Plant Archives Vol. 19 No. 1, 2019 pp. 1692-1698 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

STUDIES ON ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS OF *CLEMATIS GRATA* WALL

Ashwani Kumari, Anand Sagar and Ved Prakash*

Department of Biosciences, H.P. University, Shimla- 171005 (H.P.) India.

Abstract

In-vitro antibacterial and antioxidant activities of acetone, distilled water and methanol extracts of leaf, root and stem of *Clematis grata* Wall. were investigated. Antibacterial activity was determined by employing Agar-well diffusion method against human pathogenic bacteria: two Gram-positive (*Bacillus cereus* and *Staphylococcus aureus*) and two Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*) at different concentrations *i.e.* 25, 50, 75 and 100%. For the examination of antioxidant activity DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging activity assay was used at various concentrations *viz.,* 5, 10, 15, 20 and 25 µg/mL. Ascorbic acid was taken as a standard. The most effective plant extract was methanol extract of stem exhibiting maximum zone of inhibition (ZOI) against *Staphylococcus aureus* (20.66±0.54 mm). Remarkable antioxidant potential was exhibited by the methanol stem extract with IC_{50} value of 11.39 µg/mL, methanol leaf extract with IC_{50} value of 11.55 µg/mL and acetone extract of stem with IC_{50} value of 15.75 µg/mL. The results indicate the significant antibacterial and antioxidant potential of tested plant extracts to allow future exploration and utilization as a natural antibacterial and antioxidant agent.

Key words : *Clematis grata*, different plant extracts, Agar-well diffusion method, DPPH free radical scavenging assay.

Introduction

21st century, is the century of tech savvy generations, also the century of the most grievous diseases developed by oxidative stress and microbes becoming more and more drug resistant with time. Alone microbial infections caused 50,000 deaths everyday throughout the globe (Anonymous, 2000). For the protection of mankind, the microbes causing infectious diseases must be controlled. Frequently used antibiotics due to their repeated use resulted in drug resistant bacteria which further transmit this power of resistance vertically to same species and horizontally to other bacterial species (Pattanayak, 2018).

The second leading cause of dangerous diseases is oxidative stress caused by excess production of Reactive Oxygen Species (ROS) in the body due to redox disequilibrium in normal metabolic reactions occurring usually in mitochondria, peroxisomes and endoplasmic reticulum. Oxidative stress is a pivotal player in pathogenesis of various diseases including chronic diseases, atherosclerosis, neurodegenerative diseases, rheumatoid arthritis, diabetes and cancer initiation (Chen *et al.*, 2013). Also due to lipid peroxidation, food products get spoiled because of ROS. Synthetic antioxidants being used for slowing down oxidative damage such as Butylated Hydroxyl Toluene (BHT) and Butylated Hydroxyl Anisole (BHA) have toxic effects on humans.

This scenario of multi-drug resistant bacteria and toxicity of synthetic antioxidants prompt us to evaluate new prototype antibacterial and antioxidant agents from natural sources including plants (Ahmad *et al*., 2000) as plants are always the savior of mankind. Medicinal plants are being used from ancient times in the Ayurvedic, Unani and other traditional systems of medicine for the treatment of diseases. These effects of plants are due to the presence of phytochemicals, one class of phytochemicals is phenolic compounds having redox property such as flavonoids, phenolic acids, tannins, hydroxycinnamic acid and phenolic diterpenes which are among the best known for their medicinal properties (Prakash *et al*., 2017). Presently 80% of population of developed countries have been using drugs obtained from medicinal plants (WHO, 2002). Even developing countries are also marching on

***Author for correspondence :** E-mail : vedp685@gmail.com

the same path as they contain a number of traditionally used medicinal plants, need to be explored yet.

Investigated medicinal plant *Clematis grata* Wall. belongs to family Ranunculaceae and has been used commonly as a herbal medicine to cure skin diseases. The bioactive potential of these perennial herbs is related to their good content of phytochemical compounds including triterpenoids, saponins, flavonoids, polyphenols and alkaloids (Hao *et al*., 2004; Chen *et al*., 2009; Li *et al*., 2009). Antibacterial activity was determined against two Gram-positive and two Gram-negative bacteria, while DPPH free radical scavenging assay was used for the determination of antioxidant potential of this plant.

Materials and Methods

Collection of plant material

Plant parts (Leaves, stem and roots) of *Clematis grata* Wall. were collected in the months of March and April from a village named Kandraur situated at an altitude of 375 m above sea level of Bilaspur district of Himachal Pradesh.

Processing of plant material

All the plant parts used were washed under running tap water and then surface sterilized with 2% Mercuric Chloride. All parts were then allowed to shade dried for 5-20 days. Dried plant materials were then crushed to make fine powder with the help of mortar-pestle. Prepared fine powders were kept at room temperature in separate air tight storage containers.

Preparation of acetone, distilled water and methanol extracts

Five grams of leaf/root/stem of *C. grata* were taken in three different Erlenmeyer flasks to which 50 mL of acetone/distilled water/methanol were added, followed by covering the flasks with aluminium foils, flasks were allowed to stand for 3-5 days for extraction purposes. After extraction, the extracts were filtered with Whatman filter paper no. 1 and evaporation was done with the help of rotary evaporator at 40°C. Dried extracts were collected and weighed to prepare stock solutions of conc. 50 mg/mL.

Procurement of bacteria

Bacterial species used for analysis of antibacterial activity were *Bacillus cereus, Escherichia coli, Salmonella typhi* and *Staphylococcus aureus* which were procured from Indira Gandhi Medical College, Shimla, and Department of Microbiology and Biotechnology, Himachal Pradesh University, Summer Hill, Shimla, India.

Revival of pathogens

The revival of collected bacterial pathogens was done in nutrient broth and storage was done in nutrient agar slants at 4°C.

Screening of antibacterial activity of different extracts of *C. grata*

Different extracts *viz*., acetone, distilled water and methanol of *C. grata* were examined 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 and Distilled Water 1000 mL) was taken throughout the investigation. Autoclaving at 121.6°C for 30 minutes was done to sterilize the medium and then after a while poured into Petri plates. Bacteria were allowed to grow in nutrient broth for 24 hours. A bacterial suspension of 100 µL was spread on each solidified nutrient agar plate. Agar wells of 8 mm diameter were made in each Petri plate with the help of sterilized stainless steel borer. The wells were loaded with 25, 50, 75 and 100% concentration of prepared plant extracts in each plate. The wells taken as control contained pure solvents only. The plates were incubated at 37±2°C for 24 hours in incubation chamber. The zone of inhibition (ZOI) of bacterial growth was calculated by measuring the diameter of the inhibition zone (in mm) around the well including the well diameter. Measurements were taken in perpendicular direction in all the three replicates and the average values were tabulated. Streptomycin was used as a standard. (Prakash *et al.,* 2016; Rana *et al.,* 2016).

Screening of antioxidant activity of different extracts of *C. grata*

DPPH free radical scavenging activity assay

The percent free radical scavenging activity of plant extracts was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described by Blois (1958) with slight modifications. Briefly, to 1 mL of different concentrations *viz*., 5, 10, 15, 20 and 25 µg/mL of plant extracts, 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:

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

Where, Acontrol is the absorbance of control; Asample 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 scavenge the initial DPPH concentration by 50%) for each extract and ascorbic acid was evaluated using the following equation given below:

$$IC_{50} = \frac{50 - Y - Intercept}{Slope}$$

Results

Screening of antibacterial activity of different extracts of *C. grata*

Table 1.1-1.3 and Fig. A-F highlighted the results of antibacterial activity of leaf, root and stem of *Clematis grata* Wall. displaying gradual increase in zone of inhibition (ZOI) with increasing concentrations against all the tested bacteria. Results from Table 1.1 concluded that methanol extract of leaf showed remarkable antibacterial activity against all the tested bacteria with maximum ZOI against *Staphylococcus aureus* (19.33±1.18 mm) and minimum ZOI against *Salmonella typhi* (12.33±0.26 mm). Also, acetone extract of leaf of this plant was found to be most active against same bacterium *S. aureus* (ZOI=13.66±0.26 mm). Distilled water extract was not active against *S. typhi* and *Escherichia coli*. As per Table 1.2, methanol extract of root displayed remarkable antibacterial activity against all the tested bacteria with ZOI of 17.00±0.47, 16.00±0.82, 13.00±0.46 and 10.66±0.26 mm against *Bacillus cereus, S. aureus*, *E. coli* and *S. typhi,* respectively. The rest of the two extracts namely acetone and distilled water displayed no appreciable antibacterial activity against the tested bacteria. As per Table 1.3, acetone extract of stem showed ZOI of 8.66±0.27, 9.33±0.27, 13.66±1.44 and 16.66±15.66 mm from concentrations 25, 50, 75 and 100% respectively in case of *B. cereus*. Methanol stem extract exhibited maximum activity against *S. aureus* with ZOI of 20.66±0.54 mm.

Screening of antioxidant activity of different extracts of *C. grata*

Table 2.1-2.4 and Fig. G-J demonstrated the results

Table 1.1 : Screening of antibacterial activity of different extracts of leaves of *C. grata.*

Extract	Concentrations (%)	Zone of inhibition (mm±S.E.)			
		B. cereus	***S. aureus***	***E. coli***	***S. typhi***
Acetone extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	8.33±0.046	8.66±0.46	0.00±0.00	0.00±0.00
	50	8.66±0.26	9.33±0.26	8.66±0.26	9.00±0.00
	75	9.00±0.26	12.00±0.46	9.33±0.26	9.33±0.26
	100	11.00±0.46	13.66±0.26	10.33±0.54	11.66±0.54
Distilled water Extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	9.66±0.27	9.33±0.27	0.00±0.00	0.00±0.00
	50	11.66±0.27	11.00±0.47	0.00±0.00	0.00±0.00
	75	12.00±0.27	11.66±0.26	0.00±0.00	0.00±0.00
	100	12.33±0.72	12.66±0.26	0.00±0.00	0.00±0.00
Methanol Extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	9.00±0.00	9.66±0.26	9.00±0.00	9.00±0.00
	50	9.66±0.46	11.00±0.46	9.33±0.26	9.33±0.26
	75	10.66±0.98	13.00±0.46	11.66±0.72	12.00±1.63
	100	18.66±0.26	19.33±1.18	14.66±0.26	12.33±0.26

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

Table 1.2: Screening of antibacterial activity of different extracts of root of *C. grata.*

Extract	Concentrations (%)	Zone of inhibition (mm±S.E.)			
		B. cereus	***S. aureus***	***E. coli***	***S. typhi***
Methanol Extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	11.00±0.47	14.00±0.46	10.00±0.46	0.00±0.00
	50	13.66±0.72	15.33±0.26	11.00±0.46	0.00±0.00
	75	14.00±0.47	15.66±0.26	12.33±0.26	9.33±0.26
	100	17.00±0.47	16.00±0.82	13.00±0.46	10.66±0.26

Each mean data point represents of three replicates±S.E.

Table 1.3 : Screening of antibacterial activity of different extracts of stem of *C. grata.*

Extract	Concentrations (%)	Zone of inhibition (mm±S.E.)			
		B. cereus	***S. aureus***	***E. coli***	***S. typhi***
Acetone Extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	8.66±0.27	10.33±0.72	9.33±0.27	9.00±0.47
	50	9.33±0.27	12.33±0.27	10.00±0.047	9.66±0.54
	75	13.66±1.44	14.00±0.47	10.33±0.27	11.00±0.72
	100	15.66±0.72	14.66±0.72	12.33±0.72	12.66±0.54
Methanol extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	9.33±0.26	10.00±0.00	9.33±0.27	10.00±0.47
	50	10.66±0.27	11.66±0.26	10.00±0.47	11.66±0.54
	75	12.00±0.47	14.66±0.26	10.33±0.27	13.33±0.72
	100	17.33±1.52	20.66±0.54	14.33±0.72	15.33±0.72

Each mean data point represents of three replicates±S.E.

Table 2.1 : Percent free radical scavenging activity of ascorbic acid.

S. No.	Concentration (µg/mL)	Percent free radical scavenging activity±S.E.	IC_{50} value (µg/mL)
1	5	12.48±0.48	28.12
2	10	19.93±0.98	
3	15	27.22±0.63	
4	20	36.65±0.46	
5	25	45.37±1.8	

Each mean data point represents of three replicates±S.E.

Table 2.2 : Percent free radical scavenging activity of different extracts of leaf of *C. grata*.

Extract	Concentration (µg/mL)	Percent free radical scavenging activity±S.E.	IC_{50} value (µg/mL)
Acetone Extract	5	12.77±0.76	22.59
	10	22.07±0.68	
	15	35.60±0.43	
	20	41.86±0.09	
	25	56.44±1.09	
Distilled water Extract	5	2.88±0.40	56.85
	10	5.03±0.51	
	15	10.65±0.30	
	20	17.55±0.34	
	25	20.12±0.42	
Methanol Extract	5	24.53±0.19	11.55
	10	47.15±0.71	
	15	63.29±0.53	
	20	81.15±0.50	
	25	92.12±0.48	

Each data point represents mean of three replicates±S.E.

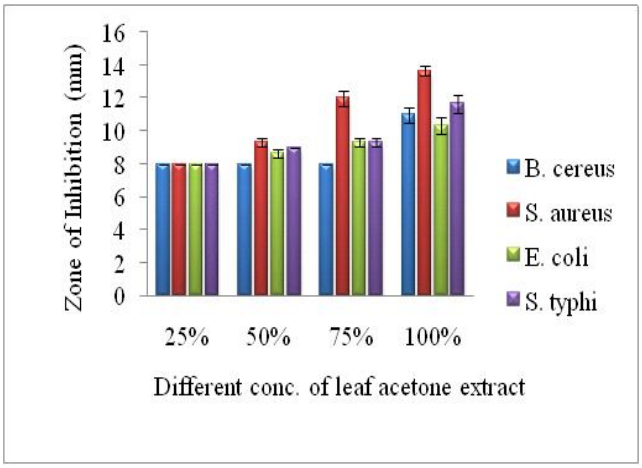

Fig. A : Antibacterial activity of acetone extract of leaf of *C. grata*.

of antioxidant activity for acetone, distilled water and methanol extracts of different parts (leaf, root and stem)

Table 2.3 : Percent free radical scavenging activity of different extracts of root of *C. grata*.

Extract	Concentration (µg/mL)	Percent free radical scavenging activity±S.E.	IC_{50} value (µg/mL)
Acetone Extract	5	2.55±0.46	49.01
	10	6.36±0.64	
	15	11.04±0.68	
	20	16.69±0.76	
	25	25.06±0.64	
Distilled water Extract	5	1.60±0.62	60.62
	10	4.69±0.47	
	15	8.23±0.71	
	20	12.74±0.37	
	25	19.81±0.60	
Methanol Extract	5	4.60±0.49	38.73
	10	10.48±0.62	
	15	16.75±0.49	
	20	24.62±0.45	
	25	31.65±0.42	

Each data point represents mean of three replicates±S.E.

Table 2.4 : Percent free radical scavenging activity of different extracts of stem of *C. grata*.

Extract	Concentration (µg/mL)	Percent free radical scavenging activity±S.E.	IC_{50} value (µg/mL)
Acetone extract	5	14.43±0.67	15.75
	10	27.80±0.51	
	15	34.46±2.32	
	20	72.96±1.26	
	25	86.17±1.08	
Distilled water extract	5	9.90±0.28	34.48
	10	15.40±0.56	
	15	21.88±0.70	
	20	27.72±1.14	
	25	38.74±1.13	
Methanol extract	5	30.38±1.45	11.39
	10	42.20±1.20	
	15	60.63±1.26	
	20	84.04±1.54	
	25	93.40±0.25	

Each data point represents mean of three replicates±S.E.

of *C. grata*. Ascorbic acid was used as a standard exhibiting IC_{50} value of 28.12 µg/mL Table 2.1. In case of leaf, out of all the extracts, methanol extract exhibited maximum percent inhibition with lowest IC_{50} value of 11.55 µg/mL followed by acetone extract with IC_{50} value of 22.59 µg/mL and distilled water extract with IC_{50} value of 56.85 µg/mL table 2.2. In case of root, methanol extract

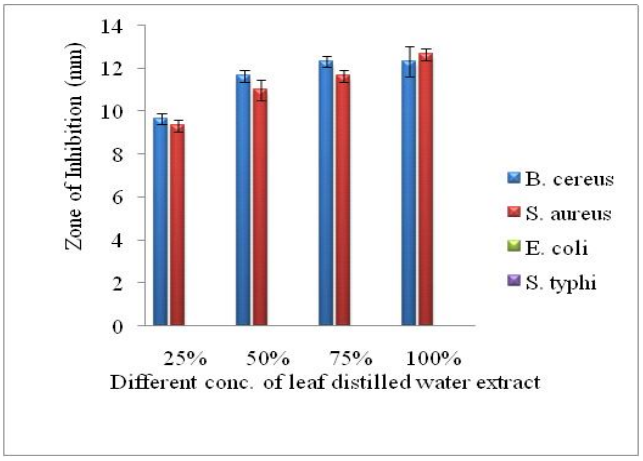

Fig. B : Antibacterial activity of distilled water extract of leaf of *C. grata.*

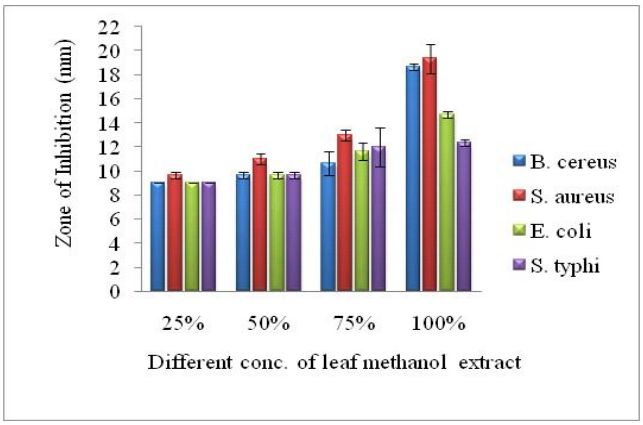

Fig. C : Antibacterial activity of methanol extract of leaf of *C. grata.*

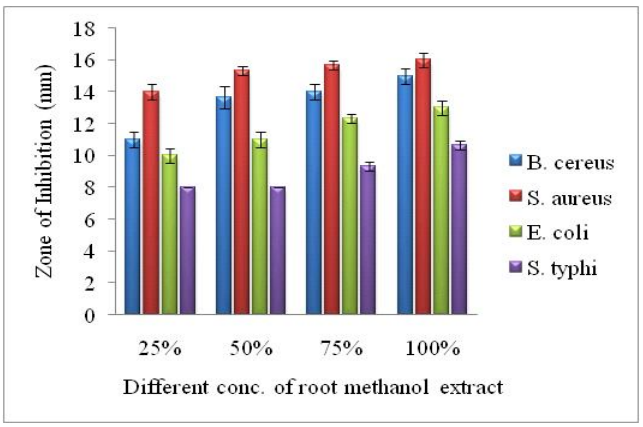

Fig. D : Antibacterial activity of methanol extract of Roof *C. grata.*

had IC_{50} value of 38.73 µg/mL while IC_{50} value of root extract prepared in acetone was 49.01 µg/mL. Stem methanol extract had IC_{50} value of 11.39 µg/mL whereas acetone extract had IC_{50} value of 15.75 µg/mL and distilled water extract had IC_{50} value of 34.48 µg/mL.

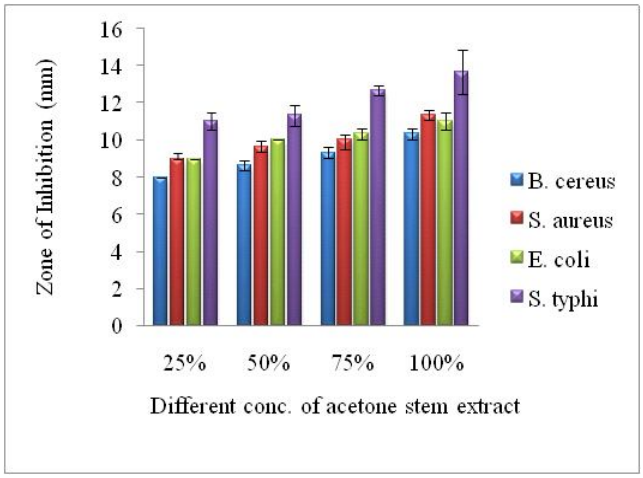

Fig. E : Antibacterial activity of acetone extract of Stem of *C. grata.*

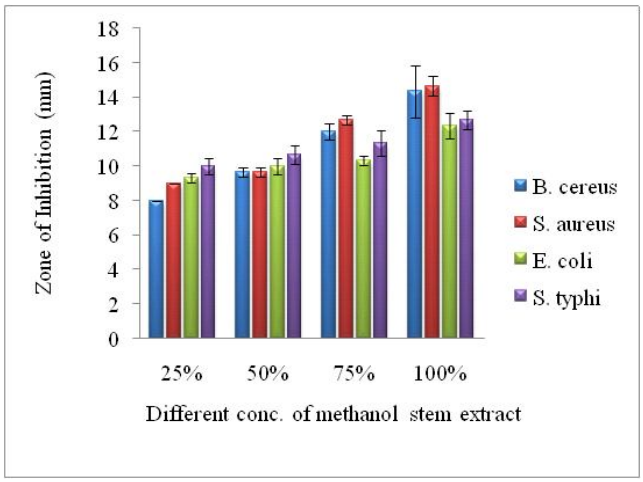

Fig. F : Antibacterial activity of Methanol extract of Stem of *C. grata.*

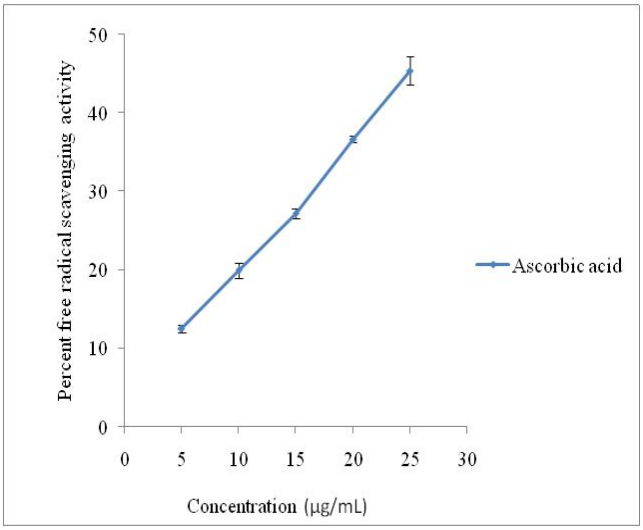

Fig. G : Percent free radical scavenging activity of ascorbic acid used as standard.

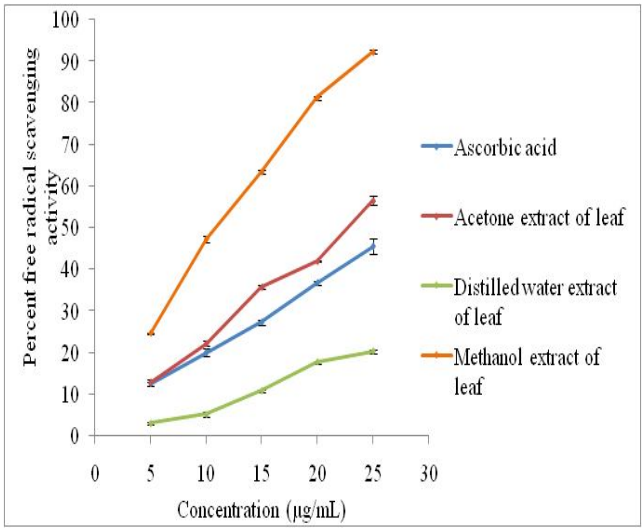

Fig. H : Percent free radical scavenging activity of different extracts of leaf of *C. grata*.

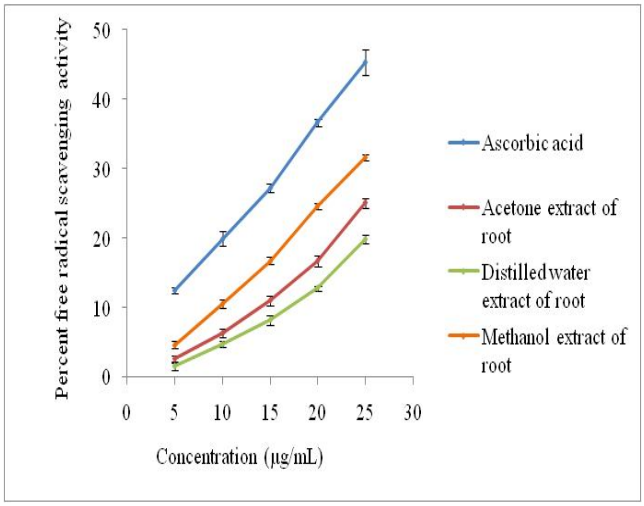

Fig. I : Percent free radical scavenging activity of different extracts of root of *C. grata*.

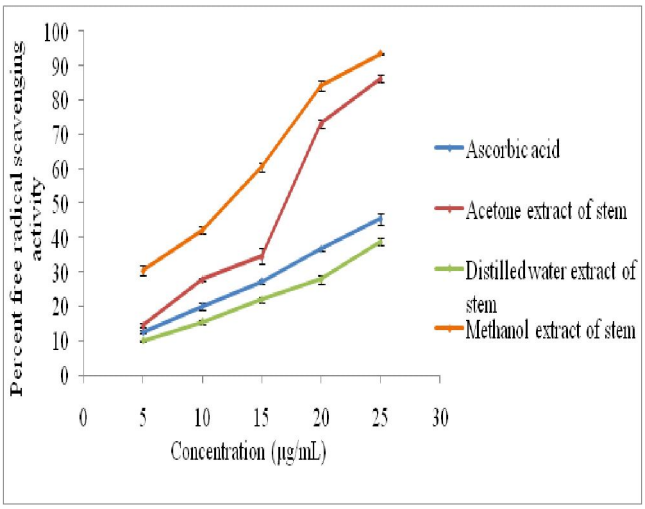

Fig. J : Percent free radical scavenging activity of different extracts of stem of *C. grata*.

Discussion

Screening of antibacterial activity of different extracts of *C. grata*

It was clear from the results that methanol extract of leaf of *C. grata* showed maximum antibacterial activity against *S. aureus* at 25, 50, 75 and 100% concentration with zone of inhibitions 9.66±0.26 mm, 11.00±0.46 mm, 13.00±0.46 mm and 19.33±1.18 mm, respectively. Acetone extract of leaf was also found to be most effective against *S. aureus* with zone of inhibition of 13.66±0.26 mm at 100% concentration. Methanol extract of root was most active against *B. cereus* with zones of inhibition of 11.00±0.47 mm, 13.66±0.72 mm, 14.00±0.47 mm and 17.00±0.46 mm at 25, 50, 75 and 100% concentrations, respectively. Methanol extract of the stem was found to be most effective against all tested bacteria *viz*., *B. cereus, S. aureus*, *E. coli* and *S. typhi* displaying ZOI of 17.33±1.52 mm, 20.66±0.54 mm, 14.33±0.72 mm and 15.33±0.72 mm respectively. No previous literature was found related to present investigations.

Screening of antioxidant activity of different extracts of *C. grata*

Present investigations showed that the methanol extracts of leaf and stem of *C. grata* possess greater free radical scavenging activity in comparison to ascorbic acid (IC_{50} value=28.12 µg/mL) with IC_{50} value of 11.29 µg/mL in case of stem methanol extract followed by methanol extract of leaf with IC_{50} value of 11.55 µg/mL. Also, the acetone extract of stem and leaf have better free radical scavenging activity than ascorbic acid with IC_{50} value of 15.75 µg/mL and 22.59 µg/mL, respectively.

Atmani *et al*., 2011 examined antioxidant potential of another species of this genus, *C. flammula* and the extracts tested were aqueous phases obtained from ethyl acetate and chloroform. The aqueous phase obtained from ethyl acetate exhibited IC_{50} value of 56.5 µg/mL and that of chloroform showed IC_{50} value of 48.4 µg/mL. Furthermore, Zhang *et al*., 2015 examined antioxidant and anti-inflammatory phenolic glycosides from *C. tashiroi* ethanol extract. Antioxidant determination was done by DPPH free radical scavenging activity assay. The results demonstrated that the plant glycosides have good antioxidant potential. These significant antibacterial and antioxidant activity were attributed to the great phenolic content of this plant *viz*., saponins, triterpenoids etc (Hao *et al*., 2004; Chen *et al*., 2009; Li *et al*., 2009).

Conclusion

From present investigation, it can be adjudicated that the methanol leaf, root and stem extracts of *Clematis*

grata Wall. were the most active extracts showing maximum ZOI against *S. aureus* (19.33±1.18 mm, 16.00±0.82 mm and 20.66±0.54 mm, respectively). Also, active antioxidant potential of plant *C. grata* was highlighted throughout the investigation. Methanol extracts of all tested plant parts exhibited great antioxidant activity as per DPPH free radical scavenging activity assay with IC_{50} value of 11.39 µg/mL< 11.55 µg/mL< 38.73 µg/mL for stem, leaf and root, respectively. Also, the acetone extract of leaf displayed remarkable free radical scavenging activity (IC_{50} value= 22.59 µg/mL). Good antioxidant and antibacterial activities as revealed in present investigation of this plant can contribute to its exploitation as a good natural antibacterial and antioxidant agent, in future.

Acknowledgement

Authors acknowledge the Chairman, Department of Biosciences, Himachal Pradesh University, Shimla for encouragement and provision of good lab facilities. Also, local people of sampling area were heartily acknowledged.

References

Ahmad, I. and A.Z. Beg (2000). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi drug resistant human pathogens. *Journal of Ethnopharmacology,* **7:** 113-123.

Anonymous. (2000). Centre of disease control and prevention. Preventing emerging infectious diseases, a strategy for 21st century, 1-3.

Atmani, D., M.B. Ruiz-Larrea, J.I. Ruiz-sanz, L.J. Lizcano, F. Bakkali and D. Atmani (2011). Antioxidant potential, cytotoxic activity and phenolic content of *Clematis flammula* leaf extracts. *Journal of Medicinal Plants Research*, **5:** 589-598.

Blois, M.S. (1958). Antioxidant determination by the use of stable free radicals. *Nature,* **26 :** 1199-1200.

Chen, J.H., Z.Z. Du., Y.M. Shen and Y.P. Yang (2009). Aporphine alkaloids from *Clematis parviloba* and their antifungal activity. *Archives of Pharmacal Research*, **32:** 3–5.

Chen, Z., R. Bertin and G. Froldi (2013). IC_{50} estimation of antioxidant activity in DPPH assay using statistical programs. *Food Chemistry,* **138:** 414-420.

Hao, P., H. Lv, Y. Wang, Y.H. Liu, C.Y. Li, F. Chen, J.K. Bao and L. Meng (2004). *Clematis montana* lectin, a novel mannose-binding lectin from traditional Chinese medicine with antiviral and apoptosis-inducing activities. *Peptides*, **30:**1805–1815.

Li, Y., S.F. Wang, Y.L. Zhao, K.C. Liu, X.M. Wang, Y.P. Yang and X.L. Li (2009). Chemical constituents from *Clematis delavayi* var. spinescens. *Molecules,* **14:** 4433–4439.

Pattanayak, S. (2018). Alternative to Antibiotics from Herbal Origin- Outline of a comprehensive Research project. *Current Pharmacogenomics and Personalized Medicine,* **16:** 9-62.

Prakash, V., S. Rana and A. Sagar (2016). Studies on antibacterial activity of leaf extracts of *Rhododendron arboreum* and *R. campanulatum. International. Journal of Current Microbiology and Applied Sciences,* **5:** 315-322.

Prakash, V., S. Rana and A. Sagar (2017). Studies on analysis of antioxidant and enzyme inhibitory activity of *Vitex negundo* Linn. *International. Journal of Pharmacognosy and Phytochemical Research*, **9:** 833-839.

Rana, S., V. Prakash and A. Sagar (2016). Antibacterial activity of *Mallotus philippensis* fruit extract. *Journal of Medicinal Plants Studies,* **4:** 104-106.

World Health Organization (WHO) (2002). Traditional Medicine strategy 2004-2005. *WHO Publication,* 1-6.

Zhang, L.J., H.T. Huang, S.Y. Haung, Z.H. Lin, C.C. Shen, W.J. Tsai and Y.H. Kuo (2015). Antioxidant and anti-inflammatory phenolic glycosides from *Clematis tashiroi. Journal of Natural Products,* **78:** 1586-1592.