



## Short Communication

### Anthelmintic Activity of *Murraya Koenigii* (L) Fruits Extract on Indian Earthworm

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#### ABSTRACT

Investigation of ethanolic and petroleum ether extracts from the *Murraya koenigii* fruits (MKF) for their anthelmintic activity against Indian earthworm (*Pheretima posthuma*). Four concentrations (50, 100, 150 and 200 mg/ml) of each extracts were studied in activity, which involved the determination of time of paralysis and time of death of the worm. Piperazine citrate (15 mg/ml) was taken as a reference standard drug and distilled water as control. When the dose of extract gradually increased *Murraya Koenigii* shows gradual increase in anthelmintic activity characterized by paralysis of earthworm and its death. The ethanolic extract showed better anthelmintic activity as compared with petroleum ether extract. From the results of the present study, it concluded that the ethanolic and petroleum ether extracts of *Murraya koenigii* fruit possesses anthelmintic activity. The present study is further supported by isolation of two carbazole alkaloids from fruit extract which may be responsible for the anthelmintic activity.

**Key words:** *Murraya koenigii* fruits, Anthelmintics, *Pheretima posthuma*, Paralysis, Death

#### INTRODUCTION

The most common and important disease in ruminants and human being are parasitic nematodes (McLeod, 1995) globally these infections cause severe health problems in man and domestic animals, especially in developing countries. More than 1 billion people are infected with *Ascaris lumbricoides* and hundreds of millions are infected with hookworms and *Trichuris* (Gyatt and Evans, 1992).

The helminths mainly subsist in human body in intestinal tract, but they are also found in tissue, as their larvae migrate towards them (Tripathi, 2003). Unfortunately many developing countries cannot afford commercially available modern anthelmintics. Therefore tradition of using medical plants, some of which have been claimed, to possess anthelmintic activities (Perry, 1980; Dharma, 1985).

*Murraya koenigii* commonly known as Kadi patta or curry leaves belongs to family rutaceae. Traditionally it is used as tonic, antibacterial, stomachic, and carminative. (Manwal and Sarin, 2011). Medicinally it has inotropic, hypoglycemic, hypolipidemic, analgesic, and antioxidant properties (Tembhrune and Sakarkar, 2012), (Tembhrune and Sakarkar, 2010a, 2010b). Many reports on *Murraya koenigii* (Linn) leaves claimed as anthelmintic (Monterio *et al.*, 1997; Kumar *et al.*, 2011; Tapas and Sundar, 2011), but the same activity on fruit extract is not yet studied.

Thus, the present study was design to evaluate the *in-vitro* anthelmintic activity of petroleum ether and ethanolic extract of *Murraya koenigii* fruits.

#### MATERIALS AND METHODS

**Plant:** The fruits of *Murraya koenigii* were collected from the region of Yavatmal district, Maharashtra, India during the month of June to September 2012. The fruits were authenticated by Dr. NM Dongarwar, Head of the Department; Botany Department, RTM Nagpur University, Nagpur. A voucher specimen (No. 9916) was deposited at Herbarium, Department of Botany, RTM Nagpur University Nagpur, India.

**Experimental worms:** Indian adult earthworms (*Pheretima posthuma*) were selected for the study due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings (Vigar, 1984; Sharma *et al.*, 2010) which were collected from moist soil and washed with normal saline to remove all fecal matter. The earthworms selected were of 5-7 cm in length & 0.1-0.2 cm in width.

**Material:** Ethanolic and petroleum ether extracts of *Murraya koenigii* fruits, Piperazine citrate (GlaxoSmith Kline Pharmaceuticals Ltd, Mumbai).

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### Extraction and isolation

*Murraya Koenigii* fruits (1kg) were dried under shade and crushed in electric blender to form coarse powder and subjected to extraction by using Soxhlet's extractor at room temperature using petroleum ether and alcohol as solvents. The percent yield of ethanolic extract was 2.9% w/w and petroleum ether (60 Grade) extract yield 5.1% w/w. Both the extracts were concentrated by evaporation at room temperature and brown-green colored viscous residue was used for pharmacological studies.

### Isolation

The ethanolic extract was evaporated under reduced pressure to give a residue (20 g), which was loaded onto a silica gel column (60-120 mesh), and the column was eluted with a gradient of a n-hexane-ethyl acetate (0-100%), which afforded several fractions, which were pooled based on their analytical TLC results. The fractions obtained with the mixture of n-hexane-ethyl acetate (92:8 v/v) were further chromatographed with a gradient elution using a mixture of n-hexane-CHCl<sub>3</sub> (88:12 and 86:14 v/v) to afford two fractions, labeled as MKF1 and MKF2. Fraction MKF1 was subjected to preparative TLC (PTLC) using n-hexane-chloroform (85:15 v/v) as the mobile phase in order to purify compound 1 (13.6 mg, Rf = 0.76, 0.00068%). PTLC of MKF2 using n-hexane-chloroform (80:20 v/v) as mobile phase yielded compound 2 (15 mg, Rf=0.54, 0.00075%).

**Compound 1:** White powder IR  $\nu_{\max}(\text{cm}^{-1})$  3309,2920, 2851,1647,1610,1496,1377,1346,1251,1198,1142,1056,979,845,746,679. MS m/z (% intensity) 331(M<sup>+</sup>,basepeak),319.3,248.1, 182.1,170.4. <sup>1</sup>H NMR: 1.25 (3H, s, 3-CH<sub>3</sub>), 1.45, (each 3H, br s, 4-CH<sub>3</sub>), 1.73- 1.79 (2H, m, H-1), 2.12-2.21 (2H, m, H-2), 2.33 (3H, s, 5-CH<sub>3</sub> J 3.10), 5.11 (1H, t, H-3, J 1.11), 5.64,5.68 (1H, d, J 1.099 Hz H-2), 6.63,6.67 (1H, d, J 1.09 Hz, H-1), 7.14-7.17 (1H, br t, J 1.42 Hz, H-8), 7.30-7.32 (1H, br t, J 0.921 Hz, H-9), 7.36 (1H, br d, J 1.0 Hz, H-10), 7.661 (1H, s, H-6), 7.861 (1H, br s, NH), 7.89-7.92 (1H, br d, J 1.079 Hz, H-7),

**Compound 2 :**White powder IR  $\nu_{\max}(\text{cm}^{-1})$  3402,2915,2848,1645,1582,1456,1206,1139,1139,1108,1055,808,718. MS m/z(% intensity) 116.1(M<sup>+</sup> base peak),293.7,278.8,266.9,244.4, 212 .<sup>1</sup>H NMR: 1.253 (3H, s, 3-CH<sub>3</sub>), 1.48, (each 3H, br s, 4-CH<sub>3</sub>), 2.32 (3H, s, 5-CH<sub>3</sub>), 3.90(s, -OCH<sub>3</sub>) 5.67,5.70 (1H, d, J 1.00 Hz H-2), 6.59,6.62 (1H, d, J 1.01 Hz, H-1),6.91( d, J 1.05 Hz) 7.40,7.41( 1H,s,) 7.62 (1H, s,) 7.69 (1H, br s, NH),

**Phytochemical analysis:** The extract was subjected to various phytochemical tests to determine the nature of constituents of the extract and two carbazole alkaloids isolated from extracts, were analysed by IR, MASS and NMR spectroscopic methods.

**Administration of piperazine citrate:** Piperazine citrate (15 mg/ml) was prepared by using 0.2% v/v of Tween-20 as a suspending agent.

**Administration of extract:** Different concentrations of both extracts of MKF (50-200mg/ml) were prepared by using 0.2% v/v of tween-20 as a suspending agent and

final volume was made to 10 ml for respective concentration.

### Experimental design

Anthelmintic activity was carried out on Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal water to remove all fecal matter. The anthelmintic assay was carried out as per our previous reported methods (Vidyarthi and Pandey, 2005; Chandan *et al.*, 2011). Ten groups of approximately equal size worms consisting of six earthworms individually in each group were released into 10 ml of desired concentration of standard drug and both the extracts of MKF in petri plate.

Observations were made for the time taken for paralysis and death of individual worms. Paralysis was said to occur when worm did not revive in normal saline and time for death was recorded after concluding that worms neither moved when shaken nor when dipped in warm water (50<sup>o</sup>c), and their body colors was found to be faded (Hood *et al.*, 2011; Kelly and Hall, 1979). All the results were expressed as mean  $\pm$  S.D. of six worms in each group.

### RESULTS

The percentage recovery of extracts (extractability) was calculated by using following formula

% recovery (for ethanolic extract) = (Collected Mass / Initial Mass) x 100% = % = (29 gm / 1000 gm) x 100% = 2.9%

% recovery (for petroleum ether extract) = (Collected Mass / Initial Mass) x 100% = % = (51 gm / 1000 gm) x 100% = 5.1%

Various phytochemical tests performed on extracts of *Murraya koenigii* fruits shows positive results for alkaloids, flavonoid and phenolic contents. Table 1 illustrates the presence of various phytoconstituents. Table 2 explains anthelmintic activity of ethanolic and petroleum ether extracts of *Murraya koenigii* fruits when compared to standard drug. Ethanolic extract of *Murraya koenigii* fruits (50, 100, 150, 200 mg/ml) showed paralysis at 51.66, 33.00, 25.00, and 23.33 min respectively and death time found at 159.33, 134.66, 101.00, and 95.33 min., for respective concentration. While at same concentration the petroleum ether extract of *Murraya koenigii* fruits shows paralysis at 65.66, 54.66, 45.33, and 30.00 min respectively and death at 200.33, 180.66, 161.33, and 112.33 min. The standard drug Piperazine citrate (15mg/ml) showed paralysis at 19.32 min and death after 56.43 min.

**Table 1:** Phytochemical evaluation of fruit extracts of *Murraya koenigii*

Sr. No.	Compound	Test	Inference
1	Flavonoids	Shinoda test	+
2	Alkaloid	Lead acetate Wagner	+
3	Phenolic content	Dragendorffs Ferric chloride test Liebermann's test	+ + +

+ indicates the positive test; - indicates negative test

**Table 2:** Anthelmintic activity of *Murraya koenigii* fruit extracts

Treatment	Group	Concentration (mg/ml)	Time of paralysis (min)	Time of death (min)
Normal saline (Control)	I	--	--	--
Piperazine citrate (Std.)	II	15	19.32± 3.51	56.43±4.50
Ethanollic extract of <i>Murraya koenigii</i>	III	50	51.66±4.10	159.33±2.86
	IV	100	33.00±1.66	134.66±3.39
	V	150	25.00±0.82	101.00±3.26
	VI	200	23.33±0.72	85.33±3.31
Petroleum ether extract of <i>Murraya koenigii</i>	VII	50	65.66±3.3	200.33±1.02
	VIII	100	54.66±1.7	180.66±0.94
	IX	150	45.33±2.05	161.33±3.39
	X	200	30±2.01	112.33±2.05

All the values are given in mean ± SD (standard deviation); N=6 earthworms individually in each group were released into 10 ml of desired concentration of respective drugs in Petri plate.

## DISCUSSION

Infections of parasitic worms are harmful to human beings. Juvenile parasites can invade human beings through skin or gastrointestinal tract (GIT) and develop into adult worms. Anthelmintics are drugs that act locally to eject worms from the GIT or systemically to eliminate adult worms or inhibit development of worms. (Jain and Jain, 1972; Dash *et al.*, 2002). From the result, it is clear that ethanolic and petroleum ether extract of *Murraya koenigii* fruits have significant anthelmintic activity in dose dependent manner when compared with standard anthelmintic drug. Data in the table no.1 illustrates that the ethanolic extracts of *Murraya koenigii* fruits took the less time to cause paralysis of the earthworm than that of petroleum ether extract.

Thus, from results the traditional claim of *Murraya koenigii* as an anthelmintic have been confirmed and fruit extracts as well displayed activity against the worm used in present study. The presence of carbazole alkaloids may be responsible for anthelmintic activities which are isolated from extracts of *Murraya koenigii* fruits. Their presence is verified by IR, MASS & NMR analysis.

The possible mechanism of the anthelmintics activity of *Murraya koenigii* cannot be explained on the basis of the present results. However, it may be due to its effect on inhibition of glucose uptake in the parasites and depletion of its glycogen synthesis (Singh *et al.*, 2002), (Goodman and Gilman, 2001).

## Conclusion

From the results of the present study, it concluded that the ethanolic and petroleum ether extracts of *Murraya koenigii* fruit possesses anthelmintic activity.

The present study is further supported by isolation of two carbazole alkaloids from fruit extract which may be responsible for the anthelmintic activity. While there is need for further study to isolate and reveal the active compound contained in the crude extract of *Murraya koenigii* fruits as well as to establish its mechanism(s) of action.

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