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Phytochemical Profiling and Broad-Spectrum Antimicrobial Potential of Meyna laxiflora Extracts against Bacterial and Fungal Pathogens

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ABSTRACT: Antimicrobial resistance poses a severe global public health threat, necessitating the discovery of novel bioactive agents from medicinal plants. *Meyna laxiflora Robyn*s (*Rubiaceae*) is a wild edible ethnomedicinal plant traditionally utilized across India to manage infectious and inflammatory conditions. This study evaluated the qualitative phytochemical profile and broad-spectrum antimicrobial efficacy of fruit (MF), leaf (ML), and stem (MS) extracts of *M. laxiflora* prepared using solvents of varying polarity: methanol: water (9:1 v/v), ethanol: water (7:3 v/v), and dichloromethane:methanol (3:7 v/v). Qualitative screening identified flavonoids, tannins, phenolics, alkaloids, saponins, glycosides, terpenoids, and steroids across extracts. Antibacterial activity against *Staphylococcus aureus* (ATCC 5345) and *Escherichia coli* (ATCC 5346) and antifungal activity against *Candida albicans* were determined using the agar well diffusion method. Among the tested plant extracts, the methanol: water stem extract (MS1) demonstrated the highest antibacterial potency, generating mean inhibition zones of 17.56 ± 0.25 mm against *E. coli* and 17.48 ± 0.36 mm against *S. aureus*, compared to standard erythromycin (21.14 ± 0.31 mm and 22.36 ± 0.31 mm, respectively). For antifungal screening, extracts MS3, MF3, ML2, and ML3 exhibited marked inhibition against *C. albicans* (18.44 ± 0.31 to 18.86 ± 0.22 mm) compared to amphotericin B (22.12 ± 0.32 mm). One-way ANOVA and Tukey's post-hoc tests revealed significant variations among extracts ($p < 0.05$), demonstrating that plant anatomical part and extraction solvent polarity critically govern antimicrobial potency. These findings validate the ethnomedicinal utility of *M. laxiflora* and underscore its potential as a sustainable natural source of therapeutic antimicrobial agents.

1. INTRODUCTION

Medicinal plants have served as cornerstone therapeutic reservoirs for centuries and remain indispensable conduits for contemporary pharmacotherapy. They synthesize diverse specialized secondary metabolites possessing multifaceted pharmacological activities, including antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory properties (Fabricant and Farnsworth, 2001; Heinrich, 2013). In recent decades, the global escalation of antimicrobial resistance (AMR) has substantially diminished the clinical efficacy of synthetic antibiotics, precipitating an urgent imperative to discover natural antimicrobial alternatives or adjuvants (Ansari et al., 2025). Crude botanical extracts characteristically harbor synergistic multi-component chemotypes capable of targeting divergent microbial cellular pathways, thereby reducing the likelihood of resistance selection (Balouiri et al., 2016; Sudhakar and Afinisha Deepam, 2025).

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Meyna laxiflora Robyns (family *Rubiaceae*) is a thorny, wild deciduous shrub or small tree indigenous to tropical and subtropical ecosystems across India and neighboring regions.

Figure 1: *Meyna Laxiflora Robyns* Stem and Leaf.

Ecologically distributed along forest margins, degraded hillsides, and roadsides, the plant occupies a distinctive ethnopharmacological niche owing to its dual utility as a wild edible fruit and an empirical folk remedy (Majaz and Khurshid, 2015; Dhenge et al., 2023). Historically classified under the genus Vangueria, recent taxonomic revisions have clearly delimited Meyna as an autonomous genus encompassing eleven valid species (Majaz and Khurshid, 2015; Singh et al., 2014). Ethnobotanical surveys across the Western Ghats and Northeast India document extensive indigenous applications of *M. laxiflora* fruit, bark, and leaves among local communities (e.g., Meitei and Pawra tribes) for gastrointestinal disorders, hepatic ailments, inflammatory conditions, and urinary calculus management (Singh et al., 2014; Khwairakpam et al., 2018; Dhande, 2016; Deore et al., 2022).

Pharmacological investigations have provided preliminary scientific substantiation for several traditional claims. Methanolic extracts of *M. laxiflora* demonstrated marked antimycobacterial activity against Mycobacterium tuberculosis H37Rv with minimum inhibitory concentrations (MIC) ranging from 50 to 100 μg/mL (Ansari et al., 2025). Pentacyclic triterpenoids such as lupeol acetate and betulinic acid isolated from allied Rubiaceae taxa are documented to perturb mycolic acid biosynthesis in the mycobacterial cell envelope (Mendes et al., 2024). Furthermore, in vitro cytotoxicity assessments utilizing MTT and SRB assays against human malignant cell lines (MCF-7 breast adenocarcinoma, HeLa cervical carcinoma, and A549 lung carcinoma) demonstrated significant dose-dependent antiproliferative activity, with IC50 values between 75 and 110 μg/mL (Majaz, 2020).

Phytochemical analyses have corroborated the presence of diverse secondary metabolites across different morphological parts of M. laxiflora, including polyphenols, flavonoids, alkaloids, saponins, condensed tannins, cardiac glycosides, and terpenoids (Bag and Devi, 2016; Bhamare et al., 2022). Bag and Devi (2016) quantified high total phenolic content (129.54 ± 0.25 mg GAE/g) in fruit pulp methanolic extract and notable flavonoid content (80.45 μg QE/100 g) in foliar extracts, correlating with robust DPPH free-radical scavenging. Proximate nutritional profiling reveals ripe fruits to be abundant in carbohydrates (65%–68%), ascorbic acid (28–35 mg/100 g), and polyphenols (110–130 mg GAE/g), drawing compositional parallels with well-characterized medicinal fruits like Morinda citrifolia and Gardenia jasminoides (Bhamare et al., 2022; Dhenge et al., 2023).

Despite these preliminary findings, there remains a critical research deficit regarding how solvent extraction polarity influences the selective solubilization of phytoconstituents and the resulting antimicrobial potency across different anatomical parts of M. laxiflora. Prior studies have predominantly evaluated single solvent extractions, precluding systematic comparisons. Therefore, the present investigation was undertaken to perform comprehensive qualitative phytochemical screening and comparative evaluation of antibacterial and antifungal activities of fruit, leaf, and stem extracts of *M. laxiflora* across three binary solvent systems: methanol: water (9:1 v/v), ethanol:water (7:3 v/v), and dichloromethane:methanol (3:7 v/v).

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2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

Fresh stem, leaf, and fruit specimens of *Meyna laxiflora Robyns* were collected during the flowering and fruiting phases (May–June) from the wild habitats of Bhor taluka, Pune district, Maharashtra, India (latitude: 18.145744° N, longitude: 73.842469° E). The plant specimens were botanically authenticated by the Botanical Survey of India (BSI), Western Regional Centre, Pune, Maharashtra, India. A voucher specimen (GBNML-1) has been deposited in the institutional herbarium under authentication certificate reference number BSI/WRC/Iden. Cer./2024/0603240025820 dated 07/03/2024.

2.2 Preparation of Botanical Extracts

Collected plant organs (fruits, stems, and leaves) were thoroughly washed with running tap water followed by deionized water to remove surface debris, shade-dried at ambient room temperature (25 ± 2 °C) for 14 days, and pulverized into a coarse powder using an electric blender. The powdered plant matrices were defatted with petroleum ether (boiling range 60–80 °C) to eliminate lipophilic pigments and fatty matter. Exhaustive sequential solvent extractions were carried out using maceration and Soxhlet extraction apparatus with three distinct solvent systems of differing polarity: methanol: water (9:1 v/v), ethanol: water (7:3 v/v), and dichloromethane: methanol (3:7 v/v). The resulting liquid extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure at 40 °C using a rotary vacuum evaporator to obtain semi-solid crude residues. The dried extracts were coded as MF1–MF3 (fruit), MS1–MS3 (stem), and ML1–ML3 (leaf), and stored at 4 °C in airtight amber glass vials until further experimental evaluation.

Table 1. Extraction codes and solvent systems used for *Meyna laxiflora* plant parts.

Entry	Extract code	Plant part and solvent system	Solvent ratio (v/v)
1	MF1	Fruit (Methanol: Water)	9:1
2	MF2	Fruit (Ethanol: Water)	7:3
3	MF3	Fruit (Dichloromethane:methanol)	3:7
4	MS1	Stem (Methanol: Water)	9:1
5	MS2	Stem (Ethanol: Water)	7:3
6	MS3	Stem (Dichloromethane:methanol)	3:7
7	ML1	Leaf (Methanol: Water)	9:1
8	ML2	Leaf (Ethanol: Water)	7:3
9	ML3	Leaf (Dichloromethane:methanol)	3:7

Table 2. Extraction yields of different solvent extracts of *Meyna laxiflora*.

Plant Part	Methanol: Water	Ethanol: Water	Dichloromethane:methanol
Fruit	3.49 ± 0.07	5.05 ± 0.07	2.43 ± 0.03
Stem	3.28 ± 0.04	6.01 ± 0.03	3.15 ± 0.02
Leaf	4.37 ± 0.08	6.42 ± 0.05	2.14 ± 0.03

2.3 Qualitative Phytochemical Screening

Qualitative chemical tests were carried out on all nine *M. laxiflora* extracts using standard phytochemical protocols (Majaz and Khurshid, 2015; Bag and Devi, 2016) to establish the presence of major secondary metabolite classes:

• Glycosides: Assessed via the Keller–Kiliani test (glacial acetic acid, ferric chloride, concentrated sulfuric acid; emergence of a reddish-brown interfacial ring and bluish-green upper layer) and Liebermann's test (acetic anhydride and concentrated sulfuric acid; green to blue-green coloration).

• Saponins: Evaluated by the froth test (vigorous agitation of 5 mL aqueous extract for 2 min; persistence of a stable honeycomb honeycomb froth layer > 1 cm for > 15 min).

• Flavonoids: Assessed using the alkaline reagent test (addition of 10% sodium hydroxide solution producing intense yellow coloration that becomes colorless upon addition of dilute hydrochloric acid).

• Tannins and Phenolics: Assessed by the ferric chloride test (5% neutral ferric chloride yielding deep blue-black or greenish coloration) and bromine water test (decolorization of bromine water).

• Alkaloids: Evaluated by acidifying extracts with 1.5% v/v hydrochloric acid, filtering, and treating aliquots with Mayer's reagent (potassium mercuric iodide; creamy-white precipitate) and tannic acid solution (buff/turbid precipitate).

• Carbohydrates: Assessed via Molisch's test (α-naphthol in ethanol followed by concentrated sulfuric acid layering; purple ring at the interface).

• Terpenoids and Steroids: Evaluated using Salkowski's test (treatment of chloroform extract solution with concentrated sulfuric acid; reddish-brown interface indicates terpenoids, golden yellow/green fluorescence indicates steroids).

2.4 Antibacterial Assay

The in vitro antibacterial activity of *M. laxiflora* extracts was evaluated against two reference bacterial pathogens: Gram-positive *Staphylococcus aureus* (ATCC 5345) and Gram-negative *Escherichia coli* (ATCC 5346) using the agar well diffusion method (Balouiri et al., 2016; Sudhakar and Afinisha Deepam, 2025). Mueller–Hinton agar (MHA) plates were prepared and inoculated with standardized bacterial suspensions adjusted to 0.5 McFarland turbidity standard (approximately 1.5 × 10^8 CFU/mL) using sterile cotton swabs. Wells of 6 mm diameter were punched equidistantly into the agar medium using a sterile cork borer. Crude extracts were reconstituted in 10% v/v dimethyl sulfoxide (DMSO) to a stock concentration of 100 mg/mL and sterilized through 0.22 µm syringe filters. Aliquots of 100 µL of each test extract were aseptically loaded into designated wells. Erythromycin (15 µg/disc or equivalent reference solution in DMSO) served as the positive standard antibiotic control, while 10% DMSO served as the negative control. The inoculated plates were pre-incubated at 4 °C for 2 h to allow uniform sample diffusion, followed by incubation at 37 ± 1 °C for 18–24 h. After incubation, zones of inhibition around the wells were measured in millimeters (mm) using a digital vernier caliper across two perpendicular diameters. All assays were executed in triplicate.

2.5 Antifungal Assay

The antifungal efficacy of *M. laxiflora* extracts was evaluated against *Candida albicans* (clinical isolate / ATCC reference strain) using the agar well diffusion method (Balouiri et al., 2016; Alsalamah et al., 2023). Sabouraud dextrose agar (SDA) / MHA plates were surface-seeded with standardized fungal inocula adjusted to 1.0 × 10^6 CFU/mL. Wells (6 mm diameter) were bored into the agar medium. Graded sample volumes (10, 20, 30, 40, and 50 µL) and standard loading volumes (100 µL) of reconstituted extracts (100 mg/mL in DMSO) were delivered into the wells. Amphotericin B (100 µg/mL) served as the positive reference antifungal drug, and 10% DMSO served as the negative vehicle control. Plates were incubated at 37 ± 1 °C for 24–48 h, and antifungal zones of inhibition were recorded in millimeters (mm). Antifungal activity was interpreted according to standardized clinical categories: resistant / less active (< 10 mm), intermediate / moderately active (10–15 mm), and susceptible / good activity (> 15 mm). Assays were conducted in triplicate.

2.6 Statistical Analysis

All bioassay experiments were performed in triplicate (n = 3), and data are reported as mean ± standard error of the mean (SEM) or mean ± standard deviation (SD). Welch's unpaired t-test was used to determine whether overall antimicrobial

susceptibility differed significantly between Gram-positive *S. aureus* and Gram-negative *E. coli*. One-way analysis of variance (ANOVA) was performed to evaluate variations in inhibitory efficacy across plant organs, solvent extraction systems, and reference standard drugs. When ANOVA revealed statistically significant differences, Tukey's honestly significant difference (HSD) post-hoc test was applied for pairwise multiple comparisons. A probability level of $p < 0.05$ was considered statistically significant (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

3. RESULTS AND DISCUSSION

3.1 Phytochemical Profiling and Chemical Diversity

Qualitative phytochemical screening revealed that *Meyna laxiflora* extracts contain a broad spectrum of bioactive secondary metabolites, including glycosides, flavonoids, saponins, alkaloids, tannins, phenolics, terpenoids, and steroids.

Table 2. Qualitative phytochemical screening of *Meyna laxiflora* extracts.

Entry	Phytochemical class	MF1	MF2	MF3	ML1	ML2	ML3	MS1	MS2	MS3
1	Glycosides	+	+	++	+	+	++	+	+	+
2	Flavonoids	++	-	+	+++	++	+	+++	+	++
3	Saponins	+	+	+	+	+	+	+	+	+
4	Alkaloids	++	-	+	++	-	++	++	-	++
5	Tannins	++	++	+	++	+	+	++	+	+
6	Phenolics	+	+	+	+	+	+	+	+	+
7	Terpenoids	+	+	+	++	+	+	+	+	++
8	Steroids	+	+	+	++	+	+	+	+	+
9	Carbohydrates	++	+	++	++	+	++	++	+	++

However, the presence and relative abundance of these phytoconstituents varied markedly across plant parts (fruit, leaf, and stem) and extraction solvent polarities. Methanol: water extracts (MF1, ML1, MS1) exhibited the most comprehensive phytochemical richness, displaying prominent amounts of flavonoids, phenolics, tannins, and carbohydrates. Specifically, flavonoids were detected at high concentrations in leaf (ML1) and stem (MS1) methanolic fractions, moderate levels in fruit (MF1) and ethanolic leaf (ML2) fractions, but were absent or diminished in ethanolic fruit (MF2) and stem (MS2) extracts. Alkaloids gave intense precipitates in dichloromethane:methanol stem (MS3) and leaf (ML3) fractions as well as methanolic fruit (MF1) and leaf (ML1) extracts, whereas they were absent in ethanol:water preparations (MF2, ML2, MS2). Saponins, phenolics, and steroids were ubiquitously detected at baseline levels across all nine preparations.

These phytochemical findings are in robust agreement with earlier literature on M. laxiflora. Bag and Devi (2016) reported that polar alcoholic solvents maximize the extraction of free polyphenols and flavonoids from *M. laxiflora* leaves and fruits. The pronounced concentration of polyphenols and flavonoids in the polar fractions provides a biochemical rationale for the traditional medicinal efficacy of aqueous and hydroalcoholic decoctions (Singh et al., 2014; Dhenge et al., 2023). Flavonoids and tannins are known to form irreversible complexes with bacterial extracellular enzymes and cell-surface adhesins, whereas alkaloids disrupt nucleic acid synthesis and cell wall integrity (Ansari et al., 2025; Savickiene, 2024).

3.2 In Vitro Antibacterial Efficacy

The antibacterial potency of *M. laxiflora* fruit, stem, and leaf extracts against *Staphylococcus aureus* (ATCC 5345) and *Escherichia coli* (ATCC 5346) was quantitatively determined and compared with the standard antibiotic erythromycin.

Table 3. In vitro antibacterial zone of inhibition (mm) against *Escherichia coli* (ATCC 5346).

Entry	Treatment	Replicate 1	Replicate 2	Replicate 3	Mean ± SEM
1	Erythromycin (Std)	21.5	20.9	21.0	21.14 ± 0.18
2	MS1	17.9	17.4	17.5	17.56 ± 0.14
3	MS2	11.6	11.1	11.2	11.23 ± 0.14
4	MS3	13.8	13.2	13.4	13.48 ± 0.17
5	MF1	15.5	14.9	14.9	15.14 ± 0.19
6	MF2	12.7	12.3	12.4	12.45 ± 0.11
7	MF3	13.9	13.5	13.4	13.59 ± 0.14
8	ML1	15.8	15.3	15.3	15.47 ± 0.14
9	ML2	13.5	13.1	13.3	13.28 ± 0.11
10	ML3	14.6	14.0	14.2	14.25 ± 0.17

Values are expressed as mean ± SEM (n = 3). Statistical significance set at $p < 0.05$.

Table 4. In vitro antibacterial zone of inhibition (mm) against *Staphylococcus aureus* (ATCC 5345).

Entry	Treatment	Replicate 1	Replicate 2	Replicate 3	Mean ± SEM
1	Erythromycin (Std)	22.7	22.1	22.2	22.36 ± 0.18
2	MS1	17.8	17.1	17.5	17.48 ± 0.21
3	MS2	14.6	13.9	13.2	14.23 ± 0.42
4	MS3	13.9	13.1	13.4	13.48 ± 0.23
5	MF1	13.3	12.7	12.7	12.89 ± 0.19
6	MF2	11.6	11.1	11.2	11.29 ± 0.14
7	MF3	9.6	9.1	9.2	9.28 ± 0.14
8	ML1	9.9	9.5	9.4	9.58 ± 0.14
9	ML2	11.6	11.1	11.2	11.28 ± 0.14
10	ML3	12.8	12.4	12.3	12.48 ± 0.14

Values are expressed as mean ± SEM (n = 3). Statistical significance set at $p < 0.05$.

Table 5. Comparative antibacterial zone of inhibition (mm) across bacterial test strains.

Entry	Sample	*Escherichia coli* (mm)	*Staphylococcus aureus* (mm)
1	Control (10% DMSO)	NA	NA
2	Erythromycin (Std)	21.14 ± 0.31	22.36 ± 0.31
3	MS1	17.56 ± 0.25	17.48 ± 0.36

4	MS2	11.23 ± 0.25	14.23 ± 0.72
5	MS3	13.48 ± 0.30	13.48 ± 0.40
6	MF1	15.14 ± 0.33	12.89 ± 0.33
7	MF2	12.45 ± 0.20	11.29 ± 0.25
8	MF3	13.59 ± 0.25	09.28 ± 0.25
9	ML1	15.47 ± 0.25	09.58 ± 0.25
10	ML2	13.28 ± 0.20	11.28 ± 0.25
11	ML3	14.25 ± 0.30	12.48 ± 0.25

Values are expressed as mean ± SD (n = 3); NA: No activity / not applicable; *p < 0.05 indicates statistically significant difference.

Meyna Laxiflora Robyns Stem Extract Methanol: water (*S. Aureus*)

Meyna Laxiflora Robyns Stem Extract Methanol: water (*E. Coli*)

Meyna Laxiflora Robyns Stem Extract Ethanol: water (*S. Aureus*)

Meyna Laxiflora Robyns Stem Extract Dichloromethane: Methanol (*E. Coli*)

Meyna Laxiflora Robyns Stem Extract Ethanol: water(*E. Coli*)

Meyna Laxiflora Robyns Stem Extract Dichloromethane: Methanol (*S. Aureus*)

Meyna Laxiflora Robyns Stem Extract Dichloromethane: Methanol (*E. Coli*)

Meyna Laxiflora Robyns Stem Extract Dichloromethane: Methanol (*E. Coli*)

Meyna Laxiflora Robyns Fruit Extract Methanol: water (*S. Aureus*)

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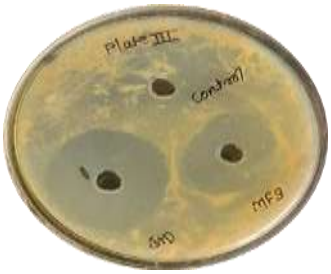
Meyna Laxiflora Robyns Fruit Extract Methanol: water (*S. Aureus*)

Meyna Laxiflora Robyns Fruit Extract Methanol: water(E. Coli)

Meyna Laxiflora Robyns Fruit Extract Ethanol: water (*S. Aureus*)

Meyna Laxiflora Robyns Fruit Extract Ethanol: water (*E. Coli*).



Meyna Laxiflora Robyns Stem Extract Dichloromethane: Methanol (*S. Aureus*)

Meyna Laxiflora Robyns Stem Extract Dichloromethane: Methanol (*E. Coli*)

Meyna Laxiflora Robyns Leaf Extract Methanol : Water (*S. Aureus*)

Meyna Laxiflora Robyns Leaf Extract Methanol : Water (*E. Coli*)

Meyna Laxiflora Robyns Leaf Extract Dichloromethane: Methanol (*S. Aureus*)

Meyna Laxiflora Robyns Leaf Extract Dichloromethane: Methanol (*E. Coli*)

Figure 2: Photographs zone of inhibition *Escherichia Coli* & *Staphylococcus Aureus* for the Antibacterial activity.

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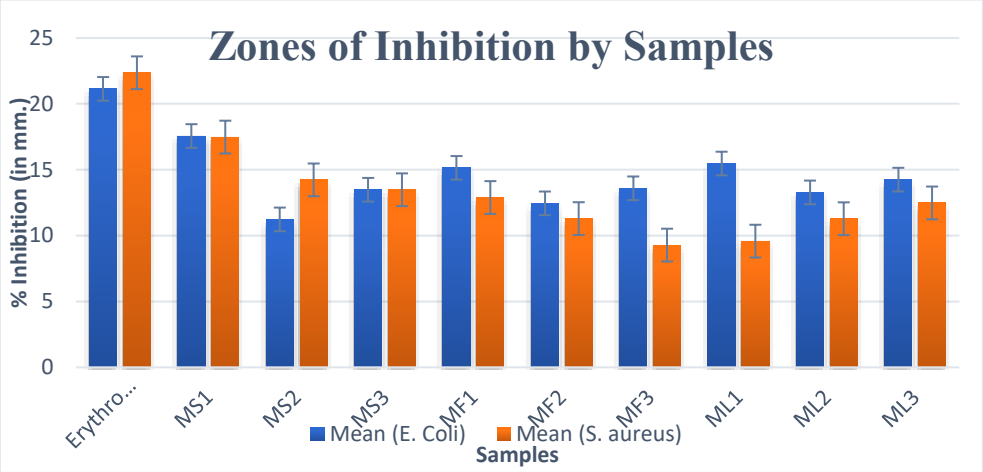

Figure 3: Graphical representation showing the antimicrobial Activity of Zone of inhibition in mm (*Escherichia Coli*, *Staphylococcus Aureus*).

Standard erythromycin produced the largest inhibition zones against *S. aureus* (22.36 ± 0.31 mm) and *E. coli* (21.14 ± 0.31 mm). The negative vehicle control (DMSO) produced no discernible inhibition zone (0.00 mm), confirming that the observed antibacterial effects were exclusively attributable to the botanical extracts.

Among all tested plant extracts, the methanol: water stem extract (MS1) demonstrated superior antibacterial efficacy against both pathogens, generating inhibition zones of 17.56 ± 0.25 mm against *E. coli* and 17.48 ± 0.36 mm against *S. aureus*. High antibacterial potency was likewise recorded for the methanol: water leaf extract ML1 (15.47 ± 0.25 mm against *E. coli*; 9.58 ± 0.25 mm against *S. aureus*) and fruit extract MF1 (15.14 ± 0.33 mm against *E. coli*; 12.89 ± 0.33 mm against *S. aureus*). In contrast, extracts prepared using less polar solvent mixtures, particularly dichloromethane: methanol (MF3, ML3, MS3) and ethanol: water (MF2, ML2), exhibited moderate to weak antibacterial inhibition (ranging from 9.28 ± 0.25 mm to 14.25 ± 0.30 mm). The superior efficacy of methanol: water extracts aligns directly with their high flavonoid and tannin content, corroborating the paradigm that hydroalcoholic systems effectively solubilize polar antibacterial phenolics (Balouiri et al., 2016; Sudhakar and Afinisha Deepam, 2025).

3.3 In Vitro Antifungal Efficacy against Candida albicans

The antifungal activity of *M. laxiflora* extracts against the opportunistic fungal pathogen *Candida albicans* was assessed via agar well diffusion and compared with amphotericin B.

Table 6. Interpretive criteria for antifungal activity against *Candida albicans*.

Entry	Activity level	Zone diameter (mm)	Clinical observation / interpretation
1	Less Active (Resistant)	< 10 mm	The sample is ineffective or demonstrates poor inhibitory activity.
2	Moderately Active (Intermediate)	10–15 mm	Intermediate inhibition; may be insufficient for complete mycological eradication.
3	Good Activity (Susceptible)	> 15 mm	The sample is considered potent and effective with pronounced antifungal action.

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Table 7. In vitro antifungal zone of inhibition (mm) against *Candida albicans*.

Entry	Treatment / Sample	Zone of inhibition (mm)
1	Control (10% DMSO)	NA
2	Amphotericin B (Std)	22.12 ± 0.32
3	MS1	17.36 ± 0.24
4	MS2	17.51 ± 0.40
5	MS3	18.86 ± 0.22
6	MF1	17.65 ± 0.27
7	MF2	17.82 ± 0.30
8	MF3	18.52 ± 0.28
9	ML1	17.77 ± 0.26
10	ML2	18.71 ± 0.26
11	ML3	18.44 ± 0.31

Standard amphotericin B produced an average zone of inhibition of 22.12 ± 0.32 mm. Remarkably, all nine *M. laxiflora* extracts produced inhibition zones exceeding 17 mm, placing them firmly within the 'good activity / susceptible' interpretive category (> 15 mm) defined in Table 6.

Zone of inhibition of test sample ML-1, ML -2 and ML-3 against *Candida albicans strain*

Zone of inhibition of test sample MF-1, MF – 2 and MF – 3against *Candida albicans strain*

Zone of inhibition of test sample MS-1, MS – 2 and MS - 3 against *Candida albicans strain*

Figure 4: Photographs representation for the antifungal activity of Zone of Inhibition of *Candida albicans*.

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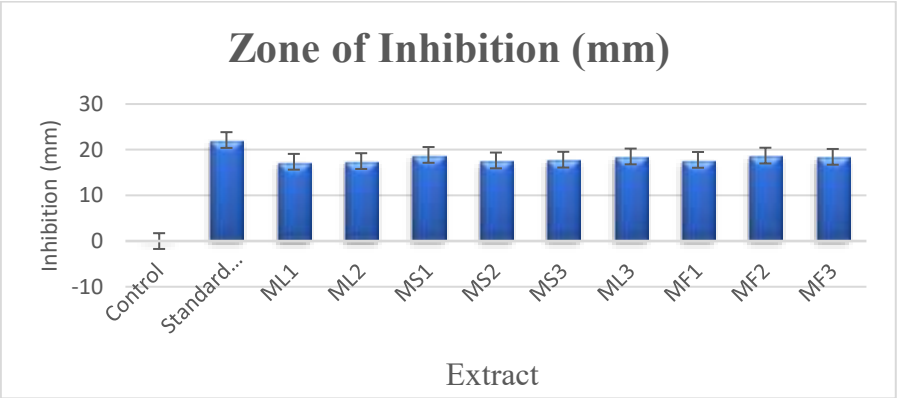

Figure 5: Graphical representation showing the antimicrobial Activity of Zone of inhibition in mm (*Candida albicans*).

In contrast to the antibacterial results where methanol: water extracts predominated, the most potent antifungal effects against *C. albicans* were recorded for dichloromethane: methanol stem extract MS3 (18.86 ± 0.22 mm), ethanol: water leaf extract ML2 (18.71 ± 0.26 mm), dichloromethane: methanol fruit extract MF3 (18.52 ± 0.28 mm), and dichloromethane: methanol leaf extract ML3 (18.44 ± 0.31 mm). The remaining extracts displayed comparable, robust inhibitory activity ranging between 17.36 ± 0.24 mm (MS1) and 17.82 ± 0.30 mm (MF2). The heightened antifungal activity of the dichloromethane: methanol fractions likely reflects the enriched extraction of lipophilic secondary metabolites, such as pentacyclic triterpenes, steroids, and lipophilic alkaloids, which readily partition into and destabilize fungal ergosterol-containing plasma membranes (Alsalamah et al., 2023; Mendes et al., 2024).

3.4 Statistical Evaluation and Comparative Significance

Welch's unpaired t-test revealed no statistically significant difference in mean zone diameters between *S. aureus* and *E. coli* across the plant extracts ($t = 0.88$, $p = 0.39$). The mean overall inhibition across all botanical extracts was 14.76 ± 2.83 mm for *E. coli* versus 13.44 ± 3.93 mm for *S. aureus*. This lack of significant strain-dependent divergence signifies that *M. laxiflora* extracts possess genuine broad-spectrum antibacterial characteristics, effectively circumventing the permeability barrier imposed by the Gram-negative outer membrane.

One-way ANOVA showed highly statistically significant differences in inhibitory zones among the various plant extracts and reference standards for both bacterial pathogens ($p < 0.001$) and *C. albicans* ($F = 68.47$, $p < 0.001$). Pairwise multiple comparisons via Tukey's HSD post-hoc test demonstrated that standard erythromycin and amphotericin B maintained significantly higher activity than all plant extracts ($p < 0.001$). Among the botanical extracts, methanol: water stem extract MS1 exhibited statistically superior antibacterial inhibition compared to leaf and fruit extracts and all dichloromethane: methanol fractions ($p < 0.05$). In the antifungal bioassay, MS3, MF3, and ML2 demonstrated statistically significant enhancements over MS1 and ML1 ($p < 0.05$). These statistical analyses confirm that both anatomical plant organ and extraction solvent polarity are critical, statistically significant determinants of antimicrobial efficacy in *M. laxiflora*.

4. CONCLUSION

The present investigation provides compelling scientific evidence supporting the traditional medicinal uses of *Meyna laxiflora Robyns* as an efficacious antimicrobial botanical. Qualitative screening established that fruit, stem, and leaf tissues are rich reservoirs of diverse secondary metabolites, notably flavonoids, phenolics, tannins, alkaloids, and terpenoids. Bioassay screening demonstrated that the methanol: water stem extract (MS1) possessed the most pronounced antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*, while moderately polar and lipophilic fractions (MS3, MF3, ML2, and ML3) displayed exceptional antifungal potency against *Candida albicans*. Statistical evaluations verified that solvent polarity and anatomical plant part significantly govern phytochemical yield and bioactivity. Future investigations should prioritize bioassay-guided fractionation, high-resolution LC-MS metabolomic characterization, and in vivo toxicological validation to facilitate the development of standardized phytotherapeutic formulations or novel lead scaffolds against multidrug-resistant infectious pathogens.

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AUTHOR CONTRIBUTIONS

G. N. Badak: Conceptualization, Methodology, Investigation, Data curation, Writing - original draft.

D. P. Kardile: Formal analysis, Validation, Writing - review & editing.

V. C. Bhagat: Supervision, Resources, Project administration, Writing - review & editing. All authors have read and approved the final submitted manuscript.

CONFLICT OF INTEREST

The authors declare that there are no financial, personal, or professional conflicts of interest associated with this study.

ETHICS STATEMENT

This study did not involve human participants or live animal subjects; therefore, formal ethical approval was not required.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

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