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Research Article

FERNS OF SHALMALA RIPARIAN FOREST IN KARNATAKA AND ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *DICRANOPTERIS LINEARIS*

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ABSTRACT

Present study, an attempt has been made to enumerate the Pteridophytic composition in Shalmala riparian forest, Sirsi taluk of Uttarkannada District, Karnataka and taken up for explore scientifically the antioxidant and antimicrobial potency of *D. linearis*. Present study indicated that, study area exhibited rich pteridophytic diversity and most of them are terrestrial, few are aquatic and epiphytic. Results from the extracts of *D.linaeris* frond have showed antimicrobial activity against the bacterial strains and ethanolic extract had good antioxidant property than chloroform extract of *D.linaeris* frond. The present work helped in making an awareness about fern and fern allies and helpful for the further studies on Pteridophytes and to take conservation strategies.

INTRODUCTION

The Western Ghats are one of the important centers of plant diversity and richness of fern flora of the World. Western Ghats supports 349 Pteridophytic species out of 1100-1200 species of ferns and fern allies in India (Manickam and Irudayaraj, 1992; Manickam, 1995) and 12,000 species of Pteridophytes occur in the world (Manickam and Rajkumar, 1999; Chandra, 2000; Dixit, 2000). Karnataka is the well-known state in India with 400km long stretch of Western Ghats region, which is rich in flora and fauna. Many authors reported significant work on the Pteridophytes in Karnataka (Match person, 1986; Blatter and Almedian, 1992; Alston, 1945; Kammathy *et al.,* 1967; Razi and Rao, 1971; Bhaskar and Razi, 1973; Deepa *et al*., 2011, 2013c, 2013d, 2013e; Natarajaet *al*., 2011). There have been some notable studies on the Pteridophytes of Central Western Ghats in Karnataka with the earliest record of 174 species of Pteridophytes mostly growing in Central western Ghats (Rajagopal and Bhat, 1998) and 75 fern species from north canara district (Matchperson, 1986). Ferns are also have an medicinal value, which have been used to cure various ailments like Diabetes, Cough, boils, cuts, wounds, Inflammations, respiratory problems, female disorders (Srivasthava, 2007).

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The medicinal value of ferns has been known to many for more than 2000 years. A systematic survey of antimicrobial activity of ferns have been done by Singh *et al.,* (2008), Sen and Ghose (2011) and Dalli *et al.,* (2007). A number of angiosperms with significant antimicrobial activity have been reported in literatures. However, very less information is available at present in the literature regarding antimicrobial activity of Pteridophytes (Deepa *et al*., 2013a). *Blechnum orientale* L. were showing good antioxidant and antibacterial activities. (Deepa *et al*., 2013a, 2013b). In this present study, an attempt has been made to enumerate the Pteridophytic composition in Shalmala riparian forest, Sirsi taluk of Uttarkannada District, Karnataka. The opinions made by the people around Shalmala riparian forest that, there is no folkloric use of *D. linearis* and they are treated it as weed. Therefore, this study was also taken up for explore scientifically the antioxidant and antimicrobial potency of *D. linearis*.

MATERIALS AND METHODS

The present study is the outcome of exhaustive field survey undertaken during year of 2014-15. Shalmala riparian forest located in the Sirsi taluk of Uttara Kannada district, Karnataka state, India. It lies in 14°70' N latitude and 74°80' E longitude. Mean annual rainfall ranges from 2000 mm to 6000mm. About 95% of the rainfall is received during the month of June to September, when the southwest monsoon is at its climax.

Table 1. Diversity and habitat of ferns from Shalmala riparian forest in Sirsi taluk

Sl. no	Name of the species	Family	Habitat
1	*Adiantum capillus-veneris* L.	Pteridaceae	T
2	*Adiantum phillipense* L.	Pteridaceae	T
3	*Angiopteris helferiana* C.Presl	Marattiaceae	T
4	*Azolla pinnata* R.Br.	Azollaceae	A
5	*Blechnum orientale* L.	Blechnaceae	T
6	*Bolbetis subcrenatoides* Fraser –Jenk.	Lomariopsidaceae	T
7	*Cyathea gigantean* (Wall.ex Hook.) Holttum	Cyatheaceae	T
8	*Dicranopteris linearis* (Burm.f.) undrew.	Gleichenaceae	T
9	*Drynaria quercifolia* Fraser-Jenk.	Lomariopsidaceae	E
10	*Huperzia hamiltoni* (Spreng.)	Lycopodiaceae	E
11	*Lepisorus nudus* (Hook.) Ching	Polypodiaceae	E
12	*Leptochilus lanceolatus* Fee	Polypodiaceae	T
13	*Palhinhea cernua* (L.) Vasc.& Franco	Lycopodiaceae	T
14	*Lygodium flexuosum* (L.) Sw	Lygodiaceae	T
15	*Marsilea minuta* L.	Marsileaceae	A
16	*Nephrolepis cordifolia* (L) C.Presl	Oleandraceae	T
17	*Nephrolepis undulate* (Afzel.ex Sw.) J.Sm.	Oleandraceae	T
18	*Odontosoria chinesis* (L.) J.Sm.	Lindsaeaceae	T
19	*Parahemionitis cordata* (Roxb.ex Hook. &Grev.) Fraser –Jenk.	Pteridaceae	T
20	*Pteris biaurata* L.	Pteridaceae	T
21	*Pityogramma calomelanos* (L.)Link	Pteridaceae	T
22	*Selaginella tenera* (Hook. &Grew.) Spring	Sellaginellaceae	T
23	*Tectaria polymorpha* (Wall.ex Hook.) Copel.	Dryopteridaceae	T
24	*Tectaria coadunate* (Wall.ex Hook. & Grev.) C.Chr.	Dryopteridaceae	T

Table 2. Inhibition zones against fungal strains

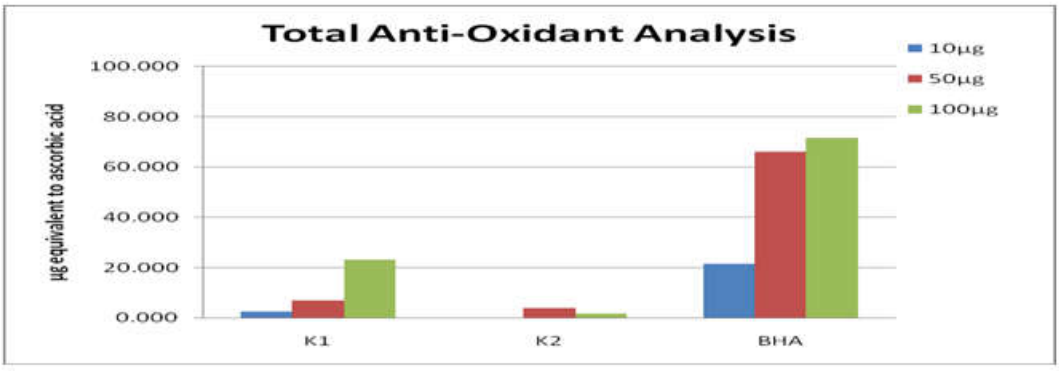
Concentration of sample	Methanol		Ethanol		Chloroform		Amphotericin	
	NC	CA	NC	CA	NC	CA	NC	CA
25 µg	-	-	-	-	-	-	-	-
50 µg	-	-	-	-	-	-	-	2
100 µg	-	-	-	-	-	-	-	7
200 µg	-	-	-	-	-	-	-	9
400 µg	-	-	-	-	-	-	7	13
800 µg	-	-	-	-	-	-	9	15
MIC µg	-	-	-	-	-	-	400	50

Note: NC – Neurospora crassa , CA – Candida albicans , '-':absent

Table 3. Inhibition zones against bacterial strains

Concentration of sample	Methanol		Ethanol		Chloroform		Gentamycin	
	SA	EC	SA	EC	SA	EC	SA	EC
25 µg	-	-	-	-	-	-	13	18
50 µg	-	-	-	-	-	-	18	20
100 µg	-	-	-	-	-	-	21	23
200 µg	-	-	-	-	-	-	25	26
400 µg	3	2	-	-	-	-	27	28
800 µg	4	3	-	-	-	-	34	31
MIC µg	400	400	-	-	-	-	25	25

Note: SA – Staphylococcus aureus, EC – Escherichia coli, '-': absent.



Note: K1- Methanol extract, K2- Chloroform extract

Fig.1. Total antioxidant activity of samples in comparison with Butylated hydroxylanisole (BHA)

Temperature range from 22°C to 36°C with relative humidity of dry month and monsoon month were less than 35% and 75% respectively. Diagnostic features of all the pteridophytic specimens were observed and relevant field notes were made on fresh plant materials. Pteridophytic species were identified with the help of standard Pteridophyte floras (Beddome, 1863, 1883, Clarke 1880, Blatter & D'Almeida, 1922 and Manickam & Iradayaraj 1992). Authentications of species were done with the help of Dr. Deepa J, Pteridologist, Panchavati Research academy for nature, Sagara. The nomenclature of taxa and species has been given according to Fraser-Jenkins (2008, 2010).

Antimicrobial assay

D. linearis selected in Shalmala Riparian forest for antimicrobial assay. The Fronds of *D.linearis* were collected and shade dried, milled to obtain a fine powder. The 50gm of dry powder sample was subjected to soxhlet extraction with 350ml of organic solvents such as Chloroform, Ethanol and Methanol separately for 24h. The extract obtained was dried at room temperature and used for the further study. The Antimicrobial activities were analyzed by agar diffusion method using above solvent extracts and were screened at various concentrations like 100µg, 200µg, 400µg, 800µg.

The microbial cultures such as *Staphylococci aureus* (MTCC 3160), *Escherichia coli* (MTCC 1554), *Neurospora crassa* (MTCC 1855) and *Candida albicans* (MTCC 3958) procured from Biogenics, Research and Training Centre in Biotechnology, Hubli. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37˚C for 18 h. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures and spread evenly on the plate. After 20 min, the wells were filled with of compound at different concentrations of extract. The control wells with Gentamycin were also prepared. All the plates were incubated at 37°C for 24 h and the diameter of inhibition zone were noted (Deepa *et al.,* 2013a). For antifungal activities, the stock cultures of fungi were grown at 27^0C for 48 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 48 h old fungal cultures and spread evenly on the plate and the wells were filled with different concentrations of extract. The control wells were filled with antibiotic. All the plates were incubated at 27^0C for 72 h and the diameter of inhibition zone were noted (Deepa *et al.,* 2013a; Chang et al., 2007).

Antioxidant assay

Methanol and Chloroform extracts are used for antioxidant assay. Various concentrations of samples (10µg, 50µg and 100 µg) were taken in a series of test tubes. Add 1.9 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 min and allowed to cool. The absorbance of the aqueous solution of each was measured at 695 nm against a blank. Antioxidant capacities are expressed as equivalents of ascorbic acid. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. Butylated hydroxyanisole (BHA) was used as reference standard. The values are expressed as ascorbic acid equivalents in µg per mg of extract (Deepa *et al.,* 2013b).

RESULT AND DISCUSSION

Sirsi taluk is one of the richest floristic areas of Uttara Kannada District located in Central Western Ghats, Karnataka. The present floristic study showed the high richness and diversity of Pteridophytes in study area. It harbours a rich Pteridophytic flora of 24 species belonging to 15 families (Table 1). Out of these, Pteridaceae reported as the dominant family with 5 species and 5 families viz., Polypodiaceae, Oleandraceae, Lomariopsidaceae, Lycopodiaceae and Dryopteridaceae are represented by 2 species. The 9 families are monospecific. According to habitats, 15 species are terrestrial ferns, 3 species are epiphytic and 2 species are aquatic. Similar observation noted by Deepa *et al.,* (2013) from Kemmangundi forest, Karnataka, South India. Ferns are widely cultivated as ornamental pot plants. Some Pteridophytes are traditionally used as medicines by the native people from the hilly region. Many of these Pteridophytes are known to be used by humankind in various ways, for example., *Lygodium flexuosum* L. – aqueous extract of rhizome cures Gonorrhoea and the paste of the rhizome is applied on piles in India (Srivastava, 2007), *Adiantum capillus-veneris* (Linn.)-Fresh fronds are astringent, stimulant, refrigerant and tonic (Srivastava, 2007), *Dicranopteris linearis*-Young circinately vernated leaves mixed with cow milk used seven days continuously to remove sterility in Women (Srivastava, 2007).

Antimicrobial assay indicated that bacterial strains in methanolic extract only shows a minimum inhibition zone at 400µg compared to other extracts. In case of fungal strains including *Neurospora crassa* and *Candida albicans were* not inhibited by any extracts compare to standard (Table.2). Smaller inhibition zones were observed in the case of methanolic extracts of *Dicranopteris linearis* frond against both gram positive and gram negative bacterial strains. The extracts were tested at 10µg/ml concentration against bacterial and fungal strains. The data obtained from the disc diffusion method (Table.3) indicated that the methanolic extract displayed an antimicrobial activity on *S. aureus* and *E.coli*. The diameter of inhibition zones produced by *D. linearis* extract against *S. aureus* was 3mm (400 µg), 4mm (800 µg); in case of gram-negative bacterial strain inhibition zones was 2mm (400 µg) and 3mm (800 µg). This shows that an *S. aureus* shows high degree of inhibition zone when compared to *E. coli*. The result of minimum concentration was also shown in Table3.

Minimum inhibitory concentration defined as the lowest concentration of test samples that result in a complete inhibition of visible growth. It indicated that methanolic extract was more potent to *S.aureus* and followed by *E.coli* at 400µg MIC. When compared with earlier reports on antibacterial activity of *D.linearis* (Samir, 2013) showed almost equal activity against both Gram-positive (*S.aureus*) and Gram-negative bacteria (*Bacillus megaterium*), while the present study showed relatively Gram-positive had high inhibition zone against *E.coli*. The investigation suggested that all the extracts have not effective against the tested organisms. Only methanolic extract active against bacterial strains. The methanolic extract may be proving good. The result mentioned is revealed that *D.linearis* possesses considerable antibacterial activity. The isolation of bioactive components of these extract responsible for the activity in progress. The extract of *D.linearis* may be useful as an alternative antibacterial agent as

natural medicine for the treatment of diseases caused by microbes. The Antioxidant assay indicated that Methanol extract had higher antioxidant potency than the Chloroform extract (Fig.1). The total antioxidant activity which expressed equivalent to Ascorbic acid has 23.25 of methanol extract, whereas in Chloroform extract it was observed that 1.65. However, the antioxidant activity of these two samples compared with Butylated hydroxylanisole gives smaller activity.

Conclusion

Present study indicated that, study area exhibited rich pteridophytic diversity and most of them are terrestrial, few are aquatic and epiphytic. Results from the extracts of *D.linaeris* frond have showed antimicrobial activity against the bacterial strains and ethanolic extract had good antioxidant property than chloroform extract of *D.linaeris* frond from Total antioxidant assay. All these indicate the scientific basis for some therapeutic uses of fern *D.linaeris* and supported ethnomedicinal importance. Many ferns are on the decline due to Over-exploitation. Excessive felling of the tree flora for various purposes has adversely affected the habitat for ferns, which is the main cause of the disappearance of many ferns. There is an urgent need for the conservation of ferns, which are important for their academic, medicinal and Ornamental values. The present survey will be helped in making an awareness about fern and fern allies and helpful for the further studies on Pteridophytes and conservation strategies.

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