IN VITRO ANTHELMINTIC PROPERTY OF VARIOUS HERBAL PLANTS EXTRACTS AGAINST *PHERITIMA POSTHUMA*

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Summary

Aqueous extracts of leaves of *Jatropha curcas*, *Vitex negundo*, *Mentha spicata* and flowers of *Delonix regia* were investigated for anthelmintic property against Pheritima posthuma (Indian Earth worm). Three concentrations (25, 50 and 100 mg / ml) of each plants aqueous extracts were studied in a bioassay, which involved the determination of time of paralysis and time of death of the worms. All the aqueous extracts exhibited significantly anthelmintic activity, but among the three plants, leaves of *Mentha spicata* demonstrated the best anthelmintic activity in both the parameters. Piperazine citrate (10 mg/ml) was included in the assay as standard reference drug.

Introduction

Diseases caused by helminthes parasites in continue to be a major productivity constraint, especially in small ruminants in the tropics and subtropics [1]. In the developing world, the greatest impact of parasitic diseases is indirect and potential productivity losses [2]. Infections by gastrointestinal helminth parasite of livestock are among the most common and economically important diseases of grazing livestock [3]. Adulteration of anthelmintic has been found to be common practice [4]. Illiteracy and unfamiliarity with synthetic anthelmintic, resulting in incorrect usage, are also a problem leading to the same consequence. Moreover, these drugs are relatively expensive. As a consequence of these problems and difficulties, pastoralists and small holder farmer have continued to use indigenous plants as livestock dewormers [5]. Considerable research has shown that some plants not only affect the nutrition of animals, but also have antiparasitic effects [6]. For Example, plants that contain condensed tannins, a class of phenolic secondary metabolite, have these effects. The leaves of *Jatropha curcas* Linn belonging to family Euphorbiaceae commonly known as Jangalierandi in Hindi [7]. Apigenin, vitexin and isovitexin. α amyrin, stigmasterol, along with two new flavonoids founds in leaves and twigs of *Jatropha curcas* Linn. [8]. It is successful local remedy for scabies, eczema and ringworm [9].

The plant *Vitex negundo* Linn. belonging to family Verbenaceae commonly known as 'Nirgundi' in Marathi[10]. The leaves of *Vitex negundo* Linn. has been reported for antibacterial, analgesic and anticonvulsant activities [11].The palnt of *Mentha spicata* Linn. belonging to the family Labiatae, Commonly known as Pudina in Hindi [12]. Diosmentin and diosmin have been found in the leaves of *Mentha spicata* Linn. [13]. It is successful local remedy for fever, bronchitis and used as an anthelmintic [14]. The plant of *Delonix regia* Rafin (Synonym *Poinciana regia* Rafin.) belonging to the family Caesalpiniaceae commonly known as Flamboyant Flame Tree [15]. The plant of *Delonix regia* Rafin. is used as catharatic, antirheumatic and flatulalence [16]. The flowers of *Delonix regia* Rafin have been folk used as an anthelmintic [17]. The present study is based to identify the potential anthelmintic property along with comparative details of various herbal plants using Pheritima posthuma (Indian Earth worm).

Material and Methods

Plants Collection and Authentification

The leaves of *Mentha spicata* Linn., *Jatropha curcas* Linn., *Vitex negundo* Linn. and flowers of *Delonix regia* Rafin. have been collected from the local area of Nandurbar (Maharashtra). All the plants are authentify by Dr. Santosh Tayade, Dept. of Botany, Art's, Science and Commerce College, Lonkheda, Shahada, Dist-Nandurbar (MS).

Plant Extraction

Collected leaves and flowers were dried and crushed to a coarse powder macerated with water. Extract was dried over anhydrous sodium sulphate and solvent was removed in vacuum at 40°C by using rotary evaporator (Rotavapour Buchii, Switzerland)**.** The aqueous extract was subjected to preliminary phytochemical testing for the presence of different chemical classes of compounds [18].

Worms Collection and Authentication

Indian earthworm *Pheritima posthuma* (Annelida) were collected from the water logged areas of soils Indian earthworms are identified at Department of Zoology, P.S.G.V.P. Mandal's, Shahada, Maharashtra.

Anthelmintic activity

The Anthelmintic assay was carried as per the method of Ajaiyeoba *et al*. with necessary modifications [19]. The assay was performed on adult Indian earthworm *Pheritima posthuma*, due to its anatomical and physiological resemblance with the intestinal round worm parasite of human being [20, 21]. Because of easy availability, earth worms have been used widely for initial evaluation of anthelmintic compounds *in* vitro [22]. 50 ml. of formulation containing different concentration of crude aqueous extract (25, 50 and 100 mg/ml in distilled water) were prepared and 6 worms of same type were placed in it. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50^0C). Piperazine citrate (10 mg/ml) was used as reference standard while distilled water as control.

Statistical Analysis [23]

The data presented as Mean ± SEM. The activities of all extracts were compared with the control. All the extracts showed significantly activity with higher duration of paralysis and death. Values of $P<0.001$ were considered statistically significant.

Results and Discussion

Phytochemical screening of crude aqueous extracts of leaves of *Jatropha curcas*, *Vitex negundo*, *Mentha spicata* and flowers of *Delonix regia* revealed the presence of alkaloids, saponins, flavonoids and tannins. As shown in Table 1, aqueous extracts of all the plants exhibited anthelmintic activity in dose dependent manner giving shortest time of paralysis (P) and death (D) with 100 mg/ml concentration, for *Pheritima posthuma* worms. The aqueous extract of leaves of *Mentha spicata* caused paralysis is 10 min and time of death is 13 min while aqueous extracts of leaves of *Jatropha curcas*, *Vitex negundo*, and flowers of *Delonix regia* Rafin. revealed paralysis of 16, 17 and 12 min. and time of death 28, 37 and 18 min. respectively

against *Pheritima posthuma*. The reference drug Piperazine citrate showed the paralysis at 22 min. and time of death at 100 mg conc. 49 min. respectively.

Table 1:- Anthelmintic activity of aqueous extracts of various herbal pants on *Pheritima Posthuma* (Indian Earth worm)

Groups	**Concentration in mg/ml**	**Time taken for Paralysis (P) and Death (D) of *Pheritima posthuma* worms in min (**Mean ± SEM**)**	
		P	**D**
Piperazine citrate	10	21.56± 0.34	48.70 ± 0.78
Aqueous extract of *Jatropha curcas* Linn.	25	54.6 ± 0.50	64.04 ± 0.57
	50	32.6 ± 0.43**	50.72 ± 0.44**
	100	16.2 ± 0.37**	27.92 ± 0.19**
Aqueous extract of *Vitex negundo* **Linn.**	25	62.50 ± 0.67	98.38 ± 0.0.43
	50	48.33 ± 0.42*	61.05 ± 0.0.56*
	100	17.50 ± 0.43**	37.17 ± 0.0.45**
Aqueous extract of *Mentha spicata* Linn.	25	61.66 ± 0.50	134.9 ± 0.78
	50	28.48 ± 0.19**	50.36 ± 0.52**
	100	10.04 ± 0.10***	13.28 ± 0.31***
Aqueous extract of *Delonix regia* Raffin.	25	82.70± 0.19	150.1± 0.11
	50	43.32± 0.33*	56.94± 0.12*
	100	11.92± 0.31 ***	18.42± 0.15***

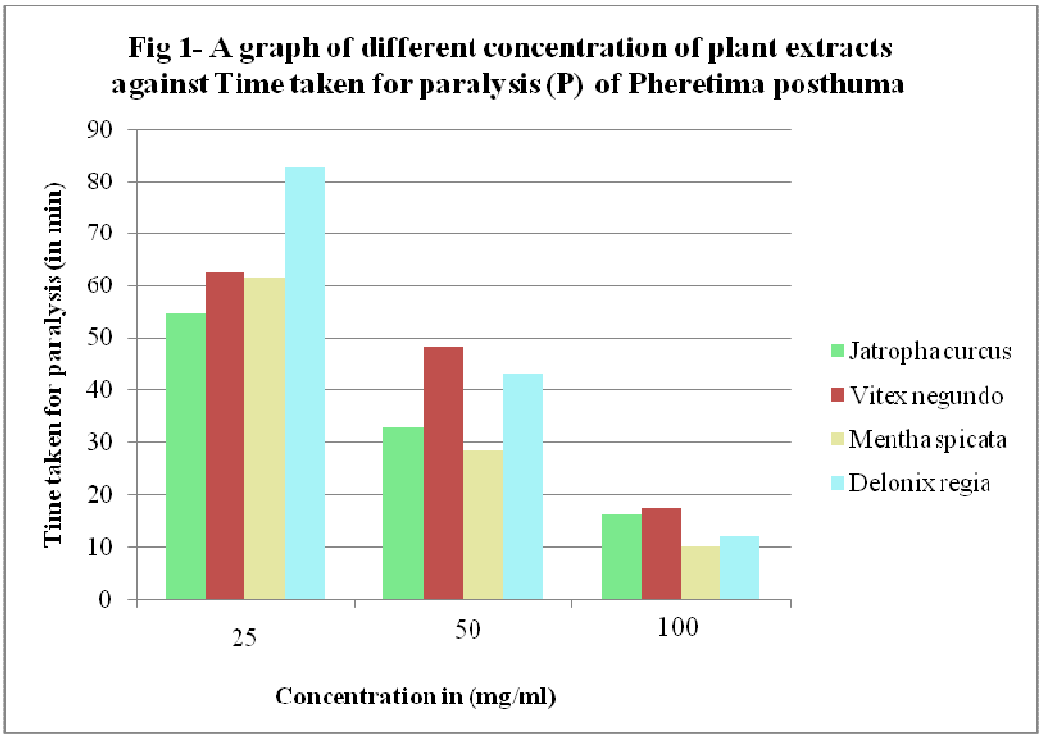
All values are Mean ± SEM; n=6 in each group. Values are significantly different from reference standard (Piperazine citrate) *p<0.05; **p<0.01; ***p<0.001

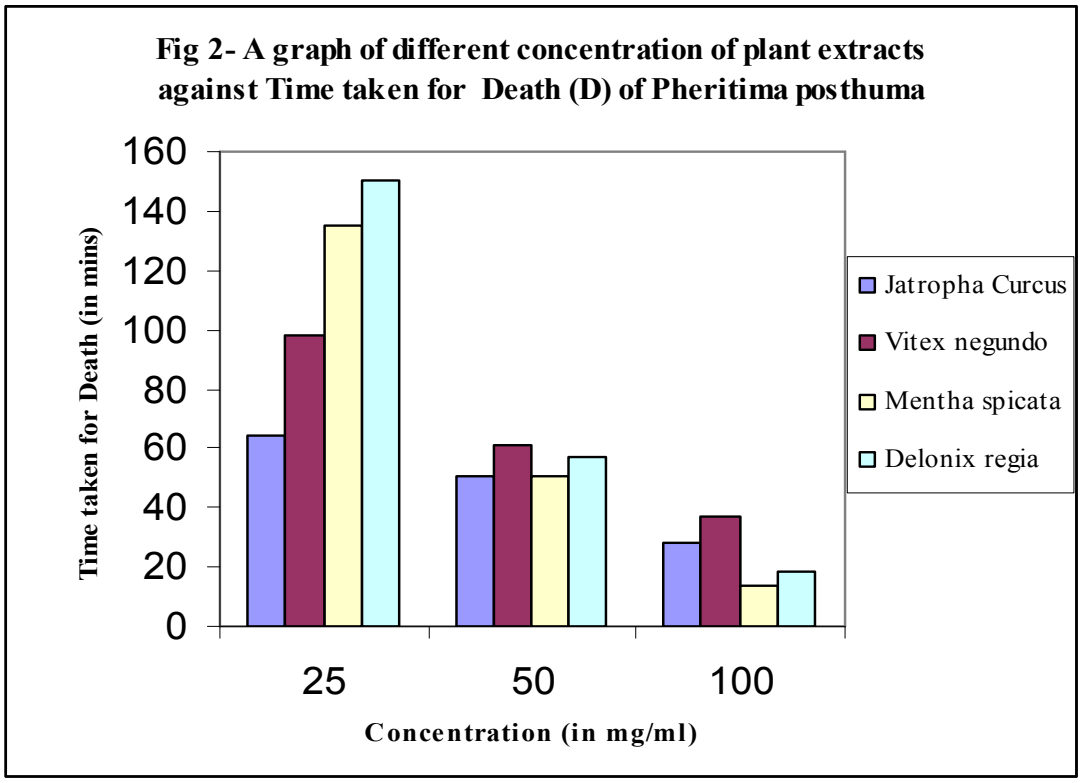
Piperazine citrate by increasing chloride ion conductance in worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis [24]. The aqueous extract of leaves of *Mentha spicata* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 100 mg/ml, in shorter time as compared to reference drug Piperazine citrate. Phytochemical analysis of the crude extracts revealed the presence of tannins among the other chemical constituent within them. Tannins were shown to produce anthelmintic activities [25]. Chemically tannins are polyphenolic compounds [26]. Some synthetic phenolic anthelmintic e.g. niclosamide, oxyclozanide, bithionol etc., are reported to interfere with energy generation in helminthes parasites by uncoupling oxidative phosphorylation [27]. It is possible that tannins contained in the aqueous extract of leaves of *Mentha spicata* produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tracts of host animal [28] or glycoprotein on the cuticle of the parasite [29] and may cause death.

The traditional medicines hold a great promise as source of easily available effective anthelmintic agents to the people, particularly in developing countries, including in India. It is in this context that the people consumed several plants or plant derived preparation to cure helminthic infections [30]. The origin of many effective drugs has been found in the traditional medicines practices and in view of this it is important to undertake studies pertaining to screening of the folklore medicinal plants for their proclaimed anthelmintic efficacy.

In Conclusion, the traditional use of plants *Jatropha curcas*, *Vitex negundo*, *Mentha spicata* and *Delonix regia* as an anthelmintic have been confirmed. As the aqueous extract of leaves of *Mentha spicata* displayed profound anthelmintic activity as compared with other herbal extracts and standard drug in the study. Further, it would be interesting to isolate the possible phytoconstituents, which may be responsible for the anthelmintic activity and to reveal the mechanism (s) of actions.

Fig 1- A graph of different concentration of plant extracts against Time taken for paralysis (P) of Pheretima posthuma





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References

1. Perryand BD, Randolph TF, McDermott JJ, Sones KR, and Thornton PK, *Vererinary Parasitology*, Nairobi: International Liverstock Research Institute; 2002. p.148.
2. Perryand BD, Randolph TF, McDermott JJ, Sones KR, and Thornton PK, *Vererinary Parasitology*, Nairobi: International Liverstock Research Institute; 1999. p. 84, 145.
3. Monteiro AM, Wanyangu SW, Kariuki DP, Bain R, Jackson F. and McKellar QA, Veterinary Record; 1998. p.142, 396.
4. Dano AR and. Bogh HO. World Animal Review; 1999; 93: 60.
5. Waghorn GC and McNabb WC. Proceedings of the Nutrition Society; 2003; 62: 383.
6. Bairacli De and Levy J. *The Complete Herbal Handbook for Farm and Stable*, 4th Ed. London: Faber and Faber; 1991. p. 471.
7. Anonymous. *The Wealth of India- A Dictionary of Indian raw material and industrial products*. Vol. V, New Delhi: CSIR; 1950. p. 293-95.
8. Joshi SG. *Medicinal Plants*, New Delhi: Oxford & IBH Publications; 2004. p.184.

9. Nadkarni AK and Nadkarni KR. *Indian Materia Medica*, Vol. I, Bombay: Popular Prakshan; 1976. p.705-6.
10. Anonymous. *The Wealth of India- A Dictionary of Indian raw material and industrial products*. Vol. X, New Delhi: CSIR; 1989. p. 522-4.
11. Shastry JN and Dravyaguna Vijnana. Vol. II, Mumbai : Chaudhambha Orientalia Publication ; 2004. p. 414-5.
12. Kirtikar KR and Basu BD. *Indian Medicinal Plants*. Vol. III, 2nd ed. Dehradun: International Book Distributors; 1995. p. 1979-80.
13. Joshi SG. *Medicinal Plants*. New Delhi: Oxford & IBH Publications; 2004. p. 226-7.
14. D'souza M. *Triable Medicine*. Ahmednagar: Social centre; 2000. p. 318.
15. Anonymous. *The Wealth of India- A Dictionary of Indian raw material and industrial products*. Vol. III, New Delhi: CSIR; 1950. p. 30.
16. Singh VK, Govin JN. Hashmi S and Singh G. *Recent Progress in Medicinal Plant*. Vol III, USA: Studium Press LLc; p. 238.
17. Khare CP. *Indian Medicinal Plants-Illustrated Dictionary*. New Delhi: Springer International Publication; 2007. p. 205-6.
18. Kokate CK. *Practical Pharmacognosy*. 3rd ed. New Delhi: Vallabh Prakshan; 1984. p. 107-13.
19. Ajaiyeoba EO, Onocha PA and Olarenwaju OT. *Phar. Biol*. 2001; 39 (3): 217-20.
20. Chatterjee KD. *Parasitology, Protozoology and Helminthology*. Calcutta: Guha Ray Sree Saraswaty press Ltd; 1967. p. 168-9.
21. Vigar Z. *Atlas of Medical Parasitology*, Singapore: P.G. Publishing House; 1984. p. 216.
22. Sollmann T, *J. Pharmacol. Exp. Ther.* 1918; 12:129-70.
23. Wayne W. *Biostatistics, A foundation for the analysis in the health sciences*, 7th ed. Singapore: John Wiley and Sons (Asia) Pvt. Ltd; 2004. p. 312.
24. .Martin R J. Br. J. Pharmacol. 1985; 84 (2): 445-61.
25. Niezen JH, Waghorn GC, Charlestone WAG and Waghorn GC, *J. Agri. Sci.* 1995: 125: 281-89.
26. Bate-Smith EC. *J. Linn. Soc. Bot.* 1962; 58: 95-173.
27. Martin RJ. *Vet. J.* 1997; 154: 11-34.
28. Athnasiadou S, Kyriazakis F, Jackson RL and coop, *Vet. Parasitol.* 2001; 99: 205-19.
29. Thomson DP and Geary TG. *The structure and function of helminth surfaces. In: J. J. Marr, eds. Biochemistry and molecular biology of parasites*, 1st ed. New York: Academic Press; 1995. p. 203-32.
30. Satyavati GV, *Use of Plants Drugs in Indian Traditional System of medicines and their relevance to Primary Health Car,* In: N. R. Farnsworth, H. Wagner, eds. *Economic and Medicinal Plant Research*, Vol IV, London: Academic Press Ltd; 1990. p.190.