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(RESEARCH ARTICLE)

Evaluation of anthelmintic activity of *dryopteris filix*-mas using earthworm

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Abstract

Evaluation of anthelmintic activity of a leaves extracts of *Dryopteris filix*-mas against earthworm with Preliminary work as physical characteristics. Finally extraction of defatted *Dryopteris filix*-mas extract was done with hydro-alcoholic solvent and % yield was found to be 6.55% w/w and their characteristics are reported. Results obtained from qualitative chemical tests are tabulated. Total alkaloid content was calculated as atropine equivalent mg/100 mg using the equation based on the calibration curve: Y=0.007X+ 0.007, R2=0.999, where X is the Atropine equivalent (AE) and Y is the absorbance. Total phenol content was expressed as mg/100 mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.017X+0.017, R2= 0.998, where X is the gallic acid equivalent (GAE) and Y is the absorbance. The present findings indicated that the usefulness of the hydroalcoholic extract of leaves of *Dryopteris filix*-mas in earthworm. This study suggested, hydroalcoholic extract of leaves of *Dryopteris filix*-mas dose-dependently produced anthelmintic activity.

Keywords: Anthelmintic; *Dryopteris filix*-mas; Earthworm; Hydroalcoholic

1. Introduction

Nature has provided a complete store-house of remedies to cure all ailments of mankind and its related diseases[1]. Charaka made fifty groups of ten herbs each of them sufficient for an ordinary physician's need. Sushruta arranged 760 herbs in 7 distinct sets based on some of their common properties[2]. Most diseases caused by helminths are chronic, debilitating in nature, they probably cause more morbidity, greater economic and social deprivation among humans and animals than any other parasites[3]. It is not only limited to tropical and subtropical countries but is also to endemic in many regions because of poor sanitation, poor family hygiene, malnutrition and crowded living condition[4]. Potent anthelminitics are available today, and treatment is frequently done by using different types of drugs[5]. However the high costs of modern anthelmintics have limited effective control of the parasites[6]. In some cases, wide spread use of low quality anthelmintics are used for the development of resistance and hence causes reduction in use of anthelmintics[7]. Recently the use of anthelmintics produces toxicity in human beings. Hence the development and discovery of new substances acting as anthelmintics are being derived through plants which are considered to be the best source of bioactive substances[8]. Various plants were used in veneral diseases, to promote healing of wounds, swellings, abscesses, rheumatism and treating pain in lower extremities, skin diseases, leucorrhoea, dysentery, dysuria and fever[9,10]. Anthelmintics are those drugs that are used in expelling out the worms that are parasitic in nature by either stunning them or by killing them. They are also known as vermifuges or vermicides[11].

Evaluations of anthelmintic activity of *Dryopteris filix*-mas drug was carried out in artificial laboratory conditions by using the earthworms (*Lumbricus terrestris*). The earthworms are ecofriendly for decomposing organic materials, feeding upon undecayed leaves and other plant materials, more geophagous.

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2. Material and methods

2.1. Preliminary Work

The plant has been selected on the basis on Availability of plant material and its wide geographical distribution globally also economic plant. Leaves of *Dryopteris filix*-mas were collected from local areas and authenticated. Fresh leaves drying were carried out in sun but under the shade. They were pulverized to make coarse powder. The coarse powder of fruit was passed through sieve No. 18 to maintain uniformity. Dried leaves were preserved in plastic bags and closed tightly and powdered as per the requirements.

2.2. Method of Extraction

Following procedure was adopted for the preparation of extracts from the shade dried and powdered herbs:

2.2.1. Defatting of plant material

Leaves of *Dryopteris filix*-mas were shade dried at room temperature. 70.50 gram of dried leaves was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

2.2.2. Extraction by maceration process

Defatted leaves of *Dryopteris filix*-mas were extracted with hydroalcoholic solvent (Ethanol: Water:75:25) using maceration process (48 hrs). The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts.

2.2.3. Determination of percentage yield

The percentage yields of each extract were calculated by using following formula:

$$\text{Percentage Yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \text{ X } 100$$

2.3. Qualitative Evaluation

Phytochemical tests were done as per the standard methods described for detection of alkaloids, carbohydrates, Saponins, phenols, flavonoids, proteins and diterpenes.

2.3.1. Quantitative studies of bioactive constituents

Estimation of total alkaloids content[12]

The plant extracts (1 mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 μg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100 mg of extract.

Estimation of total phenol content13

The total phenol content of the extract was determined by the modifiedfolin-ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol,various aliquots of 10- 50μg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanoland filter. Two ml (1 mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexes for 15s and allowed to stand for 10min for color development. The absorbance was measured at 765 nm using a spectrophotometer.

2.4. *In-vitro* Evaluation of Anthelmintic Activity with Earthworm

2.4.1. Preparation of Drug Solutions

The standard drug albendazole was received from Lee Pharma, Hyderabad and test samples of hydroalcoholic extract of *Dryopteris filix*-mas prepared in collage Laboratory. Albendazole and test drugs were prepared as 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml, and 200 mg/ml concentrations using water and ethanol as solvents, respectively.

2.4.2. Collection of Earthworms

Earthworms were collected locally from compost plant situated in a nursery of Gwalior. The average size of worms was 5–8 cm. Earthworms were identified and authenticated. The anthelmintic activity was carried out as per the method described elsewhere. The assay was performed *in-vitro* using adult earthworms owing to their anatomical and physiological resemblance with the intestinal round worms, parasites of human beings for preliminary evaluation of anthelmintic activity. A concentration of standard drug (albendazole) and synthetic test sample extract was prepared as described earlier.

2.4.3. Evaluation of Anthelmintic Activity Using Earthworms

The anthelmintic activity was performed according to the method of Ghosh[14] et al. Earthworms, each of average length of 6 cm, were placed in Petri dishes containing 2 ml of various drug concentrations, 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml, and 200 mg/ml of solutions. Albendazole solution was used as reference standard drug and distilled water as control. The worms were observed for the motility after incubating at 37°C. This was done after pouring the Petri dishes content in the wash basin and allowing the worms to move freely. By tapping the end of each worm with the index finger and applying a bit of pressure, the worms that were alive showed motility and those dead were nonmotile. The motile worms were returned to the respective Petri dishes containing drug solutions and the incubation process was carried out again. In the control, the worms were viable for at least twelve days, which is similar to the findings. The time taken for paralysis, motility activity of any sort and death time of worms were observed and recorded after ascertaining that the worms did not move neither when shaken vigorously nor when dipped in warm water (50 °C).

3. Results

3.1. Physical Characteristics of Extract

Finally extraction of defatted *Dryopteris filix*-mas extract was done with ethanol solvent and % yield was found to be 6.55% w/w and their characteristics are reported in table below

Table 1 Physical characteristics of extract

Extract	Consistency	Colour	Odour	% Yield
Hydroalcoholic	Solid	Dark Brown	Pungent	6.55

3.2. Qualitative Chemical Test

Results obtained from qualitative chemical tests are tabulated in table below

Table 2 Qualitative chemical tests of extract of *Dryopteris filix*-mas

S. No.	Bioactive constituents	Hydroalcoholic extract
1	Alkaloids	+ ve
2	Carbohydrates	+ ve
3	Glycosides	- ve
4	Saponins	- ve
5	Phenols	+ ve
6	Flavonoids	+ ve
7	Proteins	- ve
8	Diterpenes	- ve

(+ ve) – Present, (- ve) – Absent

3.3. Quantitative study

3.3.1. Estimation of total alkaloid content (TAC)

Total alkaloid content was calculated as atropine equivalent mg/100 mg using the equation based on the calibration curve: Y=0.007X+ 0.007, R^2=0.999, where X is the Atropine equivalent (AE) and Y is the absorbance.

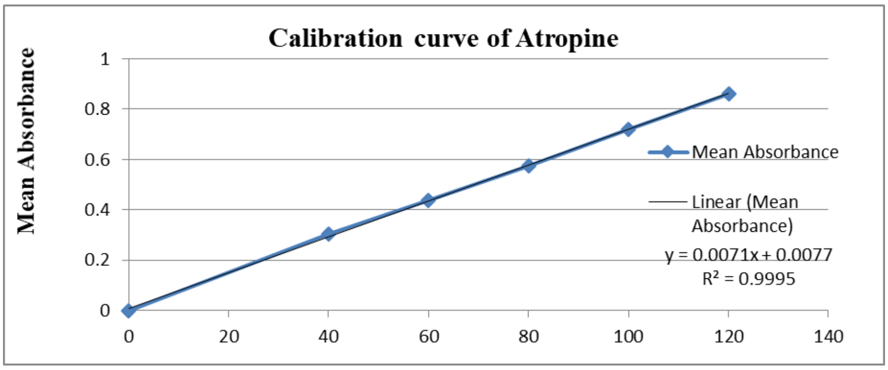

Figure 1 Graph of calibration curve of Atropine

3.3.2. Estimation of total phenol content (TPC)

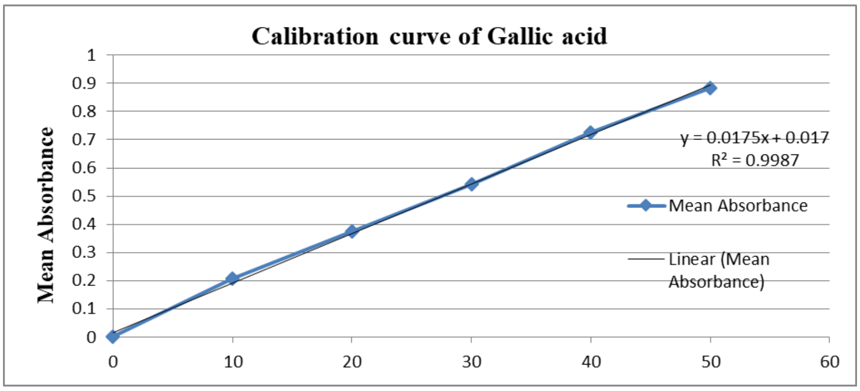

Figure 2 Graph of Calibration curve of Gallic acid

Table 3 Estimation of total alkaloids and phenol content

Total alkaloids content	Total phenol content
0.869 mg/100 mg	0.574 mg/100 mg

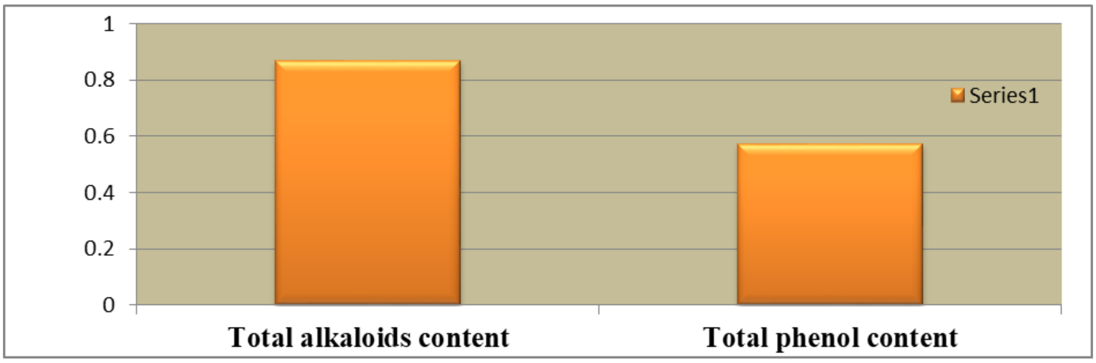

Figure 3 Graph of total alkaloids and phenol content

Total phenol content was expressed as mg/100 mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.017X+0.017, R^2= 0.998, where X is the gallic acid equivalent (GAE) and Y is the absorbance.

3.4. *In-vitro* Evaluation of Anthelmintic Activity with Earthworm

Table 4 Anthelmintic activity of Albendazole (SD) and extract in earthworms

Concentration of drug sample (mg/ml)	Albandazole (standard) treated		Extract treated	
	Time of Paralysis (sec) Mean ± SEM	Time of Death (sec) Mean ± SEM	Time of Paralysis(sec) Mean ± SEM	Time of Death (sec) Mean ± SEM
12.5	989 ± 15	1304 ± 12	1154 ± 19	1473 ± 13
25	858 ± 23	1107 ± 17	964 ± 14	1246 ± 07
50	758 ± 12	1067 ± 21	823 ± 31	1143 ± 17
100	534 ± 26	783 ± 08	614 ± 10	924 ± 15
200	394 ± 17	635 ± 02	501 ±16	784 ± 35

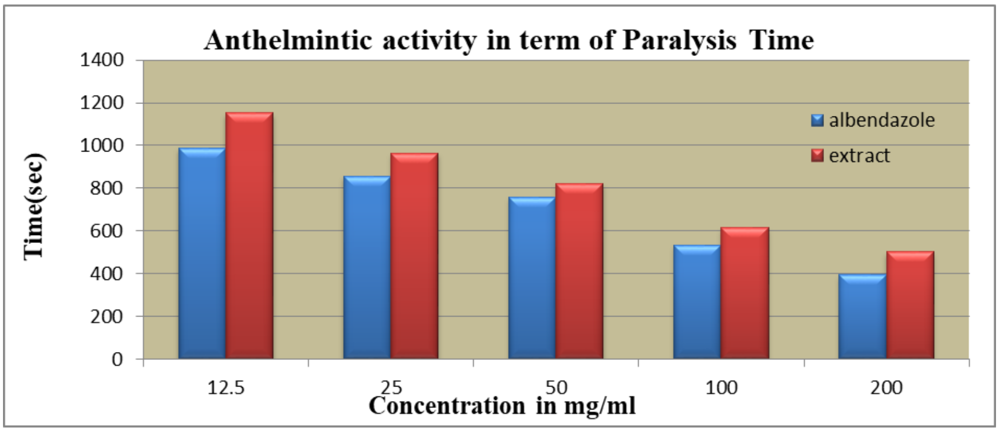

Figure 4 Comparison of Paralysis Time

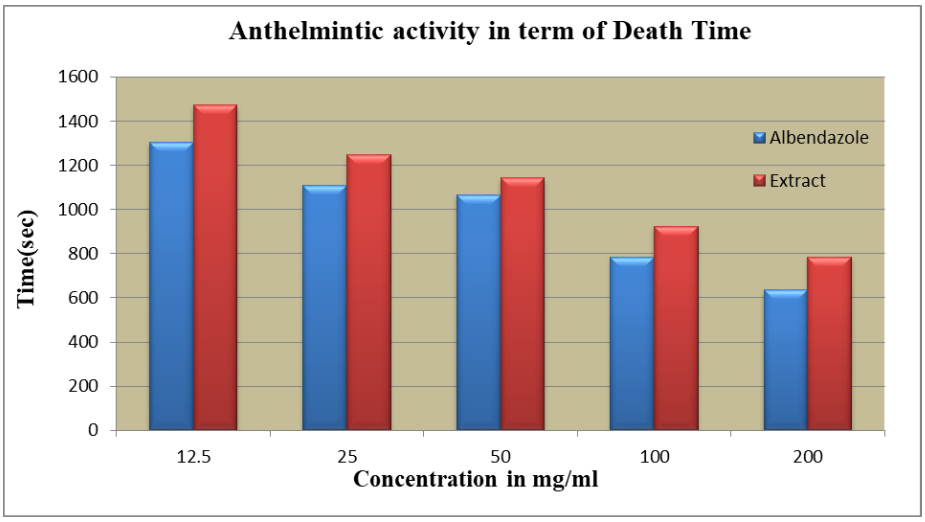

Figure 5 Comparison of Death Time

Figure 6 Extracts treated earthworms

4. Discussion

Evaluation of anthelmintic activity of a leaves extracts of *Dryopteris filix*-mas against earthworm with Preliminary work as physical characteristics. It was observed that leaves were Green, small ovate and smooth. The leaves *Dryopteris filix*-mas were dried under shade in laboratory. They were pulverized to make coarse powder. The coarse powder of fruit was passed through sieve No. 18 to maintain uniformity and stored in cool and dry place for further study. Powders of leaves *Dryopteris filix*-mas was subjected for extraction, hydroalcoholic solvent (Ethanol:Water:75:25) using maceration process (48 hrs), while petroleum ether was used for defatting of the waxy materials. The final yield with hydroalcoholic solvent was found 6.55%. Further obtained leaves extract was subjected for phytochemical analysis found that leaves extract was rich source of alkaloids, carbohydrate, phenol and flavonoids. Total alkaloid content was calculated 0.869 mg/100 mg as atropine equivalent and Total phenol content was 0.574 mg/100 mg expressed as gallic acid equivalent of dry extract sample.

In-vitro anthelmintic activity of leaves of *Dryopteris filix*-mas was performed by preparation of Albendazole and test drugs were prepared as 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml, and 200 mg/ml concentrations using water and ethanol as solvents, respectively. Earthworms were collected locally from compost plant situated in a nursery of Gwalior. The average size of worms was 5–8 cm. Earthworms, each of average length of 6 cm, were placed in Petri dishes containing 2 ml of various drug concentrations, 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml, and 200 mg/ml of solutions. Albendazole solution was used as reference standard drug and distilled water as control. The worms were observed for the motility after incubating at 37°C. This was done after pouring the Petri dishes content in the wash basin and allowing the worms to move freely. By tapping the end of each worm with the index finger and applying a bit of pressure, the worms that were alive showed motility and those dead were nonmotile. The motile worms were returned to the respective Petri dishes containing drug solutions and the incubation process was carried out again. In the control, the worms were viable for at least twelve days, which is similar to the findings. The time taken for paralysis, motility activity of any sort and death time of worms were observed and record after ascertaining that the worms did not move neither when shaken vigorously nor when dipped in warm water (50°C).

In concentration of 12.5 mg/ml worms were paralyzed in 1154sec while dead in 1473sec. In concentration of 25 mg/ml worms were paralyzed in 964 sec while dead in 1246sec. In concentration of 50 mg/ml worms were paralyzed in 823sec while dead in 1143sec. In concentration of 100 mg/ml worms were paralyzed in 614sec while dead in 924sec. In concentration of 200 mg/ml worms were paralyzed in 501sec while dead in 784sec. Results were evaluated and concluded that the potency of extract was increased with increasing of dose or concentration of extract. Therefore, it can be concluded that worms can be used successfully for the anthelmintic activity study as it is easy, prominent, an adaptable to laboratory conditions, and reproducible method in all aspects such as equal age, size and weight of the worms.

This experiment was to provide natural environment to the worms and was used for evaluating the effect different doses of the drugs on the viability of the preparasitic stages of the helminthics. In conclusion the adaptable factors known to us have influence on the anthelmintic activity of the drugs. Moreover, there was a need for an alternative method apart from the conventional method to justify anthelmintic studies in laboratory investigations. Further studies have to follow up the improved new methodology in evaluating the anthelmintic activity for any drug with potential role in worm infections.

5. Conclusion

In the last few decades eco-friendly, bio-friendly, cost effective and relatively safe, plant-based medicines have moved from the fringe to the main stream with the increased research in the field of traditional medicine. Finally extraction of defatted *Dryopteris filix*-mas extract was done with hydro-alcoholic solvent and % yield was found to be 6.55% w/w and their characteristics are reported. Results obtained from qualitative chemical tests are tabulated. Total alkaloid content was calculated as atropine equivalent mg/100 mg using the equation based on the calibration curve: $Y=0.007X+ 0.007$, $R^2=0.999$, where X isthe Atropine equivalent (AE) and Y is the absorbance. Total phenol content was expressed as mg/100 mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $Y = 0.017X+0.017$, $R^2= 0.998$, where X is the gallic acid equivalent (GAE) and Y is the absorbance. The present findings indicated that the usefulness of the hydroalcoholic extractof leaves of *Dryopteris filix*-mas in earthworm. This study suggested, hydroalcoholic extract of leaves of *Dryopteris filix*-mas dose- dependently produced anthelmintic activity.

Compliance with ethical standards

Disclosure of conflict of interest

There are no conflicts of interests.

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