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Preliminary phytochemical investigation and anthelmintic activity of *Acanthospermum hispidum* DC.

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Abstract:

Acanthospermum hispidum DC. is found in the tribal area of Koraput and Malkangiri districts and traditionally used by the tribal people as anthelmintic, antidiabetic, antihypertensive, carminative and antifungal. No systematic study was conducted on *A .hispidum* DC. The present work was conducted to investigate the preliminary phytochemical studies and anthelmentic activities on the leaves of *A .hispidum* DC against adult Indian earthworms, *Pheretima postuma.* Various concentrations (5-25 mg/ml) of each extract along with the reference samples (Piperazine citrate, Albendazole) were subjected for anthelmintic activity study. The qualitative test revealed that the petroleum ether extracts contained only terpenoids but chloroform and hydroalcoholic (Ethanol 70% v/v) extracts exhibited the presence of carbohydrates, alkaloids, glycosides, flavonoids, tannins and saponins but amino acids and steroids were absent. All the extracts showed anthelmintic activity when compared with petroleum ether and chloroform extracts. The anthelmintic activity of hydroalcoholic extract was comparable with reference drugs. The data were verified as statistically significant by using one way ANOVA at 5 % level of significance ($p < 0.05$).

Keywords: *Acanthospermum hispidum* DC, *Pheretima postuma,* Anthelmintic, Hydroalcoholic, Albendazole, Piperazine citrate.

Introduction:

Medicinal plants have served through ages, as a constant source of medicaments for the treatment of a variety of diseases [1]. The history of herbal medicine is almost as old as human civilization. The plants are known to provide a rich source of botanical anthelmintics, antibacterials and insecticides [2]. A number of medicinal plants have been used to treat parasitic infections in man and animals. Parasitic helminthes effect the human beings as well as animals leading to considerable hardship and stunted growth. The parasitic invasion is caused by mixed infections with several species of stomach and intestinal worms. Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. Despite extensive use of synthetic chemicals in modern clinical practices all over the world, interest in exploiting potential use of plants [3] as source of drugs are under study.

Acanthospermum hispidum DC belongs to family *Asteraceae* and also called as Bidigadi Kanta (Odisha), Bristly Starbur or Goathead (English), Herbe tricorne (French), Carapichno (Spanish).The plant commonly known as Bristly Starbur, is an upright annual with dichotomous (Y-shaped) branching. The Y-shaped form of branching gives the plant one of its common names, Slingshot Weed. The scientific name of the genus, *Acanthospermum*, is from the Greek words *acantha* (thorn) and *sperma* (seed) and refers to the prickly fruit. *Hispidum* is Latin, and means rough, shaggy, prickly or bristly. The stems are densely covered with hairs. Some leaves can be up to 11.5 cm long. The margins of the leaves can have irregular teeth or they may be entire and smooth. The flowers are typical of the Aster or Daisy Family. Each head has 5-9 ray flowers. The petals (corollas) of the ray flowers are pale yellow and are about 1.5 mm long. The fruits are flattened and triangular in shape. These fruits are covered with stiff, hooked hairs and have either a straight or curved pair of spines at the top. The literature survey reveals that various parts of *A. hispidum* DC have been used as a folklore medicine for

Fig. 1: Leaves and flowers of *Acanthospermum hispidum* DC.

curing various ailments. There are no sufficient reports on systematic and scientific study of anthelmintic activities of leafy extracts. As such; it is proposed to carry out the preliminary phytochemical screening and anthelmintic on the leaves of *A. hispidum* DC.

Materials and Methods:

Plant materials:

The leaves of *A. hispidum* were collected in the month of July 2009, from open field Rondapalli, Koraput (Odisha). The collected plant with complet herbarium was authenticated at Botanical survey of India, Howrah, [CNH/I-I/ (340)/2009/Tech.II/382/90]. The leaves were collected and dried under subdued sun light and then homogenized to get coarse power. The powder was stored in a dessicator for further use.

Preparation of crude extracts:

The powdered mass was exhaustively extracted successively in soxhlet apparatus using solvents like petroleum ether, chloroform and hydroalcohol (Ethanol 70 % v/v) based on their polarity. Finally extracts were concentrated under reduced pressure using rotary evaporator and stored for further analysis.

Preliminary Phytochemicals analysis:

During preliminary phytochemical screening tests were mainly concluded for alkaloids, carbohydrates, saponins, flavonoids, tannins and terpenoids. The constituents are reported in Table 1.

Preliminary Anthelmintic activity screening:

The anthelmintic activity was performed according to the method of Ghosh et al. [4] on adult Indian earthworm *Pheritima posthuma* as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. All the extracts were dissolved in minimum amount of dimethyl sulphoxide and then volume was adjusted with saline water. Three groups were prepared control (saline water), reference samples (piperazine citrate and albendazole at 10 mg/ml and the extracts of (5, 10, 20 and 25 mg/ml). The reference samples and extract solutions were prepared freshly before starting he experiment. Observations were made for the time taken to paralyze or death of individual worms. Paralysis was said to occur when the worms do not receive any sense even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body color, when dipped in warm water (50^0C). The results are shown in Table II depict the time taken for paralysis and death of worms after treatment with the extracts at the selected concentrations.

Statistical analysis

The data on biological studies were reported as mean ± Standard deviation (n = 5). For determining the statistical significance, standard error mean and analysis of variance (ANOVA) at 5 % level significance was employed. The P values < 0.05 were considered as significant [5].

Table 1: Phytochemical qualitative analysis and percentage yield of *Acanthospermum hispidum* DC leaves.

Chemical tests	Extracts		
	Petroleum ether extract (PE)	**Chloroform extract (CE)**	**Hydroalcoholic extract (HE)**
Carbohydrates	-	+	+
Alkaloids	-	+	+
Glycoside	-	+	+
Flavonoids	-	-	+
Tannins	-	+	+
Terpenoids	+	-	-
Saponins	-	-	+
Aminoacids	-	-	-
Steroids	-	-	-
% yield (w/w)	0.328	2.4	6.1

(+) indicates presence, (-) indicates absence.

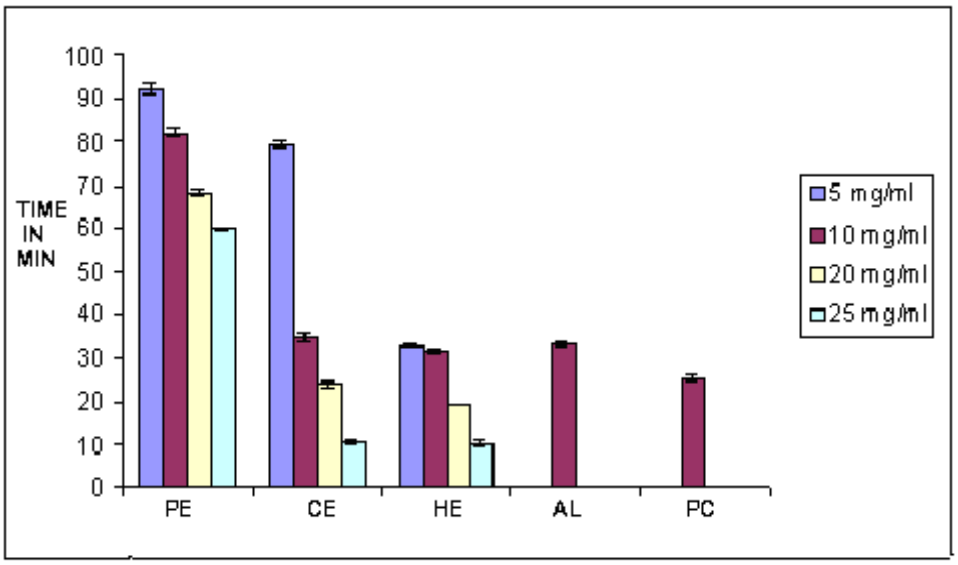
Table 2: Comparative anthelmintic activity of the extracts with reference samples.

Test substances	**Concentration(mg/ml)**	**Time taken for paralysis(min.) (X±SEM)**	**Time taken for death(min.) (X±SEM)**
Control	-	-	-
Pet ether extract(PE)	5	92.25±1.25	138.43±1.01
	10	82.13±0.58	110.25±0.49
	20	68.19±0.51	109.58±0.19
	25	59.73±0.21	98.83±0.73
Chloroform extract(CE)	5	79.35±0.68	101.03±0.61
	10	34.54±0.97	92.34±0.79
	20	23.89±1.02	62.81±0.62
	25	10.67±0.64	60.56±0.84
Hydroalcoholic extract(HE)	5	32.72±0.11	58.62±0.58
	10	31.43±0.53	48.67±0.25
	20	19.34±0.16	39.33±0.15
	25	10.33±0.98	29.82±0.53
Albendazole(AL)	10	33.12±0.51	52.16±0.82
Piperazine citrate(PC)	10	25.22±0.72	72.61±0.39

ANOVA

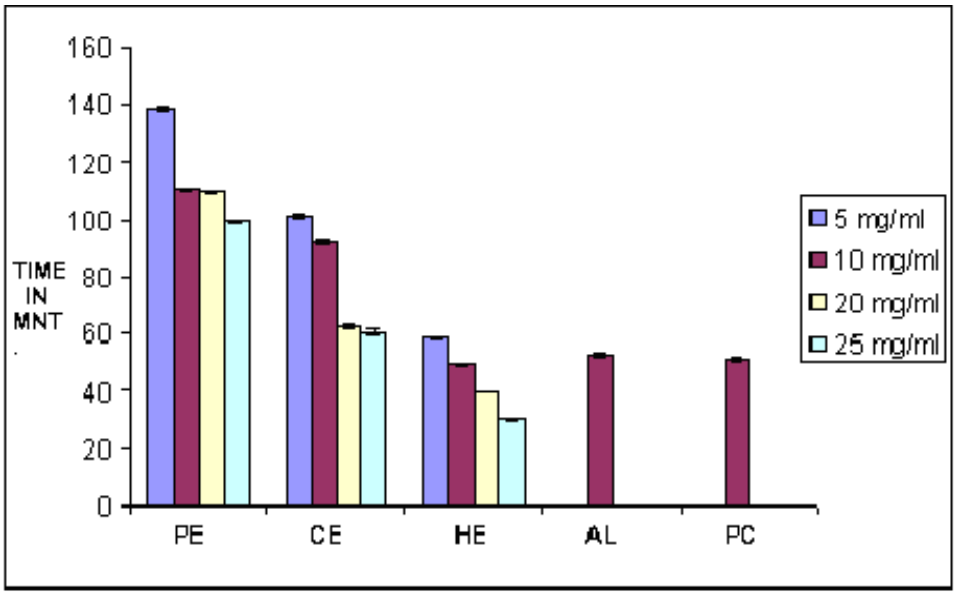
source of variation	***ss***	***df***	***MS***	***F***	***P-value***	***F crit***
Between the group	7960.955	2	3980.477	3.31208	0.04261	3.38519
Within group	23077.48	25	923.0992			
total	31038.44	27				

Results are expressed as mean ± Standard error mean (n=6).



PE: Pet. ether extract, CE: Chloroform extract, HE: Hydroalcoholic extract, AL: Albendazole, PC: Piperazine citrate. Each bar is representing mean ± S.E. (n=6).

Fig. 2: Time taken to paralyze by leafy extracts of *Acanthospermum hispidum* DC at different concentrations and reference samples.



PE: Pet. ether extract, CE: Chloroform extract, HE: Hydroalcoholic extract , AL: Albendazole, PC: Piperazine citrate. Each bar is representing mean ± S.E.(n=6)

Fig. 3: Time taken to death by leafy extracts of *Acanthospermum hispidum* DC at different concentrations and reference samples.

Results and Discussion:

Preliminary phytochemical screening indicated the presence of carbohydrates, alkaloids, saponins, glycosides, tannins, flavonoids and terpenoids (Table 1).All the extracts showed the anthelmentic activity in dose dependent manner at 5 to 25 mg/ml. The chloroform and hydroalcoholic extracts of *A. hispidum* revealed significant anthelmintic activity [6-8]. The hydroalcoholic extract shown better paralytic value and death at 10 mg/ml than the standards (Fig 2 and 3). The chloroform extract also showed satisfactory result at concentration of 10 mg/ml. The presence of alkaloids,

glycosides and tannins may be the responsible chemical constituents [9, 10,] for demonstrating anthelmintic activity. The possible mechanism of tannins may to interfere with energy generation by uncoupling oxidative phosphorylation or they may interfere with glycoprotein of cell surface. It was also possible that alkaloids may act on central nervous system and caused paralysis of the *Pheritima posthuma* worms. All data were found to be significant at 5 % level of significance ($p < 0.05$).

Conclusion:

It could be concluded and confirmed that the hydroalcoholic extract of leaves of

A. hispidum DC has anthelmintic activity comparable with standard drugs, which is a significant result. Further studies are required to identify the actual chemical constituents that are present in the crude extracts of this plant which are responsible for anthelmintic activity [11-13]. It is, however, suggested to conduct further research on pure chemical constituents to critically evaluate their activity on a large number of animals [14, 15].

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