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Extraction and evaluation of antibacterial and antifungal activities of *Morus alba* along with its Minimum Inhibitory Concentration (MIC)

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Abstract- *Morus alba* L. (mulberry), a member of the Moraceae family, is a valuable medicinal plant traditionally used to treat fever, inflammation, diabetes, and infectious diseases. The present study aimed to evaluate the antibacterial and antifungal properties of *M. alba* L. leaf extracts obtained through the Soxhlet extraction method using ethanol and distilled water as solvents, and to determine their minimum inhibitory concentration (MIC) values. Dried leaves were milled and extracted in a Soxhlet apparatus for 8 hrs, and the concentrated extracts were tested for antimicrobial activity. Antimicrobial tests using the disc diffusion and broth dilution methods showed that the ethanolic extract was effective against *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*, whereas aqueous extracts exhibited negligible inhibition. The lowest MIC was recorded against *Escherichia coli*, followed by *Staphylococcus aureus* confirming the potent antibacterial potential of the ethanolic fraction.

Keywords: *Morus alba* L.; Soxhlet extraction; Antibacterial activity; Antifungal activity; Minimum inhibitory concentration

INTRODUCTION

The rapid emergence of antibiotic-resistant bacterial strains poses a major threat to global health. Continuous evolution of pathogenic microorganisms has reduced the efficacy of conventional antibiotics, necessitating the exploration of safer and more sustainable alternatives.¹ Medicinal plants, owing to their diverse bioactive phytochemicals, represent promising sources of novel antimicrobial agents. Medicinal plants contain diverse bioactive components such as alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds, which are responsible for their therapeutic potential. These phytochemicals act as antioxidants, anti-inflammatory, antimicrobial, and anticancer agents. Due to their structural diversity and

pharmacological activities, plants are considered promising sources for developing new pharmaceutical compounds. The increasing global burden of infectious diseases and the rapid development of antimicrobial resistance have emphasized the urgent need for new therapeutic agents derived from natural sources. Medicinal plants have long been recognized for their bioactive constituents-such as flavonoids, alkaloids, tannins, and terpenoids which exhibit potent antibacterial and antifungal activities.² Ethno medicinal knowledge plays a crucial role in identifying potential plant species for such investigations.

It has been estimated by WHO that at least 80% of the world population, mainly in the developing countries, still depends on herbal medicines for their primary health care needs. Use of traditional medicine is based on its accessibility, affordability, and its firm embedment within the faith systems of people.³ *Morus alba* belongs to the

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Moraceae family, is widely cultivated and naturalized elsewhere and is one of the most important medicinal plant.⁴ Many exotic or invasive plant species, though ecologically competitive, possess substantial medicinal potential. For example, species from families such as Amaranthaceae, Apocynaceae, and Euphorbiaceae are used traditionally to treat ailments such as fever, asthma, skin disorders, and digestive problems. These plants commonly contain flavonoids, alkaloids, saponins, terpenoids, steroids, phenols, and glycosides, compounds also reported in *Morus alba* and known for their antioxidant and antimicrobial activity.⁵ Plants show a rich source of antimicrobial agents and natural oxidants. Nearly about 80% of the world's population still depend upon traditional remedie based on phytotherapy.⁶ The mulberry (*Morus alba*) fruit is widely regarded as a nutritious food. Root, stem barks, twigs and leaves of mulberry have long been used in traditional medicine to treat fever, inflammation, hepatitis, cancer, diabetes, dislipidemia, diarrhoea, dyspepsia, hypertension, anthelmintic and to protect the liver, improve eyesight, strengthen joints, facilitate discharge of urine, and lower blood pressure. Different parts of the mulberry have been extensively investigated for their various health benefits, including antioxidative, hypolipidemic and antiatherogenic effects. Wounds and skin infections represent complex biological responses of vascular tissues to microbial or chemical injury. The growing incidence of infected wounds underscores the importance of antimicrobial agents in accelerating tissue repair and preventing secondary infections.³



Fig. 1 *Morus alba* L. (Mulberry)

Phytochemical studies have revealed that *M. alba* contains numerous bioactive secondary metabolites such as flavonoids, phenolic acids, alkaloids, terpenoids, anthocyanins, and benzofuran derivatives.⁷ Important compounds including oxyresveratrol, mulberroside A, quercetin, rutin, kuwanon J, and morusin are associated with strong antioxidant, antimicrobial, anti-inflammatory, and antidiabetic activities. Inflammation is a complex biological response of vascular tissues to harmful stimuli such as pathogens, toxic chemicals, and physical injury. Medicinal plants have been traditionally used to manage inflammatory disorders due to the presence of bioactive phytochemicals, including alkaloids, flavonoids, and glycosides, which exhibit antioxidant and anti-inflammatory properties.⁸ The use of herbal resources continues to rise due to the side effects and high cost of synthetic drugs.⁹ These compounds act as natural free-radical scavengers and microbial inhibitors, explaining the wide pharmacological spectrum of *M. alba* L. The biological potential of *M. alba* strongly depends on the extraction method and solvent polarity used to isolate these compounds. It was demonstrated that ethanolic extracts of *M. alba* leaves exhibited a higher total antioxidant status (1.56 mmol Trolox Eq/L) and significant antimicrobial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* compared with aqueous extracts, which showed minimal inhibition.¹⁰ Similarly, it was reported that both ethanolic and aqueous fruit extracts of *M. alba* displayed strong antioxidant and antibacterial activities, particularly against *E. coli*, *Salmonella typhi*, *Shigella dysenteriae*, *S. aureus*, and *Vibrio cholerae*.¹¹ The aqueous extract contained higher levels of phenolic and anthocyanin compounds (23.77 mg GAE/g and 177.84 mg Cy-3-glc/g, respectively), supporting the influence of solvent type on extraction efficiency.

Although several studies have examined the antioxidant and antibacterial activities of *M. alba* L. limited information is available on its antifungal properties and minimum inhibitory concentration (MIC) values, especially in leaf extracts prepared by Soxhlet extraction. Therefore, the present study was undertaken to evaluate the antibacterial and antifungal activities of *M. alba* leaf extracts obtained using ethanol and distilled water as solvents and to determine their MIC values. This investigation aims to establish the comparative antimicrobial potential of *M. alba* extracts and to highlight their significance as natural alternatives to synthetic antimicrobial agents.

MATERIAL & METHODS

Sample collection

Fresh plant leaves are collected from local area.

Extraction Preparation

The fresh leaves were cut from the *M. alba* plant, washed thoroughly three times in water, shade dried at room temperature. After complete drying, the leaves were subjected to size reduction in a grinder and then sieved through sieve number 40 to get a coarse powder and stored in airtight glass jar. Coarse powder (70 g) was extracted with solvent (1000 ml of 99.9% ethanol) at room temperature using Soxhlet method of extraction. After the completion of Soxhlet process, the ethanol was evaporated using IKA Rotavapour evaporator at 40°C, leaving a small yield of extracted plant material (about 2-3 ml) in the glass bottom flask. The extract was further dried at room temperature until the remaining ethanol was completely evaporated. The yield obtained was 11.4 g. The extract was stored at 4°C until further microbial tests are carried out.

Source of Microbes

The bacterial and fungal cultures used in this study *Escherichia coli* (MTCC-452), *Staphylococcus aureus* (MTCC-96), *Enterococcus faecalis* (MTCC-439), *Pseudomonas aeruginosa* (MTCC-424), *Trichoderma reesei* (MTCC-164), *Fusarium oxysporum* (MTCC-2780). All strains were maintained on nutrient agar and Sabouraud dextrose agar (SDA) slants under standard laboratory conditions prior to antimicrobial and antifungal testing.

Antibacterial Activity Analysis

The antimicrobial activity of *Morus alba* leaf extracts was evaluated against selected bacterial strains: *Escherichia coli* (MTCC-452), *Staphylococcus aureus* (MTCC-96), *Enterococcus faecalis* (MTCC-439), and *Pseudomonas aeruginosa* (MTCC-424). These strains were maintained on nutrient agar slants under standard laboratory conditions prior to testing.¹¹ The antimicrobial assay was performed using the agar well diffusion method. Wells of 6 mm diameter were filled with different concentrations of *Morus alba* leaf extracts. The plates were incubated at 37°C for 24 hours. After incubation, the zones of inhibition were measured in millimeters to evaluate the effectiveness of the extracts.^{12,13}

Antifungal Activity Analysis

The antifungal potential of *Morus alba* leaf extracts was analyzed against fungal strains: *Trichoderma reesei*

(MTCC-164), *Fusarium oxysporum* (MTCC-2780), and *Aspergillus niger* (MTCC-282). All fungal cultures were obtained from the laboratory and maintained on Sabouraud dextrose agar (SDA) slants under standard laboratory conditions prior to testing.¹¹ The antifungal activity was determined using the agar well diffusion method. Wells of 6 mm diameter were made in SDA plates and loaded with various concentrations of *Morus alba* leaf extracts. The plates were incubated at 28°C for 48 hours. After incubation, the zones of inhibition around the wells were measured in millimeters to evaluate the antifungal efficacy of the extracts.^{12,13}

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of *Morus alba* leaf extracts was determined using the broth dilution method.^{14,15} This method was used to determine the lowest concentration of extract that completely inhibited visible microbial growth. Stock solutions of the ethanolic and aqueous extracts were prepared at a concentration of 125 mg/mL. Serial twofold dilutions were made in Mueller-Hinton Broth (MHB) for bacterial isolates and Sabouraud Dextrose Broth (SDB) for fungal isolates to obtain concentrations ranging from 0.98 to 125 mg/mL.¹⁴ Test microorganisms (*Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Trichoderma reesei*, *Fusarium oxysporum*, and *Aspergillus niger*) were cultured on nutrient agar and SDB agar, respectively, and incubated for 18-24 hours. A loopful of each culture was suspended in sterile saline and adjusted to 0.5 McFarland standard (approximately 1×10^6 CFU/mL) as per CLSI. Each test tube containing 1 mL of extract dilution was inoculated with 100 μ L of the microbial suspension. A positive control (broth with inoculum only) and a negative control (broth with extract only) were included. The bacterial tubes were incubated at 37°C for 24 hours, and the fungal tubes at 28-30°C for 48 hours. After incubation, the tubes were visually examined for turbidity, and the MIC was recorded as the lowest concentration showing no visible growth compared with the positive control.¹⁵

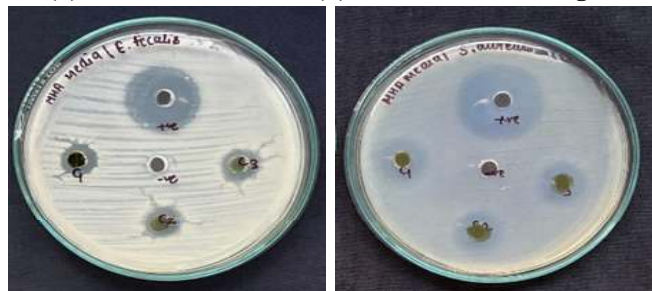
To determine the minimum bactericidal/fungicidal concentration (MBC/MFC), 100 μ L from clear tubes was subcultured on nutrient agar or Sabouraud dextrose agar and incubated under the same conditions. The lowest concentration that produced no colony growth was recorded as the MBC or MFC.¹⁴

RESULT**Antibacterial Activity Analysis:**

The ethanol-based *Morus alba* leaf extract showed antibacterial activity against the tested bacterial strains at a concentration of 100 mg/mL, suggesting its potential as a natural antimicrobial agent.



(a) *Escherichia coli* (b) *Pseudomonas aeruginosa*



(c) *Enterococcus faecalis* (d) *Staphylococcus aureus*

Fig. 2- Antibacterial activity of sample *Morus alba* Extract, Ciprofloxacin (+ve control), Methanol (-ve control) against bacteria (a), (b), (c), and (d) using Well diffusion method.

Table 1 - Antibacterial activity of sample *Morus alba* Extract, Ciprofloxacin (+ve control), Methanol (-ve control) against bacteria

Bacterial Strain	Sample	Zone of Inhibition (mm)
		Mean $\pm$ S.D.
<i>Escherichia coli</i>	<i>Morus alba</i> Extract	13.66 $\pm$ 2.36
<i>Staphylococcus aureus</i>		19.83 $\pm$ 1.44
<i>Enterococcus faecalis</i>		15.66 $\pm$ 2.31
<i>Pseudomonas aeruginosa</i>		10.50 $\pm$ 1.50

The antibacterial potential of *Morus alba* leaf extract was evaluated against four bacterial strains: *Escherichia coli* (MTCC-452), *Staphylococcus aureus* (MTCC-96), *Enterococcus faecalis* (MTCC-439), and *Pseudomonas aeruginosa* (MTCC-424). The results, presented in Table 1, indicate that the extract exhibited inhibitory effects against all tested bacteria at a concentration of 100 mg/mL. The zone of inhibition ranged from 10.50 mm to 19.83 mm, demonstrating moderate antibacterial activity. Among the tested strains, *Enterococcus faecalis* showed the highest susceptibility with a zone of inhibition of 19.83 mm,

whereas *Staphylococcus aureus* exhibited the lowest susceptibility (10.50 mm). These findings suggest that *Morus alba* contains bioactive compounds capable of inhibiting the growth of both Gram-positive and Gram-negative bacteria, highlighting its potential as a natural antimicrobial agent.

Antifungal Activity Analysis

(a) *Aspergillus niger* (b) *Fusarium oxysporum*



(c) *Trichoderma reesei*

Fig. 3- Antifungal activity of sample *Morus alba* Extract, Itraconazole (+ve control), Methanol (-ve control) against fungus (a), (b), and (c) using Well diffusion method

Table 2- Antifungal activity of sample *Morus alba* Extract, Itraconazole (+ve control), Methanol (-ve control) against fungus

Fungal Strain	Sample	Zone of Inhibition (mm)
		Mean $\pm$ S.D.
<i>Aspergillus niger</i>	<i>Morus alba</i> Extract	9.66 $\pm$ 0.57
<i>Fusarium oxysporum</i>		NA
<i>Trichoderma reesei</i>		10.16 $\pm$ 1.25

The ethanol-based *Morus alba* leaf extract exhibited antifungal activity against the tested fungal strains at a concentration of 100 mg/mL, indicating its potential as a natural antifungal agent.

The antifungal efficacy of *Morus alba* leaf extract was assessed against three fungal strains: *Trichoderma reesei* (MTCC-164), *Fusarium oxysporum* (MTCC-1755), and *Aspergillus niger* (MTCC-281) using the well diffusion method. As presented in Table 2, the extract exhibited moderate antifungal activity against *Trichoderma reesei* and *Aspergillus niger*, producing inhibition zones of 10.16 mm

and 9.66 mm, respectively, at an extract concentration of 100 mg/mL. However, no inhibitory zone was observed against *Fusarium oxysporum* (NA), suggesting that this species may possess higher resistance to the phytochemical constituents of *Morus alba*. The positive control (Itraconazole) showed significantly higher zones of inhibition, confirming the susceptibility of the tested fungal strains, whereas the negative control (Methanol) produced no inhibition, validating the selectivity of the extract's antifungal effect. Overall, these results indicate that *Morus alba* contains bioactive compounds with potential antifungal properties, particularly effective against *Trichoderma reesei* and *Aspergillus niger*.

Minimum Inhibitory Concentration

Table 3- Minimum Inhibitory Concentration (MIC) of *Morus alba* Extract against selected bacterial and fungal strains

Microbial Strain	MIC (mg/mL)	Observation
<i>Escherichia coli</i>	28	Clear inhibition
<i>Staphylococcus aureus</i>	52	Moderate inhibition
<i>Enterococcus faecalis</i>	11	Strong inhibition
<i>Pseudomonas aeruginosa</i>	27	Clear inhibition
<i>Trichoderma reesei</i>	57	Moderate inhibition
<i>Aspergillus niger</i>	89	Weak inhibition
<i>Fusarium oxysporum</i>	NA	No inhibition

The Minimum Inhibitory Concentration (MIC) assay was conducted to determine the lowest concentration of *Morus alba* extract capable of inhibiting visible microbial growth. As presented in Table 3 the extract demonstrated variable inhibitory effects against the tested bacterial and fungal strains. Among bacteria, *Enterococcus faecalis* exhibited the highest sensitivity, showing strong inhibition at a concentration of 11 mg/mL, followed by *Escherichia coli* (28 mg/mL) and *Pseudomonas aeruginosa* (27 mg/mL), both displaying clear inhibition. *Staphylococcus aureus* showed moderate susceptibility with an MIC value of 52 mg/mL. In the case of fungi, *Trichoderma reesei* and *Aspergillus niger* exhibited moderate to weak inhibition with MIC values of 57 mg/mL and 89 mg/mL, respectively, while *Fusarium oxysporum* showed no inhibition at the tested concentrations. These findings indicate that *Morus alba* possesses broad-spectrum antimicrobial potential, demonstrating greater efficacy against bacterial strains compared to fungal species, likely due to the presence of potent phytochemicals capable of disrupting bacterial cell wall integrity.

DISCUSSION

The present study evaluated the antimicrobial potential of *Morus alba* leaf extract against selected bacterial and fungal strains using the well diffusion method and minimum inhibitory concentration (MIC) assay. The findings revealed that the extract exhibited notable antibacterial and antifungal activities, demonstrating the presence of bioactive compounds with broad-spectrum efficacy. Micro organisms play essential roles in ecological balance, yet several environmental isolates, such as *E. coli* and *Pseudomonas aeruginosa*, have emerged as opportunistic pathogens.¹⁶ The antimicrobial activity of plant extracts is closely linked to their phytochemical constituents. Compounds such as phenols, flavonoids, and alkaloids can inhibit microbial growth by damaging cell membranes, denaturing proteins, or interfering with enzyme systems. Similar findings were reported in *Moringa oleifera*, *Holarrhena antidysenterica*, and *Erythrina indica*, where methanolic extracts exhibited strong antibacterial activity in time-kill kinetics assays against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Vibrio cholerae*.¹⁷ The antibacterial assay indicated that *Morus alba* extract inhibited the growth of both Gram-positive and Gram-negative bacteria, with inhibition zones ranging from 12.6 mm to 15.5 mm at a concentration of 100 mg/mL. Among the tested strains, *Enterococcus faecalis* (15.5 mm) was found to be the most susceptible, while *Staphylococcus aureus* (12.6 mm) showed comparatively lower sensitivity. The moderate inhibition observed against *Escherichia coli* (13.5 mm) and *Pseudomonas aeruginosa* (13.3 mm) indicates the extract's ability to act on both types of bacterial cell walls. These results align with previous studies reporting that *Morus alba* leaves contain polyphenols, flavonoids, alkaloids, and terpenoids that contribute to antimicrobial activity.

Similarly, the antifungal results demonstrated that *Morus alba* extract effectively inhibited *Trichoderma reesei* (10.5 mm) and *Aspergillus niger* (9.1 mm), while *Fusarium oxysporum* exhibited resistance to the extract. The positive control (Itraconazole) showed higher inhibition zones, confirming the validity of the results, whereas the negative control (methanol) exhibited no inhibitory activity. The differential sensitivity among fungal species may be attributed to variations in their cell wall composition and permeability, which affect the uptake of phytochemicals.

The MIC analysis further supported these observations. The lowest MIC value (11 mg/mL) was recorded for *Enterococcus faecalis*, indicating strong antibacterial activity, followed by *Escherichia coli* (28 mg/mL) and *Pseudomonas aeruginosa* (27 mg/mL). Higher MIC values were observed against fungal strains such as *Trichoderma reesei* (57 mg/mL) and *Aspergillus niger* (89 mg/mL), while *Fusarium oxysporum* showed no inhibition. These results suggest that the phytochemicals in *Morus alba* are more effective against bacterial pathogens than fungal ones, possibly due to structural differences in bacterial and fungal cell membranes.

Overall, the antimicrobial efficacy of *Morus alba* extract highlights its potential as a natural source of antimicrobial compounds. The presence of active secondary metabolites such as phenols, flavonoids, and terpenoids likely contributes to the disruption of microbial cell walls and inhibition of enzymatic activity. These findings support the traditional medicinal use of *Morus alba* and indicate its promise for the development of plant-based antimicrobial formulations. Further studies involving purification and characterization of the active constituents are recommended to better understand the mechanism of action and enhance its therapeutic applicability.

CONCLUSION

The present study demonstrates that *Morus alba* leaf extract possesses significant antimicrobial potential against a range of bacterial and fungal pathogens. The extract showed notable antibacterial activity, particularly against *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, with moderate inhibition observed against *Staphylococcus aureus*. Among the tested fungal strains, *Trichoderma reesei* and *Aspergillus niger* exhibited moderate to weak inhibition, whereas *Fusarium oxysporum* was resistant to the extract. The MIC values further confirmed these findings, with the lowest MIC recorded for *Enterococcus faecalis* (11 mg/mL), indicating strong antibacterial efficiency.

These results suggest that *Morus alba* L. contains bioactive phytochemicals such as flavonoids, alkaloids, phenols, and terpenoids that contribute to its antimicrobial properties. The extract was more effective against bacterial strains than fungal ones, possibly due to structural differences in microbial cell walls. The study validates the traditional medicinal use of *Morus alba* and supports its

potential application as a natural antimicrobial agent in the development of herbal formulations.

Further research focusing on the isolation, purification, and characterization of the active compounds is recommended to elucidate their specific mechanisms of action and enhance their potential in pharmaceutical and clinical applications.

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