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Screening the Antimicrobial activity of some semi-arid zone weed plants against Clinical and Environmental Microbial Pathogens in Great Thar Desert in India

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ABSTRACT

In vitro antibacterial activity of weed plants (*Tribulus terrestris* & *Ageratum conyzoides*) found in semi-arid region of Rajasthan was analysed in this study. Traditional uses for these plants treat specific conditions or diseases. The present study examined the antibacterial activity of two weed plants using the disk diffusion method using Ethanolic, aqueous & Methanolic extract as part of the process of understanding the chemistry, toxicity and efficacy of these plant extracts. Ethanol extracts of the herbs were examined using a standard antimicrobial disk diffusion method. Extracts were tested against both Gram negative (*Escherichia coli, Vibrio cholerae, Salmonella typhi* and *Pseudomonas aeruginosa*) & Gram positive (*Bacillus cereus* & *Staphylococcus aureus*) bacteria. This present pilot study data with *T. terrestris* plant extract resulted in active against *Staphylococcus aureus* and *P. aeruginosa* while *A. conyzoides* plant extract showed good antimicrobial activity against *V. cholerae*. These results served to validate our procedures and indicate the need for the present study. Implications of these results for bioactivity and drug discovery potential of plant extracts can be further explored. This study serves as basis for further research on these weed plants.

Keywords: Antibacterial activity, *T.* terrestris, *A.* conyzoides, Semi-arid region, Disk Diffusion Method.

I. INTRODUCTION

The medicinal use of plants is probably as old as mankind. Plants have continued to be a valuable source of natural products for maintaining human health, as studies on natural therapies have intensified. More than 152,000 plant species have been studied and several of them contain therapeutic substances and the use of plant compounds for pharmaceutical purposes has gradually increased. A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources.[1],[2].

According to the World Health Organization, medicinal plants are probably the best source of a variety of drugs. About 80 % of individuals in developed countries use traditional medicine containing compounds derived from medicinal plants.[3]

India is represented by rich natural biodiversity and it offers a unique opportunity for drug discovery research. A number of traditional natural products have been increased and much work has been done on selected ethno medicinal plants for antibacterial activity against pathogenic strains of bacteria in the last decades.[4]

Nowadays, an increasing number of infectious agents are becoming more resistant to commercial antimicrobial compounds.[5] The necessity to develop new drugs requires varied strategies, among them, the bioprospection of secondary metabolites produced by medicinal plants.[6] Thus, the phytomedicines derived from plants have shown great promise in the treatment of various infectious diseases.[7] Here, we investigated the antimicrobial activity of *Azeratum conyzoides and Tribulum terrestris* valuable weed plants that found in northern part of great thar dessert of India, against

clinical and environmental microbial pathogens of Gram negative and Gram positive bacterial strains.

II. METHODS AND MATERIAL

Collection of plant materials - *Ageratum conyzoides and Tribulus terrestris* two weed plants collected from agricultural field of northern part of Rajasthan state, encompassing the Asteraceae and Zygophyllaceae families were utilized and transferred in department for further searching to microbiological activity. In research laboratory all plant materials were thoroughly washed using water, dried and carefully separated into leaves, stems and roots with suitable equipment. The dried plant materials were each macerated and ground into a fine powder, using clean, dry electric blender.

Preparation of extract; the powder materials were extracted with two organic solvents methanol (50%), ethanol (50%) and one aqueous (water) extraction respectively. 30g of the accurately weighed each parts of plant powder was put in the Soxhlet thimble made by Whatman filter paper No. 1 and 300ml of the each solvents (distilled water, ethanol & methanol) in a round bottomed flask in soxhlet flask. After this samples were allowed for extraction at 20-30^{0}C. Following this the extracts were concentrated under pressure using rotary vacuum evaporator. The concentrated extract was weighed and labeled appropriately. All residues were kept in tightly stoppered bottle until used for the anti-microbial tests.

Bacterial strains and antibiotics-The antimicrobial activity was assayed utilizing two groups of well-known microorganisms. One group of MTCC gram negative, pathogenic strains: *Escherichia coli* 1692; *Vibrio cholerae* 3906; *Salmonella typhi* 0733; *Pseudomonas aeruginosa* 4676; other the gram positive *Bacillus cereus* 1272; *and Staphylococcus aureus* 7443. The microorganisms were maintained in nutrient agar at 4°C until the assays were carried out.Different (Himedia) antibacterial antibiotics (Streptomycin, Ampicillin) were used in work.

Antimicrobial Susceptibility Test

The antimicrobial screening of the bio extract were carried out by determining the zone of inhibition using disc diffusion method.[8] The sterilized Mueller-Hinton agar (MHA) plates were prepared and labeled appropriately with the name of the bacterial strains and the weed plant extracts. Using sterile forceps, sterile 6mm discs (cut from Whatman No. 1 filter paper with a paper punch device and sterilized before use) were picked and submerged in each of the graded concentrations of weed plant extracts namely; leaf, stem and root of *Ageratum conyzoides and Tribulus terrestris*. Overnight bacterial culture 1X10^{-6} CFU/mL viable count inoculums were used and applying the bio extract impregnated discs (6mm). Commercially prepared antibiotic (Himedia) disc were used as a positive control and discs soaked in distilled water as a negative control in each agar plate. The plates were allowed to stand for 30minutes and then incubated at 37°C for 24 hours. Antimicrobial activity of each extract against the test organisms were indicated by a growth-free zone around the respective discs and the diameters of the zones of inhibition to the nearest millimeter with a ruler were obtained by measuring the distance from one end of the inhibition zone, across the disc to the other end, as reported by Nwanebu et al.[9]

III. RESULT AND DISCUSSION

From this study following results were interpreted as according to following headings

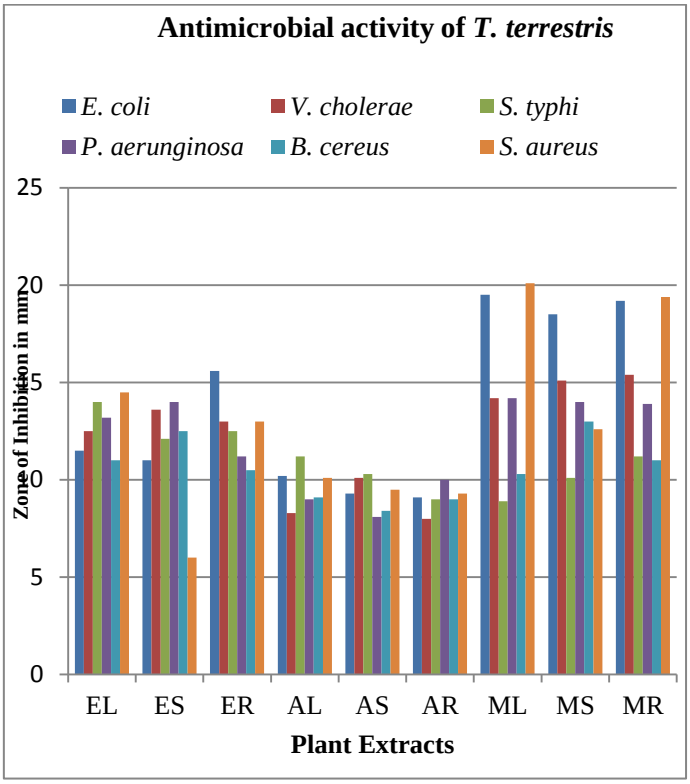
Antimicrobial activity of *Tribulus terrestris*

In the respect of ethanol extract maximum inhibition zone (15.6 mm) was observed in root extract against *Escherichia coli* while minimum (6.0 mm) was observed in stem extract against *Staphylococcus aureus.* The Yemeni *T. terrestris* had no detectable anti-bacterial activity against any of the reference bacteria[10] and all parts (fruits, stems plus leaves and roots) of Turkish *T. terrestris* showed activity against all reference bacteria,[11] but only fruit and leaf of Indian *T. terrestris* were active against exclusively *E. coli* and *S. aureus*.[12] In the present investigation, the most susceptible bacterium was *S. aureus* and *P. aeruginosa*. Present results were agreed with observations in which Methanolic leaf extracts of *Coccinia grandis* showed inhibitory effect on *S. aureus, P. aeruginosa, E. coli* and *K. pneumoniae*.[13],[14] In the respect of aqueous extract maximum inhibition zone (11.2 mm) was observed in

leaf extract against *Salmonella* typhi while minimum (8 mm) was observed in root extract against *Vibrio cholerae*. The potential antibacterial effects of the Methanolic extract of few plants are reported by Tanaka et al.[15],[16] In Methanolic extract maximum inhibition zone (20.1 mm) was observed in leaf extract against *Staphylococcus aureus* and minimum (8.9 mm) against was observed in leaf extract against *Salmonella typhi*. The activity of the *T. terrestris* plant against both gram-positive and gram-negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins in the plant. In this study, fluctuating trends of inhibition zone was found to be appear in certain extracts used media contained against some pathogens. Similar fluctuation trends of inhibition zone was reported by Kunjal Bhatt et al.[17],[18]

Table 1-Showing Antimicrobial activity of *T. terrestris* using various solvents

Micro-organisms	Ethanolic Extract			Aqueous Extract			Methanolic Extract		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
E. coli	11.5±.88	11±.90	15.6±.57	10.2±.88	9.3±.90	9.1±.57	19.5±0.25	18.5±0.52	19.2±0.49
V. cholerae	12.5±0.47	13.6±0.36	13±0.38	8.3±0.12	10.1±0.26	8±0.59	14.2±0.88	15.1±0.59	15.4±0.60
S. typhi	14±1.22	12.1±.66	12.5±1.15	11.2±1.22	10.3±.66	9±1.15	8.9±0.68	10.1±1.15	11.2±0.29
P. aeruginosa	13.2±0.25	14±0.29	11.2±0.99	9±0.26	8.1±0.49	10±0.97	14.2±0.88	14±0.38	13.9±0.52
B. cereus	11.0±.33	12.5±.95	10.5±.66	9.1±.33	8.4±.95	9.0±.66	10.3±0.49	13±1.40	11±0.97
S. aureus	14.5±.33	6±.91	13±1.45	10.1±.33	9.5±.91	9.3±1.45	20.1±0.77	12.6±0.18	19.4±1.12



EL-Ethanolic Leaf, ES-Ethanolic Stem, ES-Ethanolic Root, AL-Aqueous Leaf, AS-Aqueous Stem, AR-Aqueous Root, ML-Methanolic Leaf, MS-Methanolic Stem, MS-Methanolic Root Extract

Antimicrobial Activity of *Ageratum Conyzoides*

In the respect of ethanol extract maximum inhibition zone (14 mm) was observed in root extract against *V. cholerae* and leaf extract against *Bacillus cereus* while minimum (9 mm) inhibition zone was observed in root extract against *E. coli*. *S. typhi* showed no inhibition in this extract. The whole extract (mixture of volatile and nonvolatile extract) of *Piper betle* Linn. could inhibit *S. aureus*.[19] Okwulehie et al observed that Ethanolic extract of this plant showed inhibition zone (7mm, 6mm, 4.5mm & 5mm) against *S. aureus, E. coli, P. aeruginosa* & *S. typhi*.[20] In the respect of aqueous extract maximum inhibition zone (12.2 mm) was observed in root extract against *S. aureus* while minimum (1.2 mm) was observed in leaf extract against *S. typhi*. Water extract showed too less inhibition against *S. typhi* rather than other microorganisms. The aqueous extract was not found to be too active against all organisms tested. In the respect of methanol extract maximum inhibition zone (16.5 mm) was observed in leaf extract against *Vibrio cholerae* while minimum (5.6 mm) inhibition zone was

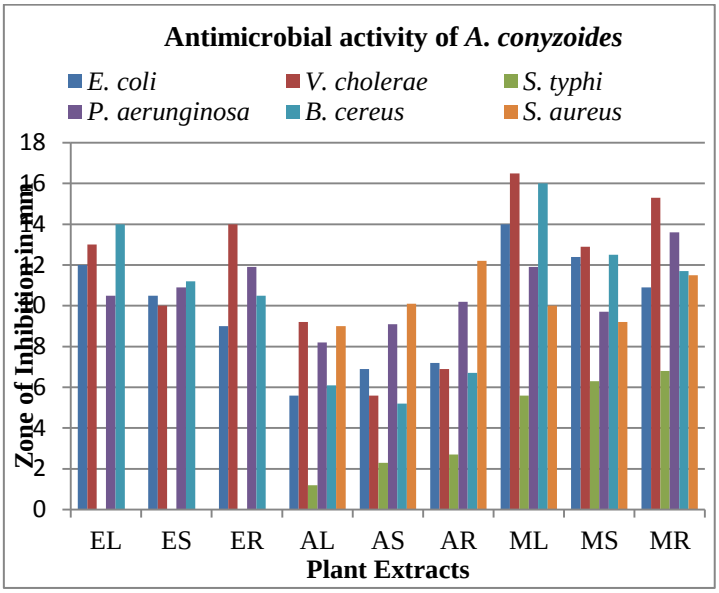
observed in leaf extract against *S. typhi*. The studies by Osho and Adetunji have reported antibacterial activity of essential oils of leaf extracts of *A. conyzoides*.[21]

Antimicrobial Activity Against Standard Drugs

In standard antibiotics maximum and minimum zone of inhibition (33.3 mm) and (13.3) were observed against *Staphylococcus aureus* & *Vibrio cholerae*. In both the cases *P. aeruginosa* showed no inhibition against these drugs.

Table 2 - Showing Antimicrobial activity of *A. conyzoides* using various solvents

Micro-organisms	Ethanolic Extract			Aqueous Extract			Methanolic Extract		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
E. coli	12 ±0.24	10.5± 0.35	9± 0.67	5.6± 0.36	6.9 ±0.47	7.2 ±0.68	14 ±0.33	12.4± 0.85	10.9± 0.96
V. cholerae	13 ±0.18	10 ±0.58	14 ±0.49	9.2± 0.12	5.6 ±0.29	6.9 ±0.86	16.5± 0.91	12.9± 1.21	15.3± 0.22
S. typhi	------	------	------	1.2± 0.43	2.3 ±0.75	2.7 ±0.89	5.6 ±0.35	6.3 ±0.66	6.8 ±0.72
P. aeruginosa	10.5± 0.53	10.9± 0.68	11.9± 0.88	8.2± 0.24	9.1 ±0.41	10.2± 0.29	11.9± 0.36	9.7 ±0.47	13.6± 0.55
B. cereus	14 ±0.36	11.2± 0.60	10.5± 0.34	6.1± 1.16	5.2 ±0.09	6.7 ±0.17	16 ±0.96	12.5± 0.78	11.7± 1.09
S. aureus	------	------	------	9± 0.34	10.1± 0.54	12.2± 0.32	10 ±0.96	9.2 ±0.38	11.5± 1.24

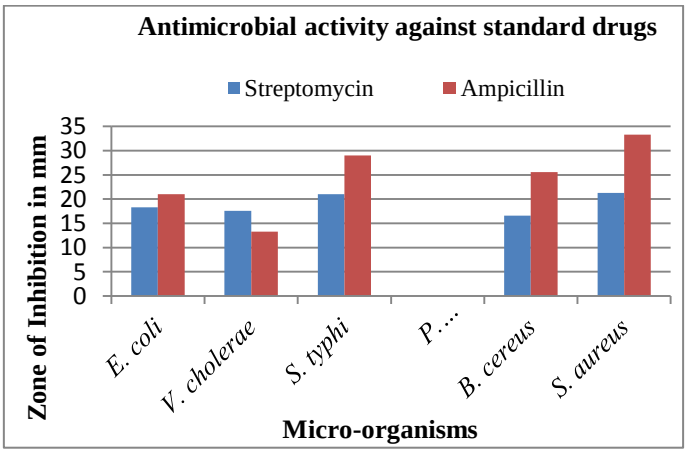


EL-Ethanolic Leaf, ES-Ethanolic Stem, ES-Ethanolic Root, AL-Aqueous Leaf, AS-Aqueous Stem, AR-Aqueous Root, ML-Methanolic Leaf, MS-Methanolic Stem, MS-Methanolic Root Extract

Table 3-Showing Antimicrobial activity of Micro-organisms against standard drugs

Micro-organisms	Streptomycin (in mm)	Ampicillin (in mm)
E. coli	18.3±.95	21±.52
V. cholerae	17.6±.56	13.3±.57
S. typhi	21±1.55	29±.90
P. aerunginosa	-------	------
B. cereus	16.6±.38	25.6±1.20
S. aureus	21.3±.92	33.3±.66

Result as per shown in Mean±S.E; -------- No inhibition



IV. CONCLUSION

The present study reveals the importance of selected weed plants (*Tribulus terrestris* & *Ageratum conyzoides*) in controlling bacterial strains. Among different solvents (ethanol, water and methanol) used for the study revealed that the Methanolic extract of the plants has maximum inhibitory effect on pathogens. The implication of the broad spectrum action of some of these extracts is that they can be useful in antiseptic and disinfectant formulation as well as in chemotherapy if the active principle can be isolated. The anti-bacterial activities of some of the effective extracts of these plants can be further explored. The differentiating activities of the extracts against variety of micro-organisms encouraged to develop novel broad spectrum antimicrobial product formulations with these extracts. So these plants could be potential medicine for many diseases. It is necessary to carry out screening of these plants in order to reveal the active principles by isolation and characterization of their antimicrobial constituents.

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