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Antimicrobial screening & phytochemical profiling of methanolic extract of *Meyna spinosa*

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Abstract- *Meyna spinosa* represents a traditionally used plant whose methanol extract's qualitative phytoconstituent analysis and antibacterial activity have been investigated in the present research using established procedures. The results of the investigation demonstrate that *Meyna spinosa* methanol extract contains phytoconstituents, including tannins, alkaloids, terpenoids, saponins, flavonoids, and phenolic compounds, but not carbohydrates, fixed oils, or lipids. Antibacterial activity was examined against *X. campestris*. In this investigation, it is observed that the stem of *Meyna spinosa* had the lowest Minimum Bactericidal Concentration (MBC) at 7.22µg/ml, followed by a methanolic extract of *Meyna spinosa* (Leaves) at 9.87µg/ml. This value is four times the minimum inhibitory concentration (MIC) against the related microorganisms and significantly lower than the MIC of the leaf extract. The stem extract of *M. spinosa* exhibited significant antibacterial activity against the test pathogens in the disc diffusion testing. The stem extract of *Meyna spinosa* exhibited significant antibacterial activity against the test pathogens in the disc diffusion testing. The maximum inhibition zone measured 10 mm for the *Meyna spinosa* (Leaves) methanolic extract (250 µg/ml/disc) and 12 mm for the *Meyna spinosa* (Stem) methanolic extract (350 µg/ml/disc). The conventional azithromycin discs had a zone of inhibition of 15 mm. Based on the aforementioned findings, it can be inferred that the methanol extract of *Meyna spinosa* leaves and stems captures rich phytoconstituents that can be used in food technology, pharmaceutical industries, ethnopharmacological fields, etc., to promote health and combat adverse health effects.

Key words: *Meyna spinosa*, Qualitative phytochemical profiling, flavonoids, alkaloids, Antibacterial activity

INTRODUCTION

From the beginning of human civilization, people have utilised plants as a source of medicine. Ethno therapy has been documented as the use of proven and tested medications derived from medicinal plants to treat a wide range of illnesses and conditions for which there is currently no cure through modern medicine. Since time immemorial, Ayurveda and Siddha systems of treatment have been practiced in India, with a variety of herbs. *Meyna spinosa*, a tiny tree or wild deciduous shrub belonging to the Rubiaceae family, is an important ethnomedicinal plant. This plant is mostly found in India, as well as in its four bordering countries, Bangladesh, China, Java, and Myanmar.[1]

It has several synonyms, including *Pyrostria spinosa* Miq., *Vangueria miqueliana* Kurz, *Vangueria pyrostria* Boerl., *Vangueria spinosa* Roxb., & *Vangueria stellata* Blanco.[2] *Meyna spinosa* leaf & stem extract contains a high concentration of phytoconstituents and has been shown to have excellent antioxidant & antibacterial activities.[3,4] The plant has been used as traditional medicine to treat a variety of health issues, including diabetes,

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diphtheria, stomach discomfort, headaches, liver difficulties, indigestion, painful urination, as well as skin problems like pimples and acne.

Fever, inflammation, biliary symptoms, and hepatic congestion can all be treated with fruits. In order to treat diphtheria and treat bone fractures, leaves are employed.[5,6] Additionally, it has been stated that the herb has historically been used to cure renal illnesses and skin irritation.[7,8] Consequently, the primary goal of this study is to evaluate the bio efficiencies of *Meyna spinosa*, including its antibacterial activity, total polyphenol content (TPC), total flavonoid content (TFC), antioxidant efficiency, and qualitative phytochemical profiling.

MATERIALS & METHODS

Plant material

Between January and April of 2024, *Meyna spinosa* plant material was gathered from the Patna district's Ganga River banks. The plant was identified at Ganga Devi Mahila College's Department of Botany, Patliputra University, India. Plant samples were carefully cleansed under running water before being rinsed with distilled water. The plant's stem and leaf portions were separated, cut into bits, air-dried, and ground into a powder for extraction.

Preparation of plant extracts

For methanol extraction, approximately 100 g of powder from the different plant sections were used. The Soxhlet apparatus was used for the process. The extracts were then collected, made solvent-free in rotavapor under reduced pressure, then stored at 5°C until use.

Yield of the extracts

The extract yield was estimated using the formula ([WE/WD]×100), where WE is the weight of the solvent-free extract and WD is the dry weight of the plant material.

Qualitative chemical profiling analysis of extracts

Standard procedures were used to assess the qualitative phytochemical screening of *Meyna spinosa* both stem and leaf extracts.[9,10] To determine the presence of starch, fixed oils, lipids, phytosterols, alkaloids, terpenoids, saponins, tannins, flavonoids, and phenolic substances, nine distinct qualitative tests were conducted.

Bacterium retrieval and culture:

The *X. campestris* BH0001 strain was obtained from the Indian Institute of Agricultural Research (ICAR) via the Indian Type Culture Collection (ITCC). To separate the colonies, the material is streaked onto an agar plate with aseptic procedures. The agar plates illustrate the growth of *X. campestris* BH0001 colonies throughout an incubation period at optimal conditions & temperature. A colony was then chosen & transferred into a liquid culture medium, where it continues to grow as well as reproduce. The pure culture of *X. campestris* BH0001 was kept in a controlled environment.

ANTIBACTERIAL ACTIVITY

Determination of Minimum Inhibitory Concentration (MIC) of the bacterium

The Minimum Inhibitory Concentration (MIC) was determined using the broth microdilution method described by Bostanci *et al.* (2022)[11], with minor changes made via serial dilution. Plant extracts were produced as stock solutions in 1.5ml microcentrifuge tubes by dissolving the dry extract in dimethylsulphoxide (DMSO) to a final concentration of 64µg/ml. Nutrient broth was used in 96-well microplates to prepare serial dilutions of the stock solution ranging from 32µg/ml to 0.25µg/ml. A bacterial solution with 5×105 colony-forming units/mL was produced from a 24-hour culture plate. 100µl of this suspension was injected into each well. Microtiter plates were incubated at 37°C for 24 hours to accommodate bacteria's prolonged growth requirements.

After the incubation period, 40 µl of a 0.4 µg/ml INT solution was added to each well to measure microbial growth. Plates were incubated at 37°C for 30 minutes with bacteria, and MIC values were visually assessed. The minimal inhibitory concentration was determined by taking the lowest dose of each extract that produced no observable growth. Positive control studies were used to assess the microorganisms' susceptibility. Ampicillin (Sigma-Aldrich) was used to treat bacterial strains at a dosage of 0.10 µg/ml in sterile water.

Determination of Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) was calculated using the microtitre broth dilution method. MBC has been defined as the minimum concentration of the extract that is capable of killing 99.9% of the bacterial inoculum after 24 hours of incubation at 37°C. The MBC determination process used the method described by Ozturk and Ercisli.[12] Ten microliters of the solution from the well with the MIC value and two wells above the MIC value were removed and distributed on Muller-Hinton Agar (MHA) plates. Colonies were counted after incubating at 37°C for 18-24 hours. The MBC value was defined as the

concentration of the sample that yielded less than 10 colonies. Each experiment was repeated three times to confirm that the results were consistent and reliable.

Antibacterial assay

Disc diffusion techniques were used to examine the antibacterial activity. Nutrient agar plates were streaked against reference pathogens from the stock, and the inoculation plates were then incubated at 37°C for the entire night. A tiny part of the subculture was transferred using a sterile loop into a test tube filled with nutrient broth, and it was then cultured (for two to four hours) at 37°C until the growth attained log phase. Petri dishes were filled with nutrient agar medium that had been seeded with a standard inoculum solution and left to harden. Using sterile forceps, insert the extract-impregnated (5 µg/ml, 10 µg/ml, and 15 µg/ml) and standard antibiotic (25 µg/ml/disc) discs on the Petri-dishes and gently press to ensure that the discs make interface with the inoculated agar surface. After 18 hours of incubation at 37°C for the inoculated plates, the zone of inhibition was measured in millimetres.

RESULTS & DISCUSSION

Table 1- Qualitative chemical profiling analyses of leaf and stem part of *Meyna spinosa*

S. No.	Name of the phytochemical	Specific test followed	Leaf	Stem
1.	Alkaloids	Wagner's test	Present	Present
2.	Fats	Spot test	Absent	Absent
3.	Flavonoids	Alkaline reagent test	Present	Present
4.	Phenolic compounds	Ferric chloride test	Present	Present
5.	Phytosterols	Liebermann–Burchard's or acetic anhydrate test	Present	Present
6.	Reducing sugars	Fehling's test	Absent	Absent
7.	Saponins	Froth test	Present	Absent
8.	Tannins	Gelatin test	Present	Present
9.	Terpenoids	Salkowski's test	Present	Present

The extract for the leaf and stem of *Meyna spinosa* was prepared using methanol as the solvent. The leaf portion had the highest yield of 3.2%, followed by the stem part at 2.4%. Secondary metabolites are biological products found in a range of plant extracts with varying therapeutic effects.[13] In the present investigation, qualitative chemical profiling of methanol extracts of *M. spinosa* was performed, which revealed the presence of plant-based constituents including alkaloids, terpenoids, saponins, tannins, phytosterols, flavonoids, and phenolic compounds, as well as the absence of carbohydrate, fixed oils, and fat. Nevertheless, various plant sections produce secondary metabolites in varying quantities.[14]

Meyna spinosa contained seven phytochemicals, including alkaloids, terpenoids, saponins, tannins, phytosterols, flavonoids, and phenolic compounds, while the stem contained six phytochemicals, including alkaloids, terpenoids, tannins, phytosterols, flavonoids, and phenolic compounds. Numerous therapeutic benefits of phenolic compounds have been documented, including their ability to combat heart disease, operate as an antioxidant, be hepatoprotective, reduce inflammation, and fight cancer.[16]

Table 2- Minimum Inhibitory Concentration values of Methanolic extract of leaves and stems of *M. spinosa*

Gram negative Bacteria ↓	Methanolic extract of *M. spinosa* (Leaves)	Methanolic extract of *M. spinosa* (Stem)
X. campestris	123	215.5

The minimum inhibitory concentration (MIC) values of medicinal plants *Meyna spinosa* were measured using the broth microdilution method, yielding the following concentrations: leaves (123µg/ml) and stems (215.5µg/ml) (Table 2). Methanolic extracts of *Meyna spinosa* had a notable bactericidal action. The lowest MBC (Minimum Bactericidal Concentration) result is less than four times the corresponding MIC (Minimum Inhibitory Concentration), indicating that the tested material has potential antibacterial action. In this investigation, the stem of *Meyna spinosa* had the lowest MBC (7.22µg/ml), followed by the methanolic extract of *Meyna spinosa* (Leaves) at 9.87 µg/ml. This result is within four times the minimum inhibitory concentration (MIC) against the related microorganisms and is somewhat lower than the MIC value of the leaf extract.

Table 3- Minimum Bactericidal Concentration of *M. spinosa* against the *X. campestris*

Gram negative Bacteria ↓	Methanolic extract of *M. spinosa* (Leaves)	Methanolic extract of *M. spinosa* (Stem)
X. campestris	9.87	7.22

Although the precise processes by which the medicinal plants included in this study combat different microbes are still not fully understood, it is conceivable that one or more of the mechanisms is essential to the plants' antibacterial action.

The stem extract of *M. spinosa* exhibited significant antibacterial activity against the test pathogens in the disc diffusion testing (Table 4). The maximum inhibition zone measured 10 mm for the *M. spinosa* (Leaves) methanolic extract (250µg/ml/disc) and 12 mm for the *M. spinosa* (Stem) methanolic extract (350 ?g/ml/disc). The conventional azithromycin discs had a zone of inhibition of 15 mm.

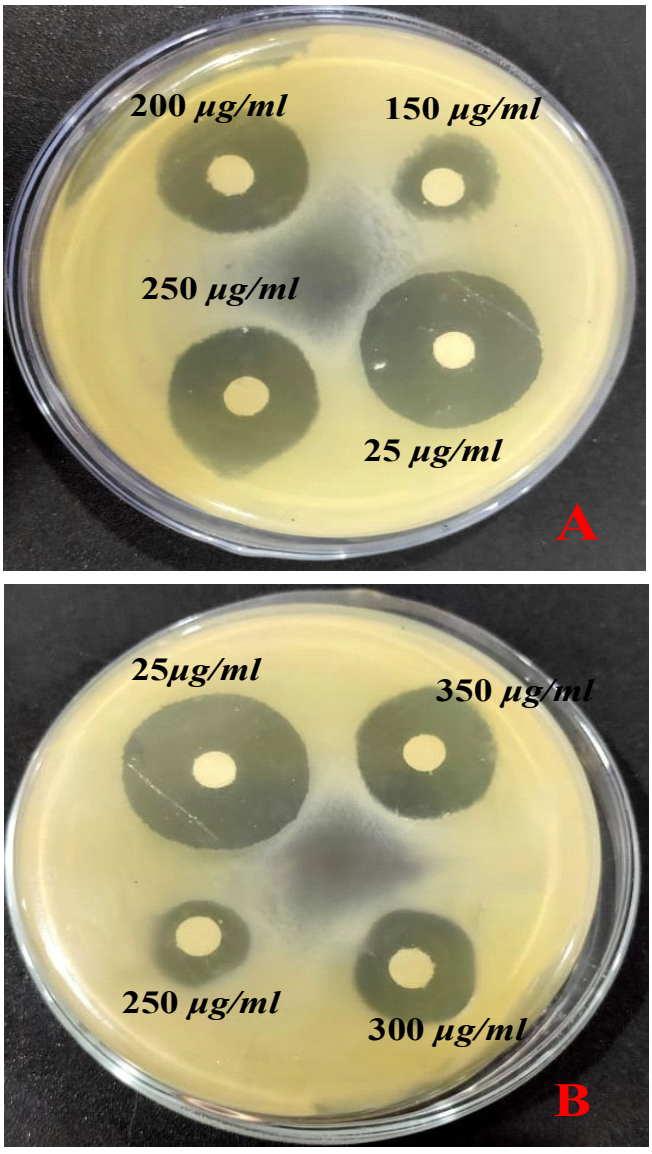

Fig. 1- Results of the disc diffusion assay of Methanolic extract of *M. spinosa* (A) stem and (B) Leaves

Table 4. Results of the disc diffusion assay of Methanolic extract of *Meyna spinosa* stem and Leaves .

Zone of inhibition	
Methanolic extract of *M. spinosa* (Leaves) (150 *µg/ml*/disc)	**3mm**
Methanolic extract of *M. spinosa* (Leaves) (200 *µg/ml*/disc)	**8 mm**
Methanolic extract of *M. spinosa* (Leaves) (250 *µg/ml*/disc)	**10 mm**
Methanolic extract of *M. spinosa* (Stem) (250*µg/ml*/disc)	**5 mm**
Methanolic extract of *M. spinosa* (Stem) (300*µg/ml*/disc)	**9 mm**
Methanolic extract of *M. spinosa* (Stem) (350 *µg/ml*/disc)	**12 mm**
Azithromycin (25 *µg/ml* /disc)	**15 mm**

CONCLUSION

The presence of phenols and flavonoids has played an essential role in validating folk claims about *M. spinosa*. Phenolics, alkaloids, steroids, saponins, terpenoids, and other naturally occurring phytochemicals with a wide range of bioactivity are abundant in nature. These results suggest that the plant extracts under analysis may operate as a source for the discovery of novel plant-based antibiotic drugs. However, it is crucial to remember that the minimum inhibitory concentration (MIC) & antibacterial activity can be modified by a variety of factors, including the technical procedures used in different laboratories, as well as the virulence or sensitivity of the bacterial strains tested. As a result, when extending *in vitro* results to clinical applications without first doing full in vivo trials, extreme caution is advised. To fulfil their potential as effective therapeutic agents against bacterial infections, these in vivo studies are essential for confirming the safety and efficiency of these plant-derived chemicals in the clinical context. Therefore, the plant product may be used in the near future to meet significant societal demand in the food and pharmaceuticals industries as well as pharmaceutical research.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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