



IN VITRO ANTHELMINTIC ACTIVITY OF LEAVES OF *JUGLANS REGIA L* AGAINST *PHERETIMA POSTHUMA*

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ABSTRACT

Among the most common infections of digestive system in human beings are helminth infections. In developing countries they pose a large threat to the society. Such parasitic diseases cause severe morbidity, including lymphatic filariasis, onchocerciasis and schistosomiasis. Present study is an attempt to evaluate anthelmintic activity of different extracts of *Juglans regia L* leaf.

Many traditional plants and herbs are reported to have anthelmintic activity. *Juglans regia L* the valuable species from Juglandaceae family has a long history of traditional use as an anthelmintic. All parts of the plant are a rich source of medicinally useful components. Present study is an attempt to evaluate anthelmintic activity of different extracts of leaves of *J. regia L*. Different extracts of the plant material were tested against adult Indian earthworms *Pheretima posthuma* (Pheritimidae) as test worms. The bioassay involved determination of the time of paralysis and time of death control. Piperazine citrate (10 mg/mL) was used as standard reference drug

The results of this study strongly support the traditional anthelmintic use of the plant material.

Key words: Anthelmintic activity, *Pheretima posthuma*, Piperazine citrate, *Juglans regia*.

INTRODUCTION

Juglans regia L (family Juglandaceae) is known as the common walnut tree. Many traditional plants and herbs are reported to have anthelmintic activity. *Juglans regia L*, the valuable species has a long history of traditional use as an anthelmintic. Green walnuts, shells, kernels and seeds, bark and leaves are used in the pharmaceutical and cosmetic industries^{1,2}. All parts of the plant are a rich source of medicinally useful components. Leaves are easily available in abundant amounts. Walnut leaves are considered to be a source of healthcare compounds and have been intensively used in traditional medicine for the treatment of venous insufficiency, hemorrhoids, hypoglycemia, diarrhea and fungal or microbial infections³. In addition to antioxidant activity, several studies have demonstrated the antimicrobial activity of phenols and/or phenolic extracts^{4,5}, making them a good alternative to antibiotics and chemical preservative. An ideal anthelmintic must have a wide margin between its toxicity to the worm and its toxic effect on the host. The drug must be effective in one dose. Anthelmintic drugs can be classified according to their chemical structure as well as to their action against the specific type of helminthes⁶. As per WHO, only few drugs are frequently used in the

treatment of these parasite infections⁷. Family Juglandaceae includes a valuable, medicinally useful species *Juglans regia* L, growing in the forests of Himalayas in India. The root, stem bark, leaves, seeds, cotyledons and seed oil are used to treat a variety of health complaints.

Helminthiasis, the condition resulting from worm infestation, is one of the major prevalent diseases in the world, particularly in the tropical countries. Lack of adequate sanitary facilities and supply of pure water coupled with poverty and illiteracy are some of the factors responsible for wide spread nature of this disease in the developing countries. Helminthiasis is prevalent globally (1/3 of world's population harbours them), but is more common in developing countries with poorer personal and environmental hygiene⁸. Anthelmintics or antihelmintics are drugs that expel parasitic worms (helminths) from the body, by either stunning or killing them⁹. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs; therefore, there is a foremost problem in treatment of helminthes diseases¹⁰. Moreover, these drugs are unaffordable because of their high cost. These factors paved the way for herbal remedies as alternative anthelmintics.

However, so far no study has been reported to evaluate anthelmintic activity in leaf part. The present study was therefore undertaken to evaluate the *in vitro* anthelmintic activity of different extracts of leaves of *Juglans regia* L against *Pheretima posthuma* (Pheritimidae).

EXPERIMENTAL

Material and methods

Plant material

Leaves of *Juglans regia* L were collected from Akhnoor, Jammu region India, and plant material was identified by Dr. S. N. Sharma of Department of Botany, IIM, Jammu. The leaves were dried under shade and powdered.

Drugs and chemicals

All the chemicals and reagents were procured from S.D.Fine Chemicals (Mumbai, India). All the chemicals were of analytical grade. The drug piperazine citrate was procured from Glaxo Smithkline Ltd, Mumbai.

Animals

Earthworms *Pheretima posthuma* (Pheritimidae) have been used widely for the initial evaluation of anthelmintic activity, because of their easy availability. Indian adult earthworms (*Pheretima posthuma*) were collected from the water logged areas of soils and washed with normal saline to remove all filthy matter. They were authenticated by Head, Department of Zoology, S.D. College, Pathankot, Punjab. 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.

Preparation of extracts

The walnut leaves were cut into small pieces and air dried for 48-72 h. Dried leaves were ground into a fine powder using an electric grinder. Coarsely powdered material (500 g) was extracted successively with petroleum ether (40-60), methanol and water by maceration process at room temperature for three days. Then all the extracts were filtered and concentrated with a rotary evaporator and kept in a refrigerator.

Phytochemical test

The freshly prepared crude extracts of leaves of *Juglans regia* L were subjected to standard phytochemical screening tests for various constituents^{11,12}.

Anthelmintic activity

The assay was performed on adult Indian earthworm *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal round worm parasite of human being. Five groups of Indian earth worms each containing six earthworms approximately of equal size was used for the study. Each group of earth worms were tested with different extract conc. (10, 25 and 50 mg/mL), distilled water (Control), and reference standard piperazine citrate (10 mg/mL in distilled water)¹³. Observations were made for the time taken for paralysis and death of individual worms. 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 the worms neither moved when shaken vigorously nor when dipped in warm water at 50 °C followed with fading of their body colour.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of different extracts of leaves of *Juglans regia L* revealed the presence of alkaloids, flavonoids, tannins and saponins. Tannins and polyphenolic compounds were shown to produce anthelmintic activities¹⁴. In the present study, it was observed that all the extracts of *Juglans regia* leaf have exhibited positive response to certain degree of anthelmintic activity. Extracts exhibited more potent activity at higher concentration (50 mg/mL) against *Pheretima posthuma* (earthworm). Evaluation of anthelmintic activity was compared with reference standard piperazine citrate as shown in Table.1.

Table: 1 Anthelmintic activity of Juglans regia L.leaf Extract.

Sr. No.	Test Substance	Conc. (mg/mL)	Time taken for paralysis (P) and death (D) of worms in min	
			P	D
1.	Distilled water (Control)	---	---	---
2.	Piperazine citrate (Standard)	10	19.36 ± 0.87	61 ± 0.68
3.	Aqueous extract	10	23.53 ± 0.79	73.83 ± 1.72
		25	15.5 ± 0.42	35.83 ± 1.16
		50	11 ± 0.25	20.66 ± 1.43
4.	Petroleum ether Extract	10	28.13 ± 0.61	85 ± 2.84
		25	19.56 ± 0.95	48 ± 1.05
		50	12.10 ± 0.87	35 ± 1.32
5.	Methanolic extract	10	18.16 ± 0.65	60.83 ± 1.32
		25	10.32 ± 0.51	28.10 ± 0.81
		50	5.48 ± 0.40	15.5 ± 0.40

Values are expressed as Mean ± SEM (n = 6)

The methanolic leaf extract of *Juglans regia L* demonstrated paralysis as well as death of worms in a less time as compared to piperazine citrate especially at higher concentration of 50 mg/mL. While water extract showed significant activity and petroleum ether extract being the least active among all the extracts.

Piperazine citrate increases chloride ion conductance of worm muscle membrane, producing hyper polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis¹². Different extracts of *Juglans regia L* leaves *not* only demonstrated paralysis, but also caused death of worms even at low concentration of 10 mg/mL as compared to standard drug piperazine citrate.

In conclusion, the traditional use of leaves of *Juglans regia L* as an anthelmintic have been confirmed as the leaf extracts displayed activity against the worms used in the study. The methanolic extract being more potent. Further studies need to establish the mechanism(s) of action are required.

CONCLUSION

It clearly indicates that the aqueous extract is more potent as compared to other extracts as it took less time to cause paralysis and death of the earthworms as compared to standard reference drug.

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REFERENCES

1. J. A. Pereira, I. Oliveira and A. Sousa et al., Walnut (*Juglans regia L*.) Leaves, Phenolic Compounds, Antibacterial Activity and Antioxidant Potential of Different Cultivars, Food Chem. Toxicol., **45**, 2287-95 (2007).
2. F. Stampar, A. Solar and M. Hudina et al., Traditional Walnut Liqueur- Cocktail of Phenolics, Food Chemistry, **95**, 627-31 (2006).
3. M. Wichtl and R. Anton, Plantes Th érapeutiques, Tec. hnique et., Documentation, Paris (1999).
4. C. Proestos, N. Choriantopoulos and G. J. A. Nychas, et al, RP-HPLC Analysis of the Phenolic Compounds of Plant Extracts, Investigation of their Antioxidant Capacity and Antimicrobial Activity, J. Agric. Food Chem., **53**, 1190-5 (2005).
5. A. Sousa, I. C. Ferreira and R. Calhelha et al, Phenolics and Antimicrobial Activity of Traditional Stoned Table Olives “alcaparra”, Bioorg. Med. Chem., **14**, 8533-8 (2006).
6. Rao, Chawathe and Shah, An Introduction to Synthetic Drugs and Dyes Second Edition, Himalaya Publishing House (June 1995) pp. 50-53.
7. Aswar Manoj, Aswar Urmila and Watkar Bhagyashri, Int. J. Green Pharm., 170-173 (2008).
8. K. D. Tripathi, Essentials of Medical Pharmacology, 6th Ed., Jaypee Brothers Medical Publishers, (P) Ltd. New Delhi (2008).
9. A. Dwivedi, S. Dwivedi, A. K. Sitoke, R. Patel and D. Jhade, Anthelmintic Activity of a Polyherbal Preparation, Ethnobotanical Leaflets., **13**, 259-62 (2009).
10. S. B. Kosalge and R. A. Fursule, Investigation of *in Vitro* Anthelmintic Activity of *Thespesia Lampas (cav.)*, Asian J. Pharm. Clin. Res., **2(2)**, 69-71(2009).

11. C. K. Kokate, Practical Pharmacognosy, 4th Edition, Vallabha Prakashan, New Delhi (1999) p. 149-156.
12. S. N. Khadatkar, J. V. Manwar and N. S. Bhajipale, *In Vitro* Anthelmintic Activity of Root of *Clitoria Ternatea* Linn, Phcog. Mag., **4(13)**, (Suppl), 148-50 (2008).
13. R. G. Mali and R. R. Wadekar, *In Vitro* Anthelmintic Activity of *Baliospermum Montanum* Muell. Arg Roots, Indian J. Pharm. Sci., 131-133 (2008).
14. E. C. Bate-Smith, The Phenolic Constituents of Plants and their Taxonomic Significance, Dicotyledons, J. Linn. Soc. Bot., **58**, 95-173 (1962).