

EVALUATION OF ANTHALMATIC ACTIVITY OF *PHYLLANTHUS AMARUS* LEAVES EXTRACT IN EARTHWORMS (*PHERETIMA POSTHUMA*)

Divya G. Thite^{1*}, Yojana A. Kunjir², Tejal B. Korde³, Pragti S. Dukare⁴, Abhilasha T. Temgire⁵ and Priyanka R. Khemnar⁶

¹Assistant Professor of Sinhgad College of Pharmacy, Pune.

²Student of Pravara Rural College of Pharmacy, Pravaranagar Tal-Rahata, Dist-Ahmednagar.

*Corresponding Author: Divya G. Thite

Assistant Professor of Sinhgad College of Pharmacy, Pune.

Article Received on 16/05/2022

Article Revised on 06/06/2022

Article Accepted on 26/06/2022

ABSTRACT

In this paper, the anthelmintic activity of Evaluation Of Anthelmintic Activity Of *phyllanthus amarus* Extract In Earthworms (*Pheretima Posthuma*) leaves extract is evaluated. Albendazole, methanol, chloroform and water were used during experimental protocol. Extraction of plant leaves was carried out by using Soxhlet apparatus and aqueous extraction is done by using maceration technique. The anthelmintic activity was evaluated using Indian adult Earthworms (*Pheretima posthuma*). The result obtained from experimental data, showed significant anthelmintic activity against Earthworms. Screening of plant extract was performed for complete death and paralysis of worms. The result shows a dose dependent increase in activity of the extract at 5, 10 and 20 mg/ml concentration. Results were comparable along the standard drug albendazole, which indicates that the *phyllanthus amarus* leaf shows the potent anthelmintic activity and has good action on worms, as compared with standard drug.

KEYWORDS: Anthelmintic activity, *phyllanthus amarus*, *Pheretima posthuma*, albendazole.

INTRODUCTION

Helminthiasis is recognized as a major problem to livestock's throughout the tropics. Helminth infections are one of the most prevalent diseases in developing and developed countries. Globally, an estimated 2 billion people are infected by intestinal nematodes. Most diseases caused by helminthes are of a chronic and debilitating in nature, they probably cause more morbidity and greater economic and social deprivation among humans and animals than any other single group of parasites. Helminth infections causes diseases such as ascariasis, hookworm infection and schistosomiasis constitutes the bulk of the 13 diseases classified as neglected tropical diseases (NTDs) by the WHO. Estimated that the global burden of helminth infections, in terms of disability-adjusted life years (DALYs), is 39 million life years which was comparable to that of tuberculosis (34.7 million DALYs) or malaria (46.5 million DALYs), the two major human infectious diseases associated with a high mortality rate. Factors that sustain the parasite life cycles and favour the proliferation of the disease vectors include poor sanitation, poverty, unsafe water, malnutrition and ignorance. Children, especially those at a preschool age less than five. years), have been identified as the most vulnerable group with very high rates of infection. Due to the asymptomatic nature of these diseases, the helminths remain undetected and children born in an endemic region may harbor the worms for the most part

of their lives. The manifestation of most parasitic diseases is due to the host responses to the presence of the parasite. Also worth considering is the fact that the immune response triggered by helminth infection may drain the body's ability to fight other diseases, making affected individuals more prone to co-infection.

MATERIALS AND METHODS**Collection and Authentication of plant**

Fresh leaves of *phyllanthus amarus* collected from Ayurvedic Chikistalay Nasrapur, pune.

Preparation of plant extract

Fresh leaves of *phyllanthus amarus* were dried under shade. Leaves were cut into small pieces and powdered in a hand mill. The extract was prepared by using 2 methods:-

- 1] Soxhlet extraction method
- 2] Maceration Method.

1] SOXHLET METHOD

In this method 30gm of powder is extracted with 100ml methanol as well as chloroform successively in a Soxhlet extractor repeatedly for 3 hours. Extract was dried by solvent evaporation in a thermostat water bath.

2] MACERATION METHOD

In this method 50gm course powder is kept in 200 ml water for 72 hours. It is filtered to separate water extract

from the marc. Extract was concentrated in a thermostat water bath at 70°C -80°C.

Drugs and chemicals

Albendazole, methanol, chloroform and water were used during experimental protocol. All chemicals used are laboratory and analytical grade.

Selection of earthworms

Indian adult earthworms (*Pheretima posthuma*) were used to carry out the experiment. Earthworms were

collected from the local suppliers. Worms were washed with saline to remove all fecal matter. The earthworms are 5-7cm in length and 0.3-0.5cm in width was used for experimental protocol. Ready availability, anatomical and physiological resemblance of *Pheretima posthuma* made it to be used initially for in-vitro evaluation of anthelmintic activity.



Indian adult earthworms (*Pheretima posthuma*)

Anthelmintic activity

Earthworm in the petridish was studied for paralysis/death as an end point of the study. The experiment was done using Indian earthworms (*Pheretima posthuma*), adult type due to their anatomical resembles with ascaris lubricoids. Aqueous extract is prepared using standard method. Albendazole was used as comparator drug. The 20 mg/ml concentration was prepared by as per the approved method. The suspension of aqueous extract of leaves of *phyllanthus amarus* concentration 5, 10, 20 mg/ml was prepared and final volume was made up to 10 ml for relevant concentration. Each type of dried extract was suspended in 1% w/v Carboxy Methyl Cellulose, prepared in normal saline water in different conc. (5, 10, 20 mg/ml). Albendazole suspension of same concentration was taken as standard and normal saline water was taken as a control. Groups of equal size worms consisting of earthworms individually in each group were released into in each 10ml of desired concentration of drug and extracts in the petridish. The anthelmintic activity was performed according to standard screening methods. Three Indian adult earth worms were placed in petridish containing 10 ml contained 20 mg/ml of aqueous extract of leaves of *phyllanthus amarus* to paralysis/death was recorded and expressed in minutes. The test results were compared with Albendazole (20 mg/ml). The procedure continued for 3 times to verify the results.

All the earthworms were washed in normal saline solution before they were released into 10ml respective formulation

Group I: Control group: Vehicle (0.05% CMC in normal saline)

Group II: Standard group: Albendazole (20mg/ml)

Group IIIa: Methanolic extract solution (5mg/ml)

Group IIIb: Methanolic extract solution (10mg/ml)

Group IIIc: Methanolic extract solution (15mg/ml)

Group IVa: Chloroform extract solution (5mg/ml)

Group IVb: Chloroform extract solution (10mg/ml)

Group IVc: Chloroform extract solution (15mg/ml)

Group Va: Aqueous extract solution (5mg/ml)

Group Vb: Aqueous extract solution (10mg/ml)

Group Vc: Aqueous extract solution (15mg/ml)

Observation

For the evaluation of anthelmintic activity of *phyllanthus amarus* following observations were made:

CONTROL

Figure 2. Group I: Normal saline solution.

STANDARD

Figure 3. Group II: Standard solution: Albendazole (20mg/ml).

IN METHANOLIC EXTRACT

Group IIIa: Methanolic extract solution (5mg/ml).



Group IIIb: Methanolic extract solution (10mg/ml).



Group IIIc: Methanolic extract solution (15mg/ml).

IN CHLOROFORM EXTRACT



Group IVa: Chloroform extract solution (5mg/ml).



Group IVb: Chloroform extract solution (10mg/ml).



Group IVc: Chloroform extract solution (15mg/ml).

IN AQUEOUS EXTRACT**Group Va: Aqueous extract solution (5mg/ml).****Group Vb: Aqueous extract solution (10mg/ml).****Group Vc: Aqueous extract solution (15mg/ml).****RESULT**

SR. NO.	GROUPS	CONCENTRATION(mg/ml)	TIME(MIN)FOR PARALYSIS	TIME(MIN)FOR DEATH
1.	Control (Normal Saline Water)	-	-	-
2.	Standard Albendazole	10	52	74
3.	Ethanol Extract	5	80	117
		10	50	80
		20	25	42
4.	Chloroform Extract	5	91	124

		10	74	110
		20	40	62
5.	Aqueous Extract	5	84	103
		10	68	94
		20	37	58

DISCUSSION

Soil-transmitted helminth infestation is widespread in rural India. *Colocasia esculenta* leaves are easily available in the local area, therefore this can be tried as an alternative remedy to already existing standard anthelmintic drugs. A number of medicinal plants have been used to treat parasitic infections in humans and animals. Anthelmintic derived from plant source can be an answer to this STH problem as they form secure and non-toxic with a modified site of action. Leaves of *phyllanthus amarus* showed marked anthelmintic activity.

CONCLUSION

Alcoholic extract *phyllanthus amarus* leaves showed noteworthy anthelmintic activity as compared to normal saline but less potent than albendazole. Additional large studies are warranted using in vivo models to prove *phyllanthus amarus* leaves as a better anthelmintic substance.

REFERENCES

1. WHO I Soil-transmitted helminths. WHO; [accessed 22 November 2012], 2012b. Available at: http://www.who.int/intestinal_worms/en/ [Ref list]
2. Curtale F, Pezzotti P, Saad YS, Aloï A. An analysis of individual, household, and environmental risk factors for intestinal helminth infection among children in Qena Governorate, Upper Egypt. *J Trop Pediatr*, 1999; 45: 14-7. [PubMed]
3. Piyushjain, Seema Singh, Sandeep K. Singh, S.K. Verma, M.D. Kharya, Sanjeev Solanki, Anthelmintic Potential of Herbal Drugs, *International Journal of Research and Development in Pharmacy and Life Sciences*, 2013; 412-419.
4. Rupa Muzumder, Souradeep Bhattacharya, Shaili Kumari, Avijit Mazumder, Pharmacognostical and Phytochemical Evaluation of the leaves of *phyllanthus amarus* Linn, *Ancient Science of life*, 2014; 30(2): 28-32.
5. Gupta Daksha, Chandrashekar, Richard Lobo and Gupta Nilesh, Anthelmintic Activity of Stem Bark of *Hauhinia purpurea* Linn, *Der Pharmacia Lettre*, 2012; 4(2): 662-664.
6. Krishnaveni Marimuthu, Ravi Dhanalakshmi, A study on Phytochemicals in *C. esculenta* l. leaf and flower, *International Journal of Pharmaceutical Science Review and Research*, 2014; 29(2): 72-76.
7. Marimuthu Krishnaveni, Phytochemical study of *C. esculenta* Stem, *Research Journal of Pharmacy and Technology*, 2015; 8(2): 1554-1558.
8. M.S. Akhtar, Zafar Iqbal, M.N. Khan, Muhammad Lateef, Anthelmintic Activity of Medicinal Plants with particular reference to their use in animals in the Indo-Pakistan subcontinent, *Small Ruminant Research*, 2000; 38: 99-107.
9. Ravindra G Mali, Anita Mehta, A Review on Anthelmintic Plants, *Natural Product Radiance*, 2008; 7(5): 466-475.
10. Prakash Pandey, Ashish Garg, Vishal Singh, Ajay shukla, Evaluation of Anthelmintic and Antimicrobial activity of Ursolic acid obtained from Tulsi, *Asian Journal of Pharmacy and Pharmacology*, 2016; 2(3): 67-71.
11. Tekeshwar Kumar, Amit Alexander, Dhansay Dewangan, Kushagra Nagori, Anthelmintic Activity of the whole plant of *phyllanthus amarus*, *Asian Journal of Pharmaceutical and Clinical Research*, 2011; 4(3): 110-111.
12. T. Kumar and K S Chandrashekar, *colocasia esculenta* Linn: A Review of its Ethnobotany, Phytochemical and Pharmacological Profile, 2011; 5(4): 420-431.
13. Annegowda H V, Mohd Nizam Mordi, S. Ramanathan, M.R. Hamdan, S.M. Mansor, Effect of Extraction Techniques on Phenolic Content, Antioxidant and antimicrobial Activity of *B. Purpurea*, *Food Analytical Methods*, 2012; 2-9.
14. M. Sugumaran and T. Vetrichelvan, Studies on some Pharmacognostic Profiles of *C. esculenta* Linn. Leaves, *Ethnobotanical leaflets*, 2008; 12: 461-468.
15. O. U. Igwe and I. E. Okeke, Leaf and Flower extracts of *Piliostigma Malabaricum*: Phytochemistry and Antibacterial applications, *International journal of applied chemical science*, 2017; 8(3): 89-94.
16. T.K. Lim, *C. esculenta* Edible Medicinal and Non-medicinal plants, 2013; 743-753.
17. S. Pathwa, R. Muzumder, S. Bhattacharya, S. Kumari, A. Mazumder, D.P. Singh, Pharmacognostical and Phytochemical Evaluation of the leaves of *colocasia esculenta* Linn, *Ancient Science of life*, 2010; 30(2): 28-32.
18. M. Sugumaran, T. Vetrichelvan, Studies on some Pharmacognostic

- Profiles of Bauhinia Purpurea Linn. Leaves, An international journal of Ethnobotanical Research, 2010, 2008(2008); 1: 461-468.
19. Mukherjee P K, Gopal T K, Subburaju T, Dhanbal S P, Duraiswami B, Elango and Suresh B, Studies on the anti-diarrhoeal profiles of colocasia esculenta Linn Leaves(Fam. Caesalpinaceae) extract, Natural Product Science, 1998; 4: 234-237.
 20. Pettit G R, Numata A, Iwamoto C, Usami Y, Yamada T, Ohishi H and Cragg G M, Antineoplastic agents. 551. Isolation and structures of bauhiniastatins of colocasia esculenta Journal of natural product sciences, 2006; 6: 323-327.
 21. Yadav R and Sodhis N, Phytochemical studies on Bauhinia racemosalam. Bauhiniapurpurea Linn, Europ Chemistry, 2007; 4: 123.