

Original Research Article

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Phytochemical Characterization of *Moringa oleifera* Leaf Extract and Assessment of its Antioxidant, Antimicrobial and Antibiofilm Activities

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ABSTRACT

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Moringa oleifera leaves are a rich source of plant secondary metabolites and have been investigated for diverse biological activities. The present study characterized a methanolic leaf extract of *M. oleifera* by qualitative phytochemical screening and thin-layer chromatography (TLC), followed by assessment of antioxidant, antimicrobial and antibiofilm activities. Fresh leaves collected in Chennai, Tamil Nadu, were shade-dried, powdered and extracted with methanol. The extract showed strong reactions for flavonoids and tannins, moderate reactions for alkaloids, terpenoids, phenols and quinones, and no detectable saponins under the test conditions. TLC produced a prominent spot with an R_f value of approximately 0.98. In the DPPH assay, radical-scavenging activity increased with extract concentration. The extract inhibited *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*, with the largest recorded inhibition zones at 100 µg/mL measuring 19, 16 and 15 mm, respectively. In the crystal-violet microtiter assay, antibiofilm activity was concentration dependent. At 100 µg/mL, inhibition reached 76.6% for *S. aureus*, 53.1% for *E. coli* and 59.0% for *C. albicans*. These findings indicate that the tested *M. oleifera* leaf extract contains detectable phytochemical constituents and expresses measurable in vitro antioxidant, antimicrobial and antibiofilm activities.

Introduction

Medicinal plants remain important sources of chemically diverse compounds with potential applications in food, pharmaceutical and biomedical research. *Moringa oleifera*, a member of the family Moringaceae, is widely cultivated in tropical and subtropical regions and is

valued for both nutritional and traditional medicinal uses. Its leaves contain phenolics, flavonoids and other secondary metabolites that have been associated with antioxidant and antimicrobial properties. The search for plant-derived antimicrobial agents has gained importance alongside the continuing problem of resistance to conventional antimicrobials. At the same time,

antioxidant screening provides a useful preliminary measure of the capacity of plant extracts to neutralize stable free radicals in vitro. Biofilm formation represents an additional microbial survival strategy because cells embedded in an extracellular matrix can show increased tolerance to antimicrobial exposure.

Phytochemical screening provides a preliminary indication of the major classes of secondary metabolites present in an extract, whereas TLC can provide a rapid chromatographic fingerprint. The present investigation therefore combined qualitative phytochemical screening, TLC profiling, DPPH radical-scavenging analysis, agar diffusion testing and a crystal-violet microtiter assay to characterize the biological properties of methanolic *M. oleifera* leaf extract.

Materials and Methods

Collection and Preparation of plant material

Fresh, fully expanded and disease-free *M. oleifera* leaves were collected during January from Chennai, Tamil Nadu, India (Fig 1). The leaves were washed with distilled water, blotted dry and shade-dried at room temperature until a constant condition was obtained. The dried material was pulverized to a fine powder and stored in an airtight container until extraction (Fig.2A)

Systematic Position of *Moringa olifera*

Kingdom: Plantae
Subkingdom: Tracheobionta (Vascular plants)
Division/Phylum: Tracheophyta (Magnoliophyta)
Class: Magnoliopsida (Dicots)
Order: Brassicales
Family: Moringaceae
Genus: *Moringa*
Species: *oleifera*

Extraction of methanolic extract

Five grams of powdered leaf material was immersed in 50 mL methanol and stirred thoroughly (Fig. 2B). The mixture was maintained at room temperature for 24 h. The extract was filtered through Whatman No. 1 filter paper, freeze-dried and stored at 4 °C until analysis.

Qualitative phytochemical screening

The extract was examined qualitatively for major

phytochemical groups using standard procedures cited in the dissertation, including tests for alkaloids, saponins, flavonoids, tannins, phenols, terpenoids and quinones.

Thin-layer chromatography

The methanolic extract was subjected to TLC to obtain a preliminary phytochemical fingerprint followed by the method of Lawrence *et al.*, 2015. The separated constituents were visualized and their retention factor (Rf) was determined from the migration distance of the compound relative to the solvent front.

Antimicrobial assay

The antimicrobial activity was examined against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Cultures were prepared in nutrient broth to approximately 0.5 McFarland turbidity. Mueller–Hinton agar plates were inoculated uniformly and the extract was tested at 50, 75 and 100 µg/mL. Streptomycin served as the reference antimicrobial. Plates were incubated at 37 °C for 24 h and inhibition zones were measured in millimetres.

DPPH radical-scavenging assay

Antioxidant activity was assessed using the DPPH radical-scavenging method. A DPPH working solution was adjusted to an absorbance of approximately 0.98 ± 0.02 at 517 nm. Extract samples were tested over the concentration range stated in the dissertation (10–500 µg/mL), incubated for 30 min in darkness at room temperature and read at 517 nm. Scavenging activity was calculated as: $[(\text{control absorbance} - \text{sample absorbance}) / \text{control absorbance}] \times 100$.

Microtiter plate antibiofilm assay

Biofilm inhibition was evaluated for *S. aureus*, *E. coli* and *C. albicans* using a 96-well crystal-violet assay. Two hundred microlitres of microbial suspension was added to each well and extract concentrations of 25, 50, 75 and 100 µg/mL were tested. After incubation at 37 °C for 24 h, non-adherent cells were removed and the wells were washed with phosphate-buffered saline. Attached biofilm was fixed with methanol, stained with 0.1% crystal violet, washed and the retained stain was solubilized. Absorbance was measured at 570 nm. Biofilm inhibition was expressed relative to the untreated control.

Results and Discussion

The whole plant of *Moringa oleifera* was successfully collected during the month of January from Chennai, Tamil Nadu, India. The collected leaves were disease-free and suitable for further experimental analysis Fig.1.

Collection and Processing of Plant Material

The collected *Moringa oleifera* leaves were initially observed in their fresh form and later subjected to shade drying (Fig. 2A). The drying process resulted in a reduction of moisture content along with slight changes in color, confirming proper dehydration. The dried leaves were further processed into a fine powder (Fig. 2B). The uniformity of the powdered sample indicates efficient grinding and suitability for extraction procedures.

Phytochemical Analysis

The qualitative phytochemical screening of *Moringa oleifera* leaf extract revealed the presence of various bioactive compounds (Table 1). Flavonoids and tannins were observed in strong abundance (+++), indicating their high concentration in the extract. Alkaloids, terpenoids, phenols, and quinones showed moderate presence (++) , whereas saponins were not detected (–). The presence of these phytoconstituents suggests the potential biological activity of the plant extract.

TLC profile

Thin Layer Chromatography analysis of *Moringa oleifera* leaf extract showed distinct spots with an Rf value of approximately 0.98 (Fig. 3). The observed separation indicates the presence of phytochemical constituents with similar polarity and confirms the presence of bioactive compounds in the extract.

Antimicrobial activity

The antimicrobial activity of the methanolic leaf extract of *Moringa oleifera* was assessed against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* (Table 2). The results demonstrated a concentration-dependent increase in the zone of inhibition. At 50 µg/mL, moderate inhibition was observed against all tested microorganisms. Increased activity was noted at 75 µg/mL, while the highest inhibition was recorded at 100 µg/mL, with zone diameters of 19 mm for *S. aureus*, 16

mm for *E. coli*, and 15 mm for *C. albicans*. The standard drug streptomycin exhibited greater inhibitory activity compared to the plant extract. These findings indicate that *Moringa oleifera* leaf extract possesses notable antimicrobial properties.

Zone of Inhibition Analysis

The antimicrobial activity was further confirmed by the presence of clear zones of inhibition in the assay plates (Fig. 4.5 A–C). The formation of these zones indicates the susceptibility of the test organisms to the plant extract. An increase in the diameter of the inhibition zones was observed with increasing extract concentration, demonstrating a dose-dependent antimicrobial effect against *E. coli*, *S. aureus*, and *C. albicans*.

Antioxidant activity

The antioxidant activity of *Moringa oleifera* leaf extract was evaluated using the DPPH free radical scavenging assay (Fig. 5). The extract demonstrated significant antioxidant potential, with increased activity observed at higher concentrations. The results indicate a dose-dependent free radical scavenging effect, suggesting that the extract can serve as a natural antioxidant source.

Antibiofilm activity

Antibiofilm Activity against *Candida albicans*

The antibiofilm effect of the extract against *Candida albicans* also showed a concentration-dependent pattern. The control recorded an OD value of 0.987, indicating substantial biofilm formation. The extract reduced biofilm formation from 37.3% inhibition at 25 µg/mL to 59.0% at 100 µg/mL. The positive control showed 80.4% inhibition. These findings suggest that the extract possesses notable antifungal antibiofilm activity, though slightly lower than the standard.

The *Moringa oleifera* leaf extract exhibited significant antibiofilm activity against all tested microorganisms, with effectiveness increasing with concentration. The highest inhibition was observed against *Staphylococcus aureus*, followed by *Candida albicans* and *Escherichia coli*. The results indicate that the extract can disrupt biofilm formation, highlighting its potential as a natural antibiofilm agent.

The qualitative phytochemical screening of *Moringa oleifera* confirmed the presence of flavonoids, tannins, alkaloids, phenols, terpenoids, and quinones, while saponins were not detected. The strong presence of flavonoids and tannins is particularly significant, as these compounds are widely reported for their antioxidant and antimicrobial properties. Similar findings have been reported by Sreelatha and Padma (2009), who demonstrated that *M. oleifera* leaves are rich in phenolic compounds contributing to their biological activity. In addition, Coppin *et al.*, (2013) also reported abundant polyphenols and flavonoids in *M. oleifera*, supporting its medicinal value. The absence of saponins in the present study may be attributed to solvent specificity or geographical variation.

The transition from fresh to dried leaves showed reduction in moisture content and slight color variation, indicating successful dehydration. Proper drying is essential to prevent microbial contamination and degradation of phytochemicals. The fine powdered form obtained ensured increased surface area for efficient extraction. These observations are consistent with standard plant processing techniques described by Harborne (1998), emphasizing the importance of sample preparation in phytochemical studies. Similarly, Azwanida (2015) highlighted that drying and particle size significantly influence extraction efficiency and phytochemical yield.

The TLC analysis revealed a prominent spot with an R_f value of approximately 0.98, indicating the presence of less polar phytoconstituents in the extract. TLC serves as a simple, rapid, and cost-effective technique for preliminary identification and separation of bioactive compounds. The observed R_f value suggests the presence of compounds such as flavonoids or phenolic derivatives, which are known to contribute to biological activity.

Similar TLC profiles of *Moringa oleifera* have been reported by Kasolo *et al.*, (2010), confirming the presence of multiple phytoconstituents responsible for its pharmacological properties. In addition, Wagner and Bladt (1996) emphasized that TLC is a reliable tool for phytochemical fingerprinting and compound identification in medicinal plant research.

The DPPH assay demonstrated that *Moringa oleifera* leaf extract exhibits significant free radical scavenging activity in a concentration-dependent manner. This activity can be attributed to the presence of phenolic compounds and flavonoids, which donate hydrogen atoms to neutralize free radicals. These findings align with the work of Anwar *et al.*, (2007), who reported strong antioxidant potential in *M. oleifera* due to its rich phytochemical composition. Similarly, Verma *et al.*, (2009) also reported significant DPPH scavenging activity of *M. oleifera* leaf extracts.

The antimicrobial assay revealed that the methanolic extract exhibited activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, with increased inhibition at higher concentrations. The greater susceptibility of *S. aureus* compared to *E. coli* may be due to differences in cell wall structure between Gram-positive and Gram-negative bacteria. Similar antimicrobial effects of *M. oleifera* leaf extracts have been documented by Rahman *et al.*, (2009), supporting its use as a natural antimicrobial agent. Furthermore, Peixoto *et al.*, (2011) also reported broad-spectrum antimicrobial activity of *M. oleifera* extracts.

The formation of clear zones of inhibition confirms the effectiveness of the extract against the tested microorganisms. The observed dose-dependent increase in inhibition zones indicates that the bioactive compounds exert stronger effects at higher concentrations. This trend is consistent with findings by Doughari *et al.*, (2007), who reported similar dose-dependent antimicrobial responses in plant extracts. Likewise, Nostro *et al.*, (2000) highlighted that plant extracts exhibit concentration-dependent inhibitory effects against microbial pathogens.

The results of the present study collectively demonstrate that *Moringa oleifera* leaf extract possesses significant phytochemical richness and notable biological activities, including antioxidant and antimicrobial effects. These properties are primarily attributed to the presence of phenolics, flavonoids, and tannins. The findings are in agreement with previous literature and support the therapeutic potential of *Moringa oleifera* as a natural source of bioactive compounds for pharmaceutical applications.

Fig.1 *Moringa olifera*



Fig.2A Fresh and Dried Leaves of *M. olifera*



Fig.2B Powder form of Leaves of *M. olifera*



Fig.3 TLC profile of the methanolic leaf extract

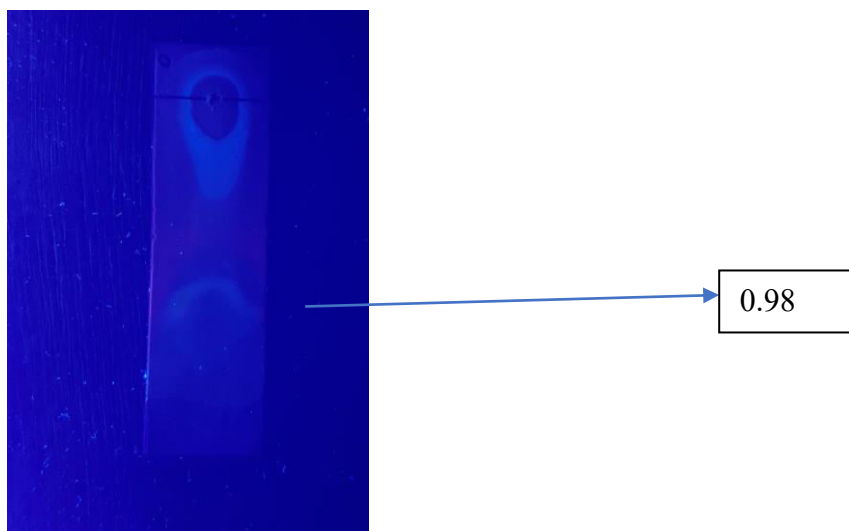


Fig.4 Antimicrobial Activity of *M. olifera* which shows Zone of Inhibition against A. *Escherichia coli*; B. *Staphylococcus aureus* and C. *Candida albicans*

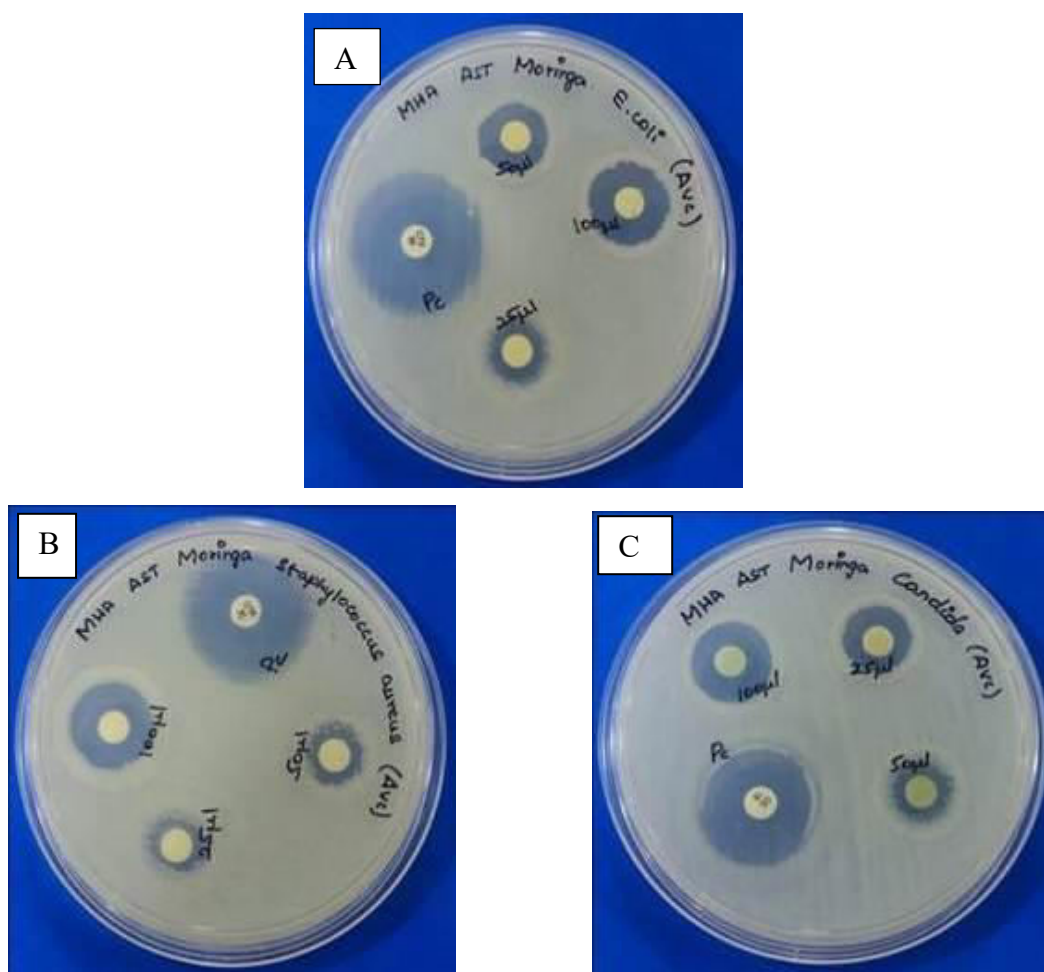


Fig.5 Antioxidant activity of Leaf extract of *M. olifera* - DPPH assay

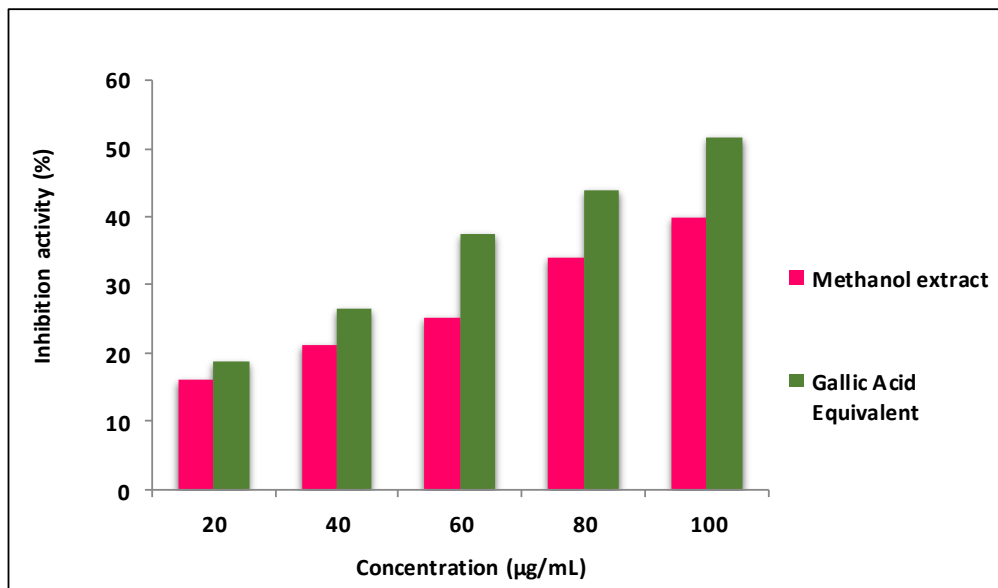


Table.1 Qualitative phytochemical profile of methanolic *Moringa oleifera* leaf extract.

Phytochemical group	Observation
Flavonoids	+++
Tannins	+++
Alkaloids	++
Terpenoids	++
Phenols	++
Quinones	++
Saponins	—

- Not detected; + Mild Positive; ++ Moderate Positive; +++ Strong Positive

Table.2 Maximum recorded inhibition zones of the methanolic *M. oleifera* leaf extract.

Extracts	Doses	<i>S. aureus</i>	<i>E. coli</i>	<i>Candida albicans</i>
	Control	-	-	-
Methanol extract	50 µg/mL	12mm	12mm	13mm
	75 µg/mL	13mm	14mm	13mm
	100 µg/mL	19mm	16mm	15mm
Streptomycin (Control drug)		26mm	27mm	27mm

The present study demonstrated that *Moringa oleifera* leaf extract exhibits significant antibiofilm activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The results showed a clear concentration-dependent inhibition of biofilm formation, with maximum activity observed at 100 µg/mL. Among the tested organisms, *Staphylococcus aureus* showed the highest susceptibility, followed by *Candida albicans* and *Escherichia coli*. The comparatively lower inhibition observed in *E. coli* may be attributed to the presence of an outer membrane in Gram-negative bacteria, which restricts the penetration of bioactive compounds.

The antibiofilm activity of *Moringa oleifera* can be attributed to the presence of phytochemicals such as flavonoids, phenolics, and tannins, which are known to interfere with microbial adhesion, quorum sensing, and extracellular polymeric substance (EPS) production. These compounds may disrupt the initial stages of biofilm formation as well as destabilize mature biofilms. Similar findings have been reported by Doughari *et al.*, (2007), who demonstrated that *Moringa oleifera* leaf extracts possess strong antimicrobial properties against a wide range of pathogens.

In addition, Vergara-Jimenez *et al.*, (2017) reported that the bioactive constituents of *M. oleifera* contribute to its antimicrobial and antibiofilm potential by inhibiting microbial growth and colonization.

Overall, the results suggest that *Moringa oleifera* leaf extract can be considered a promising natural antibiofilm agent with potential applications in controlling biofilm-associated infections.

In conclusion, Methanolic *Moringa oleifera* leaf extract showed a diverse preliminary phytochemical profile, a distinct TLC fingerprint, concentration-dependent DPPH radical-scavenging activity, antimicrobial activity against *S. aureus*, *E. coli* and *C. albicans*, and measurable antibiofilm activity. The highest recorded antimicrobial zones at 100 µg/mL were 19, 16 and 15 mm for *S. aureus*, *E. coli* and *C. albicans*, respectively, while antibiofilm inhibition at the same concentration reached 76.6%, 53.1% and 59.0%. The study provides a basis for subsequent work involving quantitative phenolic/flavonoid estimation, compound identification by HPLC or LC–MS, dose-response analysis with replication and statistical testing, and mechanistic evaluation of antibiofilm activity.

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Author Contributions

Shyamala V: Supervision, scientific guidance, validation of experimental work, interpretation of results, critical review, and manuscript editing. Grace Sofiya S: Conceptualization, sample collection, experimental work, phytochemical analysis, antioxidant, antimicrobial and antibiofilm assays, and manuscript preparation.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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