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ANALYSIS OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF *GERANIUM NEPALENSE* SWEET.

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Abstract

The efficacy and safety of herbal medicines have attracted the major pharmaceutical companies towards medicinal plants research. The current study was designed to investigate the leaf and root extracts of *Geranium nepalense* Sweet for its antibacterial and antioxidant activity. Traditionally different parts of the plant are used to cure ulcers, haemorrhoids and renal infections. The antibacterial activity of the acetone and methanol plant extracts was determined *in-vitro* against medically important pathogens such as *Escherichia coli, Shigella dysenteriae, Listeria monocytogenes and Staphylococcus aureus* following Agar-well diffusion method using different concentrations (25%, 50%, 75% and 100%) of plant extracts. Results showed low to significant antibacterial activity against the mentioned bacterial species. Leaf extracts (acetone and methanol) was found to be more effective against *Staphylococcus aureus* as compared to other bacterial species. Further, the maximum activity (*i.e.* 17.7mm) was shown by acetone root extract against *Shigella dysenteriae.* The antioxidant capacity of different extracts (methanol and acetone) of *Geranium nepalense* Sweet was evaluated by DPPH (2, 2-diphenyl-1-picrylhydrazyl) at different concentrations (20-100 µg/mL). Methanol root extract exhibited higher antioxidant capacity *i.e.* 18.79µg/mL with lesser IC_{50} value. Therefore, the leaf and root extracts of this plant need to be selected for further investigation to determine their therapeutic potential.

Key words*: Geranium nepalense* Sweet, plant extracts, Agar-well diffusion, DPPH.

Introduction

The world's leading cause of premature deaths is infectious diseases, killing almost 50,000 people every day (Ahmad *et al*., 2001). To overcome and control such a variety of diseases, several hundred genera of medicinal plants are used by a large portion of the world population, especially in developing countries (Ahmad *et al*., 1998). Medicinal plants are a source of great economic value in the Indian subcontinent. These plants contain the components of therapeutic value and have been used for centuries as remedies for human diseases (Nostro *et al*., 2000).

India with respect to the traditional knowledge is one of the leading countries in Asia (Pant *et al*., 2009). Himachal Pradesh, one of the pioneer states of the Himalaya, has great wealth of plants with medicinal value (Boktapa and Sharma, 2007). There are about 3500 known plant species present in Himachal Pradesh of which around 500 plant species have proven medicinal importance (Chauhan, 2003).

Due to the present scenario of emerging multiple drug resistance to various pathogenic bacteria, there is a strong need of searching new antimicrobial substances from the traditional medicinal plants (khan *et al*., 2017). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to screening of several medicinal plants for their potential antimicrobial activity (Martins *et al*., 2001). The antibacterial and antioxidant properties of plants are due to the presence of many active phytochemicals also known as plants secondary metabolites including flavonoids, terpenoids, carotenoids, cumarins, alkaloids, lignins, saponins etc (Calucci *et al*., 2003).

Experimental evidences show that the large numbers of diseases are caused by the free radicals (FR) and reactive oxygen species (ROS) (Richards and Sharma, 1991). Natural antioxidants possess the wide range of biochemical activities which include the alteration of intra cellular redox potential, inhibition of ROS generation and direct or indirect scavenging of free radicals (Abdollahi *et al*., 2005). So the medicinal plants with antioxidant potential are usually employed as an alternative source

of medicine to alleviate the diseases associated with oxidative stress (Sharma *et al*., 2013).

Geranium nepalense Sweet is an annual or perennial herb belonging to Geraniaceae family native to China, Japan and in India throughout Himalayas. In Himachal Pradesh, this plant has been reported from Chamba, Kangra, Kinnaur, Kullu, Lahaul & Spiti, Shimla and Sirmaur districts. The plant is used as an astringent and in certain renal diseases. Also used mainly for ulcers and haemorrhoids. The roots have been reported to contain, tannis, gallic acid, quercetin, citric acid, and are used for colouring medicinal preparation and oils. They are used in tanning industry and plant oil perfumery (Lata, 2014). It is in view of this, that the present research was set up to evaluate the antibacterial and antioxidant activity of *G. nepalense*.

Materials and Methods

Collection of plant material:

The whole plant *Geranium nepalense* Sweet was plucked and collected from Summer Hill area of District Shimla, Himachal Pradesh, India. The collected plant material was brought to the laboratory for further analysis.

Procurement of bacteria:

Different strains of bacteria (*Staphylococcus aureus, Escherichia coli, Shigella dysenteriae* and *Listeria monocytogenes*) have been procured from IGMC, Shimla and Department of Microbiology & Biotechnology, H.P. University Shimla for screening antibacterial properties of different plant extracts.

Revival of pathogen:

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

Table 1: Zones of inhibition produced by leaf extract of *Geranium nepalense* Sweet at different concentrations in acetone and methanol.

Extract	Conc. (%)	Inhibition zone diameter in mm (±S.E.)			
		S. aureus	*E. coli*	*L. monocytogenes*	*S. dysenteriae*
Acetone extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	9.8±0.17	12.3±0.33	9.8±0.17	12.3±0.17
	50	12.7±0.33	13.5±0.29	11.5±0.29	13.7±0.33
	75	14.7±0.33	15.2±0.17	13.5±0.29	15.0±0.00
	100	16.3±0.33	16.2±0.17	15.7±0.33	15.7±0.17
Methanol extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	10.7±0.33	10.2±0.17	10.7±0.33	10.8±0.17
	50	11.7±0.33	11.7±0.33	12.7±0.33	12.7±0.33
	75	13.7±0.33	13.3±0.33	14.7±0.33	14.7±0.33
	100	16.3±0.33	14.7±0.33	16.0±0.00	15.8±0.17

Each data point represents mean of three replicates± S.E. (Standard error).

Maintenance and preservation of pure cultures:

Pure cultures of all the bacteria were maintained on nutrient medium broth and preserved in refrigerator. Sub-culturing was done at regular intervals in order to maintain the cultures. Each bacterial species was transferred from parent source to maintain and preserve the cultures.

Processing of plant material:

Whole plant was first washed under tap water and then treated with 2% Mercuric chloride. After that the leaves and roots were cut into smaller pieces and allowed to shade dry for 15-20 days. After drying plant material was 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:

Acetone and methanol extracts of dried leaves and roots were prepared to screen antimicrobial activity. 3 gm of material was taken in Erlenmeyer flask to which 30 ml of required solvents (*i.e.* methanol and acetone) were added. The flask was covered with aluminium foil and placed at safe place for 3-5 days for extraction. Material was filtered using Whatman filter paper no.1 and the extract was evaporated at 40ºC using rotary evaporator. The extract was collected and weighed. At last, a stock solution of 50 mg/ml conc. was prepared.

Antibacterial activity test using Agar-well diffusion method:

Different extracts (acetone and methanol) of *Geranium nepalense* Sweet were screened using agar-well diffusion method. Nutrient agar medium (Beef extract 1g, Yeast extract 2g, Sodium Chloride 1g, Peptone 5g, Agar 20g, Distilled Water 1000mL) was used throughout the investigation. The medium was autoclaved at 121.6ºC 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% concentration of prepared plant extracts. The Petri plate kept as a control contained pure solvent only. The plates were incubated at 37 ± 2ºC 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.

Percentage of growth inhibition (%) =

$$\left(\frac{Control - Test}{Control}\right) \times 100$$

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

Antioxidant activity test by DPPH radical scavenging activity assay:

The free radical scavenging activity of plant extracts was measured using 2, 2 Diphenyl-1-picrylhydrazyl (DPPH) as described by Blois (1958). Briefly, to 1mL of different concentrations (5, 10, 15, 20 and 25 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:

DPPH scavenging effect (%) =

$$\left(\frac{Acontrol - Asample}{Acontrol}\right) \times 100$$

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

Table 2: Zones of inhibition produced by root extract of *G. nepalense* at different concentrations in acetone and methanol.

Extract	Conc. (%)	Inhibition zone diameter in mm (±S.E.)			
		S. aureus	*E. coli*	*L. monoc -ytogenes*	*S. dysen -teriae*
Acetone extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	11.3±0.33	10.8±0.17	11.3±0.33	11.2±0.17
	50	14.0±0.00	13.7±0.33	14.0±0.00	13.7±0.33
	75	15.0±0.00	13.7±0.33	15.2±0.17	14.3±0.33
	100	17.0±0.00	17.3±0.33	15.0±0.00	17.7±0.33
Methanol extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	10.3±0.33	9.7±0.33	10.2±0.44	10.8±0.44
	50	13.3±0.33	12.5±0.29	13.0±0.00	12.7±0.33
	75	14.5±0.33	15.0±0.00	14.3±0.33	14.3±0.33
	100	15.3±0.33	16.0±0.00	14.7±0.33	15.3±0.33

Each data point represents mean of three replicates ± S.E. (Standard Error)

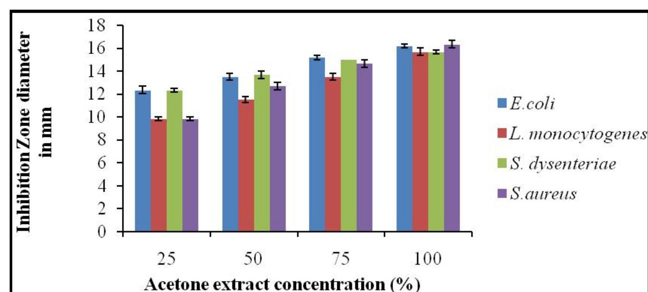

Fig. A: Antibacterial activity of Acetone leaf extract of *G. nepalense.*

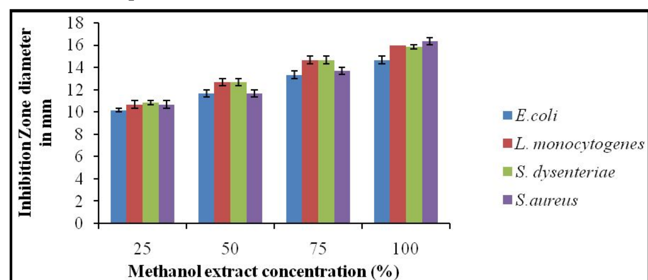

Fig. B: Antibacterial activity of Methanol leaf extract of *G. nepalense*.

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:

$$IC_{50} = \left(\frac{50 - Y - Intercept}{Slope}\right)$$

Results and Discussion

Antibacterial activity screening:

The antibacterial activity of plant extracts of *G. nepalense* was determined by Agar-well diffusion method and the results of the above assay are shown in tables 1, 2 and in Figs. A-D. Results came out from the tables concluded that at the minimum concentration (25%) of methanol extract of leaf, zones of inhibition for *S. aureus, E. coli, S. dysenteriae* and L. *monocytogenes* were 10.7 mm, 10.2 mm, 10.8 mm and 10.7 mm respectively.

Similarly at 100% concentration zones of inhibition were 16.3 mm, 14.7 mm, 15.8 mm and 16.0 mm respectively for test bacteria. At 25% concentration of acetone extract of leaf, zones of inhibition were 9.8 mm, 12.3 mm, 12.3 mm and 9.8 mm and similarly at 100% concentration, zones of inhibition were 16.3 mm, 16.2 mm, 15.7 mm and 15.7 mm respectively table 1.

Table 3: Free radical scavenging activity (%) of the control *i.e.* ascorbic acid at different concentrations.

Ascorbic acid (Control)	Concentration (µg/mL)	Free radical scavengingactivity (%)	IC_{50} Value (µg/mL)
	5	47.80±0.006	5.84
	10	53.65±0.004	
	15	59.08±0.001	
	20	61.37±0.002	
	25	63.13±0.001	

In case of methanol root extract of this plant, at 25% conc., zones of inhibition for *S. aureus, E. coli, S. dysenteriae* and L. *monocytogenes* were 10.3 mm, 9.7 mm, 10.8 mm and 10.2 mm and alike at 100% conc., zones of inhibition were 15.3 mm, 16.0 mm, 15.3 mm and 14.7 mm respectively. For acetone root extracts, zones of inhibition at 25% conc. were 11.3 mm, 10.8 mm, 11.2 mm and 11.3 mm. Similarly, for 100% conc. zone of inhibition were 17.0 mm, 17.3 mm, 17.7 mm and 15.0 mm respectively Table 2.

Antioxidant activity:

Tables 3-5 and Figs. E-G show the results for antioxidant activity of acetone and methanol extracts of selected medicinal plant. Ascorbic acid was taken as the control which exhibited IC_{50} value of 5.84µg/mL table 3.

Methanol root extract of *G. nepalense* showed higher antioxidant activity with 18.79 µg/mL IC50 value than acetone extracts with 20.23 µg/mL IC50 value table 4. But in case of leaf extract of *G. nepalense*, acetone extracts showed less IC50 value (29.64 µg/mL) than methanol extracts (31.28 µg/mL) table 5.

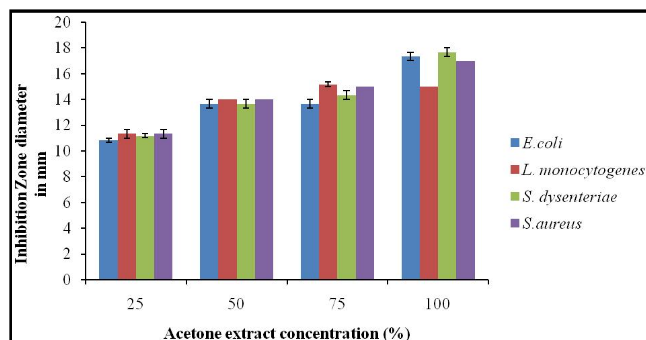

Fig. C: Antibacterial activity of Acetone root extract of *G. nepalense*.

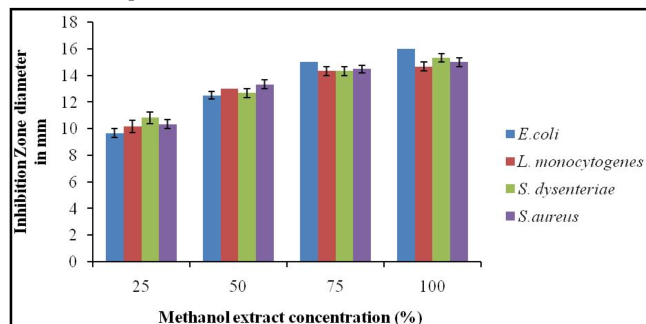

Fig. D: Antibacterial activity of Methanol root extract of *G. nepalense*.

Table 4: Free radical scavenging activity (%) of the leaf extract of the *Geranium nepalense* Sweet at different concentrations in methanol and acetone.

Name of the plant	Concentra-tion(µg/mL)	Acetone extract	IC_{50} Value (µg/mL)	Methanol extract	IC_{50} Value (µg/mL)
G. nepalense (leaf)	5	14.70±0.002	29.64	25.56±0.001	31.28
	10	21.17±0.001		29.24±0.002	
	15	28.35±0.002		32.74±0.001	
	20	34.02±0.003		38.71±0.002	
	25	44.80±0.002		45.00±0.001	

Table 5: Free radical scavenging activity (%) of the root extract of the *Geranium nepalense* Sweet at different concentrations in methanol and acetone.

Name of the plant	Concentra-tion(µg/mL)	Acetone extract	IC_{50} Value (µg/mL)	Methanol extract	IC_{50} Value (µg/mL)
G. nepalense (root)	5	18.01±0.011	20.23	13.66±0.006	18.79
	10	32.15±0.011		29.59±0.019	
	15	41.24±0.007		40.28±0.001	
	20	48.82±0.012		53.23±0.006	
	25	58.58±0.013		65.14±0.007	

Discussion

Antibacterial activity screening:

It has been observed that with increase in concentration of leaf and root extracts, the zones of inhibition also increased. In case of acetone and methanol leaf extracts maximum zone of inhibition *i.e.* 16.3 mm was reported at 100% concentration against bacterium *S. aureus*. In case of root extracts maximum diameter of zone of inhibition (17.7mm) was reported for the acetone extract against *S. dysenteriae* while for its methanol extract maximum diameter of zone of inhibition was 16.0mm against *E. coli*. Antibacterial activities of methanol and acetone extracts of the plant showed positive results against all the bacteria.

Ismail *et al*., (2012) investigated the crude extracts of rhizomes and leaves of *Geranium wallichianum.* The results indicated that the crude extracts and different fractions of rhizomes and leaves showed varied degree of antimicrobial properties.

Graca *et al*., (2016) studied the phytochemical composition and biological activities of *Geranium robertianum* L. commonly known as Herb Robert. The

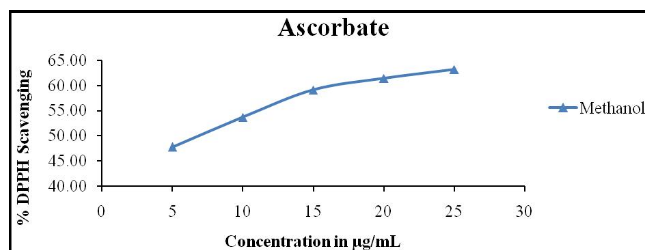

Fig. E: Scavenging activity of L-Ascorbate at different concentration in methanol.

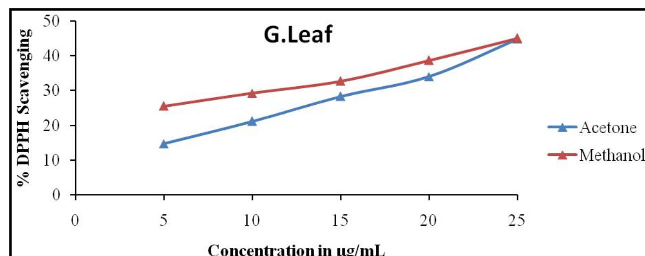

Fig. F: Free radical scavenging activity of the acetone and methanol leaf extract of the *G. nepalense*.

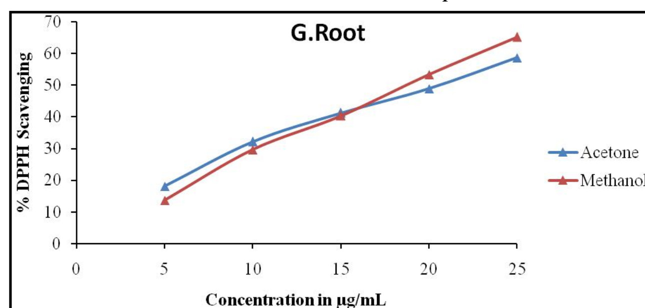

Fig. G: Free radical scavenging activity of the acetone and methanol root extract of the *G. nepalense*.

chemistry of *G. robertianum* is clearly dominated by tannins, flavonoids and phenolic acids and possesses the antimicrobial and anti-inflammatory activities. The antibacterial and antioxidant properties of plants are due to the presence of many active phytochemicals. There is a need to investigate the phytochemicals present in this plant and its antimicrobial potential can also be attributed to the presence of certain biochemicals in it.

Antioxidant activity:

As per the results, better antioxidant activity was observed in methanol root extracts, however in case of the leaf extract of *G. nepalense* acetone extract gave stronger activity than methanol extract. Xiufen *et al.*, (2004) examined the radical scavenging activity of seven traditional Japanese herbs including *Geranium nepalense var.* thunbergii. The extracts were made in water, petroleum ether and ethyl ether solvents. The radical scavenging activity was determined by the decrease of free radicals of DPPH detected by both colorimetric assay and HPLC method at 517 nm. The results showed remarkable radical scavenging activity.

Lee *et al.*, (2003) tested one hundred sixty species of Korean medicinal plants for their antioxidative potentials. Among them *Geranium nepalense* var. thunbergii showed 23.9 µg/mL value in comparison with α- tocopherol of 13.5µg/mL in $RC_{50.}$ The results on study of antioxidant potential of *Geranium nepalense* are in agreement with the studies of earlier workers and this can be attributed to presence of bioactive molecules which needs further investigation.

Conclusion

From the performed investigation it was concluded that the selected plant showed considerable activity against all the tested pathogenic bacteria. Maximum diameter of zone of inhibition (17.7mm) was reported for the acetone root extract against *S. dysenteriae.* In case of antioxidant activity, methanol root extracts showed stronger activity than the other extracts. This study suggests that the plant extracts possess potent antibacterial and antioxidant activity, which might be helpful in preventing or slowing the progress of various bacterial and oxidative stress-related diseases.

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