



## ***IN VITRO* ANTHELMINTIC ACTIVITY OF *ROTULA AQUATICA* LOUR BARK**

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### **ABSTRACT**

The World Health Organization estimates that a staggering two billion people harbor parasitic worm infections. Despite this prevalence of parasitic infections, the research on anthelmintic drug is poor. As per WHO, only few drugs are frequently used in the treatment of helminthes in human beings. Anthelmintics from the natural sources may play a key role in the treatment of these parasite infections. In view of this, an attempt has been made to study the anthelmintic potential of methanolic extract of *Rotula aquatica Lour* bark using adult earthworms. *Rotula aquatica Lour* belongs to family *borogenaceae* known in Ayurveda as pashanbed. The phytochemical investigation of methanolic extract of *Rotula aquatica Lour* bark reveals the presence of alkaloids, flavonoids and phenolic compounds. Various concentrations (10, 20, 30, 40, 50mg/ml) of methanolic extract of *Rotula aquatica Lour* bark were evaluated for the anthelmintic activity on adult Indian earthworm *Phertima posthuma* and results were expressed in terms of time for paralysis and time for death of worms. Albendazole was used as a reference standard and 1% acacia as a control group. The methanolic extract of bark exhibited not only a dose-dependent spontaneous paralysis (Vermifuge) but also kill the earthworms (Vermicidal). The result shows that the methanolic extract possesses wormicidal activity and thus, may be useful as an anthelmintic.

**Keywords:** Methanolic extract, *Rotula aquatica Lour*, bark, Anthelmintic activity, Earthworms.

### **INTRODUCTION**

Anthelmintics are drugs that either kill or expel infesting helminthes (worms). Helminthiasis is a disease in which a part of the body is infected with worms such as pinworm, roundworm or tapeworm. Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver and other organs<sup>[1]</sup>.

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*Rotula aquatica* Lour belongs to family *Borogenaceae*, known as pashanbed in Ayurveda. It is widely distributed in India from Kumoun to Assam and western to southern India. The plant is a mandatory component of many ayurvedic drug preparations and is an important traditional medicine for kidney and bladder stones. The root and tuber are astringent, bitter, diuretic and also useful in treating coughs, heart diseases, dysuria, blood disorders, fever, poisonings, ulcers and uterine diseases. Root decoctions are both diuretic and laxative and are used to treat bladder stones and sexually transmitted diseases. In Ayurveda, *Rotula aquatica* Lour has been reported to be used as diabetes <sup>[2]</sup>, antilithic effect <sup>[3]</sup>, cardiotoxic activity <sup>[4]</sup>, antiurolithiatic activity <sup>[5]</sup> and is used in stone bladder <sup>[6]</sup>. The plant contains Baunerol <sup>[7]</sup>, steroid alkaloids <sup>[8]</sup> which showed antimutagenic effect. Allantoin <sup>[9]</sup> found in root which is responsible for diuretic activity. The aqueous extract of the root of *Rotula aquatica* Lour showed antioxidant activity. It also contains sterol, rhabdiol <sup>[10]</sup> which is found to be active to induce diuresis <sup>[11]</sup>.

## MATERIAL AND METHODS

### Plant Material

The bark of *Rotula aquatica* Lour was collected from KRS dam, Mysore and authenticated by Dr.P.Santhan, Plant Taxonomist, Natural Remedies private limited (Batch no 556/2012), Bangalore. The barks are cut in to small pieces and dried under sun for seven days and powdered coarsely.

### Preparation of Extract

The powdered plant material was extracted exhaustively with methanol using soxhlet hot extraction process. The solvent was then distilled under reduced pressure, which gave brownish black coloured residue and dried under vacuum dessicator. The percentage yield of extract was found to be 1.56 and extract was subjected to Preliminary phytochemical screening <sup>[12]</sup>. Different concentrations (10, 20, 30, 40, 50mg/ml) of extract solution were prepared by diluting the stock solution in 1% gum Acacia using normal saline. 1% gum acacia in normal saline acts as control and Albendazole 20mg/kg body weight acts as standard <sup>[13]</sup>.

### Earthworms collection and authentication

Adult earthworms (*Pheretima posthuma*) were used to evaluate anthelmintic activity in *vitro*.

Healthy adult Indian earthworms (*Pheretima posthuma*; *Annilida*; *Megaskolesidae*) were collected from Microbial Resources Division, Gandhi Krushi Vignana Kendra (GKVK), Government of Karnataka, Bangalore. The earthworms in moist soil were washed with normal saline to remove all faecal matter. The earthworms of 5-7 cm in length and 0.1-0.2 cm in width were used for all experimental protocol. The earthworm resembles both anatomically and physiologically to the intestinal roundworm parasites of human beings, hence can be used to study the anthelmintic activity<sup>[14]</sup>.

### **Anthelmintic Activity**

The anthelmintic activity was evaluated on adult Indian earthworm *Phertima posthuma* due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings<sup>[15, 16, 17]</sup>. The method of Mathew et al<sup>[18]</sup> and Dash et al<sup>[19, 20]</sup> was followed for anthelmintic screening. Seven groups, each consisting of six earthworms of approximately equal size were released in to 20 ml of the extract suspended in 1% Acacia gum in normal saline. Each group were treated with one of the following: vehicle 1% gum acacia in normal saline, Albendazole 20 mg/ml and extract contains 10, 20, 30, 40, 50mg/ml in normal saline containing 1 % gum Acacia. Observation was made for the time taken to paralyse and /or death of individual worms up to four hours of test period. Paralysis was said to occur when the worms lost their motility followed with fading away of their body colour and finally death. Each experiment was carried out in triplicate and the results were recorded as shown in table No.1.

### **Statistical analysis**

The results were expressed as mean  $\pm$  standard deviation of mean (SD).

### **RESULTS**

Preliminary phytochemical screening of methanolic extract of *Rotula aquatica Lour* bark has shown the presence of alkaloids, Flavonoids and phenolic compounds. Some of these phytoconstituents may be responsible to show a potent anthelmintic activity. The result of anthelmintic activity of methanolic extract of *Rotula aquatica Lour* bark on earthworms *Phertima prosthuma* were given in Table No.1. The methanolic extract of *Rotula aquatica Lour* bark showed moderately significant dose dependent anthelmintic activity.

**TABLE 1: ANTHELMINTIC ACTIVITY OF METHANOLIC EXTRACT OF  
*ROTULA AQUATICA* LOUR BARK ON EARTHWORMS  
*PHERETIMA PROSTHUMA*.**

Treatment	Concentration (mg/ml)	Time taken for paralysis (minutes)	Time taken for death (minutes)
Control(1% acacia)	20 ml	-	-
Albendazole(Standard)	20	25.40±0.53	37.20±0.49
Methanolic extract	10	83.20±0.38	105.1±0.40
Methanolic extract	20	60.31±0.26	71.40±0.31
Methanolic extract	30	51.52±0.74	60.70±0.69
Methanolic extract	40	40.23±0.49	47.38±0.52
Methanolic extract	50	21.35±0.26	28.50±0.29

All the results were expressed as mean  $\pm$  standard deviation of mean (N=6).

## DISCUSSION

The mode of action of Albendazole is to cause paralysis of worms and to expel them in the faeces. Albendazole causes degenerative alterations in the intestinal cells of the worm. Degenerative changes in organs like endoplasmic reticulum, the mitochondria results in decreased production of adenosine triphosphate (ATP), which is the energy required for the survival of the helminthes. Due to diminished energy production, the parasite is immobilized and eventually dies.

Tannins, the secondary metabolite, occur in several plants have been reported to show anthelmintic property by several investigators (Athnasiadou et al; 2001; Waller et al.,1997; Yesilada et al., 1993). Tannins, the polyphenolic compounds, are shown to interfere with energy generation in helminthes parasites by uncoupling oxidative phosphorylation (Martin, 1997) or, binds to the glycoprotein on the cuticle of parasite (Thompson and Geary, 1995), and cause death. Coming to the chemistry of nematode surface, it is a collagen rich extracellular matrix (ECM) providing protective cuticle that forms exoskeleton, and is critical for viability, the collagen is a class of proteins that are modified by a range co-and post –translational modification prior to assembly into higher order complexes (or) ECMS (Page and Winter 2003). The mammalian skin also consists largely of collagen in the form of fibrous bundles. In leather making industry, vegetable tannins are commonly used in the tanning operation of leather processing that imparts stability to collagen of skin matrix through its reactivity and hence make the collagen molecule aggregate into fibres, this results in the loss of flexibility in the collagen matrix

and gain of mechanical property with improved resistance to the thermal (or) microbial/enzymatic attack. Similar kind of reaction is expected to take place between the nematode cuticle (the earth worm) and the tannin of *Rotula aquatica Lour* bark, possibly by linking through hydrogen bonding, as proposed in this study. This form of reactivity brings toughness in the skin and hence the worms become immobile and non-functional leading to paralysis followed by death. Hence further investigation and proper isolation of the active principles might help in the findings of new lead compounds, which will be effective against various parasitic infections.

**Control****Albendazole(20mg/ml)****Extract (10mg/ml)****Extract (20mg/ml)****Extract (30mg/ml)****Extract (40mg/ml)**



*Extract (50mg/ml)*

**Figure 1**

Anthelmintic activity of methanolic extract of *Rotula aquatica* lour bark on earthworms *Pheretima prosthuma*.

## CONCLUSION

Preliminary phytochemical screening of methanolic extract of *Rotula aquatica* Lour bark has shown the presence of alkaloids, flavonoids and phenolic compounds. Some of these phytoconstituents may be responsible to show a potent anthelmintic activity. The methanolic extract of *Rotula aquatica* Lour bark showed moderately significant dose dependent anthelmintic activity against earthworms *Pheretima prosthuma*. The further studies using *in vivo* models are required to carry out and establish the effectiveness and pharmacological rationale for the use of *Rotula aquatica* Lour as an anthelmintic drug. The drug may be further explored for its phytochemical profile to identify the active constituent responsible for anthelmintic activity.

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