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Research Article

IN VIVO AND IN VITRO PHYTOCHEMICAL, MICROBIAL AND ANTIOXIDANT EVALUATION OF SERICOSTOMA PAUCIFLORUM STOCKS EX WIGHT CALLUS

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ABSTRACT

Cell cultures of *Sericostoma pauciflorum,* a promising antidiabetic plant, have been established using Murashige and Skoog's (MS) medium supplemented with different concentrations of 2, 4-dichlorophenoxyacetic acid (2, 4-D). Callus was harvested at different time intervals of 2, 4, 6, 8 and 10 weeks, and their antimicrobial and antioxidant potentials were investigated using established protocols. Methanol extracts demonstrated appreciable antifungal activity (17.33 ± 0.34 mm in 6 weeks-old and 16.66 ± 0.34 mm in 4 weeks-old callus) against *P. chrysogenum* while antibacterial activity was recorded in 10 weeks-old callus against *B. subtilis* and *P. aeruginosa* (17.00 ± 0.57 mm and 16.33 ± 0.67 mm respectively). Total levels of phenolics and antioxidant potentials were analyzed using Folin-Ciocalteau and DPPH, FRAP methods respectively. Methanol extracts of 8 weeks-old callus showed appreciable data (total phenolics 114.00 ± 1.80 mg GAE/g extract; IC_{50} 0.07 mg/ml, % inhibition 85.71 µg/ml), followed by 6 weeks-old callus (total phenolics 110.32 ± 0.50 mg GAE/g extract; IC_{50} 0.08 mg/ml, % inhibition 74.57 mg/ml). Later, the results were compared with those of *in vivo* studies.

Keywords: Antifungal, Antibacterial, Antioxidant, Phenolics.

INTRODUCTION

Sericostoma is a small genus of family Boraginaceae. It comprises of eight species distributed through the tropical East and North East of Africa and North West India. *S. pauciflorum* Stocks ex Wight is a short straggling under-shrub growing widely throughout sea coast of Saurashtra and Maharashtra and used in making an important drug in Ayurveda named "Krishnavalli". It is anticancer, antidiabetic, used in dehydration, acidity, and health promoting drug (as described in "Nighantu Ratnakar"). Phytochemically, fernane, hopane and other type of triterpenoids[1-4] have been isolated.

The production of secondary metabolites through cell culture technology of renowned medicinal plants has been a challenging subject for many researchers. Various secondary metabolites were isolated from cell cultures of Boraginaceae family viz., from *Lithospermum erythrorhizon* and *Eritrichium sericeum* rosmarinic acid, which has biological activities including antioxidant, antiischemia reperfusion, antithrombosis, antihypertension, antifibrosis, antivirus and antitumor[5-7]; shikonin, a benzoquinone from *L. erythrorhizon*[8,9]; quinones and naphthoquinones from *Echium lycopsis* have been isolated and their antimicrobial activities were reported[10-12].

Since *S. pauciflorum* extract is one of the important herbal formulations in ayurvedic medicine, as a tonic for treating many diseases and there is no report on its biological studies. Therefore, the present work deals with comparative studies of *S. pauciforum* cell culture and plants extracts antibacterial, antifungal and antioxidant activities. This study adds further value for the possible use of this plant, especially the *in vitro*-induced callus extract, as a food additive.

MATERIALS AND METHODS

Plant material

Whole plants of *Sericostoma pauciflorum* Stocks ex Wight (Boraginaceae), aerial parts and roots, were collected from the campus of University of Rajasthan, Jaipur during the months of July-October, 2009. The botanical identity was confirmed by Herbarium, Department of Botany, University of Rajasthan, Jaipur. Voucher specimen (No. 20155) of the plant has been deposited at the Herbarium and the Laboratory for further reference.

Callus induction and maintenance

Young nodal explants were washed under running tap water for 15 min, treated with 1% tween 20 (commercial detergent, Sigma) for 10 min and cut into small pieces of 1 inches each. Explants were sterilized by successive treatment with abs. alcohol for 30 s, 0.1% (w/v) $HgCl_2$ for 4 min and later washing with sterilized distilled water (DW) four times. Sterilized explants were transferred on autoclaved Murashige and Skoog's basal culture medium[13] supplemented with different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D; 0.5, 1, 1.5 and 2 mg/l; Sigma), sucrose 30 g/l and 1% (w/v) agar (Merck). Biomass production in terms of growth index was recorded at different time interval of 2, 4, 6, 8 and 10 weeks[14]. Cultures were maintained on 25±2C° with the daily photoperiod of 16 hours. Out of the four 2, 4-D concentrations used, concentration yielding higher biomass of callus was selected and used for further study.

Preparation of extracts

Aerial parts with roots of *S. pauciflorum* was collected, dried and powdered. 50 g of this plant material was extracted with 100 ml of ethanol (3×18 hours). Similarly, callus was harvested at different growth periods of 2, 4, 6, 8 and 10 weeks, kept at 100^0 C for 5 min to inactivate the enzymatic activity and later, at 60^0C till a constant weight is achieved. 20 g of callus of each growth period was separately powdered and extracted in 100 ml of ethanol (60^0C, 3l×18 hours). Each sample was filtered using Whatman No. 1 filter paper in a Buchner funnel, the filtrate was freeze-dried and weighed. Later, the ethanolic extract was fractionated successively using pet. ether (60^0- 80^0 C), methanol and water. Subsequently, each fraction was filtered, concentrated to dryness *in vacuo* and stored at appropriate temprature for further studies.

Phytochemical studies

Thin layer chromtography (TLC) profile of callus extracts was carried out on silica G plates using heptane-benzene-alcohol (100:100:1)(I) and butanol: 27% aqueous acetic acid (1:1 v/v)(II) as solvent systems. Plates were visulized by spraying with 10% $SbCl_3$ for solvent I and 10% methanolic $AlCl_3$ for solvent II. Several spots coinciding reference markers were scrapped from unsprayed plates, eluted with methanol, filtered, evoparated to dryness, reconstituted and crystallized in methanol. The melting point of the isolated compounds was determined in capillary tubes (Toshniwal Melting point apparatus) and subjected to ir spectrum (Perkin Elmer 337, Grating Infra red spectrophotometer).

The levels of terpenoids and phenolics acids were estimated colorimetrically[15, 7].

Bioefficacies

The bacterial strains of *Bacillus subtilis* (MTCC 441), *Enterobacter aerogenes* (MTCC 111), *Escherichia coli* (ATCC 443), *Pseudomonas aeruginosa* (ATCC 741), *Raoutella planticola* (MTCC 530) and *Staphylococcus aureus* (ATCC 740) were obtained from IMTECH, Chandigarh, India, and maintained on Nutrient broth medium (NB) at 27°C for 48 hours. In fungus, *Aspergillus flavus* (ATCC 16870), *A. niger* (ATCC 322), *Candida albicans* (ATCC 4718*)*, *Penicillium chrysogenum* (ATCC 5476*)* and *Tricophyton rubrum* (ATCC 2327) were obtained from IARI, New Delhi, India, and cultured on Sabouraud dextrose agar (SDA) at 37°C for 48 hours.

Determination of antimicrobial activity

Antimicrobial tests were performed by agar well diffusion method[16]. Inoculum of each bacterial strain was suspended in nutrient broth and fungal strains in SDA broth at 25^0C for period of 24 hours to the final cell concentration of 10^6-10^7 CFU/ ml. Bacterial and fungal inoculums were inoculated in Müller-Hinton and SDA medium respectively. 4 mg extract in methanol was delivered to each well. To ensure diffusion of sample into agar, plates were incubated at 4^0C for 1 hour, which were then incubated overnight at 25^0C. The diameter of the inhibition zone around each hole was measured and recorded (Inhibition zone recorder, HiMedia, India). Streptomycin (10 mcg/disc) for bacteria and ketonocozole (10 mcg/disc) for fungus were used as positive controls.

Total phenolic contents

The total phenolic contents were determined with Folin-Ciocalteau reagent[17] by measuring optical density (OD) at 750 nm (Shimadzu, Pharmaspec UV- Vis spectrophotometer). A standard calibration curve of gallic acid (1 - 50 mg/l) was prepared and total phenolics were expressed in mg of gallic acid equivalents (mg GAE/g of extract).

Antioxidant activity

Free radical scavenging activity by 2, 2-diphenyl-1-picryl-hydrazyl radical (DPPH)

The effect on DPPH radical was determined using the method by Fogliano et al.[18]. Different concentrations of extract (0.8, 0.6, 0.4, 0.2, 0.1 mg/ml) were prepared in methanol and mixed with 2.5 mL of DPPH (2 mg/10 ml methanol). After 30 min of incubation time, absorbance was measured at 517 nm using UV-Vis spectrophotometer. Negative control (methanol) and positive control (quercetin) were also used. Capability to scavenge the DPPH radical was calculated using following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1 / A_0) \times 100]$$

Where, A_0 was the absorbance of the control reaction and A_1 was the absorbance in presence of the sample of given extract.

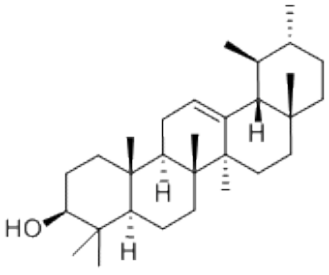
Total reduction capability by Ferric ion Reducing Antioxidant Potentials (FRAP).

Total reducing power of extracts was determined according to FRAP method[19]. Specific concentration of extracts (ascorbic acid) and extract (62.5-1000 µg/ml) was prepared in 1 mL ethanol separately, mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and $K_3Fe(CN_6)$ (2.5 ml, 1%). After incubation at 50^0C (20 min), 2.5 ml of 10% tri chloroacetic acid, 2.5 ml of distilled water and $FeCl_3$ (0.5 ml, 1%; chromogenic reagent) were added in the sequence and absorbance was measured at 700 nm. A standard calibration curve of ascorbic acid was prepared and antioxidant activity was expressed in mg of ascorbic acid equivalents (mg AAE/g) of extract. All determinations were carried out in triplicate and statistically analyzed.

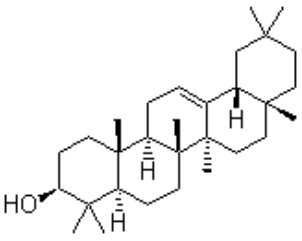
RESULTS

Callus was initiated within 15 days, where MS medium with 2,4-D, 1.5 mg/l proved better for the callus biomass production and it was used for further experimentation. Colour and texture of the callus at different growth stages was also recorded. From callus cultures, several triterpenoidal compounds viz. friedelin, α- amyrin, β-amyrin, β- sitosterol and a phenolic compound-caffeic acid, have been isolated and identified on the basis of their chromatographic behaviour, melting points and spectral analysis (Table 1; Figure 1)

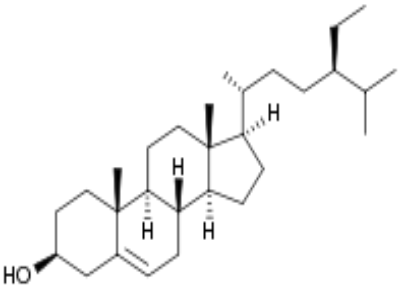
From the data, appreciable antimicrobial activity was demonstrated by pet. ether extract of 10 weeks- old callus as it is effective against *B. subtilis*, *P. aeruginosa, E. coli* (IZ 17.00±0.57, 16.33±0.67 and 15.66±0.34 mm respectively; Table 2), followed by its methanol extract against *S. aureus* (16.00±0.57 mm) and *E. coli* (15.33±0.34 mm). In antifungal activity, methanol extract of 4 weeks-old callus was more effective, showing appreciable activity against *P. chrysogenum*, *A. niger* (16.66±0.34 and 15.66±0.33 mm respectively), followed by 6 weeks-old callus methanol extract against *C. albicans*, *P. chrysogenum* and *T. rubrum* (17.33±0.34 and 14.66 mm in last two cases). Aqueous extract of callus showed negligible activity against most of the used microbes as compared to *in vivo* plant.



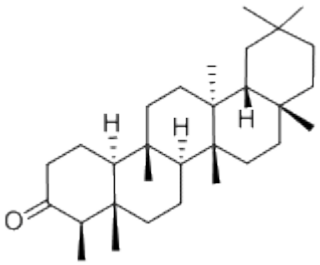
α -Amyrin



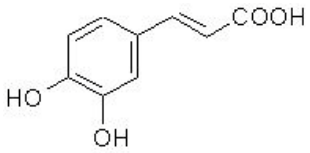
β-Amyrin



β- Sitosterol



Friedelin



Caffeic acid

Fig. 1: Isolated compounds from *S. pauciflorum* cell cultures

Table 1: Chromatographic behavior and chemical characteristics of isolated compounds from *S. pauciflorum* stem callus

Isolated compounds	R_F (× 100)		Color after spray		m.p. (°C)	IR (ν_{max}) cm^{-1} (KBr)
	I	II	I	II		
Friedelin	81	-	Pink	-	198-200	1720, 1380, 1365, 1255, 1230, 1200, 920
α-Amyrin	26	-	Pink	-	183-184	3350, 1640, 1480, 1360, 1130, 1050, 930
β- Amyrin	20	-	Pink	-	197-198	3350, 1650, 1190, 1140, 1100, 1050
β - Sitosterol	06	-	Blue	-	136-137	1730, 1640, 1240, 735, 725
Caffeic acid	-	76	-	Yellow	195-198	812, 849, 899, 972, 1118, 1172, 1212, 1448, 1640, 3440

I: Heptane-benzene-alcohol (100:100:1), spray with 10% $SbCl_3$; II: Butanol: 27% aqueous acetic acid (1:1 v/v); spray with 10% methanolic $AlCl_3$.

Table 2: **Antimicrobial activity⁺ of *S. pauciflorum* stem callus at different growth stages**

Age of callus	Type of extract	Bacteria					Fungi					
		B. subtilis	*E. aerogenes*	*E. coli*	*P. aeruginosa*	*R. planticola*	*S. aureus*	*A. flavus*	*A. niger*	*C. albicans*	*P. crysogenum*	*T. rubrum*
2 week	PE	14.33±0.34	13.33± 0.67	12.67±0.3	15.00±0.00	10.66±0.66	11.00±0.57	15.66±0.66	12.33±0.33	10.00±0.00	12.66±0.33	12.00±0.5
	Met	13.33±0.00	10.00±0.57	10.66±0.3	13.00±0.57	10.33±0.67	10.00±0.00	15.00±1.00	11.33±0.33	13.66±0.67	16.00±0.00	13.00±0.0
	Aq	11.66±1.37	14.00±0.57	8.66±0.67	13.30±1.00	12.66±0.66	-	9.33±0.67	11.33±0.33	-	-	-
4 week	PE	14.33±0.34	10.00±0.00	12.67±0.3	12.33±0.76	10.66±0.66	-	9.33±0.67	13.33±0.33	9.66±0.88	13.66±0.67	12.66±0.3
	Met	13.33±0.00	12.00±0.57	10.66±0.3	13.00±0.00	10.00±0.57	10.66±0.66	15.00±0.57	15.66±0.33	13.66±0.34	16.66±0.37	15.00±0.0
	Aq	11.66±1.37	11.66±0.34	8.66±0.67	13.33±0.76	-	10.00±0.00	13.66±0.66	8.00±0.00	-	-	-
6 week	PE	11.66±0.67	8.66±0.66	10.66±0.3	15.00±0.00	10.00±0.00	10.00±0.00	13.00±0.66	12.66±0.67	11.33±0.33	13.33±0.67	13.00±0.5
	Met	14.33±0.34	13.33±0.66	12.66±0.6	11.00±0.00	11.00±0.00	13.66±0.66	13.00±0.57	13.66±0.34	17.33±0.34	14.66±0.67	14.66±0.3
	Aq	8.00±0.00	13.33±0.76	8.00±0.00	15.33±0.32	9.00±0.57	12.66±0.34	-	-	-	-	-
8 week	PE	15.66±0.67	10.66±0.67	14.66±0.6	15.33±0.32	9.66±0.67	10.00±0.00	11.66±0.87	14.66±0.67	8.66±0.66	12.66±0.66	14.33±0.3
	Met	15.66±0.67	-	15.00±0.0	15.33±0.32	12.66±0.66	16.00±0.76	13.66±0.66	13.66±0.34	12.33±0.34	13.66±0.66	15.66±0.6
	Aq	12.33±0.33	13.00±0.57	10.00±0.0	10.33±0.33	-	8.00±0.57	-	-	-	-	-
10 week	PE	17.00±0.51	-	15.66±0.3	16.33±0.67	15.00±0.00	10.33±0.76	-	-	-	12.33±0.67	13.33±0.3
	Met	15.00±0.00	14.33±0.34	15.33±0.3	15.00±0.00	13.00±0.57	16.00±0.57	-	-	-	14.00±0.57	15.66±0.6
	Aq	8.66±0.34	12.00±0.57	8.00±0.00	11.66±0.67	11.33±0.57	-	-	-	-	-	15.66±0.6
In vivo plant	PE	12.33±0.67	13.00±0.57	11.66±0.6	14.66±0.34	11.33±0.34	8.00±0.00	12.66±0.66	11.00±0.33	12.66±0.66	11.66±0.32	10.00±0.0
	Met	12.33±0.34	13.00±0.57	10.66±0.3	13.33±0.34	8.66±0.67	12.00±0.57	10.00±1.00	10.00±0.00	11.33±0.66	11.66±0.32	10.00±0.0
	Aq	15.00±0.57	10.33±0.32	10.33±0.3	15.66±0.66	10.33±0.32	10.33±0.32	10.00±0.00	12.66±0.74	11.66±0.32	13.33±0.32	9.33±0.34

Antimicrobial activity (in terms of inhibition zone in mm including the diameter of well; 6 mm); Mean ± S.E. (Standard error); Standard: Streptomycin (10 mcg/ml) for bacteria, Ketonocozole, (10 mcg/disc) for fungi; PE = pet. ether, Met = methanol, Aq = aqueous.

In methanol extracts, 114.00±1.80 mg GAE/g of total phenolics was recorded in 8 weeks-old callus followed by 6 weeks-old callus (110.32 ± 0.50 mg GAE/g). In the present study, enhanced antioxidant activity was demonstrated by methanol extract of 8 weeks-old callus with lowest IC_{50} value (0.07 mg/ml, % inhibition 85.71 on the concentration of 0.8 mg/ml; Table 3) followed by 6 weeks old-callus methanol (IC_{50} 0.08 mg/ml, % inhibition of 75.51 in 0.8 mg/ml concentration). In FRAP method, methanol extract 10 weeks-old callus showed higher values of 60.00 ± 0.00 AAE/g dw antioxidant potentials (Table 3).

Table 3: Total phenolics and antioxidant assay by DPPH and FRAP method

Age of callus	Type of extract	Total phenolics (mg GAE/g)	IC_{50}	% Inhibition in DPPH method (concentration in mg/ml)					Antioxidant activity in FRAP method (in terms of AAE/g dw)				
				0.1	0.2	0.4	0.6	0.8	62.5	125	250	500	1000
2 week	**PE**	45.00±0.61	0.47	45.51	46.50	48.07	55.25	55.12	14.66 ± 1.34	21.66 ± 2.79	23.33 ± 1.97	26.00 ± 1.66	30.00 ± 0.00
	Met	80.00± 1.25	0.62	32.68	34.78	46.10	48.76	62.27	43.33 ± 2.02	194.33 ± 1.49	214.33 ± 4.67	230.00 ± 5.78	300.00 ± 3.47
	Aq	59.66±0.32	-	35.26	55.78	60.82	61.30	65.29	20.66 ± 3.33	20.33 ± 1.44	21.66 ± 5.78	25.66 ± 5.41	23.33 ± 1.72
4 week	**PE**	51.32±0.32	0.11	49.16	54.40	60.00	60.36	60.61	21.66 ± 3.33	20.00 ± 0.00	25.00 ± 0.00	26.66 ± 5.78	36.66 ± 4.41
	Met	102.66±0.72	0.09	54.97	63.55	65.90	69.15	74.62	95.00 ± 7.67	153.33 ± 1.49	165.00 ± 0.00	178.33 ±1.67	320.00 ± 0.00
	Aq	78.00±0.73	0.55	70.43	70.89	73.88	74.82	77.58	18.33 ± 0.02	21.66 ± 0.57	23.33 ± 1.98	23.33 ± 1.66	35.00 ± 0.00
6 week	**PE**	52.32±1.58	0.085	58.29	58.85	59.59	60.31	61.46	18.33 ± 1.61	23.33 ± 5.78	25.00 ± 0.00	33.33 ± 6.62	36.66 ± 1.61
	Met	110.32±0.50	0.08	53.41	58.77	63.40	66.57	75.58	121.66 ± 1.72	126.66 ± 2.97	226.66 ± 1.97	231.66 ± 1.66	340.66 ± 3.33
	Aq	78.00±0.73	0.17	34.67	46.10	51.82	50.16	52.59	19.66 ± 7.55	24.00 ± 0.00	26.00 ± 0.00	28.66 ± 1.97	38.66 ± 0.33
8 week	**PE**	53.66±0.15	0.14	47.17	53.02	60.51	61.28	66.36	19.00 ± 0.88	20.00 ± 1.51	25.66 ± 8.68	20.66 ± 7.64	41.66 ± 3.35
	Met	114.00±1.80	0.07	68.94	69.79	74.87	83.00	85.71	25.00 ± 0.00	126.66 ± 7.27	235.00 ± 0.00	236.66 ± 5.78	360.00 ± 0.00
	Aq	73.00±0.73	0.08	57.78	60.84	63.86	68.23	73.01	28.33 ± 1.66	31.66 ± 6.12	34.33 ± 4.94	35.00 ± 0.00	43.33 ± 5.79
10 week	**PE**	47.32±0.72	0.095	51.98	52.64	55.22	55.50	56.04	20.00 ± 0.00	23.33 ± 7.82	23.33 ± 4.23	28.33 ± 3.78	42.33 ± 7.72
	Met	75.00±0.60	0.10	49.60	63.25	65.06	67.33	74.57	125.00 ± 0.00	126.66 ± 7.27	235.00 ± 0.00	236.66 ± 5.78	360.00 ± 0.00
	Aq	71.00±0.73	-	28.36	30.13	34.98	37.64	89.99	30.00 ± 0.00	35.00 ± 0.00	46.66 ± 3.33	53.33 ± 6.67	54.33 ± 6.01
***In vivo* plant**	**PE**	31.5 ± 1.67	-	68.61	70.61	70.61	74.40	73.75	18.33 ± 4.41	21.00 ± 6.69	25.00 ± 0.00	31.66 ± 3.78	46.66 ± 3.33
	Met	84.93 ± 0.15	0.15	47.32	51.80	72.60	72.93	80.04	110.00 ± 3.33	211.66 ± 7.24	220.00 ± 1.95	275.00 ± 0.00	351.66 ± 9.36
	Aq	104 ± 0.44	0.075	68.48	76.92	92.51	93.40	93.78	32.56 ± 5.66	32.66 ± 4.56	43.99 ± 3.33	51.99 ± 3.48	51.11 ± 5.69

PE = pet. ether, Met = methanol, Aq = aqueous.

On quantification, caffeic acid was estimated to be maximum in the methanolic extract of 6 weeks- and 8 weeks-old callus (4.54 ± 1.56 mg/g dw and 4.42 ± 3.15 mg/g dw respectively). Out of the terpenoids extracted, levels of β-sitosterol was found to be maximum (12.8 ± 2.71 mg/g dw) as compared to other compounds (Table 4).

Table 4: Quantification of isolated secondary metabolites

Nature of extract		Concentration (mg/g dw)	
		β – Sitosterol	**Caffeic acid**
Callus	2 weeks-old	3.00 ± 4.42	1.58 ± 2.78
	4 weeks-old	5.70 ± 0.71	2.56 ± 0.25
	6 weeks-old	7.00 ± 3.94	4.54 ± 1.56
	8 weeks-old	7.30 ± 1.44	4.42 ± 3.15
	10 weeks-old	12.8 ± 2.71	3.32 ± 1.33
Whole plant		14.0 ± 1.66	2.24 ± 5.64

DISCUSSION

In the developing countries, infectious diseases due to development of antibiotic resistance related to several health problems[20]. Epidemiological studies suggested that phytosterols reduces the risk of colon cancer[21, 22]. β-Sitosterol found in almost all plants and work against human breast cancer cells[23]. Gram +ve bacteria were more susceptible to phenolic acids (*p*-coumaric-, sinapic- and caffeic acids) than Gram -ve bacteria, possibly due to the presence of their outer

membrane spaces[24, 25]. Moreover, *S. aureus* and *B. subtilis* were the most susceptible Gram +ve bacteria, an observation that may be attributed to the presence of a single membrane of the organisms which makes it more accessible to permeation by active principles of *S. pauciflorum* active extracts.

In the present study, methanol extract demonstrated greater antioxidant efficacy as compared to pet. ether and water extracts, where the levels of phenolic contents were more then the others. Similar results were obtained by FRAP method also which were better then the *in vivo* plant. Free radicals are responsible for several disorders in humans such as atherosclerosis, arthritis, ischemia and central nervous system injury[26, 27]. It is known that compounds called as antioxidants, scavenge these free radicals and protect our body system. In recent years due to toxic effect of commercially available antioxidants, medicinal plants as antioxidants have been used and they are found more effective then their isolated forms[28]. Phytochemical screening indicated the presence of phenolic acids, which are mainly responsible for the antimicrobial and antioxidant effect of this plant callus.

CONCLUSION

The successful establishment of callus cultures of *S. pauciflorum* for the secondary metabolites production, antimicrobial and antioxidant potentials has been demonstrated. Terpenoids and phenolic metabolites play an important role in human health and can be strong scientific evidence to use this plant as a useful source of both biological and pharmacological references. Establishment of standard protocols for the production of these potent antimicrobial and antioxidant compounds could give rise to the commercial production of valuable metabolites. Further studies on phytochemical investigation are underway to isolate the bioactive phytochemicals leading to isolation of effective drug(s) from this plant callus.

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