INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANT EXTRACTS OF AGERATUM CONYZOIDES

Sarvesh Kumar[*1,2], **Vijay Jyoti Kumar**[2], **Ranjit Singh**[1]
[1]*AVIPS Shobhit University, Gangoh Saharanpur (UP).*
[2]*Department of Pharmaceutical Sciences, H.N.B. Garhwal (A Central University) Srinagar (Garhwal), Uttarakhand.*

ARTICLE INFO

Article history
Received 30/06/2020
Available online
09/07/2020

Keywords
Ageratum Conyzoides,
Gram Positive,
Gram Negative,
Minimum Inhibitory
Concentration.

ABSTRACT

The plant *Ageratum conyzoides* is widely used in folk medicine in the Villages in west UP and Uttarakhand, India. The aim of this study is evaluate the anti bacterial study of the n-hexane, Chloroform, ethyl acetate, methanol and water extract of the Ageratum conyzoides (Linn.) leaves belonging to family Asteraceae were investigated against Two Gram positive bacteria like *Staphylococcus aureus* and streptococcus pneumoniae as well as Three Gram negative bacteria like *Escherichia coli,* Pseudomonas aeruginosa and klebisella pneumoniae. Because of such concerns, the necessity to find potentially fruitful, healthy safer and natural alternative preservatives is increased. Plant extracts have been used to control food poisoning defect and preserve foodstuff. Agar disc diffusion techniques were used for in-vitro antibacterial Screening. Methanolic extract was potentially effective with variable efficiency against the tasted four bacterial stains *Staphylococcus aureus, streptococcus pneumoniae, Pseudomonas aeruginosa* and *klebisella pneumoniae* at concentration of 70 mg/m. Methanolic extract was the most effective plant extract and showed bacteriostatic and bactericidal activities against the highly susceptible strains of food borne pathogenic bacteria with MIC's ranged from 16.0 to 32.0 mg/ml. Antibacterial activity of total five bacteria were used for evaluation of n-hexane chloroform, ethyl acetate, methanol and water extracts of Ageratum conyzoides. Result of zone of inhibition showed in both tables. Water extract negative test for all five bacterial strain. Antibacterial activity was measured by noting zone of inhibition in disc diffusion. Minimum inhibitory concentration was also noted. Methanolic extract of Ageratum conyzoides for use as antibacterial drug.

Corresponding author
Sarvesh Kumar
AVIPS Shobhit University,
Gangoh Saharanpur (UP).
sarveshlohan@gmail.com
9719832971

*Please cite this article in press as **Sarvesh Kumar** et al. Antibacterial Activity of Medicinal Plant Extracts of Ageratum Conyzoides. Indo American Journal of Pharmaceutical Research.2020:10(06).*

INTRODUCTION

Food poisoning is considered as one of the most common cause of disease and death in developing countries [1-3]. Most of these reports have been found to be due to food poisoning of gram-negative bacteria, mainly salmonella typhi Escherichia coli and Pseudomonas aeruginosa with bacterial contamination [4,5]. Other Gram positive bacteria including *Staphylococcus* aureus and *Bacillus* cereus have been also identified as the causal agents of food borne illness or food spoilage [6]. Prevention of food spoilage and their etiological agent is traditionally achieved by the use of chemical preservatives [7,8]. Despite the proven efficiency of these chemical preservatives in the prevention and outbreak control of food poisoning diseases, their repeated applications have led to the accumulation of chemical residues in the food and forage chain, the acquisition of microbial resistance to applied chemicals and the unpleasant side effects on human health Chemistry [9,10]. Because of such musing, efforts have been focused on developing potentially effective, healthy, safe and natural food preservatives. In this reference the use of plant extracts as antimicrobial agents for food preservation [11-13]. Extracts from these plants are naturally considered as a natural source of antimicrobial agents, which are considered safe and easily degrading as nutrition [14-17]. The antimicrobial activity show by plant extracts against food poisoning bacteria has been demonstrated by many researchers [18-21].Gupta et al. (2010) investigated antibacterial activity of n-hexane, chloroform, ethyl acetate, methanol and water extracts of Ageratum conyzoides against *S. Ethanolic extracts of the four plants Aureus, Pseudomonas aeruginosa and Bacillus subtilis and their results showed that the fight against disease-causing microbes are mostly secondary metabolites in the tissue of plant species*[22].Many plant species tissues contain secondary metabolites with the potential to fight against disease-causing micro-organisms. These amalgams include flavonoids, saponins, glycosides, steroids, terpens, alkaloids and tannins [23]. The plant organs including roots, leaves, bark, flowers, fruits and seeds, comprise diverse phytochemicals with antibacterial and antifungal activity [24]. In folk medicine, a single plant species is often used to treat more than one type of disease or infection [25]. The plant extracts with a history of traditional use should be tested using modern methods for activities against human pathogens, with the aim of discovering potential new drugs.

Natarajan et al., 2010examined the *in-vitro* antibacterial potential of *Biophytum sensitivum* leaves against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Klebsiella pneumonia*, *Salmonella typhi*, *Proteus vulgaris* and *Escherichia coli* using the agar well diffusion method. All the extracts inhibited the growth of selected bacteria in the range of 7-25 mm, showed antimicrobial activity of *Biophytum sensitivum* dried leaves extract [26]. For most herbs, the definite ingredient that causes a therapeutic effect is not known. Modern formulators favor using whole plants rather than extracting and isolating single components from them with an objective to produce synergistic therapeutic effects and also diminish the chances of side effects from any one isolated component. Several herbs are often used together to enhance effectiveness and synergistic actions and to reduce toxicity [27].
Ageratum conyzoides (Asteraceae) commonly called as goat or billy goat weed is an herbaceous plant that grows annually and has been used in several countries of the world for numerous medicinal purposes [28]. Aqueous extracts of leaves or whole plants have been used to treat colic, colds, fever, diarrhea, rheumatism, spasms, or as a tonic. *Ageratum conyzoides* has a quick and effective action in burn wounds and is recommended by Brazilian drugs central as an anti rheumatic [29]. The curative properties of the leaves especially in the treatment of ailments such as burns, wounds, arthritis, headaches, dyspnea, and pneumonia have been documented along with its analgesic, anti-hypertensive, wound hilling activity, pneumonia, analgesic, anti inflammatory, anti asthmatic, antispasmodic anti-inflammatory, anti asthmatic, antispasmodic and haemostatic effects [30].

MATERIAL AND METHODS

Collection and Identification

Leaves of Ageratum conyzoides was collected from the Village and Post- Libberheri, District- Haridwar, Uttarakhand (INDIA) respectively in the month of March-April 2017. Collected plants were shade dried at room temperature and ground separately and herbarium was prepared for the authentication. Plant was identified by Dr. R. M. Painuli, Department of Botany and Microbiology Hemvati Nandan Bahuguna Garhwal (A Central University) University Srinagar (Garhwal), Uttarakhand. The voucher specimen number GUH 20753 were deposited in herbarium. Plant was shade dried in room temperature, ground to moderately fine powder.

Prepration of Extracts

Dried leaves of Ageratum conyzoides sample was powdered mechanically to obtained coarse powder. Dry powder (1 Kg) was extracted separately with n-Hexane, Chloroform, Ethyl acetate, Methanol and water in the increasing polarity for 72 Hrs. with each solvent and then extract was filtered. The solvents were evaporated under reduced pressure to obtain a semi solid mass and then vacuum dried to yield a residue [31].

Chemicals used:

Nutrient Agar (Hi Media), Nutrient broth (Hi Media), n-Hexane, Chloroform, Ethyl acetate and Methanol.

Preparation of plates

Eight petridishes were sterilized in hot air oven at 160^0Cfor 2 hours. Out of these, four petridishes were used for preparation of plates using Nutrient Agar as a media for well diffusion assay.

Microorganisms used:

Bacterial strains i.e. *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and *Klebisella pneumoniae* had been obtained from the Department of Botany and Microbiology, H.N.B. Garhwal (A Central University) Srinagar Garhwal Uttarakhand India. The test organisms were further sub cultured at 37^0C for 24 hours. The cultures of bacteria were maintained in their appropriate agar slants at 4^0C throughout the study and used as stock cultures.

Antibacterial activity

The antimicrobial susceptibility test (AST) is an essential technique in modern biological science. AST standard tests are broadly classified into diffusion and dilution methods for convenience. Diffusion tests include agar well diffusion, agar disc diffusion, poison food technique and Bioautography; while dilution methods include agar dilution, broth micro-dilution and broth macro-dilution technique.

Agar well diffusion method was used to the screening of antibacterial activities for the different solvent extracts [32]. Four Petridis which were earlier prepared using 25 ml sterile molten Nutrient Agar as a media, stabilized at 45^0C, seeded with 0.1ml of a 24 hour nutrient broth sub-culture of the individual specific test organism (E. coli, pseudomonas aeruginosa, staphylococcus aureus, klebisella pneumoniae and streptococcus pneumoniae) containing approximately 108 cfu /ml in each sterile petridish and allowed to set. In each of these seeded plates six wells were punched by using sterile cork borer (5 mm diameter).By using micropipette fitted with sterile tip, first well was poured with 100 µl of the n- Hexane, Chloroform, Ethyl acetate and methanolic extract of Ageratum conyzoides. (1:1 ratio, suspended in DMSO) used as standard antibiotic (positive control).After pre-incubation at RT for one hour they were incubated for 24 hours at temperature of 37^0C, after which the diameter (in mm) of the zone of inhibition was measured and recorded. The results were shown in Table 01 given below. The *in vitro* antibacterial response to the n-Hexane, Chloroform, Ethyl acetate, Methanol and water extract of *Ageratum conyzoides* leaves was evaluated by measuring the diameter of the zones of inhibition.

Assay for antibacterial activity

Antibacterial activity of then-Hexane, Chloroform, Ethyl acetate, Methanol and water extracts of the *Ageratum conyzoides* leaves was determined using the agar well diffusion method [26]. Petri plates were prepared by pouring 75 mL of seeded MH agar and allowing the agar to solidify. Freshly prepared bacterial inoculum was evenly spread using a sterile cotton swab on the entire agar surface. A hole was then punched with a sterile cork borer (6 mm) and 100 µL of each crude extract was poured into the well. Petri plates were then allowed to stand at room temperature for 1 h and incubated at37°C overnight. Controls were run in parallel whereby solvent was used to fill the well. The plates were observed for zones of inhibition after 24 h and the results compared with those of the positive control, streptomycin (30µg/mL).

Determination of minimum inhibitory concentration (MIC)

The determination of MIC of the n-Hexane, Chloroform, Ethyl acetate, Methanol and water extracts of the *Ageratum conyzoides* was carried out by the micro dilution method using nutrient broth [33]. Plant extracts were dissolved in 10% DMSO and two-fold dilutions were prepared with culture broth. Each test sample and growth control (containing broth and DMSO, without plant extract/antimicrobial substance) was inoculated with 10 µL of bacterial suspension containing 5 × 106 CFU/mL. A 10-µL solution of resazurin (270 mg resazurin tablet dissolved in 40 mL of sterile water) was also added to each sample and incubated for 24 h at 37°C. Bacterial growth was detected by reading absorbance at 500 nm. Bacterial growth was indicated by a color change from purple to pink or colorless (assessed visually). MIC was defined as the lowest plant extract concentration at which the color changed, or the highest dilution that completely inhibited bacterial growth. The test dilutions were further sub-cultured on fresh solid media and incubated for 18 h to determine the MBC values. The lowest plant extract concentration that killed all the bacteria was defined as MBC. Tests were carried out in triplicate to test each dilution for each bacterial strain to determine MIC values.

RESULT AND DISCUSSION

Anti bacterial activity of total five bacteria, two Gram positive and three Gram negative bacteria like E. coli, pseudomonas aeruginosa, staphylococcus aureus, klebisella pneumoniae and streptococcus pneumoniae were used for evaluation of n-Hexane, chloroform, ethyl acetate, methanol and water extracts of *Ageratum conyzoides* leaves. The result of zone of inhibition showed in Table No. 1.

Table No. 1: zone of inhibition in antibacterial activity of *Ageratum conyzoides*.

S. No.	Name of the sample	Sample concentration (Microgram/ml)	117 (mm)	193	154	Kp	sp	Control
1	AGHXN	70	-	-	9	5	5	8
		100	-	-	10	6	9	8
		150	-	-	11	7	10	8
		200	-	-	12	8	11	8
		350	-	-	14	9	12	8
2	AGCHL	70	-	-	7	7	8	8
		100	-	-	11	9	9	8
		150	-	-	12	14	12	8
		200	-	-	16	15	13	8
		350	-	-	18	17	16	8
3	AGETH	70	-	-	-	-	-	8
		100	-	-	-	-	-	8
		150	-	-	-	-	-	8
		200	-	-	-	-	-	8
		350	-	-	-	-	-	8
4	AGMOH	70	9	-	18	20	7	8
		100	10	-	23	23	10	8
		150	11	-	29	25	15	8
		200	12	-	31	27	18	8
		350	19	-	32	29	24	8
5	AGWAT	70	-	-	-	-	-	8
		100	-	-	-	-	-	8
		350	-	-	-	-	-	8
		200	-	-	-	-	-	8
		350	-	-	-	-	-	8

Where; AGHXN- n-Hexane extract, AGCHL- chloroform extract, AGETH- ethyl acetate extract, AGMOG- methanol extract and AGWAT- water extract. Sp- streptococcus pneumoniae, Kp- klebisella pneumoniae, 193- E. coli, 117- pseudomonas aeruginosa and 154- staphylococcus aureus.

Minimum Inhibitory concentration (MIC)-

Anti bacterial activity of total five bacteria, two Gram positive and three Gram negative bacteria were used for evaluation of n-Hexane, chloroform, ethyl acetate, methanol and water extracts of *Ageratum conyzoides* leaves. The result of minimum inhibition concentration (MIC) showed in Table No. 2.

Table No. 2: Minimum inhibition concentration in antibacterial activity of extracts of *Ageratum conyzoides*.

Extract	Kp	Sp	0154	0193	0117
AGMOH	32µg/ml	32µg/ml	32µg/ml	-	16µg/ml
AGCHL	32µg/ml	8µg/ml	4µg/ml	-	-
AGHXN	128µg/ml	8µg/ml	128µg/ml	-	-

Statistical Analysis:

The values were expressed as mean ± SEM and was 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 $p < 0.05$.

RESULTS AND DISCUSSION

In vitro antibacterial activity of *Ageratum conyzoides*, the n-hexane, chloroform, ethyl acetate, methanol and water extract of *Ageratum conyzoides* Linn. was given in Table – 1. Results showed that n-Hexane and Chloroforms exerted anti bacterial activity at all concentration in *staphylococcus aureus*, *klebisella pneumoniae* and *streptococcus pneumoniae* per disc, ethyl acetate extract not showed anti bacterial activity of all tested bacteria. Methanolic extract of *Ageratum conyzoides* showed four bacterial strains like *pseudomonas aeruginosa*, *staphylococcus aureus*, *klebisella pneumoniae* and *streptococcus pneumoniae* positive in all concentration. Water extract negative test for all five bacterial strain. N-Hexane, chloroform and methanol extract of Ageratum conyzoides 350 µg/ml concentration showed large zone of inhibition in disc diffusion was found out. Table – 2 indicates results of minimum inhibitory concentration of n-Hexane, chloroform, ethyl acetate, methanol and water extract of *Ageratum conyzoides* Linn. *Ageratum conyzoides* family, Asteraceae is a medicinal plant, distributed lower and middle hill in Sikkim and Darjeeling up to 6000 ft and throughout India. The plant has erect hairy annual 30 – 90 cm high leaves. Different vernacular names are given to the plant [34].

In Nepali the plant is called as 'Elame'; in Lepcha 'Namyew' and in English the plant is known as 'Goat weed' [35]. 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 [36]. 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*.[37].recently we have noticed anti bacterial activity of leaves of *Ageratum conyzoides* 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* responsible for anti bacterial activity. n-Hexane, chloroform, ethyl acetate, methanol and water extract from the leaves of *Ageratum conyzoides* Linn. Negative control containing water had no MIC value. Thus, it has not been shown Antibacterial property of any extract of *Ageratum conyzoides* was evaluated against Two Gram – positive and Three Gram – negative bacteria. Anti bacterial activity was measured by noting zone of inhibition in disc diffusion. Minimum inhibitory concentration was also noted. We are now interested to note the mechanism of anti bacterial property of Ageratum conyzoides. Work is now in progress.

CONCLUSION

Anti bacterial activity of n-Hexane, chloroform, ethyl acetate, methanol and water extract from the leaves of *Ageratum conyzoides* was examined against Two Gram-positive and Three Gram-negative bacteria. Kanamycin was employed as control drug. AGHXN, AGCHL and AGMEOH showed anti bacterial activity against all the tested bacteria exceptional one bacteria. Maximum activity was found against *staphylococcus aureus* and minimum activity was noted for *pseudomonas aeruginosa.* Results were comparable to that of Kanamycin. Methanolic extract of Ageratum conyzoides thus provides a scientific rationale for use as anti bacterial drug.

ACKNOWLEDGMENT

The author thankful to Dr Sarla Saklani HOD, Department of Pharmaceutical Chemistry, HNB Garhwal (A Central University) Srinagar Garhwal for providing laboratory Resources and Dr RM Painuli Department of Microbiology and Botany, HNB Garhwal (A Central University) Srinagar Garhwal for identify the plant.

Conflict of interest

Nil

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