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Full Length Research Paper

In vitro antibacterial activity of fronds (leaves) of some important pteridophytes

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The main objective of this research work is to screen various unexploited plants for their antimicrobial activity as these unexploited or pteridophytic plants are being used ethanomedicinally but, very little work has been done on antimicrobial aspects. So, to explore the efficacy of these plants, the following research has been carried out. Bacterial strains of *Agrobacterium tumefaciens, Escherichia coli, Salmonella arizonae, Salmonella typhi* and *Staphylococcus aureus* were procured from the Institute of Microbial Technology (IMTECH), Chandigarh and the aqueous and alcoholic leaves extract of twelve important pteridophytic plants were prepared and tested for their antimicrobial activity against the bacteria selected by Disc diffusion method as suggested by Bauer et al. (1966). It has been observed that, nearly all the leaves extracts have shown inhibitory effect against the bacterial strains selected and some of the extracts were more competent than the selected antibiotic. Our findings provide the novel insights with regards to antimicrobial agents and these could be further enhanced through *in vivo* studies and isolation and characterization of active constituents for human health. In the present *scenario*, the use of herbs and herbal medicine is at its peak and majority of researchers are screening higher plants for the same but, very few researchers are considering the lower plants for their antimicrobial potential. Since, these pteridophytic plants are considered to be the disease free plants and are being used ethanobotanically by various tribal communities. These plants are further screened for their *in vivo* potential as well as for their drug properties.

Key words: Antimicrobial activity, pteridophytic plants, leaves extracts, bacteria.

INTRODUCTION

Now-a-days, the study of the drugs and drug plants has progressed steadily and at present pharmacology is the essential branch of medicine, and Botany and medicine have gone hand in hand and the majority of Botanists of past had a knowledge of medicinal plants. Although, antimicrobial properties of the drugs are not mentioned in early literature but therapeutically, properties of drugs may be due to presence of chemical substances. Some of which either individually or collectively may be effective as antimicrobial for gram positive as well as gram-negative bacteria, fungi, actinomycetes, protozoa etc.

India is profusely rich in the history of medicinal plants and its 75% folk population is still using herbal preparations in the form of powder, extracts and decoction because these are easily available in nature and the natives have stronger faith on traditional knowledge (Dixit, 1974). Pteridophytes (fern and fern allies) by virtue of possessing great variety and fascinating foliage have drawn the attention and admiration of horticulturists and plants breeders for centuries.

They are represented by about 305 genera, comprising more than 10,000 species all over the world. About 191 genera and more than 1000 species are reported from India (Kirtikar and Basu, 1935; Nayar, 1957).

As folk medicine, the pteridophytes which constitute ferns and ferns allies, have been known to man for more than 2000 years, and also been mentioned in ancient literature (Chopra et al., 1958; Kumar and Roy, 1972; Watt, 1972; Dixit and Bhatt, 1975). It has been observed that, pteridophytes are not infected by microbial patho-gens, which may be one of the important factors for the

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evolutionary success of pteridophytes and the fact that, that, they survived for more than 350 million years (Sharma and Vyas, 1985).

Considering the rich diversity of Indian medicinal plants including pteridophytes, it is expected that, the screening of plant extract for antibacterial activity may be beneficial for humans and plants diseases. The synergistic interaction among crude extracts or the active compounds may be useful in the preparation of improved herbal or drug formulations. Therefore, the aims of the present investigation were the *in vitro* antibacterial activity of leaf extracts of 12 pteridophytes harvested at Rajasthan against four gram-negative and one gram-positive human and plant pathogenic bacteria.

MATERIALS AND METHODS

Collection and identification of plants

The specimens of plant, that is, *Adiantum capillus-veneris* L. (Adiantaceae), *Adiantum incisum* forsk. (Adiantaceae), *Adiantum lunulatum* Burm. F. (Adiantaceae), *Actiniopteris radiata* (Swartz.), Link (Actiniopteridaceae), *Araiostegia pseudocystopteris* Copel. (Davalliaceae), *Athyrium pectinatum* (Wall ex Mett.) T. Moore (Athyriaceae), *Chelienthes albomarginata* Clarke (Sinopteridaceae), *Cyclosorus dentatus* (Forsk.) Ching (Thelypteridaceae), *Dryopteris cochleata* (Don.) C. Chr. (Dryopteridaceae), *Hypodematium crenatum* (Forsk.) Kuhn (Hypodematiaceae), *Marsilea minuta* L. (Marsileaceae) and *Tectaria coadunata* (J. Smith) C. Chr. *(T. macrodonta)* (Aspediaceae) were collected from Aravalli ranges in Rajasthan during the month of August -December 2001 and their identity was confirmed through specimens, herbaria and literature available in the Department of Botany, J. N. Vyas University, Jodhpur.

Preparation of plant extracts

5 g of fresh leaves were washed 2 - 3 times with tap water and distilled water and then surface sterilized with 90% ethanol. Subsequently, the plant materials were grounded in 50 mL of distilled water and methanol separately for aqueous and alcoholic extracts, respectively. The methanolic macerates were kept for 24 h at room temperature to evaporate the alcohol. In the remaining residue, 50 mL of distilled water was added. Macerates were squeezed through double-layered muslin cloth and filtered through filter paper. After filtration, aliquot was centrifuged at 10,000 rpm for 20 min at room temperature. The supernatants were filtered through Whatman No. 1 filter paper and then sterilized by passing through 0.2 µm disposable filters. The extracts (10%) thus, obtained were used for the *in vitro* studies (Parihar et al., 2007a; Parihar et al., 2007b).

Antibacterial assay

Agrobacterium tumefaciens (MTCC No. 431), *Escherichia coli* (MTCC No. 443), *Salmonella arizonae (*MTCC No.660), *Salmonella typhi* (MTCC No. 734) and *Staphylococcus aureus* (MTCC No. 96) were procured from the Institute of Microbial Technology (IMTECH), India and were used as indicator strains disc diffusion method and between strains and dics (Bauer et al., 1966) was used to test antimicrobial activity against bacteria. Solutions of known concentration (10% that is, dilution of the in water to a final concentration of 10%) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents.

Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amounts of the test substances using micropipette. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the test micro-organisms. Standard antibiotic discs and blank discs (impregnated with solvents) were used as a positive and negative control. These plates were then kept at low temperature (4ºC) for 24 h to allow maximum diffusion. There was a gradual change in concentration in the media surrounding discs. The plates were then incubated at 37ºC for 24 h to allow maximum growth of the organisms. The test materials having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium.

The antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment was carried out three times and the results were the mean of three replicates (Bauer et al., 1966).

RESULTS AND DISCUSSION

From Table 1, it was found that aqueous and alcoholic extracts of leaves of *A. lunulatum* and *A. pectinatum,* aqueous extract of leaves of *D. cochleata* and *M. minuta* and alcoholic extract of *C. dentatus* and *H. crenatum* have not shown any inhibition against *A. tumefaciens* while the rest of the extracts were found effective. Except aqueous and alcoholic extract of *A. pectinatum,* aqueous extract of *A. incisum* and *H. crenatum* and alcoholic extract of *C. dentatus,* all other extracts of leaves were found effective against *E. coli.* It was also found that the aqueous and alcoholic extracts of leaves of *M. minuta* were found more effective than the reference standard antibiotic (tetracycline) against the pathogenic strain of *E. coli.*

It was also observed that aqueous and alcoholic extract of leaves of *A. incisum* and *D. cochleata,* aqueous extract of leaves of *A. capillus-veneris* and *M. minuta* and alcoholic extract of leaves of *C. albomarginata, C. dentatus* and *H. crenatum* were not found effective against the growth of *Salmonella arizonae.* Rest of all the extracts was effective against the bacteria. It was also found that except aqueous and alcoholic extract of leaves of *A. pectinatum* and aqueous extract of leaves of *C. albomarginata, all* other extracts have shown inhibitory effect against *S. typhi.*

It has also been found that, only aqueous and alcoholic extract of leaves of *A. pectinatum*, aqueous extract of leaves of *A. incisum* and *D. cochleata* and alcoholic extract of leaves of *C. dentatus* and *D. cochleata* have not shown any inhibitory effect against the growth of *S. aureus.*

DISCUSSION

Very less work has been done on the antimicrobial activity of pteridophytes, yet ethanobotanical importance of these plants have been investigated and studied by various authors. They reported that these plants are of

Table 1. Antibacterial activity of leaves extracts of some important pteridophytic plants.

Plant species	Extract	*A. tumefaciens*	*E.coli*	*S. arizonae*	*S. typhi*	*S. aureus*
Adiantum capillus-veneris	Aqueous	10	09	00	06	07
	Alcoholic	13	15	07	04	10
Adiantum incisum	Aqueous	09	00	00	07	02
	Alcoholic	06	09	00	09	10
Adiantum lunulatum	Aqueous	00	19	16	15	11
	Alcoholic	00	09	08	13	15
Actiniopteris radiate	Aqueous	09	08	10	07	11
	Alcoholic	06	11	09	12	07
Araiostegia pseudocystopteris	Aqueous	09	16	02	07	06
	Alcoholic	09	15	09	09	09
Athyrium pectinatum	Aqueous	00	00	09	00	00
	Alcoholic	00	00	09	00	00
Chelienthes albomarginata	Aqueous	08	15	04	00	09
	Alcoholic	06	07	01	14	10
Cyclosorus dentatus	Aqueous	09	08	05	09	05
	Alcoholic	04	00	00	09	00
Dryopteris cochleata	Aqueous	00	22	00	12	00
	Alcoholic	08	18	00	13	00
Hypodematium crenatum	Aqueous	06	03	05	09	07
	Alcoholic	09	16	03	10	11
Marsilea minuta	Aqueous	00	25	00	14	16
	Alcoholic	16	24	04	15	12
Tectaria macroconta	Aqueous	13	16	10	11	16
	Alcoholic	06	11	12	09	09
Tetracycline (Antibiotic)	10%	24	21	23	40	22

Inhibition zone against the bacteria by methanol: 0 mm. Inhibition zone against the bacteria by ethanol: 6 mm.

great medicinal importance and are used by the tribal and local people for remedy against various ailments (Chopra et al., 1956; Vyas, 1987; Manickam and Irudayaraj, 1992; Hansraj, 1996; Kaushik and Dhiman, 1995; Chandra, 2000; Kumar et al., 2003). Similar results have been found for their biological activity (Dhar et al., 1968). The phytochemical composition of *A. radiata* has been studied and found that the isolated phytochmicals were effective against the growth of microorganisms. Bhabbie et al. 1972 in 1980, antibiotic activity of pteridophytes has been studied (Banerjee and Sen, 1980) while the antiviral activity of crude extracts of some pteridophytes have also been analyzed (Pandey and Bhargava, 1980). The effect of leaf extracts of some pteridophytes has been studied against the conidial germination of *Drachslera oryzae*. Ganesan, 1993 was on the opinion that, these extracts could be used to minimize the plant diseases. The antibacterial activity of *A. capillus-veneris* was also been studied and found that, nearly all the extracts were effective against the selected microorganisms which is comparable with our results (Kumar and Kaushik, 1999; Guha et al., 2004).

Antifungal effect of pteridophytic plant part extracts have been studied against the dermatophytes (Davvamani et al., 2005) and that of leaf glands of pteridophytes have also been studied (Manickam et al., 2005). Similar results have also been evaluated (Parihar and Bohra, 2003; Parihar and Bohra, 2004; Parihar et al., 2005; Parihar and Parihar, 2006a; Parihar and Parihar, 2006b; Parihar et al., 2006). Besides, pteridophytes, antimicrobial activity of crude extracts of Bryophytes and Gymnosperms have also been evaluated (Parihar et al., 2002; Parihar et al., 2005).

It is concluded that, antibacterial activity of root extracts of these pteridophytic plants and their active constituents would be helpful in treating various kinds of diseases. Crude

extracts and their interactions with different active fractions of the plants are needed to explore the exact mechanism of the interaction among the active phyto-constituents. Similarly, the efficacy of crude extracts or polyherbal preparations needs to be studied *in vivo* to assess their therapeutic utility.

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