

IN-VITRO ANTHELMINTIC ACTIVITY OF ACORUS CALAMUS LEAVES

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ABSTRACT

Objectives: The present study was aimed to investigation of the anthelmintic potential of crude MeOH extract, 50% EtOH and aqueous extract of the leaves of *Acorus calamus* on Indian earth-worm (*Pheretima posthuma*). **Methods:** Three concentrations (25, 50, 100 mg/ml) of each extract were studied in activity which involved the determination of time of paralysis (vermifuge) and time of death (vermicidal) of the worms. Albendazole in same concentration as those of extract was included as standard reference and normal saline water with 1% CMC as control. **Results:** All the extracts exhibited significant anthelmintic activity at a concentration of 100 mg/ml. Peak activity was exhibited by the MeOH extract at a concentration of 100 mg/ml. **Conclusion:** Findings of the present investigations confirms that, the ethno-medicinal claim of anthelmintic activity of this plant is genuine.

Keywords: *Acorus calamus*, Anthelmintic activity, Albendazole, Vermifuge, Vermicidal.

INTRODUCTION

Parasitic infection including Helminthiasis is a critical serious problem in the tropical regions including the Asian countries which affects more than two billions of people worldwide. Helminths produce serious problem in human and other animals around the world specifically to the third world countries [1]. Different type of helminths infects the human and animals out of which intestinal round worms (*Ascaridia sp.*) are most common [2]. Approximately 300 million people suffer severe morbidity associated with these parasites and half of which are school-going children affected by massive infections. Variety of several clinical symptoms arises due to this infection include dysentery, diarrhoea, nausea-vomiting, loss of appetite and weight, acidity and sometimes anaemia [2]. Other manifestations of helminthic infections include respiratory symptoms, dermatological consequences and epilepsy as a result of neurocysticercosis. Helminthic infections may also subvert immune responses to pathogens of other diseases such as tuberculosis, HIV, and malaria [1].

Acorus calamus commonly known as 'Vacha' belonging to the family Araceae is a volatile oil containing bushy herb or medicinal plant. The plant has a branched and aromatic root or rhizome from which raise its long erect leaves. The roots have a sweet fragrance and the leaves smell similar to lemon. They are sharp pointed and have a ridged midrib running their entire length [3, 4]. The different parts of this plant are used traditionally as abortifacient, aphrodisiac, aromatic, carminative, diaphoretic, emmenagogue, febrifuge, hallucinogenic, analgesic and anti-inflammatory, odontalgic, sedative, stimulant, memory enhancer, stomachic, tonic, vermifuge etc. [3,5]. Different parts of the plant have been subjected to exhaustive extraction and the isolated constituents were reported in the literature which includes Terpenoids, Steroids, Xanthenes, Lignans, Flavones and traces of alkaloids [6]. Entire plant parts containing volatile oil including leaves. β -Asarone is the major constituent in the leaves (27.4 to 45.5%), whereas acorenone is dominant in the rhizomes (20.86%) followed by isocalamendiol (12.75%). Methyl Eugenol, cis-methylisoeugenol, β -asarone, geranylacetate, β -farnesene, shyobunone, epishyobunone and isoshyobunone are the most abundant chemical compounds which are present in 20% of the essential oil [5]. The juice of young leaves is used in the treatment of different diseases including in the different worm infections according to traditional Ayurveda and

several other folk medical practices [3]. Anthelmintic activity of the roots & rhizomes of this herb is already reported earlier [5, 6] The present study was aimed to investigation of the anthelmintic potential of crude MeOH extract, 50% EtOH and aqueous extract of the leaves of *Acorus calamus* in three different concentrations (25, 50, 100 mg/ml) on Indian earth-worm (*Pheretima posthuma*) taking Albendazole as a standard drug.

MATERIALS AND METHODS

Plant Material

Fresh *Acorus calamus* leaves were collected from the Medicinal Plant garden of Maharaja Bir Bikram College; College Tilla, Agartala, Tripura in the month of March 2012 and authenticated by the renowned taxonomist Prof. B. K. Datta, Dept. of Botany, Tripura University (A Central University). A voucher specimen of dried sample (AC002/RIPSAT/TU/2012) was submitted for the future reference and experiment.

Drugs and Chemicals

Albendazole suspension [Zentel, GSK Pharmaceuticals Ltd. Bangalore], EtOH [Bengal Chemicals, Kolkata, WB], MeOH and CMC [Rankem Lab.] were used during the experimental protocol. All the chemicals used are laboratory and analytical grade.

Preparation of Plant Extract [7]

The fresh leaves were dried under shade. The dried leaves were cut into small pieces and powdered in a hand mill. Then 300 gm of coarse powder was extracted with 900 ml of extra pure MeOH and 300 gm of coarse powder with 900 ml of 50% EtOH (WHO recommended) successively in a Soxhlet extractor repeatedly for 48 hours. The extract was dried by solvent evaporation in a thermostat water bath at 50-60 °C temperature.

Another 300 gm coarse powder was macerated with 900 ml of distilled water for 48 hours. After completion of 48 hours it is filtered to separate the water extract from the marc. The extract was concentrated in a thermostat water bath at 70-80 °C temperature and all the extracts were kept in dessicator for the experiment.

Preliminary Phyto-chemical Investigation [7]

The percentage yield of MeOH, EtOH & aqueous extract obtained was calculated and all the extracts were subjected to number of proximate qualitative phyto-chemical analysis. These procedures are already reported by number of workers and used without any

modification.

Selection of Experimental Model [8]

Indian adult earthworms (*Pheretima posthuma*) were used to carry out the experiment. The earthworms were collected from the local supplier. Worms were washed with normal saline to remove all faecal matter. The earthworms of 8-10 cm in length and 0.3-0.4 cm in width were used for all the experimental protocol. Ready availability, anatomical and physiological resemblance of *Pheretima posthuma* made it to be used initially for *in-vitro* evaluation of anthelmintic activity.

Experimental Design [9-12]

The anthelmintic activity was carried out as described by Ajaiyeoba *EO. et. al*, 2001, with minor modifications. The Indian earthworm

(*Pheretima posthuma*) of nearly equal size, six in each group was taken for the experiment. Each type of dried extract was suspended in 1% w/v Carboxy Methyl Cellulose, prepared in normal saline water in three different conc. (25, 50, 100 mg/ml). Albendazole suspension of same conc. was taken as standard and normal saline water with 1% CMC was taken as a control. Worms were placed in petridish containing 15 ml of sample (drug) solution. Time for paralysis was noted either when any movement could not be observed except when the worms were shaken vigorously or when dipped in warm water (50 °C). Death was included when the worms lost their motility followed by white secretions and fading away of their body colour.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of crude extract of the leaves of *Acorus calamus* revealed the presence of tannins, steroids, flavonoids, saponin, terpenoids etc. The presence of phyto-constituents is reported in Table 1. The crude extracts of the leaves of *Acorus calamus* produced a significant anthelmintic activity on *Pheretima posthuma* in a dose dependent manner as shown in Table 2.

Table1: It shows- (Preliminary Phyto-chemical screening of various extracts of *Acorus calamus* Leaves).

Sl. No.	PYTOCHEMICAL TESTS	MLE	ELE	ALE
1.	Alkaloids	-	-	-
2.	Glycosides	+	+	-
3.	Saponins	+++	+++	++
4.	Flavonoids	+++	+++	++
5.	Tannins	+++	++	+
6.	Steroids	++	++	-
7.	Terpenoids	+++	+++	+

(+++) – Abundant, (++) – Moderately present, (+) – Slightly present, (-) – Absent

[MLE- MeOH Leaf Extract, ELE- EtOH Leaf Extract, ALE- Aqueous Leaf Extract]

Table2: It shows- (Anthelmintic activity of various extracts of *Acorus calamus* Leaves).

GROUPS/TREATMENT	CONCENTRATION (mg/ml)	TIME (Min)	
		PARALYSIS	DEATH
Control (1% CMC in Normal Saline)	-	-	-
Albendazole(Standard)	25	2.43 ± 0.22	19.13 ± 0.28
	50	1.25 ± 0.16	11.93 ± 0.58
	100	0.33 ± 0.21	06.83 ± 0.47
MeOH Leaf extract (MLE)	25	22.7 ± 0.32	79.3 ± 0.46
	50	16.62 ± 0.25	66.62 ± 0.22
	100	09.16 ± 0.12	33.11 ± 0.11
EtOH Leaf extract (ELE)	25	26.19 ± 0.44	111.3 ± 0.44
	50	18.05 ± 0.42	87.62 ± 0.28
	100	11.22 ± 0.11	55.71 ± 0.21
Aqueous Leaf extract (ALE)	25	66.9 ± 0.12	141.33 ± 0.16
	50	47.6 ± 0.11	112.9 ± 0.9
	100	31.16 ± 0.16	89.31 ± 0.55

[Values are expressed a mean ± SEM (n = 6)]

The percentage yield of MeOH, EtOH & aqueous extract was obtained 14.15, 9.5 & 3% w/w. Alcoholic (MeOH & EtOH) extract exhibit more concentration of phyto-constituents than aqueous extract. Preliminary phyto-chemical screenings confirms that the presence of tannins, steroids, flavonoids, saponin, terpenoids etc. The peak anthelmintic activity exhibited by the MeOH extract at highest concentration (100 mg/ml) which takes 09.16 ± 0.12 minute for paralysis and 33.11 ± 0.11 minute for death of the worms, followed by EtOH crude extract which includes 11.22 ± 0.11 for paralysis and 55.71 ± 0.21 minute for death of the worms. Aqueous extract exhibit less activity in comparison to alcoholic extract at all the tested doses. Potency of the extract was inversely proportional to the time for paralysis (vermifuge) and death (vermicidal) of the worms.

CONCLUSION

The *Acorus calamus* leaf extracts has showed significant anthelmintic activity at all the tested doses when compared to control as vermifuge and vermicidal while highest activity exhibited by the higher conc. (100 mg/ml) which assures the ethno-medicinal claim.

Hence, we can think about this herb as alternate source of anthelmintic drugs and also can generate new active lead for suitable anthelmintic drug.

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CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest.

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