

# Phytochemical Constituents and Antibacterial Activity of Aqueous and Ethanolic Extracts of *Moringa oleifera* Leaves

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**Abstract**—The present study aimed to evaluate the antibacterial activity of *Moringa oleifera* (family: Moringaceae) leaf extracts and to perform phytochemical screening. *Moringa oleifera* is a widely utilized plant in tropical regions, valued in Ayurvedic medicine for its therapeutic properties. Its leaves are known for their nutritional benefits and potential antibiotic effects. In this study, aqueous and ethanolic extracts of *M. oleifera* leaves were prepared and tested against a panel of bacterial strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Streptococcus mutans*, *Bacillus subtilis*, and *Staphylococcus epidermidis*. Phytochemical analysis of both extracts revealed the presence of bioactive compounds including flavonoids, tannins, steroids, alkaloids, and saponins. The antibacterial activity was assessed using the agar well diffusion method. Both extracts exhibited inhibitory effects against all tested bacterial strains. Notably, the ethanolic extract demonstrated maximum antibacterial activity against *Streptococcus mutans*, whereas the aqueous extract showed the highest activity against *Proteus vulgaris*. These findings suggest that *M. oleifera* leaves possess broad-spectrum antibacterial potential, likely due to the presence of multiple phytoconstituents. Further studies focusing on compound isolation and mechanism of action are warranted to explore its potential in antimicrobial drug development.

**Keywords**—Antibacterial Activity, *Moringa oleifera*, Phytochemical Screening

## I. INTRODUCTION

The exploration of medicinal plants for therapeutic purposes has garnered significant attention in recent years, particularly in response to the increasing threat of antimicrobial resistance<sup>[1]</sup>. Among the vast array of botanicals with documented medicinal properties, *Moringa oleifera* Lam. (family: Moringaceae) stands out due to its broad spectrum of pharmacological activities. Commonly known as the drumstick tree, *M. oleifera* is native to the Indian

subcontinent and is widely cultivated in tropical and subtropical regions<sup>[2,3]</sup>. Traditionally, various parts of the plant—including leaves, seeds, bark, roots, and flowers—have been used in Ayurvedic and folk medicine for treating a range of ailments such as infections, inflammation, and malnutrition<sup>[4]</sup>.

The leaves of *M. oleifera* are especially valued for their rich nutritional content and presence of bioactive compounds, including flavonoids, alkaloids, tannins, and saponins<sup>[5]</sup>. These phytoconstituents are known to possess significant antimicrobial, antioxidant, and anti-inflammatory properties<sup>[6]</sup>. With rising concerns over the efficacy and safety of synthetic antibiotics, natural products like *M. oleifera* are being explored as alternative sources for novel antibacterial agents<sup>[7]</sup>.

Several studies have indicated the antimicrobial potential of *M. oleifera*, yet there remains a need for comparative analysis of its effectiveness in different solvent systems<sup>[8]</sup>. Solvent polarity can significantly influence the extraction efficiency of phytochemicals, thereby impacting biological activity<sup>[9]</sup>. This study aims to evaluate the phytochemical profile and antibacterial activity of *M. oleifera* leaf extracts prepared using aqueous and ethanolic solvents<sup>[10]</sup>. The antibacterial potential will be tested against selected gram-positive and gram-negative bacteria using standard microbiological methods<sup>[11]</sup>. The findings from this investigation could contribute to the development of plant-based antimicrobial formulations and validate the ethnopharmacological applications of *M. oleifera*<sup>[12]</sup>.

## II. MATERIALS AND METHODS

### Collection of Plant Materials

The experiment was conducted in 2025 in the college laboratory. Fresh, healthy, and disease-free leaves of *Moringa oleifera* were collected from the

institutional herbal garden. The leaves were thoroughly washed under running tap water to remove dust and surface contaminants, then shade-dried for further processing.

#### Preparation of Leaf Extracts

Fresh leaves of *Moringa oleifera* (20–30 g) were collected and shade-dried at room temperature (32–35 °C) for a period of five days until a constant weight was achieved, indicating complete moisture removal. The dried leaves were then ground into a fine powder using a clean mortar and pestle to ensure uniform particle size suitable for extraction<sup>[13,14]</sup>.

For the preparation of extracts, 25 g of the powdered leaves were separately transferred into 500 mL conical flasks containing 90% ethanol and distilled water to obtain ethanolic and aqueous extracts, respectively<sup>[15]</sup>. The flasks were sealed with sterile rubber corks to prevent contamination and placed on a rotary shaker at 120 revolutions per minute (rpm) for 30 minutes to initiate the extraction process. Subsequently, the flasks were left undisturbed at room temperature for five days to allow thorough extraction, with occasional manual agitation every 24 hours using a sterile glass rod to enhance solvent penetration and compound release<sup>[16]</sup>.

After the extraction period, the contents of each flask were filtered through sterile Whatman No. 1 filter paper to separate the liquid extracts from plant residues. The resulting ethanolic and aqueous extracts were collected in sterile containers, labeled accordingly, and stored at refrigerated temperatures until further phytochemical and antibacterial analyses were performed<sup>[17]</sup>.

#### Phytochemical Analysis

Phytochemical analysis of extract for qualitative detection of alkaloids, flavonoids, steroid, volatile oil, glycoside, reducing sugar, tannins and saponins was performed by the extracts.

##### Alkaloids

- Wagner's test
- Dragandroff test
- Hager test
- Baljet test

##### Flavonoids

3ml of each extract was added to 10ml of distilled water and the solution was shaken. 1ml of 10% NaOH solution was added to the mixture.

##### Saponins

Frothing Test: 3ml of each extract and dilute with 2ml of distilled water was added in a test tube. The mixture was shaken vigorously.

##### Steroids

Salkowski Test: 5 drops of concentrated H<sub>2</sub>SO<sub>4</sub> were added to 1ml of each extract in a separate test tube.

##### Tannins

2ml of each extract in a separate test tube were boiled gently for 2min and allowed to cool. 3 drop of ferric chloride solution were added to each extract.

##### Glycosides

25ml of dilute sulphuric acid was added to 5ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10%NaOH, then 5ml of Fehling solution added.

##### Reducing Sugars

To 0.5ml of plant extracts, 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath.

##### Volatile Oil

2ml of Extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl<sup>[18]</sup>.

#### Source of Microorganisms

The bacterial strains used in this study included *Escherichia coli* (MTCC 40), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC 87), *Proteus vulgaris* (MTCC 742), *Streptococcus mutans* (MTCC 497), *Bacillus subtilis* (MTCC 441), and *Staphylococcus epidermidis* (MTCC 9041). All strains were procured from the Microbial Type Culture Collection (MTCC), Bangalore, India. Upon receipt, the organisms were maintained under recommended conditions as per MTCC guidelines to preserve viability and purity. Sub-culturing was performed at regular intervals of 15 days to ensure the continuous availability of active cultures for antibacterial testing.

#### Determination of Antibacterial Activity

The antibacterial activity of *Moringa oleifera* leaf extracts was evaluated using the agar well diffusion method following standard protocols. Nutrient agar plates were inoculated by uniformly spreading bacterial suspensions over the surface. Wells of 6 mm diameter were aseptically punched into the agar, and each well was filled with the respective plant extract. Negative control wells containing the

respective solvents without extracts were included on the same plates. The inoculated plates were incubated at 37°C for 24 hours. Antibacterial efficacy was determined by measuring the diameter of the zones of inhibition surrounding the wells. The activity of the plant extracts was compared to that of the standard antibiotic streptomycin to assess relative antibacterial potency<sup>[19,20]</sup>.

Solvent used for extraction	Ethanol	Water
Alkaloid	+	+
Flavonoid	+	+
Saponin	+	+
Steroid	+	+
Tannin	+	+
Glycoside	-	-
Reducing sugar	-	-
Volatile oil	-	+

Table 1: Qualitative Phytochemical Screening of Ethanol and Aqueous Leaf Extract of *Moringa oleifera*

The antibacterial activity of *Moringa oleifera* leaf extracts was evaluated against several bacterial strains, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Streptococcus mutans*, *Bacillus subtilis*,

### III. RESULT AND DISCUSSION

The present study demonstrates that *Moringa oleifera* leaf extracts contain various phytochemical constituents, including alkaloids, flavonoids, carbohydrates, glycosides, proteins, saponins, tannins, terpenoids, and anthraquinones, as detailed in Table 1.

and *Staphylococcus epidermidis*. The ethanolic leaf extract exhibited the highest antibacterial activity against *Streptococcus mutans*, whereas the aqueous extract showed maximum efficacy against *Proteus vulgaris*, as summarized in Table 2.

Name of Microorganism	Zone of Inhibition (mm)		Standard (Streptomycin)
	Water Extract	Ethanol Extract	
<i>Escherichia Coli</i>	01	-	05
<i>Staphylococcus aureus</i>	03	-	06
<i>Proteus vulgaris</i>	05	02	05
<i>Pseudomonas aeruginosa</i>	-	04	07
<i>Bacillus subtilis</i>	04	03	05
<i>Staphylococcus epidermidis</i>	-	02	04
<i>Streptococcus mutans</i>	02	05	06

Table 2: Antibacterial Activity of Ethanol and Aqueous Leaf Extract of *Moringa oleifera*

Alkaloids are naturally occurring compounds characterized by the presence of basic nitrogen atoms. These substances often exhibit significant pharmacological properties and are widely utilized in both medicine and recreational drug use. Flavonoids, known for their antioxidant activity, enhance the action of Vitamin C and display a broad spectrum of biological activities, including protection against liver toxins, tumors, viruses, and various microbes. Terpenoids, another major class of plant compounds, are widely valued for their aromatic properties and are commonly used in fragrances and flavorings.

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These compounds play a significant role in traditional herbal remedies and are currently being

studied for their antibacterial, antineoplastic, and other pharmaceutical applications. Tannins have demonstrated promising antiviral, antibacterial, and antiparasitic properties, while saponins are known to induce haemolysis of red blood cells. Due to their notable medicinal potential against pathogenic microorganisms, the antibacterial activity of various compounds was evaluated. The medicinal plant *Moringa oleifera* has shown strong antibacterial effects against several pathogens, including *Staphylococcus aureus*, *Pseudomonas*, *Bacillus*, *Klebsiella* and *E. coli*, as reported in earlier studies.

### IV. CONCLUSIONS

The present study clearly establishes that *Moringa oleifera* is a rich source of various phytochemicals,

including alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, and terpenoids. The antibacterial potential of *Moringa oleifera* was effectively demonstrated against a range of test organisms such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Streptococcus mutans*, *Bacillus subtilis*, and *Staphylococcus epidermidis*. These preliminary findings highlight the need for further comprehensive investigations to explore the potential of *Moringa oleifera* as a promising therapeutic agent for the treatment of various infectious diseases.

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