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INVITRO ANTIHELMINTHIC ACTIVITY OF RAUWOLFIA SERPENTINA ON PHERETIMA POSTHUMA

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Abstract:

Antihelmintics are the drugs used to eradicate or reside the number of helmintic parasites from intestinal tract or tissue of humans and other animals. The antihelmintic activity of Rauwolfia serpentina root extracts were evaluated separately on adult Indian earthworm (Pheretima posthuma). It was found that methanolic extract and aqueous extract of Rauwolfia serpentina showed antihelmintic activity at various concentration (50,100 and 200 mg/ml). The standard drug used was Albendazole (50, 100 and 200 mg/ml). The methanolic extract of Rauwolfia serpentina showed better result when compared to aqueous extract.

Keywords: *Antihelmintics, Rauwolfia serpentina, Albendazole and Pheretima posthuma.*

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INTRODUCTION:

Helminthes infections, repeatedly entitled helminthiasis are among the most pervasive infection and a foremost degenerative disease distressing a large proportion of world's population. In developing countries, they pose a large threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia and pneumonia [1]. Herbal medicines are made from all-natural ingredients; they are much safer than lab-made drugs. Studies show that manufactured items – including synthetic drugs – are not superior or effective than natural items, such as herbal meds. Synthetic meds are made from cocktails of chemicals that do more harm than good. These medications are less stable than herbal drugs.

Helminthiases are classified as follows (the disease names end with "-sis" and the causative worms are in brackets):

Roundworm infection (nematodiasis)

- Filariasis (*Wuchereria bancrofti*, *Brugia malayi* infection)
- Onchocerciasis (*Onchocerca volvulus* infection)
- Soil-transmitted helminthiasis – this includes ascariasis (*Ascaris lumbricoides* infection, trichuriasis (*Trichuris* infection), and hookworm infection (includes necatoriasis and *Ancylostoma duodenale* infection)
- Trichostrongyliasis (*Trichostrongylus* spp. infection)
- Dracunculiasis (guinea worm infection)
- Baylisascaris (raccoon roundworm, may be transmitted to pets livestock and humans)

Tapeworm infection (cestodiasis)

- Echinococcosis (*Echinococcus* infection)
- Hymenolepiasis (*Hymenolepis* infection)
- Taeniasis/cysticercosis (*Taenia* infection)
- Coenurosis (*T. multiceps*, *T. serialis*, *T. glomerata*, and *T. brauni* infection)

Trematode infection (trematodiasis)

- Amphistomiasis (amphistomes infection)
- Clonorchiasis (*Clonorchis sinensis* infection)
- Fascioliasis (*Fasciola* infection)
- Fasciolopsiasis (*Fasciolopsis buski* infection)
- Opisthorchiasis (*Opisthorchis* infection)
- Paragonimiasis (*Paragonimus* infection)
- Schistosomiasis/bilharziasis (*Schistosoma* infection)

Acanthocephala infection

- *Moniliformis* infection

Classification of antihelmintics

➢ **Benzimidazoles (BZ – WHITE)**

Examples: Albendazole, Fenbendazole, Oxfendazole

➢ **Tetrahydropyrimidines/imidazothiazoles (LEV – YELLOW)**

Examples: Levamisole

➢ **Macrocyclic lactones (ML – CLEAR)**

Examples: Ivermectin, Moxidectin, Doramectin

Table 1: Plants Posessing Anthelmintic Activity

Plant name	Family name	Part used
Buchholzia coriaceae	Capparaceae	Leaves and stems
Gynandropsis gynandra	Capparidaceae	Leaves and stems
Adhatoda vasica	Acanthaceae	Roots
Curcuma longa	Zingiberaceae	Rhizome
Zingiber officinale	Zingiberaceae	Rhizome
Caesalpinia crista	Fabaceae	Seed kernel
Chenopodium album	Amaranthaceae	Whole plant
Senna occidentalis	Fabaceae	Leaves
Leonotis ocymifolia	Lamiaceae	Aerial parts
Leucas martinicensis	Lamiaceae	Aerial parts
Rumex abyssinicus	Polygonaceae	Aerial parts
Albizia schimperiana	Leguminosae	Stem bark
Annona senegalensis	Annonaceae	Whole plant
Euphorbia thymifolia	Euphorbiaceae	Whole plant
Hibiscus furcatus	Malvacea	Whole plant
Gymnema sylvestre	Apocynaceae	Leaves
Holarrhena Antidysenterica	Apocynaceae	Bark
Luffa cylindrical	Cucurbits	Leaves
Morus alba	Moraceae	Bark
Plumbago zeylanica	Plumbaginaceae	Roots
Pavonia odorata	Malvacea	Roots
Quisqualis Indica	Combretaceae	Leaves
Sapindus emarginatus	Sapindaceae	Pericarps

LITERATURE REVIEW

- **D. S. K. Sarma[2] (2015)** Anthelminthics are the drugs used to eradicates or reside the number of helminthic parasites from intestinal tract or tissue of humans and other animals. The Anthelminthic activities of different leaf extracts of *QuisqualisIndica* were evaluated separately on adult Indian earthworm(*Pheritima posthuma*). It was found that methanolic extract and aqueous extract of *Quisqualis Indica* showed anthelminthic activities at a concentration of 60 mg/ml of each. The reference standard was Albendazole (60 mg/ml).The methanolic extract of *Quisqualis Indica* showed better result compared to aqueous extract.
- **Kshitij Agarwal [3](2016)** Different extracts of Morusalba bark were taken for anthelmintic activity against Indian earthworm Pheretimaposthuma. Three concentrations (2, 4, and 10 mg/ml) of various extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. Piperazine hydrate (2 and 4 mg/ml) was used as reference standard and normal saline as control group. Dose dependent activity was observed in the plant extracts but hydroalcoholic extract exhibited more activity as compared to others. The anthelmintic activity of Morusalba bark extract has therefore been demonstrated for the first time.
- **Prashant Kumar Dhakad [4](2016)** Rajasthan state is rich in flora of xerophytic plants. Capparis decidua (Forssk.) Edgew. is a wild bushy plant found in hot arid regions. This plant has wide range of distribution in many parts of India. Ethnobiologically, this plant is useful as its various parts like immature flower buds, semi-mature fruits, young shoots with small leaves are pickled for use as a condiment and traditional people use this plant in treating ailments like digestive diseases, anodyne, sudorific, constipation, gout, cough, flu, dropsy, palsy, asthma, and intestinal worms, lumbago, odontalgia. Capparis decidua (Forssk.) Edgew. contains chemical compounds like alkaloids, flavanoids, terpenoids, phenolic compounds, steroids, vitamins, quarternary ammonium compounds and many more phytoconstituents that are responsible for its medicinal value. Different parts of this plant like seed, root, stem, flowers, fruits and leaves have medicinal importance and has shown numerous pharmacological activities like antimicrobial, antibacterial, antifungal, anti-inflammatory, antioxidant, hepatoprotective, anthelmintic, antidiabetic, antisebum, antihyperlipidemic, antisclerotic, antitermite, antiplaque, analgesic, sedative and anticonvulsant. Economic importance of this plant has tended for harvesting, yield and marketing specifically in Thar Desert. Future potential of this crop is very

promising as it is a drought and heat tolerant plant which makes it a good weather forecasting species; also it provides people with food (pickle & vegetable), fodder, wood and fuel. The presence of numerous phytoconstituents makes it a medicinally important crop for treating deadly diseases. This review covers taxonomy, distribution, phytochemicals, and nutritional value, and commercial value, traditional and pharmacological aspects of Capparisdeciduas.

- **ManjuMadhavan[5] (2017)** Coriander sativum traditionally used in treatment of several diseases (inflammation, diabetes, hypertension, wounds, pain and fever).Its seeds are used for herbal drink preparation in Kerala and consumed as part of diet. The present study was carried out to investigate the phytochemical profile, total phenol content, antioxidant and anthelmintic activity of hot water extracts of seeds of Coriander sativum L. Phytochemical analysis showed the presence of flavonoids, tannins triterpenoids, saponins, steroids, alkaloids and carbohydrates. The total phenol content in 5 µg/ µl hot water extract is 36 µg/ µl phenol expressed as catechol equivalent. The radical scavenging potential showed dose dependent increase on increasing the concentration of coriander seed extracts 1-5 µg. IC50 value of Coriander sativum seeds was 3.78µg/µl. Anthelmintic activity also was dose dependant increase. At the highest concentration tested ie 75mg/ml it is almost comparable to the standard Albendazole

OBJECTIVE AND PLAN OF WORK

Objective

Objective of the present work is to extract the root of *Rauwolfia serpentina* with different solvent system and evaluate these extracts by phytochemical and pharmacological screening using different methods.
Ascertain the utility of fraction showing significant antihelmintic activity for future development of herbal formulation.
The present study shall undertake:

- Selection of plant for antihelmintic activity based on review of literature.
- Collection of plant material from field and roadsides.
- Identification and authentication of the plant from Department of Botany, Osmania University, Hyderabad.
- Preparation of suitable extracts of the plant under investigation.
- To undertake phytochemical investigation of the promising plant extract.
- Evaluation of the promising extract for antihelmintic activity in pheretima posthuma (earthworms).

Plan of work

Review of literature

A systematic attempt shall be made to review the literature including source, synonyms, botanical description, taxonomical characteristics, phytochemistry, toxicity study, pharmacological actions, and ethnomedicinal uses.

Selection of plant

The criteria for selection of plant shall be based on the following guidelines:

- The available literature like folklore, Ayurvedic, Pharmacognosy, etc. mentioned about the use of the plant material by tribal people, vaidyas or other practioners of medicine.
- A scientific investigation using laboratory models of screening antihelmintic activity has not been carried out.
- The phytochemical investigation has not been reported.
- Pharmacological (efficacy) and toxicity (safety) data is not available.

Collection of the plant

The selected plant shall be collected in sufficient quantity either from natural source or local dealer. The plant material shall be authenticated from authorized taxonomist and voucher specimen number shall be obtained.

Preparation of extracts

The suitable extracts like aqueous and methanolic extracts of the plant Rauwolfia serpentina shall be prepared.

Phytochemical investigation

The promising extract shall be screened for presence of various phytoconstituents (Kokate et al., 1985; Evans et al., 1994).Also quantitative determination of various phytochemicals by various reported method**.**

Preliminary screening of extract for antihelmintic activity:

The extracts shall be screened for preliminary antihelmintic activity at various doses, using Pheretima posthuma (earthworms) by:

- Invitro antihelmintic activity

EXPERIMENTAL PROCEDURE

Preparation of aqueous extract

In this process, the crushed roots are boiled in specified volume of water for a defined time; This procedure is suitable for extracting water-soluble, heat-stable constituents. This process is typically used in the preparation of Ayurvedic extracts called “quath” or “kawath”. the starting ratio of crude drug to water is fixed, e,g. 1:4 or :16; the volume is

brought down to one-fourth its original volume by boiling during the extraction procedure. Then, the concentrated extract is filtered and used as such or processed further.

Preparation of methanolic extract

Procedure:

- In this process, the dried roots needs to be crushed, using a pestle and mortar, to provide a greater surface area.
- The dried roots should be sufficient to fill the porous cellulose thimble.
- All equipment should be assembled.
- The solvent (Methanol) is added to a round bottom flask, which is attached to a Soxhlet extractor and condenser on an isomantle.
- The powdered plant material (Dried roots powder) is loaded into the thimble, which is placed inside the Soxhlet extractor.
- The side arm is lagged with glass wool.
- The solvent is heated using the isomantle and will begin to evaporate, moving through the apparatus to the condenser.
- The condensate then drips into the reservoir containing the thimble.
- Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again.
- The process should run for a total of 12 hours
- The obtained extract was evaporated to dryness by placing the petri plates containing extract over boiling water bath.
- Brown coloured powder was obtained which will be used further.

Qualitative phytochemical screening

Table 2: Qualitative phytochemical screening of aqueous and methanolic extract of *Rauwolfia serpentina*

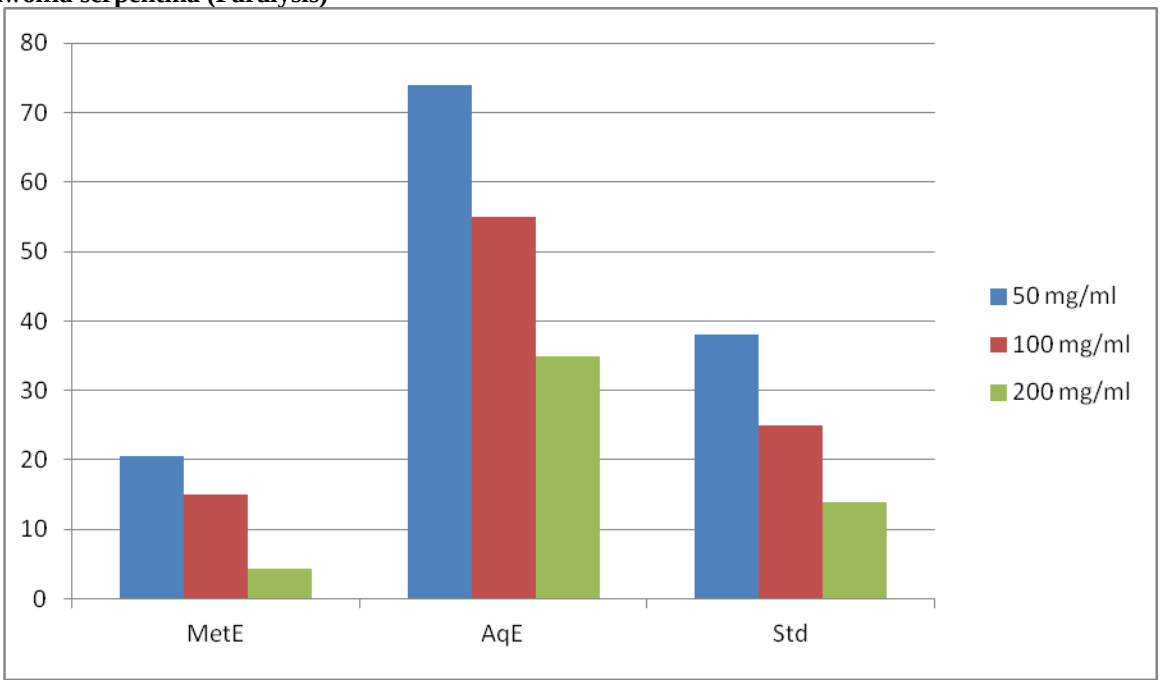
Phytochemical Screening	Aqueous Extract		Methanolic Extract	
	Observation	Inference	Observation	Inference
Alkaloid	Colored precipitate	Presence of alkaloids (+)	Colored precipitate	Presence of alkaloids (+)
Saponin	No froth formation	Absence of saponins (-)	Froth formation	Presence of saponins (+)
Tannin	No blue black precipitate	Absence of tannins (-)	Blue-black precipitate	Presence of tannins (+)
Flavanoids	Flavonoids dissolved giving yellow color	Presence of flavonoids (+)	Flavonoids dissolved giving yellow color	Presence of flavonoids (+)
Phenolics	No dark green	Absence of phenols (-)	Dark green	Presence of phenols (+)
Phytosterols	No green- blue color	Absence of phytosterols (-)	No green-blue color	Absence of Phytosterols (-)
Terpenoids	Reddish brown color	Presence of terpenoids (+)	Reddish brown color	Presence of terpenoid (+)
Carbohydrates	Blue color of Fehling's solutions turned brick red ppt.	Presence of reducing sugar (+)	Blue color of Fehling's solutions turned brick red ppt.	Presence of reducing sugar (+)
Carbohydrates	Green color	Presence of reducing sugar (+)	Green color	Presence of reducing sugar (+)
Protein	No violet color	Absence of proteins (-)	No violet color	Absence of proteins (-)

RESULTS AND DISCUSSION:

Table 3: Results

Treatment	Concentration mg/ml	Time taken for paralysis (minutes)	Time taken for death (minutes)
Control (normal saline)	-	-	-
Methanolic extract (MetE)	50	20.58 ± 0.56	41.28 ± 0.42
	100	15.07 ± 0.49	25.27 ± 0.35
	200	4.27 ± 0.23	18.32 ± 0.15
Aqueous extract (AqE)	50	74 ± 0.47	130 ± 0.66
	100	55 ± 0.71	96 ± 0.55
	200	35 ± 0.33	50 ± 0.29
Albendazole	50	38 ± 0.60	73 ± 1.52
	100	25 ± 0.57	75 ± 1.51

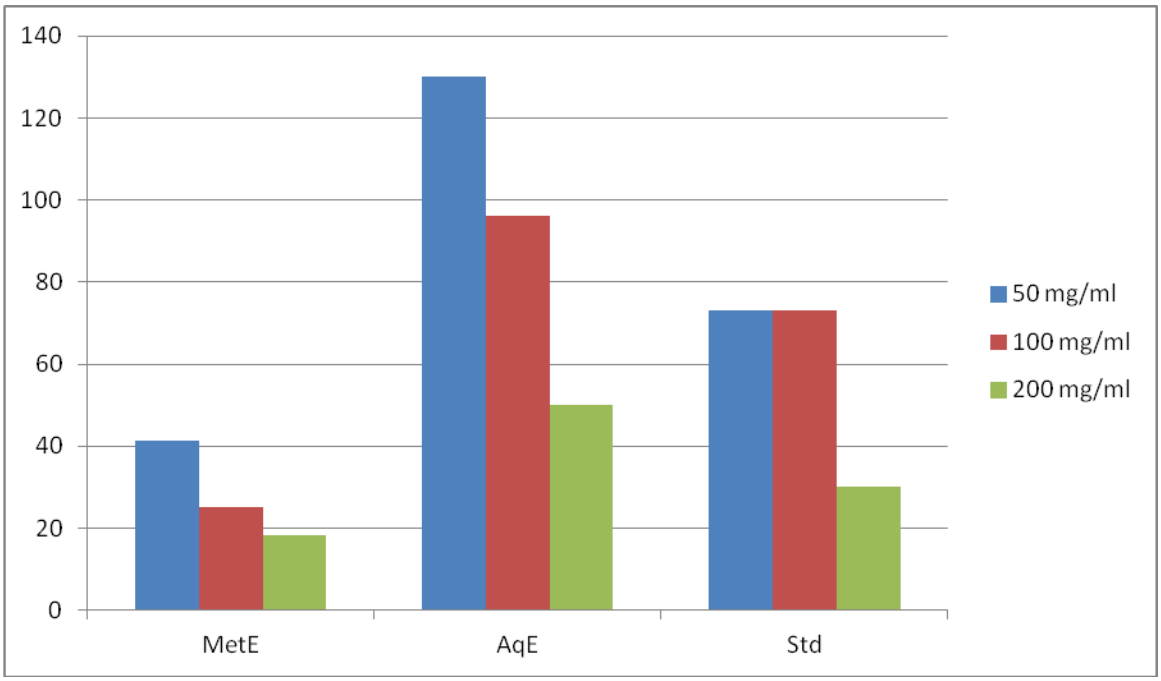
Rauwolfia serpentina (Paralysis)



Graph 1: *Time taken for paralysis after treatment with different extracts*

Where MetE- Methanolic extract, AqE- Aqueous extract, Std- standard Albendazole drug and the number represents paralysis time in minutes.

Rauwolfia serpentina (Death)



Graph 2:*time taken for death after treatment with different extracts*

Where MetE- Methanolic extract, AqE- Aqueous extract, Std- standard Albendazole drug and the number represents death time in minutes.

Preliminary phytochemical screening of plant of Rauwolfia serpentina showed the presence of alkaloids, tannins, flavonoids, terpenoids and carbohydrates

Methanoilc and aqueous extract of Rauwolfia serpentina exhibited the antihelmenthic activity in dose dependent manner giving shortest time of paralyses (p) and death (d) with 200mg/ml concentration. The methanoilic extract of Rauwolfia serpentina caused paralysis of 4.27 min and time of death of 18.32 min while aqueous extract reveiled paralysis and death of 35 and 50 min respectively against the earthworm Pheretima posthuma. The std. Drug Albendazole showed the same at 14 min and 30 min respectively

In anti helmenthic assay the methanolic and aqueous extract showed paralysis and death of worms. The effect was dose dependent and the effect was observed in case of methanolic extract at 200mg per ml conc. with potent activity aganist Pheretima posthuma and the results were comparable with std. drug.

As displayed in graph 1 and graph 2 , methanolic and aqueous extract of Rauwolfia serpentine exhibited significant antihelmenthic activity in dose dependent when compared with std. drug Albendazole. Methanolic extract in conc. of 200 mg/ml was found to be paralysed and death of worm in 4.27 min and 18.32 min respectively which is potentially more effective when compared to AqE with std. drug Albendazole.

The compound constituents responsible for antihelmintic activity were not investigated. However, preliminary phytochemical screening of MetE gave positive test for alkaloids, tannins, flavonoids, terpenoids and carbohydrates. The activity of root methanolic extract may attribute due to presence of tannins, flavonoids, terpenoids and carbohydrates which are reported of Rauwolfia serpentina.

These factors paved the way for herbal remedies as alternative anthelmintic evaluation of activities of medicinal plants claimed for possessing the anthelmintic property is getting attention these days. Screening and proper evaluation of the claimed medicinal plants could offer possible alternatives that may be both sustainable and environmentally acceptable. The results of this study showed the promising anthelmintic activity suggesting possible use of Rauwolfia serpentina extracts in intestinal nematode control.

CONCLUSION:

The results of present study clearly indicate that crude methanolic extract of Rauwolfia serpentina root that produce anthelmintic activity against Indian earthworm (Pheretima posthuma). The plant possesses significant anthelmintic activity at 200 mg/ml concentration measured by time taken for paralysis or death of earthworms. The current investigations leads to conclusion that the root of Rauwolfia serpentina have potent anthelmintic activity when compared with conventionally used drugs.

The present study was carried out to evaluate anthelmintic activities of two different extract of Rauwolfia serpentina. Methanolic extract of Rauwolfia serpentina showed better anthelmintic activity when compared to aqueous extract.

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