

IN VITRO HELMINTHOLYTIC ACTIVITY OF LEAVES OF *CITRULLUS COLOCYNTHIS*

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ABSTRACT

Citrullus colocynthis(L) Schrad (Cucurbitaceae) is commonly known as "Indrayan or Bitter Apple" distributed throughout the tropics which It is used in the treatment of cough, ophthalmia, neuralgia, migraine bronchitis, abortifacient, cathartic, purgative and for the treatment of fever, cancer, amenorrhea, jaundice, leukemia, rheumatism and tumour. Authenticated plant leaves were taken for the study includes extraction of leaves using different solvents and evaluation of in vitro helmintholytic activity. Coarse dried leaves of the plant were extracted successively by Soxhlet using petroleum ether, ethanol and water as solvents according to their increasing polarity. Dried extracts were tested for helmintholytic activity using *Pheretima posthuma* as a species of earth worm and compare the paralysis time and death time with standard drug Albendazole. Ethanol (40mg/ml) and aqueous extract (40mg/ml) shows comparable anthelmintic Activity with standard drug Albendazole (40mg/ml).

Keywords: *Citrullus colocynthis*, helmintholytic activity, *Pheretima posthuma*, Albendazole.

INTRODUCTION

Citrullus colocynthis (L.) Schrad. (Cucurbitaceae), commonly known as 'bitter apple', 'colocynth', 'vine-of-Sodom', 'tumba' or 'wild gourd' is a tropical plant that grows abundantly in the south of Iran, and widely in other parts of the world¹. Monoecious root, Perennial Stem diffuse or creeping, slender, angled, branched, hirsute or scabrid. Tendrils are simple or 2-fid, slender, hairy. Leaves are very variable, 3.8-6.3 by 2.5-5cm in the wild form, usually deltoid in outline pale green above ashy beneath, scarbid on both surfaces. Leaves are 5-7 lobed or very commonly 3-lobed^{2, 3}. Helminthiasis is among the most important animal diseases inflicting heavy production losses. The disease is highly prevalent particularly in third world countries due to poor management Helminthiasis practices⁴. A number of medicinal plants have been used to treat parasitic infections in man and animals.⁵⁻⁷. The plants are known to provide a rich source of botanical helmintholytic. The ethnobotanical uses of this

plant include its use as an abortifacient, cathartic, purgative and vermifuge, and for the treatment of fever, cancer, amenorrhea, jaundice, leukemia, rheumatism and tumour^{8,9}. In Syria, it has also been used as an insect repellent. The Ayurvedic Pharmacopoeia of India indicated the use of the fruit in jaundice; the root in diseases of the liver and spleen and the leaf in cutaneous affections and alopecia^{10,11}. In the present study, anthelmintic potential of petroleum ether, ethanol, and aqueous extract of leaf have been evaluated by comparing with the standard drug albendazole.

MATERIALS AND METHODS

Plant material

Fresh Leaves of *C. colocynthis* were collected from Ahmednagar district of Maharashtra in September 2009 and authenticated by Mr. S.C. Majumdar, Botanical Survey of India, Pune, where a sample specimen (Voucher number: BSI/ 501) has been deposited.

Extraction

Dried and coarsely powdered Leaves of *C. colocynthis* were subjected to successive solvent extraction in Soxhlet extractor using petroleum ether, ethanol as solvent and the marc left was refluxed with water.

Phytochemical tests

The preliminary phytochemical tests revealed the petroleum ether, ethanol, and aqueous extract of the leaves shows the presence of alkaloids, steroids, flavonoids, glycosides.

Worms

Indian adult earthworms (*Pheretima Posthuma*) collected from moist soil and washed with normal saline to remove all faecal matter, were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.

Drugs and Chemicals

The following drugs and chemicals were used.

1. Drugs: Albendazole
2. Chemicals: Ethanol A.R., Petroleum ether, Saline solution, water

Pharmacological Study Helmintholytic Activity

All the extracts of *C. colocynthis* were dissolved in minimum amount of DMF and the volume was adjusted to 10 ml with saline water. All drugs and extract solutions were freshly prepared before starting the experiment. In each case, six earthworms were released into 10 ml of desired formulations as follows; vehicles (5% DMF in

normal saline), Albendazole (20 mg/ml), or extracts of leaf of *C. colocynthis* (40 mg/ml, each) in normal saline containing 5% DMF. Observations were made for the time taken to paralysis and death of individual worm.

Paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colors as our previous method¹²⁻¹⁴.

Statistical significance

The data was analyzed for statistical significance using t-test, one way ANOVA $P < 0.05$ was considered as statistically significant.

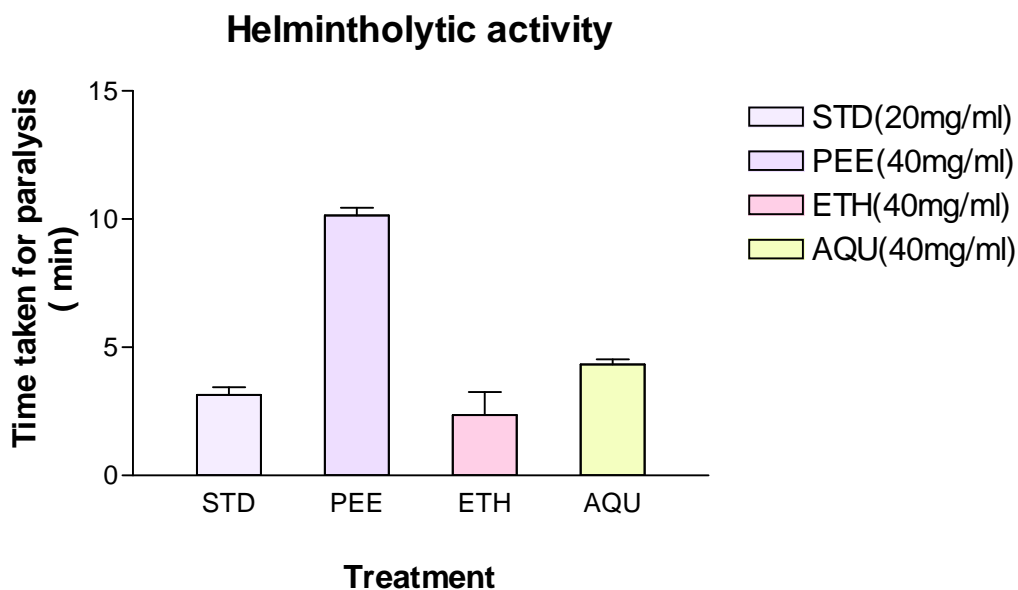
RESULT AND DISCUSSION

It is evident from the experimental data that, the ethanol and aqueous extracts of the *C. colocynthis* showed significant helmintholytic activity at 40 mg/ml. Results were comparable with the standard drugs, Albendazole, at concentration 20 mg/ml. Table 1 reveals that ethanol and aqueous extracts of leaf of *C. colocynthis* showed better helmintholytic activity. These extracts required the least time for causing paralysis and death of the earthworms followed by other extracts. As shown in Table 1 leaf of *C. colocynthis* displayed intrinsic helmintholytic properties with 40 mg/ml giving a shortest time of paralysis and death. The function of the anthelmintic drugs like Albendazole is to cause paralysis of worms so that they are expelled in the feces of man and animals. The extracts not only demonstrated this property, they also caused death of the worms, especially at 40 mg/ml as compared with the Albendazole.

Table 1: Effect of various extracts of leaves of *C.colocynthis* on *PheretimPosthuma*

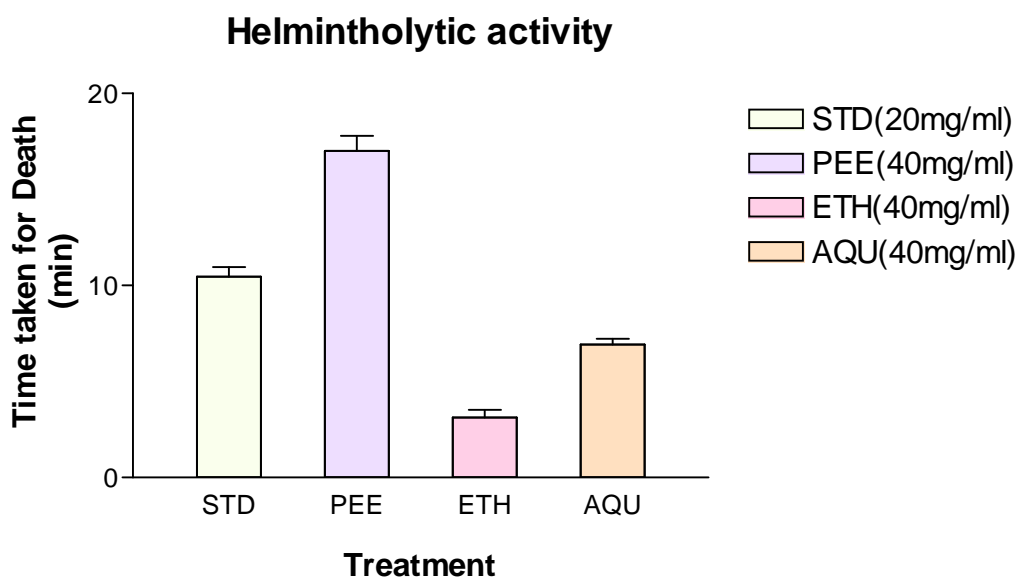
Group	Sample	* Time taken for paralysis (min)	* Time taken for Death (min)
I	Control	No paralysis (upto 25min)	No death (upto 25min)
II	Albendazole (20mg/ml)	3.14±0.3	10.46±0.5
III	PEE (40mg/ml)	10.14±0.3	17.00±0.8
IV	ETH (40mg/ml)	2.36±0.9	3.12±0.4
V	AQU (40mg/ml)	4.33 ±0.2	6.92±0.3

* Results are expressed as Mean from eight observations; Control worms were alive up to 24 hrs of observation. Extracts of leaves of *C. colocynthis* used for the study were designated as PEE, ETH, AQU for petroleum ether, ethanol and aqueous respectively.



Graph 1: Time Taken for Paralysis of *Pheretima Posthuma*

All values are expressed as mean \pm SEM; * $<$ 0.05.



Graph 2: Time Taken for Death of *Pheretima Posthuma*

All values are expressed as mean \pm SEM; * $<$ 0.05.

CONCLUSION

From the above result, it is concluded that Ethanol and Aqueous extracts of leaves of *C.colocynthis* have potent anthelmintic activity when compared with the conventionally used drug & is equipotent to standard helmintholytic drug.

Further studies using in vivo models are required to carry out & established the effectiveness & pharmacological rationale for the use of *C.colocynthis* as an helmintholytic drug. The drug may be further explored for its phytochemical profile to identify the active constituent.

REFERENCES

1. GRIN Database, USDA, ARS, National Genetic Resources Program, Germplasm Resources Information Network (GRIN), National Germplasm Resources Laboratory, Beltsville, Maryland. 2006; 10674
2. Kirtikar KR and Basu BD. Indian Medicinal Plants, Vol.I, International Book Distributors, Dehradun. 1991; 2nd Edn:1147-1149
3. Nadkarni KM. Indian Materia Medica. Vol.I, Bombay Popular Prakashan, 1982; 3rd Edn:335-337.
4. Dhar DN, Sharma RL, Bansal GC. Gastrointestinal nematodes in sheep in Kashmir. *Vet. Parasitol*; 1982, 11: 271-7.
5. Chopra RN, Nayyar SL, Chopra IC. *Glossary of Indian Medicinal Plants*. Council of Scientific and Industrial Research 1956:160
6. Said M. Hamdard Pharmacopeia of Eastern Medicine. Hamdard National Foundation, Karachi, Pakistan; 1969.
7. Akhtar MS, Iqbal Z, Khan MN, Lateef M. Anthelmintic activity of medicinal plants with particular reference to their use in animals in Indo-Pakistan subcontinent. *Small Ruminants Res*; 2000, 38: 99-107
8. Dr. Duke's Phytochemical and Ethnobotanical Databases, Ethnobotani Dr. Duke's Phytochemical and Ethnobotanical Databases, Ethnobotanical uses of *Citrullus* (Cucurbitaceae). 2006.
9. Ambasta SP. The Useful Plants Of India, New Dehli, 1992:127
10. Agrawal SS and Paridhavi M. Herbal Drug Technology, University press, Hyderabad, 2007:52
11. Khare CP. Indian medicinal plants, springer publication, New Delhi; 2007:152-153.
12. Nirmal SA, Malwadkar G and Laware RB. Anthelmintic Activity of *Pongamia Glabra* Songklanakarin, J. Sci. Technol, 2007; 29(3):755-757
13. Raut DN, Pal SC and Mandal SC. Anthelmintic Potential of *Dendrophthoe Falcata* Etting. (l.f) Leaf, IJPRD 2009.
14. Borkar VS, Gangurde HH, Gulecha VS., Bhojar PK and Mundada AS. Evaluation of *In Vitro* Anthelmintic Activity of Leaves of *Butea Monosperma*. Int. J. Phytomedicine, 2010:31-35.