaceae. It is widely distributed throughout the tropical and subtropical regions of India, Sri Lanka, and Southeast Asia. The plant has been extensively utilized in traditional systems of medicine, including Ayurveda, Siddha, and folk medicine, for the treatment of various diseases. Different parts of the plant, such as leaves, roots, bark, fruits, and seeds, have been reported to possess significant therapeutic properties.Traditionally, Morinda tinctoria has been used to manage fever, inflammation, skin diseases, ulcers, diabetes, arthritis, and microbial infections. The medicinal value of the plant is mainly attributed to the presence of diverse phytochemical constituents, including alkaloids, flavonoids, phenolic compounds, anthraquinones, tannins, saponins, glycosides, and terpenoids. These bioactive compounds exhibit a broad spectrum of biological activities such as antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, antidiabetic, anticancer, and wound-healing effects. Due to these pharmacological properties, Morinda tinctoria has emerged as an important medicinal plant and has attracted considerable attention in the field of natural product research.

Fungal infections continue to be a major global health concern, affecting millions of individuals annually. The increasing incidence of fungal diseases, coupled with the emergence of antifungal resistance, has created a significant challenge in modern healthcare. Among the fungal pathogens, Candida albicans is one of the most prevalent opportunistic fungi responsible for oral candidiasis, denture stomatitis, angular cheilitis, and systemic candidiasis. In healthy individuals, C. albicans exists as a commensal organism within the oral cavity; however, under favorable conditions such as immunosuppression, prolonged antibiotic therapy, diabetes mellitus, xerostomia, and poor oral hygiene, it can transform into a pathogenic form and cause infection. Recent studies have also highlighted the involvement of C. albicans in the progression of dental caries. The fungus can interact synergistically with cariogenic bacteria, particularly Streptococcus mutans, leading to the formation of highly virulent biofilms that enhance acid production and accelerate enamel demineralisation.Consequently, controlling Candida growth has become an important aspect of oral disease prevention and management.

The currently available antifungal drugs, including azoles, polyenes, and echinocandins, have proven effective in treating fungal infections; however, their prolonged use is often associated with adverse effects, toxicity, high treatment costs, and the development of of resistant fungal strain and the emergence of drug-resistant Candida species has emphasized the urgent need for alternative antifungal agents that are safe, effective, and economically feasible. Medicinal plants have gained considerable importance in this regard because they represent a rich source of structurally diverse bioactive compounds with antimicrobial potential. Plant-derived compounds often exhibit multiple mechanisms of action, reducing the likelihood of resistance development and making them attractive candidates for therapeutic applications.Numerous studies have demonstrated that medicinal plants possess significant antifungal activity against a variety of pathogenic fungi, including Candida species, due to the presence of phenolics, flavonoids, alkaloids, and terpenoids that interfere with fungal growth and metabolism.

Among medicinal plants, species belonging to the genus Morinda have been widely investigated for their antimicrobial properties.

Furthermore, fractionation of methanolic extracts can help isolate and concentrate active compounds responsible for antifungal activity, thereby improving their therapeutic potential.

In recent years, advanced analytical techniques such as Gas Chromatography-Mass Spectrometry (GC-MS) have become valuable tools for identifying bioactive compounds present in medicinal plants. GC-MS analysis provides detailed information regarding the chemical composition of plant extracts and facilitates the identification of compounds responsible for biological activities. Correlating antifungal activity with GC-MS-identified phytochemicals enables a better understanding of the mechanisms underlying the therapeutic effects of medicinal plants and supports the development of novel plant-based drugs.

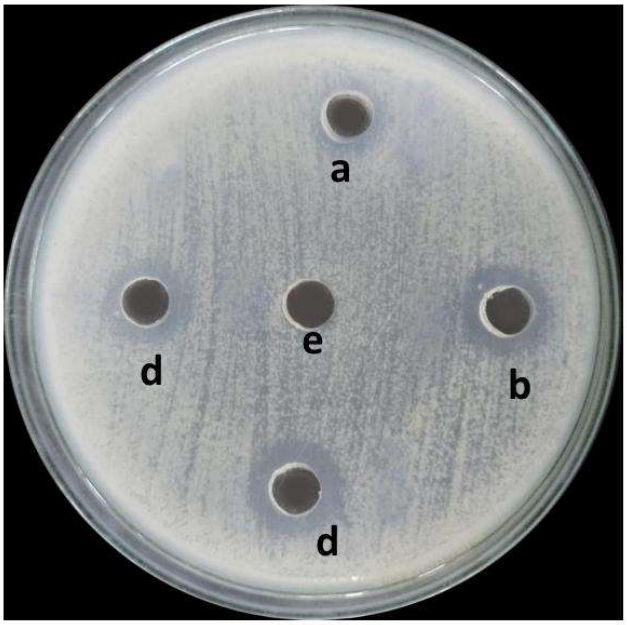
Therefore, the present study was undertaken to evaluate the antifungal activity of methanolic leaf extracts and fractions of Morinda tinctoria against Candida albicans using the agar well diffusion method. In addition, GC-MS analysis was performed to identify the bioactive compounds present in the most active fraction. The findings of this study may provide scientific evidence for the traditional use of Morinda tinctoria and contribute to the development of natural antifungal agents for the management of oral candidiasis, dental caries-associated biofilms, and other fungal infections.

MATERIALS AND METHODS :

The study was conducted at Saveetha Dental College and Hospital, Chennai. For this study *M. tinctoria* plant leaves were taken. *M. tinctoria* leaves were first rinsed with tap water and then distilled water to remove all the dust and unwanted visible particles. Then the leaves were dried at room temperature to remove the water from the surface of the leaves. About

50 g of finely incised dried leaves were taken and then added to 500 ml Methanol and incubated shaker over night. The aqueous extract was filtered using Whatman filter paper No.1 to remove the particulate matter. A dark brown colour solution is obtained and passthrough open column C18 and eluted with Acetonitrile 20% (Fraction 1), 40% (Fraction 2), 60% (Fraction 3) and 100% (Fraction 4). All fractions were subjected to anti-dental bacterial activity. Positive fraction selected and proceed with GC-MS analysis.

RESULTS :

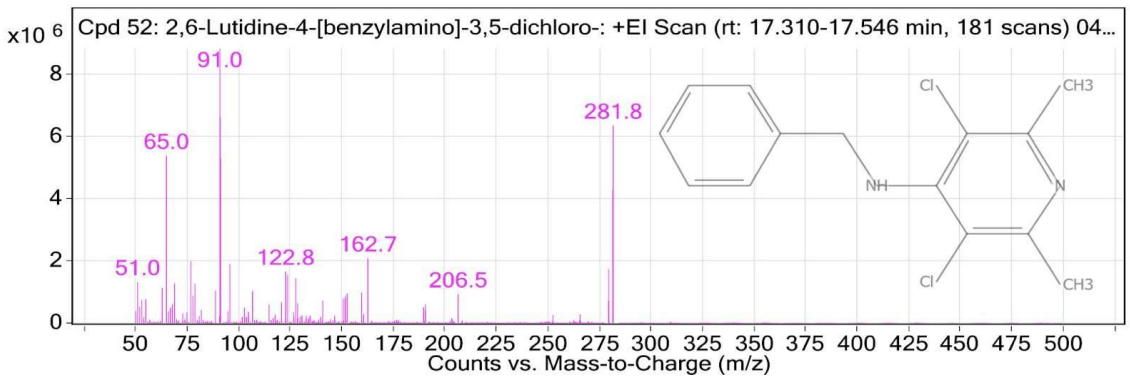


a. Fraction 1 (50ul)
b. Fraction 2 (50ul)
c. Fraction 3 (50ul)
d. Fraction 4 (50ul)
e. DMSO control (50ul)

M. tinctoria **extract Fraction 3 was effectively inhibited the growth of** ***C. albicans*** **compared with other fractions**

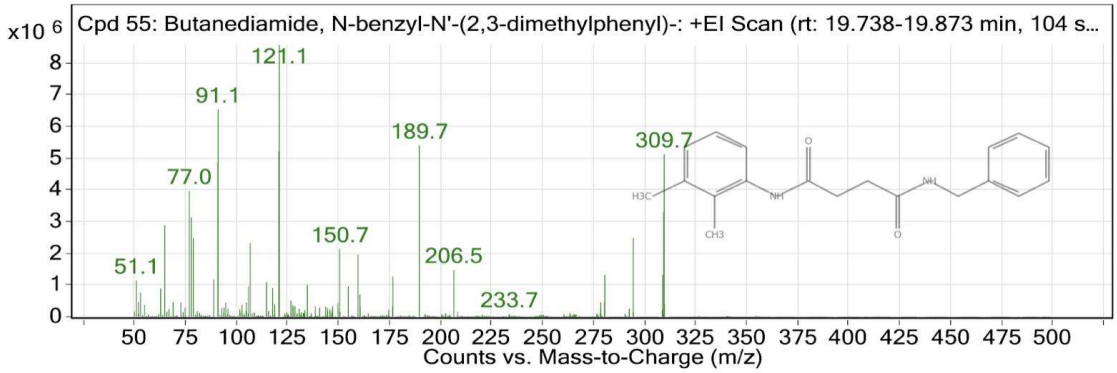
GC-MS identified molecule

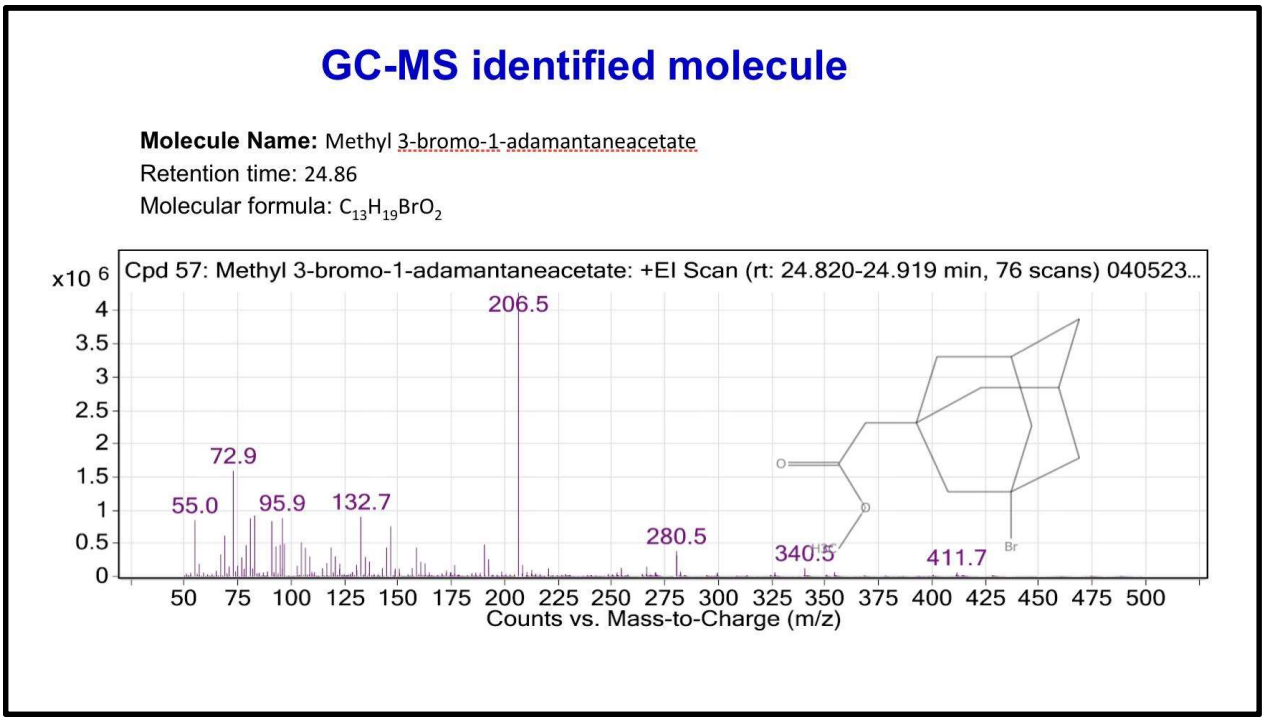
Molecule Name: 2,6-Lutidine-4-[benzylamino]-3,5-dichloro
Retention time: 17.442
Molecular formula: $C_{14}H_{14}Cl_2N_2$



GC-MS identified molecule

Molecule Name: Butanediamide, N-benzyl-N'-(2,3-dimethylphenyl)-
Retention time: 19.816
Molecular formula: $C_{19}H_{22}N_2O_2$





The methanolic leaf extract of Morinda tinctoria was fractionated and subjected to C-MS analysis. Several bioactive compounds were identified in the active fractions.

Fraction 1 showed the presence of 2,6-Lutidine-4-[benzylamino]-3,5-dichloro (RT:17.442 min; Molecular Formula: GrataCIN2).This compound contains chlorinated aromatic and amine groups, which are often associated with antimicrobial and antifungal properties.

Fraction 2 was identified as Butanediamide, N-benzyl-N'-(2,3-dimethylphenyl) - (RT:19.816 min; Molecular Formula: CoH22N202).Amide-containing compounds have been reported to possess biological activities including antimicrobial effects through interference with cellular metabolism.

Fraction 3 contained Methyl 3-bromo-1-adamantaneacetate (RT: 24.86 min;Molecular Formula: CasHhsBrO2). The presence of bromine and an adamantane skeleton may contribute to enhanced biological activity, as halogenated compounds are known to exhibit antimicrobial effects.

The antifungal activity was evaluated using the agar well diffusion method against the test fungus. The assay plate showed clear zones of inhibition around wells containing the fractions, whereas the DMSO control exhibited little or no inhibition. Among the tested fractions, the fraction producing the largest inhibition zone demonstrated the strongest antifungal activity, indicating the presence of potent antifungal constituents.

DISCUSSION:

The present study demonstrated that the methanolic leaf extract of Morinda tinctoria exhibited antifungal activity against Candida albicans, with Fraction 3 producing the greatest zone of inhibition among all fractions tested. The absence of inhibition in the DMSO control confirmed that the observed activity was due to the bioactive constituents present in the extract.GC-MS analysis of Fraction 3 identified Methyl 3-bromo-1-adamantaneacetate as a major compound. The strong antifungal activity observed in this fraction may be attributed to the presence of the brominated adamantane derivative. Halogenated compounds,particularly brominated molecules, are known to possess enhanced antimicrobial properties due to their ability to alter membrane permeability and interfere with essential cellular processes in microorganisms. The adamantane nucleus has also attracted attention in pharmaceutical research because of its broad-spectrum biological activities,including

antimicrobial effects.The antifungal activity observed against Candida albicans is consistent with previous studies on species of the genus Morinda. Barani et al. reported that extracts of Morinda citrifolia, a closely related species, significantly inhibited the growth of C. albicans in a dose-dependent manner, demonstrating the antifungal potential of Morinda-derived phytochemicals. Furthermore, a recent study by Medrano-Colmenares et al. showed that methanolic extracts of Morinda citrifolia produced measurable inhibition zones against C. albicans and exhibited both fungistatic and fungicidal activities.The higher activity of Fraction 3 compared with the other fractions suggests that fractionation enhanced the concentration of active compounds responsible for antifungal action. In addition to Methyl 3-bromo-1-adamantaneacetate, minor phytoconstituents present in the fraction may act synergistically to inhibit fungal growth. Such synergistic interactions among phytochemicals have been widely reported in medicinal plant extracts and are often responsible for stronger antimicrobial effects than those produced by individual compounds alone.The results obtained in this study are particularly significant considering the growing incidence of antifungal resistance among Candida species. The emergence of resistant strains has reduced the effectiveness of many conventional antifungal drugs, creating a demand for novel therapeutic agents from natural sources. Plant-derived compounds offer several advantages, including structural diversity, lower toxicity, and reduced likelihood of resistance development.Previous studies have demonstrated that methanolic extracts are particularly effective in extracting phenolic compounds, flavonoids, alkaloids, and other secondary metabolites responsible for antimicrobial activity. The strong anticandidal activity observed in the present investigation may therefore result not only from the major compound identified by GC-MS but also from synergistic interactions among multiple phytochemicals present in the fraction. Synergism among plant metabolites has been reported to enhance antifungal efficacy by targeting multiple cellular pathways simultaneously.Therefore, Morinda tinctoria represents a promising candidate for the discovery of new antifungal molecules.Although the present study demonstrates promising antifungal activity, further investigations are required to fully characterize the active principles. Isolation and purification of the bioactive compounds, determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC), toxicity evaluation, and mechanistic studies would provide a better understanding of their therapeutic potential. Advanced analytical techniques such as HPLC, LC-MS/MS, and NMR spectroscopy may also be employed to confirm the identity and structure of the active constituents.

CONCLUSION :

The present study demonstrated that the methanolic leaf extract of Morinda tinctoria possesses notable antifungal activity against Candida albicans, with Fraction 3 exhibiting the greatest inhibitory effect. GC-MS analysis of the active fraction revealed the presence of bioactive compounds, particularly Methyl 3-bromo-1-adamantaneacetate, which may contribute to the observed anticandidal activity. The findings suggest that Morinda tinctoria is a promising natural source of antifungal agents and supports its traditional medicinal use. As Candida albicans is a major causative agent of oral candidiasis and has been implicated in the progression of dental caries through its interaction with cariogenic bacteria, the antifungal potential of Morinda tinctoria highlights its possible application in oral healthcare. The plant extract may serve as a potential candidate for the development of herbal oral formulations such as mouthwashes, gels, and other preventive therapies. However, further studies involving the isolation of active compounds, toxicity evaluation, determination of MIC and MFC values, and clinical investigations are necessary to validate its efficacy and safety for therapeutic use.

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CONFLICT OF INTEREST:
The authors declare that there was no conflict of interest in the present study.

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