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Anthelmintic activity of leaves of *Alocasia indica* Linn.

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ABSTRACT: The study was designed to evaluate the anthelmintic activity of hydroalcoholic extract of leaves of *Alocasia indica* Linn. (Araceae) and its two different fractions namely petroleum ether and ethyl acetate using *Pheretima posthuma* as test worms. Different concentrations (10, 25 and 50 mg/ml) of hydroalcoholic extract and its various fractions were tested in the bioassay, from which time of paralysis (P) and time of death (D) of the worms were determined. Piperazine citrate was used as standard reference and distilled water as control. All the extracts were found not only to paralyze (Vermifuge) but also to kill the earthworms (Vermicidal). The hydroalcoholic extract was found to be more effective to execute the earthworm at higher concentration of 50 mg/ml, as compared to standard reference Piperazine citrate. Further, systematic scientific studies are essential for screening different activities.
Keywords: Anthelmintic, *Pheretima posthuma*, *Alocasia indica* Linn.

NTRODUCTION

The World Health Organization reveals that over two billion people are suffering from parasitic worm infections. Livestock and crops are also affected by parasitic worms also infect, affecting food production with a resultant economic impact. Parasitic diseases cause severe morbidity, including lymphatic filariasis (a cause of elephantiasis), onchocerciasis (river blindness), and schistosomiasis. These infections can influence most populations in endemic areas with major economic and social consequences. [1] Although the majority of infections due to worms are generally limited to tropical regions, they can occur to travelers who have visited those areas and some of them can develop in temperate climates. [2] Despite this prevalence of parasitic infections, the research on anthelmintic drug is poor.

Because of the increasing anthelmintic resistance and the impact of conventional anthelmintics on the environment, it is important to look for alternative strategies against gastrointestinal nematodes. Phytotherapy could be one of the major options to control these pathologies. Many Indian ethno botanic traditions propose a rich repertory of medicinal plants used by the population for treatment of parasitic worm infections. [3] Despite the steady increase in demand for herbal medicine over the past decade worldwide, a great majority of herbal products are not pharmacologically assessed for their quality, safety and efficacy. [4] Also there were not enough scientific investigations on the anthelmintic activities conferred to these plants. One of such plant from Indian flora is *Alocasia indica* Linn. Different parts of this plant are used in inflammation and in diseases of abdomen and spleen. [5] The juice of the leaves of the plant is used as digestive, laxative, diuretic, astringent and traditionally used for the treatment of rheumatic arthritis. It has antifungal properties. [6] This plant contains flavonoids, cynogenetic glycosides, ascorbic acid, gallic acid, mallic acid, oxalic acid, alocasin, amino acids, succinic acid, and β-lectines. [7] Since no scientific data are available to justify the traditional anthelmintic potential of the plant, present study was planned to validate the therapeutic use of this plant in treatment of parasitic worm infections.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Alocasia indica* Linn. family: Araceae were collected from different places at Karad Dist – Satara (Maharashtra). Authentication was done by Prof. S. K. Patil, a voucher specimen was deposited in the herbarium of the department of Botany, Yashwantrao Chavan College of Science, Karad Dist-

Satara (Maharashtra). The fresh leaves of *Alocasia indica* Linn.– [AI] were separated from plant, washed under running tap water and then with isopropyl alcohol (5%) followed by distilled water. Leaves were cut into small pieces and allowed it to shed dry (30 ^{o} C, 45 % relative humidity) for 15 days and then homogenized to get a coarse powder. This powder was stored in an air tight container and used for further successive extraction.

Preparation of Extract

Hydroalcoholic extract (by cold maceration method)

About 250 gm of the powder was extracted with hydroalcohol (ethanol- 95% and water in 1:1 proportion) at room temperature by cold maceration method. [8] The filtrate was collected and concentrated on heating mantle at 45˚c till a syrupy mass was obtained. Then the extract was again dried by using rotary evaporator under controlled condition of temperature and pressure. The extract thus obtained was preserved at – 4˚c. The percentage yield was found to be 7.56 g.

Solvent Extraction

The petroleum ether extracts and ethyl acetate extracts of leaves of *Alocasia indica* Linn. were prepared by soxhletion. The powdered plant material (250 g) was repeatedly extracted in a 1000 mL round bottomed flask with 500 mL solvents of increasing polarity starting with petroleum ether, ethyl acetate. The reflux time for each solvent was 40 cycles for complete extraction. The extracts were cooled at room temperature, filtered, and evaporated to dryness under reduced pressure in a rotary evaporator. [9] The percentage yield of ethyl acetate and petroleum ether was found to be 0.79 % and 0. 53 % respectively.

Animals

Indian adult earthworms-*Pheretima posthuma* (Annelida, Megascolecidae) were used to evaluate anthelmintic activity. The earthworms were collected from moist soil, washed with normal saline to remove all fecal matter and identified at Zoology Dept. Yashwantrao Chavan College of Science; Karad Dist-Satara (Maharashtra). The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all experimental protocol.

Drugs and Chemicals

Ethyl acetate, petroleum Ether used was of analytical grade and procured from Loba Chemicals Pvt. Ltd., Mumbai. Piperazine citrate was procured from G. Amphray Laboratories, India. The solvents and other chemicals used were of analytical grade.

Preparation of Test Sample

Samples for in-vitro study were prepared by dissolving and suspending 0.5 gms of each hydroalcoholic extract, its petroleum ether and ethyl acetate in 10 ml of distilled water to obtain a stock solution of 50 mg/ml. From this stock solution, different working dilutions were prepared to get concentration range of 10, 25 and 50 mg/ml.

Anthelmintic activity

The anthelmintic activity was evaluated using adult Indian earthworm due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. [10], [11], [12], [13] Earthworms are easily available and used as a suitable model for screening of anthelmintic drug was advocated earlier. [14], [15], [16], [17], [18], [19] Forty ml formulations containing 10, 25 and 50 mg/ml of hydroalcoholic extract and its various fractions were prepared and six worms (same type) were placed in above formulations and they were observed for their spontaneous motility and evoked responses. Time for paralysis was noted at different time intervals when no movement of any sort could be observed except the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50 ^{0}C (16,17). Piperazine citrate (10 mg/ml) was used as reference compound.

RESULTS

After a brief stimulant effect, earthworms lost their motility on exposure to crude extracts of leaves of *Alocasia indica* Linn. Each crude extract containing 10, 25 and 50 mg/ml, produced dose-dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death. As shown in fig. no. 1, hydroalcoholic extract of leaves of *Alocasia indica* Linn. and its different fractions exhibited anthelmintic activity in dose-dependant manner giving shortest time of paralysis (P) and death (D) with 50 mg/ml concentration. At 10 mg/ml concentration hydroalcoholic extract caused paralysis of 4.27 min. and time of death of 10.58 min. while ethyl acetate and petroleum ether (40-60 C) fractions caused paralysis of 6.28 and 5.63 min and time of death of 21.59 and 11.92 min. respectively as shown in fig no. 2 and 3. The reference drug Piperazine citrate showed the same at 19.26 and 63.25 minutes, respectively at concentration of 10 mg/ml. The higher concentrations of each crude extract produced paralytic effect much earlier and the time of death was shorter. Hemorrhagic and necrotic spots were observed externally on the worms, with the higher concentrations. The effect of each crude extract was compared with Piperazine citrate, which was found to produce Grade 3 paralyses within 90 min.

DISCUSSION

Parasitic worm infections of the gastrointestinal tract of human beings and animals have been renowned to

have adverse effects on health standards with a consequential lowering of resistance to other diseases. To evaluate compounds with anthelmintic activity, a number of substances were analyzed using different species of worms, for example, earthworms, *Ascaris*, *Nippostrongylus and* Heterakis. From all these species, earthworms have been used extensively for the preliminary evaluation of anthelmintic compounds *in vitro* because they are similar to intestinal "worms" in their reaction to anthelmintics and are easily accessible. It has been verified that all anthelmintics which are toxic to earthworms are creditable to study as an anthelmintic. [20] Earthworms have the ability to move by ciliary movement. The outer layer of the earthworm is a mucilaginous layer and composed of complex polysaccharides. This layer being slimy enables the earthworm to move freely. Any damage to the mucopolysaccharide membrane will expose the outer layer and this restricts its movement and can cause paralysis. This action may lead to the death of the worm by causing damage to the mucopolysaccharide layer. This causes irritation leading to paralysis.

Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyper polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis. [21] Preliminary Phytochemical analysis of extracts of leaves of *Alocasia indica* Linn. and its different fractions showed the presence of flavonoids, cynogenetic glycosides, ascorbic acid, hydrolysable tannins-gallic acid, mallic acid, oxalic acid, alocasin, amino acids, succinic acid, and β-lectines. [9] Tannins which are polyphenolic compounds were known to have anthelmintic activities. [22] [23] Reported anthelmintic effect of tannins is due to binding of tannins to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may be responsible for death. [24], [25] Also some flavonoids and other secondary metabolites known to have anthelmintic activities but exact mechanisms are not clearly established. [26] It is possible that tannins, flavonoids, cynogenetic glycosides present in the extracts of *Alocasia indica* Linn. produced similar anthelmintic effects. As variety of above secondary metabolites along with flavonoids, hydrolysable tannins-gallic acid are soluble in hydroalcoholic medium, hydroalcoholic extract of *Alocasia indica* Linn. not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 50 mg/ml, in shorter time as compared to reference drug Piperazine citrate and showed maximum efficacy. From study it can be concluded that hydroalcoholic extract of *Alocasia indica* Linn. possess anthelmintic activity. Further studies are in process to identify the possible phytoconstituents responsible for anthelmintic activity.

Figure no. 1: Graph of time for paralysis (PT) and for death (DT) of earthworms vs different concentrations of hydroalcoholic extract of AI and Piperazine citrate (PC, 10 mg/ml)

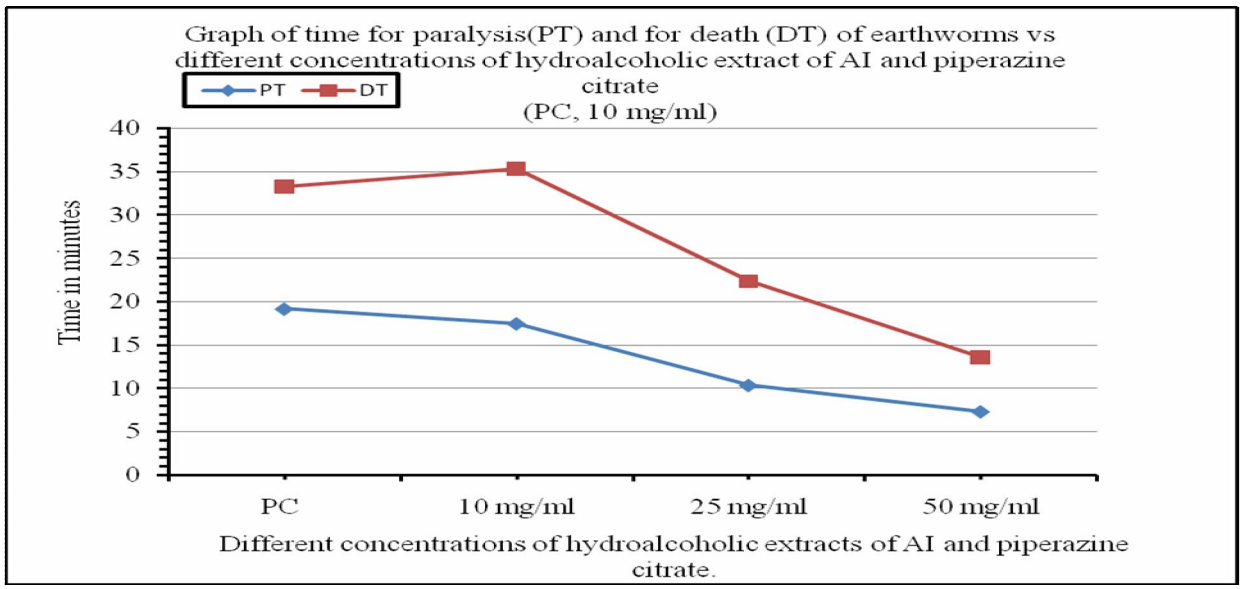


Figure no. 2: Graph of time for paralysis (PT) and for death (DT) of earthworms vs different concentrations of petroleum ether fraction of AI and Piperazine citrate (PC, 10 mg/ml)

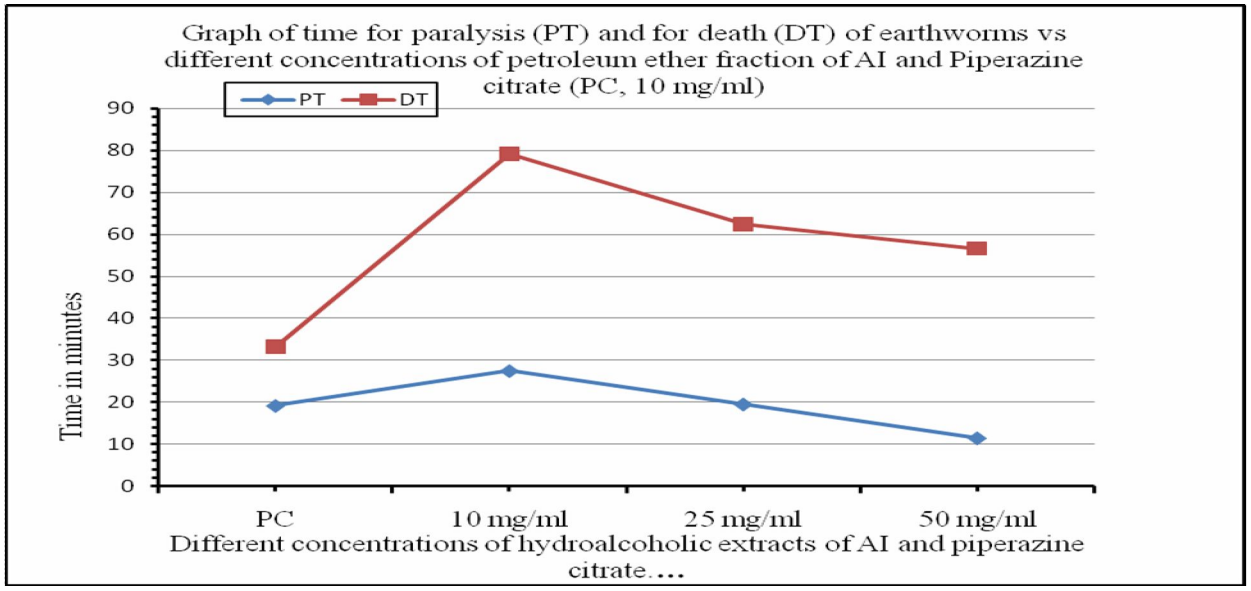
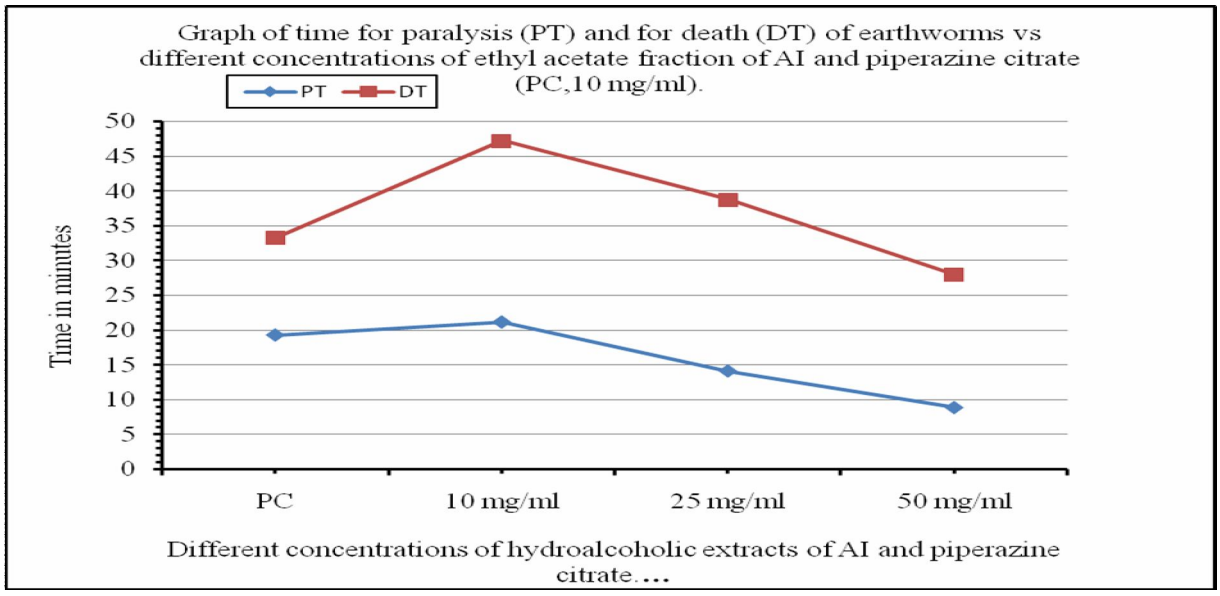


Figure no.3: Graph of time for paralysis (PT) and for death (DT) of earthworms vs different concentrations of ethyl acetate fraction of AI and Piperazine citrate (PC,10 mg/ml).



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REFERENCES

1. Tagboto S., Townson S., Antiparasitic properties of medicinal and other naturally occurring products, Adv Parasitol, 50, 2001, 199-295.
2. Bundy D.A., Immunoepidemiology of intestinal helminthic infection I: The global burden of intestinal nematode disease, Trans Royal Soc Trop Med Hyg, 8, 1994, 259-61.
3. Prakash V. and Mehrorta B. N., Anthelmintic plants in traditional remedies in India. Ind J of History of Science, 22, 1987, 332-340.
4. Kirtikar K.R., Basu B.D., Ind. Med. Plants, 2nd ed., M/s Bishen Singh Mahendrapal Singh, New Cannaught place, Dehradun, 1975, 2617-2618.
5. Nadkarni K.M., Indian Materia Medica, Popular Prakashan, Mumbai, 1996, 72-73.
6. Prajapati N.D., A Handbook of Medicinal plants, Agrobois Publication, India, 2003, 32.
7. Quality control methods for medicinal plants materials, World Health Organization, Geneva, 1998, 32.
8. Didry N., Duberwil L., Tratin F., Pinkas M., Antimicrobial activity of aerial arts of *Drossera pelitata* Smith on oral bacteria, J Ethnopharmcol, 60, 1998, 215-28.
9. Nirmal S.A., Malwadkar G., Laware R.B., Anthelmintic activity of *Pongamia glabra*, J Sci Technol, 29, 2007, 755.
10. Ajaiyeoba E.O., Onocha P.A. and Olarenwaju O.T., In-vitro anthelmintic properties of *Buchholzia coiaceae* and *Gynandropsis gynandra* extract, Pharm Biol, 39, 2001, 217-220.
11. Thorn G.W., Adams R.D., Braunwald E., Isselbacher K.J. and Petersdorf R.G., Harriasons Principles of Internal Medicine, McGraw Hill Co, New York, 1997, 1088 - 1089.
12. Vigar Z., Atlas of Medical Parasitology, P.G. Publishing House, Singapore, 1984, 216- 218.
13. Chatterjee K.D., Parasitology, Protozoology and Helminthology, Calcutta, Guha Ray Sree Saraswaty Press Ltd., Calcutta, 1967, 168-169.
14. Dash G.K., Mishra B., Panda A., Patro P. and Ganpaty S., Anthelmintic activity of *Evolvulus nummularis*, Ind J Nat Prod, 28, 2003, 19-24.
15. Tambe V.D., Nirmal S.A., Jadhav R.S., Ghogare P.B., Bhalke R.D., Girme A.S. and Bhamber RS, Anthelmintic activity of *Wedelia trilobata* leaves, Ind J Nat Prod, 22, 2006, 27-29.
16. Mali R.G., Mahajan S.G. and Mehta A.A., In-vitro anthelmintic activity of stem bark of *Mimusops elengi* Linn, Phcog Mag, 3, 2007, 73-76.
17. Dash G.K., Suresh P., Sahu S.K., Kar D.M., Ganpaty S. and Panda S.B., Evaluation of *Evolvulus alsinoids* Linn. for anthelmintic and antimicrobial activities, J Nat Rem, 2, 2002, 182- 85.
18. Szewezuk V.D., Mongelli E.R. and Pomillo A.B., Antiparasitic activity of *Meli azadirach* growing in Argentina, Mole Med Chem, 1, 2003, 54-57.
19. Shivkar Y.M., Kumar V.L., Anthelmintic activity of latex of *Calotropis procera*, Pharm Biol, 41, 2003, 263-265.
20. Sollmann T., Anthelmintics: Their efficiency as tested on earthworms, J Pharm Exp Ther 12, 1918, 129-133.
21. Martin R.J., Y-Aminobutyric acid and Piperazine activated single channel currents from *Ascaris suum* body muscle, Br J Pharmacol, 84, 1985, 445-461.
22. Bate-Smith E.C., The Phenolic constituents of plants and their taxonomic significance, Dicotyledons, J Linn Soc Bot, 58, 1962, 173-176.
23. Niezen J.H., Waghorn G.C., and Charleston W.A., Growth and gastrointestinal nematode parasitism in lambs grazing either Lucerne (*Medicago sativa*) or (*Hedysarum coronarium*), which contains condensed tannins, J Agri Sci, 1995, 125, 281-289.
24. Athnasiadou S., Kyriazakis F. and Jackson R.L., Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep, *In vivo* studies, Vet Parasitol, 99, 2001, 19-21.
25. Thompson D.P. and Geary T.G. The structure and function of helminth surfaces, In: Marr J J, editors, Biochemistry and Molecular Biology of Parasites, 1st ed., Academic Press, New York, 1995, 203-232.
26. Kerboeuf D., Riou M. and Guegnard F., Flavonoids and related compounds in parasitic disease control, vol. 8, Bentham Science Publishers, USA, 2008, 116-128.

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