International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 5(1): January-February 2015

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences **Research Article……!!!**

Received: 11-01-2015; Revised: 24-01-2015; Accepted: 25-01-2015

IN-VITRO ANTHELMINTIC ACTIVITY OF *CALOTROPIS GIGANTEA* AGAINST INDIAN EARTH WORM *PHERETIMA POSTHUMA*

Sujata D. Dongare*, Sachin S. Mali, Pooja P. Dhanawade, Savita S. Mali, Prasad V. Patrekar

Department of Pharmaceutics, Adarsh Institute of Pharmacy, Vita, Maharashtra, India

Keywords:

Calotropis gigantea, Soxhlet extraction, Indian earthworms, Anthelmintic activity etc.

For Correspondence:

Sujata D. Dongare

Department of Pharmaceutics, Adarsh Institute of Pharmacy, Vita, Maharashtra, India

E-mail:

sujata_pore@rediffmail.com

ABSTRACT

Helminthes is debilitating disease and it has features such as subcutaneous nodules, conjunctivitis, retinitis, blindness, dysentery, urticaria, diarrhea, cough, wheeze, fever and abdominal pain. Now a day's various allopathic medicines have anthelmintic property but they produce resistance and very serious side effects. So peoples in the world increasing turning towards indigenous system of medicine which mainly derived from plant because of their safety, economy and easy availability. But however use of these herbal drugs demand correct identification and characterization, so primary object of this work is to study morphological, microscopic characters and identification of phyto constituents of *Calotropis gignata*. In these work we screen Calotropis gignatea for anthelmintic activity against Indian earth worm *Pheretima posthuma*. *Pheretima posthuma* has anatomical resemble with intestinal Parasite (ascaris) .In this work Methanolic extract was collected by soxhlet extraction method and compared with standard Albendazole and result shows that *Calotropis gigantea* has equivalent anthelmintic activity as albendazole.

INTRODUCTION

Ayurveda is science of longevity (long life) it contains list of various medicinal plants and their therapeutic uses. *Calotropis gigantea* is an important drug of ayurveda. Arka is ancient name of the plant which occurs in the Vedic literature, in the form of leaves, which was used in the sacrificial rat.[14] There are two common species of Calotropis viz. *Calotropis gigantea* (Linn.) R. Br. and *Calotropis procera* (Ait.) R.Br described by the Sanskrit writers. *Calotropis gigantea*n (Linn.) called as crown flower, *Calotropis procera* (Ait.) called as Sodom apple[15]. *C. gigantea* is a common wasteland weed and commonly known as giant milkweed. This plant is a native of Bangladesh, Burma, China, India, Indonesia, Malaysia, Pakistan, Philippines, Thailand and Sri Lanka. C. gigantean is frequently available in India and used for several medication purposes in traditional medicinal system. Most recently C. Gigantea is scientifically reported for several medicinal properties. The flowers are reported to possess analgesic, antimicrobial and cytotoxic activity. Leaves and areal parts of the plant are reported for anti-diarrhoeal, anti-Candida, antibacterial activity and antioxidant activity. Roots are reported to contain anti-pyretic activity and cytotoxic activity [1]. But in previous literature no reports were found for its anthelmintic activity so recent study was carried out for its anthelmintic property[14].

MATERIAL AND METHOD

Collection of leaves

Leaves of *Calotropis gigantea* were collected from college garden. Plant material was taxonomically identified in Department of Botany,Shivaji University, Kolhapur, India (M.S.).

Macroscopy

Morphological examination of leaf reveals following interpretations. *Calotropis gigantea* is weed plant having 6m height, after cutting all parts excrete white milkey exudition. Leaves are subsessile, ovate or elliptical in shape,10-15cm long & 4.5to6.5cm broad, pubescent & glabrous on maturity. Bark is yellowish-brown, thick, rough, corky containing fer like hairs. Flowers are small triangular, dirty white color, contain 5 sepals, 5 thick ovate petals, white at base & purple at top, contain 5 stamens & 5 lobed stigma. They have faint odour and present as cluster. Fruits of this plant are green, spongy and ovoid in nature. About 15x10 cm in size. Seeds are papery & light brown in color. Roots are whitish, grey in color, wrinkled in fresh condition[14].

Figure No.1. Leaves of *Calotropis gigantea*

MICROSCOPY

Microscopical study of leaf showed the following characters[1]:- When Transverse section of leaf passing through midrib was taken it shows single layer of upper and lower epidermis covered with cuticle. It also contains ground tissue having intercellular spaces, setel composed of bicolateral and open vascular bundles. Xylem contains vessel and trachieds, cambium strip present between xylem and phloem tissue. Palisade mesophyll present at lamina contain 3 rows of irregular closely arranged central cells which contain scattered vascular bundles, palisade cells differentiate between palisade spongy tissue which are radialy elongated cells with intracellular spaces.

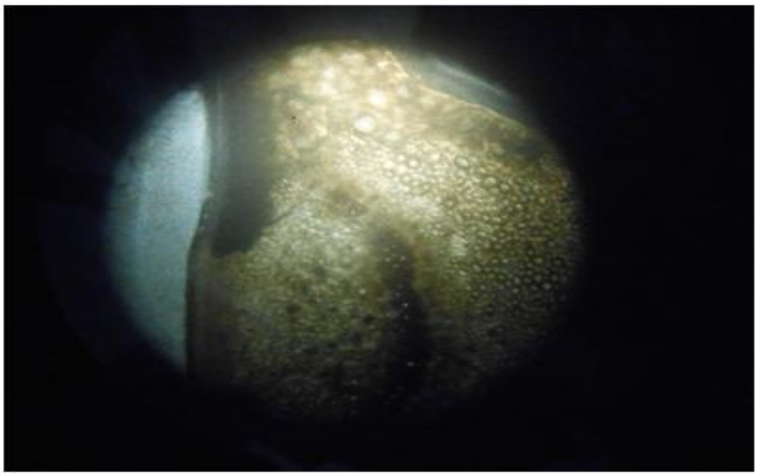

Figure No.2. TS of *Calotropis gigantea*

EXPERIMENTAL STUDY

Preparation of extract

Extract was prepared by soxhlet extraction method. Then filtered, evaporated to dryness and finally dried in electronic water bath [10,6 11].

Figure No.3. Soxhlet extraction apparatus and Electronic water bath

Selection of Indian earthworms: - Earthworms required for experiment were collected from moist soil and washed with distilled water to clean the worms. Healthy earthworms were selected and two worms were placed in each Petri plates i.e. standard, control, test solution.**(7)**

Preparation of standard solution: (7)

Albendazole (20mg/ml) was prepared using 2% v/v tween 80 as suspending agent.

Preparation of extract for activity: (7)

The suspension of Methanolic extract of leaves of *Calotropis gigantea* of different concentration (10,15,20mg/ml) were prepared using 2% v/v of tween 80 as suspending agent and final volume was made up to 10 ml of normal saline for respective concentration. The practical yield of methanolic extraction was found to be 31.5%.

Procedure for anthelmintic activity: (7)

Group of two equal sized worms released into 10 ml of standard drug and extract solution in Petri dish and was observed for paralyzing and death time. Time for paralysis was noted when no movement of any sort, except when the worm was shaken vigorously. Then death time was recorded after ascertaining that worm's did not move neither when shaken nor when given external stimuli. The fading away of body colour were noted. Hemorrhagic and Necrotic spots were observed **(9).** The test result was compared with standard Albendazole.

Phytochemical investigation

Phytochemical studies on *Calotropis gigantea* have afforded several types of compounds such as Cardenolide, triterpinoids, alkaloids, resins, anthocyanins, and proteolytic enzymes in latex, flavonoids, tannins, sterol, saponins, cardiac glycosides. Flowers contain terpenes, multiflorenol, and cyclisadol. [14] and it was confirmed by performing various qualitative tests for these phytoconstituents[11]

Table No 1. Anthelmintic potency of methanolic extract of *Calotropis gigantea* leaves

Sample	Concentration (mg/ml)	*Pheretima posthuma*	
		Paralise time	**Death time**
Methanol	10	20±0.51	25±1.45
	15	14±0.70	22±o.76
	20	11±0.49	16±1.54
Standard	10	18±2.22	22±0.69
	15	12±0.98	20±1.32
	20	10±0.34	15±o.79
Control	-	-	-

Figure No.4. Photographic representation of anthelmintic activity of *Calotropis gigantea* by soxhlet extraction (methanolic extract) a) Test solution (Plant Extract 20mg/ml), b) Standard solution (Albendazole 20 mg/ml), c) Control (Normal saline)

RESULT AND DISSCUSION

Morphological characterization and microscopic study essential for identification and proper authentication of drug and also helpful to avoid adulteration[1,2]. Phytochemical screening reveals that *Calotropis gigantea* leaves contains secondary metabolites like Volatile oil, Alkaloids, carbohydrate, Steroids, Glycoside, Tannins, resin, saponins, flavonoids. *Calotropis gigantea* contains major chemical constituent amyrin, amyrin acetate (alkaloids)[14] which incorporate anthelmintic activity to extract. Alkaloidal 'N' atom helps to enhance penetration of active constituents in earthworm. Hence from results it clear that methanolic extract has an equivalent activity as Albendazole. Earthworms in both solutions died within 16 min.

CONCLUSION

From the result it is clear that methanolic extract of *Calotropis gigantea* leaves has prominent anthelmintic activity against earthworm. The activity was found to be inversely proportional to concentration of extracts. Indian system has great history for Ayurveda and researchers can focus on development herbal formulation.

ACKNOWLEDGEMENT

Authors would like to thankful to our principal, Adarsh Institute of Pharmacy, for providing Research facilities, also we are thankful to Miss. Bhagyashree Kumbhar for her co-operation.

REFERENCES

1. C.K. Kokate, A.P. Purohit, S.B. Gokhale. Pharmacognosy; Nirali Publication, 45th edition 3.83-3.84.
2. Vinod D. Rangari. Pharmacognosy and phytochemistry, part II, Career publication, Ist edition, 181.
3. S.S. Handa and V.K. Kapoor. Text book of Pharmacognosy ,Vallabh prakasion ,IInd revised and enlarge Edition ,165-66.
4. Momhad Ali. Text book of Pharmacognosy, CBS publisher, IInd edition, 355-56.
5. J.S. Qadry. Pharmacognosy, B.S.Shah Publication, 12th edition, 384-85.
6. W.C. Evans. Trease and Evans Pharmacognosy, saunders Elsevior Science, 15th edition 52,471.
7. Sangh Partap, Saurabh Kumar, Amit Kumar. *In-vitro* Anthelmintic Activity of *Luffa cylindrica* Leaves in Indian Adult Earthworm, Journal of pharmacognosy and phytochemistry, ISSN No. 2278-4136,vol1 No.2 .2012 ,27-30.
8. Kamarapu Pallavi, Momula Swathi, Thati Swetha. Anthelmintic Activity of *Clitoria Ternatea* against *Pheretima posthuma*, Indo American Journal of Pharmaceutical Research, Issn No. 2231-6876 ,3935-3939.
9. V Rajesh, P.Perumal, V. Chinthakindhi, S.Prabhakarng,G.Hymavathi, T.Guntupalli. *In-vitro* Evaluation of *Smilax zeylanica* Linn. Leaves for Anthelmintic Activity, Internet Journal of Pharmacology 200,9 Vol.9, No.1, 1/11-11/11.
10. Mohini Upadhye, Parikshit Gandhi, Mohini Phanse. Pharmacognostical, Phytochemical Studies and Comparative Extraction Techniques of *Daucus carota*, Research Journal of Pharmacognosy and Phytochemistry, 6(3), July- September,2014, 115-117.
11. Hammuel C.Anyim B.P. Raplong H.H. ,Inekwe U.V., Batari M.L., Phytochemical Screening and In-Vitro Antibacterial Activity Of Methanol and Aqueous Extract of *Acaiypha racemisa* Leaves, Research Journal of Pharmacognosy and Phytochemistry ISSN 0975-2331, 6(3), July- September,2014,118-121.
12. Arabind Kumar,Vipink Garg,. Pharmacognostic Study and Establishment of Quality of Leaves of *Adhatoda vasica*. Journal of Medicinal Plant Studies ISSN 2320-3862 Vol1, Issue 3.

13. Santosh Kumar Singh, Jay Rampate. Pharmacognostic Study and. Phytochemical Screening *Adhatoda vasica*. ISSN 2320-3862 Vol1, Issue 3.
14. S.Sarkar et.al., *Calotropis gigantea* linn. A complete basket of Indian Traditional Medicine. Int. J.Pharm.Res. Sci.,2014,02(1), 7-17
15. B.Premkumar,et.al., medicinal plants for inflammatory Arthritis Research journal of Pharmacognosy and Phytochemistry.ISSn-0975-23316(2).April-June 2014, 51-65.