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Evaluation of Anthelmintic Activity of Leaves of *Callistemon citrinus* Curtis

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The anthelmintic activities of different extracts of leaves of *C. citrinus* curtis were evaluated separately on adult Indian earthworm (*Pheretima posthuma*) and the activities were compared with standard drug piperazine citrate and albendazole. It was found that petroleum ether, ethyl acetate and ethanol extract of *C. citrinus* (PECC, EACC, ECC, respectively) exhibited, respectively dose dependent action and inhibition of spontaneous motility (paralysis) and death of earthworms. The results indicated that the PECC was more potent than EACC and ECC.

Key Words: Anthelmintic activity, *C. citrinus*, Piperazine citrate and Albendazole.

INTRODUCTION

Helminthic infections are now being recognized as cause of much chronic ill health and sluggishness amongst the tropical people. More than half of population in the world suffers from worm infection of one or the other. Helminths also affect the domestic animals and livestock causing considerable economic losses. Traditional system of medicine reports the efficacy of several natural products eliminating helminths. Keeping this in view, the present communication deals with the evaluation of anthelmintic activity of leaves of *Callistemon citrinus* curtis.

C. Citrinus curtis (crimson bottle brush, lemon bottlebrush, family: myrtaceae) is an evergreen, ornamental shrub distributed throughout Australia. It is also found near Sagar, M.P and Northern India[1,2]. The leaves of the plant are a tea substitute and have a delightfully refreshing flavour of lemon[3,4]. A tan dye is obtained from the flowers and does not require a mordant[5]. The leaf extract has strong and broad fungitoxic, insecticidal and antimicrobial activities[6-11]. The plant also possess anti-thrombin property[12]. On literature survey, it was found that no detailed study has yet been done regarding the anthelmintic property of leaves of *C. citrinus* curtis. On preliminary testing, it was found that petroleum ether, ethyl acetate and ethanolic extract of leaves of this plant (PECC, EACC, ECC, respectively)

showed significant anthelmintic activity compared to other extracts of it. Hence in the present study, we have evaluated the anthelmintic activity of PECC, EACC and ECC to substantiate the folklore claims.

EXPERIMENTAL

The leaves of *C. citrinus* curtis were collected from Lucknow, India, during the month of September and were authenticated by Dr. H. J.Chaudhary, Joint Director, Central National Herbarium, Botanical Survey of India, Howrah, West Bengal. A voucher specimen has been preserved in our laboratory for further references. After collection, the plant leaves were washed properly and fungal infected leaves were discarded.

Prepration of extracts: Shade dried, powdered, sieved (40 mesh size) plant materials were extracted in succession with petroleum ether (60-80°C), ethyl acetate and ethanol using soxhlet apparatus. The extract was evaporated to dryness. The trace amount of solvent, which may be present with the solid mass of respective extracts, was removed under vaccum. The yield of petroleum ether (PECC), ethyl acetate (EACC) and ethanol (ECC) extraction were 3.9, 3.83 and 21.46 % w/w, respectively, with respect to the dry starting material. On preliminary phytochemical analysis PECC, EACC and ECC showed positive tests for steroid, triterpenoid, steroidal glycosides, flavonoids, alkaloid, glycoside, saponin, tannin, respectively[13-19].

Evaluation of anthelmintic activity: Anthelmintic activity was evaluated for PECC, EACC and ECC separately. The activity was tested according to method described in detail by Kailashraj and Kurup[20]. *Pheritima posthuma* (earthworm obtained from Horticulture Department) of nearly equal size (8 ± 1 cm) were selected for present study due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings[21,22].

Each group was treated with one of the following: vehicle (3% Tween 80 in normal saline), piperazine citrate (15 mg/mL), albendazole (10 mg/mL) and extracts (2.5, 5, 10, 25 and 50 mg/mL) in normal saline containing 3% Tween 80. Observations were made for the time taken to paralyse and/or death of individual worms up to 4 h of test period. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colour[23].

RESULTS AND DISCUSSION

The anthelmintic activity[24,25] of the title compounds on *P. posthuma* is exhibited in Table-1. The perusal of the data reveals that PECC and EACC showed anthelmintic activity at a concentration of 2.5 mg/mL, whereas

ECC showed only paralysis but no mortality at similar concentration. The other test concentration of PECC, EACC and ECC showed considerable degree of anthelmintic activity. The anthelmintic effect of PECC and EACC at 25 mg/mL concentrations is comparable with that of the effect produced by the reference standards albendazole and piperazine citrate. However, the ECC showed the effect at 50 mg/mL concentration that is comparable with the reference standards.

TABLE- 1
ANTHELMINTIC ACTIVITY OF LEAVES OF *CALLISTEMON CITRINUS* CURTIS

Compound	Concentrative (mg/mL)	Time (min)	
		For paralysis	Death
Control (Normal saline)	9	–	–
Albendazole	10	34.63 ± 0.71	63.80 ± 0.75
Piperazine citrate	15	18.59 ± 0.33	–
PECC	2.5	56.25 ± 0.55	65.32 ± 0.61
	5	43.21 ± 0.31	50.05 ± 0.52
	10	34.32 ± 0.50	44.15 ± 0.51
	25	19.02 ± 0.21	32.23 ± 0.54
	50	14.36 ± 0.17	24.02 ± 0.26
EACC	2.5	135.84 ± 2.30	145.40 ± 3.15
	5	107.41 ± 0.82	135.20 ± 2.38
	10	69.17 ± 0.75	107.32 ± 1.99
	25	22.00 ± 0.21	41.15 ± 0.22
	50	11.32 ± 0.15	23.37 ± 0.19
ECC	2.5	150.23 ± 3.50	–
	5	116.14 ± 2.01	171.48 ± 4.00
	10	66.23 ± 0.76	138.59 ± 2.39
	25	46.02 ± 0.32	84.20 ± 0.29
	50	19.58 ± 0.23	27.51 ± 0.27

Results are expressed as mean ± SEM from six observations.
PECC = petroleum ether extract of *C. citrinus*, EACC = ethyl acetate extract of *C. citrinus*, ECC = ethanolic extract of *C. citrinus*.

The present study therefore reveals that the PECC was more potent than the EACC and ECC, even though all the three extracts were endowed with anthelmintic property. The activity reveals concentration dependent nature of the different extracts. Potency of the extracts was found to be inversely proportional to the time taken for paralysis/death of the worms.

The above findings justify the anthelmintic properties of the leaves, which augment its use by the tribes of Northern India. Further studies regarding the isolation and characterization of the active principle(s) respon-

sible for anthelmintic activity and their mode of action are currently under progress.

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