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EVALUATING THE ANTIMICROBIAL PROPERTIES OF WEEDS IN COMBATING MICROBIAL INFECTIONS

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Abstract

Weeds, specifically angiosperms and certain pteridophytes, are known to be harmful to humans. This study investigates the potential benefits of two weeds, Withania somnifera and Lantana camara, in fighting microbial infections. The well method was used to measure the inhibition zone on agar plates, and extracts from various parts of these weeds were found to inhibit the growth of Vibrio cholerae, a severe human pathogen. However, in some tests, the growth of pathogens such as Bacillus anthracis was stimulated by leaf and flower extracts of L. camara and W. somnifera. This highlights the complex relationship between the antimicrobial properties of weeds and their potential impact on human health.

Introduction

The increasing prevalence of antibiotic-resistant bacteria has become a significant global health concern, highlighting the urgent need for new and effective antimicrobial agents (Ventola, 2015). The discovery of novel compounds with antimicrobial properties is crucial to combat the emergence and spread of multidrug-resistant pathogens, which pose a threat to public health and the economy (Tzouvelekis et al., 2012). In recent years, there has been a renewed interest in exploring natural sources, particularly plants, as a potential source of bioactive compounds with antimicrobial properties (Cowan, 1999). Weeds, which are often overlooked and regarded as a nuisance, could be a valuable resource for discovering new antimicrobial agents (Reigosa et al., 2018). This study aims to evaluate the antimicrobial properties of weeds in combating microbial infections.

Plants have evolved various defense mechanisms to protect themselves from pathogens, including the production of secondary metabolites with antimicrobial activities (Newman & Cragg, 2012). These compounds have been found to possess a wide range of biological activities, including antibacterial, antifungal, and antiviral properties (Cushnie & Lamb, 2005). Moreover, several studies have reported the successful isolation and identification of bioactive compounds from various plant species, highlighting the potential of plants as a source of new antimicrobial agents (Balandrin et al., 1985; Rates, 2001).

Weeds, being abundant and diverse, could be a potential source of novel antimicrobial compounds. Previous studies have reported the antimicrobial activities of various weed species against a range of pathogens, including Gram-positive and Gram-negative bacteria, fungi, and viruses (Abu-Darwish et al., 2016; Chanda & Baravalia,

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2011; Saxena et al., 2014). The bioactive compounds identified from weeds belong to various chemical classes, such as alkaloids, flavonoids, terpenoids, and tannins, which have been found to exhibit significant antimicrobial activities (Cowan, 1999; Górniak et al., 2019).

However, the antimicrobial potential of weeds has not been extensively explored, and the majority of studies have focused on individual weed species or a limited number of pathogens (Reigosa et al., 2018). Therefore, a comprehensive evaluation of the antimicrobial properties of weeds, including their effectiveness against a wide range of microbial pathogens, is necessary to identify and characterize potential bioactive compounds. Additionally, understanding the mechanisms of action of these compounds can contribute to the development of new therapeutic strategies for treating infections caused by antibiotic-resistant bacteria (Lavermicocca et al., 2018).

In this study, we aim to evaluate the antimicrobial properties of weeds in combating microbial infections by investigating a panel of weed species for their potential antimicrobial activities against a range of clinically relevant pathogens. Furthermore, we will attempt to isolate and identify the bioactive compounds responsible for the observed antimicrobial activities and elucidate their mechanisms of action. The outcomes of this study could contribute to the discovery of novel antimicrobial agents and provide valuable insights into the potential applications of weeds in the development of new therapeutic strategies for treating microbial infections.

Material and Methods

Plant collection and its extraction: The plants of *Withania somnifera* and *Lantana camara* were collected from the barren fields of ICAR-Indian Agricultural Research Institute, New Delhi. The different plant parts like, leaves, roots, flowers, fruits and stem bark collected were initially rinsed with distilled water to remove soil and other contaminants, shade dried using tray under controlled temperature at 37^0 C for a week.

Extraction of plant material by Soxhlet apparatus: All these parts of plants were powdered using mechanical pulverize and powdered materials were preserved in the sterilized polythene bags until further use. For extraction of crude drugs, 250g of shade dried powdered plant material was weighed and subjected to successive Soxhlet extraction with different solvents such as Petroleum ether, Chloroform, Ethyl acetate, Methanol and Distilled water (Aqueous) in the order of increasing polarity of solvents for a period of 18-22 h. The extracts obtained were concentrated to dryness in evaporating dish at 40^0 C and stored the dried extract at 4^0 C in the refrigerator until further use.

Test microorganisms and preparation of inoculums: The pure axenic cultures of bacteria were procured from the stock culture of Division of Microbiology, ICAR-Indian Agricultural Research Institute, New Delhi and were further maintained on nutrient agar slants at 4^0C until further use. For preparation of inoculum, 48 hours old bacterial culture grown in nutrient broth (Himedia, M002) at 37^0 C and maintained on nutrient agar slants at 4^0 C was used for experimental studies.

Antibacterial activity: The assay was conducted by agar well diffusion method. About 15 to 20 ml of nutrient agar medium was poured in the sterilized Petri dishes and allowed to solidify. Bacterial lawn was prepared using 5 days old culture strain. The bacterial strains were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards (108 CFU/ml). 1 ml of bacterial strain was spread over the medium using a sterilized glass spreader. Using flamed sterile borer, wells of 10 mm diameter were punctured in the culture medium. Required fractions of extracts were added to the wells. The plates thus prepared were left for diffusion of extracts into media for one hour in the refrigerator and then incubated at 370C. After incubation for 18 h, the plates were observed for zones of inhibition. The diameter of zone of inhibition was measured and expressed in

millimeters. Dimethyl sulphoxide (DMSO) was used as a negative control. Streptomycin for bacteria was used as positive control (500µg/ml). The experiments were conducted in triplicates.

Table 1. Growth inhibition of bacteria in different extracts of *W. somnifera.*

Organisms	**Zone of inhibition in mm* in the extracts** (average of three values)**						**Control**
	1	**2**	**3**	**4**	**5**	**6**	
Baccilus anthracis (+)	12	22	14	00	00	00	30
B. pumilis (+)	28	22	16	18	00	24	38
B. subtilis (-)	28	36	29	36	20	20	22
Salmonella paratyphii (-)	28	22	14	14	22	12	28
Staphylococcus albus (+)	00	20	22	18	22	16	22
Vibrio cholerae (+)	28	24	24	20	24	20	22
Xanthomonas compestris (-)	22	22	00	00	18	16	26
X. *malvaacearum* (+)	00	20	16	00	20	12	28

*Diameter of well 10 mm is included. **_Withania somnifera_ **1. Root bark, 2. Root, 3. Stem, 4. Leaves, 5. Flower, 6. Fruit. + is gram-positive bacteria. - is gram-negative bacteria

Table 2. Growth inhibition of bacteria in different extracts of *L. camara*.

Organisms	**Zone of inhibition in mm* in the extracts** (average of three values)**			**Control**
	1	**2**	**3**	
Baccilus anthracis (+)	16	00	00	30
B. pumilis (+)	28	24	30	38
B. subtilis (-)	16	00	00	22
Salmonella paratyphii (-)	24	20	20	28
Staphylococcus albus (+)	16	22	18	22
Vibrio cholerae (+)	24	12	18	22
Xanthomonas compestris (-)	22	22	24	26
X. *malvaacearum* (+)	24	12	14	28

*Diameter of well 10 mm is included, **_Lantana camara._

1. Stem extract, 2. Leaf extract, 3. Flower extract, + is gram positive, - is gram negative.

Results and Discussion

Antimicrobial properties of *Withania somnifcra:*

It was found that out of eight test bacteria, five were strongly inhibited by the root-bark extract. Root bark extract caused a good inhibition against two Bacilli i.e., *B. pumilis* and *B. subtilis.*

This extract also caused a satisfactory inhibition against human pathogen i.e., *Salmonela paratyphii* and *Vibrio cholerae*. While X. *malvacearum., Staphylococcus albus* and *B. anthracis* were not inhibited by root bark extract. *V. cholerae* was severely inhibited by all the extracts, even the inhibition was found more than control. *V. choleree*

and *B. subtilis* were found susceptible to all the extracts while *B. anthracis* was found highly resistant against extracts of leaves, flowers and fruits. Extracts of leaves and stem failed to inhibit the bacterial growth. Similar results were recorded with fruit extract except against *B. pumilis* where comparatively a mild inhibition was observed.

Antimicrobial properties of *Lantana camara*:

Aromatic shrub showed inhibition against all the test organisms, except *B. subtilis* and *B. anthracis* where the inhibition was almost nil. In case of *S. albus,* leaf-extract showed an equal inhibition as that of the control. Though all three extracts showed prominent inhibition against *B. pumilis,* none of them reached upto that of the control. Though *S. paratyphii* was not inhibited up to the extent of control, the results were promising. Specially in case of stem extract it was found comparatively more inhibitory against *V. choleree.* Results of *X. campestris,* against three extracts, were more satisfactory than those of *X. malvacearum.* Amongst tested Baccilli, *B. anthracis* was found to show similar response to extracts of stem, leaves and flowers as were reported by Trivedi *et al.* (1980a) for the extracts of *Orthrosiphon pallidus.* Out of two *Xamhomonas,* X. *malvacearum* was found resistant against leaf extracts of both weeds, their results are also in conformity with those of *O. pallidus* (Trivedi *et al.* 1980a). As far as *Vibrio cholerae* - a severe human pathogen is concerned, stem extracts of both weeds are found strong inhibiting agent. Similar results were observed with extracts of whole plants of *Azolla sp.* and *Salvinia sp.* (Trivedi *et al.* 1980b) and stem extract of O. *pallidus* (Trivedi *et al.* 1980a). *S. paratyphii* another human pathogen is found highly resistant to the stem extract of *W. somnifera,* while a slight susceptibility observed against stem extract of *L. camara.* Contrary to those results, a very strong inhibition was reported by stem extract of *O. pallidus* (Trivedi *et al.* 1980a).

Conclusion

Concisely it can be said that nearly all extracts from *W. somnifera* are controlling the growth of *V. cholerae. B. pumilis* is succeptible to extracts of *L. camara,* especially to flower extract while B. *anthracis* is quite resistant to all the extracts from both weeds.

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