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In-Vitro anthelmintic activity of *Kaempferia rotunda*S. Agrawal^{1*}, A. Bhawsar², P. Choudhary², S. Singh², N. Keskar³ and M. Chaturvedi²

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Abstract

Alcoholic extract from the rhizomes of *Kaempferia rotunda* Linn was investigated for their anthelmintic activity against *Pheretima posthuma* and *Ascardia galli*. Three concentrations (25, 50 and 100 mg/ml) of extract were studied in activity, which involved the determination of time of paralysis and time of death of worm. The alcoholic extract exhibited significant Anthelmintic activity at highest concentration of 100mg/ml Piperazine citrate in same concentration as that of extract was included as standard reference and distilled water as control. The Anthelmintic activity of alcoholic extract of *Kaempferia rotunda* has therefore been demonstrated for the first time.

Key-Words: Anthelmintic activity, *Ascardia galli*, *Kaempferia rotunda*, *Pheretima posthuma*

Introduction

The plant *Kaempferia rotunda* Linn belongs to the family Zingiberaceae also named bhuichampaka (Sanskrit), bhuchampa (Hindi), and blackhorm (English). It is a fragrant aromatic herb with a tuberous rhizome distributed throughout India. In some districts of Maharashtra the powder root is popular in mumps and also said to be used in the form of poultice, promotes suppuration. The main constituent crotopoxide is useful for the inhibition of tumors¹.

Phytochemical the plant has been attributed to contain flavonoids, crotopoxide, chalcones, quercetin, flavonols, β -sitosterols, stigmasterol, syringic acid, protocatechuic acid and some hydrocarbons have been previously reported². Literature survey revealed that the plant extract has yet not been investigated screened for its traditional claim of Anthelmintic activity. Therefore the objective of this work was to explore the Anthelmintic activity of *Kaempferia rotunda* Linn³.

Material and Methods**Extraction of plant material**

The tuberous rhizomes of *Kaempferia rotunda* Linn were collected during July-August from the various regions of Sikkim Himalayan region and authenticated by Botanical survey of India, Gangtok, Sikkim. The dried, powdered rhizomes were subjected to soxhlet extraction successively using methanol. The extract was filtered, concentrated in vacuum under reduced pressure. The yield value was found to be 8.5%. The extract was subjected to qualitative chemical investigation for phytochemical constituents like flavonoids, steroids, triterpenoids, and crotopoxide.

Experimental

Methanolic extract from the rhizomes of *Kaempferia rotunda* were investigated for their Anthelmintic activity against *Pheretima posthuma* and *Ascardia galli*. Various concentrations (10-100 mg/ml) of extract were tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. Piperazine citrate was included as standard reference and distilled water as control.

The Anthelmintic assay was carried as per the method of Deore S.L.⁴ with minor modifications. The assay was performed on adult Indian earthworms, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings^(5,6,7,8) because of easy availability, earthworms have been used widely for the initial evaluation of Anthelmintic compounds in vitro (11,12,13,14,15).

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Indian adult earthworms (*Pheretima posthuma*) collected from the moist soil and washed with normal saline to remove all faecal matter were used for the Anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol. *Ascaridia galli* worms are easily available in plenty from freshly slaughtered fowls and their use, as a suitable model for screening of Anthelmintic drug was advocated earlier^(16,17). In the first set of experiment, six groups of six earthworms were released in to 50 ml of solutions of Piperazine citrate, methanolic extracts of rhizomes of *Kaempferia rotunda* Linn (25,50,100 mg/ml) in distilled water. Piperazine citrate was used as reference standard while distilled water as control.

Observations were made for the time taken to paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms are shaken vigorously. Death was concluded when the worms lost their mortality followed with fading away of their body colors. Same experiment was done for *Ascaridia galli* worms only the difference was solution were prepared in normal saline solutions.

Results and Conclusion

Preliminary phytochemical screening of methanolic extract revealed the presence of flavonoids, crotopoxide, chalcones, quercetin, flavonols, β -sitosterols, stigmasterol, syringic acid, protocatechuic acid and some hydrocarbons compounds. From the results shown in table no 1, the predominant effect of Piperazine citrate on the worm is to cause a 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.

The methanolic extract of rhizomes of *Kaempferia rotunda* Linn was demonstrated paralysis as well as death of worms in a less time as compared to Piperazine citrate especially at higher concentration of 100 mg/ml. While water extract also shown significant activity. Phytochemical analysis of the crude extract revealed presence of flavonoids as one of the chemical constituent. Polyphenolic compounds show Anthelmintic activity⁽¹⁷⁾. Some synthetic phenolic Anthelmintics e.g. niclosamide, oxiclozanide and bithionol are shown to interfere with energy generation in helminthes parasites by uncoupling oxidative phosphorylation⁽¹⁸⁾. It is possible that phenolic content in the extract of *Kaempferia rotunda* produced similar effects.

In conclusion, the traditional use of rhizomes of *Kaempferia rotunda* as an Anthelmintic have been confirmed as the rhizomes extract displayed activity against the worms used in the study. Further studies need to establish the mechanisms of action are required.

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Table 1: Anthelmintic activity of extract of *Kaempferia rotunda*

Extract	Concentration mg/ml	<i>Pheretima poshtuma</i>		<i>Ascaridia galli</i>	
		P	D	P	D
ME					
	25	65 ± 0.4	72±0.44	64.04±0.9	79.5±0.23
	50	43 ± 0.3	66±0.11	49.7±0.1	68.2±0.1
	100	33 ± 0.8	33±0.45	34.2±0.6	45.75±0.23
PC	25	1.5 ± 0.7	54.5±0.4	41.23±0.14	54.5±0.4
	50	0.9 ± 0.12	30.2±0.1	29.75±0.5	30.2±0.1
	100	0.5 ± 0.17	18.5±0.8	20.05±0.9	23.5±0.8
Control	-	-	-	-	-

Where, AE: Methanolic extract, PC :Piperazine citrate, P: Time taken for paralysis (min) ,D: Time taken for death of worms (min)