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Original Article

IN VITRO ANTIBACTERIAL POTENTIAL OF DIFFERENT COMBINATION OF PLANT EXTRACT OF SOME COMMON WEED PLANTS AGAINST XANTHOMONAS ORYZAE AND AGROBACTERIUM SPP

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ABSTRACT

The present study investigates the antibacterial activity of various polyherbal combinations against two significant plant pathogens, Xanthomonas oryzae (ATCC 35933) and Agrobacterium spp. (ATCC 12210), both of which are known to cause major crop losses. Using the agar well diffusion method, five different polyherbal combinations (F-1 to F-5) were evaluated at a concentration of 100 mg/ml. The results showed a progressive increase in antibacterial activity from F-1 to F-5, with F-5 exhibiting the largest zones of inhibition—21 mm against X. oryzae and 25 mm against Agrobacterium spp. This enhanced activity is attributed to the synergistic action of phytochemicals within the polyherbal mix. The findings highlight the potential of polyherbal combinations as eco-friendly biopesticides and suggest that such natural alternatives could play a significant role in sustainable agricultural practices. Further studies are recommended to validate these results under field conditions and optimize formulations for commercial use.

Keywords: Polyherbal Combination, Antibacterial Activity, Xanthomonas Oryzae, Agrobacterium Spp., Biopesticide, Phytochemicals, Sustainable Agriculture, Zone of Inhibition

INTRODUCTION

Pathogenic bacteria present a major threat to global agriculture, causing significant yield losses in economically important crops and threatening food security. To combat these pathogens, chemical bactericides are widely employed. While effective, their indiscriminate use poses severe ecological risks.

Chemical pesticides not only eliminate harmful microorganisms but also disrupt the balance of beneficial microbes and insects in the environment. This contributes to biodiversity loss, soil degradation, and the emergence of resistant microbial strains, challenging long-term agricultural sustainability.

In response to these concerns, biopesticides have gained attention as safer, eco-friendly alternatives. Derived from natural sources such as microbes, plant extracts, and biochemical compounds, biopesticides offer targeted pathogen control with minimal harm to non-target organisms and the environment.

Among natural sources, weed plants are particularly promising. These species are widespread, low-maintenance, and often resistant to herbivores and microbial infections due to their inherent chemical defenses Saha and Modi (2024). Their bioactive compounds offer potential for use in natural plant protection formulations.

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Transparency: The authors affirm that this manuscript presents an honest, accurate, and transparent account of the study. All essential aspects have been included, and any deviations from the original study plan have been clearly explained. The writing process strictly adhered to established ethical standards.

The present study investigates the antibacterial efficacy of aqueous extracts from seven commonly occurring weed species—Datura metel, Alternanthera sessilis, Calotropis gigantea, Argemone mexicana, Tridax procumbens, Lantana camara, and Sida acuta. These extracts were tested against Agrobacterium spp and Xanthomonas oryzae, to assess their potential as natural alternatives to synthetic bacteriocides, aiming to promote sustainable agricultural practices.

MATERIAL AND METHOD

COLLECTION OF PLANT MATERIAL

In the present study, a total of seven weed plants were selected: Datura metel, Alternanthera sessilis, Calotropis gigantea, Argemone mexicana, Tridax procumbens, Lantana camara, and Sida acuta. These plants were chosen based on their well-documented antimicrobial properties, as reported in previous research studies. The plant materials were collected from areas in and around Bhopal, Madhya Pradesh, India. After collection, the plants were taxonomically identified and authenticated by Dr. Suman Mishra, CEO of the Herbal Testing and Research Laboratory, Bhopal, Madhya Pradesh, India. Detailed information about the selected weed plants is presented in Table 1.

PROCESSING OF PLANT MATERIAL FOR EXTRACTION

After collecting the weed plants, the next step is to prepare them for extraction. First, the collected plant material is thoroughly washed and cleaned to remove dirt and impurities. The cleaned material is then dried in the shade to preserve the phytochemicals, which may degrade under direct sunlight or high temperatures. Once the plant samples are properly dried, they are ground into a fine powder. This powdered form is then used in the subsequent step to obtain the plant extract.

EXTRACTION PROCEDURE

Defatting Process: Prior to extraction, the powdered plant samples were subjected to a defatting process to remove lipophilic substances such as oils, waxes, and greasy materials that may interfere with subsequent extraction. Petroleum ether was used as the defatting solvent. The powdered samples were thoroughly mixed with petroleum ether, filtered, and the residue was dried and sieved to ensure uniform particle size before further use.

Soxhlation Extraction Method: The defatted leaf powders of seven different weed species were used for aqueous extraction via the Soxhlation method. For each plant sample, 20 grams of defatted leaf powder were placed in a Soxhlet extractor (250 mL capacity), and distilled water—a polar solvent—was used as the extraction medium. The Soxhlation was carried out at a temperature range of 60°C to 80°C for a continuous period of 24 hours. Handa et al. (2008)

After completion of the extraction, the aqueous extract was concentrated by evaporating the excess solvent using a boiling water bath until a semi-solid or solid residue was obtained. The percentage yield of each extract was calculated using the following formula

$$\%\ \text{yield} = \frac{\text{Weight of Extract.}}{\text{Weight of crude subtract take before extraction}} \times 100$$

PRELIMINARY PHYTOCHEMICAL TEST

1) Organoleptic Properties:-After obtaining the phytochemical extracts, the organoleptic properties—such as taste, color, and aroma—are assessed through visual and sensory evaluation. These observations provide valuable insights into the quality and authenticity of herbal products Heinrich et al. (2009). The Organoleptic property is given in Table 2.
2) Phytochemical Analysis:-This section involves the qualitative analysis of various phytochemicals such as alkaloids, flavonoids, terpenoids, and saponins, following the standard methods outlined by Harborne (1998). Additionally, the presence of trace elements in the plant extracts was also examined. These phytochemicals are known for their potential role in pest control Isman (2000). The results of the phytochemical analysis are represented by graphics Figure 1.

Table 1

Table 1 Selected Weed Plants and Their Reported Antimicrobial Properties

S.No.	Botanical name	Common name	Family	Reported anti microwave activity	Reference
1	*Datura metel*	Thorn Apple	Solanaceae	Antibacterial and antifungal activity against clinical pathogens	Sharma et al. (2019)

2	*Alternanthera sessilis*	Sessile Joyweed	Amaranthaceae	Effective against E. coli, S. aureus, and P. aeruginosa	Rao and Pillai (2018)
3	*Calotropis gigantea*	Crown Flower	Asclepiadaceae	Inhibits fungal growth and bacterial infections	Kumar and Singh (2020)
4	*Argemone mexicana*	Mexican Poppy	Papaveraceae	Broad-spectrum antimicrobial properties	Mehta et al. (2017)
5	*Tridax procumbens*	Coat Buttons	Asteraceae	Effective against Gram-positive bacteria	Jain et al. (2015)
6	*Lantana camara*	Wild Sage	Verbenaceae	Antibacterial and antifungal activities	Verma and Rao (2016)
7	*Sida acuta*	Wireweed	Malvaceae	Antibacterial, anti-inflammatory, antipyretic	Okoli et al. (2002), Nworu et al., (2010)

Figure 1

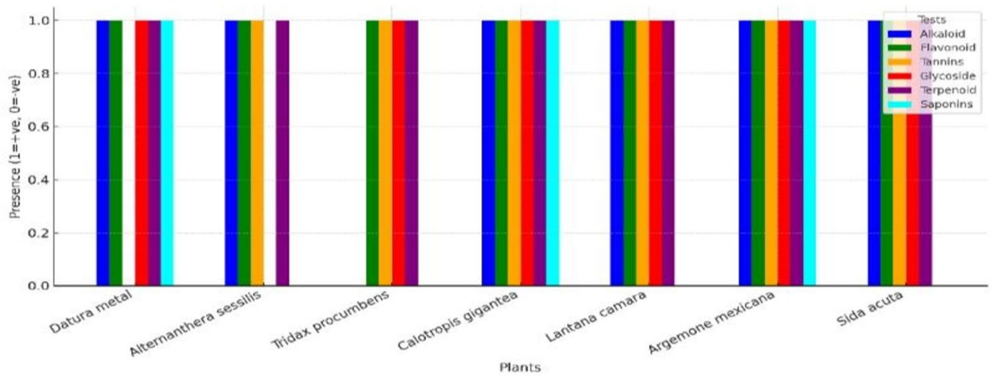

Figure 1 Comparative Analysis of Phytochemical Constituents in Seven Medicinal Plant Species

Table 2

Table 2 The Results of the Phytochemical Analysis and Organoleptic Property

S.N.	Aqueous Extract of	Organoleptic Properties			
		Colour	**Texture**	**Odor**	**Percentage Yield**
1	***Datura metal***	Dark Brown	Greasy texture	Intense, Aromatic, Unpleasant	26.13%
2	***Alternanthera sessilis***	Chocolate Brown	Crystal Texture,	Intense, Aromatic, Unpleasant	27.86%
3	***Tridax procumbens***	Chocolate Brown	Hard Sticky paste, rough texture	Intense, Aromatic, Unpleasant	26.53%
4	***Calotropis gigantea***	Black	Hard Sticky paste, rough texture	Intense, Aromatic, Unpleasant	27.06%
5	***Lantana camara***	Black	Crystal texture	Intense, Aromatic, Unpleasant	21.06%
6	***Argemone mexicana***	Chocolate Brown	Hard Sticky paste, rough texture	Intense, Aromatic, Unpleasant	32.26%
7	***Sida Acuta***	Chocolate Brown	Hard Sticky paste, rough texture	Intense, Aromatic, Unpleasant	26%

PREPARATION OF COMBINATIONS OF PLANT EXTRACTS

Aqueous extracts of individual plants were prepared and used to formulate a stock solution at a concentration of 100 mg/mL using distilled water. This stock solution served as the basis for preparing various combinations of plant extracts. Binary, ternary (tri-herbal), and polyherbal combinations were formulated by mixing the individual extracts in a 1:1 ratio. These prepared combinations were subsequently evaluated for their antimicrobial activity. Detailed information on the specific phytochemical extract combinations is presented in Table 3.

Table 3

Table 3 Herbal Formulations Overview

S.No.	Combination Name	Combination Code	Aqueous Extracts Used
1	Triherbal	Formulation – 1	Datura metel, Sida acuta, Alternanthera sessilis
2	Polyherbal (4 herbs)	Formulation – 2	D. metel, S. acuta, A. sessilis, Tridax procumbens
3	Polyherbal (5 herbs)	Formulation – 3	D. metel, S. acuta, A. sessilis, T. procumbens, Calotropis gigantea
4	Polyherbal (6 herbs)	Formulation – 4	D. metel, S. acuta, A. sessilis, T. procumbens, C. gigantea, Lantana camara
5	Polyherbal (7 herbs)	Formulation – 5	D. metel, S. acuta, A. sessilis, T. procumbens, C. gigantea, L. camara, Argemone mexicana

TEST BACTERIA

For the present investigation, bacterial cultures were initially revived in nutrient broth. Specifically, Agrobacterium spp. and Xanthomonas oryzae were inoculated into nutrient broth (HiMedia) using lyophilized cultures obtained from ATCC. All procedures were carried out under aseptic conditions within a laminar airflow cabinet. The inoculated broths were incubated at 37 °C for 24 to 48 hours. Upon observation of turbidity, indicating active bacterial growth, inoculum was aseptically transferred using an inoculation loop onto nutrient agar plates and slants. These cultures were incubated under the same conditions to allow colony development on a solid medium. The resulting cultures were designated as stock cultures and maintained for use throughout the research Table 4

Table 4

Table 4 List of Microorganisms Used in Present Work for Antimicrobial Studies

S.N.	Microorganism	Pathogen Type	Accession Number
1	*Agrobacterium spp.*	Pathogenic Bacteria	*ATCC-12210*
2	*Xanthomonas oryzae*	Pathogenic Bacteria	*ATCC-35933*

ANTIBACTERIAL ASSAY

The bacterial cultures used in the present investigation were initially revived in nutrient broth. Specifically, nutrient broth (HiMedia) was inoculated with lyophilized cultures of Agrobacterium spp. and Xanthomonas oryzae, obtained in vials from ATCC. All procedures were conducted aseptically under a laminar air flow cabinet. The inoculated broths were incubated at 37°C for 24 to 48 hours. Upon observing turbidity, indicating microbial growth, inocula were taken using an inoculation loop and streaked onto nutrient agar plates and slants. These were further incubated under the same conditions to develop visible bacterial colonies on solid media. These cultures were then maintained as stock cultures for use throughout the research.

RESULT

The antibacterial activity of five polyherbal formulations (F-1 to F-5) was evaluated against Xanthomonas oryzae (ATCC 35933) and Agrobacterium spp. (ATCC 12210) using the agar well diffusion method at a stock concentration of 100 mg/ml. The results indicated a progressive increase in the zone of inhibition with each formulation, suggesting enhanced antibacterial efficacy with successive formulations. Among all, F-5 exhibited the highest activity, producing zones of inhibition of 21 mm and 25 mm against X. oryzae and Agrobacterium spp., respectively Image 1. The lowest activity was observed in F-1, with inhibition zones of 11 mm and 9 mm against the respective bacterial strains. These findings demonstrate that F-5 was the most effective formulation, followed by F-4, F-3, F-2, and F-1 in descending order of activity. All necessary information related to the result is given in Table 5.

Table 5

Table 5 Results of antibacterial activity of Polyherbal Combination

S.N.	Sample Stock (100 mg/ml)	Zone of inhibition (Φ in mm) against test microbes	
		Xanthomonas oryzae	***Agrobacterium***

1	F-1	11 mm	9 mm
2	F-2	13 mm	12 mm
3	F-3	15 mm	17 mm
4	F-4	17 mm	21 mm
5	F-5	21 mm	25 mm

Image 1

A. B.

Image 1 Antibacterial Activity of Plant Extracts Combination on Bacterial Sample.
A. Agrobacterium spp., B. Xanthomonas oryzae.

DISCUSSION

The current study assessed the antibacterial potential of various polyherbal combinations against Xanthomonas oryzae and Agrobacterium spp., two major bacterial pathogens in agriculture. A clear trend of increasing antibacterial activity was observed from combination F-1 to F-5, as reflected by the progressively larger zones of inhibition. Among these, F-5 demonstrated the strongest effect, suggesting a higher concentration or more effective synergy of bioactive compounds.

The enhanced performance of F-5 highlights the advantage of using polyherbal combinations, which allow multiple phytochemicals to act together against microbial targets. This synergistic action not only boosts efficacy but may also reduce the risk of resistance. These findings are consistent with earlier reports Nisar et al. (2017), Upadhyay et al. (2020) that emphasize the superior antimicrobial properties of combined herbal extracts compared to individual ones.

Overall, the antibacterial activity demonstrated by the polyherbal combinations—especially F-5—underscores their potential as effective, plant-based alternatives to chemical pesticides. Their natural origin, environmental safety, and promising efficacy position them as valuable tools in sustainable crop protection. Further work on formulation optimization and field validation is recommended to support their practical application Mishra et al. (2018), Pandey et al. (2019).

CONCLUSION

The results of this study clearly demonstrate the antibacterial potential of polyherbal combinations against key agricultural pathogens, Xanthomonas oryzae and Agrobacterium spp. Among the tested combinations, F-5 showed the highest efficacy, indicating the critical role of synergistic interactions among bioactive plant compounds. These findings support the use of polyherbal combinations as promising natural alternatives to synthetic pesticides, offering a sustainable and environmentally friendly approach to crop protection. Further research, including field trials and formulation refinement, is essential to translate these laboratory results into practical agricultural applications.

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