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Research Article

Anthelmintic efficacy of selected ferns in sheeps (Ovis aries.Linn)

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Abstract

Ethnobotany of *Actinopteris radiata* (Sw.) Link, *Acrostichum aureum* (Linn), *Dryopteris cochleata* (Buch. Ham. ex D. Don), *Drynaria quercifolia* (L.) J. Smith, *Hemionitis arifolia* (Burm.) Moore and *Pityrogramma calomelanos* (L.) Link is perhaps the first report on the phytochemical analysis, clinical trials for anthelmintic efficacy of ferns in naturally infected sheeps (*Ovis aries*) against *Haemonchus contortus*. Qualitative phytochemical analysis among the three solvents used viz., Aqueous, Ethanolic and Petroleum ether, ethanolic fern extract performed well to express the phytoconstituents. Quantitative phytochemical analysis indicated a higher tannin and phenolic content in *Pityrogramma calomelanos* (L.) Link and lower content in *Drynaria quercifolia* (L.) J. Smith. *In vitro* study confirmed lesser time taken for paralysis and death of *Haemonchus contortus* worm in *Pityrogramma calomelanos* (L.) Link extract unlike the other ferns studied. *In vivo* clinical trials using Faecal Egg Count Reduction Test on day 0 (pre-treatment) and on day 5, 7, 9 (post-treatment) with ethanolic fern extracts revealed *Pityrogramma calomelanos* (L.) Link (91%) had better efficacy than *Actinopteris radiata* (Sw.) Link (84%), *Dryopteris cochleata* (Buch. Ham. ex D. Don) (78%), *Drynaria quercifolia* (L.) J. Smith (65%), *Acrostichum aureum* (Linn) (56%) and *Hemionitis arifolia* (Burm.) Moore (49%).

Keywords: Ethnobotany, Fern, Phytochemical, Anthelmintic, *In vitro*, *In vivo*, Sheeps

Introduction

Tropical forests are one of the most threatened habitats in the world and are the most prolific when considering plant communities [1]. In India, these forests are restricted to a few pockets in the Eastern Himalayas and Western Ghats, both of which are

recognized as biodiversity hotspots [2]. The low land forests in the foothills of Western Ghats are tropical evergreen or semi-evergreen and are repositories of Pteridophytic flora. Pteridophytes constitute the primitive vascular cryptogamic plants which occupy a position between the lower non-seed bearing and higher seed bearing plants from generally much neglected group of plants [3]. Ferns are represented by about 305 genera, comprising more than 12,000 species all over the world. About 70 families, including 191 genera and 1000 species are likely to occur in India. Pteridophytes form a conspicuous element of vegetation all over the earth's surface. Pteridophytes grow luxuriantly in moist tropical and temperate forest and their occurrence in different eco-geographically threatened regions from sea level to the highest mountain are of such interest. They are a group of plant similar to Bryophytes important from phylogenetic and evolutionary point of view; the lines are evolved from hypothetical land plants of primitive archegonia group [4]. Pteridophytes (fern and fern allies) are called as reptile group of plants and are one of the earliest groups of vascular plants. Most of the indigenous people are not well known about the uses of Pteridophytes since it is not easily available like flowering plants. Economic and medicinal values of higher plants have been investigated thoroughly; unfortunately Pteridophytes have been ignored [5, 6].

Ethnobotanical studies in human and veterinary medicine is an emerging trend in the treatment of chronic infectious diseases, skin infections, tumors, parasitic infections with no untoward symptoms, least toxic and are low cost effective. There are limited research activities in the medicinal plants of lower taxonomic group of vascular cryptogams, "The Pteridophytes". Pteridophytic medicinal plants are declining and are destroyed due to human habitation, natural landslides, soil erosion, heavy rainfall and many of them are in threatened list and some are in endangered status in the Red Data book of IUCN. The limited knowledge of these medicinal plants for disease control and their weed habitat, these fern are destroyed by the human. The ferns had an important role in folklore medicine. These plants have been successfully used in the different systems of medicines like Ayurvedic, Unani, Homeopathic and other systems of medicines.

Parasitic disease mostly helminthiasis is the condition resulting from round worm infestations especially *Haemonchus contortus* in small ruminants and is one of the major prevalent diseases in the world, particularly in the tropical countries, India [7, 8]. Problems have emerged with the use of anthelmintics, notably the development of resistant in helminthes [9] to various anthelmintic compounds and classes, as well as chemical residue and toxicity problems. Consequently there is an urgent and ever present need to control infections caused by *H.contortus* in ruminants. The frequent use of anthelmintics over many years has inevitably led to the development of drug resistance to each class in parasitic nematodes. *H.contortus* has been documented to be resistant to all three broad spectrum families of anthelmintics viz., benzimidazole, imidazothiazole and ivermectin. The medicinal plants selected for endoparasitic infection produce fewer unanticipated side effects and apparently do not trigger parasitic chemo-resistance. The emergence of resistance to anthelmintic drugs and the increased awareness of consumers about drug residues that potentially enter the food chain have stimulated investigation into alternative to commercially available anthelmintics such as medicinal plants [10]. For centuries, medicinal plants have been used to combat parasitism and in many parts of the world are still used for this purpose. The use of medicinal plants for the prevention and treatment of gastro-intestinal parasitism has its origin in ethno-veterinary medicine. The problems connected with the use of herbal medicine, the largest being the lack of scientific evaluation. The most effective approach to obtain such evaluation is the ethno medicinal approach, which assumes that indigenous uses of plant indicate the presence of biologically active compounds in the plants.

Phytochemical investigations of the ferns have been studied to a lesser extent as compared to the higher plants. The phytochemical composition responsible for the anthelmintic activity among ferns may be

the first report in the field of ethno medicine. The phytochemical analyses of naturally available plants and controlled endoparasitic drug trials along with contemporary knowledge of parasite control strategies may offer new opportunities for effective and economical control of parasitic diseases. This may uplift the poor farmers holding the livestock sector and the economic status of our country. The application of ferns both *in vitro* and *in vivo* in ethno-veterinary medicine may put forth a foundation for taxonomists and research botanist of the world to limelight tremendous work in ethnobotany of these vascular cryptogamic plants.

Materials and methods

The study was carried during the period from, January, 2014 to July, 2014 in A.V.V.M. Sri Pushpam College, Thanjavur Dt. Pteridophytic plants *viz., Actinopteris radiata* (Sw.) Link, *Acrostichum aureum* (Linn), *Hemionitis arifolia* (Burm.) Moore and *Pityrogramma calomelanos* (L.) Link were collected from part of Kanyakumari district (77º7´ - 77º35´E and 8º5´ - 8º35´N), *Dryopteris cochleata* (Buch. Ham. ex D. Don) and *Drynaria quercifolia* (L.) J. Smith, collected from Kolli hills, part of Eastern Ghats (11º10´– 30º0´ N and 75º15´– 75º30´ E) were selected for the study.

Identification of ferns

The herbarium of the ferns collected were confirmed with the herbarium of Scott Christian College, Nagercoil, Kanyakumari District with the help of different floras [11, 12, 13] and a sample specimen for each fern collected were preserved in A.V.V.M Sri Pushpam college.

Preparation of fern extracts

Collected ferns were washed in running tap water and cut into small pieces and were shade dried and then in hot air oven at 55-60oC. Dust was prepared by pulverizing the ferns with the help of mixer. A 25- mm mesh diameter sieve was used to obtain fine dust and preserved them into airtight plastic container, labelled, till their use in extract preparation.

Phytochemical studies of collected ferns

Phytochemical studies were carried out in Poonga Biotech Research Laboratory, Arumbakkam, Chennai. Phytochemical screening of the extracts was carried out according to standard reference [14]. The Qualitative phytochemical analysis was carried out from the whole parts of plant material powdered and was extracted by the distillation method using soxhlet apparatus [15]. Different solvent systems were used for the separation of chemicals especially to study the anthelmintic property of all the six ferns collected according to the polarity (Aqueous, Ethanol and Petroleum ether), to identify the major natural chemical groups such as Tannins, Saponins, Flavonoids, Quinones, Phenols, Terpenoids, Alkaloids, Glycosides, Cardio glycosides, Coumarins, Betacyanin, Anthocyanin and Steroids. Quantitative phytochemical analysis was studied for Total Phenol and Total Tannin content, responsible for the major anthelmintic activity of the ferns under study. For the quantification 4 ferns were used, having strong positive for phenol and tannin content after qualitative analysis (*Actinopteris radiata* (Sw.) Link, *Dryopteris cochleata* (Buch. Ham. ex D. Don), *Drynaria quercifolia* (L.) J. Smith and *Pityrogramma calomelanos* (L.) Link). Tannin content of the given sample was estimated as per the method of [16, 17]. The Folin–Ciocalteau reagent method has been used for the estimation of total phenolic extracts quantities according to [18].

In vitro screening with adult Haemonchus contortus

10 gram of powdered fern were taken in 500 ml beaker and separately mixed with 100 ml ethanol. Then the mixture was stirred for 30 minutes by a magnetic stirrer (1000 rpm) and left stand for next 24 hrs. The mixture was then filtered through Whatman filter paper, No 1. The filtered materials were taken into a round bottom flask and then condensed by evaporation of solvent from filtrate in a water bath at 50oC for ethanol up to final volume of 10 ml [19]. After the evaporation of solvent from filtrate, the condensed extracts were preserved in tightly corked-labelled bottle and stored in

refrigerator until their screening for anthelmintic property.

Adult live nematodes, *Haemonchus contortus* were collected from Perambur slaughter house, Chennai, from the g/I tracts (abomasm) of Sheeps (*Ovis aries*). They were opened in a plastic bucket separately and the contents were washed up in tap water. The process was repeated for several times until the sediment becoming transparent. Then the adult g/I worms were collected with the help of a needle and placed in a petridish containing PBS (Phosphate Buffer Saline). Petridish containing the worms was kept in incubator at 38oC until required for experiment on the same day. *In vitro* screening with pharmacological preparations (ethanolic extracts) of different ferns (6 Nos) was performed using the g/I nematodes, *Haemonchus contortus* [19]. The ethanolic fern extracts were used at various concentrations i.e. 25 mg/ml (2.5%), 50 mg/ml (5%) and100 mg/ml (10%), 0.1% Tween in normal saline (control) and reference standard Piperazine citrate (2.5%, 5% and 10%) using adult *Haemonchus contortus* worms (n=6) in each petridish. Observations were made for time taken to paralysis and death of individual worms. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colour. Statistical analysis was carried out and the results were expressed as mean ± SEM [20, 21].

In vivo screening of fern extracts in sheeps (Ovis aries*.Linn*)

The ethanolic extracts of above mentioned selected ferns which were showing potential wormicidal effects following *in vitro* screening were further tested for *in vivo* study using a selected group of naturally infected sheep (*Ovis aries*) flocks with endoparasites [19]. The *in- vivo* test were performed after getting the complete willingness of sheep flock owners and were conducted in the presence of registered Government Veterinary Doctor. The sheeps were selected based on random analysis of faecal Egg Per Gram (EPG) count, among the flocks surveyed. Twenty-one (21) sheeps aged between 13- 24 months, irrespective of sex were selected for the study. The sheeps selected for the anthelmintic trials were based on the highest worm burden due to nematodes during EPG (Egg Per Gram) count on 0 day before anthelmintic treatment. The sheeps selected for the trials were divided into Seven (7) groups each containing three animals with one group as the control. The control group was not given any drug. The clinical group were treated with ethanolic extracts of the 6 ferns under study (10%) at 100 mg/ml concentration (highest concentration under *in vitro* efficacy trials), orally with the help of 10 ml injectable syringe. EPG count was done on day 5, 7, and 9 post-treatment by McMaster egg counting technique [8]. The efficacy of different treatment was determined by faecal egg count reduction test (FECRT) following the formula.

$$\text{Efficacy} = \frac{\text{EPG prior to treatment} - \text{EPG post-treatment} \times 10}{\text{EPG prior to treatment}}$$

Two grams of faecal samples were taken in a beaker and 30 ml of tap water was poured and stirred to dilute the sample. Then 30 ml of saturated salt solution was added and shaken. A small amount of the diluted sample was withdrawn by a pipette and run into the counting chamber to fill all the space. The slide was then put to stand for sometimes allowing the eggs to float under the surface of the upper slide of the McMaster chamber. Similarly the other chamber were filled and counted. The slide was then examined under microscope using low power objective (X10) and eyepiece (X6) and the total eggs within each ruled area were counted. The number of eggs per gram of faeces was calculated by using the following formula. EPG of faeces was counted on day 0 (pre-treatment), and on day 5, 7 and 9 of post-treatment.

$$\text{Number in one gram} = \frac{\text{Number in two chamber} \times \text{dilution factor}}{0.3}$$

$$\text{Dilution factor} = \frac{\text{Total volume of suspension in ml}}{\text{Total volume of faeces}}$$

Table 1: Qualitative phytochemical analysis of six fern species

Plants name	Solvents	Tannins	Saponins	Flavonoids	Quinones	Glycosides	Cardio glycosides	Terpenoids	Phenol	Coumarins	Steriods	Alkaloids	Anthocyanin	Betacyanin
A.radiata	**A**	+	-	+	+	-	+	-	+	+	-	+	-	+
	E	++	+	++	++	-	+	+	++	+	+	+	-	+
	P	-	+	-	-	-	-	+	-	-	+	-	-	-
A.aureum	**A**	+	+	+	+	-	+	+	+	+	+	-	-	+
	E	+	-	+	++	-	+	++	++	+	++	+	-	+
	P	-	-	-	+	-	-	+	-	-	+	-	-	-
D.quercifolia	**A**	+	+	+	+	-	+	-	+	-	-	-	-	+
	E	++	++	+	++	-	+	+	++	+	+	-	-	+
	P	-	-	-	-	-	+	-	-	-	+	-	-	-
D.cochleata	**A**	++	+	+	++	-	+	-	+	+	-	-	-	+
	E	++	-	+	++	-	++	-	++	-	+	+	-	+
	P	-	-	-	-	-	-	-	-	-	-	-	-	-
P.calomelanos	**A**	+	+	+	+	-	-	+	+	+	-	-	-	+
	E	++	+	+	+	+	+	++	++	+	++	+	-	+
	P	-	-	-	-	-	-	-	-	-	-	-	-	-
H.arifolia	**A**	+	+	+	+	-	+	+	+	+	-	-	-	+
	E	+	+	+	+	-	+	+	+	+	-	-	-	+
	P	-	-	-	-	-	-	-	-	-	-	-	-	-

++ **Strong Positive** + **Positive** - **Negative**

Results

Qualitative phytochemical analysis

Phytochemical analysis of the six ferns was performed using aqueous, ethanolic and petroleum ether solvent extracts (Table 1). Among the three solvents used, ethanolic extract performed well to show strong positivity (++) for major phyto constituents like Tannin and Phenol in these ferns studied. However extract of *Hemionitis arifolia* (Burm.) Moore showed only positivity (+) for Tannin and Phenol. The 13 phytochemicals were well expressed during the qualitative analysis based on the order of solvent Ethanol > Aqueous > Petroleum ether. The qualitative analysis showed that the chemical constituents like tannin, phenol, alkaloids and flavonoids contributing for anthelmintic property in these ferns were well expressed using ethanol. Among the six (6) ferns for phytochemical study, better anthelmintic activity was noticed in *Pityrogramma calomelanos* (L.) Link followed by *Actinopteris radiata* (Sw.) Link, *Dryopteris cochleata* (Buch. Ham. ex D. Don), *Drynaria quercifolia* (L.) J. Smith, *Acrostichum aureum* (Linn) and *Hemionitis arifolia* (Burm.) Moore.

Quantitative phytochemical analysis

Quantitative phytochemical analysis was performed for Total tannin and Total phenol constituents. The study was conducted among 4 best anthelmintic ferns after the qualitative analysis viz., *Pityrogramma calomelanos* (L.) Link, *Actinopteris radiata* (Sw.) Link, *Dryopteris cochleata* (Buch. Ham. ex D. Don) and *Drynaria quercifolia* (L.) J. Smith respectively and the results were interpreted (Table 2).

In vitro anthelmintic trials

The efficacy of ethanolic extract of the 6 ferns under study against the nematode *Haemonchus contortus* of sheep (*Ovis aries*) at varied concentration @ 2.5% (25 mg/ml), 5% (50 mg/ml) and 10% (100 mg/ml) showed, the ethanolic extract at 10% concentration had higher anthelmintic efficacy against the nematode (Table 3). Evaluation of anthelmintic activity was compared with the standard drug Piperazine citrate, which however showed better efficacy that the fern extracts used. The *in vitro* efficacy showed *Pityrogramma calomelanos* (L.) Link had lesser time taken for both paralysis and death of the nematodes (in minutes) than the other ferns.

In vivo anthelmintic activity in sheeps (Ovis aries*.Linn*)

The ethanolic extract of all the 6 ferns showed that they have more or less wormicidal effect. The *in vivo* study @ 100 mg/ml of ethanolic extract showed *Pityrogramma calomelanos* (L.) Link (91%), followed with *Actinopteris radiata* (Sw.) Link (84%), *Dryopteris cochleata* (Buch. Ham. ex D. Don) (78%), *Drynaria quercifolia* (L.) J. Smith (65%), *Acrostichum aureum* (Linn) (56%) and *Hemionitis arifolia* (Burm.) Moore (49%) had anthelmintic efficacy (Table 4).

Table 2: Quantitative analysis of phytochemicals (mg/g) in four fern species

Phytochemicals	*Ar*	*Dq*	*Dc*	*Pc*
Total tannin	12.189 ± 0.258	6.332 ± 0.187	9.405 ± 0.299	17.181 ± 0.441
Total phenol	10.962 ± 0.327	7.131 ± 0.184	8.912 ± 0.310	13.581 ± 0.481

Ar ***Actinopteris radiata* (Sw.) Link**
Dq ***Drynaria quercifolia* (L.) J. Smith**
Dc ***Dryopteris cochleata* (Burm.) Moore**
Pc ***Pityrogramma calomelanos* (L.) Link**

Table 3: *In vitro* anthelmintic activity

Test Substance	Conc (mg/ml)	Time taken for paralysis (minutes)	Time taken for death (minutes)
Distilled Water (Control)	-	-	-
Piperazine citrate (Standard)	25	24.333 ± 1.382	57.500 ± 1.962
	50	18.000 ± 1.095	37.500 ± 3.074
	100	6.833 ± 0.792	23.833 ± 1.621
***Actinopteris radiata* EE**	25	33.833 ± 0.980	73.167 ± 0.833
	50	28.833 ± 0.703	47.667 ± 1.174
	100	14.500 ± 1.204	30.500 ± 1.765
***Acrostichum aureum* EE**	25	56.500 ± 1.839	123.000 ± 1.673
	50	39.667 ± 1.520	87.333 ± 1.687
	100	36.833 ± 0.792	75.333 ± 1.945
***Drynaria quercifolia* EE**	25	45.500 ± 1.384	99.833 ± 2.496
	50	38.167 ± 1.249	75.167 ± 1.869
	100	31.667 ± 0.678	61.833 ± 1.167
***Dryopteris cochleata* EE**	25	43.000 ± 3.578	92.167 ± 1.222
	50	35.667 ± 0.843	70.833 ± 2.007
	100	29.333 ± 1.085	54.000 ± 1.461
***Pityrogramma calomelanos* EE**	25	25.167 ± 1.641	58.333 ± 0.955
	50	20.833 ± 0.792	38.000 ± 1.367
	100	9.167 ± 0.600	24.667 ± 1.333
***Hemionitis arifolia* EE**	25	67.667 ± 2.985	158.000 ± 2.049
	50	54.167 ± 1.515	127.333 ± 2.186
	100	42.667 ± 1.308	105.667 ± 2.092

EE-Ethanolic Extract

Discussion

The qualitative phytochemical analysis and the *in vitro* study of Panda *et al.*, was similar to our phytochemical and *in vitro* study, who said that the potency of the extract was found to be inversely proportional to the time taken for paralysis/ death of the worms[22]. However the activity was compared to a single reference drug Piperazine citrate unlike the study of Panda *et al.*, compared to the standard reference drug Piperazine citrate and albendazole[22]. Both the findings substantiate the anthelmintic properties of the plant as suggested by the folklore practice. Examination for evidences of phyto-constituents showed ethanolic extract performed well to express the phytochemicals in the 6 ferns studied. There was strong positivity (++) for almost major phyto-constituents of the ferns studied. Tannin, phenol showed strong positivity except *Hemionitis arifolia* (Burm.) Moore which showed positivity (+). Aqueous extract performed well next to ethanolic extract showed positivity (+) among the phytochemical in all the 6 ferns. Aqueous extract of *Dryopteris cochleata* (Buch. Ham. ex D. Don) showed strong positivity (++) for Tannins and Quinones.

Table 4: Comparative *In vivo* anthelmintic efficacy

Botanical Name	EPG pre-treatment (0day)	EPG post-treatment (percentage reduced)		
		5th day	7th day	9th day
Actinopteris radiata	864	485 (44%)	272 (69%)	140 (84%)
Acrostichum aureum	970	764 (21%)	586 (40%)	424 (56%)
Drynaria quercifolia	1148	662 (42%)	558 (51%)	406 (65%)
Dryopteris cochleata	1042	672 (36%)	468 (55%)	226 (78%)
Pityrogramma calomelanos	1536	914 (40%)	485 (68%)	138 (91%)
Hemionitis arifolia	852	650 (24%)	538 (37%)	432 (49%)

The Petroleum ether extract showed negativity (-) to express the phyto-constituents in *Dryopteris cochleata* (Buch. Ham. ex D. Don), *Pityrogramma calomelanos* (L.) Link, *Actinopteris radiata* (Sw.) Link showed positivity for Saponins, Terpenoids and Steroids. *Acrostichum aureum* (Linn) showed positivity for Quinones, Terpenoids and Steroids. *Drynaria quercifolia* (L.) J. Smith showed positivity for Cardio glycosides and Steroids. *Hemionitis arifolia* (Burm.) Moore showed positivity for Cardio glycosides. Mithraja *et al.,* performed phytochemical screening with acetone, benzene, chloroform, ethanol, petroleum ether and aqueous extracts of whole plants of *B.orientale, C.thalictroides, D.heterophyllum, D.linearis, H.arifolia, L.ensifolia, N.multiflora, P.calomelanos, P.confusa* and leaves and rhizomes of *Drynaria quercifolia,* revealed that the presence or absence of the phyto-constituents depend upon the solvent medium used for extraction and the physiological property of individual taxa [23].

The present study on the phytochemical analysis of *Actinopteris radiata* (Sw.) Link, *Acrostichum aureum* (Linn) and *Dryopteris cochleata* (Buch. Ham. ex D. Don) was not performed in the study of Mithraja *et al.,* [23]. Our research was in opinion with the study of Mithraja *et al.,* stated that tannin containing drugs are used in medicine as astringent and have been found to posses antiviral, antibacterial and anti-parasitic effects for possible therapeutic applications [24]. Since Tannin was present in the aqueous extract and ethanolic extract of all the 6 ferns under study. However petroleum ether showed negativity, indicative that tannin could not be expressed well in petroleum ether. It was a confirmation for the anthelmintic property in these ferns, but the potent action of response varied on their positivity. Kumudhavalli et al., evaluated the petroleum ether, chloroform, acetone, ethanol and aqueous extracts of the fern *Hemionitis arifolia* (Burm.) Moore, for preliminary phytochemical screening[25].

Our study was not subjected for quantifying *Hemionitis arifolia* (Burm.) Moore, since on qualitative analysis showed lesser positivity towards total tannin and total phenol content. Similarly Gracelin *et al.,* conducted qualitative and quantitative phytochemical analysis in five *Pteris* fern species [26]. Qualitative analysis of methanol extract exhibited positivity for 10 phytochemical tests. Our study on the qualitative analysis of ethanolic, petroleum ether and aqueous extract of 6

ferns species showed ethanolic extract performed well to exhibit positivity for anthelmintic activity. The quantitative analysis of the extract of *Pteris* species showed flavonoids content were highest followed by alkaloids and phenolic compounds. The amount of tannin and saponin was very low in the fern extract. However our quantitative study showed total phenolic and total tannin content were highest in *Pityrogramma calomelanos* (L.) Link followed by *Actinopteris radiata* (Sw.) Link, *Dryopteris cochleata* (Buch. Ham. ex D. Don) and *Drynaria quercifolia* (L.) J. Smith. reported that the ethanolic extract showed most potent anthelmintic activity[27]. The other two extract e.g. petroleum ether and aqueous extract, exhibited lesser anthelmintic activity than the ethanolic extract, revealed that the anthelmintic activity increases with increasing polarity which was in opinion with our *in vitro* study. However further studies were required to identify the actual chemical constituents that were present in the crude extracts of this plant which were responsible for anthelmintic activity which were in confirmation with our present study.

Siraj et al., studied on the *in vitro* and *in vivo* anthelmintic activity of *Ferula costata* against gastrointestinal nematodes and reported that the crude powder and crude methanolic extract of *F.costata* exhibited time and close dependant *in vivo* anthelmintic activity against mixed culture of gastrointestinal nematodes[28]. Both the administrations showed maximum activity at highest dose (3 g/kg b.w.) on day 14 PT. Maximum reduction in EPG detected for crude powder @ 3 g/kg b.w at day 14 was 30.71% while at same dose and day PT it was noted for crude methanolic extract at 47.90%. Though both the oral administrations considerably decreased the egg counts in faeces but this activity was not comparable with that of positive control levamisole @ 7.5 mg/kg b.w (99.39% reduction in EPG), which was in confirmation with our study, in which highest dose concentration (100 mg/ml) of ethanolic fern extract was selected after subjected to *in vitro* trials and the result was best achieved for *Pityrogramma calomelanos* (L.) Link (91%) and least effective for *Hemionitis arifolia* (Burm.) Moore (49%). But this activity was not compared with positive control in our present study.

Various studies reported that filicin and filmarone were active vermifuge and were toxic to tapeworms and the taenids were expelled within hours, however purgative typically was ingested concomitantly with the vermifuge to aid expulsion[29, 30, 31]. The oleoresin paralyzes intestinal voluntary muscle and the analogous muscles of the tapeworm, which was then readily eliminated by the action of purgative. The components of the plant have been used as veterinary vermifuges should be used with caution as in large doses it was poisonous and reported to be hepatotoxic and nephrotoxic. Our present studies dealing with Dryopteris cochleata (Buch. Ham. ex D. Don) was very safe and potent drug and were proved to be more effective against the nematode *Haemonchus contortus*, but were less effective than *Pityrogramma calomelanos* (L.) Link and *Actinopteris radiata* (Sw.) Link. It might be attributed due to the tannin (9.458 mg) and phenolic content (8.917 mg) of *Dryopteris cochleata* (Buch. Ham. ex D. Don). However, detailed study was needed on the combination of this drug with anthelmintics and isolation and validation of the active principles was essential.

It has been postulated that the beneficial effects of tanniferous plants against internal parasites could be due to one, or a combination, of the following factors, Tanniferous plants increase the supply and absorption of digestible protein by animals. This is achieved by tannins forming non-biodegradable complexes with protein in the rumen, which dissociate at low pH in the abomasums to release more protein for metabolism in the small intestine of ruminants. In other words,"natures protected protein." This indirectly improves host resistance and resilience to nematode parasite infections. Tannins have a direct anthelmintic effect on resident worm populations in animals. Tannins and/or metabolites in dung have a direct effect on the viability of the free-living stages (development of eggs to infective larval stages). Although there is some evidence to support each of these above

claims [32, 33] tannin can interact with proteins in the nematode cuticle, changing its chemical and physical properties [34]. Recent study has shown flavonoids that also were observed to aqueous extract of immature mango, to possess action against *Haemonchus contortus* [35]. Research showed that inclusion in the diet of the condensed tannin in Quebracho extract reduces egg output and worm burden in sheep infected with *Trichostrongylus colubriformis* and studies suggested that quebracho tannin was acting through a direct toxic effect against the nematodes, similar to our research findings might be one of the reason that the rich tannin content after quantitative analysis, attributed to the reduction in EPG count in the *in vivo* study and also the reduction in paralysis and death time during the *in vitro* study.

Sujon *et al.,* studied on 10 indigenous medicinal plants having anthelmintic action using ethanolic extracts for both *in vitro* and *in vivo* study against gastro-intestinal nematodes in goat and showed 4 plants had >70% efficacy at a concentration of 100 mg/ml[19]. However our first reported study on ferns (6 Nos) with the same methodology of Sujon et al., showed 3 ferns had >70% efficacy with ethanolic extract as solvent against GI nematodes of sheeps[19].

Singh et al., stated that phloroglucinol group of compounds were responsible for anthelmintic activity of *Dryopteris.filix-mas* and the major phloroglucinol of *D.Filix-mas* was filicin or filicid acid (1.5%) as the active principle[36]. The other phloroglucinols present in the rhizome of *D.filix-mas* includes aspidin, desaspidin, paraspidin, margaspidin, aspidine, filixic acid, flavaspidic acid and flumarone. In these clinical trials the varying degree of anthelmintic efficacy was rule out after *in vitro* and *in vivo* anthelmintic trials. The *in vitro* and *in vivo* study were in the same confirmation that *Pityrogramma calomelanos* (L.) Link had greater than 90% anthelmintic efficacy and *Hemionitis arifolia* (Burm.) Moore had less than 50% anthelmintic efficacy. The research finding was the first report for anthelmintic efficacy in sheeps with the comparison of 6 Pteridophytic plants both *in vitro* and *in vivo*.

However, Marathe *et al.,* conducted an endoparasitic survey in goats and had taken *Haemonchus contortus* as the sole worm for both *in vitro* and *in vivo* anthelmintic trials using *Drynaria quercifolia* (L.) J. Smith had suggested and given direction for future studies required and isolation of biochemical compound and studying their efficacy singularly or synergistically against anthelmintic properties of rhizome of *Drynaria quercifolia* (L.) J. Smith. Our study was also in opinion with the study of Marathe et al., for the *in vitro* and *in vivo* efficacy against *Haemonchus contortus* in sheep populations rather than goat population and proved to be very much effective with any toxic symptoms and side effects[37]. However our study did not dealt with the blood profile picture and pharmacokinetics of ferns used to judge the toxic levels unlike Marathe et al., Lu et al., [37-38] performed *in vivo* anthelmintic activity of *Dryopteris crassirhizoma* against *Dactylogyrus intermedius* (Monogenea) in gold fish (*Crassius auratus*) using petroleum ether, chloroform, ethyl acetate, acetone and methanol extracts and found methanolic extract of *D.crassirhizoma* was observed the most effective with EC50 value of 22.97 mgL-L after 48 h of exposure. However, our study dealt with only ethanolic extracts for *in vivo* anthelmintic property. Hence a comparative study on various solvent extracts might be required for effective interpretation of anthelmintic drugs.

Shokier et al., conducted *in vivo* anthelmintic efficacy using five allopathic anthelmintic against natural fasciola species infection in cattle based on FECRT[39]; Siraj etal., conducted *in vitro* and *in vivo* anthelmintic activity of *Ferula costata* (Kor.) against GI nematode of sheep using various solvent extracts[28]; Sourov et al., conducted *in vitro* biological investigations of methanolic extracts of *Enhydra flucttuans* Lour[21]; Sreejith et al., performed anthelmintic of various leaf extracts of *Flacourtia sepiaria* Roxb[20]; which was clearly indicative of various research activities of angiospermic plants in the field of veterinary

medicine using various solvent extract. But there was no detailed work of anthelmintic application trials in human and veterinary medicine using Pteridophytic plants. The main content of these anthelmintic application trials was taken from the review work of Manickam et al., who reported various anthelmintic usage in the tribal community of western Ghats as a folklore medicine for them and their livelihood especially children and was an attempt to the baseline application study of these Pteridophytic plant species in the field of veterinary medicine, especially sheeps[12].

However, ferns were toxic in nature especially ferns like *Pteridium aquilinum* and subsequent doses of the same extract for longer therapy might be attributed to toxicity. The clinical trials was an attempt on ferns anthelmintic property using the whole plant, powdered extract as a single dose for *in vivo* study (100 mg/ml) showed no side effects and symptoms of toxicity. Also the study was planned to conduct in small ruminants (sheep), because nature by itself permitted ruminants to synthesize vitamin B1 (Riboflavin) in rumen. Therefore ruminants were not subjected to thiaminase enzyme inhibitors after consuming ferns and were free from thiaminase poisoning. However the ferns itself were lesser toxic in ruminants especially sheep and goats, because of the browsing nature. Hence these baseline studies need a comparative and detailed research investigation in other ruminants like cattles, buffaloes and goats was needed using different solvent extract. Also the study need subsequent analysis with chronic and varied doses of the fern extract and their blood profile picture was thoroughly investigated for toxicity.

The use of chemical anthelmintic drugs for controlling animal parasitic nematodes was rapidly losing popularity due to a number of disadvantages. Anthelmintic resistance in the parasites was spreading and the inefficacy of chemical anti-parasitic compounds was threatening animal health. New plants with medicinal properties against parasites of ruminants were being investigated around the world with promising results. In the near future natural products obtained from plants extracts seems that likely will become a viable alternative of control of parasitizes of veterinary importance. When plant/plant extracts were being selected for use as anti-parasitic drugs in sheep particular attention should be given to the fact that the bio-active compound could be found in stems, roots, leaves, flowers, fruits or even in the entire plant. This means that obtaining plant extracts was a laborious and complex process. Also, the mode of extraction and the solvent used can determine the success in isolating the expected bioactive compounds; since a wide variety of compounds can be hidden into the structural parts of the plants and the only way they could be isolated is through exploring the use of a range of organic solvents. On the other hand, a rigorous effort to identify possible side effects due to the administration of plant extracts should be established before carrying *in vivo* assays. It was remarkably important to consider that using plant/plant extracts as a unique method of control was insufficient to control itself the parasitosis in the animals. So, an alternated or combined method with other methods of control should be considered as an integrated method which would lead to reduce the use of chemical anthelmintic drugs.

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