

Antimicrobial Activity and Antifungal Activity of Morus Alba Leaf Methanolic Extract

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ABSTRACT

The growing resistance of pathogenic microorganisms to existing antibiotics has become a global concern, demanding exploration of novel natural antimicrobial sources. *Morus alba* (white mulberry), belonging to the family Moraceae, is a medicinal plant known for its rich phytochemical content and diverse therapeutic properties. The present study investigates the antimicrobial and antifungal potential of *Morus alba* leaf methanolic extract against selected bacterial and fungal strains. Phytochemical screening revealed the presence of flavonoids, alkaloids, phenolic acids, and terpenoids. The antimicrobial activity was determined using the agar well diffusion method, and results showed significant inhibition zones against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and fungal strains like *Candida albicans* and *Candida glabrata*. GC–MS analysis confirmed the presence of major bioactive compounds such as palmitic acid, rutin, scopoletin, and chalcocomoracin. These findings support the traditional use of *Morus alba* and suggest its potential for developing plant-based antimicrobial agents.

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I. INTRODUCTION:

Plants have long been a valuable source of bioactive compounds with therapeutic potential. Traditional medicine systems such as Ayurveda, Unani, and Traditional Chinese Medicine (TCM) rely on plant extracts to treat various infectious diseases. With the rise of drug-resistant pathogens, the search for plant-derived antimicrobial agents has gained renewed attention. Methanol is a widely utilized solvent for extracting bioactive compounds from plant material due to its ability to dissolve a broad range of phytochemicals, including polar and semi-polar molecules. The resulting methanolic extract of *Morus alba* leaves is therefore an important subject for scientific investigation. Research has indicated that these extracts possess significant antibacterial activity against both Gram-positive and Gram-negative bacteria, including common pathogens such as *Staphylococcus aureus* and *Escherichia coli*. The extract is also effective against a range of pathogenic fungi, with demonstrated efficacy against species like *Candida albicans* and *Aspergillus niger*. The antimicrobial action of the methanolic leaf extract is primarily attributed to its rich phytochemical composition. Flavonoids, such as quercetin and kaempferol, and alkaloids, like moracin, can disrupt bacterial cell membranes, inhibit nucleic acid synthesis, and interfere with essential metabolic enzymes. The presence of tannins and other phenolic acids further contributes to these effects. The potential of this extract as a natural antimicrobial agent is promising, offering a safer and biodegradable alternative to synthetic chemicals for applications in fields ranging from pharmaceuticals to food preservation.

Morus alba, commonly known as white mulberry, is native to South and East Asia. It is a fast-growing plant widely cultivated for its fruits, leaves, and bark. Beyond its agricultural use in sericulture, the plant is known for its pharmacological properties, including antioxidant, antidiabetic, anti-inflammatory, and antimicrobial effects. The leaves of *Morus alba* are particularly rich in flavonoids (quercetin, rutin), phenolic acids, alkaloids, terpenoids, coumarins, and stilbenoids. These compounds contribute to the plant's bioactivity, especially its antimicrobial and antifungal potential. The leaves, in particular, are an abundant source of bioactive compounds and have been used for centuries in traditional medicine. A methanolic extract of *Morus alba* leaves is a subject of significant research, especially for its antimicrobial and antifungal potential. The growing resistance of pathogenic microbes to conventional antibiotics has driven a global search for novel antimicrobial agents from natural sources.



II. MATERIALS REQUIRED AND METHODS:

This experiment aims to investigate and quantify the antimicrobial and antifungal properties of the methanolic extract from *Morus alba* (mulberry) leaves against specific bacterial and fungal pathogens. By testing the extract's effectiveness, the study seeks to confirm its potential as a natural source for developing new therapeutic agents and to validate its traditional use. The central objective is to determine the zone of inhibition produced by the extract against target microorganisms using the agar disc-diffusion method.

Procedure

1. Preparation of *Morus alba* leaf methanolic extract-

Plant material collection and processing- Collect healthy, fresh leaves of *Morus alba*. Wash them thoroughly to remove dirt, then shade-dry the leaves for several days. Once dry, grind the leaves into a fine powder using an electric blender. The percentage yield of the methanolic extract was 5.5%.

Extraction:- Weigh a specific amount of the powdered leaves and place it into a Soxhlet apparatus. Add methanol as the solvent. Heat the apparatus to allow the solvent to cycle through and extract the phytochemicals from the leaf powder. This process should run for several hours until the solvent in the siphoning tube becomes colorless.

Filtration and concentration: Once extraction is complete, filter the resulting crude extract through filter paper to remove any remaining plant material. The filtrate is then concentrated using a rotary evaporator under reduced pressure and moderate temperature to remove the methanol. This leaves a semi-solid or solid crude extract. The extract is then weighed and stored in an airtight container at a cool temperature until use.

Plant Material Collection:

Fresh *Morus alba* leaves were collected from Erragadda, Hyderabad, during March 2025. The specimen was authenticated by the Department of Botany, Government Degree College, Kukatpally.

2. Preparation for antimicrobial and antifungal assay-

Culture media preparation: Prepare the MHA and SDA/PDA media according to the manufacturer's instructions. Sterilize the media by autoclaving and then pour it into sterile petri dishes. Allow the media to solidify on a level surface.

Inoculum standardization: Prepare a suspension of each test microorganism in sterile saline solution. The turbidity of the suspensions should be adjusted to match the 0.5 McFarland standard, corresponding to a specific cell density.

Disc preparation: Prepare sterile filter paper discs. The extract is dissolved in a minimal amount of sterile methanol to create different concentrations. The sterile filter paper discs are then impregnated with these different concentrations of the extract and allowed to air dry in a sterile environment. Positive control discs (with commercial antibiotics/antifungals) and negative control discs (with sterile methanol) are also prepared.

3. Agar disc-diffusion bacterial assay- The agar well diffusion method was used. Mueller-Hinton Agar (MHA) was poured into Petri plates and inoculated with test organisms. Wells were filled with different concentrations of the extract (25, 50, 75, and 100 mg/mL). Plates were incubated at 37°C for 18–24 hours, and the diameter of inhibition zones was measured in millimeters. Using a sterile cotton swab, spread the standardized bacterial inoculum evenly over the entire surface of the MHA plates to create a "lawn" of bacteria.

Fungal assay: Repeat the same process, but with the fungal inoculum on the SDA/PDA plates.

Disc placement: Place the prepared discs

(with extract, positive control, and negative control) onto the surface of the inoculated agar plates using sterile forceps, ensuring uniform spacing. Incubation: Incubate the bacterial plates upside down at 37°C for 24 hours. For fungi, incubate the plates at 30°C for 48–72 hours.

Phytochemical Screening

A preliminary phytochemical screening is a qualitative analysis performed to identify the major classes of bioactive compounds present in a plant extract. This process involves a series of chemical tests that produce characteristic color changes or precipitates, indicating the presence or absence of specific phytochemicals. Since the methanolic extract of *Morus alba* leaves is known to contain various compounds, this screening is a crucial step to link the observed antimicrobial and antifungal activity to specific groups of phytochemicals like flavonoids, tannins, saponins, and alkaloids.

The methanolic extract was subjected to qualitative tests:

Mayer's and Dragendorff's tests → Presence of alkaloids.

Molisch's test → Carbohydrates present.

Shinoda test → Flavonoids confirmed.

Microorganisms Used are Gram-positive bacteria are *Staphylococcus aureus*, *Bacillus subtilis*. Gram-negative bacteria are *Escherichia coli*, *Pseudomonas aeruginosa*

Fungal strains are *Candida albicans*, *Candida glabrata*.

RESULTS AND DISCUSSION

The methanolic extract of *Morus alba* leaves exhibited noticeable antimicrobial activity. The zone of inhibition increased proportionally with extract concentration, confirming a dose-dependent effect.

Antibacterial Activity:

The extract showed strong activity against both Gram-positive and Gram-negative bacteria. *Staphylococcus aureus* and *Bacillus subtilis* were more susceptible compared to *E. coli* and *P. aeruginosa*.

Antifungal Activity:

Antifungal studies revealed inhibition against *Candida albicans* and *Candida glabrata*, indicating that the phytochemicals may interfere with fungal membrane integrity or inhibit spore germination. GC–MS Analysis: GC–MS profiling identified multiple bioactive compounds including palmitic acid, scopoletin, rutin, and chalconoracin, all known for antimicrobial and antioxidant activity.

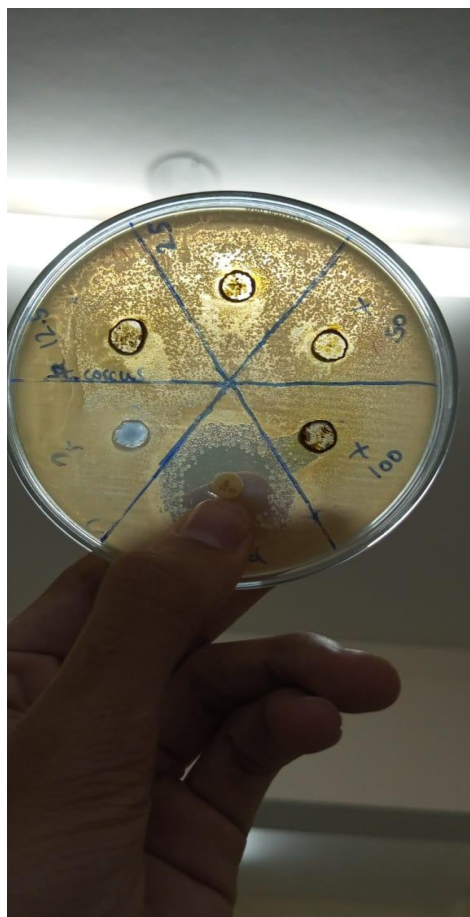
III. RESULT



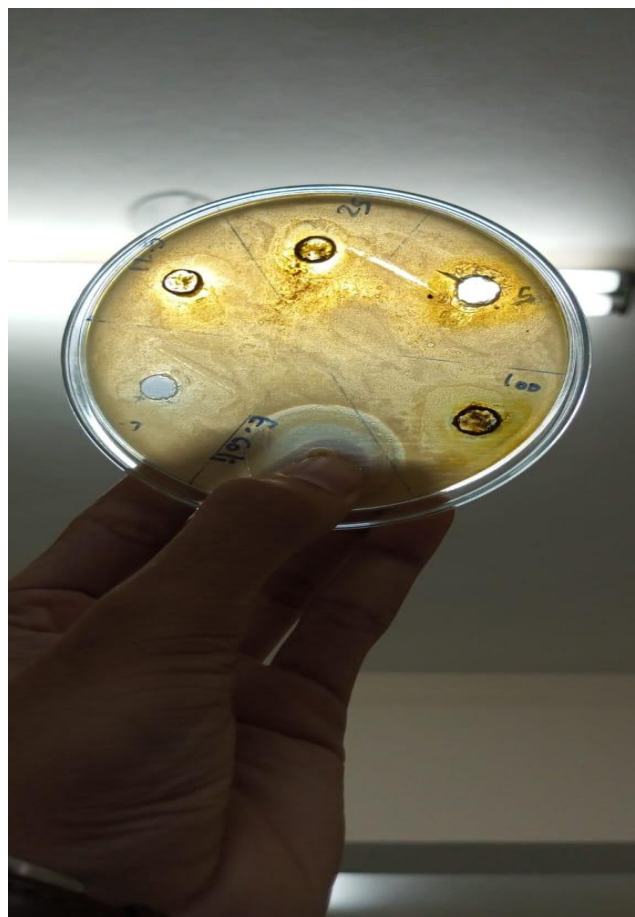
(A)



(B)



(C)



(D)

Mechanism of Action:

Morus alba's antibacterial effects are driven by its phytochemicals interfering with bacterial cell structures and metabolic pathways.

Flavonoids and alkaloids, abundant in *Morus alba*, bind to and disrupt the integrity of the bacterial cell wall and membrane. This causes the leakage of essential intracellular components, like nucleic acids and proteins, leading to cell death. The bioactive compounds can interfere with the activity of bacterial enzymes critical for metabolism, thus inhibiting bacterial growth and proliferation. Certain compounds, such as the flavonoid kuwanon O, have been shown to inhibit bacterial efflux pumps. These pumps are used by bacteria to expel antibiotics, and by inhibiting them, *Morus alba* can increase the effectiveness of traditional antibiotics. Extracts from *Morus alba* can prevent the adhesion of pathogenic bacteria to surfaces, thereby inhibiting the formation of biofilms, which are dense bacterial communities that are highly resistant to antimicrobial agents.

IV. SUMMARY AND CONCLUSION

The present study validates the traditional use of *Morus alba* leaves in treating microbial infections. Methanolic extract of the leaves contains various bioactive phytochemicals that exhibit significant antibacterial and antifungal activities. The GC–MS analysis confirmed the presence of compounds responsible for these effects. Therefore, *Morus alba* methanolic extract can serve as a promising natural source for developing new antimicrobial agents, offering a sustainable alternative to synthetic antibiotics, especially against resistant microbial strains.

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