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PHYTOCHEMICAL CONTENTS AND ANTIMICROBIAL EFFECTS OF ETHNOMEDICINAL PLANT *ADIANTUM INCISUM* FORSK.

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ABSTRACT:

The aim of the present study was to find out the antimicrobial activity of crude and phenol extracts obtained from different parts of the sporophytic plant of some selected ethno-medicinal ferns of Arunachal Pradesh, India.Out of which thefern *Adiantum incisum* Forsk. showed antibacterial activity against both Gram+(*Bacillus subtilis*AR-2)and gram (Escherichia coli XL-1 Blue) bacterial strains. The antimicrobial activity was measured using 'agar cup' method.The extracts from roots revealed better result. Both crude extract and phenol extract showed antibacterial activity. However, the crude extracts were found more effective than phenol extracts.

Keywords: Antibacterial activity, phytochemical content, extracted phenol, crude extract, Gr+ve and Grve bacteria, *AdiantumincisumForsk.*

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Introduction:

Adiantum incisum Forsk.belongs to the family Adiantaceae and is a common medicinal plant in Bangladesh which is generally used in cough and cold and diabetes. It grows on shaded humid rocks as a lithophyte and is distributed in Zimbabway, South Africa, Namibia and India also. In India it is found as lithophyte or terrestrial in nature and may grow in the rock crevices also in the state of Arunachal Pradesh. It is small fern with tufted appearance havingfronds of 18-30 cm long with simple pinnae and roundedsori. This fern is found in between 500-1400m 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 (Asolkaret. al., 1992; Guhaet. al., 2004; Puri and arora, 1961; Sharma, 198 and Ganguly et.al., 2011) however, their antimicrobial properties are not known.

Materials and Methods:

CRUDE EXTRACT: The sporophytic plants of *Adiantum incisum* Forsk. were collected from different parts of North-Eastern Himalayas at the altitudes of 500-1400m from the states of Arunachal Pradesh, India.Different parts of the plant body (leaves, rachis, root and rhizome) were taken into consideration for the study. The experiments were done by fresh plant materials. In one set, 100 mg of each of root, rhizome, rachis, sterile and fertile leaves were collected in winter (Dec.,2014) and summer (April, 2015)). 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 4000rpm 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 40oC 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 two seasons i.e. winter and summer.

TOTAL PHENOL: In second set of experiment, total phenols from each 100 mg fresh wt of different plant parts collected in winter and summer were extracted and established according to the method of Bray & Thorp, 1954. The biochemical analysis of the crude extracts was done according to (Brittoet.al., 1994; Patric et.al., 1995; Vyas, et.al., 1989). 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 McCreadyet al., 1950. For all the biochemical analysis, 10 replicates were made for each season. The treatment consisted of three factors- 1) Sporophyte 2) Plant parts (Root, rhizome, rachis, sterile and fertile leaves 3) Bacteria (Bacillus subtilisAR-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(as in the figure 1-4). One set was inoculated with Bacillus subtilisAR-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 37oC. 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 6 replicates were made.

RESULTS AND DISCUSSION:

From the present study it has been revealed that the different parts of AdiantumincisumForsk,accumulate different amounts of secondary metabolites in different climatic conditions (winter and summer). The rhizome accumulates highest average amount of phenols (256.43 µg/mg fresh wt) followed by root (230.32 µg/mg fresh wt).Fertile leaf stood third position in respect to accumulation of phenol (195.23 µg/mg fresh wt.) (Table:2). However, the fertile leaves showed highest concentration of proteins followed by rhizome and roots. As far as average concentration of soluble and insoluble carbohydrates are concerned the rhizomes revealed the highest amount which is little higher than roots. In this species the sterile leaves reflected not so good amount of accumulation in respect of secondary metabolites.

The result found in antibacterial activity was that, both crude extracts and extracted phenols showed bacterial lyses against gram positive (*Bacillus subtilis*AR-2) and gram negative (*Escherichia coli* XL-1 Blue) bacteria as well. Here crude extracts showed higher inhibitory property than extracted phenols. It has been found that gr +ve bacteria are more prone to extracted phenol than gr ve one (Table: 1). Inhibition zone (hallow area) created by root extract was larger than any other parts of the plant in respect to crude extracts as well as extracted phenols.

From the above results it is found that, the plant extracts of *Adiantum incisum* Forsk, have significant inhibitory activity against both

bacterial strains (grm +ve and gr ve). 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.

Antibacterial property of this plant might be attributed to the presence of high levels of phenolic compounds. This is supported by the presence of high phenol concentration (177.32-256.43µg/mg wt) in each parts of the plant as reflected in Table: 2.

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. *Adiantum incisum* Forsk., showed potential evidence for its ethno-pharmacological use and promising broad spectrum antibacterial drug. Further work is needed to isolate those active principles responsible for antibacterial properties.

From the above result and discussion we can conclude that *Adiantum incisum* Forsk., 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.

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Table: 1. Effect of crude extracts and extracted phenols of Adiantum incisum on hallow zone size in the plates of Bacillus subtilis AR-2 and Escherichia coli XL-1 Blue.

Plant parts	**Area of hallow zones (in sq.cm.)**			
	Crude plant extract of A. *incisum*		Extracted phenols of A. *incisum*	
	Bacillus subtilis	***Escherichia coli***	***Bacillus subtilis***	***Escherichia coli***
1. Root	2.11	2.17	2.00	1.82
2. Rhizome	1.23	2.00	1.23	1.10
3. Rachis	1.17	1.00	1.10	1.00
4. Sterile leaf	0.20	0.15	0.18	0.17
5. Fertile leaf	1.24	1.21	1.04	1.27

Table: 2. Average Total phenol, total protein, soluble and insoluble carbohydrate contents in plant parts of Adiantum incisum of two seasons i.e. in winter and summer

Plant parts	***Adiantum incisum***
	Average Concentration of Phenols (µm/mg fresh wt) of two seasons
1. Sterile leaf	177.32
2. Fertile leaf	195.23
3. Rhizome	256.43
4. Root	230.32
LSD at 5%	**1.50**
	Average Concentration of Protein (µm/mg fresh wt) of two seasons
1. Sterile leaf	11.24
2. Fertile leaf	15.32
3. Rhizome	12.51
4. Root	12.40
LSD at 5%	**0.78**
	Average Concentration of Soluble Carbohydrate (µm/mg fresh wt) of two seasons
1. Sterile leaf	10.44
2. Fertile leaf	16.24
3. Rhizome	23.72
4. Root	23.32
LSD at 5%	**0.85**
	Average Concentration of Insoluble carbohydrate (µm/mg fresh wt) of two seasons
1. Sterile leaf	13.41
2. Fertile leaf	17.32
3. Rhizome	20.73
4. Root	18.79
LSD at 5%	**0.77**

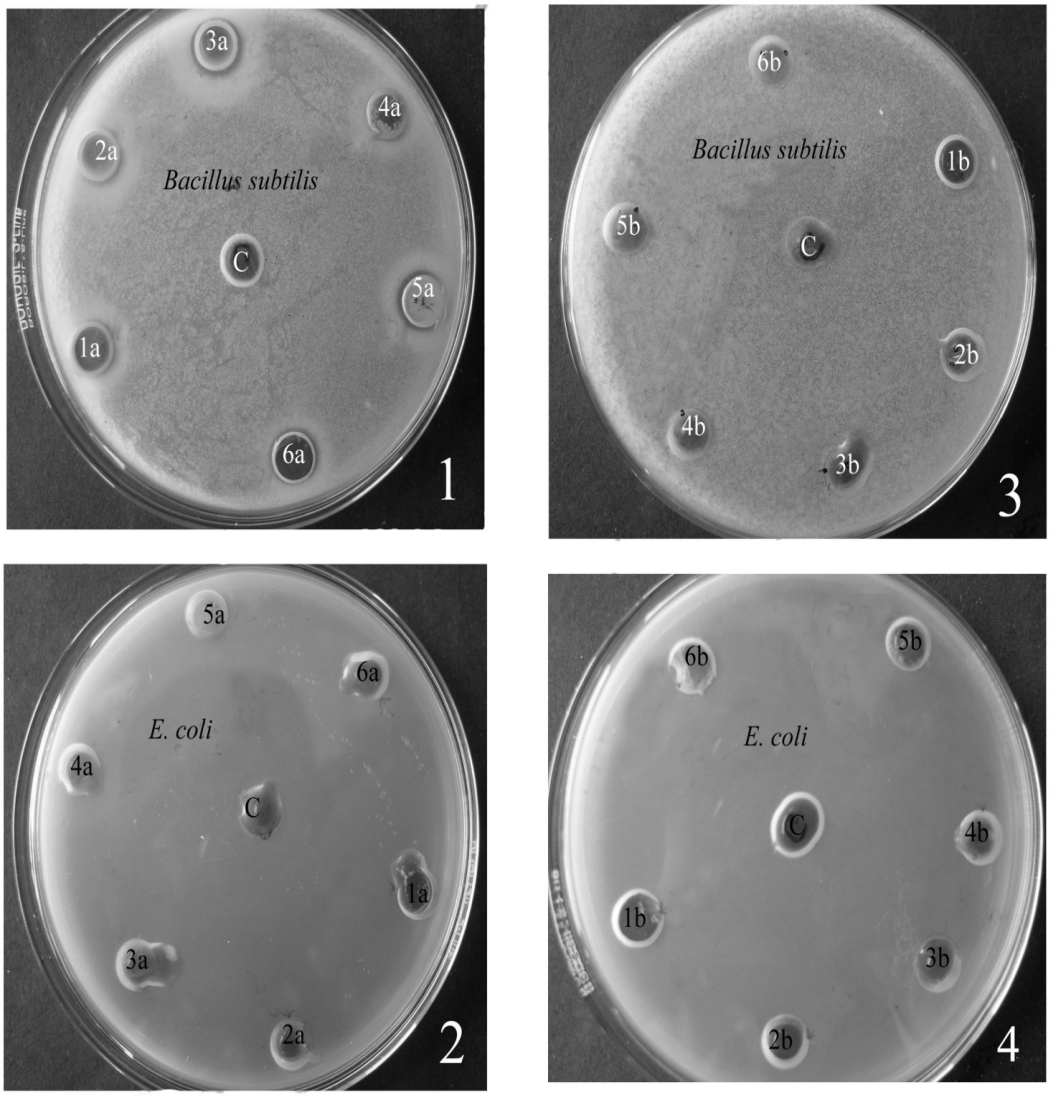

Fig 1&3.
Effect of crude extract of *Adiantum incisum* on Bacillus (1a-b, 2a-b,3a-b)
Effect of extracted phenol of *Adiantum incisum* on Bacillus (4a-b,5a-b,6a-b)

Fig 2&4.
Effect of crude extract of *Adiantum incisum* on E.coli (1a-b, 2a-b, 3a-b)
Effect of extracted phenol of *Adiantum incisum* on E.coli (4a-b, 5a-b,6a-b)

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