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Antibacterial Activity of an Isolated Compound (AC-1) from the Leaves of *Ageratum conyzoides* Linn.

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Antibacterial activity of an isolated compound (AC-1) from the leaves of *Ageratum conyzoides* Linn. was evaluated against four Gram-negative bacteria like *Escherichia coli, Shigella dysenteriae, Pseudomonas aeruginosa* and *Salmonella typhi* as well as four Gram - positive bacteria viz. *Bacilliu subtilis, Bacilus megaterium, Staphylococcus aureus* and *Streptococcus pyogenes.* Disc diffusion technique was used for *in vitro* antibacterial screening. Result showed that compound AC-1 had large zone of inhibition in disc diffusion against the said bacteria. Antibacterial activity was more in Gram - positive bacteria than Gram - negative bacteria. Highest activity was noted against *Staphylococcus aureus* and lowest was found for *Salmonella typhi.* The MIC (minimum inhibitory concentration) values of AC-1 against the bacteria ranged from 8 – 32 microgram/mL. Results, thus, suggests that the compound (AC-1) isolated from the leaves of *Ageratum conyzoides* Linn. had good anti bacterial activity against the tested bacteria.

Keyword: Antibacterial activity, *Ageratum conyzoides* Linn. Disc diffusion technique, Zone of inhibition, Minimum inhibitory concentration.

1. Introduction

Multiple antibiotic resistance in bacterial populations is a pervasive and growing clinical problem, which is recognized as a threat to public health. Various bacteria like *Staphylococcus aureus, Pseudomonas aeruginosa* etc. are inherently resistant to many antimicrobial agents, mainly due to the energy between multi-drug efflux system or a type1 AmpC beta lactamase and low outer membrane permeability[1-5] . There is thus continuous effort for synthesis of new chemicals having antimicrobial activity[6-8] . But most of these chemicals are potentially toxic and are not free of side effects on the host[9] .This has urged microbiologist for formulation of new antimicrobial agents and evaluation of the efficacy of natural plant products as the substitute for chemical antimicrobial agents[10].

Several plants were screened to know their antimicrobial property[11-18] . We also examined various plants of North - East Himalayas to know their anti microbial property and noted that leaves of *Ageratum conyzoides* Linn. had anti bacterial activity against *Bacilliu subtilis, Bacilus megaterium* etc. Adopting solvent extraction and chromatographic techniques a compound (AC-1) was isolated from the leaves of *Ageratum conyzoides* Linn.[19]. Antibacterial activity of AC-1 was studied against few Gram-positive and Gram-negative bacteria. In this communication

results of the experiments are being reported.

2. Materials and Methods

2.1 Plant Material:

Leaves of *Ageratum conyzoides* Linn.were collected from the medicinal plants garden of the University of North Bengal and authenticated by the experts of the department of Botany of the said University. A voucher specimen was kept in the department for future reference. Leaves were sundried and powdered. The powder was used as the test drug.

2.2 Isolation of AC-1 from the leaves of *Ageratum conyzoides* Linn:

Process of Isolation of AC-1 from *Ageratum conyzoides* Linn was reported in somewhere else [19]. In short, sundried powdered leaves of *Ageratum conyzoides* Linn was extracted with chloroform. Supernatant thus obtained was refluxed with hydrochloric acid. Solution was evaporated to dryness. The dry mass was extracted with ethyl acetate and the solution was chromatographed using silica gel G as adsorbent. The eluant was again chromatographed using polyamide as adsorbent. Fractions were repeatedly crystallized with n-butanol, ethyl acetate mixture when one fraction yielded crystal (AC-1).

2.3 Bacteria:

Four Gram - positive bacteria *viz. Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus* and *Streptococcus pyogenes* and four Gram-negative bacteria *viz. Escherichia coli, Shigella dysenteriae, Pseudomonas aeruginosa* and *Salmonella typhi* were employed to determine antibacterial activity and minimum inhibitory concentration. All these bacteria were collected from the department of Microbiology, North Bengal Medical College Hospital.

2.4 Media:

Nutrient agar media (Difco laboratories) pH 7.2 and nutrient broth media (Difco laboratories) pH 6.8 were used for antibacterial screening and minimum inhibitory concentration determination.

2.5 Antibacterial screening:

In vitro antibacterial screening was carried out by disc diffusion method [20]. According to this method , 20 ml quantities of nutrient agar were placed in a petri dish with 0.1 ml of 10^{-2} dilution of bacterial culture of 20 hours old. Filter paper discs (6 mm diameter) impregnated with 60 µg per disc and 120 µg per disc concentration of the solution prepared from AC-1, isolated compound from *Ageratum conyzoides* Linn., were placed on test bacteria-seeded plates. Blank disc impregnated with water was used as negative control. Zone of inhibition was recorded after 18 hours of incubation. Diameters of zone of inhibition produced by AC-1 were compared with that of standard antibiotic kanamycin 40 µg per disc. Each sample was used for five times for determination of anti bacterial activity.

2.6 Minimum inhibitory concentration (MIC) determination:

Minimum inhibitory concentration is defined as the lowest concentration of antibiotic completely inhibiting visible growth of bacteria after 18 – 24 hours of incubation at 37 ^{0}C. This was done by the method of [21]. According to this method, 1.0 mg of AC-1, the compound isolated from *Ageratum conyzoides* Linn. was dissolved in 2 ml nutrient broth media to obtain a stock solution of concentration 500 µg/ml. 3 drops of Tween 80 was added in nutrient broth to facilitate dissolution. Serial dilution technique was followed to obtain 250 µg/ml concentration of the compound. One drop

(0.02ml) of prepared suspensions of organism (10^6organism/ml) was added to each broth dilution. These dilutions were then incubated for 20 hours at 37^0C. Growth of bacteria was examined by noting turbidity of the solution. The nutrient broth media with 3 drops of Tween 80 was used as negative control while kanamycin was used as positive control.

2.7 Statistical Analysis:

The values were expressed as mean ± SEM and were analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS). Differences between means were tested employing Duncan's multiple comparison test and significance was set at $p < 0.05$.

3. Results and Discussion

In vitro antibacterial activity of AC-1, the compound isolated from *Ageratum conyzoides* Linn. and kanamycin is given in Table – 1. Results showed that AC-1 exerted anti bacterial activity both at 60 µg per disc and at 120 µg per disc concentrations for all the tested bacteria
which were comparable to that of reference drug kanamycin at 40 µg per disc concentration.
Large zone of inhibition in disc diffusion was found out. Antibacterial activity was more in Gram - positive bacteria than Gram - negative bacteria. Highest activity was noted against *Staphylococcus aureus* and lowest activity was found for *Salmonella typhi*.
Table – 2 indicates results of minimum inhibitory concentration of AC-1, the compound isolated from *Ageratum conyzoides* Linn, and kanamycin. The MIC (minimum inhibitory concentration) values of AC-1 against Gram-positive and Gram-negative bacteria ranged from 8 to 16 and 16 to 32 microgram/mL respectively. MIC with kanamycin, however, came 2 to 8 for Gram-positive bacteria and 4 to 16 for Gram –negative bacteria.

Table 1: *In vitro* antibacterial activity of AC-1, the compound isolated from the leaves of *Ageratum conyzoides* Linn. and kanamycin
[Zone of inhibition (diameter in mm)]

Bacteria	Strain	AC-1 (60 µg per disc)	AC-1 (120 µg per disc)	Kanamycin (40 µg per disc)
Gram – positive				
Bacillus subtilis	ATCC 19659	24 ± 0.9	32 ± 0.8	36 ± 1.1
Bacillus megaterium	NBMC 1122	20 ± 0.8	28 ± 1.5	34 ± 1.2
Staphylococcus aureus	ATCC 25923	28 ± 1.1	36 ± 1.7	38 ± 1.3
Streptococcus pyogenes	NBMC 1321	22 ± 0.7	30 ± 0.9	33 ± 0.8
Gram – negative				
Escherichia coli	ATCC 25922	21 ± 0.8	28 ± 1.1	29 ± 1.0
Shigella dysenteriae	NBMC 1127	19 ± 1.0	26 ± 1.2	30 ± 1.2
Pseudomonas aeruginosa	NBMC 1243	20 ± 1.2	27 ± 1.0	31 ± 1.4

Salmonella typhi	MTCC 733	17 ± o.8	24 ± 0.8	32 ± 1.0

Data was in mean SEM (n = 5). Control was made with water. It had no zone of inhibition. So data has not been shown.

Ageratum conyzoides Linn.(family, asteraceae) is a medicinal plant, distributed throughout India, lower and middle hill in Sikkim and Darjeeling up to 6000 ft. The plant has erect hairy annual 30 – 90 cm high leaves. Different vernacular names are given to the plant. In Nepali the plant is called as 'Elame'; in Lepcha 'Namyew' and in English the plant is known as 'Goat weed'. Flowering time of the plant is throughout the year. Purple white flower appears. Leaves, root, stem and flower of *Ageratum conyzoides* Linn. have medicinal use. Leaves are styptic effective in healing of wounds, used in boils and prevent tetanus. Leaf juice is also used as eye lotion. The root juice has antibiotic property. The plant is boiled with oil and applied externally in rheumatism. Phenol, essential oil, friedolin, sitosterol, stigmasterol and unidentified esters are active components of *Ageratum conyzoides* Linn[22-25].

Recently we have noticed anti bacterial activity of leaves of *Ageratum conyzoides* Linn. As leaves of *Ageratum conyzoides* Linn. are widely used in folk medicine in Sikkim and adjoining area we tried to isolate the active compound(s) from the leaves of *Ageratum conyzoides* Linn. responsible for anti bacterial activity. A compound (AC-1) has been isolated from the leaves of *Ageratum conyzoides* Linn.

Table 2: Minimum inhibitory concentration of AC-1, the compound isolated from the leaves of *Ageratum conyzoides* Linn. and kanamycin

Bacteria	**AC-1` (microgram/mL)**	**MIC values of kanamycin (microgram/mL)**
Gram – positive		
	16	2
Bacillus subtilis	16	4
Bacillus megaterium	8	8
Staphylococcus aureus	16	8
Streptococcus pyogenes		
Gram – negative	16	8
	16	4
Escherichia coli	16	16
Shigella dysenteriae	32	8
Pseudomonas aeruginosa		
Salmonella typhi		

Negative control containing water had no MIC value. Thus, it has not been shown

Antibacterial property of AC-1 was evaluated against four Gram – positive and four Gram – negative bacteria. Anti bacterial activity was measured by noting zone of inhibition in disc diffusion. Minimum inhibitory concentration was also noted. Standard antibiotic kanamycin was kept as control drug. It was found out that AC-1 isolated from the leaves of *Ageratum conyzoides* Linn. exerted antibacterial activity against the tested bacteria. Maximum activity was found in case of

Staphylococcus aureus while minimum activity was noted for *Salmonella typhi*. The results were comparable to that of standard antibiotic kanamycin. We are now interested to note the mechanism of anti bacterial property of AC-1. Work is now in progress.

4. Conclusion

Anti bacterial activity of AC-1, a compound isolated from the leaves of *Ageratum conyzoides* Linn., was examined against four Gram-positive and four Gram-negative bacteria. Kanamycin was employed as control drug. AC-1 showed anti bacterial activity against all the tested bacteria. Maximum activity was found against *Staphylococcus aureus* and minimum activity was noted for *Salmonella typhi*. Results were comparable to that of kanamycin. Compound AC-1. thus provides a scientific rationale for use as anti bacterial drug..

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