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Phytochemistry and Antibacterial Activity of Crude Extracts and Extracted Phenols of *Selaginella bisulcata* Spring

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ABSTRACT

Phytochemical analysis and Antibacterial activities of crude extracts and extracted phenols from sporophytic parts of *Selaginella bisulcata* Spring belonging to the family Selaginellaceae were studied in summer and winter seasons against *Bacillus subtilis* AR-2 (Gr +ve) and *Escherichia coli* XL1-Blue (Gr -ve). Both the crude extracts and extracted phenols from sporophytic plant parts showed antibacterial activities. In summer, the phenol content is minimum and in winter the phenol content is maximum. Each and every sporophytic plant parts showed antibacterial activities by crude extract as well as by extracted phenols. Detailed observations revealed that crude extract shows better antibacterial activity than extracted phenol. HPLC analysis for extracted total phenol reveals that 6 types of phenolic compounds were present in *Selaginella bisulcata* Spring.

INTRODUCTION

Selaginella a member of the family Selaginellaceae, is a very common fern-ally and according to the modern concept belongs to the Lycophytes (Smith *et al*., 2006), lithophytic in nature, mainly grows on shaded humid rocks and is distributed in India throughout the Himalayan region from Eastern Himalayas to Western Himalayas and also found in the Western and Eastern Ghats. This genus is represented by about 700 species throughout the world mainly in the tropical and subtropical climates (Alston, 1945; Jermy, 1990; Mukhopadhyay, 2017). This species isfound in between sea level to 2700 m altitude. Current microbial resistance to antibiotics has been a global concern, as all known classes of natural compounds for antimicrobial therapy are becoming resistant (Boller, 1995). Many fern species are important medicinal plants (Puri and Arora, 1961; Sharma, 1981; Asolkar *et. al.*, 1992; Guha *et. al*., 2004;Ganguly et. al. 2011, 2013). However, some of these plants have been tested for antimicrobial properties (Khandelwal *et. al*., 1985; Hain *et. al.*, 1993). As per literature concerned, there is no report of antimicrobial property of this species of *Selaginella*. Only Homoeopathic medicines are available in the market but not as antimicrobial drug.So,there is urgent need of new compounds for antimicrobial therapy. This study was conducted to test potentiality of this plant as an antimicrobial agent. The present study was conducted to reveal thephytochemical properties, types of phenolic compounds and antibacterialeffects of extracted phenols and

crude extracts of different parts of sporophytic plant body of *Selaginella bisulcata.*

MATERIALS AND METHODS

The study material *Selaginella bisulcata* Spring was collected from different parts of Darjeeling Himalayas at the altitudes of 1900 -2100m and identified with the help of Alston's key (1945) and with the help of the book of Dixit (1992).

CRUDE EXTRACT: Different parts of the sporophytic plant body (Leaves, sporophyll, stem, rhizophore and root) were takenfrom *Selaginella bisulcata* Sring. All the experiments were done byextracts of fresh plant materials. In one set, 100 mg of each of leaf, sporophyll, stem and rhizophore were collected in summer. Each sample of this 100 mg plant parts was crushed with mortar and pestle and extracted in 80% boiled ethanol. This ethanolic mixture was centrifuged at 4000 RPM for 10 min. Then the supernatant was taken out and its total volume was made to 5 ml with 80% boiled ethanol. Then 4 ml distilled water was added to this alcoholic extracts and was kept on a hot plate at 40^{o}C to evaporate the alcohol. Thus the crude extract comes in water solution with a concentration of 2.5% v/v. For each extraction of plant parts, 10 replicates were made for summer and winter.

TOTAL PHENOL: In second set of experiment, total phenols from each 100 mg fresh wt of different plant parts collected and extracted according to the method of Bray & Thorp, 1954. The biochemical analysis of the crude extracts was done (Vyas, *et.al.*, 1989; Britto *et.al.*, 1994; Patric *et.al.*, 1995;). Plant materials were extracted in 80% boiled ethanol, the extract contains plants total protein, total phenols and total soluble and insoluble carbohydrates. As extracted phenols and crude extracts were made from 100 mg plant tissue, the phenol contents were same in both. Total protein content was determined by method of Moore and Stein,1948. Total carbohydrate content was determined following the methods of Mc Cready *et al*., 1950. For all the biochemical analysis, 10 replicates were made for each season. The treatment consisted of three factors- (1) Sporophytic plant body (2) Plant parts (Rhizophore, stem, sporophyll, leaves, root) (3) Bacteria (*Bacillus subtilis* AR-2 and *Escherichia coli* XL-1 Blue).

ANTIBACTERIAL ACTIVITY: Antibacterial activity was measured using 'Agar cup' method (Tortura, *et. al.*, 2001). In agar cup assay, nutrient agar plates of 2 cm thickness were prepared. One set was inoculated with *Bacillus subtilis* AR-2 and the other with *Escherichia coli* XL-1 Blue. Cups of 9 mm diameter were made in the plates in a systematic manner with cork borer. The 0.1 ml water extract of the different parts of sporophytic plant body and their extracted phenols were applied in separate cups and incubated at 37^{o}C. After 24 hrs, diameter of the hallow zones formed due to bacterial lyses were measured. Distilled water was used as control. In each of the experiments 5 replicates were made.

PHYTOCHEMISTRY: Total protein was estimated following Moore & Stein, (1948) and Berenbaum& Zangri's, (1996) method. Soluble and insoluble carbohydrates were estimated

bythe methods of Mc Cready *et al.* (1950).

HPLC: HPLC analysis was performed by using available standards of phenolic compounds following Boudet (2007) and Boligon & De Brum (2012). The extracted total phenol from *Selaginella bislcata*was dissolved in 10 ml HPLC grade methanol and was passed though Whatman membrane filter before injecting in the C18 column. Analysis of extracted total phenol was performed by using 515 HPLC pump and 2489 UV/VIS detector of Waters Company. HPLC analysis of crude extract was not performed as several unknown compounds may be present and requirement of standards to identify them. Further work is needed in this respect.

RESULTS

From the present study it has been revealed that the different parts of *Selaginella bisulcata* Spingaccumulate different amounts of secondary metabolites in different climatic conditions (summer and winter) (Table: 1). Stem and Rhizophore contain maximum amount of phenols in both the seasons. In two seasons*Selaginella bisulcata* stem contains 250.43 µg/mg fresh wt phenol in winter, where as in summer it contains 240.51 µg/mg fresh wt phenol, which is also highest amount found in different parts of the plant parts studied in *S. bisulcata*. In both the seasons the sporophylls contain significant amount of phenols, it is about 195.23 µg/mg fresh wt phenols in summer and 211.17 µg/mg fresh wt phenols in winter followed by leaf which contain 187.32 µg/mg fresh wt in summer and 199.41 µg/mg fresh wt in winter respectively and root 147.41 µg/mg fresh wt in summer and 141.44 µg/mg fresh wt in winter.

Spophyllsand sterile leaves of *Selaginella* are small in size, microphyllous, very thin in textureand probably for these reasons both sporophylls and sterile leaves accumulate very little amount of proteins, it is about 11.21 µg/mg fresh wt in summer and 10.98 µg/mg fresh wt in winter.In both seasons *Selaginella bisulcata* accumulates highest amount of phenols (250.43 µg/mg fresh wt in summer and 240.51 µg/mg fresh wtin winter) in stem followed by rhizophore (230.32µg/mg fresh wt in summer and 235.47 µg/mg fresh wt in winter) and root (10.91µg/mg fresh wt in summer and 10.0791µg/mg fresh wt in winter).Sporophylls stood third position in respect to accumulation of phenols (195.23 µg/mg fresh wt in summer and 211.17 µg/mg fresh wt in winter. In rhizome a little variation is found in accumulation of phenols among the two seasons, which is 230.32 µg/mg fresh wt of in summer and 235.47 µg/mg fresh wt in winter. The total concentration of phenols was highest in summer season i.e. in heat stress condition and protein in winter i.e. in cold stress condition. On the other hand, total concentrations of soluble and insoluble carbohydrates were maximum in winter i.e. in heat stress condition (Table: 1).

Accumulation of biochemical compounds in different parts of the sporophytic plant body of *Selaginella bisulcata*Spring shows a definite seasonal trend. Phenols and proteins accumulation was highest in heat stress condition i.e. in summer season, whereas the

condition was reverse i.e. in cold stress condition in cases of soluble and insoluble carbohydrates. Increase in protein content was very little in winter in respect to summer season. Here also the Sporophyll accumulates highest concentration of protein 15.32µg/mg fresh wt in summer and 15.78 µg/mg fresh wt in winter, followed by leaf (12.51-13.00 µg/mg fresh wt) > Rhizophore (13.12-12.40 µg/mg fresh wt) >Stem (47.27-53.87 µg/mg fresh wt). Soluble and insoluble carbohydrates follow the reverse trend i.e. increase in cold stress condition in respect to phenols and proteins. Rhizophore which is the main accumulator of soluble carbohydrate contains 23.32-26.30 µg/mg fresh wt, followed by stem(21.72-14.30 µg/mg fresh wt) >sporophyll (12.24-10.60 µg/mg fresh wt) > leaf (11.67-10.44µg/mg fresh wt). Accumulation of insoluble carbohydrates was greater than soluble carbohydrates in leaf and sporophyll but reverse in other parts. Sporophyll accumulates maximum amount of insoluble carbohydrates (23.31-1732 µg/mg fresh wt) during winter and summer respectively among the plant parts studied, followed by leaf (14.41-18.62 µg/mg fresh wt) >stem (19.73-18.21 µg/mg fresh wt) >rhizophore (20.32-18.79µg/mg fresh wt) (Table: 1).

The results found in antibacterial activity was that the, both crude extracts and extracted phenols showed bacterial lyses against gram positive (gr +ve) and gram negative (gr –ve) bacteria. Here, crude extracts showed higher inhibitory property than extracted phenols. It has also been found that gr+ve bacteria are more prone to extracted phenols than gr –ve bacteria (Figure: 1). Inhibition zone (hallow area) created by fertile leaf extract was larger than any other parts of the plant in respect to crude extracts as well as extracted phenols (Fig: 1).

DISCUSSIONS

From the above results it is found that, the plant extracts of *Selaginella bislcata* Springshowed significant inhibitory activity against bacterial grm +ve bacterial strain, but show less significant inhibitory activity againstand gr – ve bacterial strain. Crude extracts of the plant parts were more potential than extracted phenols regarding antimicrobial property. It is probably due to the presence of some unknown compounds and phenols which cumulatively inhibit bacterial growth.

HPLC analysis of extracted total phenol reveals that there are about six phenolic compounds present in the plant extract namely Ascorbic acid, Gallic acid, Resorcinol, Catechol, Vanillinand Benzoic acid.

Antibacterial property of this plant might be attributed due to the presence of number of phenolic compoundsatmoderate levels. This is supported by the presence of moderate phenol concentration (240.51-250.43µg/mg wt) in each parts of the plant.

Now a day's microbial resistance to antibiotics has been a global concern, as it spans nearly all known classes of natural compounds. So, in this alarming situation, need of new compounds for antibacterial therapy is very urgent. It has been found that phenolic compounds in plants have strong antibacterial properties against gr+ve and gr–ve bacteria. Pharmacological,

pharmaceutical, phytopathological and food processing industries are some of the fields where phenolic compounds can be applied as bio- preservatives. *Selaginella bisulcata* Springshowed potential evidence for presence nine different kinds of phenolic compounds which has ethno-pharmacological use and promising broad spectrum antibacterial drug. Further work is needed to isolate those active principles responsible for antibacterial properties present in crude extract.

CONCLUSION

From the above result and discussion we can conclude that *Selaginella bisulcata* Spring have the potentiality to establish itself as a broad spectrum ethno-pharmacological antibacterial drug. Current microbial resistance to antibiotics has been a global concern, as it spans nearly all known classes of natural compounds. So, this type of research work for searching new sources of antibacterial agents and new antibacterial compounds is required to enrich the medical science. Further work is in progress to isolate the causal compounds responsible for antimicrobial properties.

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Table: 1- Total phenols, total protein, soluble and insoluble carbohydrate contents in different plant partsof*Selaginella bisulcata* Spring.

Plant Parts	*Selaginella bisulcata* Spring	
	SUMMER	**WINTER**
	Concentration of Phenols (µg/mg fresh wt)	
1. Leaf	187.32	199.41
2. Sporophyll	195.23	211.17
3. Stem	250.43	240.51
4. Rhizophore	230.32	235.47
5. Root	147.61	141.92
LSD at 5%	1.5	1.2
	Concentration of Protein (µg/mg fresh wt)	
1. Leaf	11.21	10.98
2. Sporophyll	15.32	15.78
3. Stem	12.51	13.00
4. Rhizophore	12.40	13.12
5. Root	10.91	12.07
LSD at 5%	0.78	0.69
	Concentration of Soluble carbohydrate (µg/mg fresh wt)	
1. Leaf	10.44	11.67
2. Sporophyll	12.24	10.60
3. Stem	21.72	14.30
4. Rhizophore	23.32	26.30
5. Root	19.25	21.71
LSD at 5%	0.95	0.78
	Concentration of insoluble carbohydrate (µg/mg fresh wt)	
1. Leaf	14.41	18.62
2. Sporophyll	17.32	23.31
3. Stem	19.73	18.21
4. Rhizophore	18.79	20.32
5. Root	09.32	10.47
LSD at 5%	0.87	0.91

Table: 2. Retention times of phenolic compounds present in ethyl acetate extract of *Selaginellabisulcata* Spring.

Phenolic compounds	Retention time	Area	Height	Concentration
Ascorbic acid	2.875	52,900	16,881	84.446
Gallic acid	6.097	3353	227	0.534
Resorcinol	12.850	1638	154	0.625
Catechol	16.200	559,222	26,580	24.276
Vanillin	28.254	294,220	14,324	22.544
Benzoic acid	39.809	517,865	19,835	348.303

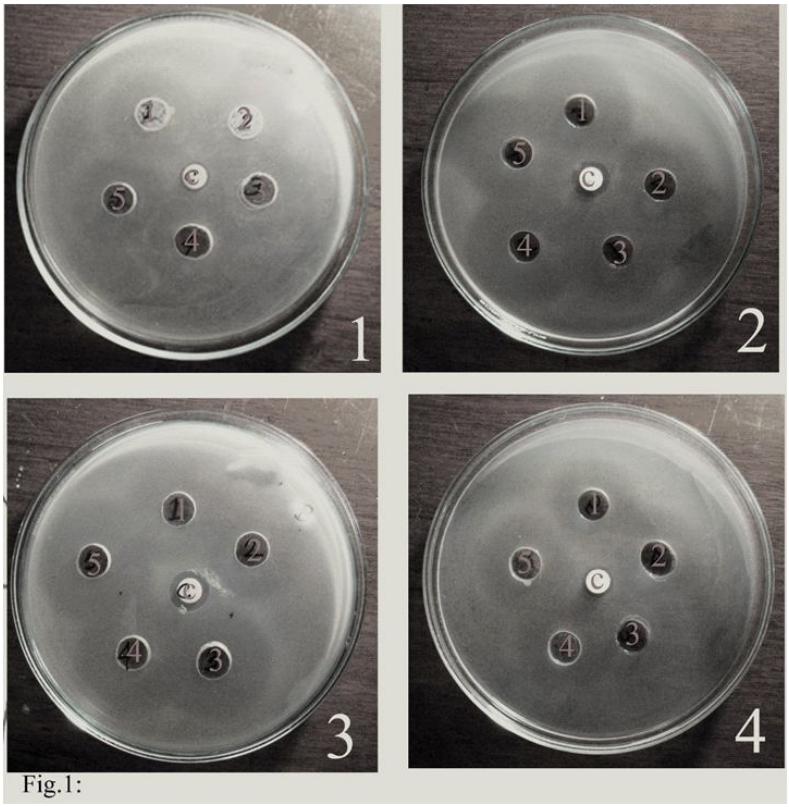

Fig.1:

Legend to the Figure: 1

1: Effect of Crude extract from different parts of *Selaginella bisulcata* on *Bacillus subtilis* AR-2

2: Effect of Crude extract from different parts of *Selaginella bisulcata* on *Escherichia coli* XL-1 Blue

3: Effect of Extracted Phenol from different parts of *Selaginella bisulcata* on *Bacillus subtilis* AR-2

4: Effect of Extracted Phenol from different parts of *Selaginella bisulcata* on *Escherichia coli* XL-1 Blue

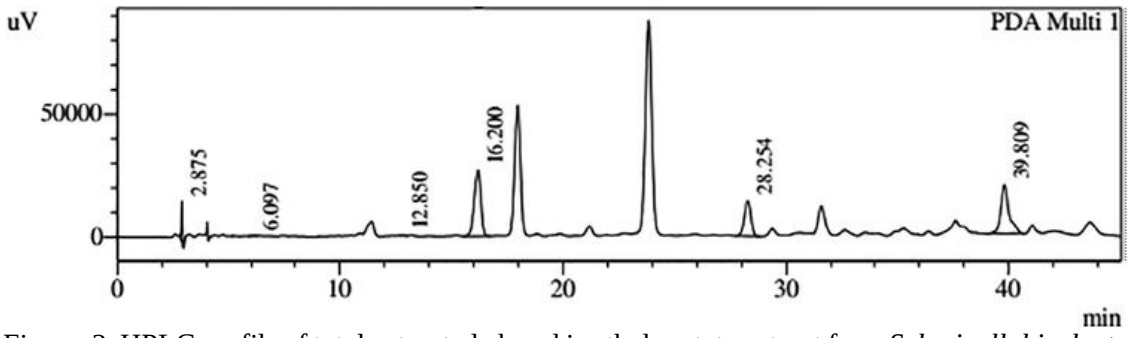

Figure: 2. HPLC profile of total extracted phenol in ethyl acetate extract from *Selaginellabisulcata* Spring

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