

## **In Vitro Anthelmintic Property of Gulmohar Flowers against *Pheritima Posthuma***

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### **Summary**

The anthelmintic property of fresh methanol and aqueous extracts of flowers of *Delonix regia* Raffin. belonging to family caesalpiniaceae was studied for anthelmintic activity against *Pheritima posthuma* (Indian Earth worm). Three concentrations (25, 50 and 100 mg / ml) of both extracts were studied in a bioassay, which involved the determination of time of paralysis and time of death of the worms. Both the extracts of flowers of *Delonix regia* Raffin. exhibited considerable anthelmintic activities. The methanol extract of flowers of *Delonix regia* Raffin. was found to be the most active. Piperazine citrate (10 mg /ml) and distilled water were included in the assay as standard reference drug and control, respectively.

**Keywords:-** *Delonix regia*, anthelmintic, *Pheritima posthuma* and Piperazine citrate.

### **Introduction**

Diseases caused by helminth parasites in continue to be a major productivity constraint, especially in small ruminants in the tropics and subtropics <sup>1</sup>. In the developing world, the greatest impact of parasitic diseases is indirect and potential productivity losses <sup>2</sup>. Infections by gastrointestinal helminth parasite of livestock are among the most common and economically important diseases of grazing livestock <sup>3</sup>. Adulteration of anthelmintics has been found to be common practice<sup>4</sup>. Illiteracy and unfamiliarity with synthetic anthelmintics, 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 <sup>5</sup>. Considerable research has shown that some plants not only affect the nutrition of animals, but also have antiparasitic effects <sup>6</sup>. For Example, plants that contain condensed tannins, a class of phenolic secondary metabolite, have these effects.

The plant of *Delonix regia* Rafin. (Synonym *Poinciana regia* Rafin.) belonging to the family caesalpiniaceae commonly known as Flamboyant Flame Tree<sup>7</sup>. It is a beautiful tree which is an erect, large having flowers are red in simple or branched racemes<sup>8</sup>. It is well known as 'Gulmohar.' in Hindi. The plant of *Delonix regia* Rafin. is used as cathartic, antirheumatic and flatulalence<sup>9</sup>. The flowers of *Delonix regia* Rafin have been folk used as an anthelmintic<sup>10</sup>. The present study is based to identify the potential anthelmintic property against *Pheritima posthuma* (Indian Earth worm).

## Material and Methods

### Plant material

The flowers of *Delonix regia* Rafin. have been collected from the local area of Nandurbar (Maharashtra). This plant is authenticating by Dr. Santosh K. Tayade, Dept. of Botany, Art's, Science and Commerce College, Lonkheda, Shahada, Dist-Nandurbar (MS).

### Preparation of extract

Collected flowers were dried and crushed to a coarse powder and subjected to extraction in Soxhlet extractor using methanol and 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<sup>11</sup>.

### 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<sup>12</sup>. 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<sup>13,14</sup>. Because of easy availability, earth worms have been used widely for initial evaluation of anthelmintic compounds *in vitro*<sup>15</sup>. 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°C). Piperazine citrate (10 mg/ml) was used as reference standard while distilled water as control.

### Statistical Analysis<sup>16,17</sup>

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

### Results and Discussion

Phytochemical screening of both extract of flowers *Delonix regia* revealed the presence of alkaloids, saponins, flavonoids and tannins. As shown in Table 1, methanolic extract 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 methanolic extract of flowers *Delonix regia* caused paralysis is 11 min and time of death is 17 min while aqueous extract of flowers *Delonix regia* revealed paralysis of 12 min. and time of death 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.

Piperazine citrate by increasing chloride ion conductance in worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis<sup>18</sup>. 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<sup>19</sup>. Chemically tannins are polyphenolic compounds<sup>20</sup>. Some synthetic phenolic anthelmintics e.g. niclosamide, oxyclozanide, bithionol etc., are reported to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation<sup>21</sup>. 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<sup>22</sup> or glycoprotein on the cuticle of the parasite<sup>23</sup> 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<sup>24</sup>. 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 *Delonix regia* as an anthelmintic have been confirmed. As the methanolic extract of flowers *Delonix regia* displayed profound anthelmintic activity as compared with aqueous extract 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.

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Table No-1 Anthelmintic activity of Methanolic extract of *Delonix regia* Raffin. Flowers.

Test Substance	Concentration in mg/ml	Time taken for Paralysis (P) and Death (D) of worms in minute	
		<i>Pheritima posthuma</i>	
		P	D
Control (in normal saline)	-	-	-
Methanolic extract	25	75.55± 0.19*	145.5 ± 0.19*
Methanolic extract	50	40.68± 0.17**	54.40 ± 0.18**
Methanolic extract	100	11.33± 0.11 ***	17.44 ± 0.15***
Piperazine citrate	10	21.56± 0.34***	48.70 ± 0.78***

Table No-2 Anthelmintic activity of aqueous extract of *Delonix regia* Raffin. flowers.

Test Substance	Concentration in mg/ml	Time taken for Paralysis (P) and Death (D) of worms in minute	
		<i>Pheritima posthuma</i>	
		P	D
Control (in normal saline)	-	-	-
Aqueous extract	25	82.70± 0.19*	150.1± 0.11*
Aqueous extract	50	43.32± 0.33**	56.94± 0.12**
Aqueous extract	100	11.92± 0.31 ***	18.42± 0.15***
Piperazine citrate	10	21.56± 0.34***	48.70 ± 0.78***

Results are expressed as Mean ± SEM from eight observations;  
Control worms were alive up to 24 hrs of observation.

### References

1. Perryand, B. D., Randolph, T. F., McDermott, J. J., Sones, K. R. and Thornton, P. K. 2002. *Vererinary Parasitology*. In: International Livestock Research Institute, Nairobi, p. 148.
2. Perryand, B.D. and Randolph, T.F. 1999. *Vererinary Parasitology*. In: International Livestock Research Institute, Nairobi, p. 84, 145
3. Monteiro, A. M., Wanyangu, S. W., Kariuki, D.P., Bain, R., Jackson, F. and McKellar, Q.A. 1998. *Veterinary Record*. p.142, 396
4. Dano, A.R. and Bogh, H.O. 1999. *World Animal Review*. p. 93, 60.

5. Waghorn, G.C. and McNabb, W.C. 2003. *Proceedings of the Nutrition Society*. p. 62, 383
6. De Bairacli and Levy, J. 1991. *The Complete Herbal Handbook for Farm and Stable*. In: 4<sup>th</sup> Ed., Faber and Faber, London, p. 471
7. Anonymous. 1950. *The Wealth of India- A Dictionary of Indian raw material and industrial products* In: Vol. III, (CSIR) New Delhi, p. 30.
8. Pullaih, T. 2002. *Medicinal Plants in India*. In: Vol 1, Regency publications, New Delhi, p. 212-213.
9. Singh, V.K., Govin, J.N., Hashmi, S. and Singh G. *Recent Progress in Medicinal Plants*. In: Vol 3, Studium Press LLC, USA, p. 238.
10. Khare, C.P. 2007. *Indian Medicinal Plants-Illustrated Dictionary*. Springer International Publication, New Delhi, p. 205-206.
11. Kokate, C.K. 1984. *Practical Pharmacognosy*. In: 3<sup>rd</sup> Ed., Vallabh Prakashan, New Delhi, p. 107-113.
12. Ajaiyeoba, E.O., Onocha, P.A. and Olarenwaju O.T. 2001. *In vitro* anthelmintic properties of *Buchholzia coriacea* and *Gynandropsis gynandra* extract. *Phar. Biol.* **39** (3), 217-220.
13. Chatterjee, K.D. 1967. *Parasitology, Protozoology and Helminthology*. Guha Ray Sree Saraswaty press Ltd., Calcutta, p.168-169.
14. Vigar, Z. 1984. *Atlas of Medical Parasitology*. P.G. Publishing House, Singapore, P. 216.
15. Sollmann, T. 1918. Anthelmintics: Their efficiency as tested on earth worms. *J. Pharmacol. Exp. Ther.* **12**, 129-170.
16. Wayne, Daniel, W. 2004. *Biostatistics, A foundation for the analysis in the health sciences*. In: 7<sup>th</sup> Ed., John Wiley and Sons (Asia) Pvt. Ltd, Singapore, p. 312.
17. Gold stain, A. 1967. *Biostatistics*. Macmillan co, New York, p. 70-72.
18. Martin, R.J. 1985.  $\gamma$ - Aminobutyric acid and Piperazine activated single channel currents from *Ascaris suum* body muscle. *Br. J. Pharmacol.* **84** (2), 445-461.
19. Niezen, J.H., Waghorn, G.C., Charlestone, W.A.G. and Waghorn, G.C. 1995. Growth and gastrointestinal parasitism in lambs grazing either Lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*), which contains condensed tannins. *J. Agri. Sci.* **125**, 281-289.
20. Bate-Smith, E.C. 1962. The phenolic constituents of plants and their taxonomic significance, Dicotyledons. *J. Linn. Soc. Bot.* **58**: 95-173.
21. Martin R.J. 1997. Mode of actions of anthelmintic drugs. *Vet. J.* **154**, 11-34.
22. Athnasiadou, S., Kyriazakis, F., Jackson R.L. and coop. 2001. Direct anthelmintic effect of condensed tannins towards different gastrointestinal nematodes of sheep: *In vitro* and *in vivo* studies. *Vet. Parasitol.* **99**, 205-219.
23. Thomson, D.P. and Geary, T.G. 1995. *The structure and function of helminth surfaces*. In: J.J. Marr, eds. *Biochemistry and molecular biology of parasites*. 1<sup>st</sup> ed., Academic Press, New York, p. 203-232
24. Satyavati, G.V. 1990. *Use of Plants Drugs in Indian Traditional System of medicines and their relevance to Primary Health Care*. In: N. R. Farnsworth, H. Wagner, eds. *Economic and Medicinal Plant Research*, Vol IV. Academic Press Ltd, London, p. 190