

## COMPARATIVE ANTHELMINTIC ACTIVITY OF *CLERODENDRUM INDICUM* & *ALPINIA GALANGA* LEAVES

PRASHANTA KR. DEB<sup>1</sup>, RANJIB GHOSH<sup>2</sup>, SANKARI DAS<sup>3</sup>, TEJENDRA BHAKTA<sup>1\*</sup>

<sup>1</sup>Regional Institute of Pharmaceutical Science & Technology, Abhoynagar, Agartala-799 005, <sup>2</sup>Dept. of Pharmacology, TMC & Dr. BRAM Teaching Hospital, Hapania, Agartala 799 014, <sup>3</sup>Dept. of Human Physiology, Women's College, Govt. of Tripura, Agartala 799 001, Tripura (W), India. Email: shaandeb2010@gmail.com

Received: 18 Apr 2013, Revised and Accepted: 30 May 2013

### ABSTRACT

**Objective:** The current study was aimed to investigation of the anthelmintic potential of different crude extracts (MeOH extract and aqueous extract) of the leaves of *Clerodendrum indicum* & *Alpinia galanga* on Indian earth-worm (*Pheretima posthuma*).

**Methods:** Three different concentrations (25, 50, 100 mg/ml) of each extract (MeOH and aqueous 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 (0.9% NaCl) water with 1% CMC as control.

**Results:** All the extracts exhibited significant anthelmintic activity at a concentration of 100 mg/ml. In both the cases it was found that MeOH extract gives better activity in comparison to aqueous extract. Results showed that *Clerodendrum indicum* leaf extract gives better anthelmintic activity than *Alpinia galanga* leaves at all the tested dosage.

**Conclusion:** Findings of the experiment confirms that, both the plants having significant anthelmintic activity and hence the traditional claims of these plants are genuine.

**Keywords:** *Clerodendrum indicum*, *Alpinia galanga*, Anthelmintic activity, Albendazole, Vermifuge, Vermicidal.

### INTRODUCTION

*Clerodendrum indicum* L. which belongs to the family Verbenaceae is a small shrub grows up to 2 meters in height with soft succulent branches and simple broad leaves. Commonly it is known as 'Bamanhati' and cultivated as an ornamental throughout India [1,2]. This species is widely available in moderate temperature zones India and other Asian countries [1,3]. Leaves and roots of this plant exert very bitter taste and are used for various medicinal purposes. In traditional system of medicine (Ayurveda) this plant is mainly used in the treatment of asthma, bronchitis, cold fever, intestinal worms, arthritis, epilepsy, convulsion, gastric tumor, hematuria, hysteria, impotence, nasal polyps, painful micturation and rheumatism [1,2,3]. From ancient time Indian peoples are using the paste of leaves of this plant in external wounds and inflammation for quick relieve [3]. Also, the juice of leaves and roots are used as blood purifier and to treat digestive disorders and other GI-related ailments. The Antinociceptive, Anti-diarrheal and Antimicrobial activities of leaf extracts of *Clerodendrum indicum* has been reported [4]. Earlier phytochemical investigation reports that the presence of saponins, alkaloids, flavonoids, tannins etc. Two flavonoids pectolinarigenin and hispidulin were isolated from the different solvent fraction of stem and roots of this plant which exhibit antimicrobial activity against a wide range of organisms [5]. The juice of fresh leaves is prescribed by the folk practitioner, empty stomach in the morning for treating the worm infections and it is very effective [3].

*Alpinia galanga* which is commonly known as 'Mahabali Vacha' is a Zingiberaceous perennial herb, about 2m high with lower portion covered with smooth leaf sheaths. The leaves are broadly lanceolate, 30-60cm long and 10-15cm broad [1]. *Alpinia galanga* is especially useful in flatulence, dyspepsia, vomiting and sickness at stomach, rheumatism, catarrh and enteritis, being recommended as a remedy for sea-sickness and motion sickness. It tones up the tissues and is sometimes prescribed in fever. Homoeopaths use it as a stimulant [1,2,3]. Recently researches on this plant have proved that regular administration of powder or decoction of this rhizome increases sperm count and acts as an Aphrodisiac. It is used as a snuff to treat cold and flu symptoms. It also possesses tonic and antibacterial qualities and is used for these properties in veterinary and homeopathic medicine. In Ayurvedic medicine this plant is used in

Helminthiasis [3,4,5]. Recently many pharmacological studies have been conducted on *Alpinia galanga* which informs that the plant having potent antibacterial, antioxidant, anti-HIV, immunomodulator, hepatoprotective, anti-diabetic, anti-ulcer activity [5]. The active principles isolated from the different parts are the volatile oil and acrid resin been reported. The root contains a volatile oil, resin, galangol, kaempferid, galangin and alpinin, starch, etc [4].

The present study was aimed to investigation of the anthelmintic potential of crude MeOH extract and aqueous extract of the leaves of *Clerodendrum indicum* and *Alpinia galanga* in three different concentrations (25, 50, 100 mg/ml) on Indian earth-worm (*Pheretima posthuma*) taking Albendazole as a standard drug.

### MATERIALS & METHODS

#### Plant Materials

Fresh leaves of *Clerodendrum indicum* and *Alpinia galanga* 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 (C1003/RIPSAT/TU/2012) & (AG004/RIPSAT/TU/2012) was submitted for the future reference and experiment.

#### Drugs and Chemicals

Albendazole suspension [Zentel, GSK Pharmaceuticals Ltd. Bangalore], Methanol and CMC [Rankem] were used during the experimental protocol. All the chemicals used are laboratory and analytical grade.

#### Preparation of Plant Extract

The fresh leaves of *Clerodendrum indicum* & *Alpinia galanga* were dried in shade. The dried leaves were cut into small pieces and powdered in a hand mill. Then 300 gm each type of coarse leaf powder was extracted with 900 ml of extra pure MeOH successively in a Soxhlet extractor repeatedly for 48 hours. Both the extract was dried by solvent evaporation in a thermostat water bath at 45-60 °C temperature.

Another 300 gm of each type of coarse leaf powder was macerated with 900 ml of distilled water for 48 hours. After completion of 48 hours it was filtered to separate the water extract from the marc. The extract was concentrated in a thermostat water bath at 60-80 °C temperature and all the extracts were kept in dessicator for the experiment.

#### Preliminary Phyto-chemical Investigation [8]

The percentage yield of MeOH & 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

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 10-12 cm in length and 0.3-0.6 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 [9].

#### Experimental Design [10-14]

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 (CMC) suspension, 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 & DISCUSSION

Preliminary phytochemical screening of crude extract of the leaves of *Clerodendrum indicum* and *Alpinia galanga* leaves revealed the presence of different phytoconstituents which are reported in Table 1. The crude extracts of both the leaves produced a significant anthelmintic activity on *Pheretima posthuma* in a dose dependent manner as shown in Table 2 & Table 3.

**Table 1: Preliminary Phyto-chemical screening of *C. indicum* & *A. galanga* Leaves.**

S. No.	Pytochemical tests	<i>Clerodendrum indicum</i>		<i>Alpinia galanga</i>	
		MLE	ALE	MLE	ALE
1.	Alkaloids	+	-	-	-
2.	Glycosides	+	+	+	-
3.	Saponins	+	+	+	+
4.	Flavonoids	+	+	+	+
5.	Tannins	+	+	+	+
6.	Steroids	-	-	+	-
7.	Terpinoids	-	-	+	-

MLE- MeOH Leaf extract, ALE - Aqueous Leaf extract (+) - Present, (-) - Absent

**Table 2: Anthelmintic activity of *Clerodendrum indicum* leaves.**

Groups	Concentration (mg/ml)	Time (min) Paralysis	Death
Control	-	-	-
MeOH Leaf extract (MLE)	25	16.33 ± 0.32	34.26 ± 0.44
	50	09.62 ± 0.25	22.62 ± 0.28
	100	04.16 ± 0.12	13.71 ± 0.21
Aqueous Leaf extract (ALE)	25	36.55 ± 0.15	140.11 ± 0.23
	50	25.62 ± 0.11	129.29 ± 0.18
	100	19.16 ± 0.22	87.71 ± 0.27
Standard (Albendazole)	25	05.43 ± 0.22	24.13 ± 0.28
	50	03.22 ± 0.16	16.93 ± 0.58
	100	02.45 ± 0.21	09.83 ± 0.47

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

**Table 3: Anthelmintic activity of *Alpinia galanga* leaves.**

Groups	Concentration (mg/ml)	Time (min) Paralysis	Death
Control	-	-	-
MeOH Leaf extract (MLE)	25	36.05 ± 0.13	161.23 ± 0.24
	50	25.62 ± 0.55	103.62 ± 0.17
	100	14.16 ± 0.29	61.71 ± 0.11
Aqueous Leaf extract (ALE)	25	67.11 ± 0.17	236.33 ± 0.29
	50	40.15 ± 0.17	188.22 ± 0.37
	100	29.33 ± 0.09	131.13 ± 0.18
Standard (Albendazole)	25	05.43 ± 0.22	24.13 ± 0.28
	50	03.22 ± 0.16	16.93 ± 0.58
	100	02.45 ± 0.21	09.83 ± 0.47

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

The percentage yield of *Clerodendrum indicum* leaves MeOH & aqueous extract was obtained 13.11 & 3% w/w respectively and 10.5 & 2.7% for *Alpinia galanga* leaves. Alcoholic extract exhibited more phyto-constituents than aqueous extract in both the cases. Preliminary phyto-chemical screenings confirms that the presence of saponins, alkaloids, flavonoids, tannins etc. in *Clerodendrum indicum* leaves and saponins, steroids, flavonoids, tannins in *Alpinia galanga* leaves. The peak anthelmintic activity exhibited by the MeOH extract at highest concentration (100 mg/ml) which includes 04.16 ± 0.12 min. for paralysis and 13.71 ± 0.21 min. for death of worms in case of *Clerodendrum* leaves as well as 14.16 ± 0.29 min. for paralysis and 61.71 ± 0.11 min. in case of *Alpinia* leaves. Aqueous extract exhibit less activity in comparison to alcoholic extract at all the tested doses. Alcoholic extract exhibit good vermifuge activity whereas aqueous extract exhibits significant vermifuge activity at highest concentration, which was 19.16 ± 0.22 min and 29.33 ± 0.09 min. respectively for *Clerodendrum indicum* & *Alpinia galanga* leaves. Potency of the extract was inversely proportional to the time for paralysis (vermifuge) and death (vermicidal) of the worms.

#### CONCLUSION

Finally it can concluded that, both *Clerodendrum indicum* & *Alpinia galanga* leaf extracts has showed significant anthelmintic activity at all the tested doses when compared to control as vermifuge and vermicidal which assures the ethno-medicinal claim and it is effective against helminthiasis.

#### ACKNOWLEDGEMENT

A special thanks to renowned taxonomist Prof. B. K. Datta (Dept. of Botany, Tripura University; A Central University) for identification and authentication of the plant material and Adarini Vermi Fertilizer & Research Centre, Agartala, Tripura for supplying the earth worms.

#### REFERENCES

1. Kirtikar KR, Basu BD, Indian Medical plants. 2nd ed. Dehradun, International Book Publication Distribution, 1975.
2. Chopra RN, Chopra IC, Handa KL and Kapur LD: Indigenous Drugs of India, Calcutta, UN Dhar & Sons Ltd. 1950.
3. Bhattacharjee AS. Chiranjivi Vanawsadhi. Calcutta-9, Ananda Publishers Private Ltd., 1982.
4. Pal A, Zobaer AM, Akter N, Islam MS, Bachar SC. Evaluation of Antinociceptive, Antidiarrheal and Antimicrobial Activities of Leaf Extracts of *Clerodendrum indicum*. Pharmacognosy Journal 2012; 4(30):41-46.
5. Rahman MAA, Azam ATM, Gafur MA. In vitro antibacterial principles of extracts and two flavonoids from *Clerodendrum indicum* Linn. Pakistan J Biol Sci. 2000; 3(10): 1769-1771.
6. Chudiwal AK, Jain DP, and Somani RS. *Alpinia galanga* Willd. – An overview on phytopharmacological properties. Indian Journal of Natural product and Resources 2010; 1(2): 143-149.
7. Verma RK, Mishra G, Singh P, Jha KK, Khosa RL. *Alpinia galanga* – An Important Medicinal Plant: A review. Der Pharmacia Sinica, 2011; 2 (1): 142-154.
8. Kokate CK, Purohit AP, Gokhale SB: Textbook of Pharmacognosy. 41st ed. Pune; Nirali Prakashani, 2008.
9. Chatterjee KD. Parasitology, Protozoology and Helminthology. Calcutta: Guha Ray Sree Saraswati Pres Ltd.; 1967. p. 168-169.
10. Ajaiyeoba EO, Onocha PA and Olarenwaju OT. In vitro anthelmintic properties of *Buchholzia coriacea* and *Gynandropsis gynandra* extract. Pharm Bio. 2001; 39: 217- 220.
11. Kumari S, Jain S, Pal R, Nain S and Paliwal S: Pharmacognostical, Phytochemical and Pharmacological Investigation on Leaves of *Tridax procumbens* Linn. International Journal of Pharmaceutical Science & Research, 2013; 4(2): 792-795.
12. Kumar T, Alexander A, Dewangan D, Nagori K: Anthelmintic Activity of the Whole Plant of *Bauhinia purpurea* (Linn.). Asian J Pharm Clin Res 2011; 4(3): 110-111.
13. Chandan HS, Tapas AR, Sakarkar DM: Anthelmintic activity of extracts of *Coriandrum sativum* Linn. in Indian earthworm. International Journal of Phytomedicine 2011; 3: 36-40.
14. Nayak BS, Jena PK, Sahu NP, Nayak UK and Patro KB: Comparative study of Anthelmintic activity between Aqueous and Ethanolic Extract of *Solanum surattense* Linn. International Journal of Pharmacy and Pharmaceutical Sciences 2009; 1(1):103-107.