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In-Vitro Anthelmintic Activity Evaluation of Seed Extract of Calamus leptospadix Griff.

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ABSTRACT

Calamus leptospadix Griff. (Arecaceae) is a well known and widely used ethnomedicinal plant in Assam and is effectively used amongst Assamese community people as folk remedy in the form of infusions and decoctions for treatment of variety of infectious ailments. No detail exploration has been made on this vital plant till date. Present work circumspects an extensive study on the seed extract of the plant with a view to substantiate the therapeutic potential of the plant in terms of its anthelmintic activity against *Pheretima posthuma* using Albendazole as a reference standard. Methanolic, Ethanolic and Aqueous extracts of *Calamus leptospadix* Griff. were freshly prepared with concentrations of 25mg/ml, 50mg/ml, 100mg/ml for each and tested for their anthelmintic activity. Results indicate that the different concentrations of various extracts under study exhibit significant anthelmintic activity and among the various extracts, the methanolic extract has shown better activity as compared to the other extracts as well as the standard. From the investigation, conclusion can be drawn that *Calamus leptospadix* Griff. (Arecaceae) used by the people of Assam traditionally to treat intestinal worm infections, possesses significant anthelmintic activity.

KEYWORDS: *Calamus leptospadix*, Seeds, Anthelmintic, *Pheretima posthuma*, Paralysis

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INTRODUCTION

Helmenthiasis causes infestation to a part of the body with worms such as pinworm, roundworm or tapeworm. Typically the worms reside in gastrointestinal tract but may also invade the liver and other organs¹. In developing countries, they pose a large threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia and pneumonia². Parasitic diseases may cause severe morbidity, including lymphatic filariasis (a cause of elephantiasis), onchocerciasis (river blindness), and schistosomiasis³. Most diseases caused by helminths are of a chronic nature⁴. Anthelmintics are drugs that act either locally to expel worms from the gastrointestinal tract or systemically to eradicate adult helminthes or developmental forms that invade organs and tissues⁵. Most of the existing anthelmintics produces side effects such as abdominal pain, loss of appetite, nausea, vomiting, head ache and diarrhea⁴. Albendazole is a well tolerated drug, however gastrointestinal side-effects, dizziness have been noted in few patients. Also prolonged use in hydatid or in cysticercosis, causes headache, fever, alopecia, jaundice and neutropenia⁶. In order to counter the harmful side-effects of these synthetic anthelmintic drugs, it is important for us to promote the studies of traditionally used anthelmintic plants which will lead to the development of new anthelmintic substances with ease of availability and lesser side-effects⁷. *Calamus leptospadix* [Synonym: *Palmijuncus leptospadix* (Griff.) Kuntze] belonging to the family Areaceae is commonly known as Lejaibet (Ass.), Jeyying (Adi.), Kukhre bet (Bhu.) and is an important ethnomedicinal plant of Assam and is traditionally used by the local community since ages in intestinal worm infections. However no detailed study or reports along with scientific evidence are available for this important plant. In this research work, an exhaustive study was carried out on the seed extract of this plant in order to substantiate the therapeutic potential of the plant in terms of its anthelmintic activity against *Pheretima posthuma* (Indian adult worms) using Albendazole as a reference standard.

MATERIALS AND METHODS

Collection and authentication of plant materials

The seeds of *Calamus leptospadix* were collected from Jokai, Dibrugarh, Assam, India. The plant was identified and authenticated by Dr. N. Odyuo, Botanical Survey of India, Eastern Regional Centre, Shillong. A voucher specimen (Specimen no. Du/SB/2012/06, Reference no. BSI/ERC/2013/Tech/Plant identification/636) is kept in Department of Pharmaceutical Sciences, Dibrugarh University, Assam for future references. The seeds were cut into pieces, washed thoroughly

with water and then dried partially under sunlight and partially under the shade for a week. These were then pulverized in to course powder by using a dry grinder and passed through the sieve before being stored in closed vessel for further use.

Preparation of extract

Powdered crude drug of *Calamus leptospadix* Griff. (Arecaceae) seeds were weighed accurately and subjected to extraction in a soxhlet apparatus (Continuous hot extraction) at room temperature using methanol, ethanol and water for 18hours after pretreatment with petroleum ether. After the extracts were concentrated, preliminary phytochemical tests were carried out with all the extracts in order to evaluate for the presence of different phytochemical constituents.

Model for experiment

The anthelmintic activity was carried out in Indian Adult Earthworms (*Pheretima posthuma*) due to their easy availability and anatomical and physiological resemblance with the intestinal roundworm parasites of human beings⁸. The worms were collected from moist soil and washed with 0.9% w/v of normal saline to remove all dirt and faecal matter. The worms measure 3-5 cm in length and 0.2-0.3 cm in width⁹.

Drugs and chemicals

0.9% w/v of normal saline was prepared. Albendazole (Lupin Pharmaceuticals Ltd.) was prepared at three different concentrations of 25 mg/ml, 50 mg/ml and 100mg/ml in distilled water and this was used as standard drug. Similarly MECL, EECL and AECL were prepared at the concentrations of 25mg/ml, 50mg/ml, 100mg/ml in distilled water and these were used as test drugs for activity evaluation.

Anthelmintic activity testing

The anthelmintic assay was carried out as per the method of Panda *et al.*¹⁰ with slight modifications in the process. Indian Adult Earthworms were collected and divided into twelve groups. 10ml of each different concentrations of standard drug Albendazole and test drugs MECL, EECL and AECL were taken in twelve different petridishes. Four earthworms were placed in each of the twelve petridishes after they have been washed with 0.9% w/v of normal saline solution. Motility of the worms were

observed and time for paralysis and time for death of the worms were noted down. Time for paralysis was noted when the worms showed no movements when shaken vigorously. Time for death was recorded when the worms showed no movements even when dipped in warm water at 50- 60 °C temperature also with fading of their body colour. The results are expressed as Mean \pm SEM of four worms in each group.

RESULTS AND DISCUSSION

The different phytoconstituents present in the seed extract of *Calamus leptospadix* are shown in indicative tabular form as Table. 1

Table. 1: Phytoconstituents present in seed extract of *Calamus leptospadix*

Phytoconstituents	MECL	EECL	AECL
Alkaloids	+	+	+
Glycosides	+	+	+
Carbohydrates	+	+	+
Saponins	+	+	+
Lipids	+	+	+
Flavonoids	-	-	-
Tannins	+	+	+
Steroids	+	+	+
Lignins	+	-	-
Triterpenoids	+	-	-

MECL: Methanolic Extract of *C. leptospadix*, EECL: Methanolic Extract of *C. leptospadix*, AECL: Aqueous Extract of *C. leptospadix*

In-vitro Anthelmintic activity shown by different concentrations of the test drugs and standard drug are depicted in tabular form in Table. 2

The activity data as illustrated in Table. 2 showed that methanolic extract of the seeds of *Calamus leptospadix* Griff. gave shorter paralysis and death time in comparison to the ethanolic and aqueous extracts. Crude methanolic, ethanolic and aqueous extracts with concentrations of 25mg/ml, 50mg/ml, 100mg/ml produced dose- dependent paralysis.

Table. 2: Anthelmintic activity of seed extract of *Calamus leptospadix*

Drug treatment	Dose (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Standard drug (Albendazole)	25	25.25 ± 0.21	28.25 ± 0.54
	50	20.50 ± 0.43	24.75 ± 0.21
	100	18.25 ± 0.21	23.25 ± 0.54
Methanolic extract	25	26.45 ± 0.15	28.75 ± 0.21
	50	23.25 ± 0.20	27.30 ± 0.44
	100	17.75 ± 0.12	20.50 ± 1.45
Ethanolic extract	25	40.68 ± 0.55	45.00 ± 0.25
	50	34.42 ± 0.22	38.22 ± 0.30
	100	33.12 ± 0.44	37.75 ± 0.12
Aqueous extract	25	52.42 ± 0.10	56.50 ± 0.25
	50	48.50 ± 0.44	50.48 ± 1.32
	100	40.22 ± 0.32	44.68 ± 0.24

It is evident from the results obtained from this evaluation that this plant could be used and further explored judiciously in controlling gastrointestinal helminthic infections. It is also suggestive from the phytochemical investigations of the methanolic, ethanolic and aqueous extracts of *Calamus leptospadix* Griff. seeds, that the presence of alkaloids¹¹ might be the possible reason for the paralysis of the worms, however presence of triterpenoids and tannins in the methanolic extract could be the reason for the faster death of the worms¹²⁻¹³ as compared to that of ethanolic and aqueous extracts.

CONCLUSION

It can be comprehensively concluded from the research work that the methanolic extract of the seeds of *Calamus leptospadix* exhibited shortest anthelmintic activity against Indian adult earthworm (*Pheretima posthuma*). From the biological assay performed and observations noted, it can be concluded that *Calamus leptospadix* Griff. (Arecaceae) used by the people of Assam traditionally to treat intestinal worm infections, possesses significant anthelmintic activity when compared with the normally used drug and hence can pave the way for development of very potent anthelmintic agent. Further isolation and characterization of the methanolic extract needs to be carried out in order to establish the possible active compound responsible for the use of *Calamus leptospadix* as an anthelmintic drug and in-vivo studies could also be carried out in the future in order to establish the effectiveness of this plant as an anthelmintic drug.

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