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STUDIES ON ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF *AGERATUM CONYZOIDS* LINN.

[1*]Shelly Rana, [2]Ved Prakash & Anand Sagar[3]
Department of Biosciences, Himachal Pradesh University, Shimla, (H.P.) 171005, India
*Corresponding author email: shellyrana87@rediffamil.com

ABSTRACT
Ageratum conyzoides Linn. (Family: Asteraceae) a medicinal plant which is commonly known as Goat weed or App grass is widely distributed over the tropical and subtropical regions of the world. The antibacterial activity of methanol, acetone and aqueous leaf extracts of *A. conyzoids* was determined *in vitro* against medically important pathogens *i.e. Escherichia coli*, *Yersinia pestis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Staphylococcus aureus* following agar well diffusion method using different concentrations (30%, 50%, 70% and 100%). Results showed low to significant antibacterial activity against the mentioned bacterial strains. Methanol leaf extract was found to be more effective against selected pathogenic bacteria as compared to acetone and aqueous leaf extracts. The antioxidant capacity of the different extracts of this plant was evaluated by DPPH (1, 1-diphenyl-2-picrylhydrazyl) and reducing power tests. The plant exhibited noticeable DPPH radical scavenging and reducing power activity .Therefore, the leaf extracts of this plant can be selected for further investigation to check their therapeutic potential.

KEYWORDS: *Ageratum conyzoids*, leaf extracts, pathogenic bacteria, agar well diffusion, DPPH, reducing power.

INTRODUCTION

Plant based antimicrobial agents represent a vast and unexplored source of drugs. Plants are one of the best gifts of nature. Plants have many therapeutic properties combined with nutritive value which have made their use in pharmaceutical industries. The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs into Asian continent (Akinyemi *et al*., 2005). Medicinal plants are considered as an effective source of both traditional as well as modern medicines and about more than 80% of rural populations depend on them as their primary health care (Ilori *et al*., 1996). In recent years, popularity and demand of medicinal plants has been increased extensively. Now- a-days, much attention is being given to the antioxidant property and other health benefits associated with it. Till date plants are one of the potent antioxidants found in nature. Since ancient times, plants are being considered as rich source of medicinal agents. Scientific exploration of traditional knowledge and its use in treatment of various ailments is one of the thrust areas of research. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal benefits, higher safety margins and lesser costs. Researchers have explored the antioxidant potential of many plants that reduce the risk for chronic diseases including cancer and heart diseases (Ali *et al.*, 2008).

A large number of medicinal plants belonging to Asteraceae family which contain many chemical compounds exhibit antimicrobial properties. The family Asteraceae includes about 25,000 species, many of which have liberal amount of secondary metabolites with various biological activities (Gyamfi *et al.*, 2007). Various studies have been carried out on some plants of Asteraceae family worldwide (Noda *et al.*, 1997). *Ageratum conyzoides* is widely utilized in traditional medicinal systems wherever it grows, although its applications vary by region (Shekhar *et al.*, 2012). *Ageratum conyzoides* Linn. is an annual herb with a rich history of traditional medicinal use in the tropical and sub-tropical regions of the world. The stems and leaves of the plant are covered with fine white hairs. The leaves are ovate in shape and grow up to 7.5 cm long. The flowers are arranged in terminal inflorescences which are white or pink in colour. The fruits are achene and anemophilous. The mature plant is used for its anti-inflammatory, antispasmodic, antiasthmatic properties for the treatment of wounds and also in bacterial infections (Kokwaro, 1976; Gonzalez *et al*., 1991). Many essential oils have been found in this plant that can inhibit the growth and production of toxigenic strains of *Aspergillus parasiticus* (Patil *et al*., 2010; Nogueira *et al.*, 2010). Now-a-days, researchers are channelizing great deal of efforts to find effective antioxidants for the treatment or prevention of free-radical mediated deleterious effects (Rieter, 1985). This study gives good information on antibacterial and antioxidant properties of *Ageratum conyzoides*. At present, there is an urgent need of exploration and development of cheaper and effective plant based drugs with better bioactive potential and least side effect.

MATERIALS & METHODS

Fresh leaves of *A. conyzoides* were collected from Kaloha village, District Kangra Himachal Pradesh, India. Plant was properly identified and authenticated in Ethno-botany Laboratory, Department of Bio sciences, Shimla Himachal Pradesh.

Processing of Plant Material

Leaves of *A. conyzoides* were washed thoroughly under tap water and then with 2% Mercuric chloride. After that

the leaves were cut into smaller pieces for quick drying. Cleaned leaves were shade dried for 15-20 days. The dried plant material was crushed into fine powder with the help of pestle mortar. Finally the fine powder was stored in an air tight container at room temperature.

Preparation of Plant Extracts

5 gm dried leaves of *A. conyzoides* were taken in separate Erlenmeyer flasks to which 50 mL of required solvents i.e. methanol, acetone and aqueous were added. The flasks were covered with aluminium foil and allowed to stand for 3-5 days for extraction. These extracts were filtered through Whatman filter paper no. 1 and evaporated at 40°C using rotary evaporator. The extracts were collected and weighed. Finally, stock solution of concentration 50 mg/mL was prepared.

Procurement of Bacteria

Bacterial spp. used for antibacterial studies were procured from Department of Biotechnology, Himachal Pradesh University, Summer Hill Shimla, India. Pathogens used in the study were *Escherichia coli, Pseudomonas aeruginosa, Yersinia pestis, Staphylococcus aureus, Bacillus cereus* and *Listeria monocytogenes.*

Revival of Pathogen

For antibacterial testing, fresh inoculum was prepared for each bacteria and they were incubated at 37 °C for 24 h.

Screening the Antibacterial Activity of *A. conyzoides*

Screening of plant extracts (methanol, acetone and aqueous) of *A. conyzoides* was done using agar well diffusion method. Nutrient agar medium (Beef extract 1g, Yeast extract 2 g, Sodium Chloride 1 g, Peptones 5 g, Agar 20 g, Distilled Water 1000 mL) was used throughout the investigation. The medium was autoclaved at 121.6°C for 30 minutes and poured into Petri plates. Bacteria were grown in nutrient broth for 24 hours. A 100 µL of bacterial suspension was spread on each nutrient agar plate. Agar wells of 8 mm diameter were prepared with the help of sterilized stainless steel cork borer in each Petri plate. The wells in each plate were loaded with 30%, 50%, 70% and 100% concentration of prepared leaf extracts of. *A. Conyzoides.* The Petri plate kept as a control contained pure solvent only. The plates were incubated at 37± 2°C for 24 hours in the incubation chamber. The zone of growth inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in perpendicular direction in all the three replicates and the average values were tabulated. Percentage inhibition of bacterial species was calculated after subtracting control from the values of inhibition diameter using control as standard (Hemashenpagam *et al*., 2010).

$$\text{Percentage of growth inhibition (\%)} = \left(\frac{\text{Control}-\text{Test}}{\text{Control}}\right) \times 100$$

Control = average diameter of bacterial colony in control.
Test = average diameter of bacterial colony in treatment sets . (Kannan *et al*., 2009)

ANTIOXIDANT ACTIVITY TEST

DPPH Radical Scavenging Activity Assay

The free radical scavenging activity of plant extracts was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described by Blois 1958. Briefly, 1mL of different concentrations (20, 40, 60, 80 and 100 µg/mL) of plant or test extract, 1 mL of DPPH (0.1 mM in methanol) was added. Corresponding blank sample was prepared and ascorbic acid was used as reference standard. Mixture of 1mL methanol and 1 mL DPPH solution (without plant extract) was used as control. All the tests were carried out in triplicate and the decrease in absorbance was measured at 517 nm after 30 minutes in dark using UV-VIS spectrophotometer. The percentage of inhibition was calculated using the following formula:

$$\text{DPPH scavenging effect (\%)} = \left(\frac{\text{Acontrol}-\text{Asample}}{\text{Acontrol}}\right) \times 100$$

Where, $A_{control}$ is the absorbance of control; A_{sample} is the absorbance of sample

Reducing Power Assay

The reducing power was determined according to the method described by Oyaizu 1986. Different concentrations of plant extract (20, 40, 60, 80 and 100 µg/mL) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 minutes. A portion (2.5 mL) of Trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as the standard. Phosphate buffer (pH 6.6) was used as blank solution. Higher absorbance of the reaction mixture indicated greater reductive potential. Experiment was performed in triplicates at each concentration to evaluate percent reducing power. The % reducing power (antioxidant activity) was calculated by using the formula:

$$\text{\% reducing power} = \left(\frac{\text{Acontrol}-\text{Asample}}{\text{Acontrol}}\right) \times 100$$

Where, $A_{control}$ is the absorbance of control; A_{sample} is the absorbance of sample**.**

RESULTS & DISCUSSION

The present study revealed that methanol, acetone and aqueous leaf extracts of *A. conyzoides* proved themselves as good antibacterial agents. The methanol extract of *A. conyzoides* showed considerable growth inhibition of test bacteria at different concentrations (30%, 50%, 70%, 100%) as compared to acetone and aqueous leaf extract of the plant. The methanol extract *w*as found to be most effective against *S. aureus* at (22 mm at 100%) followed by (14 mm at 70%), (12 mm at 50%), (10 mm at 30%) and it offered minimum inhibition in *aqueous* extract as given in table 1. The acetone extract of *A. conyzoides* was found to be most effective against at *P. aeruginosa* (20 mm at 100%) followed by (16 mm at 70%), (14 mm at 50%), (12 mm at 30%) and showed minimum inhibition towards *E. coli* at all concentrations as shown in table 1. The aqueous extract of *A. conyzoides* was found to be most effective against at *L. monocytogenes* (14 mm at 100%) followed by (12 mm at 70%), (10 mm at 50%), (10 mm at 30%) and it showed minimum inhibition towards *E. coli* at all concentrations as shown in table 1.

TABLE 1.1 Zones of inhibition (ZOI) produced by leaf extracts of at different *A. conyzoides* concentrations

Plant Extract	Concentration in %	Inhibition zone diameter in mm (±SD)					
		E.coli	*Y.pestis*	*P.aeruginosa*	*B.cereus*	*L.monocytogenes*	*S.aureus*
Methanol	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	0.00±0.00	10.00±0.10	11.00±0.10	10.00±0.00	11.08±0.09	10.00±2.05
	50	0.00±0.00	12.20±0.05	11.02±0.19	12.00±0.00	12.80±1.20	12.15±0.95
	75	0.00±0.00	14.60±0.46	12.20±2.20	14.44±0.25	15.70±1.76	14.00±0.00
	100	0.00±0.00	16.00±0.00	16.10±0.20	16.66±1.70	21.30±0.88	22.20±0.70
Acetone	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	0.00±0.00	10.90±2.00	12.00±0.00	0.00±0.00	10.30±1.92	10.00±0.065
	50	0.00±0.00	11.57±0.33	14.20±1.80	0.00±0.00	13.24±0.28	11.70±1.30
	75	0.00±0.00	12.00±1.97	16.90±1.57	0.10±0.54	15.50±2.30	12.00±1.40
	100	0.00±0.00	15.00±0.49	20.04±0.40	0.11±0.32	18.03±1.00	13.90±0.10
Aqueous	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	10.00±2.20	0.00±0.00
	50	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	10.90±1.60	0.00±0.00
	75	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	12.57±0.20	0.00±0.00
	100	0.00±0.00	10.00±0.00	9.08±1.10	10.00±0.00	14.00±0.80	10.00±0.00

TABLE 1.2 Free radical (DPPH) scavenging activity (%) of the tested medicinal plants at different concentrations

Concentration (µg/mL)	Methanol extract	Acetone extract	Aqueous extract	Ascorbic acid
20	10.10±1.00	10.04±1.27	10.40±0.56	36.24±010
40	20.80±1.60	15.80±0.05	17.00±0.02	52.50±0.12
60	34.70±0.76	20.00±0.02	25.80±0.16	60.30±1.10
80	40.30±1.52	28.00±0.10	31.30±1.06	76.10±0.00
100	49.02±0.78	36.15±1.27	38.10±1.03	82.06±2.00

Values are given as mean ± SD

It is evident from the results that methanol as well as acetone leaf extract of *A. conyzoides* were quite effective in inhibiting the growth of bacteria as compared to aqueous. Possible reason for this antibacterial activity is presence of alkaloids, phenolics and flavanoids in the leaves of *A. conyzoides* (Ekundayo *et al.,* 1988). Majority of phytochemical components are known to produce the therapeutic activities like antibacterial, antifungal and antioxidant (Sahoo *et al.,* 2010). These findings are in accordance with the work carried out by Salie (1996) and Kannabiran (2009). It has been established that our work also coincides to the work already reported by Aliero and Afolaya (2006). In the present study, leaf extracts of *A. conyzoides* in three different solvents (methanol, acetone and aqueous) were tested for their free radical scavenging ability by using DPPH assay and it was observed that the plant extracts showed moderate potency for scavenging free radicals as shown in Table 1.2. The extracts were tested on a concentration range (20-100 µg/mL) and it was found that the activity altogether increased with increase in concentration of plant extracts (Fig. 1.1). In all cases, methanol extracts proved to be better antioxidants than the corresponding acetone and aqueous extracts. A pattern of increasing antioxidant activity with increasing polarity has been reported (Goze *et al.*, 2009)

Silva *et al.* (2000) employed DPPH free radical scavenging assay on aqueous extract of *A. conyzoides* and reported 13.28-64.24% inhibition. But in our study, *A. conyzoides* exhibited 36.15% and 38.10% inhibition of free radicals by aqueous and methanol extracts respectively.

TABLE 1.3 Antioxidant activity percentages (%) of tested medicinal plants by reducing power method at different concentrations

Concentration (µg/mL)	Methanol extract	Acetone extract	Aqueous extract	Ascorbic acid
20	24.00±1.00	17.05±1.10	18.45±0.50	25.50±2.15
40	34.70±0.10	25.50±0.25	28.00±0.01	40.14±0.25
60	42.40±0.12	38.40±0.30	44.80±0.16	52.80±1.10
80	56.30±2.19	55.00±0.10	56.30±0.36	72.10±0.52
100	75.05±0.15	68.15±1.20	68.60±1.40	84.12±1.10

Values are given as mean ± SD

The reducing capacity of compounds serves as an important indicator of their potential antioxidant activity. The plant having high reducing power generally reported to carry high antioxidant potential too (Mier *et al.,* 1995). We investigated the reducing capacity of by *A. conyzoides* measuring Fe^{3+}-Fe^{2+} conversion as given in Table 1.3 and Fig. 1.2. In this experiment, Ferric ions reduced to ferrous ions with the colour of the reaction mixture changes from yellow to bluish green. Reducing power potential of extracts increased with the dose, however, plant extracts exhibited low reducing power than that of standard ascorbic acid. The methanol extract showed more reductive ability than the acetone and aqueous extracts, which was capable for neutralizing the free radicals. All the extracts of *A. conyzoides* showed greater than 50.00% reducing power when tested on a concentration range of

20-100 µg/mL (Gulfishan *et al.,* 2014) tested the methanol extract of *A. conyzoides* reducing power assay and observed 76.60% antioxidant activity. While in present study, 75.05% (maximum) antioxidant activity was reported for the methanol extract of *A. conyzoides* Thus it serves as an encouragement towards development of new drugs for the benefit of mankind.

CONCLUSION

It was concluded from the above experimental observations that the plant *A. conyzoides* showed satisfactory antibacterial and antioxidant activity at different concentrations. Methanol leaf extract was found to be more effective followed by acetone and aqueous leaf extracts. The leaf extract of *A. conyzoides* showed more inhibitory effects in gram-positive bacteria than in gram-negative bacteria. This study also suggests that the plant extracts possess potent antioxidant activity, which might be helpful in curing the progress of various oxidative stress-related diseases. Further investigation on the isolation and identification of antioxidant components in this plant may prove to chemical entities with potential for clinical use.

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