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Anthelmintic Activity of Alcoholic and Aqueous Extract of *Anisochilus carnosus* (Wall).

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ABSTRACT

The present investigation was undertaken to evaluate the anthelmintic activity of ethanolic and aqueous leaf extracts of *Anisochilus carnosus*, using Indian earthworm *Pheretima posthuma*. Three different concentrations (50, 25 and 12.5 mg/ml) of each of ethanolic and aqueous extract was used for bioassay, which involved determination of time of paralysis (T_p) and time of death (T_D) of the worms. 1% gum acacia in saline solution and Mebendazole (15mg/ml) were used as control and standard respectively in the study. The results obtained in the present study indicated potential activity at a concentration of 50mg/ml of the crude ethanolic extract when compared to reference standard Mebendazole in terms of T_p and T_D . To conclude, the leaves of *Anisochilus carnosus* can be used as a natural anthelmintic however, advanced studies on the plant can reveal the active principles responsible for the activity.

Keywords: *Anisochilus carnosus*, anthelmintic activity, Mebendazole, *Pheretima posthuma*.

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INTRODUCTION

Anisochilus carnosus (L.F.)Wall. belongs to the family Lamiaceae (Mint family), is an annual herb found in the various high altitude regions of Western Ghats, Tamil Nadu, Maharashtra and Rajasthan [1]. When the leaves of plant rubbed, it emanates a strong, sweet and aromatic odour [2] due to the presence of volatile oils. Volatile oil of *Anisochilus carnosus* contains carvacrol, camphor and α -cis bergamotene [3].The plant is used to treat gastrointestinal diseases such as peptic ulcer, cough, cold and fever. The anti-ulcer and hepatoprotective activities have been reported [4,5].

MATERIAL AND METHODS

Plant material

Anisochilus carnosus leaves were collected in September-October 2011 from Udyavar,Udupi, Karnataka, India, and the plant was identified by Dr. Gopala Krishna Bhat, taxonomist,Poorna Prajna College, Udupi, Karnataka. A voucher specimen (PP 573) has been deposited in the department of Pharmacognosy, Manipal College of Pharmaceutical sciences, Manipal, Karnataka, India.

Preparation of ethanolic and aqueous extract

The leaves of *Anisochilus carnosus* were shade dried and powdered. 100 g of the powder was extracted exhaustively with ethanol by hot extraction process (Soxhlet). The solvent from the extract was recovered by distillation and was concentrated in *vacuo*. Aqueous extract from the plant material was prepared by maceration; soaking 100g of the powdered leaves in chloroform water (2.5 ml in 1000 ml) for 5 days. The macerate was filtered, concentrated on a water bath to dryness and stored in desiccator until further use.

Preparation of the test and standard samples

The test samples from ethanolic and aqueous extracts were prepared at different concentrations, 50mg/ml, 25mg/ml and 12.5mg/ml of each in normal saline containing 1% gum acacia. A 15mg/ml suspension of Mebendazole in normal saline containing 1% gum acacia was used as a standard, while normal saline with 1% gum acacia served as normal control.

Worm collection and authentication

Pheretima posthuma (Indian earthworm, phylum: Annelida) were obtained from Vermiculture lab Manipal. It was identified by Professor N.A. Madhyastha, Poorna Prajna College, Udupi, Karnataka, India.

Antihelmintic activity

The anthelmintic assay was carried as per the method of Mathew *et al.*, [6] and Dash *et al.* [7,8] with necessary modifications. Five groups of approximately equal size Indian

earthworms consisting six worm sin each group were released in 50 ml of different concentrations of crude extracts (50, 25, and 12.5 mg/ml),15mg/ml suspension of Mebendazole and normal saline containing 1% gum acacia. The time of paralysis (T_p) was noted when no movement of the worm was observed except when the worms were shaken vigorously. Time for death (T_D) was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50 °C).

Statistical analysis

The data obtained was statistically analyzed by oneway analysis of variance (ANOVA), followed by a post hoc sheffe’s test using SPSS statistical software version 11. The results are expressed as Mean \pm SD.

RESULTS AND DISCUSSION

The ethanolic extract of *Anisochilus carnosus* exhibits significant anthelmintic activity in dose dependent manner. At a dose of 50 mg/ml the ethanolic extract caused paralysis at a T_p of 13.55 ± 0.49 and death at T_D 27.56 ± 0.427 .The aqueous extracton the other hand showed a T_p of 21.56 ± 0.743 min and death at T_D 29.87 ± 0.525 min.Mebendazole, the reference standard at 15mg/ml showed similar results as that of aqueous extract with T_p and T_D of 20.06 ± 0.050 min and 28.15 ± 0.306 min respectively(results are shown in Table 1 and represented in Fig1).

Fig 1. Anthelmintic activity of ethanolic and aqueous extracts of *A. carnosus*

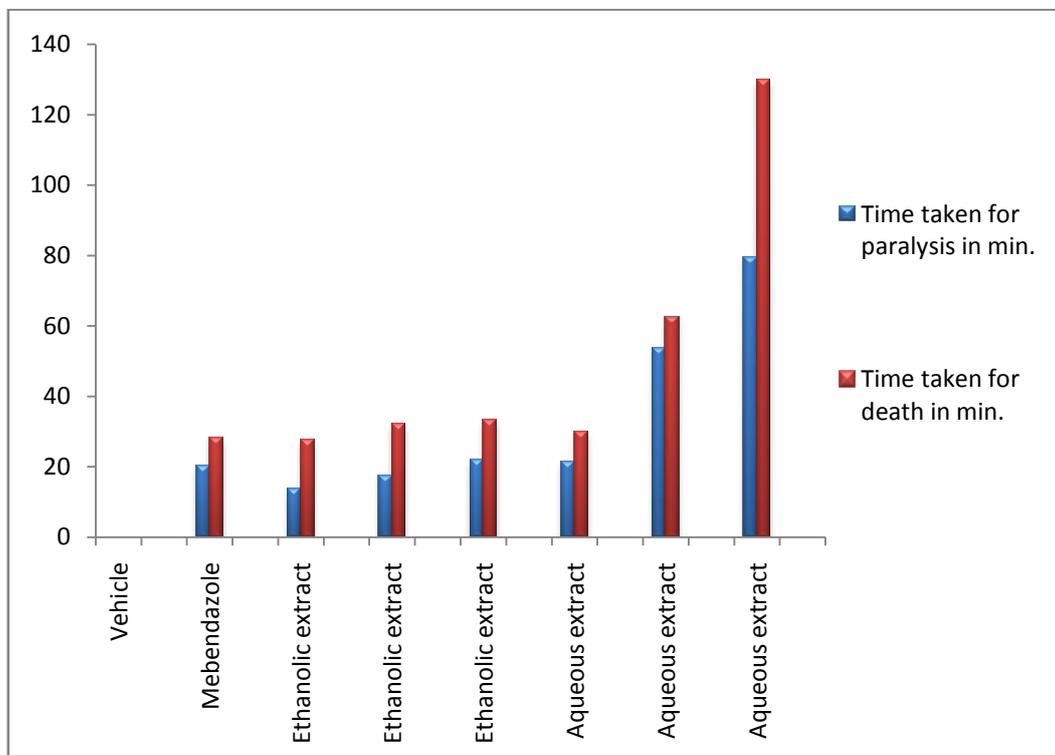


Table 1: Anthelmintic activity of ethanolic and aqueous extracts of *A. carnosus*

Test substance	Concentration in mg/ml	Time taken for paralysis in min.	Time taken for death in min.
Vehicle	-	-	-
Mebendazole	15	20.06 ± 0.050	28.15 ± 0.306
Ethanolic extract	50	13.55 ± 0.499 ^a	27.56 ± 0.427
Ethanolic extract	25	17.43 ± 0.425 ^c	32.04 ± 0.060
Ethanolic extract	12.5	21.9 ± 0.302	33.02 ± 0.030
Aqueous extract	50	21.56 ± 0.743	29.87 ± 0.525
Aqueous extract	25	53.56 ± 0.475	62.59 ± 0.976
Aqueous extract	12.5	79.47 ± 0.952	129.59 ± 0.664

All the value are expressed as mean ± SD (n = 6) a=p<0.001Vs Mebendazole c=p<0.05 Vs Mebendazole

The antihelminthic assay was carried on adult Indian earthworm *Pheretima posthuma* due to anatomical and physiological resemblance with the intestinal roundworm, a parasite that causes infestation in human beings [9,10]. Because of easy availability, earthworms have been widely used for the initial evaluation of anthelmintic activity by *in vitro* methods [11,12].

Mebendazole is known to cause degenerative alteration in the tegument and intestinal cells of worm by binding to the tubulin protein and inhibits its polymerization or assembly into microtubules. It also causes degenerative changes in the endoplasmic reticulum and mitochondria of the germinal layer and decreases the production of ATP, which is required for the survival of the helminth. Due to diminished energy production, the parasite is immobilized and eventually dies [13].

CONCLUSION

In our study ethanolic extract of *A. carnosus* at 50 mg/ml showed a better T_p and T_D when compared to mebendazole, while aqueous extract at 50 mg/ml imparted same effect as that of the standard. Thus the findings suggest that the ethanolic extract of *A. carnosus* can be a significant alternative in the treatment of helminthiasis.

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