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PHYTOCHEMICAL PROFILING AND MECHANISTIC EVALUATION OF BIOACTIVE COMPOUNDS FROM ETHNOMEDICINAL PLANTS: TARGETING EFFLUX PUMP-MEDIATED MULTIDRUG-RESISTANT BACTERIA

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ABSTRACT
The increasing prevalence of multidrug-resistant bacteria underscores the need for effective alternative therapy. Traditional herbal extracts represent a promising therapeutic option due to their broad spectrum of bioactive compounds. The present study aims to evaluate the effectiveness of *Curcuma longa* (Haldi), *Azadirachta indica* (Neem Chhal), *Saraca asoca* (Ashok Chhal), *Vitex negundo* (Shambhu leaves), *Urtica dioica* (nettle leaves), *Tridax procumbens* (Coat Button), *Artemisia vulgaris* (Tite Pati), *Ageratum conyzoides* (Goat weed), *Solanum virginianum* (Kateri), and *Hibiscus sabdariffa* (Roselle hibiscus) against multidrug-resistant bacteria. Phytochemical screening revealed the presence of diverse bioactive components, including saponins, phenols, flavonoids, Phlobatannins, amino acids, and carbohydrates. Antibiotic susceptibility profiling showed that all isolates exhibited high resistance to commonly used antibiotics, including amoxicillin, cefixime, and penicillin, while retaining susceptibility to norfloxacin. All plant extracts demonstrated antibacterial activity, with *H. sabdariffa* displaying the highest inhibition, followed by *S. asoca and A. indica.* Minimum inhibitory concentration analysis revealed the lowest MIC values for *H. sabdariffa* (61–121.5 µg/mL); however, synergistic interaction assays with norfloxacin showed that *C. longa* exhibited the strongest synergistic effect, followed by *Hibiscus sabdariffa.* These findings highlight the potential of selected medicinal plants, particularly *C. longa, H. sabdariffa,* and *S. asoca, as* promising candidates for developing plant-based antibacterial agents and antibiotic adjuvant therapies against multidrug-resistant pathogens.

KEYWORDS: Alternative therapy, Multidrug resistance, Phytochemical screening, Herbal plant extract, antimicrobial susceptibility profiling

1. INTRODUCTION
The rapid rise in infectious diseases has become a major global health problem, driven in part by the extensive use and misuse of antibiotics. A wide range of antimicrobial agents has become less effective because of the emergence of antimicrobial resistance. It has been observed that most pathogenic bacteria rapidly acquire resistance to antimicrobial drugs [1]. This results in multiple-drug resistance, the main cause of treatment failure in infectious diseases, thereby highlighting the need for alternative therapeutic options that may be more effective at controlling resistant bacteria. One feasible approach is the use of traditional herbal extracts as natural antimicrobial agents, which are often more accessible and affordable and have fewer side effects than conventional antibiotics [2].
Herbs such as *C. longa*, *A. indica*, *S. asoca, V. negundo*, *U. diocia, T. procumbens*, *A. vulgaris*, *A. conyzoides*, *S. virginianum, and H. sabdariffa have been shown to offer* tremendous health benefits. The ability to target pathogens through multiple biochemical mechanisms, often with lower toxicity and fewer side effects, further strengthens their relevance as complementary therapeutic agents. Thereby, showing a promising alternative in combating multidrug resistance [3-7].
C. longa, known as the Golden spice of India, is known for its tremendous health benefits. Most of the therapeutic activity of *C. longa* is due to Curcumin, and various studies have confirmed its antimicrobial, anticancer, anti-inflammatory, gastroprotective, and antioxidant properties [8]. Similarly*, A. indica has been* used in Ayurveda since time immemorial, as every part of this plant possesses therapeutic potential. *Azardirachta* is the main compound and is well known for its anti-inflammatory, antimicrobial, anticancer, antiulcer, antioxidant, and antidiabetic potential, making neem one of the most valuable nontoxic biological sources for the production of modern drugs [9]. *A. indica is* popularly known as the Indian neem or margosa tree. It has been used extensively in Ayurvedic, Unani, and Homeopathic medicine since time immemorial.

Building on these well-established herbal remedies, *Saraca asoca* is another widely known magic bullet in Ayurveda. This plant has been reported to possess versatile pharmacological activities, including anticancer, antimicrobial, antihemorrhagic, and treatment for skin and genitourinary infections. Leaves of *S. asoca* contain proteins, saponins, tannins, and carbohydrates, which primarily contribute to its antibacterial potential. In addition, flowers are known to possess antidiabetic activity due to the presence of steroids and tannins [10]. In addition to these plants, *V. negundo* has been described in ancient Ayurvedic texts. Phytochemical screening of *V. negundo* has revealed the presence of various secondary metabolites, including diterpenes, triterpenes, flavonoids, and various polyphenols. These secondary metabolites contribute to the medicinal potential in rheumatism, irritable bladder, and depression [11].

Phytochemical screening of *V. negundo* has revealed the presence of various secondary metabolites, including lignans, volatile oils, flavonoids, triterpenes, diterpenes, sesquiterpenes, glycosidic iridoids, and polyphenolic compounds. [12]. Similarly, *Urtica dioica* has been used in herbal medicine from ancient times to the present. This herb contains a high concentration of bioactive substances, including vitamins, minerals, flavonoids, and phenolic compounds, which contribute to its anti-inflammatory, antihistamine, and antioxidant properties [13]. *T. procumbens*, another widely used herb that contains compounds such as polysaccharides, chromone glycosides, flavone glycosides, and sterols, contributes to wound-healing, hepatoprotective, antimicrobial, immunomodulatory, anti-inflammatory, antioxidant, and antiprotozoal potential. Aqueous leaf extract of *T. procumbens* exhibits cardiovascular effects and anti-inflammatory activity comparable to those of ibuprofen and aspirin [14].

The therapeutic potential of *A. vulgaris* is attributed to its versatile chemical composition, which includes flavonoids, phenolic acids, coumarins, sesquiterpene lactones, and essential oil. Due to its rich chemical composition, this plant exhibits hypolipidemic, antifungal, antibacterial, hypotensive, and hepatoprotective activity [15]. Likewise, *A. conyzoides* is valued for its anti-inflammatory, antimicrobial, gynecological, and antirheumatic applications and is also used as manure, a pesticide, and an herbicide in agriculture [16]. The phytochemical profile of S virginianum revealed the presence of solasonoine, solamargine, diosgenin, and campesterol, which contribute to therapeutic effects such as antianaphylactic, antipiles, and immunomodulatory activities. Additionally, *H. sabdariffa* contains substantial amounts of protocatechuic acid, anthocyanins, flavonoids, gossypetine, and γ-tocopherol, which contribute to its antioxidant, anti-inflammatory, hepatoprotective, and cardioprotective properties. Additionally, the anthocyanin compound is known to inhibit the growth of human cancer cells and LDL oxidation [17].

The documented pharmacological activity and rich phytochemical diversity of these medicinal plants highlight their significant potential as natural antimicrobial agents. The growing scientific evidence and their traditional use emphasize their relevance in confronting the global challenge of multidrug resistance. These herbal resources offer a promising, safer, and more sustainable alternative compared to conventional antibiotics. Therefore, amid the growing challenge of multidrug resistance, this research aims to explore the antimicrobial potential of extracts from the aforementioned medicinal plants, quantify their minimum inhibitory concentrations, and evaluate synergistic effects to identify effective natural alternatives to conventional antibiotics.

2. MATERIALS & METHODS

2.1 Plant Materials

The plant material *C. longa* (Haldi), *A. indica* (Neem Chhal), *S. asoca* (Ashok Chhal), *V. negundo* (Shambhu leaves), *U. diocia* (nettle leaves), *T. procumbens* (Coat Button), *A. vulgaris* (Tite Pati), *A. conyzoides* (Goat weed), *S virginianum* (Kateri), and *H. sabdariffa* (Roselle hibiscus) were procured from the local market of Nepal.

2.2 Preparation of plant extract

The plant material was air-dried in an oven at 40°C for 48 h, and extraction was performed by maceration. 10 g of dried plant powder was added to 120 mL of water in a glass bottle and incubated in a water bath at 65-75°C for 30 minutes. The bottles were kept at room temperature overnight, and the plant sample was filtered through Whatman filter paper. The plant extract was then concentrated by rotary evaporation at 50°C until complete dryness [18].

2.3 Phytochemical screening

The qualitative screening for common phytochemicals, including Saponins, flavonoids, starch, Phlobatannins, carbohydrates, amino acids, phenols, alkaloids, reducing agents, glycosides, inulin, tannins, steroids, terpenoids, and naphthoquinones, was performed as per standard protocols [19-21].

2.4 Test organisms

Total 167 bacterial strains were isolated from shared cosmetic products in lab out of these 14 strains LRV6(R2), LRV8(R1), LRV8(R3), LRV9(R3), LRV22(R5), FRV6(R1), FRV6(R2), FRV6(R3), FRV14(R2), FRV15(R2), FRV16(R2), KRV13(R2), KRV14(R1) and KRV16(R2) showed maximum drug resistance against the antibiotic Amoxicillin, Cefixime, Cefuroxime, Ceftazidime, Penicillin-G, Meropenem, Ampicillin, Tetracycline, Streptomycin, Ofloxacin, Sparfloxacin, Norfloxacin as per antibiotic susceptibility test, so these 14 isolated were used for further study.

2.5 Antibacterial activity of the extract

The antibacterial activity of the extract was evaluated using the agar well diffusion assay. Briefly, MH agar plates were inoculated with 100 µl of an actively grown bacterial culture (10^5 CFU/ml), and wells (8 mm) were punched aseptically

in the agar plates. 20 µl of extract from different plants was added to each well, and 20 µl of Norfloxacin served as the control. Plates were incubated at 37°C for 24 h and monitored for zones of inhibition (mm)[22].

2.6 Determination of minimum inhibitory concentration (MIC)

MICs of the plant extracts against bacterial strains were determined by micro-broth dilution assay using MH broth. The concentration of the extracts used was 60 µg/mL. Consequently, 180 µL of a 1.5×10 ^5 CFU/mL culture of all bacterial strains was transferred into each well of 96-well plates. 20 µL from the 2-fold dilution of the plant extract was loaded in triplicate for each strain. The plates were then incubated at 37°C for 24 hours and observed for any visible bacterial growth. The lowest extract concentration that did not result in visible growth on the agar surface was considered the MIC [23].

2.7 Synergistic antibacterial assay

To study the synergistic effect, extracts with higher antibacterial effects were selected. The synergistic potential was evaluated using the broth microdilution checkerboard method [24]. Briefly, 50 µL of each plant extract and antibiotic and 100 µL of bacterial inoculum (5×10^5 CFU/mL) were added to the wells. Plates were incubated at 37 ◦C for 18–20 hours and were assessed for turbidity at OD_{600}.

3. RESULTS AND DISCUSSION

Extensive misuse of antibiotics in clinical, agricultural, and community settings has dramatically escalated multidrug resistance. This prompts researchers' interest in developing a reliable alternative therapy. Plant-based herbal extracts, known for their diverse phytochemicals and antimicrobial properties, represent a viable strategy to combat multidrug resistance worldwide. *C. longa*, *A. indica*, *S. asoca*, *V. negundo, U. diocia, T. procumbens*, A. vulgaris*, A. conyzoides*, *S. virginianum,* and *Hibiscus sabdariffa.*

3.1 Phytochemical screening of plant extracts

Qualitative analysis of various phytochemicals was carried out according to the standard protocol. It revealed the presence of saponins in *T. procumbens, A. indica, S. asoca, C. longa;* flavonoids in *Hibiscus sabdariff, T. procumbens, A. conyzoides, S virginianum, C. longa;* starch in A. vulgaris*, T. procumbens, U. diocia;* Phlobtannins in A. vulgaris*, V. negundo, C. longa, U. diocia*, carbohydrates in A. vulgaris*, V. negundo,* amino acids in *Hibiscus sabdariff, A. conyzoides, U. diocia*; phenols and alkaloids were present in all the plant extract except *S. asoca, V. negundo and C. longa* showed absence of alkaloids (Table 1). Similarly, recent studies on under-investigated medicinal plants have also reported the presence of various phytochemicals, such as saponins and flavonoids, and are consistent with our findings [12, 26].

Table. 1: The table summarizes a qualitative phytochemical screening of ten medicinal plant species for eight

Plants	Saponins	Flavonoides	Starch	Phlobtannins	Carbohydrates	Amino acids	Phenols	Alkaloides
Hibiscus sabdariff	-	+	-	-	-	+	+	+
Artemisia Vulgaris	-	-	+	+	+	-	+	+
Trides procumbers	+	+	+	-	-	-	+	+
Ageratum Conyzoides	-	+	-	-	-	+	+	+
Solanum virginianum	-	+	-	-	-	-	+	+
Saraca asoca	+	-	-	-	-	-	+	-
Azardirachta Indica	+	-	-	-	-	-	+	+
Vitex Negundo	-	-	-	+	+	-	+	-
Curcuma longa	+	+	-	+	-	-	+	-
Urtica dioica	-	-	+	+	-	+	+	+

metabolite classes, using standard presence (+) / absence (−) chemical tests.

3.2 Antibiotic susceptibility test against isolated bacterial strains

Isolated bacterial strains such as LRV6(R2), LRV8(R1), LRV8(R3), LRV9(R3), LRV22(R5), FRV6(R1), FRV6(R2), FRV6(R3), FRV14(R2), FRV15(R2), FRV16(R2), KRV13(R2), KRV14(R1) and KRV16(R2) showed maximum antibiotic resistance against Amoxicillin, Cefixime, Cefuroxime, Ceftazidime, Penicillin-G, Meropenem, Ampicillin, Tetracycline, Streptomycin, Ofloxacin, Sparfloxacin, Norfloxacin (Table 2). Strains LRV8(R1) and LRV22(R5) showed susceptibility against cefuroxime whereas FRV6(R1), FRV6(R2), FRV6(R3), FRV15(R2), KRV13(R2), KRV14(R1), KRV14(R2) and KRV16(R2) showed susceptibility against ceftazidime. Strains LRV6(R2), LRV8(R1), LRV8(R3), LRV9(R3), LRV22(R5), FRV6(R2), FRV15(R2) and FRV16(R2) showed susceptibility against Meropenem. Additionally, strains LRV6(R2), LRV8(R1), LRV8(R3), LRV9(R3), LRV22(R5), KRV13(R2), KRV14(R1) showed susceptibility against tetracycline and strains LRV6(R2) and FRV6(R3) showed susceptibility to streptomycin. The high prevalence of resistance to penicillin and cephalosporins in our findings correlates with the reported patterns from clinical

isolate studies, which document MDR bacteria resistant to amoxicillin, cefixime, cefuroxime, and ceftazidime [26]. Meanwhile, the retained susceptibility to meropenem and norfloxacin is consistent with this finding, indicating that these drugs remain useful against multidrug-resistant pathogens [24].

Antibiotic	Susceptible Isolates	Overall Observation
Amoxicillin	None	Complete resistance observed
Cefixime	None	Complete resistance observed
Cefuroxime	LRV8(R1), LRV22(R5)	Partial susceptibility
Ceftazidime	FRV6(R1), FRV6(R2), FRV6(R3), FRV15(R2), KRV13(R2), KRV14(R1), KRV16(R2)	Moderate susceptibility
Penicillin-G	None	Complete resistance observed
Meropenem	LRV6(R2), LRV8(R1), LRV8(R3), LRV9(R3), LRV22(R5), FRV6(R2), FRV15(R2), FRV16(R2)	Variable susceptibility
Ampicillin	Few/none	High resistance observed
Tetracycline	LRV6(R2), LRV8(R1), LRV8(R3), LRV9(R3), LRV22(R5), KRV13(R2), KRV14(R1)	Moderate susceptibility
Streptomycin	LRV6(R2), FRV6(R3)	Limited susceptibility
Ofloxacin	Most isolates	High susceptibility
Sparfloxacin	Most isolates	High susceptibility
Norfloxacin	Most isolates	Highest susceptibility observed

Table 2: Bacterial isolates showed complete resistance to Amoxicillin, Cefixime, and Penicillin-G, and high resistance to Ampicillin. Moderate to variable susceptibility was seen with Cefuroxime, Ceftazidime, Meropenem, and Tetracycline, while Streptomycin showed limited effect. Fluoroquinolones (Ofloxacin, Sparfloxacin, Norfloxacin) remained highly effective, with Norfloxacin performing best, indicating an overall MDR phenotype with fluoroquinolones as the most reliable treatment.

3.3 Antibacterial activity of the extract

Plant extracts were tested for their antibacterial potential against isolated bacterial strains. It was interesting to find that all the plant extracts exhibited antibacterial potential against all the strains, with *H. sabdariffa* showing maximum antibacterial efficacy, followed by *S. asoca, A. indica, C. longa, Urtica dioica, A. vulgaris, A. conyzoides, V. negundo, T. procumbens* and *S virginianum* (Fig. 1). This finding is in consistent with earlier studies where *H. sabdariffa* and other medicinal plants demonstrated significant inhibition zone, reinforcing that these medicinal plants are promising sources of natural antimicrobial agents [27,28].

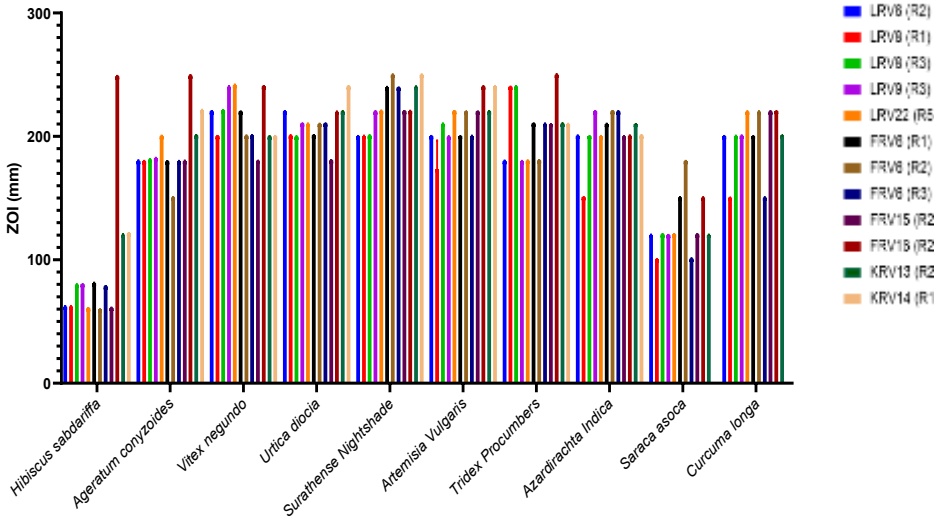

Figure 1: This bar graph depicts the antibacterial activity of ten plant extracts against multiple bacterial isolates, assessed via Zone of Inhibition (ZOI, mm). Each bar represents a distinct bacterial isolate tested per plant extract.

3.4 Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) values of the evaluated plant extracts varied significantly across the tested microbial strains, as shown in the heat map (Fig. 2). Among all extracts, *H. sabdariffa* exhibited the lowest MIC range (61-121.5 µg/mL), indicating the highest inhibitory potential. This was followed by *S. asoca,* which also exhibited a comparatively low MIC ranging from 100.4 to 150.5 µg/mL. Extracts of *A. conyzoides*, *T. procumbens*, and *A. indica* demonstrated moderate inhibition (MIC range 150-220 µg/mL), whereas *Urtica dioica*, A. vulgaris, *V. negundo*, and *S virginianum* exhibited a higher MIC range 200-250 µg/mL, indicating the lowest inhibitory potential (Fig. 2). These findings are consistent with previous studies reporting that *H. sabdariffa* extract inhibited bacterial pathogens at MIC values around 128-256 µg/mL confirming the high antibacterial potential of medicinal plants against multiple bacterial strains [29].

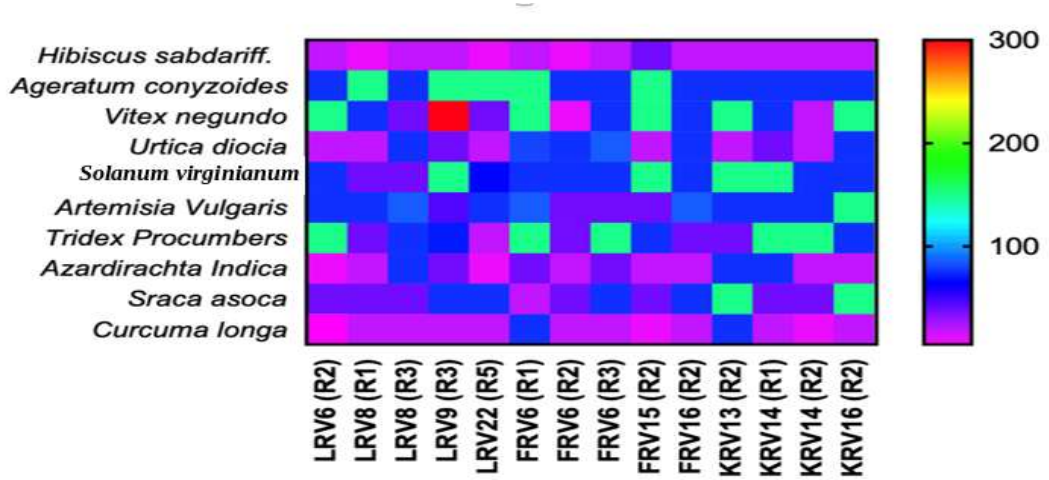

Figure 2: This heatmap depicts the Zone of Inhibition (ZOI) intensity, on a color scale from magenta/blue (low, ~0–100 mm) through green/cyan (moderate, ~100–200 mm) to red (high, ~250–300 mm), for ten plant extracts (rows) tested against thirteen bacterial isolates (columns: LRV, FRV, KRV strains).

3.5 Synergistic activity

Synergism is basically a phenomenon in which two antimicrobial agents, when used in combination, produce a greater inhibitory effect than either agent alone, thereby enhancing therapeutic effectiveness. Based on the MIC results from the preliminary screening, plant extracts of Hibiscus sabdariffa, C. longa, and S. asoca demonstrated comparatively strong antimicrobial activity and were therefore selected for further evaluation of their synergistic effects with Norfloxacin against the isolated strains. *C. longa* demonstrated FIC values ranging from 7–9 µg/mL*, H. sabdariffa* showed FIC values ranging from 6-11 µg/mL, whereas *S. asoca* exhibited FIC values between 8–10 µg/mL across all the bacterial isolates. Overall, *C. longa* exhibits the strongest synergistic activity with norfloxacin (Fig. 3). The observed synergistic enhancement of antibiotic efficacy when combined with plant extracts and standard antibiotics aligns with prior reports showing that plant-antibiotic combinations significantly lower MICs against multidrug-resistant bacteria [30,7].

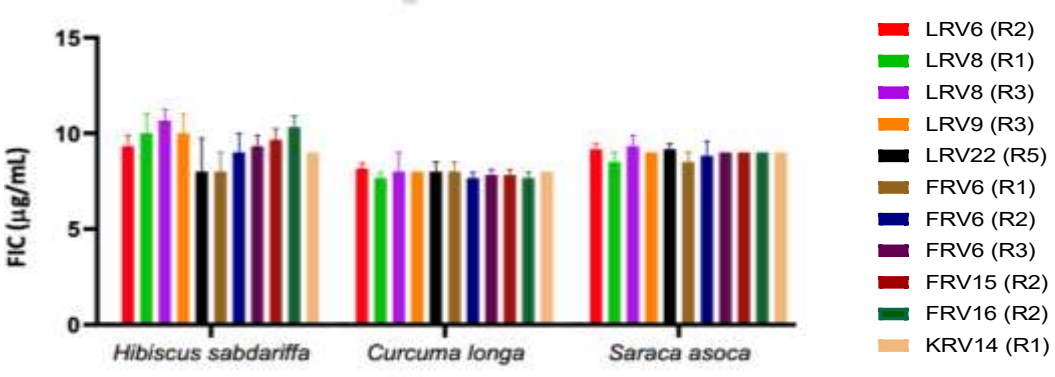

Figure 3. This bar graph depicts the Fractional Inhibitory Concentration (FIC) values (µg/mL) of three plant extracts-*Hibiscus sabdariffa*, *Curcuma longa*, and *Saraca asoca* evaluated against multiple bacterial isolates, typically used to assess synergistic, additive, or antagonistic interactions in combination antibacterial assays (e.g., checkerboard/FIC index method). Each coloured bar represents FIC values against a distinct bacterial isolate, with error bars indicating the mean ± SD of replicate measurements.

CONCLUSION

Taken together, this study highlights that medicinal plant extracts exhibit significant antibacterial activity against multidrug-resistant isolates, offering a valuable alternative for combating antibiotic resistance. *Hibiscus sabdariffa*, *S. asoca*, and *C. longa* showed the strongest bacteriostatic potential, as reflected by their low MIC values. Further, *C. longa* emerged as a particularly valuable species due to its pronounced synergistic activity with norfloxacin. Integrating plant-derived bioactive compounds with conventional antibiotics may offer an effective strategy to enhance antibiotic efficacy.

In total, this is the first comparative study to demonstrate the strong antimicrobial and synergistic activity of selected medicinal plants, particularly *C. longa,* against MDR clinical isolates.

Abbreviations
LRV- Lipstick, Recovered, Variant
FRV- Foundation, Recovered, Variant
KRV- Kajal, Recovered, Variant

Acknowledgement
None

Data Availability
All datasets generated or analyzed in this study are included in the manuscript.

Conflict of Interest
The authors declare that they have no conflicts of interest.

Ethical Approval
Not applicable.

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