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Anthelmintic activity of *Sesbania grandiflora* against gastrointestinal nematodes of sheep

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ABSTRACT

The study was carried out to validate the anthelmintic efficacy of *Sesbania grandiflora* and to standardize the effective dose of the plant extract required for worm control in livestock. *In vitro* and *in vivo* studies were conducted to determine the direct anthelmintic effect of ethanolic and aqueous extracts of *S. grandiflora* towards mixed ovine gastrointestinal nematodes. Egg hatch assay for ovicidal and larval migration inhibition and larval development assay for larvicidal properties were used to investigate *in vitro* effect of extracts on strongyle egg and larvae. Faecal egg count reduction test was conducted *in vivo* to evaluate the therapeutic efficacy of the extacts administered orally @ 125, 250, 500 mg/kg to sheep naturally infected with mixed gastrointestinal nematodes. Aqueous extract of *S. grandiflora* demonstrated significant inhibition of egg hatch and larval migration @ 40 and 80 mg/ml. The ED_{50} value of egg hatch inhibition and LM_{50} value for larval migration inhibition were 1.489 and 0.683 mg/ml respectively. In faecal egg count reduction test (FECRT), aqueous extract of *S. grandiflora* at 500 mg/kg caused significant reduction in eggs per gram (98.10 %) higher than the result obtained with albendazole (93.25 %). Although there were slight variations in the haematological parameters (PCV, haemoglobin, RBC and WBC) in all the groups between day 0 and 12, all the parameters were within the normal range reported for sheep. Except for blood urea nitrogen, overall mean of all the serum biochemical profile was within the normal range for sheep. Based on the results obtained by *in vitro* and *in vivo* assay, the aqueous extract of *S. grandiflora* possess anthelmintic activity and could offer an alternative source for the control of gastrointestinal nematodes of sheep.

Key words: Anthelmintic, Evaluation, GI nematodes, *Sesbania grandiflora,* Sheep

Sheep production plays a vital role in augmenting socio - economic status particularly of the small land holders and landless farmers, who rely on these animals for their animal protein source and income for their livelihood (Lateef 2003). However, mismanagement, poor hygiene and precarious housing conditions all contributed to the incidence of disease and high mortality (Niekerk and Pimentel 2004). Parasitic diseases especially gastrointestinal nematodes are main factors limiting small ruminant production worldwide due to retarded growth (Luscher *et al*. 2005), weight loss, reduced food consumption, lower milk production, impaired fertility and, in cases of massive infections, high mortality rates (Cavalcante *et al*. 2009). Currently, nematode control programmes in small ruminants seek not only to cure the clinical disease, which is characterized by high mortality rates, but mainly to reduce the losses caused by subclinical parasitism.

Control of these nematodes is mainly through the use of anthelmintics. Development of anthelmintic resistance (Taylor *et al*. 2009), increased public awareness over the drug residues in animal products and toxicity problems (Muhammad *et al*. 2004), has necessitated to find an alternative endoparasite control strategies. A large number of medicinal plants were used to treat parasitic infections in animals (Akhtar *et al*. 2000). Hence, the present study was envisaged to assess the anthelmintic properties of *Sesbania grandiflora* against gastrointestinal nematodes of sheep.

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MATERIALS AND METHODS

Collection of plant materials and extraction: The leaves of *Sesbania grandiflora* was collected from the local market/field and certified by an expert. The collected plant materials were shade dried, powdered and stored in air tight container for further extraction. Aqueous extract was prepared as prescribed by Onyeyili *et al*. 2001. The dried extract was collected in stoppered vials and stored at 4°C until use. Ethanolic extract was prepared as described by Wang and Waller (2006).

GC-MS analysis: GC-MS analysis was carried out on a system comprising an AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS)

instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID × 1µM df, composed of 100% dimethyl poly diloxide, operating in electron impact mode at 70eV; helium (99.99%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 µl was employed (split ratio of 10:1), injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10 °C/min, to 200 °C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 sec and fragments from 40 to 450 Da. Total GC running time is 36 min. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Preparation plant/drug stock solution: Pure thiabendazole and levamisole (0.1 g) was transferred into a 100 ml volumetric flask through a small glass funnel and rinsed twice, each with 10 ml of dimethyl sulfoxide (DMSO); 20 ml of DMSO was added and the total volume was made up to 100 ml with distilled water. Using the stock solution, a suitable working solution of thiabendazole with final concentration of 200 µg /ml was prepared and used as positive control. Stock solutions of crude aqueous and ethanolic extracts.

S. grandiflora initially were prepared by dissolving the crude extracts in dimethyl sulfoxide (DMSO) so, as to improve their solubility in water. Aliquots of stock solution (100 mg/ml) were further diluted to obtain final concentrations of 10 (1%), 20 (2%), 40 (4%) and 80.0 (8%) mg/ml for each extract.

In vitro *tests*: Egg hatch assay was performed in 24 well plates as per Jackson *et al.* (2001). The percentage of hatch for each concentration was calculated and the results were subjected to probit analysis to obtain ED_{50} values.

In larval development assay, eggs were harvested from the pooled faecal samples and the concentration of eggs was estimated in 100 µl samples and adjusted to 100 eggs per 100 µl. The assay was conducted as per Hubert and Kerboeuf (1992). The mean larval development for each drug concentration and the LD_{50} value were determined by plotting the percentage larval development and drug concentration.

Larval migration assay was conducted as per Jackson *et al*. (2001). The number of larvae retained by the mesh (Nr) and those that have migrated (Nm) through the mesh were counted. The drug concentration against percentage migration was plotted over a graph and the LM_{50} values were derived.

In vivo *tests*: Vembur lambs (30) of 6–12 months age, which showed eggs per gram of faeces (EPG) from 1,000 to 2,700 before treatment were selected and randomly distributed into 5 treatment groups each comprising 6 animals. Three groups were treated with doses of plant extracts at 125, 250 and 500 mg/kg, respectively, while the fourth and the fifth group served as positive and negative controls. Faecal samples were collected from each animal on day 0 and at day 12 post treatment and faecal egg count reduction (FECR) was assessed as recommended by WAAVP (Coles *et al.* 1992).

Estimation of haematological and serum parameters: Blood samples were collected on day 0, 3 and 12 post treatment from each animal and haematological parameters were determined as per Schalm *et al*. (1975) and Jain (1986). Serum biochemical profiles were determined using standard diagnostic kits. Pooled faecal samples were cultured and identified (MAFF 1971).

Statistical analysis: For *in vitro* assays, probit transformation was performed to transform a typical sigmoid dose-response curve to linear function (Hubert and Kerboeuf 1992). Faecal egg count, haematological and serum biochemical parameters were analysed by the statistical methods (Snedecor and Cochran 1976). All the experimental procedures described in this research were in compliance with the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals, India) for the care and use of animal for scientific purposes.

RESULTS AND DISCUSSION

Aqueous extract of *S. grandiflora* induced significant egg hatch inhibition at 40 and 80 mg/ml and ethanolic extract induced only marginal inhibition at all the concentrations tested (Table 1). On the other hand, both aqueous and ethanolic extracts of *S.grandiflora* did not induce significant inhibition of larval development and migration. Only aqueous extract of *S.grandiflora* proved to be effective in *in vitro* was tried *in vivo*.

The mean eggs per gram counts (EPG) and percentage reduction in faecal egg counts of sheep treated with different doses of aqueous extract of *S. grandiflora* and albendazole are presented (Table 2). The aqueous extract of *S. grandiflora* produced a dose dependent reduction in EPG on 12 days post treatment with higher reduction of 98.10 % at 500 mg/kg. Sheep drenched with albendazole (Albomar - positive control) @ 7.0 mg/kg showed 93.25 % reduction in EPG.

Coproculture: *Oesophagostomum columbianum* was the primary gastrointestinal nematode infecting animals with 71 %. *Haemonchus contortus* was the second averaging 27 % followed by *Bunostomum* spp. (1 %).

Effect of plant extracts on haematological values of sheep: The mean values of haemogram in sheep treated with aqueous extract of *S. grandiflora* and albendazole (Albomar) (positive control) are presented (Table 3). There was not much variation in haemogram levels before and after treatment at all the doses tested.

There was a slight variation in the serum biochemical profile (Table 4) before and after treatment with aqueous

Table 1. Mean % efficacy of aqueous and ethanolic extracts of *Sesbania grandiflora* on nematode egg hatch, larval migration and larval development inhibition

Name of the plant extracts	Concentration of the plant extracts				Positive control	Negative control
	1 %	2 %	4 %	8 %		
Egg hatch assay						
AE of *S. grandiflora*	$21.50^{c}\pm2.25$	$80.33^{b}\pm6.74$	$97.17^{a}\pm1.30$	$99.33^{a}\pm0.49$	$97.03^{a}\pm0.82$	$8.79^{d}\pm1.05$
EE of *S. grandiflora*	$1.33^{c}\pm0.33$	$1.67^{c}\pm0.88$	$2.67^{c}\pm2.50$	$17.33^{b}\pm0.88$	$97.03^{a}\pm0.82$	$8.79^{b}\pm1.05$
Larval migration inhibtion assay						
AE of *S. grandiflora*	$74.50^{b}\pm4.50$	$74.50^{b}\pm0.50$	$93.00^{a}\pm2.00$	$97.00^{a\pm}0.50$	$96.22^{a}\pm0.35$	$8.27^{c}\pm0.98$
EE of *S. grandiflora*	$13.00^{b}\pm2.00$	$12.00^{b}\pm1.00$	$15.50^{b}\pm1.50$	$15.00^{b}\pm0.00$	$96.22^{a}\pm0.35$	$8.27^{c}\pm0.98$
Larval development assay						
AE of *S. grandiflora*	$1.70^{b}\pm1.10$	$4.30^{b}\pm2.50$	$6.30^{b}\pm3.50$	$10.40^{b}\pm1.20$	$93.46^{a}\pm1.82$	$5.30^{b}\pm1.12$
EE of *S. grandiflora*	$19.40^{b}\pm1.80$	$25.20^{b}\pm3.60$	$16.40^{c}\pm2.08$	$24.60^{b}\pm4.00$	$93.46^{a}\pm1.82$	$5.30^{d}\pm1.12$

AE, aqueous extract; EE, ethanolic extract; ** Values sharing any one common superscript in a row (overall) do not differ significantly (P<0.01); NS, not significant.

Table 2. Effect of *Sesbania grandiflora* aqueous extract on per cent faecal egg count reduction (mean±SE) in sheep naturally infected with gastrointestinal nematodes

Dose (mg/kg)	AE of *S. grandiflora*	Albendazole (7 mg/kg)
125	$78.60^{bB\pm}1.83$	$93.25^{a\pm}1.15$
250	$90.26^{aB\pm}2.11$	$93.25^{a\pm}1.15$
500	$98.10^{aA\pm}1.21$	$93.25^{b\pm}1.15$

AE, Aqueous extract; EE, Ethanolic extract.** Values sharing any one common superscript in a row (small letters) and column (capital letters) do not differ significantly.

extract of *S. grandiflora*.

Phytocomponents: Urea, propyl (79.71 %), 3–Methyl-5-nonylpyrrolizidine (10.14 %), 2-azetidinone, 3,4,4-trimethyl (5.80 % and xylose (4.35 %) were identified in phytochemical screening of the aqueous extracts of *S. grandiflora* (Table 5).

Aqueous extracts of *S. grandiflora* demonstrated significant (P<0.05) inhibition of egg hatching at 40 and 80 mg/ml. Increasing concentration of the plant extracts resulted in increased inhibition of egg hatching indicating dose dependent activity. Similar dose dependent *in vitro* egg hatching inhibition was evaluated by using *Myracrodruon urundeuva* leaf extract (Lorena and Bevilaqua 2011) and aqueous and methanolic extract of *Ocimum sanctum* (Sujitha *et al*. 2015) against *Haemonchus contortus*. Our results are in accordance with findings obtained with aqueous and hydroalcolholic extracts of the seeds of *Coriandrum sativum* (Eguale *et al*. 2007), methanolic extract of *A. peniculata* and *D. metal* (Kamaraj *et al*. 2011), aqueous extract of *Caryocar brasiliense Camb*

Table 3. Effect of *Sesbania grandiflora* aqueous extract on blood parameters (mean±SE) in sheep naturally infected with gastrointestinal nematodes

Serum parameters	Period	Dose (mg/kg)			Positive control	Negative control
		125	250	500		
Packed cell volume (%)	0 Day	26.50±0.29	26.17±0.60	24.33±1.59	26.73±2.01	27.47±2.11
	3 rd day	26.17±1.17	26.33±0.33	24.97±1.80	27.77±2.10	28.07±1.65
	12th Day	26.87±1.99	26.33±0.33	24.97±1.52	29.00±2.45	29.07±1.77
	Overall	$26.51^{bcd}\pm0.68$	$26.28^{bcd}\pm0.22$	$24.76^{cd}\pm0.83$	$27.83^{abc}\pm1.14$	$28.20^{ab}\pm0.95$
Hb concentration (g/dL)	0 Day	8.73±0.32	9.40±0.31	8.90±0.17	9.33±0.73	9.57±0.88
	3 rd day	8.73±0.37	9.53±0.30	8.73±0.18	9.77±0.74	9.97±0.86
	12th Day	8.73±0.45	9.07±0.03	9.00±0.30	10.13±0.78	10.23±0.88
	Overall	$8.82^{de}\pm0.20$	$9.33^{bcde}\pm0.14$	$8.88^{cde}\pm0.12$	$9.74^{abcd}\pm0.39$	$9.92^{abc}\pm0.45$
TEC (10^6 / µl)	0 Day	8.00±0.31	8.70±0.06	8.17±0.12	8.63±0.76	8.47±0.75
	3 rd day	8.17±0.30	8.80±0.10	8.17±0.03	8.76±0.76	8.69±0.80
	12th Day	8.20±0.32	9.00±0.40	8.27±0.17	8.96±0.76	9.07±0.72
	Overall	$8.12^{e}\pm0.16$	$8.83^{abcd}\pm0.13$	$8.20^{de}\pm0.06$	$8.78^{abcde}\pm0.38$	$8.74^{abcde}\pm0.39$
TLC (10^3 /µl)	0 Day	9.77±1.72	10.40±0.40	11.00±1.30	6.77±0.52	8.67±0.79
	3 rd day	9.77±1.72	10.50±0.44	11.30±1.11	7.60±0.25	9.33±0.66
	12th Day	9.77±1.72	11.70±1.08	10.77±1.33	8.00±0.21	9.23±0.33
	Overall	9.77abc±0.86	10.87a±0.41	11.02a±0.63	7.46e±0.25	9.08cd±0.33

AE, Aqueous extract; EE, Ethanolic extract;**Values sharing any one common superscript in a row (overall) do not differ significantly (P<0.01); NS, not significant (P<0.05).

Table 4. Effect of *Sesbania grandiflora* aqueous extract on serum parameters (mean±SE) in sheep naturally infected with gastrointestinal nematodes

Serum parameters	Period	Dose (mg/kg)			Positive control	Negative control
		125	250	500		
BUN	0 Day	44.00±0.00	39.33±2.40	37.33±2.33	43.37±1.78	47.23±6.89
	3rd day	55.33±2.96	53.00±1.53	49.33±4.18	52.90±2.25	42.47±11.11
	12th Day	46.33±0.33	45.67±3.53	45.67±0.88	47.10±1.46	49.43±1.15
	Overall	48.56^{abc}±1.93	46.00^{abcd}±2.37	44.11^{bcd}±2.26	47.79^{abcd}±1.67	46.38^{abcd}±3.93
Serum creatinine	0 Day	1.40±0.00	1.17±0.12	1.03±0.07	1.40±0.06	1.40±0.12
	3rd day	1.87±0.12	1.77±0.07	1.57±0.19	1.53±0.09	1.57±0.09
	12th Day	1.40±0.06	1.30±0.06	1.40±0.10	1.43±0.03	1.47±0.03
	Overall	1.56^{bc}±0.09	1.41^{cd}±0.10	1.33^{d}±0.10	1.46^{cd}±0.04	1.48^{cd}±0.05
SGOT	0 Day	87.50±12.92	65.07±6.58	64.27±1.86	124.67±2.11	109.20±12.01
	3rd day	152.87±2.13	196.00±14.62	172.40±12.74	103.97±3.98	97.53±12.16
	12th Day	69.20±2.51	75.33±5.65	62.13±1.41	113.47±17.02	90.27±5.49
	Overall	103.19±13.71	112.13±21.58	99.60±18.58	114.03±5.90	99.00±5.87
SGPT	0 Day	14.57±2.29	16.00±1.21	15.00±3.08	27.83±5.98	20.70±1.88
	3rd day	38.07±1.32	36.43±2.17	34.40±2.46	23.40±3.80	18.90±3.09
	12th Day	14.47±1.42	20.13±0.98	14.77±1.85	21.10±1.00	17.00±0.61
	Overall	22.37±4.02	24.19±3.21	21.39±3.49	24.11±2.29	18.87±1.19

** Values sharing any one common superscript in a row (overall) do not differ significantly (P<0.01); NS, not significant (P<0.05). AE, aqueous extract; EE, ethanolic extract.

Table 5. Phytocomponents identified in the aqueous extract of *Sesbania grandiflora*

RT	Name of the compound	Molecular formula	MW	Peak area %
3.24	2-Azetidinone, 3,4,4-trimethyl-	$C_6H_{11}NO$	113	5.80
11.45	3–Methyl-5-nonylpyrrolizidine	$C_{17}H_{33}N$	251	10.14
13.74	Xylose	$C_5H_{10}O_5$	150	4.35
21.58	Urea, propyl-	$C_4H_{10}N_2O$	102	79.71

(Nogueira *et al*. 2012) and aqueous extract of *Annona muricata* (Ferreira *et al*. 2013) against gastrointestinal nematodes in sheep.

Larval migration inhibition induced by aqueous extract of *S. grandiflora* at 40 and 80 mg/ml was comparable with the findings of Bendixsen *et al*. (2005) who reported larvicidal activity by LMIA with aqueous extracts obtained from *Caliandra* spp., *Leucaena glauca* and *Acacia farnesiana* at 0.8 mg/ml.

Larval development was not inhibited by both aqueous and ethanolic extract of *grandiflora* which was comparable with the results recorded by Ademola *et al*. (2007) when aqueous and ethanolic extracts of *N. latifolia* were used.

The highest per cent faecal egg count reduction (98.10 %) recorded with aqueous extract of *S. grandiflora* was in agreement with Soro *et al*. (2013) who recorded a 81 % faecal egg count reduction at a single oral dose of 80 mg/kg 3-week post-treatment using ethanolic extract of *Anogeissus leiocarpus* in sheep naturally infected with gastrointestinal nematodes. Similar findings were also recorded by Mesquita and Batista (2013) with *Eucalyptus staigeriana* essential oil Nogueira and Fonseca (2012) with banana crop residues and Ahmed *et al*. (2014) by using *Lespedeza cuneata.*

Haematological parameters were not significantly affected in both the treated and untreated groups. The increase in PCV level recorded in this study was in agreement with Githiori *et al*. (2004) who opined that the improvement in PCV might be due to stimulatory effect on haematopoietic system of sheep. Similarly, the observed increase in the haemoglobin levels in animals was in consistent with the findings of Hossain *et al*. (1996) who reported increased haemoglobin content in sheep when treated with neem leaves and which might be due to increased absorption of iron. Similarly, increase in PCV, Hb and TEC following treatment with neem, betel leaf and jute leaves in goats (Rahman 2002) and aqueous extract of neem leaves in sheep (Rob *et al*. 2004) was reported.

The increased BUN and serum creatinine level recorded in the present study might be due to retention of urea and creatinine in kidney tubules. Earlier, increased urea and creatinine levels following treatment with *Alstonia boonei* at 200 mg/kg in guinea pigs (Oze *et al*. 2007) was reported. A transient increase in the value of serum AST may be due to accumulation of the extract in the liver during metabolism and intoxication. The result was in agreement with Ogbonnia *et al*. (2009) who recorded a significant increase in AST in animals treated with hydro alcoholic extract of *S. angustifolia* implying the deleterious effect of the extract on heart tissue.

Phytochemical analysis of aqueous extract of *S.*

grandiflora revealed that the mechanism of action is not fully understood. However, the collective or individual presence of bioactive compounds in the extract may possibly constitute the basis for the profound anthelmintic activity exhibited by the plant extract as opined by Ruben *et al*. (2011).

In conclusion, aqueous extracts of *S. grandiflora* possess potential anthelmintic activity and offer an alternative source for the control of gastrointestinal nematodes of sheep. In addition, the cost effectiveness of *S. grandiflora* as compared to commercial drenches makes it more useful for low resource farmers without compromising the performance and productivity.

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