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Synergistic Antibacterial Activity of Moringa oleifera and Hibiscus rosa-sinensis Leaf Extracts

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Abstract:
The emergence of antimicrobial resistance has intensified the need for alternative therapeutic strategies, particularly plant-based combinations exhibiting synergistic effects. The present study investigates the antibacterial potential of ethanolic leaf extracts of Moringa oleifera and Hibiscus rosa-sinensis, individually and in combination. Extracts were prepared by maceration and evaluated for phytochemical constituents, antibacterial activity using agar well diffusion, and possible synergistic interactions. The combined extracts demonstrated enhanced inhibitory effects against Staphylococcus aureus and Pseudomonas aeruginosa compared to individual extracts. FT-IR analysis suggested phytochemical interactions in the combination extract. The findings support the development of polyherbal antibacterial formulations

Keywords: *Moringa oleifera*, Hibiscus rosa-sinensis, Ethanolic extract, Leaf Extracts, FT-IR analysis

Introduction Natural products have historically provided a substantial proportion of pharmacologically active agents. Numerous antibiotics, anticancer drugs, and anti-inflammatory compounds were originally isolated from botanical or microbial sources. Unlike synthetic pharmaceuticals that typically contain a single purified molecule targeting a specific receptor or enzyme, plant extracts consist of complex mixtures of chemically diverse secondary metabolites. These constituents including alkaloids, flavonoids, tannins, terpenoids, phenolic acids, glycosides, and saponins function through multiple biochemical and cellular pathways. Such diversity enables plants to exert broad-spectrum biological effects, antioxidant, immunomodulatory, and anti-inflammatory activities.

Anti-bacterial Activity of Moringa with Hibiscus

Antibacterial activity refers to the ability of a compound (natural, synthetic) to kill bacteria (bactericidal) or inhibit their growth (bacteriostatic). The mechanisms involve interference with essential bacterial processes such as cell wall synthesis, protein synthesis, nucleic acid replication, metabolic pathways, and cell membrane integrity.

Fig.1: Moringa oleifera

Geographical Distribution

Moringa oleifera originated in the foothills of the Himalayas in the Indian subcontinent. It is now cultivated extensively throughout tropical and subtropical regions including South Asia, Africa, Southeast Asia, Central America, and parts of South America. The plant thrives in warm climates and exhibits strong tolerance to drought conditions.

Traditional and Medicinal Uses

- Traditionally, Moringa leaves have been used for:
- Treatment of infections
- Nutritional supplementation
- Management of inflammatory conditions
- Support of liver and digestive health
- Enhancement of immunity

Fig.2: Hibiscus rosa-sinensis

Geographical Distribution

Hibiscus rosa-sinensis is widely cultivated in tropical and subtropical climates across Asia, Africa, the Caribbean, and parts of the Americas. Although believed to have originated in

Southeast Asia, it is now globally distributed as both an ornamental and medicinal plant.

Traditional and Medicinal Uses

Traditionally, Hibiscus rosa-sinensis has been utilized for:

- Management of skin infections
- Wound healing
- Hair growth promotion
- Anti-inflammatory remedies

Materials and Methods

Materials required:

- Plant Materials
- Fresh leaves of Moringa oleifera
- Fresh leaves of Hibiscus rosa-sinensis

Chemicals and Reagents:

- Ethanol (95%) / Methanol / Distilled water
- Nutrient agar
- Nutrient broth
- Dimethyl sulfoxide (DMSO) / sterile water (solvent)
- Standard antibiotic (e.g., Ciprofloxacin / Ampicillin)

Microorganisms

- Staphylococcus aureus (Gram-positive)
- Escherichia coli (Gram-negative)

Equipment

- maceration setup
- Hot air oven
- Rotary evaporator / water bath
- Autoclave
- Incubator
- Laminar airflow
- Sterile Petri plates
- Micropipettes

Methodology

1. **Collection and Authentication of Plant Material**

Fresh leaves of Moringa oleifera and Hibiscus rosa-sinensis were collected from a local area, washed thoroughly with water to remove impurities, shade-dried at room temperature, and authenticated by a botanist.

2. Preparation of Plant Extracts

Drying and Powdering

The dried leaves were powdered separately using a mechanical grinder and stored in airtight containers.

Fig.3

Extraction Method (Maceration)

Materials Required

- Shade-dried powdered leaves of Moringa oleifera
- Shade-dried powdered leaves of Hibiscus rosa-sinensis
- Ethanol (95%) / Methanol / Distilled water
- Conical flasks with stoppers
- Measuring cylinder
- Whatman filter paper No.1
- Rotary evaporator / water bath
- Analytical balance

Procedure

Weighing of Plant Material

About 50 g of coarsely powdered Moringa oleifera leaves was weighed accurately using an analytical balance.

2. Soaking in Solvent: The powdered material was transferred into a clean conical flask and 500 ml of ethanol was added (solvent to drug ratio 1:10).

Fig.4

3. Maceration Process

- The flask was closed with a stopper and kept at room temperature for 72 hours.
- The mixture was shaken intermittently every 6–8 hours to enhance extraction.

4. Filtration

After 72 hours, the mixture was filtered using Whatman filter paper No.1 to obtain the extract.

5. Concentration of Extract

The filtrates were concentrated using a rotary evaporator or water bath at temperatures below 50°C.

6. Drying and Storage

The dried extract was weighed, transferred to airtight containers, and stored at 4°C until further use.

7. Same Procedure for Hibiscus Leaves

The above procedure was repeated separately for Hibiscus rosa-sinensis leaves.

3. Preparation of Combined (Synergistic) Extract

The dried extracts of Moringa and Hibiscus were mixed in different ratios:

A. 1:1
B. 1:2
C. 2:1

The mixtures were dissolved in DMSO to obtain required concentrations (e.g., 25, 50, 100 mg/ml).

4. Preparation of Bacterial Cultures

Selected bacterial strains were inoculated into nutrient broth and incubated at 37°C for 18–24 hours to obtain fresh cultures.

5. Antibacterial Activity Study

Agar well diffusion method was employed.

Procedure

- Sterile nutrient agar plates were prepared and inoculated with bacterial cultures
- Wells were made using a sterile cork borer

- Individual extracts and combined extracts were introduced into the wells
- Standard antibiotic served as positive control
- Solvent served as negative control
- Plates were incubated at 37°C for 24 hours

6. Evaluation of Antibacterial Activity

After incubation, the zone of inhibition (mm) was measured using a ruler.

Individual extracts were compared with combined extracts

Increased zone of inhibition in combined extract indicated synergistic antibacterial activity

Phytochemical screening

Phytochemical Screening of Moringa oleifera Leaves

Phytochemical screening is carried out to identify the presence of various bioactive constituents in Moringa oleifera leaves, which are responsible for its medicinal and nutritional properties.

- Phytochemical Screening Result Table
- Plant: Moringa oleifera
- Part used: Leaves
- Extract: Ethanolic / Aqueous

Results and Discussion

FTIR Analysis: The FTIR analysis confirmed the presence of phenols, flavonoids, alkaloids, and tannins in both individual and combined extracts. The observed peak shifts in the combined extract suggest interaction between functional groups of the two plant extracts. These phytochemical interactions may contribute to the enhanced antibacterial activity observed in the synergistic formulation.

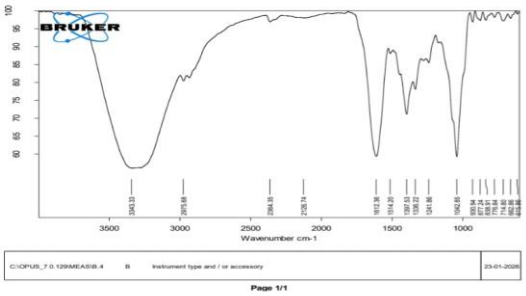

Fig.5: Sample A

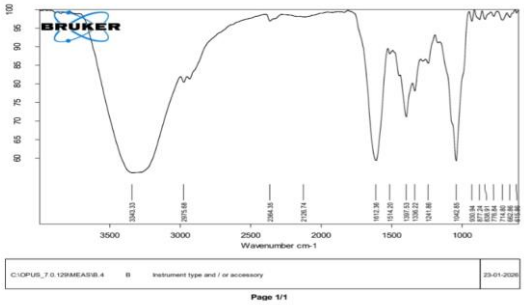

Fig.6: Sample B

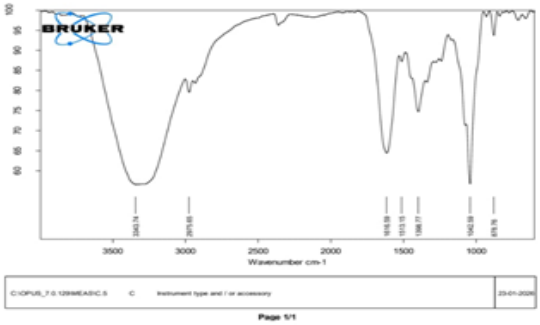

Fig.7: Sample C

Anti-microbial activity Procedure:

The antimicrobial activity of the compounds was evaluated by agar well diffusion method. Bacteria were grown in Nutrient Agar media to be inoculated on Nutrient Agar media. After inoculation, plates were dried for 15 min, and the wells were punched using sterile cork borers. Once wells were formed, they were filled with 50µL of samples (250 µg/ml, 500µg/ml and 1000µg/ml) and blank. Commercially available ampicillin was used as a positive and negative control in this study. Plates were incubated for 24 h at 37 °C to allow diffuse through the agar media to form zones of inhibition. The diameters of the zone of inhibition for different isolates against different bacteria were measured in millimetre for further analysis. An agar well showing zone of inhibition was considered as antimicrobial activity.

Table.1: Staphylococcus aureus (Gram +Ve)

S.NO	Name of the sample	Concentration		
		250 µg/ml	500µg/ml	1000µg/ml
1	**A**	ZNA	ZNA	ZNA
2	**B**	ZNA	6mm	8mm
3	**C**	ZNA	6mm	9mm
4	**Standard**	1mm (1 µg/ml)	9mm (10µg/ml)	10mm (100µg/ml)
5	**Blank**	ZNA	ZNA	ZNA

***Abbreviations:** ZNA- Zone Not Observed

Table.2: Pseudomonas aeruginosa (Gram -Ve)

S.NO	Name of the sample	Concentration		
		250 µg/ml	500µg/ml	1000µg/ml
1	**A**	ZNA	ZNA	ZNA
2	**B**	4mm	6mm	10mm
3	**C**	1mm	2mm	4mm
4	**Standard**	3mm(1 µg/ml)	12mm (10µg/ml)	14mm(100µg/ml)
5	**Blank**	ZNA	ZNA	ZNA

***Abbreviations:** ZNA- Zone Not Observed

Conclusion

The results of the present study indicate that the tested samples exhibit synergistic antibacterial activity, with enhanced zones of inhibition observed at higher concentrations against both Staphylococcus aureus (Gram-positive) and Pseudomonas aeruginosa (Gram-negative). The combined or synergistic effect is evident from the increased antibacterial response of Sample B and Sample C, particularly when compared to their minimal or absent activity at lower concentrations. Among all samples, Sample B demonstrated the strongest synergistic effect, showing a concentration-dependent increase in the zone of inhibition against both bacterial strains. Sample C exhibited moderate synergistic activity, whereas Sample A did not show any antibacterial or synergistic effect. The enhanced inhibition observed at higher concentrations suggests a possible interaction of active constituents leading to improved antibacterial efficacy. The standard drug (ampicillin) confirmed the validity of the assay by producing significant inhibition zones, while the blank showed no activity. Overall, the study concludes that Sample B possesses promising synergistic antibacterial potential and warrants further investigation to identify the compounds responsible for this synergism and to evaluate their possible therapeutic applications.

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