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STUDIES ON ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS OF *SPILANTHES ACMELLA* L.

Shavnam Thakur, Anand Sagar and Ved Prakash*

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

Abstract

For a long period of time, nature has been a great source of medicinal agents. In present study, antibacterial and antioxidant activity of different extracts of *Spilanthes acmella* L. were evaluated *in-vitro* by using Agar-well diffusion method and DPPH free radical scavenging assay respectively. Acetone, methanol and water extracts of different plant parts (leaf, flower and stem) were screened for their antibacterial activity against four pathogenic bacteria (*Bacillus cereus*, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*) using different concentrations *i.e.* 25, 50, 75 and 100%. Results showed low to significant antibacterial activity against tested bacterial species. *S. typhi* was the most susceptible bacteria. In addition, the antioxidant potential of the different extracts of this plant was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. Methanol extracts of all plant parts exhibited higher antioxidant capacity with lesser IC_{50} values than acetone and water extracts. The results provided evidence confirming the fact that the studied plant could act as a potent source of antimicrobials and antioxidants.

Key words **:** *Spilanthes acmella*, plant extracts, Agar-well diffusion, DPPH.

Introduction

Medicinal plants have a great economic value all over the world. Use of plants as a source of medicine has been inherited and is an important part of the health care system in India. About 70% of India's medicinal plants are found in tropical regions spread across the Western and Eastern Ghats, the Vindhyas, Chotta Nagpur plateu, Aravalis and Himalayas. The world Health organization (WHO) estimated that 80% of world's population relies on traditional medicines (Kumar and Nagarajan, 2012). Among major health problems, infectious diseases account for 41% of the global disease burden (Noumedem *et al*., 2013). Today multiple drug resistance has developed due to the extensive use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. Antibiotics may also sometimes associated with adverse effects on the host including allergies, hypersensitivity and immune-suppression (Ahmad *et al*., 1998). Therefore there is a need to develop antimicrobial drugs from other sources including plants.

Medicinal plants serve as a rich source of antimicrobial agents. Various parts of medicinal plants are used for the extraction of raw drugs by local communities and folk healers for local use while many other raw drugs are collected in large quantities and traded in the market as the raw material for many herbal industries (Uniyal *et al*., 2006). Plant derived products have been reported to exert multiple biological effects (Prakash *et al*., 2016) including antioxidant, free radical scavenging abilities, anti-carcinogenic etc. (Miller, 1996). The medicinal value of these plants lies in various bioactive constituents or secondary metabolites such as alkaloids, tannins, flavonoids and phenolic compounds (Larson, 1988; Kahkonen *et al*., 1999) that produce a definite physiological action on the human body. The main cause of oxidative stress is reactive oxygen species (ROS) such as hydroxyl radicals, peroxides and super oxide anions (Wang *et al*., 2003). Free radicals are the main causes of more than one hundred ailments in human including atherosclerosis, arthritis, cancer and neurodegenerative disorders. Antioxidants inhibit the oxidation process (Prakash *et al*., 2017) or delay it by blocking the initiation or multiplication of oxidizing chain reactions and therefore protect the human body from oxidative stress related

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

diseases (Behera *et al.*, 2006). Several studies indicated that medicinal plants have a wide variety of natural antioxidant such as phenolic acids, flavonoids and tannins (Prakash *et al*., 2017).

Most of the Asteraceae family members are medicinal plants which have therapeutic applications. *Spilanthes acmella* L. commonly known as toothache plant is an annual hairy herb, up to 32-60 cm tall belonging to the family Asteraceae. It is found all over the world and widely distributed throughout the tropics and subtropics (Ramsewak *et al.*, 1999). This plant has been used as a spice, antiseptic, antibacterial, antimalarial and also as a medicine for toothache, flu, cough and tuberculosis (Haw and Keng, 2003).

Materials and Methods

Collection of plant material

Balyana Village of District Mandi in Himachal Pradesh was selected for the collection of study material *i.e.* leaf, flower and stem. The collections were made during the month of April.

Processing of plant material

Firstly the plant parts *i.e.* leaf, flower and stem were washed under tap water and then with 2% Mercuric chloride. The plant parts were allowed to shade dried for 10-15 days. The dried plant materials were crushed into fine powder with the help of pestle mortar. Prepared fine powders were then transferred to airtight containers at room temperature.

Preparation of plant extracts in different solvents

5 g dried powder of leaf, flower and stem of *S. acmella* were taken in separate Erlenmeyer flasks to which 50 mL of different solvents *i.e.* water, methanol and acetone were added. The flasks were covered with aluminium foil and allowed to stand for 3-5 days for extractions. After extraction, the extracts were filtered through the Whatman filter paper no. 1 and evaporated to 40ºC by using rotary evaporator. The extracts were collected and weighted. Then stock solutions of concentration 50 mg/mL were prepared.

Procurement of bacteria

Bacterial species used for antibacterial studies were *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* which were procured from IGMC, Shimla and Department of Biotechnology, HPU Shimla, India.

Revival of pathogen

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

Screening antibacterial activity of acetone, methanol and water extracts of leaf, flower and stem of *S. acmella*

Different extracts (acetone, methanol and water) of medicinal plant were screened using Agar-well diffusion method. Nutrient agar medium (Beef extract 1 g, Yeast extract 2 g, Sodium Chloride 1 g, Peptones 5 g, Agar 20

Table 1.1 : Zones of inhibition (ZOI) produced by leaf extracts of *Spilanthes acmella* at different concentrations.

Extract	Concentrations (%)	Inhibition zone diameter in mm (±S.E.)			
		B. cereus	***S. aureus***	***E. coli***	***S. typhi***
Acetone extract	Control	00.00±00.00	00.00±00.00	00.00±00.00	00.00±00.00
	25	13.3±0.27	14.3±0.27	14.0±0.47	17.6±0.27
	50	15.3±0.27	15.0±0.47	14.6±0.27	18.6±0.27
	75	16.6±0.27	16.3±0.27	15.3±0.27	19.3±0.27
	100	22.3±0.27	17.3±0.27	16.3±0.27	21.0±0.47
Methanol extract	Control	00.00±00.00	00.00±00.00	00.00±00.00	00.00±00.00
	25	13.0±0.47	14.3±0.27	12.0±0.47	17.3±0.27
	50	14.3±0.27	14.6±0.27	15.3±0.27	19.3±0.27
	75	15.6±0.27	16.6±0.27	16.3±0.27	21.0±0.47
	100	16.3±0.27	17.6±0.27	17.3±0.27	25.3±0.27

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

Table 1.2 : Zones of inhibition (ZOI) produced by flower extracts of *S. acmella* at different concentrations.

Extract	Concentrations (%)	Inhibition zone diameter in mm (±S.E.)			
		B. cereus	***S. aureus***	***E. coli***	***S. typhi***
Acetone extract	Control	00.00±00.00	00.00±00.00	00.00±00.00	00.00±00.00
	25	14.3±0.27	13.6±0.27	13.3±0.27	15.6±0.27
	50	15.0±0.47	14.6±0.27	13.6±0.27	17.0±0.47
	75	15.3±0.27	15.0±0.47	14.0±0.47	17.3±0.27
	100	16.0±0.47	16.3±0.27	15.3±0.27	19.0±0.47
Methanol extract	Control	00.00±00.00	00.00±00.00	00.00±00.00	00.00±00.00
	25	14.6±0.27	13.3±0.27	13.6±0.27	16.6±0.27
	50	15.3±0.27	14.6±0.27	15.3±0.27	17.3±0.27
	75	16.0±0.47	15.3±0.27	16.0±0.47	18.0±0.47
	100	16.3±0.27	17.0±0.47	17.3±0.27	23.0±0.47

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

g, Distilled Water 1000 mL) 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% concentrations of prepared plants extracts. The wells 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 (Prakash *et al.*, 2016; Rana *et al.,* 2016).

Antioxidant activity evaluation of acetone, methanol and water extract of the leaf, flower and stem of *S. acmella*

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) with slight modifications. Briefly, to 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 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 decrease 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 antibacterial activity of acetone, methanol and water extracts of leaf, flower and stem of *S. acmella*

In this study, antibacterial activity of leaf, flower and stem extracts of *Spilanthes acmella* was evaluated against different bacteria namely *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* by using Agar-well diffusion method. In case of methanol leaf extract of *S. acmella*, the maximum inhibition against *S. typhi*, *S. aureus*, *E. coli* and *B. cereus* was 25.3 ± 0.27, 17.6 ± 0.27, 17.3 ± 0.27 and 16.3 ± 0.27 mm respectively at 100% concentration while minimum zone of inhibition was 17.3 ± 0.27, 14.3 ± 0.27, 12.0 ± 0.47 and 13.0 ± 0.47 mm respectively at 25% concentrations. Acetone leaf extract showed maximum inhibition of 22.3 ± 0.27 mm at 100% conc. against *B. cereus* (Table 1.1 and Fig. 1.1).

Methanol flower extract inhibited all the tested bacteria. The diameter of zone of inhibition (ZOI) ranged from 13.3 to 23.0 mm (maximum ZOI = 23.0 ± 0.47 mm against *S. typhi*). Similarly acetone flower extract gave maximum zone of inhibition of 19.0 ± 0.47 mm against *S. typhi* (Table 1.2 and Fig 1.2). In case of methanol stem extract, again maximum activity was shown against *S. typhi* with zone of inhibition of 13.3 ± 0.27 mm. Acetone stem extract showed highest antibacterial activity against *S. typhi* with zone of inhibition of 13.0 ± 0.47 mm at 100% conc. and lowest antibacterial activity was reported against *B. cereus* with zone of inhibition of 9.0 ± 0.47 mm at 25% concentration (Table 1.3 and Fig 1.3). Aqueous extract of all the plant parts was found to be ineffective against all the tested bacteria.

Antioxidant activity evaluation of acetone, methanol and water extract of the leaf, flower and stem of *S. acmella*

DPPH radical scavenging activity assay

The free radical scavenging activity of different extracts of *S. acmella* was analysed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay as described by Blois (1958) with slight modifications. (Table 1.5-1.7 and Fig. 1.5-1.7) showed the results for antioxidant activity of acetone, methanol and water extracts of leaf, flower and stem of *S. acmella.* Ascorbic acid was taken as a control with IC_{50} value of 29.56 µg/mL (Table 1.4). Methanol leaf extract of *S. acmella* showed higher antioxidant activity with IC_{50} value of 127.19 µg/mL than acetone (156.16 µg/mL) and water extracts (374.49 µg/mL) (Table 1.5 and Fig. 1.5). In case of flower extract, methanol extract showed least IC_{50} value (67.34 µg/mL)

Table 1.3 : Zones of inhibition (ZOI) produced by stem extracts of *S. acmella* at different concentrations.

Extract	Concentrations (%)	Inhibition zone diameter in mm (±S.E.)			
		B. cereus	*S. aureus*	*E. coli*	*S. typhi*
Acetone extract	Control	00.00±00.00	00.00±00.00	00.00±00.00	00.00±00.00
	25	9.0±0.47	10.0±0.47	9.3±0.27	11.0±0.47
	50	9.3±0.27	10.3±0.27	9.6±0.27	11.3±0.27
	75	10.0±0.47	11.3±0.27	10.3±0.27	12.6±0.27
	100	10.3±0.27	11.6±0.27	10.6±0.27	13.0±0.47
Methanol extract	Control	00.00±00.00	00.00±00.00	00.00±00.00	00.00±00.00
	25	10.0±0.47	10.3±0.27	9.0±0.47	11.3±0.27
	50	11.3±0.27	11.6±0.27	10.3±0.27	12.3±0.27
	75	11.6±0.27	12.0±0.47	11.3±0.27	13.0±0.47
	100	12.0±0.47	12.3±0.27	12.6±0.27	13.3±0.27

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

Table 1.4 : Free radical scavenging activity (%) of control *i.e.* ascorbic acid at different concentrations.

Concentrations (µg/mL)	Methanol extract (%)	IC_{50} value (µg/mL)
20	44.6±0.72	29.56
40	54.98±0.86	
60	67.43±1.00	
80	78.17±0.55	
100	87.02±0.06	

Table 1.5 : Free radical scavenging activity (%) of the leaf extracts of *Spilanthes acmella* at different concentrations in acetone, methanol and water solvents.

Conc. (µg/mL)	Acetone extract (%)	IC_{50} value (µg/mL)	Methanol extract	IC_{50} value (µg/mL)	Water extract	IC_{50} value (µg/mL)
20	18.08±1.15	156.16	11.39±0.64	127.19	13.09±1.08	374.49
40	27.93±0.53		13.73±0.86		15.63±1.04	
60	31.68±1.17		32.63±0.49		17.07±0.70	
80	34.22±0.44		37.36±1.12		19.24±0.38	
100	36.14±0.45		47.09±0.99		21.67±0.69	

Table 1.6 : Free radical scavenging activity (%) of the flower extracts of *S. acmella* at different concentrations in acetone, methanol and water solvents.

Conc. (µg/mL)	Acetone extract (%)	IC_{50} value (µg/mL)	Methanol extract	IC_{50} value (µg/mL)	Water extract	IC_{50} value (µg/mL)
20	23.34±1.49	146.28	21.26±0.76	67.34	6.90±1.99	447.76
40	27.56±0.87		28.44±1.41		8.41±1.54	
60	29.90±0.61		37.17±3.99		10.20±1.42	
80	34.15±0.43		62.76±2.00		13.06±0.94	
100	41.68±0.45		74.53±1.09		14.73±0.88	

than acetone (146.28 µg/mL) and water extracts (447.76 µg/mL) as shown in Table 1.6 and Fig. 1.6. Also in case of stem extract, methanol extract showed higher antioxidant activity with IC_{50} value of 84.29 µg/mL than acetone (182.56 µg/mL) and water extracts (536.68 µg/mL) (Table 1.7 and Fig 1.7). In all cases, methanol extracts proved to be better source of antioxidants than the acetone and aqueous extracts.

Discussion

Screening antibacterial activity of acetone, methanol and water extracts of leaf, flower and stem of *S. acmella*

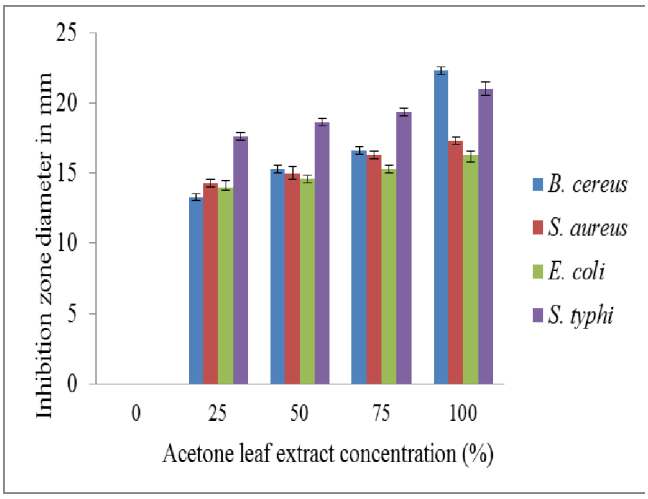
Results obtained in the present study revealed that the tested plant extracts possess potent antibacterial activity against *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Salmonella typhi*. It was observed that methanol leaf extract of *S. acmella* showed higher antibacterial activity against *S. typhi* at 100% conc. with 25.3 ± 0.27 mm zone of inhibition. While in case of acetone leaf extract, maximum inhibition was recorded as 22.3 ± 0.27 mm at 100% conc. against *B. cereus*. No antibacterial activity was recorded in the water extract of all the plant parts (leaf, flower and stem). However, both the acetone flower extract and methanol flower extract showed highest inhibition against *S. typhi i.e.* 19.0 ± 0.47 mm at 100% conc. and 23.0 ± 0.47 mm at 100% conc. respectively. In case of acetone stem extract, the minimum inhibition zone was shown against *B. cereus* (9.0 ± 0.47 mm at 25%) and maximum inhibition was shown against *S. typhi* (13.0 ± 0.47 mm at 100%) while in case of methanol stem extract the minimum zone of inhibition was shown against *E. coli* (9.0 ± 0.47 mm at 25%) and maximum inhibition was shown against *S. typhi* (13.3 ± 0.27 mm at 100%).

The antimicrobial activity of *S. acmella* flower head extract was evaluated by Rani and Murty (2005) against six microorganisms namely *Bacillus sphaericus*, *B. subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebesiella aerogenes* and *Chromobacterium violaceum*. Our results regarding antibacterial activity also correspond to the results obtained by Rani and Murty.

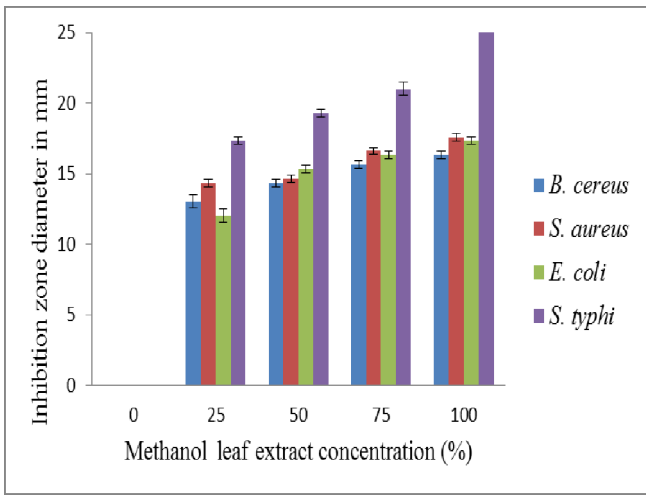
Table 1.7 : Free radical scavenging activity (%) of the stem extracts of *S. acmella* at different concentrations in acetone, methanol and water solvents.

Conc. (µg/mL)	Acetone extract (%)	IC_{50} value (µg/mL)	Methanol extract	IC_{50} value (µg/mL)	Water extract	IC_{50} value (µg/mL)
20	6.21±1.12	182.56	2.89±0.32	84.29	2.52±0.24	536.68
40	9.19±0.94		7.69±1.12		4.70±0.81	
60	12.31±0.59		19.83±2.01		5.83±0.73	
80	23.79±1.15		41.93±2.52		6.94±0.46	
100	26.91±0.69		72.41±2.13		10.61±1.85	

activity of *S. acmella* by DPPH and SOD assays. All tested fractions exhibited antioxidant properties in both DPPH and SOD assays. *In vitro* and *in vivo* comparative study of primary metabolites and antioxidant activity in *S. acmella* was studied by Tanwer *et al.,* (2010). The result revealed that methanol stem extract of *S. acmella*

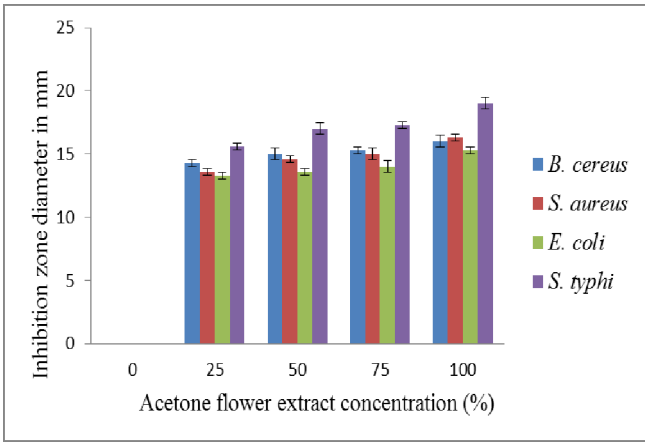


A



B

Fig. 1.1 : Antibacterial activity of leaf extract of *Spilanthes acmella* (A) Acetone leaf extract (B) Methanol leaf extract.



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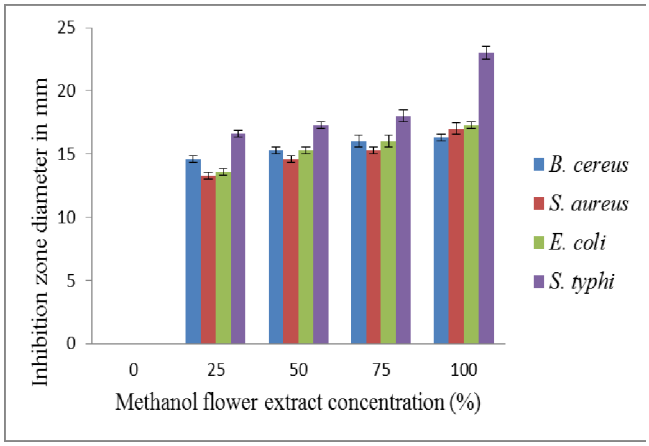

B

Fig. 1.2 : Antibacterial activity of flower extract of *S. acmella* (A) Acetone flower extract (B) Methanol flower extract.

Antioxidant activity evaluation of acetone, methanol and water extract of the leaf, flower and stem of *S. acmella*

According to our results, methanol extracts proved to be better antioxidants than the acetone and water extracts. The methanol flower extract of *S. acmella* showed highest antioxidant activity with IC_{50} value of 67.34 µg/mL.

Prachayasittikul *et al.* (2009) analysed the antioxidant showed highest superoxide radical scavenging activity however leaves showed maximum DPPH scavenging activity than other plant parts and callus. Rao *et al*., (2012) also carried out the phytochemical analysis and quantification of total phenolics and alkaloid contents of *S. acmella* extracts (70% ethanol, methanol, ethyl acetate and hexane). The antioxidant activity was analysed by using three free radicals (Superoxide, Hydroxyl and DPPH). Methanol extract showed better activity compared to other extracts. The result indicated that *S.*

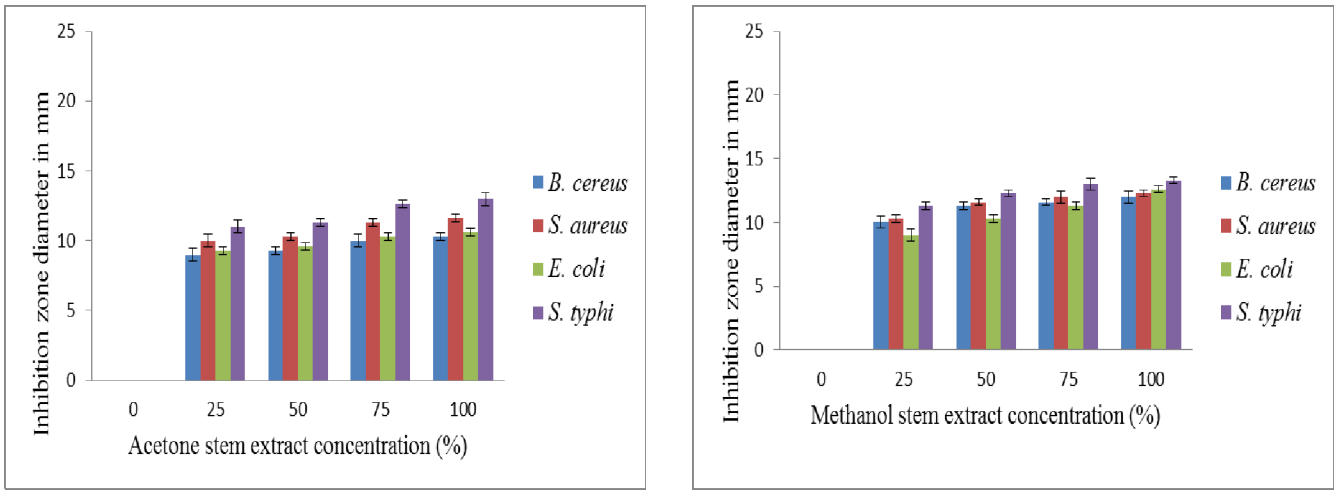

Fig. 1.3 : Antibacterial activity of flower extract of *S. acmella* (A) Acetone stem extract (B) Methanol stem extract.

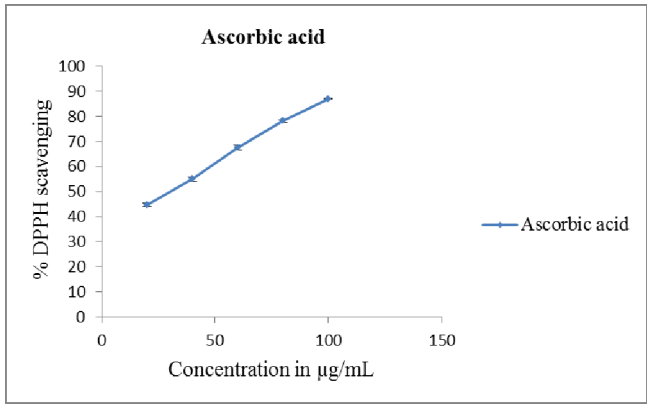

Fig. 1.4 : Free radical scavenging acivity of ascorbic acid as a control.

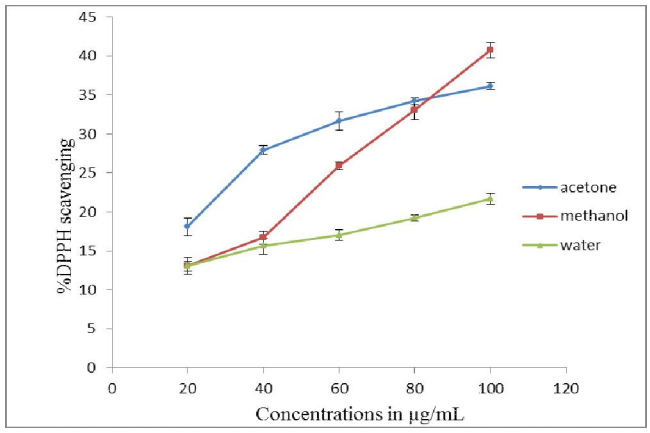

Fig. 1.5 : Free radical scavenging acivity of acetone, methanol and water leaf extract of *S. acmella*.

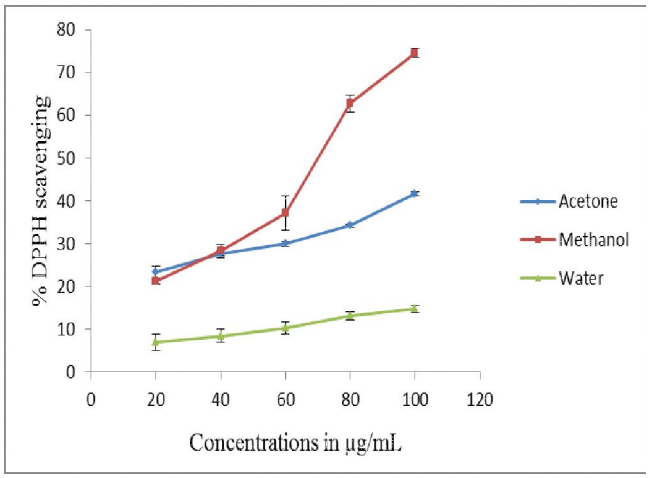

Fig. 1.6 : Free radical scavenging acivity of acetone, methanol and water flower extract of *S. acmella*.

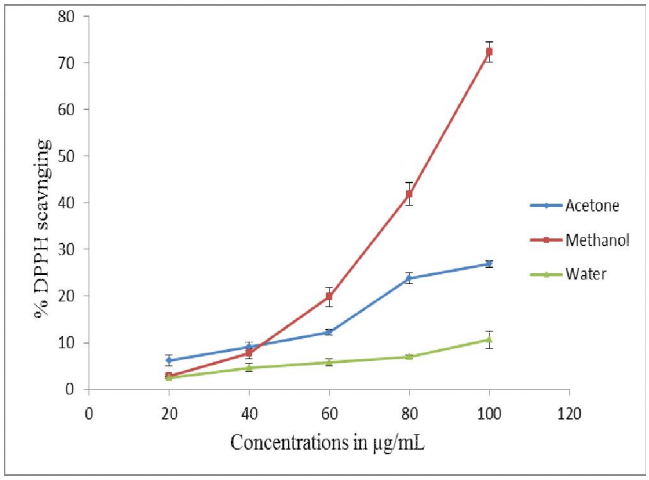

Fig. 1.7 : Free radical scavenging acivity of acetone, methanol and water stem extract of *S. acmella*.

acmella extracts exhibited better antioxidant activity. The antibacterial and antioxidant properties of the medicinal plant may be due to presence of highly valuable bioactive compounds such as phenolics, coumarins and triterpenoids (Prachayasittikul *et al*., 2009).

Conclusion

On the basis of the results, it can be concluded that the plant *Spilanthes acmella* showed significant

antibacterial and antioxidant activity at different concentrations. Methanol extracts of all plant parts (leaf, flower, stem) was found to be more effective against all the tested bacteria than acetone extracts. *S. typhi* was the most susceptible bacteria. No antibacterial activity was recorded in the water extract of all parts. This study also revealed that the plant extracts possess potent antioxidant activity. Methanol extracts of all the plant parts exhibited higher antioxidant activity than acetone and water extracts. The result justifies the claimed uses of *S. acmella* in the traditional system of medicine to treat various infectious diseases caused by microbes. Findings of present work are preliminary and further investigations are required to determine the actual nature of the bioactive compounds which may be present in the different plant parts.

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