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Evaluation of *In-Vitro* Antibacterial Potential of Selected Indian Medicinal Plants against Human Pathogens

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***Keywords*— Antimicrobial, Antibacterial, Medicinal, Pharmaceutical.**

***Abstract*—** *The increasing prevalence of antibiotic resistance necessitates the exploration of alternative antimicrobial agents. This study evaluated the antibacterial efficacy of methanolic leaf extracts of nine Indian medicinal plants, including Cassia fistula, Millettia pinnata, and Adhatoda vasica. Antibacterial activity was assessed against two Gram-negative (Escherichia coli, Enterobacter aerogenes) and two Gram-positive (Bacillus subtilis, Staphylococcus aureus) bacteria using the agar well diffusion method. Among the tested extracts, Justicia adhatoda exhibited the highest inhibition against E. aerogenes (16 mm), while Rauvolfia serpentina was most effective against B. subtilis (14 mm). These findings support the traditional use of these plants and suggest their potential as sources for novel antibacterial compounds.*

I. INTRODUCTION

Antibiotics have been the cornerstone of medical care for infectious illnesses in the modern era, greatly lowering death rates all around the world. However, an international problem of antimicrobial resistance (AMR) has been brought about by the careless and irresponsible use of these life-saving medications [1]. In 2024, the World Health Organization (WHO) revised its Bacterial Priority Pathogens List (BPPL), designating key gram-negative bacteria as serious dangers in need of immediate new therapies. Multi-drug resistant (MDR) "superbugs" have become more prevalent in developing countries like India due to high population density and poor antibiotic stewardship [2]. Throughout history, nature has served as a valuable source of medicinal substances. In the Indian medical system, including Ayurveda and Siddha, plants are widely utilized for addressing different health issues. A significant number of individuals in India are believed to continue depending on traditional herbal remedies for their main healthcare requirements [3]. Medicinal plants hold a range of bioactive secondary metabolites such as alkaloids, flavonoids, tannins, and terpenoids, which exhibit potent antimicrobial effects [4]. In contrast to synthetic antibiotics, these herbal medicines are typically regarded as safer, more affordable, and environmentally friendly, with fewer adverse effects. Thus, the evaluation of native medicinal plants for antibacterial properties is essential for creating new drugs [5]. Although many Indian medicinal plants have been studied, there is a continuous need to evaluate the local flora against specific pathogenic bacteria. The aim of the present study is to evaluate the antibacterial activity of methanolic leaf extracts of nine selected medicinal plants: *Cassia fistula* (Golden shower tree), *Millettia pinnata* (Karanj), *Rauvolfia serpentina* (Sarpagandha), *Mimosa pudica* (Touch-me-not), *Adhatoda vasica* (Adhusi), *Salvadora persica* (Miswak), *Justicia adhatoda* (Adhulsa), *Terminalia chebula* (Harad), and *Balanites roxburghii* (Ingoria). The efficacy of these extracts was tested against two gram-negative bacteria (Escherichia coli, Enterobacter aerogenes) and two gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) to determine their zone of inhibition and validate their traditional use scientifically.

II. MATERIALS AND METHODS

Collection and Preparation of Plant Material

Fresh material was collected from Gandhidham Tehsil, Gujarat, India, fresh leaves of nine medicinal plants viz. *Cassia fistula*, *Millettia pinnata*, *Rauvolfia serpentina*, *Mimosa pudica*, *Adhatoda vasica*, *Salvadora persica*, *Justicia adhatoda*, *Terminalia chebula*, and *Balanites roxburghii* were gathered. Taxonomic identification and authentication were performed on the plant samples. To get rid of dust and filth particles, the gathered leaves were carefully cleaned with tap water and then distilled water. After being shade-dried for ten to fifteen days at room temperature, the leaves were ground into a coarse powder using a mechanical grinder [6].

Extraction Process

The bioactive compounds were extracted using a Soxhlet apparatus. About 25 g of the powdered dried leaves was placed into the thimble. The extraction was conducted using methanol as the solvent for 10 hours in a extraction assembly [7]. The Soxhlet extraction technique was selected to guarantee the thorough depletion of raw plant extracts. The collected extracts were evaporated to dryness using a water bath and preserved in airtight containers at 4°C in a refrigerator for subsequent antibacterial testing prepared extracts are shown in **Fig.1** [8].

Fig.-1. Prepared Plant Extracts and its storage.

Antibacterial Susceptibility Assay

The Agar Cup Borer (Well Diffusion) method was used to assess the antibacterial potential. To make a lawn culture, a 3-hour-old bacterial culture inoculum was evenly swabbed on the agar plate surface. Wells were punched into the agar medium using an 8 mm diameter sterile cork borer. The corresponding wells were filled with the plant extracts. The positive control was gentamicin, while the negative control was pure methanol [09]. To promote bacterial growth and extract diffusion, the plates were incubated at 37°C for 24 hrs.

Determination of Zone of Inhibition

After the incubation period, the antibacterial activity was assessed by measuring the Zone of Inhibition (ZOI) around the wells. The diameter of the inhibition zones was measured in millimeters (mm) to determine the susceptibility of the bacterial strains to the plant extracts [10].

III. RESULTS AND DISCUSSION

After conducting the experiment data observed of zone of inhibition is depicted in **Table 1**. Among the tested extracts, *J. adhatoda* exhibited the highest antibacterial potential against *Enterobacter aerogenes* with a ZOI of 16 mm. *S. persica* also showed significant activity against *E. aerogenes* (15 mm) but failed to inhibit the growth of gram-positive bacteria. *R. serpentina* was found to be most effective against *B. subtilis* with a ZOI of 14 mm. In the case of *S. aureus*, *C. fistula* and *B. roxburghii* showed moderate activity with a ZOI of 14 mm. The standard antibiotic, Gentamicin (Positive Control), showed a ZOI of 15 mm against *S. aureus*, while the negative control (Methanol)

showed no inhibition, confirming the validity of the extraction protocol.

Table-1 Antibacterial Activity of Methanolic Plant Extracts (Zone of Inhibition in mm)

Sr. No.	Name of Medicinal Plant	Local Name	E. coli	E. aerogenes	B. subtilis	S. aureus
Controls						
1.	**Methanol** (Negative Control)	-	-	-	-	-
2.	**Gentamicin** (Positive Control)	-	-	-	-	**15**
Extracts						
3.	*Cassia fistula*	Golden Shower	8	10	6	**14**
4.	*Rauvolfia serpentina*	Sarpagandha	**12**	8	**14**	-
5.	*Mimosa pudica*	Touch-me-not	9	10	12	10
6.	*Adhatoda vasica*	Adhusi	12	13	9	7
7.	*Salvadora persica*	Miswak	8	**15**	-	-
8.	*Justicia adhatoda*	Adhulsa	10	**16**	-	-
9.	*Terminalia chebula*	Harad	8	10	4	5
10.	*Balanites roxburghii*	Ingoria	9	12	13	**14**

Note: "-" indicates No Zone of Inhibition. Values are mean diameter of inhibition zones including the well diameter of 8 mm

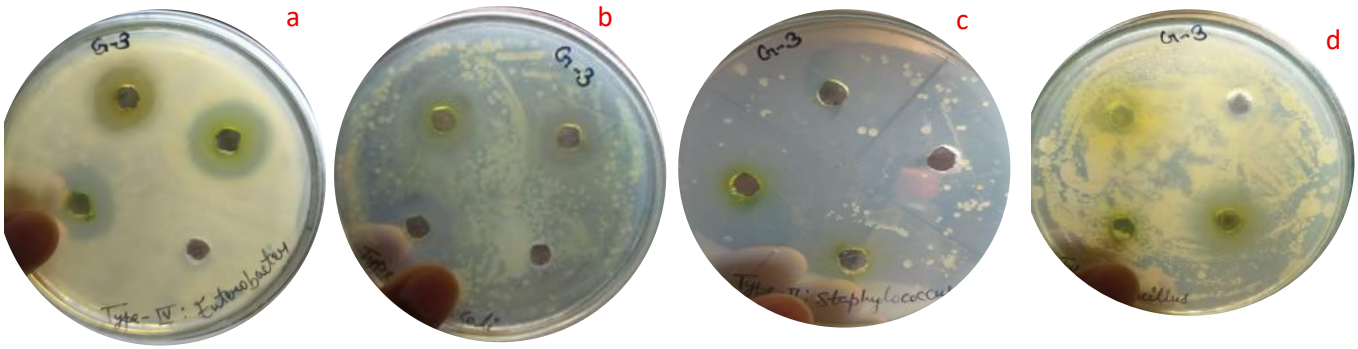

Fig.2 Antimicrobial susceptibility test showing Zone of Inhibition (ZOI) against test bacteria. (a) for E. aerogenes (b) for E. coli (c) for S. aureus (d) for B. subtilis

Table-1 and Fig.2 demonstrates that the methanolic extracts of selected Indian medicinal plants possess varying degrees of antibacterial activity against human pathogens. The results revealed that gram-negative bacteria *E. aerogenes* were generally more susceptible to the specific plant extracts tested compared to gram-positive strains. The maximum inhibitory activity was observed in *J. adhatoda* against *E. aerogenes* (16 mm). This finding is in accordance with the work of Shalini et al. (2021), who reported that the alkaloids present in Justicia species are highly effective against gram-negative respiratory pathogens [11]. Similarly, *A. vasica* showed a ZOI of 13 mm against *E. aerogenes* and 12 mm against *E. coli*. These results support the traditional use of Adhusi in treating respiratory tract infections and are consistent with the observations of Kumar and Singh (2022), who demonstrated the bactericidal effect of Vasicine alkaloid from this plant [12]. *C. fistula* extracts showed a prominent ZOI of 14 mm against *S. aureus*. This result is in agreement with the findings of Al-Jameil et al. (2020), who observed that Cassia species effectively disrupt the cell wall synthesis of gram-positive bacteria [13]. However, our results regarding *T. chebula* showed relatively lower activity (4-5 mm) against gram-positive organisms. This is in contradiction of the study by Sharma et al. (2019), which

reported *T. chebula* as a potent broad-spectrum antibacterial agent [15]. This variation might be due to differences in the geographical location of the plant collection or the solvent extraction method used in our study. It is interesting to note that *S. persica* showed significant activity against gram-negative bacteria but failed to inhibit gram-positive strains in this study. This selective activity is partially in accordance with the report of Hassan (2023), who suggested that the efficacy of Miswak extracts is highly dependent on the concentration of benzyl isothiocyanate, which may vary seasonally [16].

Overall, the study validates the ethnobotanical importance of these plants. The presence of secondary metabolites in the methanolic extracts is likely responsible for the observed antibacterial effects, suggesting their potential use as natural alternatives to synthetic antibiotics.

IV. CONCLUSION

The antibacterial properties of a few Indian medicinal herbs are scientifically validated in this study. With a 16 mm Zone of Inhibition against *E. aerogenes*, *J. adhatoda* had the most activity among the studied extracts, whereas *R. serpentina* demonstrated efficacy against gram-positive pathogens. These results corroborate the historic usage of these herbs and point to their potential as eco-friendly, alternative medicinal agents in the worldwide battle against antibiotic resistance.

AUTHORS' CONTRIBUTION

All authors actively contributed to this research work. Dodia K. carried out the experimental procedures. Tandel R. and Pandya N. verified the results and assisted in the interpretation of the data. Kher G. was involved in the standardization of the experimental protocols. Data compilation and manuscript preparation were completed by Bhanushali U.

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CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

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