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Research Article

ANTHELMINTIC ACTIVITY OF *Aeschynomene aspera* and *Aeschynomene indica.*

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ABSTRACT

Objectives: Many of the Fabaceae members are possessing anthelmintic property may be due to the presence of Phytoconstituents like tannins and phenolic compounds which acts as astringents against worm infestation. Hence *A.aspera* and *A.indica* were screened for their helminthic activity as used by the local herbalists.

Materials and Methods: Plant materials *A.aspera* and *A.indica* were collected from Renigunta Mandal of Chittoor Dt, and identified as per Jain and Rao. Anthelmintic bioassay was carried out as per the Gosh method against *Pheretima posthuma* with leaf extracts.

Results: *A.aspera* and *A.indica* leaf alcohol, methanol and aqueous extracts showed most effective anthelmintic activity than the standard drug *Albendazole* as in the time taken for paralysis and death. Out of all extracts *A.aspera* alcohol and methanol at 5, 10 and 15 mg proved its efficiency with in short time for paralysis 10-6 min and 20-11 min for death.

Conclusion: *A.aspera* and *A.indica* aqueous, alcohol and methanol extracts showed most promising anthelmintic activity both in terms of lowest concentrations and at faster rates of controlling the worms than the standard drug and also with other extracts. It also observed as the most effective anthelmintic drug than the other species of the family.

Keywords: Worm infestations, Phytoconstituents, *Albendazole*, Paralysis, *Pheretima posthuma.*

INTRODUCTION

Aeschynomene aspera (Neti jilugu) and *A. indica* (Tella jeeluga) are the sub shrubs of swamps. *A.aspera* root used to cure jaundice [1]; leaves used to relieve joint pains and swellings [2]; In Ayurveda system it is recommended for painful micturition and to break uric acid calculi [3]; Root paste applied on mumps[4] . Aerial parts juice to relieve cold, cough and fever, dried young shoot powder to increase consistency of semen [5].

A.indica leaf used as spermicidal [6]; and also used as genetical stimulant, antidote to snake bite, mosquito repellent [7]; In Ayurveda it is used against biliary calculi.[8] ; In siddha used to treat leprosy [9]. Kani tribe used as antidote against snake bite [10]; Chenchu tribe used to cure kidney and urinary troubles.[11] ; Piliyar tribes used against cattle skin eczema [12].

Preliminary screening of secondary metabolites resulted alkaloids, terpenoids, tannins, flavonoids, steroids and glycosides as main compounds in both the species. Qualitative analysis of phenols represented nearly 11 compounds in *A.aspera* as neo chlorogenic acid, homo-Protocatechuic acid, caffeic acid, trans-p-Coumaric acid and cinnamic acid; flavonoids as apigenin, myrecetin and quercetin. Where as in *A. indica* 13 phenols as iso-chlorogenic acid, scopoletin and aesculetin are the additional phenolic compounds, homo proto catechuic acid and fumaric acid are absent and flavonoid kaempferol replaced quercetin to that of *A.aspera* [13,14].

Aeschynomene sensitiva and *A. indica* are reported to possess insecticidal activity [15]; spermicidal[16-17]. From *A.indica* reynoutin and aeschynomate compounds are isolated [18]. Roots of *A.mimosifolia* yielded neoflavonoids, mimosofolial and mimosifolenone [19]. *A. aspera* hepatoprotective activity was reported. [20]

MATERIALS AND METHODS

Collection of Plant material

Plant material *A.aspera* and *A.indica* were collected from Mallemadugu dam along the water hedges near dodlamitta area of Renigunta Mandal. The botanical identity of the plant was authenticated from the literature available in the Department of Botany and the voucher specimens (CA.27, CA28) were deposited in the Department Herbarium as per the standard methods [21]. The present work was carried out in the Department of Botany, S.V.University, Tirupati. Leaf material was thoroughly washed and dried under shade at 28 ± 2°C for about 10 days. The dried leaves were ground well into a fine powder in a mixer grinder and sieved to give particle size of 50-150mm. The powder was stored in air sealed polythene bags at room temperature.

Preparation of aqueous extracts

Dried leaf powders (70 g) were extracted with cold and hot water successively. The plant material was soaked for 72 hr. and the filtrate was dried on water bath and stored at 4^0C in refrigerator.

Preparation of organic solvent extracts

Dried leaf powders (40 g) were extracted in a Soxhlet apparatus using alcohol and methanol each 200 ml respectively. The filtrates were concentrated on rotavapour, dried and stored at 40C in refrigerator.

Worm collection:

Earthworms *Pheretima posthuma* of approximately equal size were collected from Ram Mohan Organic Inputs, (Licence No: 4447/2006 issued by C&DA (A.P).Hyderabad) Brahmanakalva (Vil), Pathi Puttur (Post) Ramachandrapuram (M), Chittoor Dist., A.P.

Preparation of Desired Formulation of Plant Drug and *Albendazole* (Reference Drug)

By dissolving 5, 10, 15mg of cold and hot water, Alcohol, Methanol and *Albendazole* extracts each in 25 ml of Distilled Water.

Experimental procedure

The anthelmintic assay was carried out as per the standard method Gosh [22]. *A.aspera* and *A.indica* cold water, hot water, alcohol and methanol leaf extracts were investigated for their anthelmintic activity against *P. posthuma*. Various concentrations as 5, 10, 15 mg of each extract was tested in the bioassay, which involved the determination of time of paralysis and time of death. Albendazole used as standard reference drug and distilled water as control. Worms were washed with normal saline to remove all fecal matters and selected approximately each worm of 8cm in length and 0.5-0.8 cm in width. Seventeen groups consisting two worms in each were released into 25ml of desired formulation. Five groups were prepared as control distilled water, warm water, reference drug *Albendazole* 5, 10,15mg and remaining as drug cold water, hot water, alcohol and methanol extracts each 5, 10, 15mg of *A. aspera and A.indica*. Observations were made the time taken for paralysis

and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body color.

RESULTS

A.aspera alcohol and methanol extracts showed most effective activity at 5, 10, 15 mg concentrations than that of the standard drug, in addition its activity was most promising with in short time for paralysis 10 to 5 min and 20 to11 min for the death of worms. Where as *A.indica* alcohol and methanol extracts time for paralysis 32-19 min and 45-25 min for death, with *Albendazole* paralysis time 91- 34 min and 110- 41 min for death. Hot and cold water extracts of *A.aspera* also showed most effective activity 25-15 min for paralysis, 30-19 min for death; and *A.indica* 62-36 min for paralysis, 98-40 min for death are equally effective to that of standard drug *Albendazole*.

Table I: Anthelmintic Activity

S. No.	Extracts	Conc. in mg	Time Taken for Paralysis		Time Taken for Death	
			A.aspera	***A.indica***	***A.aspera***	***A.indica***
1	Coldwater	5	20.2±0.32	61.6±0.5	30.0±0.12	98.2±0.49
		10	14.7±0.35	51.8±0.64	20.7±0.26	65.3±0.29
		15	10.5±0.32	40.9±0.24	14.5±0.33	57.9±0.81
2	Hot water	5	25.9±0.61	79.1±0.49	40.8±0.24	120.0±0.32
		10	19.2±0.28	60.1±0.16	22.6±0.20	88.7±0.57
		15	14.5±0.28	35.6±0.35	19.3±0.41	40.2±0.32
3	Alcohol	5	10.3±0.33	32.1±0.30	21.1±0.37	45.5±0.75
		10	8.2±0.28	22.4±0.33	16.1±0.61	32.4±0.36
		15	6.3±0.33	18.5±0.12	11.3±0.45	25.4±0.38
4	Methanol	5	11.4±0.63	39.2±0.49	19.8±0.36	45.9±0.77
		10	8.7±0.12	30.1±0.24	15.0±0.69	40.3±0.37
		15	5.5±0.24	26.3±0.38	11.1±0.63	32.4±0.32
5	Albendazole	5	90.9±0.84		110.1±0.12	
		10	62.1±0.12		71.2±0.16	
		15	34.4±0.21		41.0±0.43	
6	Distilled Water	15ml	-		-	
7	Warm Water	15ml	-		-	

All the Values are represented in Mean ± S.D; n=2 in each group.

DISCUSSION

Aqueous leaf extracts of *Abrus precatorius* exhibited significant dose dependent effective anthelmintic activity equal to that of the control drug *Albendazole* at 4 and 8mg/ml with 37 to 25 min for time taken for paralysis and 76 to 45 min for the death of the worms. [23] Alcoholic extracts of *Butea monosperma* at 100mg/ml effective activity as 17 min for paralysis and 20min for death of the worms to that of the *Piperazine citrate* with 15mg/ml, 12 min for paralysis and 12.6min for death. [24]

Ethanolic leaf extracts of *Clitoria ternatea* at 10 and 25mg/ml showed dose dependant anthelmintic activity as 129 to 122min for paralysis and 239-192 min for death of the worms to that of the standard drug *Piperazine citrate* 15mg/ml 97min and 116min for paralysis and death respectively.[25] Chloroform and methanolic leaf extracts of *Crotalaria pulchra* with 10-50mg/ml against *Pheritima posthuma* and *Ascaridia galli* to that of the *Albendazole* resulted most effective on *A. galli* as 5 and 22 min for the paralysis and death 14 and 45 min equal to that of control drug. *P.posthuma* 14 and 56 min for paralysis and death [26].

Aqueous leaf extracts of *Erythrina variegata* with 10-50mg/ml on *Eicinia foetida* (Earthworms). *Railletina spiralis* (Tape worm) and *Ascaridia galli* (round worm) resulted effective anthelmintic activity at 10mg/ml on *E. foetia*, 20mg/ml on *A.galli* and 50 mg/ml on *R.spiralis* equal to that of the control drug *Piperazine citrate* at 10mg/ml, 69-132 min; 31 and 52 min, 27 and 45 min for paralysis and death of the worms respectively. [27] *Flemengia strobilifera* leaf alcoholic and chloroform extracts produced significant anthelmintic activity compared to other extracts to that of *Piperazine citrate* the standard drug. [28]

In vitro anthelmintic activity of leaf aqueous extracts of *Gliricidia sepium* on the inhibition percentage of *Haemonchus contortus* egg hatching and on the larval development resulted as effective ovidical effect (ED_{50}) at 18.6mg/ml; upto 72 % between 12.5 to 50mg/ml. Larvicidal at 6.25 to 50mg upto 91.5 % and ED_{50} dose 25 mg/ml.[29] Anthelmintic activity of *Vigna unguiculata* seed aqueous and ethanol extracts at 50mg on *Eudrilus euginiae* (worms) showed paralysis and death time 14 and 22 min equal to that of the control drug Piperazine citrate. [30]

CONCLUSION

A.aspera anthelmintic activity was more effective than *A. indica* at low concentrations (5-15mg) of Methanol, alcohol and aqueous extracts respectively than the control drug *Albendazole* as in the time taken for paralysis and death against *Pheretima posthuma* worms. It is also proved to have equal activity to that of the other species of Fabaceae against gastro intestinal nematodal parasites as leaf extracts of *Crotalaria pulchra*, *Erythrina variegata*, *Abrus precatorius*, *Gliricidia sepium* and seed extracts of *Vigna unguiculata*. Presence of tannins in both species may also support its anthelminitic activity. Hence both species may be recommended as single drug anthelmintics. Further studies required to isolate and establish the standard drug from bioactive constituents of *A.aspera* and *A.indica* species which may possess effective anthelmintic activity.

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