



# Investigation of *in-vitro* Anthelmintic Activity of *Calamus leptospadix* Griff. Shoot in Indian Adult Earthworm ( *Pheretima posthuma* )

Sudarshana Borah\*, Bibhuti Bhusan Kakoti, Kabita Mahato, Mritunjay Kumar

Department of Pharmaceutical Sciences, Dibrugarh University , Dibrugarh- 786004, Assam, India.

---

## ARTICLE INFO

### Article history:

Received on: 11/04/2013

Revised on: 09/05/2013

Accepted on: 05/06/2013

Available online: 27/06/2013

### Key words:

*Calamus leptospadix*,

Arecaceae, Anthelmintic,

Extract, *Pheretima posthuma*,

Paralysis.

---

## ABSTRACT

*Calamus leptospadix* Griff. (Arecaceae), is an important ethnomedicinal plant of Assam known as Lejaibet by the local Assamese community and is being widely used by various communities in Assam. No detailed study or reports along with scientific evidence are available with this important plant. An exhaustive study was carried out 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. 0.9% w/v of normal saline solution, 25mg/ml, 50mg/ml, 100mg/ml each of standard drug Albendazole and Methanolic, Ethanolic and Aqueous extracts of *Calamus leptospadix* Griff. were freshly prepared. Forty eight Indian Adult Earthworms were collected, divided into twelve groups containing four worms in each group. Time for paralysis and time for death were recorded for each group. All the three extracts with concentrations of 25mg/ml, 50mg/ml, 100mg/ml produced dose-dependent paralysis. Methanolic extract of *Calamus leptospadix* Griff. gave shortest paralysis and death time at 100mg/ml as compared to that of standard and other two extracts. Results are expressed as Mean  $\pm$  SEM ( $P < 0.05$ ) of 4 worms in each group. 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.

---

## INTRODUCTION

In helminthiasis disease a part of the body is infested with worms such as pinworm, roundworm or tape worm. Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver and other organs (Jaya and Yesuf, 2010). In developing countries, they pose a large threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia and pneumonia (Bundy, 1994) . The parasitic worms are divided into three groups: cestodes or tapeworms; nematodes or roundworms; and trematodes or flukes (Sharma, 2007). Parasitic diseases may cause severe morbidity, including lymphatic filariasis (a cause of elephantiasis), onchocerciasis (river blindness), and schistosomiasis (Lukhoba *et al.*, 2006). Most diseases caused by helminths are of a chronic nature; they

probably cause more morbidity and even economic and social deprivation among humans and animals than any single group of parasites (Partap *et al.*, 2012). 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 tissues (Brunton, 2001). Most of the existing anthelmintics produces side effects such as abdominal pain, loss of appetite, nausea, vomiting, head ache and diarrhea (Bundy, 1994) . 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 neutropenia (Tripathi, 2008). In order to exterminate 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-effects (Khan *et al.*, 2011).

---

### \* Corresponding Author

Department of Pharmaceutical Sciences, Dibrugarh University,

Dibrugarh- 786004, Assam, India.

E-mail: - shonapharma@gmail.com

Phone : - +919401613080

The anthelmintic activity was evaluated on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings and easy availability (Vidyasarathi, 1977).

*Calamus leptospadix* [Synonym: *Palmijuncus leptospadix* (Griff.) Kuntze] belonging to the family Arecaceae is commonly known as Lejaibet (Ass.), Jeyying (Adi.), Kukhre bet (Bhu.) and is an important ethnomedicinal plant of Assam. The plant is a slender, cluster forming climber with stems climbing to 25 m long and 2 cm diameter. Leaf sheaths are green with grayish brown hairs, with scattered to densely arranged, brownish, flattened to 2.5 (to 5 at sheath apices) cm long spines, those at sheath apices needlelike yellowish; ocreas present to 1.5cm long densely bristly, with long, needlelike spines; flagella present; leaf rachis to 2 m long with 50- 55 linear leaflets per side, these are regularly arranged, cirri absent. Inflorescence to 4m long, flagellate; bracts tubular, briefly open and spreading at the apices; flowering branches very short; fruits globose to 1.5 cm diameter, white or yellowish. Flowering and Fruiting season is in the month of March- October.

It is distributed in Bangladesh, Bhutan, Northeastern India (Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, Sikkim, Tripura, West Bengal), Myanmar (Kachin) and Nepal; mostly on lowland or mountain rainforest along river margins to 1300 m elevation (Henderson, 2009; Haridasan, 2002). In Ayurvedic system *Calamus* species are used in fever, piles, dyspepsia, biliousness; Flowers are used as antiseptic, anti-bacterial, externally for cuts, burns, bruises, scalds (Khare, 2007). Young shoots of the plant are used as vegetable (Sarmah, 2010). In Assam, it is traditionally believed by the folk people that the tender shoots, leaves and seeds of this plant are used as vermicide. This plant is widely used by various communities in Assam, however no detailed study or reports alongwith scientific evidence are available with this important plant.

An exhaustive study was carried out in order to substantiate the therapeutic potential of the plant in terms of its anthelmintic activity against *Pheretima posthuma* using Albendazole as a reference standard.

## MATERIALS AND METHODS

### Plant material

The shoots of *Calamus leptospadix* were collected from Jokai, Dibrugarh, Assam, India during the month of July, 2012. 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. Young and tender shoots were cut into pieces, washed thoroughly with water and then dried partially under sunlight and partially under the shade for a week. The dried shoot pieces were then pulverized in mechanical grinder and stored in airtight containers free from moisture.

### Preparation of extract

250 g of powdered crude drug of *Calamus leptospadix* Griff. (Arecaceae) shoots were extracted by soxhlation (Continuous hot extraction) with 1000 ml of methanol, 1000ml of ethanol and 1000ml of 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. The MECL (Methanolic extract of *Calamus leptospadix*) contains the major phytoconstituents like alkaloids, carbohydrates, fats & oils, glycosides, lignin, steroids, saponins, triterpenoids, tannins and phenolic compounds. The EECL (Ethanol extract of *Calamus leptospadix*) contains the major phytoconstituents like alkaloids, fats & oils, carbohydrates, glycosides, steroids, saponins and tannins. The AECL (Aqueous extract of *Calamus leptospadix*) contains the major phytoconstituents like alkaloids, flavonoids, carbohydrates, glycosides, steroids, saponins and tannins.

### Indian adult earthworm as model for the experiment

The anthelmintic activity was carried out in Indian Adult Earthworms (*Pheretima posthuma*) due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings and easy availability. The worms were collected from moist soil and washed with 0.9% w/v of normal saline to remove all dirt and faecal matters. The worms measure 3-5 cm in length and 0.2-0.3 cm in width (Pillai and Nair, 2011).

### 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 the activity.

### Evaluation of anthelmintic activity

The anthelmintic assay was carried out as per the method of (Panda *et al.*, 2011) with slight modifications in the process. Forty eight Indian Adult Earthworms were collected and divided into twelve groups containing four earthworms in each group. 10ml of each different concentrations of standard drug Albendazole and test drugs MECL, EECL and AECL were taken with pipette 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 away of their body colour.

### Statistical analysis

The results are expressed as Mean  $\pm$  SEM of four worms in each group. Comparisons have been made between standard against test treated groups,  $P < 0.05$  was considered significant. The observation table is shown in Table no. 1.

**Table. 1:** *In-vitro* Anthelmintic activity of shoot extracts of *Calamus leptospadix*.

Drug Treatments	Doses (Mg/MI)	Time Taken for Paralysis (Min)	Time Taken for Death (Min)
Standard drug (Albendazole)	25	25.25 $\pm$ 0.21	28.25 $\pm$ 0.54
	50	20.5 $\pm$ 0.43	24.75 $\pm$ 0.21
	100	18.25 $\pm$ 0.21	23.25 $\pm$ 0.54
Methanolic extract of <i>Calamus leptospadix</i> Griff.	25	27.5 $\pm$ 0.25	29.75 $\pm$ 0.21
	50	22.25 $\pm$ 0.41	24.5 $\pm$ 0.43
	100	18.75 $\pm$ 1.29	22.5 $\pm$ 1.25
Ethanollic extract of <i>Calamus leptospadix</i> Griff.	25	43.5 $\pm$ 0.75	50.5 $\pm$ 0.25
	50	37.5 $\pm$ 0.43	41.25 $\pm$ 0.41
	100	31.5 $\pm$ 0.43	35.75 $\pm$ 0.21
Aqueous extract of <i>Calamus leptospadix</i> Griff.	25	54.5 $\pm$ 0.25	59.5 $\pm$ 0.25
	50	50.5 $\pm$ 0.43	56.5 $\pm$ 0.43
	100	47.25 $\pm$ 0.41	51.5 $\pm$ 0.25

The result was expressed as Mean  $\pm$  SEM. Statistical analysis was carried out with comparisons between standard and treated groups.  $P < 0.05$  was considered statistically significant and  $n=4$  was taken in each group.

### RESULT AND DISCUSSION

From the observation table given in table no.1, it was found that higher the concentration of the extract faster was the paralytic effect and shorter was the death time for all the earthworms. Crude methanolic, ethanollic and aqueous extracts with concentrations of 25mg/ml, 50mg/ml, 100mg/ml produced dose- dependent paralysis. The data given in the observation table no.1 showed that methanolic extract of the shoots of *Calamus leptospadix* Griff. gave shorter paralysis and death time at 100mg/ml as compared to that of ethanollic and aqueous extracts. Even at the minimum dose of 25mg/ml anthelmintic effect of extracts was found as compared to that of standard drug Albendazole (Das *et al.*, 2011). This study was performed on Indian Adult Earthworms as these are easily available and possess anatomical and physiological resemblance with that of intestinal round worm parasites of human beings (Vidyasarathi, 1977). These parasitic helminths affect mankind and animals causing relentless infections to them. Many synthetic compounds and their derivatives have been developed but the problems associated with the use of such drugs leads to serious side-effects. Also sometimes these parasites develop resistance to the drugs leading to more severe infections. Thus, steps have been taken towards developing herbal medicines as a safer remedy to cure helminths (Partap *et al.*, 2012). Researches and studies are going on in different parts of the world on medicinal plants. The results obtained in this study have shown hopeful results on anthelmintic activity. This plant could be used by human beings in controlling gastrointestinal nematode infections. Albendazole is variably and erratically absorbed after oral administration; absorption is enhanced by the presence of fatty foods and possibly by bile salts as well. After a 400mg oral

dose, albendazole cannot be detected in plasma, because the drug is rapidly metabolized in the liver and possibly in the intestine as well to albendazole sulfoxide which has potent anthelmintic activity (Marriner *et al.*, 1986; Redondo *et al.*, 1999). Mean  $\pm$  SEM values were calculated for the extracts and standard. All the three extracts showed anthelmintic activity in a dose- dependent manner taking shortest time for paralysis (18.75) and death (22.5) for MECL. Time taken by EECL at 100mg/ml concentration is (31.5) for paralysis; (35.75) for death and time taken by AECL at 100mg/ml concentration is (47.25) for paralysis; (51.5) for death in a dose-dependent manner as compared with that of standard drug Albendazole for paralysis (18.25) and death (23.25) at 100mg/ml respectively. As phytochemical investigation of the methanolic, ethanollic and aqueous extracts of *Calamus leptospadix* Griff. showed the presence of alkaloids (Acharya *et al.*, 2011) which might be the 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 worms (Balamurugan and Selvaragavan, 2009; Sravani, 2011) as compared to that of ethanollic and aqueous extracts. Therefore from the present study conducted it can be suggested that the methanolic extract revealed higher anthelmintic activity possibly due to alkaloids, tannins and triterpenoids. 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.

### CONCLUSION

The methanolic extract of the shoots 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 provides a basis for the traditional use of this plant as an anthelmintic.

### ACKNOWLEDGEMENT

Authors are grateful to the Department of Pharmaceutical Sciences, Dibrugarh University for providing us with all the laboratory facilities to perform this research work in the department.

### REFERENCES

- Acharya S, Dash GK, Brahma DK, Chhetree RR. Preliminary phytochemical investigation and anthelmintic activity of *Acacia suma* (Roxb) barks. *Int Res J Pharm*, 2011; 2(1):136-141.
- Balamurugan G, Selvaragavan S. Preliminary phytochemical investigation and anthelmintic activity of *Indigofera tinctoria* Linn. *Int J Drug Dev Res*, 2009; 1(1): 157- 160.

Brunton L. 2001. Goodman & Gilman's The Pharmacological Basis of Therapeutics. New York, U.S.A: McGraw- Hill Medical Publishing Division.

Bundy DA. Immunoepidemiology of intestinal helminthic infection: The global burden of intestinal nematode disease. *Trans Royal Soc Trop Med Hyg*, 1994; 8: 259-61.

Das SS, Dey M, Ghosh AK. Determination of anthelmintic activity of the leaf and bark extract of *Tamarindus indica* Linn. *Indian J Pharm Sci*, 2011; 73(1):104-7.

Haridasan K, Sarmah A, Hegde SN, Bhuyan LR. 2002. Field Manual for Propagation and Plantation of canes in Arunachal Pradesh. State Forest Research Institute, Department of Environment and Forests, Govt. of Arunachal Pradesh, Itanagar- 791111,

Henderson A. 2009. Palms of Southern Asia. Princeton, U.S.A: Princeton University Press.

Jaya RN, Yesuf AE. Evaluation of Anthelmintic Activity of *Rumex abyssinicus* Jacq and *Rumex nervosus* Vahl. *International Journal of Pharmaceutical Sciences Review and Research*, 2010; 5(2): 55.

Khan RP, Karthikeyan M, Kannan M, Rajasekar S. Anthelmintic activity of *Nerium olender* flower extract in Indian adult earthworm. *Scholars Research Library J. Nat. Prod. Plant Resour*, 2011; 1 (4):40-46.

Khare CP. 2007. Indian Medicinal Plants. Berlin, Germany: Springer Science + BusinessMedia.

Lukhoba CW, Simmonds MSJ, Paton AJ. *Plectranthus*: A review of ethnobotanical uses. *Ethnopharmacol*, 2006; 103: 1-24.

Marriner SE, Morris DL, Dickson B, Bogan JA. Pharmacokinetics of albendazole in man. *Eur. J. Clin. Pharmacol*, 1986; 30: 705-708.

Panda SK, Das D, Tripathy NK. Evaluation of Anthelmintic activity of *Chlorophytum borivilianum* santapau & fernandes. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 2011; 2(2):676-679.

Partap S, Kumar S, Kumar A, Sharma NK, Jha KK. In-Vitro Anthelmintic Activity of *Luffa Cylindrica* Leaves in Indian Adult Earthworm. *Journal of Pharmacognosy and Phytochemistry*, 2012; 1(2): 30.

Pillai LS, Nair BR. A comparative study of the anthelmintic potential of *Cleome viscosa* L. And *Cleome burmanni* W. *Indian J Pharm Sci* 2011; 73(1): 98-100.

Redondo PA, Alvarez AI, Garcia JL, Larrode OM, Merino G, Prieto JG. Presystemic metabolism of albendazole: Experimental evidence of an efflux process of albendazolesulfoxide to intestinal lumen. *Drug Metab. Dispos*, 1999; 27: 736-740.

Sarmah R. Commonly used non- timber forest products (NTFPS) by the Lisu Tribe in Changlang district of Arunachal Pradesh, India, 2010; 05 : 68-77.

Sharma VN. 2007. Essentials of Pharmacology. New Delhi, India: CBS Publishers Distributors.

Sravani K, Ramya J, Pradeepkumar A, Ashajyothi V. Phytochemical studies and anthelmintic activity of *Gymnema sylvestre*. *Int J Res Ayur Pharm*, 2011; 2(3): 931-935.

Tripathi KD. 2008. Essentials of medical pharmacology. New Delhi, India: Jaypee brothers medical publishers (P) LTD.

Vidyasarathi RD. 1977. A Text Book Zoology. New Delhi, India: S. Chand and Co.

#### How to cite this article:

Sudarshana Borah, Bibhuti Bhusan Kakoti, Kabita Mahato, Mritunjay Kumar., Investigation of *in-vitro* Anthelmintic Activity of *Calamus leptospadix* Griff. Shoot in Indian Adult Earthworm (*Pheretima posthuma*). *J App Pharm Sci*, 2013; 3 (06): 156-159.