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In-Vitro Evaluation of Anthelmintic Activity of *Nephrolepis cordifolia* Leaves

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ABSTRACT

Nephrolepis cordifolia, is a fern native to northern Australia and Asia. It has many common names including fishbone fern, tuberous sword fern, tuber ladder fern, erect sword fern, narrow sword fern and ladder fern, and herringbone fern. *Nephrolepis cordifolia* is a wood fern that typically grows in woodland areas. Both fertile and sterile fronds are pinnate, up to 3 feet in length and 3 inches wide. There are many leaflets, or pinnae, ranging from 40-100 mm (1.5 to 4 inches) on each side of the rachis. Each pinna is oblong to lanceolate with an auricle that overlaps rachis. Rhizomes are orange/brown to pale brown with linear scales having hair like tips. stolonis straw coloured and produce small underground tubers. The presence of tubers distinguishes sword fern from the native *Nephrolepis cordifolia* fern. The aim of the present study was to evaluate the anthelmintic activity of aqueous extract of leaves of *Nephrolepis cordifolia*. The time of paralysis and time of death were studied and the activity was compared with albendazole as reference standard. The aqueous extract of leaves of *Nephrolepis cordifolia* exhibited anthelmintic activity as evidenced by decreased paralyzing time and death time at the concentration of 10 mg/ml.

1. Introduction

The plants used as medicines predates written in human history. Many of the herbs and species used by human in seasons also serves as the medicinal compounds. There are large number of archaeological evidences exist which indicates that humans were using medicinal plants during the paleolithic, approximately 60,000 years ago [1].

The stomach worm infection (Helminthiasis)is one of the global public health problems mostly in tropical countries [2]. Helmenthiasis rarely fatal but its major cause of ill health development of resistance has not been a problem in the clinical use of Antihelmentic. There are literally thousands of different types of worms in the animal kingdom. They generally have long, cylindrical bodies with no separate limb. The word parasite itself comes from the old Greek word “parastos” meaning a person or thing which eats at someone else’s Table [3]. This is exactly what a parasite dose: it feeds off the host’s body wheather this is animal, plant or human. Worm can cause various Gastro intestinal disorder and general symtoms. In addition, some of them can cause blood loss, nutritional deficiency, urticaria and other allergic manifestation intestinal obstruction and hepatoplantory [4,5].

Nephrolepic cordifolia is one of the popular plants of north-eastern Australia. There are many different chemicals present the in it, such as terpenes, steroids, steroidal saponins, alkaloids, etc., [6]. and it is used for different pharmacological activity like antioxidant activity [7], anticancer [8], antidiabetic [9,10], antiviral activity [11], anti-inflammatory activity, antimicrobial activity [11].

2. Materials and Methods

2.1 Collection of Plant

The fresh leaves of the plant *Nephrolepis cordifolia* were collected in the month of February to March 2019 from F.R.I (Forest Research Institute), Dehradun. The species was authenticated by in the Systemic Botany Discipline, Forest Research Institute, Dehradun.The fresh leaves of *Nephrolepis cordifolia* were washed by running water and dried under the shade. The dried leaves were subjected to grinder and the powdered was obtained. The dried powdered was packed in the paper bags & stored in an airtight container until use.

2.2 Drugs and Chemicals

Albendazole (ABZ Karehealth specialities Pvt. Ltd.), Methanol AR (Thomas Baker Chemical Pvt. Ltd.), nitric acid, sodium hydroxide, HCl, conc. HNO_3, NH_4OH, $FeCl_3$, potassium dichromate, all other solvents were used during the experimental protocol were of analytical grade and procured from Merck-India.

3. Morphological Study

The morphological study includes colour, odour, taste, and shape were carried out on fresh leaves (Figure 1). The result of the morphological features was determined and given in the Table 1.

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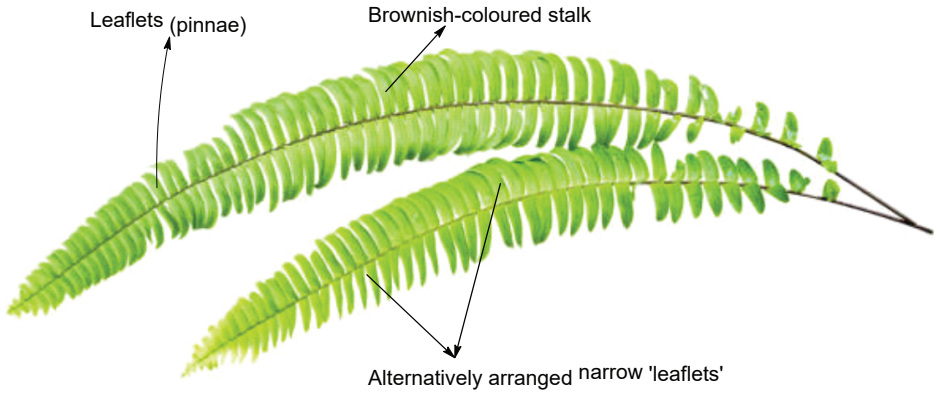

Figure 1: Morphological features of the leaves of *Nephrolepis cordifolia*

4. Extraction Process

The plant material (dried leaves), 10 gm was taken with different solvent (Ethanol, Hydroalcoholic, and Aqueous) (Figure 2) and cold extraction method was used for extraction. After 24 h the extract was properly dried by the use of the water bath. The dried extract was stored in the air tight container. The yield of different solvent extract was given in the Table 2.

5. Phytochemical Investigations

1. **Test for carbohydrates**

- **Fehling's test:** 1ml of Fehling's solution A and B was mixed and boil for 1minutes. Equal volume of extract was added then it was heated in boiling water bath for 5-10 minutes, first a yellow, brick red ppt was observed.
- **Benedict's test:** 2ml of Benedict's reagent and test solution was mixed in test tube. It was heated in boiling water bath for 5 minutes. The solution was appeared to be green in colour which indicates the presence of reducing sugar in test solution.

2. **Test for monosaccharide**

- **Barford's test:** Take equal volume of test solution and barford's reagent. Kept for 1-2 min in boiling water bath and allowed to cool. Red ppt was obtained.

3. **Test for hexose sugars**

- **Tollen'sphloroglucinol test:** take 1-2 ml of test solution and add 3 ml of conc. HCl and 4 ml of 0.5% phloroglucinol, heat the mixture. Yellow to red colour appeared.

4. **Test for reducing polysaccharides (starch)**

- **Iodine test:** To the 3ml of test solution add few drops of dil. Iodine solution, appears which was disappeared on boiling and reappears on cooling.
- **Tannic acid test:** With test solution add 20% of tannic acid solution, gives yellow ppt.

5. **Tests for alkaloids**

Each extract was dissolved in HCl and filtrates were obtained. They were used for testing.

- **Mayer's test:** Mayer's reagent (Potassium Mercuric Iodide) was treated with filtrates. The yellow colour ppt are appeared which give indicated the presence of alkaloids.
- **Wagner's Test:** Filtrates were Taken and treated with wagerer's reagent (solution of Potassium Bismuth Iodide). Brown/reddish ppt was formed that indicated the presence of alkaloids.
- **Dragendroff's Test:** Filtrates were taken and treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Reddish ppt was obtained which indicates the detection on alkaloids.
- **Hager's Test:** Filtrates were Taken and treated with Hager's reagent (saturated picric acid solution). Detection of alkaloids was confirmed by the formation of ppt of yellow colour.

6. **Test for flavonoids**

- **Alkaline Reagent Test:** The Extracts was taken and treated with few drops of sodium hydroxide solution. Indicated for flavonoids was detected by formation of yellow colour and on addition of dilute acid it turns colourless.
- **Lead acetate Test:** Extracts were treated with few drops of lead acetate solution. Indicated the presence of flavonoids by the formations of yellow colour precipitate.
- **Shinoda test:** To the extract 95% of ethanol was added with few drops of conc. HCl and 0.5 g of magnesium turnings were also added. The presence of flavonoids was confirmed by observation of pink colour.

7. **Test for cardiac glycoside**

- **Legal's test (cardenoloids):** Took extract, add pyridine and 1ml of nitroprusside. The confirmation of cardiac glycoside was indicated by appearance of pink to red colour.

8. **Test for tannins and phenols**

- **Ferric chloride test:** To the extract solution few drops of 5% of $FeCl_3$ solution were added the presence of phenolic compounds was determined by dark green colouration.
- **Lead acetate test:** To the extract solutions add few drops of 10% of lead acetate solution formation of white ppt indicate the presence of phenolic compounds.
- **Gelatine test:** To the extract solution few drops of 10% gelatine were added which results the formation of white ppt, indication for the presence of phenolic compounds.
- **Bromine water test:** To 2-3 ml of the extract solution add few drops of bromine water. The decolouration of water indicates the presence of phenolic compounds.
- **Acetic acid test:** To 2-3 ml of the extract solutions add few drops of acetic acid, solution becomes red in colour which indicated the presence of phenolic compounds.

9. **Test for proteins**

- **Biuret test (general's test):** To 3ml of the extract solution add few drops of 4% NaOH and few drops of 1% $CuSO_4$ solution. The appearance of pink or violet colour indicated the presence of proteins.
- **Xanthoprotein test (for protein containing tyrosine or tryptophan):** To 2-3 ml of the extract solution add 1ml of conc. H_2SO_4, white ppt appears which turns yellow on boiling. Now add NH_4OH ppt turns orange.
- **Test for proteins containing sulphur:** To 2-3 ml of the extract solution add few drops of 40% NaOH and few drops of lead acetate solution (10%). Black or brownish colour appeared due to PbS formed which confirmed protein.

Figure 2: Different extract of *Nephrolepis cordifolia*
(A) Hydroalcoholic, (B) Aqueous, and (C) Ethanolic extract

6. Pharmacological Studies

6.1 Animal

The Indian adult earthwormswere used to determine the anthelmintic activity of the aqueous extract of *Nephrolepis cordifolia* leaves. The collection of earthworms was done from moist soil and washed with normal saline to remove all dust and faecal matter. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all experimental protocol. The earthworm resembles both anatomically and physiologically to the intestinal roundworm parasites of human beings, hence can be used to study the anthelmintic activity.

6.2 Procedure for Anthelmintic Activity

To determine the anthelmintic activity of the aqueous extract of *Nephrolepis cordifolia* leaves. The Indianadult earthworms (*Pheretima posthuma*) of 3-5 cm in length and 0.1-0.2 cm in width were used and collected from Nursery. The worms of equal size were divided into 4 groups containing 2 worms in each group. The extracts were dissolved in normal saline and the volume was adjusted to 10ml with saline water to desired concentration (20 mg/ml). Standard drug solution was prepared freshly before experiment. Different extracts and the standard drug solution were poured in petri dishes, labelled with extract and concentrations. Normal saline, 10ml was used as vehicle control. Two worms of equal size were introduced into each petri dish and time was noted. The Observations were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colours [12].

7. Results

7.1 Morphological features of the leaves of *Nephrolepis cordifolia*

The lower part (i.e., stipes) of the leaves were n glossy brown elongated (i.e., linear-lanceolate) scales whereas, its upright 'leaves' (i.e., fronds) have a brownish-coloured stalk (i.e., stipe) up to 15 cm long. The stalk was divided into numerous alternatively arranged narrow 'leaflets' (i.e., pinnae). These 'leaflets' (usually 10-35 mm long and 4-11 mm wide, but rarely to 6 cm long) have irregularly and often finely scalloped (i.e., crenate or crenulate) margins and are usually hairless (i.e., glabrous). The different morphological features are listed in the Table 1.

Table 1: Morphological features of the leaves of *Nephrolepis cordifolia*

S.No.	Characteristic features	Observation
1	Colour	Dull green to yellow green
2	Odour	Characteristic
3	Taste	Bitter to taste less
4	Shape	10-35 mm long and 4-11 mm wide

7.2 Extractive value of leaves of *Nephrolepis cordifolia* (decoction method)

Table 2: Morphological features of the leaves of *Nephrolepis cordifolia*

S.No.	Solvents	Weight of plant material (g)	Colours of extract	Weight of extract	Extractive value (%w/w)
1	Ethanol	10	Dark green	1.08	10.8
2	Hydroalcoholic	10	Light green	0.46	4.6
3	Aqueous	10	Yellow green	0.85	8.5

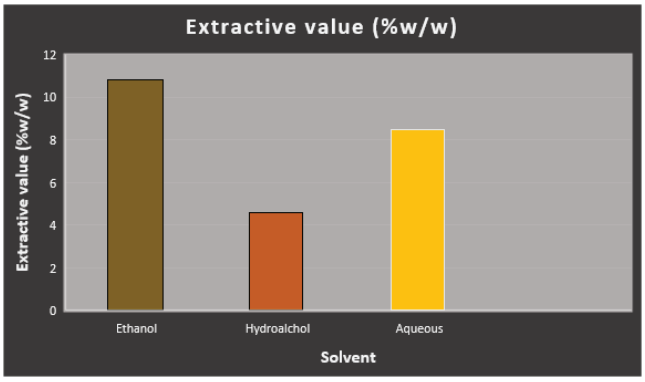

Figure 3: Graph showing the different extractive value of different solvent used for extraction

7.3 The Phytochemical Investigation Result was Tabulated in the Table 3

The different plant extracts were used for the entablement of phytochemicals. The chemical tests such as flavonoids, alkaloids, tannins, phenols, carbohydrate etc were tested. the results for the different chemical test were given in the table 3. The flow diagram for the test procedure was depicted in the Figure 4.

7.4 The result of Pharmacological activity was given in Table 4

The anthelmintic activity was performed using albendazole as standard. Aqueous extract of *Nephrolepis Cordifolia* was used for the evaluation. At the concentration of 4 and 8 mg/ml showed significant result with time taken for paralysis (80 and 50 min) and time taken for death (155 and 140 min) (Figure 5). The result of the different concentration was tabulated in the table 4and graphically presented in the Figure 6.

8. Discussion

This study suggests that the plant used to treat intestinal worm infections showed less significant anthelmintic activity. The experimental evidence obtained in the laboratory model could not provide a rationale use of this plant as anthelmintic. The aqueous extract of leaves did not display a significant anthelmintic activity in dose in dose dependent manner. The anthelmintic activity of aqueous extract was compared with that of albendazole (reference). This drug is effective in a broad range of helminth infections including round worms, hookworms, whipworms and pinworms. It may be due to its effect on inhibition of tubulin polymerization, glycogen synthesis and glucose uptake in the parasites leading to a lethal depletion of energy reserves in the helminthic.

Table 3: Phytochemical investigation of *Nephrolepis cordifolia*

Phytoconstituent	Method	Ethanolic extract	Aqueous extract	Hydro -alcholic extract
Flavonoids	Shinoda Test	+ve	+ve	+ve
	Lead acetate Test	+ve	-ve	-ve
Alkaloids	Wagner Test	+ve	+ve	+ve
	Hager's Test	+ve	+ve	+ve
	Dragendroff's test	-ve	+ve	+ve
	Mayer's test	+ve	+ve	-ve
Tannins and Phenols	Lead acetate test	+ve	+ve	+ve
	Bromine water test	+ve	+ve	+ve
	Acetic acid solution test	+ve	-ve	+ve
	Dil.potasium permanganate test	+ve	-ve	+ve
	Dil. Iodine solution test	+ve	+ve	-ve
	Dil. HNO3 test	-ve	+ve	+ve
	Potassium dichromate test	+ve	+ve	-ve
Carbohydrates	Fehling's test	+ve	+ve	-ve
	Benedict's test	+ve	+ve	-ve
	Barfoed's test	+ve	+ve	-ve
	Tannic acid test	+ve	-ve	-ve
	Iodine test	-ve	+ve	+ve
	Tollen's test	-ve	+ve	+ve
Cardiac glycoside	Legal test	+ve	+ve	-ve
Proteins	Biuret test	+ve	+ve	+ve
	Xanthoprotein test	-ve	-ve	-ve
	Test for protein containing sulphur	+ve	-ve	-ve

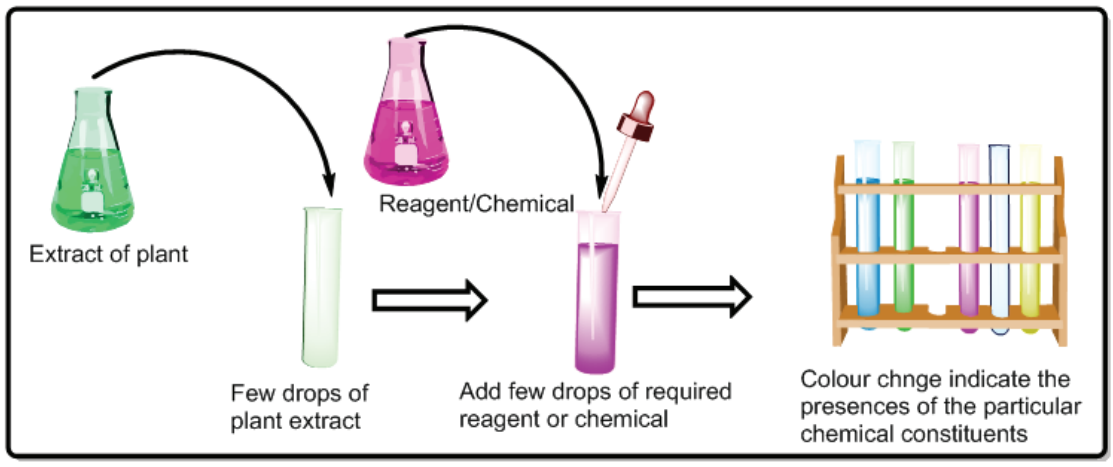

Figure 4: Flow diagram depicting the different chemical test

Table 4: Result of Evaluation of Anthelmintic Activity

Evaluation of Anthelmintic Activity				
S. No	**Treatment**	**Concentration (mg/ml)**	**Time taken for paralysis(min)**	**Time taken for death (min)**
1.	Control (Normal saline)	-	No Paralysis	No Death
2.	Albendazole	2	48	75
		4	21	60
3.	Aqueous Extract of *Nephrolepis Cordifolia*	4	90	170
		8	80	155
		10	50	140

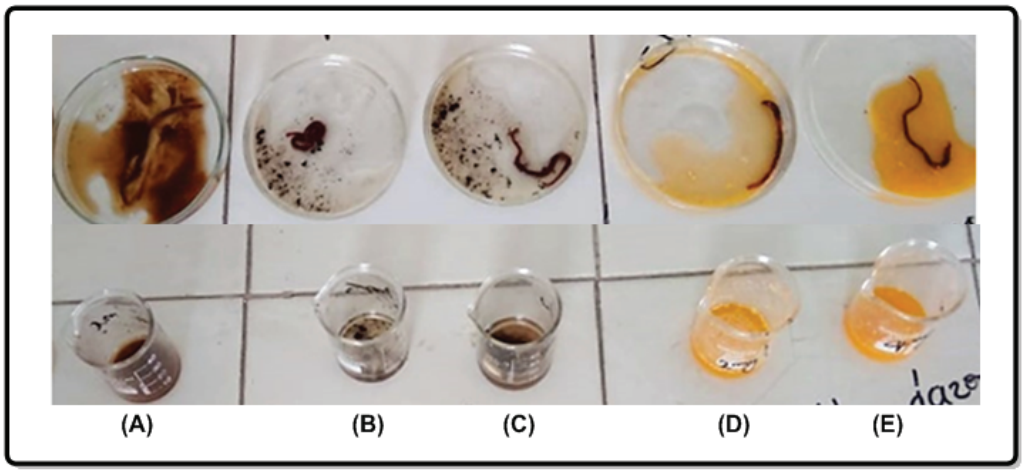

Figure 5: Different concentration of plant extract and the standard albendazole **(A)** 2, **(B)** 4, **(C)** 8 mg/ml and **(D)** 2, **(E)** 4 mg/ml and their effects on earthworm

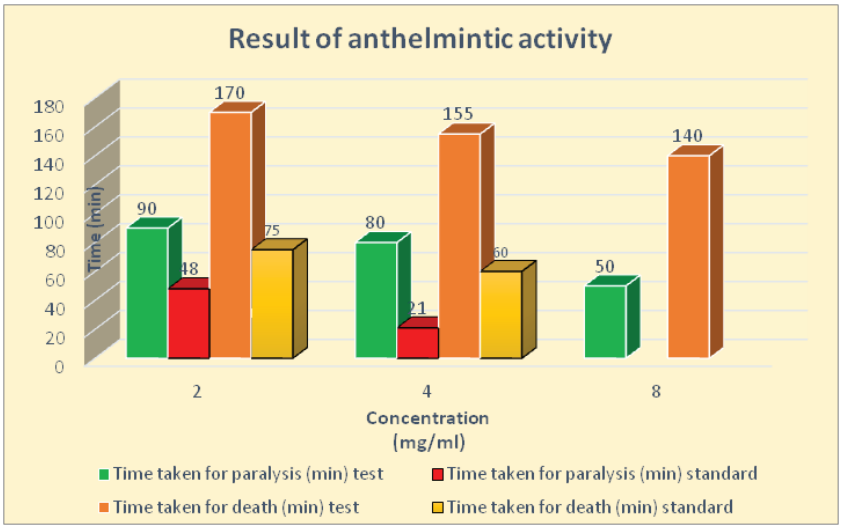

Figure 6: Graphical representation of results of anthelmintic activity

9. Conclusion

Modern synthetic medicine is very effective in curing a number of diseases but also causes serious side effects. Crude drugs are efficient with respect to cure of disease but are relatively free from side effects. Parasites have been of concern to the medical field from centuries and the helminths considered to cause problems for both humans and animals. A large number of medical plants are claimed to possessed anthelmintic property in traditionally system of medicine. In the present study the plant *Nephrolepis cordifolia* was selected for its flavonoid content and attempt was made to evaluate anthelmintic potential. The various extract of *Nephrolepis cordifolia* showed were prepared and subjected for the phytochemical screening. The result showed that the leaves contain flavonoids, alkaloids in all the three extract. The test for carbohydrate was positive only for ethanolic and aqueous extract. The aqueous extract was tested for anthelmintic activity. The result was not that significant. The extract showed slightly significant result at the concentration of 10 mg/ml with time taken for paralysis and time taken for death 50 and 140 min respectively.

Conflicts Of Interest

No conflict of interest declared.

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