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Anthelminitic activity of the whole plant of Sphaeranthus indius Linn.

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The aim of present study was to evaluate the anthelmintic activity of ethanolic and aqueous extracts of the whole plant of Sphaeranthus indicus Linn. against Pheretima posthuma and Ascardia galli . Each extracts were studied in the bioassay at concentrations of 10, 50, 100 mg/ml, which involved determination of time of paralysis and time of death of the worms. Both extracts exhibited anthelmintic activity in a dose-dependent manner. The most significant activity was observed at the highest concentration of 100 mg/mL against both types of worms. Albendazole (10 mg/ml) and distilled water were included as standard reference and control, respectively. The present study indicates that the whole plant of Sphaeranthus indicus Linn. does possess anthelmintic activity.

KEY WORDS: Sphaeranthus indicus Linn. , anthelmintic activity , *Pheretima posthuma, Ascardia galli* , Albendazole.

INTRODUCTION

Sphaeranthus indicus Linn.(Asteraceae) commonly known as Mundi, is a spreading aromatic herb, distributed throughout in India up to 1500 m from Kumaon hills to Sikkim on the Himalayas chiefly on damp habitats(1). The survey of literature reveals that the medicinal plant S.indicus Linn has been used as a nervine tonic, diuretic, febrifuge, stomachic, depurative, diabetes, anthelmintic and in case of piles(2). The flower heads of Sphaeranthus indicus Linn. have been reported to have immunomodulatory activity and anxiolytic activity(3,4). It does possess antioxidant activity (5). Decoction of the whole plant is reported to be used as diuretic in urethral discharges (6) while root is used as a stomachic and anthelmintic in doses of about 40 grains daily in the form of powder. The leaves dried in the shade and powdered are used in doses of

20 grains twice a day in chronic skin diseases as antisyphilitic and nervine tonic (7). In Ayurveda, the whole plant of S.indicus are reported to be useful in insanity, tuberculous glands, indigestion, bronchitis, diseases of the spleen, elephantiasis, anaemia, pain in the uterus and vagina, piles, strangury, biliousness, epileptic convulsions and reputed as an anthelmintic(8). Chemical constituents like alkaloids, sesquiterpenoids, isoflavone glycoside and essential oil have been reported from this plant (8-11). A new eudesmenolide-7α-hydroxyeudesm-4-en-6, 12-olide (7-hydroxy-frullanolide has been isolated from flowers of S.indicus and reported (12).

However, the anthelmintic activity of S. indicus Linn has not been scientifically investigated. The present study was, therefore undertaken to evaluate the traditionally claimed anthelmintic activity of ethanolic and aqueous extracts of the whole plant of S.indicus against worms *Pheretima posthuma* and *Ascardia galli*.

MATERIALS AND METHODS

Plant material

The whole plant of Sphaeranthus indicus was collected from local areas of Hoshangabad, Madhya Pradesh and authenticated by the Department of Botany, R.L.Science Institute, Belgaum, Karnataka.. The collected whole plants were washed under running tap water and shade dried and then milled into coarse powder with the help of an mechanical grinder. The powder was passed through sieve number 40 and used for further studies.

Preparation of extract

The powdered plant material was then exhaustively extracted with ethanol (95%) using Soxhlet apparatus. Fresh powdered plant material was taken and kept for maceration with chloroform water for 24 hours to obtain the aqueous extract. The solvents were then removed under reduced pressure, which obtained sticky residues (13,14). The dried extracts were suspended in normal saline containing 1% Tween 80 (vehicle) and used for the anthelmintic activity.

Drug used

Albendazole was used as standard reference drug for anthelmintic study.

Worms Collection

Indian earthworm *Pheretima posthuma* (Annelida) were obtained from School of Agricultural Training & Research, Belgaum, Karnataka. *Ascardia galli* (Nematode) were obtained from freshly slaughtered fowls (Gallus gallus) and were identified at the School of Agricultural Training & Research, Belgaum, Karnataka.

Screening for Anthelmintic Activity

The anthelmintic activity of the whole plant of Sphaeranthus indicus was determined by using the method of Srinivasa U *et al* (15).The activity was evaluated on adult Indian earthworm, Pheretima posthuma , due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings(16). Because of easy availability, earthworms have been widely used in the evaluation of anthelmintic drugs (17-20). Ascardia galli worms are easily available in plenty from freshly slaughtered fowls and their use, as a suitable model for screening of anthelmintic drug was mentioned in earlier research work (21, 22).

Four groups of approximately equal size worms consisting of six worms in each group were released in 50 ml of desired formulation. This procedure was done for both types of worms. Each group was then treated with one of the following: vehicle (1%Tween 80 in normal saline), albendazole (10, 50 and 100 mg/ml) and extracts of different concentrations (10, 50 and100 mg/ml). Observations were made for the time taken to paralyze and/or death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lost their motility, followed with fading away of their body colour (15).

RESULTS AND DISCUSSION

Preliminary phytochemical investigation of the extracts **Table -1**

Ethanol extract	:	Carbohydrates (reducing sugars), Flavonoids, Alkaloids, Volatile oils, Fats & Oils, Saponins Tannins and phenolic compounds
Aqueous extract	:	Carbohydrates (reducing sugars), Flavonoids, Tannins and phenolic compounds

Table-1 Preliminary phytochemical investigation of the extracts of *S. indicus* Linn whole plant

showed the presence of carbohydrates, tannins and phenolic compounds, flavonoids, alkaloids, saponin, volatile oils, fixed oils. As shown in **Table -2**,

Groups	Concentration mg/ml	Time taken for Paralysis(P) and Death (D) in minute			
		P.Posthuma		A galli	
		P	D	P	D
Control	-	-	-	-	-
Aqueous	10	81.2±0.5	102±0.3	74.2±0.1	85±0.1*
Extract	50	56±0.1*	81.8±0.4	49.1±0.2**	72±0.3**
	100	49±0.2**	65.2±0.1	32.9±0.3***	59±0.7
Ethanolic	10	62±0.3**	88.7±0.6	58.2±0.5**	70±0.5
Extract	50	39.4±0.2	51.6±0.3**	33±0.1	42±0.4**
	100	27.4±0.4***	37±0.3	22±0.4	29±0.7***
Albendazole	10	36.4±0.7	63.8±0.6	19.2±0.8	39±0.5

Table-2 Anthelmintic activity of the whole plant of *S.indicus* Linn.

Results expressed as Mean±SEM from six observation ; n=6 in each group.. $^{*}p< 0.05$, $^{**}p< 0.01$, $^{***}p< 0.001$. One way ANOVA, followed by Dunnett's multiple comparison tests

the ethanolic and aqueous extracts of S. indicus Linn. whole plant displayed significant anthelmintic activities at higher concentrations. Both the extracts showed anthelmintic activities in dose dependant manner for both types of worms. The ethanolic extract of S.indicus caused paralysis 27.4 min and death in

37 min, while aqueous extract showed paralysis and death in 49 min and 65.2 min against the worms *Pheretima posthuma and Ascardia galli*. Potency of the extracts was inversely proportional to the time taken for paralysis/ death of the worms. The reference drug Albendazole showed the same at 36.4 min and 63.8 min, respectively.

In case of *A.galli* worms, the ethanolic extract caused paralysis in 22 min, death in 29 min and the aqueous extract displayed paralysis and death in 32.9 and 59 min respectively, at higher concentration of 100 mg/ml. Albendazole did the same at 19.2 and 39 min. but having a concentration of 10mg/ml.. It was observed that the ethanolic extract was more potent than the aqueous extract, even though both the extracts were endowed with anthelmintic property. The worms in the control group were remain alive up to 24 hours of observation

Albendazole acts by binding to the parasite's ß-tubulin, inhibiting its polymerization and impairing glucose uptake, causing death (23). The mode of action of benzimidazoles is based on their binding to ß-tubulin of parasite cells. Binding of benzimidazoles to the vacant end of ß-tubulin ("capping") prevents polymerisation of tubulin to microtubules. At the same time depolymerisation is going on at the other side of microtubules. This mechanism leads to the loss of structure and length of microtubules. Therefore, microtubules are unable to function, which causes decreased uptake of nutrients and decreased intracellular transport of essential substrates. Especially a reduction of glucose uptake leads to a decreased consumption and a reduced synthesis of endogen glycogen (a highly branched glucose polymer). In addition, adenosintriphosphate (ATP)-synthesis is highly reduced because the resource for this nucleotide is glucose. ATP is an energy rich compound which is used for different energy consuming processes of the cell metabolism. After exhaustion of all endogenous glycogen reserves, parasites die due to lack of energy (24). The whole plant extract of S.indicus not only showed paralysis, but also caused death of worms especially at higher concentration of 100 mg/ml, as compared to reference drug Albendazole. Phytochemcial analysis of the ethanolic extracts revealed the presence of volatile oil as one of the chemical constituent. Volatile oils were shown to possess anthelmintic activities against worms (25-27). The possible anthelmintic activity of volatile oil is that they can inhibit the glucose uptake and deplete the glycogen content in *Ascaridia galli* or interaction of volatile

oils with glycolytic enzymes and succinic acid production in worms is postulated as possible mechanism of action (28).

CONCLUSION

The present study justifies the folkloric claims of the potential anthelmintic activity of Sphaeranthus indicus Linn.whole plant which may be due to the combined effects of flavonoids, carbohydrates, alkaloids, saponins, volatile oils, tannins and phenolic compounds. But there is probability that volatile oils may be most responsible constituents for anthelmintic activity. Because as per literature volatile oils does posses anthelmintic activity (25-27).

Further studies are required in order to isolate the active chemical constitutent (s) present in the crude extracts of Sphaeranthus indicus which are responsible for the anthelmintic activity and to establish the mode of action for it.

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