

STUDY OF ANTIMICROBIAL ACTIVITY OF SOME SELECTED WEED SPECIES

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Abstract: A large number of angiosperms and certain pteridophytes exist in the form of weeds, in nature. It is a well known fact that their abundant growth is hazardous to mankind. With the idea, that how they can be rendered useful to human the present study was taken up. Two weeds namely *Withania somnifera* and *Lantana camara* were picked up to determine the antimicrobial properties. The well method was followed to measure the inhibition zone on agar plates. *Vibrio cholerae*, a severe human pathogen was inhibited by the extracts obtained from various organs of two weeds e.g. root bark of *W. somnifera*, and stems of *L. camara*. In some of the tests pathogen, the growth got stimulated e.g., *Bacillus anthracis* against leaf and flower extracts of *L. camara*, and *W. somnifera*.

Introduction

Microorganisms are frequently developing resistance to common drugs and antibiotics and this pose an enormous threat to the treatment of a wide range of serious infections (Taylor *et al.*, 2002; Sibanda and Okoh, 2007). In the present scenario of emergence of drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants. Weeds are also found to be resistant to most of the microbial disease when compared to the crops, which shows disease symptoms (Singh *et al.*, 2019). In all regions of the World, history shows that medicinal plants have always held an important place. Plants that are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, have antimicrobial properties in vitro (Lewis and Ausubel, 2006). There are several reports on the antimicrobial activity of different herbal extracts (Bonjar, 2004; Boer *et al.*, 2005 and Islam *et al.*, 2008). The activity of plant extracts on bacteria and fungi has been studied by a very large number of researchers in different parts of the world (Bhengraj *et al.*, 2008; Vuuren and Naidoo, 2010). Antimicrobial properties of certain Indian medicinal plants were reported based on folklore information (Srinivasan *et al.*, 2001), and a few attempts were made on inhibitory activity against certain pathogenic bacteria.

*Received Jan 23, 2020 * Published Feb 2, 2020 * www.ijset.net*

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 37⁰ 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 40⁰ C and stored the dried extract at 4⁰ 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 4⁰C until further use. For preparation of inoculum, 48 hours old bacterial culture grown in nutrient broth (Himedia, M002) at 37⁰ C and maintained on nutrient agar slants at 4⁰ 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	
<i>Bacillus anthracis</i> (+)	12	22	14	00	00	00	30
<i>B. pumilis</i> (+)	28	22	16	18	00	24	38
<i>B. subtilis</i> (-)	28	36	29	36	20	20	22
<i>Salmonella paratyphii</i> (-)	28	22	14	14	22	12	28
<i>Staphylococcus albus</i> (+)	00	20	22	18	22	16	22
<i>Vibrio cholerae</i> (+)	28	24	24	20	24	20	22
<i>Xanthomonas compestris</i> (-)	22	22	00	00	18	16	26
<i>X. malvaacearum</i> (+)	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	
<i>Bacillus anthracis</i> (+)	16	00	00	30
<i>B. pumilis</i> (+)	28	24	30	38
<i>B. subtilis</i> (-)	16	00	00	22
<i>Salmonella paratyphii</i> (-)	24	20	20	28
<i>Staphylococcus albus</i> (+)	16	22	18	22
<i>Vibrio cholerae</i> (+)	24	12	18	22
<i>Xanthomonas compestris</i> (-)	22	22	24	26
<i>X. malvaacearum</i> (+)	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 somnifera*:

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., *Salmonella*

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. cholerae* 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. cholerae*. 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 *Xanthomonas*, *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 susceptible 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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