Evaluation of *Rhynchosia minima* (Linn.) DC Leaves for Anthelmintic Activity

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Introduction

A large number of medicinal plants are claimed to possess anthelmintic activity in traditional systems of medicine and also utilized by ethnic groups worldwide. Following the folk claims, several medicinal plants, products thereof and isolated phytoprinciples have been scrutinized for their anthelmintic activity to achieve lead molecules in the search of novel anthelmintic drugs (Satyavati, 1990)

Rhynchosia minima (Linn.) DC (Fabaceae) commonly known as Turvel, is a twining or trailing annual distributed throughout India, Sri Lanka and United States (Kirtikar & Basu, 1999). The plant is reported as toxic to fish and used by fishermen. The seeds are bitter and poisonous and seed extract shows specific agglutinating action on human RBC (Patil, 2003). In the folkloric system of medicine leaves of the plant are reported as abortifacient. Decoction prepared from leaves is used by tribals of North Maharashtra region as an abortifacient (Mali et al., 2006). The tribals of Sikkim are using leaves of the plant in the treatment of wounds, helminthic infections and as an abortifacient (Tarafdar, 1983). In Saurashtra region of Gujarat, leaves are utilized by the aborigines for the treatment of asthma and piles (Shah et al., 1981). The phytochemical studies on the plant revealed the presence of steroidal glycoside, ergosterol peroxide, stigmasterol and lupeol (Ahmed et al., 1992). It was also reported to contain C-glycosylflavones, sitosterol, gallic acid, protocatechuic acid (Besson et al., 1977) and hydroquinone diacetate (Krishnamurthy et al., 1975). Continuing our screenings of indigenous medicinal plants for their anthelmintic activity (Mali et al., 2004, 2005, 2007) in the present communication we evaluated the anthelmintic potential of *R.minima* leaves.

Materials and Methods

The leaves of *R.minima* were collected from the fields near Chopda in October, 2005 and were authenticated at Botanical Survey of India, Koregaon Road, Pune (Voucher specimen No. 107337). A specimen voucher of the plant has been deposited in the Department of Pharmacognosy, College of Pharmacy, Chopda. The leaves were shade dried and then milled to coarse powder by mechanical grinder. The resulting powdered leaf material was then successively extracted with petroleum ether ($40\text{-}60^0$), chloroform, ethanol and water in a soxhlet apparatus. The liquid extracts were concentrated separately under vacuum and the resulting dried extracts were preserved in a desiccator until further use. Preliminary phytochemical tests of all extracts were performed by using specific reagents through standard procedures.

The adult Indian earthworm *Pheretima posthuma* L.Vaill (Annelida) were collected from the water logged areas of soil and *Ascardia galli* Schrank (Nematode) worms were obtained from freshly slaughtered fowls *Gallus gallus* Spadiceus (Phasianidae) and immediately transferred to Tyrode solution. Both worm types were identified at the P.G. Department of Zoology, Pratap College, Amalner, Maharashtra

Anthelmintic Assay

The anthelmintic assay was carried as per the method of Ajaiyeoba et al., (2001) with necessary modifications. Formulations (50 ml) containing different concentrations of crude extracts (25, 50 and 100 mg/ml in distilled water) were prepared and six worms (same type) were placed in them. This was done for both worms. Time taken by worms for paralysis was noted when no movement of 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.

Results and Discussion

The qualitative phytochemical investigation of different extracts of *R.minima* showed the presence of an array of active chemical constituents including alkaloids, glycosides, flavonoids, sterols and tannins (**Table 1**). The results of anthelmintic activity revealed that petroleum ether, chloroform, ethanol and aqueous extracts exhibited varying degree of activity against both the worms and caused paralysis followed by death at all tested concentrations. However, aqueous and ethanolic extracts of the plant exhibited more potent activity at higher concentration (100mg/ml) when compared to the reference standard piperazine citrate (10 mg/ml).

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As shown in **Table 2** both the extracts showed anthelmintic activity in dose-dependant manner giving shortest time of paralysis (P) and death (D) with 100 mg/ml concentration, for both the worms. The ethanolic extract of *R. minima* caused paralysis in 13 min and death in 31 min while aqueous extract showed P and D in 11 and 41 min, respectively against the earthworm *P. posthuma*. The reference drug piperazine citrate (10 mg/ml) showed the same at 23 and 60 min.

Ascardia galli worms were also shown sensitivity to the ethanolic and aqueous extracts of *R.minima*. The ethanolic extract caused paralysis in 11min and death in 21 min and aqueous extract displayed P and D in 12 and 31 min, respectively at higher concentration of 100 mg/ml. Piperazine citrate did the same in 16 and 33 min.

The assay was performed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite *Ascaris lumbricoids*, of human beings (Vidyarthi, 1967; Thorn et al., 1977; Vigar, 1984; Chatterjee, 1967). Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in *vitro* (Sollmann, 1918; Jain et al., 1972; Dash et al., 2002; Szewezuk et al., 2003; Shivkar et al., 2003). A*scardia galli* worms are easily available from freshly slaughtered fowls and its use, as a suitable model for screening of anthelmintic drug was advocated earlier (Kaushik et al., 1974; Lal et al., 1976; Tandon et al., 1997). The predominant effect of piperazine citrate on worm is to cause flaccid paralysis that result in expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis (Martin, 1985). In earlier report, alcoholic extract of *R.minima* was evaluated for its molluscicidal activity against the snail *Biomphalaria alexandrina* and showed moderate activity when compared with reference standard niclosamide (Abdel-Hamid, 1997). The aqueous extract (0.25-50 mg/ml) of the plant was tested for anthelmintic activity using the nematode *Caenorhabditis elegans* and found less effective in the model test system used (Ibrahim, 1992).

Table 1 Preliminary phytochemical screening of various extract of *R.minima.*

Tests	Pet.ether extract	Chloroform extract	Ethanol extract	Aqueous extract
Akaloids	–	–	+	+
Flavonoids	+	–	+	+
Tannins	–	–	+	+
Terpenoids	+	+	+	+
Steroids	+	+	–	–
Glycosides	–	–	+	+

(+) = Present, (–) = Absent

Table 2 Anthelmintic activity of various extract of *R.minima.*

Test substance	Concentration mg/ml	Time taken for Paralysis (P) and Death (D) of worms in minute			
		P. posthuma		*A. galli*	
		P	D	P	D
Petroleum ether extract	25	34 ± 0.3	84 ± 0.7	2 ± 0.1	48 ± 0.9
	50	28 ± 0.2	65 ± 0.7	21 ± 0.8	39 ± 0.1
	100	22 ± 0.9	58 ± 0.5	17 ± 0.1	32 ± 0.2
Chloroform extract	25	37 ± 0.3	69 ± 0.2	29 ± 0.8	49 ± 0.3
	50	29 ± 0.4	60 ± 0.1	23 ± 0.6	37 ± 0.4
	100	24 ± 0.5	59 ± 0.3	18 ± 0.7	30 ± 0.1
Ethanol extract	25	28 ± 0.2	65 ± 0.3	19 ± 0.1	40 ± 0.2
	50	19 ± 0.2**	44 ± 0.5***	15 ± 0.1	29 ± 0.3**
	100	13 ± 0.1***	31 ± 0.2***	11 ± 0.5***	21 ± 0.2***
Aqueous extract	25	28 ± 0.1	68 ± 0.7	22 ± 0.6	44 ± 0.2
	50	25 ± 0.7	59 ± 0.2	19 ± 0.8*	38 ± 0.1
	100	11 ± 0.4***	41 ± 0.3***	12 ± 0.2**	31 ± 0.4*
Piperazine citrate	10	23 ± 0.7	60 ± 0.5	16 ± 0.4	33 ± 0.4

All values represent 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

However, in the present assay the extracts of *R.minima* not only demonstrated paralysis, but also caused death of worms especially at higher concentration, 100 mg/ml in shorter time as compared to reference drug piperazine citrate. The anthelmintic activity of *R. minima* may be attributed to the presence of active components such as flavonoids, tannins and terpenoids, the anthelmintic properties of which are well documented (Niezen et al., 1995; Lahlou, 2002). From the results reported here, it can be concluded that the plant *R.minima* has significant anthelmintic activity. Further it would be interesting to isolate the possible constituents those are responsible for anthelmintic activity and establish the possible mechanism (s) of action. We are working on isolation of anthelmintic compounds from these extracts and this will be reported at a later date.

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