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Research Article

**COMPARATIVE STUDIES OF PHYTOCHEMICAL SCREENING
AND ANTIMICROBIAL ACTIVITY ON ETHANOLIC
EXTRACT OF MUSA ACUMINATA AND MANGIFERA INDICA
(CUP PLATE METHOD)****Kakani Dorka Florence*, Bagu Santhosh Kumar, Kondaveeti Sivaiah, Marapaka Yelishayi,
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Abstract:

This study evaluates the antimicrobial and antifungal properties of Musa acuminata and Mangifera indica. Both plants exhibited significant antimicrobial activity, with M. indica demonstrating a stronger antibacterial effect, especially against Salmonella spp. and E. coli. In contrast, M. acuminata showed greater inhibition of Candida albicans. The presence of bioactive phytochemicals in both plants suggests their potential as natural antimicrobial agents. Further research, including the isolation of active compounds and in vivo testing, is necessary to validate these findings and explore their therapeutic applications for treating bacterial and fungal infections.

Key Words: *Musa acuminata, Mangifera indica, Cup plate method, reflux condensation, antimicrobial, antifungal, phytochemicals.*

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INTRODUCTION:

Microorganisms, or microbes, are microscopic organisms found in diverse environments like soil, water, air, and the human body. They play essential roles in ecological balance, nutrient cycling, and disease causation. Beneficial microbes aid in processes such as digestion, fermentation, and biotechnology, while harmful ones cause infections. Despite their small size, they are vital for oxygen production and decomposition. The field of microbiology expanded significantly after Antonie van Leeuwenhoek's invention of the microscope in the 17th century. Microorganisms are classified based on their structural, functional, and genetic characteristics, driving advancements in medicine, agriculture, and industry.

AIM AND OBJECTIVE

Antibiotics have played a vital role in treating bacterial infections, greatly improving human health. However, their effectiveness is declining due to issues like toxicity and the rise of resistant bacteria. This increasing resistance underscores the need for drug-discovery plants, which have historically been key in drug discovery, aiding in the treatment of infectious diseases and cancer. Despite their success, the discovery of new bioactive compounds has slowed.

Higher plants are a promising source of antimicrobial agents with distinct mechanisms. Numerous studies, especially in India, have investigated the antibacterial properties of medicinal plants. This study specifically examines the antimicrobial potential of ethanolic extracts from *Musa acuminata* and *Mangifera indica* fruits against pathogenic bacteria and fungi. Plants contain bioactive secondary metabolites, such as tannins, alkaloids, flavonoids, and glycosides, that possess antimicrobial properties. Traditional herbal medicines have been used for centuries, with the World Health Organization reporting that 80% of the global population relies on plant-based treatments. The widespread use of synthetic antibiotics has contributed to the rise of multidrug-resistant bacteria,

presenting significant clinical challenges. Despite ongoing efforts in the pharmaceutical industry to develop new antibiotics, bacterial resistance continues to be a major concern, highlighting the urgent need for natural alternatives to effectively combat resistant microbial strains.

METHOD AND METHODOLOGY:**7.1 Collection and Authentication of Plant Material**

The plant materials *Musa acuminata* and *Mangifera indica* were collected from the local market in Perecherla in December 2024. These materials were authenticated to ensure their identity and purity before proceeding with extraction and analysis.

7.2 Preparation of Ethanolic Extract**Method: Reflux Condensation Process****1. Weighing & Preparation:**

- 200g of fresh *Musa acuminata* and *Mangifera indica* fruits were carefully weighed and placed in a 500 mL round-bottom flask.

2. Extraction Process:

- 200 mL of ethanol was added to the flask containing the plant material.
- The mixture was refluxed at 40°C for 8 hours to extract the bioactive compounds.

3. Filtration & Concentration:

- After refluxing, the mixture was filtered through fine muslin cloth to remove solid residues.
- The solvent was then evaporated to concentrate the extract by heating it until three-fourths of the volume was reduced.
- The remaining solvent was allowed to evaporate at room temperature.

4. Final Product:

- A semi-solid ethanolic extract was obtained, which was used for further phytochemical screening and antimicrobial testing.

TABLE 1 : Phytochemical Screening Data (Table)

Test	Reagents Used	Observation	Inference
Glycosides	Legal's Test: Pyridine, Sodium Nitroprusside, NaOH	Pink to red color	Glycosides present
	Borntrager's Test: Chloroform, Dil. Ammonia	Pink color in the ammonia layer	Glycosides present
Saponins	Froth Test: Water	Stable froth (foam)	Saponins present

Test	Reagents Used	Observation	Inference
Alkaloids	Dragendorff's Test: Potassium Bismuth Iodide	Reddish-brown precipitate	Alkaloids present
	Mayer's Test: Potassium Mercuric Iodide	Cream-colored precipitate	Alkaloids present
Tannins	Chromic Acid Test: Chromic acid	No precipitate	Tannins present
Flavonoids	Shinoda Test: Magnesium turnings, Conc. HCl	Pink scarlet/crimson red/green to blue color	Flavonoids present
	Zinc Hydrochloride Test: Zn dust, Conc. HCl	Red color after a few minutes	Flavonoids present
Mucilage	Ruthenium Red Test: Ruthenium red solution	Pink color	Mucilage present
Carbohydrates	Molisch Test: Alcoholic α -naphthol, Conc. H_2SO_4	Violet ring at the junction	Carbohydrates present
Proteins	Xanthoproteic Test: Conc. Nitric acid, 40% NaOH	Yellow precipitate turning orange	Proteins present
Phytosterols	Salkowski Test: Conc. H_2SO_4	Red color (lower layer) or yellow color	Phytosterols present

Antimicrobial and Antifungal Estimation Method

The antimicrobial and antifungal activity was evaluated using specific media and standardized methods. For bacterial studies, Soybean Casein Digest Agar (SCDA) was prepared by dissolving 40g of media in 1000mL of purified water and autoclaving it at 121°C for 15 minutes. For fungal studies, Sabouraud Dextrose Agar (SDA) was prepared similarly, dissolving 35g of media in 1000mL of purified water and autoclaving at the same conditions.

For bacterial studies, the total bacterial count (TBC) was estimated using five petri plates: two for the test samples, one for the standard antibiotic (Ampicillin), one as a blank, and one as a control. One milliliter of the test sample was added to the respective plates and incubated at 22°C for five days. The zone of inhibition was measured to assess antimicrobial activity.

Similarly, for fungal studies, five petri plates were prepared: two for the test samples, one for the standard antifungal (Nystatin), one as a blank, and one as a control. One milliliter of the test sample was added to each plate, followed by incubation at 32°C for seven days. The zone of inhibition was then analysed to determine antifungal activity.

TABLE 2:

The antimicrobial and antifungal activity was tested using the cup-plate method. For bacterial testing, 1mL of the test sample was added to petri plates containing SCDA media with pathogens such as *Escherichia coli*, *Bacillus subtilis*, *Salmonella enterica*, and *Staphylococcus aureus*. For fungal testing, 1mL of the test sample was added to SDA media with *Candida albicans* and *Aspergillus brasiliensis*. Controls of Ampicillin and Nystatin were used as standards. The results were evaluated by observing the zone of inhibition around each sample, indicating antimicrobial or antifungal efficacy.

S. No	Media Type	Technique	Sample Induced (Test)	Pathogen	No. of Plates	Temperature (°C)	Incubation Period
1	SCDA	Cup-plate	1 mL each	<i>Escherichia coli</i>	2	30-35	24-72 hrs
2	SCDA	Cup-plate	1 mL each	<i>Bacillus subtilis</i>	2	30-35	24-72 hrs
3	SCDA	Cup-plate	1 mL each	<i>Salmonella enterica</i>	2	30-35	24-72 hrs
4	SCDA	Cup-plate	1 mL each	<i>Staphylococcus aureus</i>	2	30-35	24-72 hrs
5	SDA	Cup-plate	1 mL each	<i>Candida albicans</i>	2	32	5 days
6	SDA	Cup-plate	1 mL each	<i>Aspergillus brasiliensis</i>	2	32	5 days
7	SCDA	Cup-plate	1 mL each	Ampicillin (Std for antibacterial)	1	22	24-72 hrs
8	SDA	Cup-plate	1 mL each	Nystatin (Std for antifungal)	1	32	5 days

TABLE 3: Antimicrobial and Antifungal Activity Estimation

Parameter	Media Used	Preparation Method	Incubation Conditions	Observation
Bacterial Media Preparation	Soybean Casein Digest Agar	40g media + 1000mL purified water, autoclaved at 121°C for 15 min	-	-
Fungal Media Preparation	Sabouraud Dextrose Agar	35g media + 1000mL purified water, autoclaved at 121°C for 15 min	-	-
Estimation of Total Bacterial Count (TBC)	Soybean Casein Digest Agar	5 Petri plates: 2 test, 1 standard (Ampicillin), 1 blank, 1 control	Incubate at 22°C for 5 days	Zone of inhibition observed
Estimation of Total Fungal Count (TFC)	Sabouraud Dextrose Agar	5 Petri plates: 2 test, 1 standard (Nystatin), 1 blank, 1 control	Incubate at 32°C for 7 days	Zone of inhibition observed

Results and discussion**TABLE 4: Percentage Yield of the Extract**

Plant Name	Yield (%)
Musa acuminata	12.3%
Mangifera indica	9.8%

This table shows that *Musa acuminata* yielded more extract (12.3%) than *Mangifera indica* (9.8%).

TABLE 5: Phytochemical Screening of Plant Extracts

Plant Name	Alk	Carb	Gly	Tan	Phytos	Flav	Sapo	Pro	Muci
Musa acuminata	+	+	-	+	+	+	+	-	+
Mangifera indica	-	+	-	+	+	+	+	+	+

Legend:

- Alk = Alkaloids
- Carb = Carbohydrates
- Gly = Glycosides
- Tan = Tannins
- Phytos = Phytosterols
- Flav = Flavonoids
- Sapo = Saponins
- Pro = Proteins
- Muci = Mucilage (+) = Present, (-) = Absent

Antimicrobial and Antifungal Activity**TABLE 6 : Estimation of Antimicrobial Activity Against E. coli**

Extract	Zone of Inhibition (mm)
Musa acuminata	3.4
Mangifera indica	4.6
Ampicillin (Standard)	5.1

TABLE 7 : Estimation of Antimicrobial Activity for Staphylococcus aureus

Extract	Zone of Inhibition (mm)
Musa acuminata	3.7
Mangifera indica	4.6
Ampicillin (Std)	5.1

TABLE 8 : Estimation of Antimicrobial Activity

Extract	E. coli (mm)	S. aureus (mm)	B. subtilis (mm)
Musa acuminata	3.4	3.7	2.2
Mangifera indica	4.6	4.6	3.9
Ampicillin (Std)	5.1	5.1	5.8

TABLE 9: Estimation of Antimicrobial Activity for Salmonella

Extract	E. coli (mm)	S. aureus (mm)	B. subtilis (mm)	Salmonella (mm)
Musa acuminata	3.4	3.7	2.2	1.3
Mangifera indica	4.6	4.6	3.9	5.1
Ampicillin (Std)	5.1	5.1	5.8	5.6

TABLE10: Estimation of Antifungal Activity for Aspergillus brasiliensis

Extract	Zone of Inhibition (mm)
Musa acuminata	4.8
Mangifera indica	5.1
Nystatin (Standard)	6.2

TABLE 11: Estimation of Antifungal Activity for Candida albicans

Extract	Zone of Inhibition (mm)
Musa acuminata	3.8
Mangifera indica	2.6
Nystatin (Standard)	6.2

TABLE 12: Percentage Inhibition of Bacterial Growth

Bacteria	Musa acuminata (%)	Mangifera indica (%)
Staphylococcus aureus	72.54%	90.19%
Bacillus subtilis	37.93%	67.24%
Escherichia coli	66.66%	90.19%
Salmonella	23.21%	91.07%

TABLE 13 : Percentage Inhibition of Fungal Growth

Fungi	Musa acuminata (%)	Mangifera indica (%)
Aspergillus brasiliensis	77.41%	82.25%
Candida albicans	61.29%	41.93%

DISCUSSION:**Phytochemical Screening**

The phytochemical analysis of the ethanolic extracts revealed the presence of various bioactive compounds, including alkaloids, carbohydrates, flavonoids, glycosides, tannins, saponins, and phytosterols.

- Both plant extracts contained flavonoids, saponins, tannins, and phytosterols, which are known to contribute to antimicrobial activity.
- *Musa acuminata* contained alkaloids, whereas *Mangifera indica* was rich in proteins.
- The presence of flavonoids and tannins in both extracts supports their role in antimicrobial and antifungal properties.

The study aimed to evaluate the antimicrobial and antifungal activities of the ethanolic extracts of *Musa acuminata* and *Mangifera indica* against selected bacterial and fungal pathogens. The findings provide valuable insights into the potential of these plant extracts as natural antimicrobial agents.

Antibacterial Activity

The antibacterial activity was assessed against four bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Salmonella spp.* using the zone of inhibition method. The results demonstrated that both plant extracts exhibited notable antibacterial effects, although *Mangifera indica* showed superior inhibition compared to *Musa acuminata*.

- *Mangifera indica* exhibited the highest inhibition against *Salmonella spp.* (5.1 mm), followed by *E. coli* (4.6 mm) and *S. aureus* (4.6 mm).
- *Musa acuminata* showed moderate inhibition, with the highest effect against *S. aureus* (3.7 mm) and *E. coli* (3.4 mm).
- *Ampicillin* (standard antibiotic) exhibited the highest antibacterial activity across all bacterial strains, confirming its efficacy.
- The percentage inhibition results further supported these observations, where *Mangifera indica* had a higher inhibitory percentage, particularly against *Salmonella spp.* (91.07%) and *E. coli* (90.19%), while *Musa acuminata* showed the least effect on *Salmonella spp.* (23.21%).

These results suggest that *Mangifera indica* may contain potent bioactive compounds responsible for its strong antibacterial properties.

Antifungal Activity

The antifungal activity was tested against *Aspergillus brasiliensis* and *Candida albicans*. The extracts were compared with *Nystatin*, a standard antifungal drug.

- *Mangifera indica* exhibited higher antifungal activity against *Aspergillus brasiliensis* (5.1 mm) and *Candida albicans* (2.6 mm), whereas *Musa acuminata* showed moderate inhibition with zones of 4.8 mm and 3.8 mm, respectively.
- *Nystatin* exhibited the highest antifungal activity, with a zone of inhibition of 6.2 mm for both fungal strains.
- The percentage inhibition results indicated that *Mangifera indica* had a better inhibitory effect against *Aspergillus brasiliensis* (82.25%), whereas *Musa acuminata* showed greater inhibition against *Candida albicans* (61.29%).

These findings suggest that both plant extracts possess antifungal properties, with *Mangifera indica* being more effective against *Aspergillus brasiliensis* and *Musa acuminata* exhibiting better inhibition against *Candida albicans*.

CONCLUSION:

The results show that both *Musa acuminata* and *Mangifera indica* exhibit notable antimicrobial and antifungal activity. However, *Mangifera indica* displayed a more potent antibacterial effect, especially against *Salmonella spp.* and *E. coli*, whereas *Musa acuminata* was more effective in inhibiting *Candida albicans*. The presence of bioactive phytochemicals indicates that these plants could potentially be valuable sources of natural antimicrobial agents. Further research, including the isolation of active compounds and in vivo testing, is recommended to validate these findings and explore their potential therapeutic uses.

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