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Research Article

Antibacterial Activity of an Isolated Compound from *Ageratum conyzoides* L. Leaves

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Received: December 06, 2018; **Published:** May 04, 2019

Abstract

Aageratum conyzoides Linn (*A. conyzoides* L.) is a medicinal plant. Leaves of the plant have several pharmacological properties including antibacterial activity. Recently we have isolated a compound from *A. conyzoides* L. leaves. Antibacterial property of the isolated compound was checked against four Gram-positive bacteria *(Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus* and *Streptococcus pyogenes)* as well as four Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Shigella dysenteriae* and *Salmonella typhi).* Disc diffusion technique was used for *in vitro* antibacterial screening. Result showed that the isolated compound exerted antibacterial activity against all the bacteria used. Antibacterial activity was more in Gram-positive bacteria than in Gram-negative bacteria. Highest activity was noted against *Bacillus subtilis* and lowest activity was found for *Shigella dysenteriae*. MIC (minimum inhibitory concentration) values of the isolated compound against the bacteria ranged from 4 - 64 microgram/ml. Results, therefore, suggest that the isolated compound is responsible for antibacterial activity of the plant leaves.

Keywords: *Aageratum conyzoides L.; Isolation of a Compound; Antibacterial Property; Disc Diffusion Technique; Zone of Inhibition; Minimum Inhibitory Concentration*

Introduction

A. conyzoides L. (family, Asteraceae) is a plant that thrives in any garden soil and grows commonly in the proximity of habitation, in waste places and on ruined sites [1]. The plant is distributed throughout India, lower and middle hill in Sikkim and Darjeeling up to 6000 ft. *A. conyzoides* L. 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'. Throughout the year the plant gives flower. Purple white flower appears.

A. conyzoides L. is a medicinal plant. The medicinal value of this plant in treatment of a large number of human ailments is mentioned in Ayurveda, Charaka Samhita and Sushruta Samhita [2]. Leaves, root, stem and flower of *A. conyzoides* L. are widely used in traditional medicine. 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.

Phytochemical screening showed that *A. conyzoides* L. contains glycosides, flavonoids, tannin, saponin, resins, alkaloids, ascorbic acid etc. It also contains many different compounds like hexamethoxy flavone, coumarin compounds such as lycopsamine caffeic acid,

chromane, pyrrolizidine alkaloids, fumaric acid, kaempferol, quercetin, stigma-7-en-3-ol, scutellarein, benzopyrone, lycopsamine, disifropirrrolizidinic acid etc. Phenol, sitosterol, stigmasterol, essential oil, friedolin and unidentified esters are active components of *A. conyzoides* L [3].

Modern researchers claimed that *A. conyzoides* L. has antibacterial [4] and wound healing effect [5]. It has neurological activity [6] and possesses gastro protective property [7]. The plant acts as analgesic [8] and has effect on circulation [9]. It gives protection against gamma radiation [10]. The plant has antitumor activity [11] and has allopathic effects [12]. Ita., *et al.* (2009) demonstrated hepato protective activity of this plant [13]. The plant is also known for its anti-oxidant activity [14].

Recently we have isolated a compound from leaves of *A. conyzoides* L. In present communication we report method of isolation of the compound and results of studies on anti-bacterial activity of the isolated compound against four Gram-positive and four Gram- negative bacteria.

Materials and Methods

Collection of plant material

A. conyzoides L. leaves were collected in morning hours (9 - 10 AM) from the medicinal plants garden of the University of North Bengal, Siliguri ($26^0 41' 30.9984''$ N, $88^0 27' 4.5756''$ E, elevation, 410 ft), Dist. Darjeeling, West Bengal, India randomly during the months of July - August 2017. Leaves were authenticated by the experts of the department of Botany of the said University. A voucher specimen was kept in the Department of Medical Biotechnology, SMIMS, Sikkim Manipal University, Sikkim, India for future reference.

Figure 1: *Ageratum conyzoides L.*

Preparation of leaves for isolation work

Leaves of *A. conyzoides* L. were shed dried and powdered. This powder was used for isolation work.

Isolation work

This was done by the following scheme. Principles of standard isolation procedures of chemical compounds from plant sources were followed [15].

Chemicals

Chemicals required for the study were purchased from Loba Chem. Lab, Himedia Lab, India and from Merck, Germany.

Bacteria

Four Gram-positive bacteria *viz. Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus* and *Streptococcus pyogenes* and four Gram-negative bacteria *viz. Shigella dysenteriae, Escherichia coli, 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, Siliguri, West Bengal.

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.

Antibacterial screening

In vitro antibacterial screening was carried out by disc diffusion method [16]. 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 40 µg per disc concentration of the compound isolated from *A. conyzoides* L. leaves 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 the solution prepared from *A. conyzoides* L. were compared with that of standard antibiotic kanamycin 40 µg per disc, Each sample was used for five times for the determination of antibacterial activity.

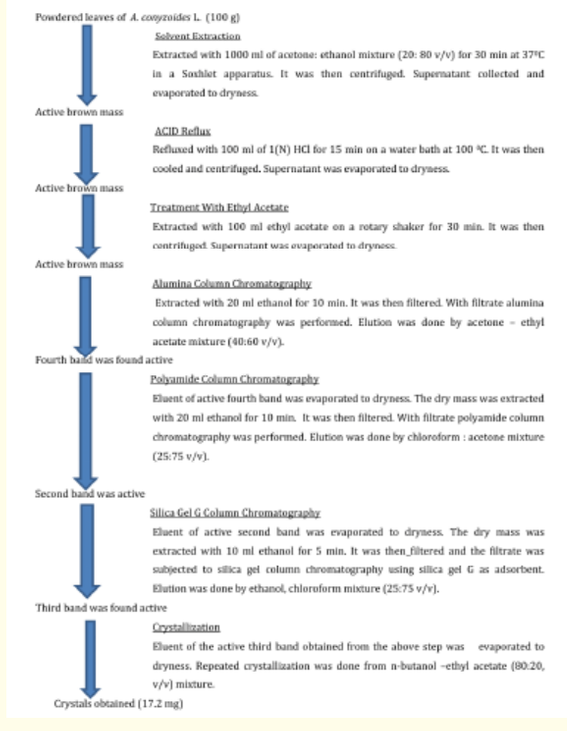
Minimum inhibitory concentration (MIC) determination

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°C. This was done by the method of Mosaddik and Haque [17]. According to this method, compound isolated from *A. conyzoides* L. (1.0 mg) 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 (0.02 ml) of prepared suspensions of organism (10^6 organism/ml) was added to each broth dilution. These dilutions were then incubated for 20 hours at 37°C. 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.

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) 20th versions. Differences between means were tested employing Duncan's multiple comparison test and significance was set at $p < 0.05$.

Diagrammatic scheme for isolation of a compound from leaves of *A. conyzoides* L.



Results

Isolation of compound

A compound was isolated from the leaves of *A. conyzoides* L.

Anti-bacterial activity of the isolated compound

Table 1 shows results of disc diffusion method. Results indicated that the isolated compound *from A. conyzoides* L. leaves at 40 µg per disc concentrations exerted anti-bacterial activity against all four Gram positive bacteria viz. *Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus* and *Streptococcus pyogenes* and all four Gram-negative bacteria *viz*. *Shigella dysenteriae, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi.* Activity was comparable to that of reference drug kanamycin in 40 µg per disc concentration. Antibacterial activity of the isolated compound was more in Gram-positive bacteria than in Gram-negative bacteria. Highest activity was noted against *Bacillus subtilis* for Gram positive bacteria and *Pseudomonas aeruginosa* for Gram-negative bacteria. Lowest anti-bacterial activity of the isolated compound, however, was noted against *Streptococcus pyogenes* and *Shigella dysenteriae* for Gram positive and Gram-negative bacteria respectively.

Bacteria	Strain	Compound isolated from *A. conyzoides* L. leaves (40 µg per disc)	Kanamycin (40 µg per disc)
Gram-positive			
Bacillus subtilis	ATCC 19659	34 ± 1.1	36 ± 1.2
Bacillus megaterium	NBMC 1122	30 ± 1.0	28 ± 1.0
Staphylococcus aureus	ATCC 25923	28 ± 0.9	26 ± 0.7
Streptococcus pyogenes	NBMC 1321	26 ± 0.6	24 ± 0.5
Gram - negative			
Escherichia coli	ATCC 25922	24 ± 0.6	27 ± 0.6
Shigella dysenteriae	NBMC 1127	16 ± 0.4	20 ± 0.7
Pseudomonas aeruginosa	NBMC 1243	25 ± 0.7	30 ± 0.8
Salmonella typhi	MTCC 733	23 ± 0.5	27 ± 1.0

Table 1: *In vitro antibacterial activity of the isolated compound from A. conyzoides L. leaves and kanamycin [Zone of inhibition (diameter in mm)] against Gram positive and Gram negative bacteria.*

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

Table 2 indicates results of minimum inhibitory concentration of the isolated compound from *A. conyzoides* L. leaves and kanamycin against the said Gram positive and Gram negative bacteria. The MIC (Minimum Inhibitory Concentration) of the isolated compound ranged from 4 to 16 for Gram positive bacteria and 8 to 64 microgram/mL for Gram negative bacteria respectively. For kanamycin MIC value for Gram positive bacteria was found to vary between 2 and 8 and for Gram negative bacteria it came between 4 and 16.

Bacteria	MIC values of the compound isolated from *A. conyzoides* L. leaves (microgram/mL)	MIC values of kanamycin (microgram/mL)
Gram - positive		
Bacillus subtilis	4	2
Bacillus megaterium	8	4
Staphylococcus aureus	16	8
Streptococcus pyogenes	16	8
Gram-negative		
Escherichia coli	8	4
Shigella dysenteriae	64	16
Pseudomonas aeruginosa	16	8
Salmonella typhi	16	8

Table 2: *Minimum inhibitory concentration of the isolated compound from A. conyzoides L. leaves and kanamycin against Gram positive and Gram negative bacteria.*

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

Discussion

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 type 1 AmpC beta lactamase and low outer membrane permeability [18]. Therefore, there is continuous effort for synthesis of new chemicals having antimicrobial activity [19]. But most of these chemicals are potentially toxic and are not free from side effects on the host [20]. 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 [21].

In this context numerous plants were screened to know their antimicrobial property and several plants were identified possessing antibacterial activity. Few of them are *Terminalia bellerica* (Family, Combretaceae), *Tinospora cordifolia* (Family, Menisoermaceae), *Woodfordia fruticosa* Kurz. (family, Lythraceae), *Bergenia ligulata* Wall (Family, Saxifragaceae), *Mangifera indica* (Family, Anacardiaceae), *Bombax ceiba* (Family, Bombacaceae), *Kalanchoe pinnata* (Family, Crassulaceae), *Abutilon indicum* (Family, Malvaceae), *Alangium salvifolium* (Family, Alangiaceae.), *Allium sativum* (Family, Alliaceae), *Emblica officinalis (*Family, Euphorbiaceae), *Ficus benghalensis* (Family, Moraceae), *Glycyrrhiza glabra (*Family, Leguminoceae*), Mangifera indica* (Anacardiaceae), *Mimosa pudica* (Family, Mimosaceae), *Syzygium cumini* (Family, Myrtaceae), *Carica papaya* (Family, Caricaceae), *Centella asiatica* (Family, Apiaceae), *Coriandrum sativum* (Family, Apiaceae), *Curcuma longa* (Family, Zingiberaceae), *Morinda citrifolia* (Family, Rubiaceae), *Ricinus communis* (Family, Euphorbiaceae), *Sida cordifollia* (Family, Malvaceae) etc [22].

Isolation studies on antimicrobial compounds from plants which may act as the substitute for chemical antimicrobial agents were undertaken by several workers. Freiburghaus., *et al.* isolated berberine from *Hydrastis Canadensis* [23]. Trichorabdal A was isolated from the medicinal plant *Rabdosia trichocarpa* [24]. Vohora., *et al.* isolated allicin from *Allium cepa* [25]. Totarol was isolated from the plant *Podocarpus nagi* [26]. Bose isolated anthemic acid from the medicinal plant *Matricaria chamomilla* [27]. All these isolated compounds exerted anti-bacterial activity against several Gram positive and Gram negative bacteria.

In the present study we have isolated a compound from *A. conyzoides* L. and noted its antibacterial activity against four Gram positive and four Gram negative bacteria. Antibacterial activity was more in Gram-positive bacteria than in Gram-negative bacteria. Highest activity was noted against *Bacillus subtilis* and lowest activity was found for *Shigella dysenteriae* (Figures 2 and 3). The compound now needs characterization. Work in this direction is presently in progress in our laboratory.

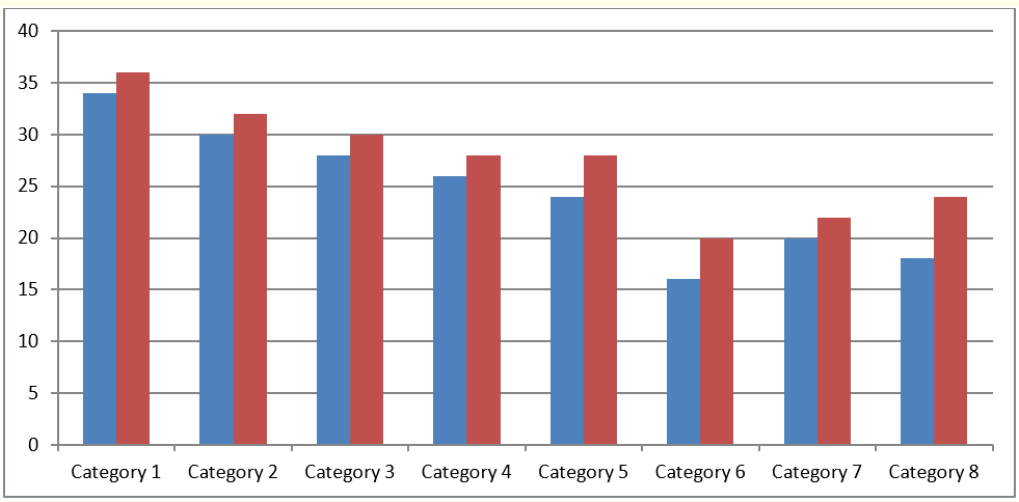


Figure 2: *In vitro antibacterial activity of the isolated compound from A. conyzoides L. leaves and kanamycin [Zone of inhibition (diameter in mm)] against Gram positive and Gram negative bacteria.*

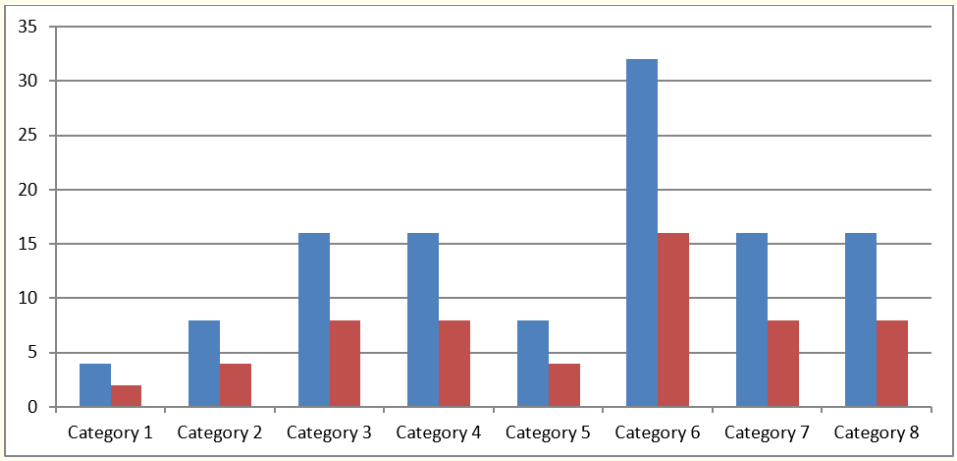


Figure 3: *Minimum inhibitory concentration of the isolated compound from A. conyzoides L. leaves and kanamycin against Gram positive and Gram negative bacteria.*

Category 1: Bacillus subtilis Category, 2: Bacillus megaterium Category, 3: Staphylococcus aureus Category, 4: Streptococcus pyogenes Category, 5: Escherichia coli Category, 6: Shigella dysenteriae Category, 7: Pseudomonas aeruginosa Category, 8: Salmonella typhi.

■ *Isolated compound* ■ *Kanamycin*

Conclusion

In the present study the compound isolated from the leaves of *A. conyzoides* L. showed anti-bacterial activity against several bacteria. The antibacterial activity was found comparable to that of kanamycin, a standard antibiotic. The isolated compound, therefore, may act as antibiotic and take a significant role to fight against multiple antibiotic resistance in bacterial populations in near future.

Acknowledgement

Identification of the leaves of *A. conyzoides* L. by the taxonomists of the department of Botany, University of North Bengal, Siliguri, Dist. Darjeeling, West Bengal is gratefully acknowledged.

Conflict of Interest

There is no conflict of interest.

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Volume 15 Issue 5 May 2019