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ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF TWO MEDICINAL PLANTS : *ERIGERON ALPINUS* L. AND *GENTIANELLA MOORCROFTIANA* WALL. EX G. DON

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Abstract

Antibacterial and antioxidant activity of the two medicinal plants namely *Erigeron alpinus* L. and *Gentianella moorcroftiana* Wall. ex G. Don were evaluated by using agar-well diffusion method and antioxidant activity by DPPH free radical scavenging assay. Antibacterial activity of acetone and methanol plant extracts was tested against four pathogenic bacteria (*Escherichia coli, Yersinia pestis, Staphylococcus aureus* and *Listeria monocytogenes*) using different concentrations i.e. 25, 50, 75 and 100%. Antioxidant activity was also examined for both types of plant extracts at 20, 40, 60, 80 and 100 µg/mL concentrations. Results showed that both the plants exhibited significant antibacterial and antioxidant activities. Methanol leaf-flower mixture extract of *G. moorcroftiana* showed maximum zone of inhibition and again methanol extracts of both the plants exhibited higher antioxidant capacity with lesser IC_{50} values than acetone extracts except in case of stem extracts of *G. moorcroftiana* where results for antioxidant activity were opposite. Medicinal value of the selected plants was proved by the present study thus plant extracts can be used for further investigation for gaining their proper therapeutic knowledge.

Key words **:** *Erigeron alpinus, Gentianella moorcroftiana,* plant extracts, agar-well diffusion, DPPH.

Introduction

India is one of the leading countries in Asia, in terms of wealth of traditional knowledge (Pant *et al*., 2009). Ayurveda, Siddha, Homeopathy, Unani, Naturopathy etc. are traditional systems prevalent in India (Samal, 2016). These medicinal systems are provided with 25,000 herb-based formulations for the treatment of various diseases. Himachal Pradesh, one of the pioneer states of the Himalaya, has great wealth of medicinal flora (Boktapa and Sharma, 2007). 500 plant species have been reported having medicinal value here (Chauhan, 2003). As the art of the herbal healing is most prevalent among tribal cultures, Lahaul-Spiti is also one of the tribal districts of Himachal Pradesh. Amchis of Spiti and Larjes of Lahaul valley are the local practitioners of Lahaul-Spiti tribe and prescribe the herbal medicines to local people (Singh, 2012).

In these days, infections have been increased to greater extent with increasing resistance against antibiotics (Austin *et al*., 1999). So there is an urgent need for novel antimicrobial compounds. Thus researchers are focusing their interest on natural products to identify these bioactive compounds (Prakash, 2016). In now a day, there is an increased interest in plant derived drugs as they provide the number of antimicrobial agents and also the natural medicines are safe and without any side effects (Jigna and Sumitra, 2006). Thus defense against invading microorganisms is served by plants derived drugs which are actually formed from secondary metabolites of plants (Balandrin *et al*., 1985).

A number of antioxidants obtained from the medicinal plants reduce the risk of diseases and oxidative damages (Prior and Cao, 2000; Kris-Etherton *et al*., 2002). These diseases and damages are due to free radicals especially reactive oxygen species (ROS). Which are the chemical species containing one or more unpaired outer shell electrons and generally highly reactive and unstable and include superoxide radicals, hydrogen peroxides, hydroxyl radicals etc. (Martinez-Cayuela, 1995). Although free radicals play positive role in cell physiology but they also

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have negative role like membrane lipid peroxidation, disturbing membrane fluidity, gene mutation etc. (Cerulti, 1991; Harman, 1994; Ames, 1998; Finkel and Holbrook, 2000). Antioxidants obtained from the plants are natural, least toxic and highly effective and increase the antioxidant capacity of plasma. Phenolics and flavonoids are the phytochemicals which are reported to have antioxidant activity (Chanda and Dave, 2009).

In fact, the natural substances which are being used for the medicinal purposes and treatment of many diseases are present in one or more organs of a plant or in a whole plant. These substances are phytochemicals like saponins, tannins, flavonoids, alkaloids, phenolics, essential oils etc. which have curative properties (Oloyede *et al*., 2010). These phytochemicals are present in mixture inside the medicinal plants and act either individually or additively against diseases. Basically the combinations of these secondary metabolites are the real cause of the medicinal effect.

Investigated two medicinal plants namely *Erigeron alpinus* L. and *Gentianella moorcroftiana* Wall. ex G. Don are commonly used as herbal medicines by healer of Lahaul-Spiti and distributed in western Himalayan Region. *E. alpinus* belonging to Asteraceae family have common names like blue fleabane, fleabane, sharp erigeron, farewell to summer etc. At its collecting site this plant is known as Bashakar. Plants are perennial herbs and consumed in powder form for cold and cough treatments (Singh *et al*., 2009) and also utilized for rheumatism (Nath, 1997).

G. moorcroftiana belongs to family Gentianaceae having common names moorcroft's gentian, airy shaw etc. In the locality of collecting site it is known as Tikta. Local practitioners utilize the plant for cough, cold, nausea, gastric and rheumatism (Nath, 1997).

Materials and Methods

Collection of plant material

Aerial parts of *Gentianella moorcroftiana* and *Erigeron alpinus* were collected from the Beeling village of Lahaul-Spiti at 3150 m altitude of Himachal Pradesh.

Processing of plant material

Aerial parts were first washed under tap water and then treated with 2% Mercuric chloride. Leaves and flowers of *G. moorcroftiana* were separated from stem and then leaves and stem of *G. moorcroftiana* and whole plant of *E. alpinus* were allowed to shade dried for 15-20 days. After drying plant materials were crushed to form a fine powder with the help of pestle mortar. Prepared fine powders were stored at room temperature, in air tight containers.

Preparation of Acetone and Methanol extracts

3 g of leaf-flower mixture and stem of *G. moorcroftiana* and whole plant of *E. alpinus* were taken in three different Erlenmeyer flasks to which 30 mL of the acetone/methanol were added. After covering the flasks with Aluminium foils, they were allowed to stand for 3-5 days for extraction purposes. After extraction, the extracts were separated with the help of the Whatman filter paper no. 1 and by using rotary evaporator evaporation occurred at 40^0C. The dried extracts were collected and weighted. Then stock solutions of conc. 50 mg/mL were prepared.

Procurement of bacteria

Bacterial strains used for antibacterial studies were *Escherichia coli, Yersinia pestis, Staphylococcus aureus* and *Listeria monocytogenes* which were procured from Department of Microbiology and Biotechnology, Himachal Pradesh University, Summer Hill, Shimla, India.

Revival of pathogen

The collected pathogens were revived in nutrient broth and stored in nutrient agar slants at 4°C.

Screening antibacterial activity of acetone and methanol extracts of the *E. alpinus* whole plant and *G. moorcroftiana* leaf-flower mixture and stem

Different extracts (methanol and acetone) of medicinal plants were screened using agar-well diffusion method. Nutrient agar medium (Beef extract 1 g, Yeast extract 2 g, Sodium Chloride 1 g, Peptone 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 25, 50, 75 and 100% concentrations of prepared plant extracts. 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 zone diameter using positive control as

standard (Prakash *et al.,* 2016).

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

Control = average diameter of bacterial colony in control.

Test = average diameter of bacterial colony in treatment sets (Rana *et al*., 2016).

Antioxidant activity evaluation of acetone and methanol extracts of the *E. alpinus* whole plant and *G. moorcroftiana* leaf-flower mixture and stem

DPPH radical scavenging activity assay

The free radical scavenging activity of plant extracts was measured using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) as described by Blois (1958). Briefly, to 1 mL 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 1 mL 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:

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

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

Graphs were plotted against percent inhibition v/s conc. of plant extracts and standard ascorbic acid in order to find out the values of slope and y-intercepts. IC_{50} value (the amount of antioxidant required to decrease the initial DPPH concentration by 50%) for each extract and ascorbic acid was evaluated using the following equation given below:

IC_{50} =

Results

Antibacterial activity screening

Tables 1-3 and figs. 1-3 showed the results for antibacterial activity of *Erigeron alpinus* and *Gentianella moorcroftiana*. Results came out from the Tables concluded that methanol leaf-flower mixture extract of *G. moorcroftiana* showed highest antibacterial activity among all plant extracts with zone of inhibition of 12.73 mm at 25%, 16.46 mm at 50%, 18.53 mm at 75% and 23.60 mm at 100% concentration against *Y. pestis* (table 1). Both acetone and methanol leaf-flower mixture extracts and acetone stem extract did not showed any

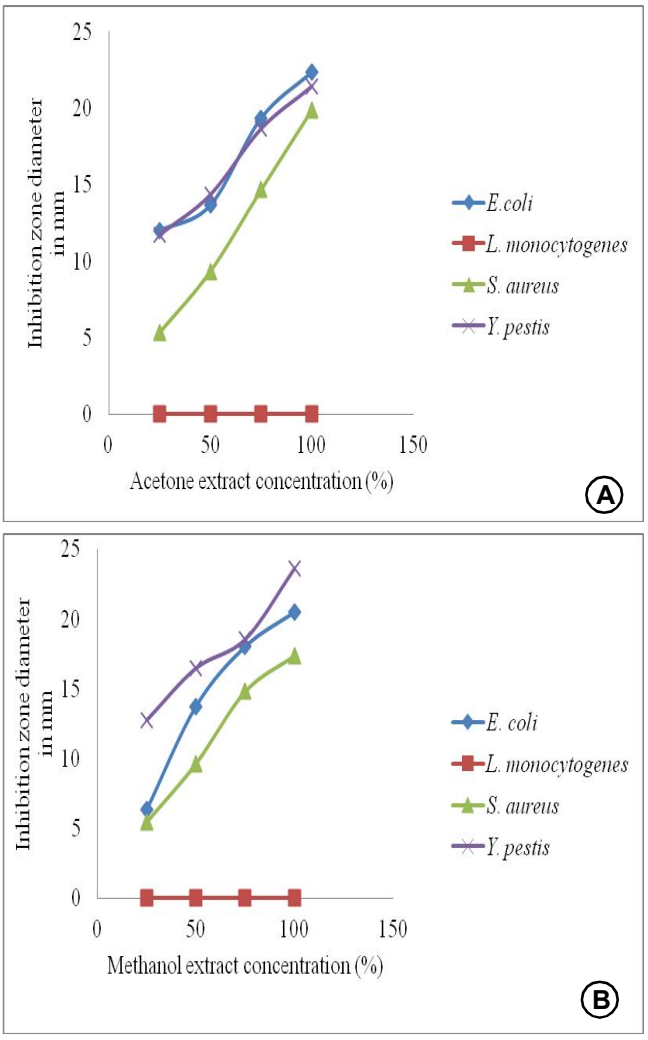

Fig. 1 : Antibacterial activity of *G. moorcroftiana* acetone (A) and methanol (B) leaf and flower mixture extracts.

inhibition against *L. monocytogenes*. According to table 2, methanol stem extract of the *G. moorcroftiana* exhibited maximum activity with 12.33 mm, 15.98 mm, 18.60 mm and 23.07 mm zones of inhibition at 25, 50, 75 and 100% concentration respectively against *Y. pestis*. In case of *Erigeron alpinus* again maximum activity was shown against *Y. pestis* by methanol whole plant extract with zone of inhibition of 12.66 mm at 25%, 16.20 mm at 50%, 19.40 mm at 75% and 22.93 mm at 100% concentration and the minimum inhibition against *E. coli* with 3.86 mm, 4.73 mm, 5.73 mm and 7.70 mm zones of inhibition at 25, 50, 75 and 100% concentrations respectively (table 3).

Antioxidant activity screening

Tables 4-7 and figs. 4-7 showed the results for antioxidant activity of acetone and methanol extracts of the two selected medicinal plants. Ascorbic acid was taken as the control which exhibited IC_{50} value of 27.17 µg/mL (table 4). Methanol leaf and flower extract of *G.*

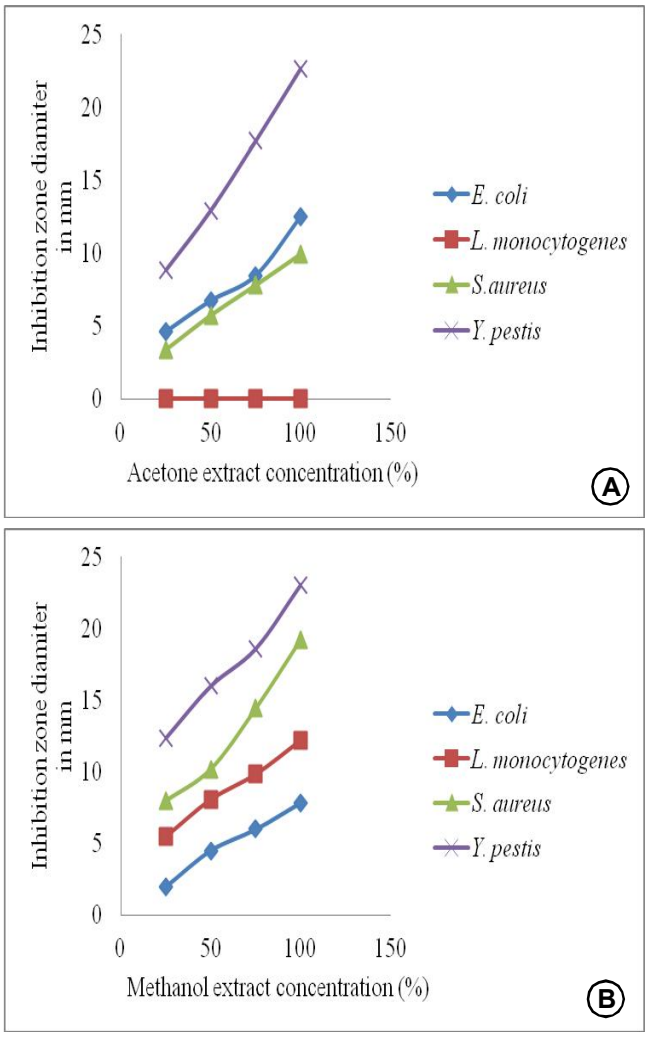

Fig. 2 : Antibacterial activity of *G. moorcroftiana* acetone (A) and methanol (B) stem extracts.

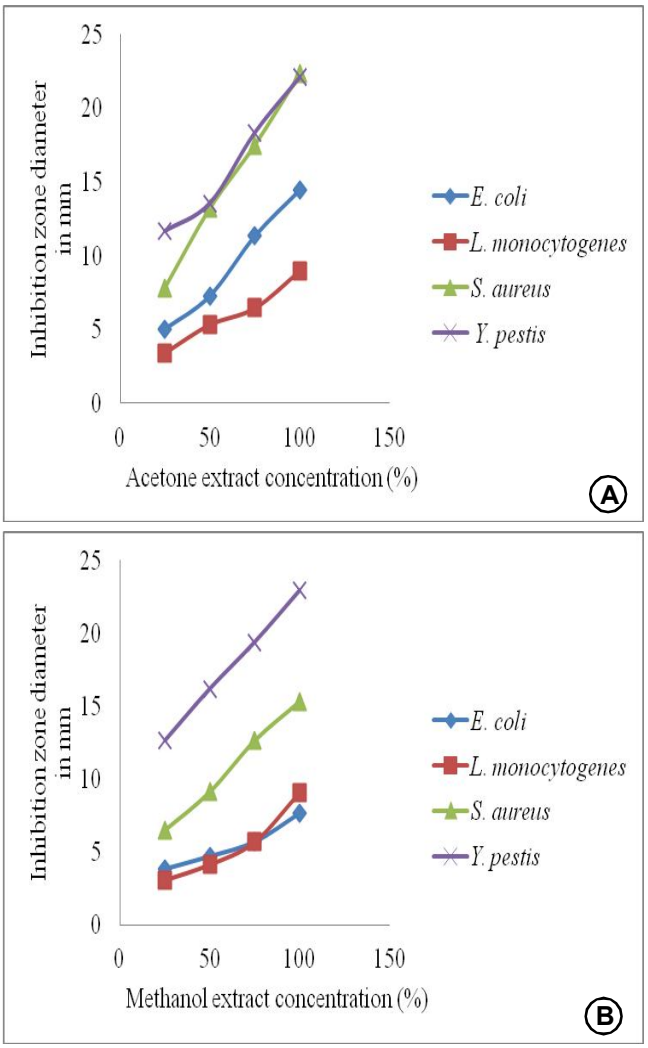

Fig. 3 : Antibacterial activity of *E. alpinus* acetone (A) and methanol (B) whole plant extracts.

Table 1 : Zones of inhibition produced by acetone and methanol flower and leaf extract of *G. moorcroftiana* at different concentrations.

Extract	Concentration (in %)	Inhibition zone diameter in mm (± S.E.)			
		E. coli	*L. monocytogenes*	*S. aureus*	*Y. pestis*
Acetoneextract	Control	00.00±00.00	00.00±00.00	00.00±00.00	00.00±00.00
	25	12.00±01.00	00.00±00.00	05.33±00.57	11.70±00.52
	50	13.66±02.51	00.00±00.00	09.33±00.57	14.33±00.51
	75	19.33±00.57	00.00±00.00	14.66±00.57	18.66±00.57
	100	22.33±0.1.15	00.00±00.00	19.88±00.34	21.43±00.51
Methanol extract	Control	00.00±00.00	00.00±00.00	00.00±00.00	00.00±00.00
	25	06.33±00.57	00.00±00.00	05.46±00.50	12.73±00.64
	50	13.73±00.64	00.00±00.00	09.66±00.58	16.46±00.50
	75	18.00±01.00	00.00±00.00	14.83±00.29	18.53±00.81
	100	20.46±00.50	00.00±00.00	17.40±00.53	23.60±00.69

Table 2 : Zones of inhibition produced by acetone and methanol stem extract of *G. moorcroftiana* at different concentrations.

Extract	Concentration (in %)	Inhibition zone diameter in mm (± S.E.)			
		E. coli	*L. monocytogenes*	*S. aureus*	*Y. pestis*
Acetoneextract	Control	00.00±00.00	00.00±00.00	00.00±00.00	00.00±00.00
	25	04.60±00.60	00.00±00.00	03.36±01.10	08.83±00.67
	50	06.73±00.31	00.00±00.00	05.73±00.64	12.93±00.47
	75	08.46±00.47	00.00±00.00	07.80±00.34	17.70±00.61
	100	12.50±01.35	00.00±00.00	09.93±00.12	22.66±00.58
Methanol extract	Control	00.00±00.00	00.00±00.00	00.00±00.00	00.00±00.00
	25	01.96±00.96	05.46±00.55	07.98±02.25	12.33±00.58
	50	04.51±00.25	08.06±00.35	10.20±01.52	15.98±00.03
	75	06.00±00.46	09.83±00.76	14.44±02.30	18.60±00.53
	100	07.86±00.42	12.20±00.85	19.25±01.04	23.07±00.89

Table 3 : Zones of inhibition produced by acetone and methanol whole plant extract of *E. alpinus* at different concentrations.

Extract	Concentration (in %)	Inhibition zone diameter in mm (± S.E.)			
		E. coli	*L. monocytogenes*	*S. aureus*	*Y. pestis*
Acetone extract	Control	00.00±00.00	00.00±00.00	00.00±00.00	00.00±00.00
	25	05.00±01.00	03.40±00.53	07.81±00.73	11.66±00.58
	50	07.26±00.64	05.33±00.58	13.21±01.07	13.56±00.51
	75	11.33±01.52	06.46±00.50	17.50±01.80	18.36±00.55
	100	14.46±00.50	09.00±01.00	22.33±00.58	22.13±00.81
Methanol extract	Control	00.00±00.00	00.00±00.00	00.00±00.00	00.00±00.00
	25	03.86±00.23	03.10±01.01	06.50±0.46	12.66±00.57
	50	04.73±00.46	04.16±00.80	09.13±00.81	16.20±00.23
	75	05.73±00.46	05.70±00.52	12.66±00.58	19.40±00.53
	100	07.70±00.51	09.06±00.90	15.33±00.58	22.93±00.90

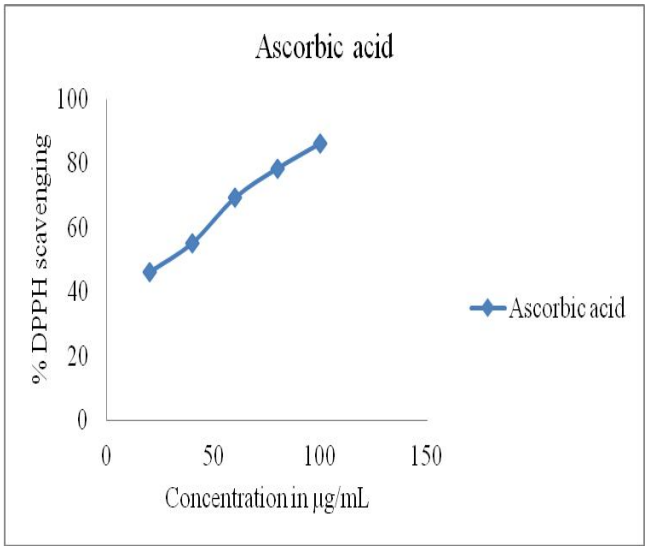

Fig. 4 : Free radical scavenging activity of ascorbic acid as a control.

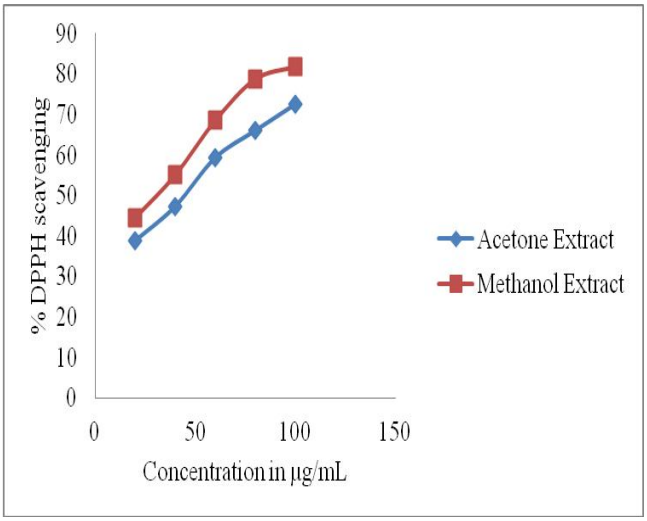

Fig. 5 : Free radical scavenging activity of the acetone and methanol leaf and flower extract of the *G. moorcroftiana*.

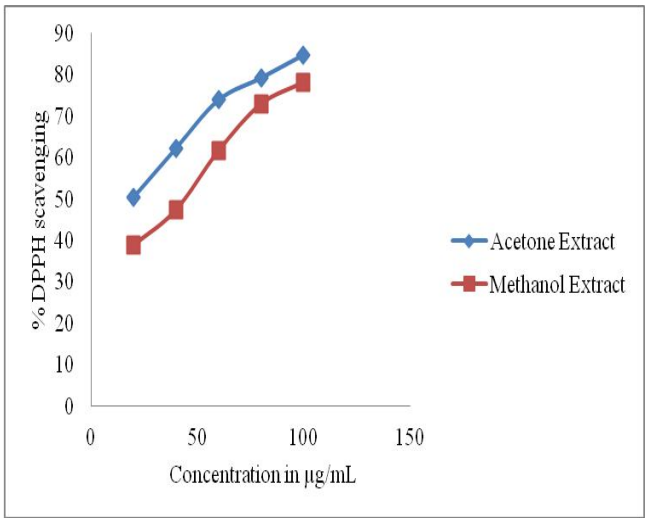

Fig. 6 : Free radical scavenging activity of the acetone and methanol stem extract of the *G. moorcroftiana*.

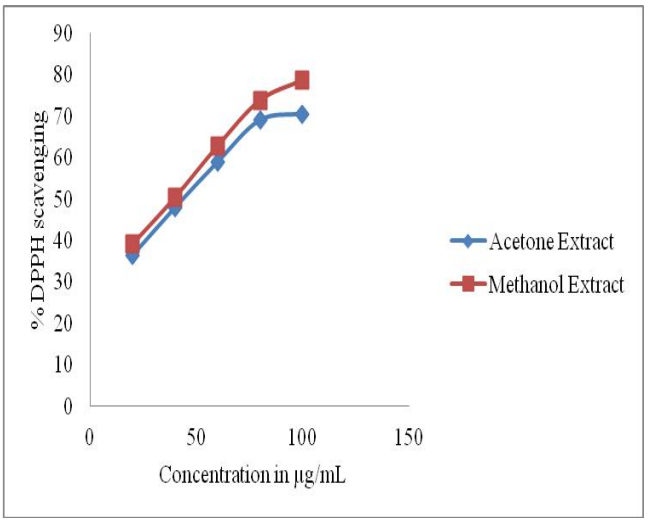

Fig. 7 : Free radical scavenging activity of the acetone and methanol whole plant extracts of the *E. alpinus*.

Table 4 : Free radical scavenging activity (%) of the control *i.e.* ascorbic acid at different concentrations.

Concentration (µg/mL)	Methanol extract (%)	IC_{50} value (µg/mL)
20	46.00±00.52	27.17
40	55.10±01.20	
60	69.25±00.75	
80	78.45±00.45	
100	86.22±01.32	

Table 5 : Free radical scavenging activity (%) of the leaf and flower extract of the *Gentianella moorcroftiana* Wall. ex G. Don at different concentrations in acetone and methanol solvents.

Conc. (µg/mL)	Acetone extract (%)	IC_{50} value (µg/mL)	Methanol extract (%)	IC_{50} value (µg/mL)
20	38.81±02.38	44.09	44.46±00.47	28.07
40	47.32±01.34		55.04±00.97	
60	59.40±00.79		68.56±00.55	
80	66.13±01.73		78.82±00.83	
100	72.67±01.18		81.92±00.95	

moorcroftiana mixture showed higher antioxidant activity with 28.07 µg/mL IC_{50} value than acetone extracts with 44.09 µg/mL IC_{50} value (table 5). But in case of stem extract of *G. moorcroftiana,* acetone extract showed least IC_{50} value (13.32 µg/mL) than methanol extracts (41.20 µg/mL) (table 6). In case of *E. alpinus,* methanol extract showed lesser IC_{50} value (38.75 µg/mL) than acetone extract (45.34 µg/mL) (table 7) thereby confirming it as good antioxidant agent.

Table 6 : Free radical scavenging activity (%) of the stem extract of the *G. moorcroftiana* at different concentrations in acetone and methanol solvents.

Conc. (µg/mL)	Acetone extract (%)	IC_{50} value (µg/mL)	Methanol extract (%)	IC_{50} value (µg/mL)
20	50.29±00.37	13.32	38.92±01.14	41.20
40	62.17±00.66		47.39±00.70	
60	73.86±01.19		61.56±01.07	
80	79.20±00.98		72.80±00.70	
100	84.75±00.96		78.01±00.76	

Table 7 : Free radical scavenging activity (%) of the whole plant extracts of the *E. alpinus* at different concentrations in acetone and methanol solvents.

Conc. (µg/mL)	Acetone extract (%)	IC_{50} value (µg/mL)	Methanol extract (%)	IC_{50} value (µg/mL)
20	36.43±00.84	45. 34	39.08±00.81	38.75
40	47.92±00.93		50.32±00.49	
60	58.81±01.02		62.70±00.79	
80	68.91±00.59		73.77±00.62	
100	70.49±00.76		78.66±01.10	

Discussion

Antibacterial activity screening

Present investigation showed results for antibacterial activity and it was observed that the methanol extracts of the both plants exhibited maximum zone of inhibition i.e. 23.60 mm by leaf-flower mixture extract of the *G.*

moorcroftiana, 23.07 mm by stem extract of the *G. moorcroftiana* and 22.93 mm by whole plant extract of *E. alpinus* against *Y. pestis.* Acetone and methanol leaf-flower mixture extract and acetone stem extract of *G. moorcroftiana* showed no inhibition against *L. monocytogenes,* while in case of *E. alpinus* minimum inhibition was shown by methanol extract against *E. coli*.

Rahman and Kang (2008) investigated the antibacterial activity of the *Erigeron ramosus* against many bacterial strains and found that methanol extract showed antibacterial activity against *S. aureus*, *L. monocytogenes*, *B. subtilis*, *P. aeruginosa* and *E. coli*. *Gentianella nevadensis* species was also investigated for antibacterial activity against *S. aureus*, *E. coli*, *P. aeruginosa* and *B. subtilis* by the Baez *et al.* (1999) and found to be active against *S. aureus* and *B. subtilis.* Bussmann *et al*. (2008) also examined the antibacterial activity of the *Gentianella alborosea, Gentianella bicolor* and *Schkuhria pinnata* against *E. coli* and *S. aureus* and reported that only *G. alborosea* and *S. pinnata* exhibited activity against *S. aureus.*

Antioxidant activity screening

According to our results, better antioxidant activity was observed in methanol extracts, however in case of the stem extract of *G. moorcroftiana* acetone extract gives stronger activity than methanol extract.

E. annuus was examined for antioxidant activity by the Lee and Seo (2006) using DPPH free radical scavenging assay and it was found that the aqueous, methanol and n-butanol showed higher activity. Lima *et al*. (2012) investigated antioxidant activity of *G. multicaulis* in different extracts and proposed that dichloromethane and methanol extracts had strongest activity among all extracts. Five species of *Gentiana (G. asclepiadea, G. cruciata, G. olivieri, G. septemfida, G. verna)* and *G.caucasea* were examined by Senol *et al*. (2012) using DPPH free radical scavenging assay and result that came out from investigation indicated that methanol extracts of all the species showed significant free radical scavenging activity.

Conclusion

Conclusion that came out from the performed investigation was that both the medicinal plants (*Gentianella moorcroftiana* and *Erigeron alpinus*) showed considerable activity against tested pathogenic bacteria except acetone and methanol leaf and flower mixture, and acetone stem extracts of the *G. moorcroftiana* against *L. monocytogenes. Y. pestis* was found inhibited by both the plant extracts at maximum extent. In case of antioxidant activity, methanol extracts of both the plant extracts exhibited higher antioxidant activity than acetone extract except in case of stem extract of *G. moorcroftiana* where acetone extract showed higher antioxidant activity than methanol extract.

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