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Pharmacological Screening of *Sesbania grandiflora* L. Poiret Extracts

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Abstract – In the present study, the roots of *Sesbania grandiflora* L. Poiret (Papilionaceae) were successively extracted with petroleum ether (PE), chloroform (CE), methanol (ME) and water (AE) by soxhlet extraction. The extracts were vacuum dried and screened for analgesic, antidiarrhoeal, antibacterial (*Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus luteus, Bacillus cereus*, and *Klebsiella pneumonia*) and antifungal (*Candida albicans* and *Aspergillus niger*) activity. All the extracts exhibited potent, dose dependant (40 and 80 mg/kg) and significant analgesic and antidiarrhoeal activity in the order of AE>PE>CE>ME and ME>PE>AE>CE respectively. AE at the experimental dose was found to exhibit more potent analgesic activity than the standard drug. All the extracts exhibited significant antibacterial (100 μg/ml) and antifungal activity (50 and 100 μg/ml). ME exhibited the most potent antibacterial activity.

Key words – *Sesbania grandiflora*, analgesic, antidiarrhoeal, antibacterial, antifungal

Introduction

Sesbania grandiflora L. Poiret (Papilionaceae) is claimed (Anonymous, 1995; Nadkarni and Nadkarni, 1976; Sivarajan and Balachandran, 1996) to be used as expectorant, antidiarrhoeal, anti-inflammatory, antihistaminic, emetic and in the treatment of dysentry, small pox, malaria, fever, sinusitis and wound healing in the Indian system of medicine. *S. grandiflora* leaves are also consumed as food by Indians and as fodder for cattle. The analgesic (Tamboli *et al.*, 2000), anti-inflammatory (Tamboli *et al.*, 1996), antipyretic (Tamboli *et al.*, 2000) activity of the flowers of *S. grandiflora* were reported. The leaves of *S. grandiflora* were reported to be associated with hemolytic (Kumar *et al.*, 1982), antimicrobial and cytotoxic properties (Mackeen and Ali, 1997). To our knowledge, there are no scientific reports on the bio-activity of *S. grandiflora* roots. In the present study, the roots of *S. grandiflora* were successively extracted with petroleum ether, chloroform, methanol and water by soxhlet extraction. The extracts were vacuum dried and screened for analgesic (writhing reflex and tail immersion method), antidiarrhoeal (castor oil induced diarrhoea model), antibacterial (*Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus luteus, Bacillus cereus*, and *Klebsiella pneumonia*) and antifungal (*Candida albicans* and *Aspergillus niger*) activity.

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Materials and Methods

Plant material and extraction – The roots of *Sesbania grandiflora* L. Poiret were collected from Tirunelveli, India during Sep' 2002 and identified by Dr. E. Sasikala, Botanist, Central Research Institute for Sidda, Chennai. The roots were dried in shade (7 days), powdered and extracted successively with petroleum ether (60-80°C) (PE), chloroform (CE), methanol (ME) and water (AE) by soxhlet extraction (36 h) to yield the respective extracts. The extracts were vacuum dried in a rotary vacuum film evaporator and the extractive yields of PE, CE, ME and AE were 3.1, 4.3, 17.1 and 44.4 % (w/ dry w of roots) respectively.

Animals – Inbred Swiss albino mice (20-25 g) of either sex were used for the evaluation of pharmacological activities. They were kept in colony cages at 25±2°C, relative humidity 45-55% under 12 hours light and dark cycle (06:00 to 18:00 h-light and 18:00 to 06:00 h-dark). All the animals were acclimatized for a week before use. They were fed with standard animal feed (Hindustan Lever Ltd.) and water *ad libitum*. The test compounds and the standard drugs were administered in the form of a suspension using 0.1% carboxymethylcellulose as vehicle. Each group consisted of six animals. All the pharmacological experimental protocols were performed according to the recommendation of the institutional animals ethics committee.

Analgesic activity

Writhing reflex method – The analgesic activity (Ghosh, 1984) was determined by acetic acid induced writhing reflex method using swiss albino mice of either sex selected by random sampling technique. Rofecoxib at a dose level of 30 mg/kg was administered as a standard drug. The extracts (PE, CE, ME and AE) at 2 dose levels (40 and 80 mg/kg) was administered orally by gavage 30 min prior to administration of the writhing agent (1% v/v aqueous acetic acid, i.p-1 ml/100g). The writhings produced in the animal were observed for 20 min and percentage protection was calculated for analgesic activity. The results are presented in Table 1.

Tail immersion method – The analgesic activity (Krishnaveni *et al.*, 1997) was determined by tail immersion method using swiss albino mice of either sex selected by random sampling technique. Rofecoxib at the dose of 30 mg/kg was administered as standard drug for comparison. The extracts (PE, CE, ME and AE) at 2 dose levels (40 and 80 mg/kg) were administered orally. The animals were held in position by a suitable restrainer with the tail extending out and the tail (upto 5 cm) was then dipped in a beaker of water maintained at 55±0.5°C. The time in seconds taken to withdraw the tail clearly out of water was taken as the reaction time. The first reading (0 min) was taken immediately after the administration of the test compound and subsequent reaction time was recorded at 60, 120 and 180 min after the administration of compounds. A cut off point of 15 sec was observed to prevent the tail damage. The results are presented in Table 1.

Antidiarrhoeal Activity – Antidiarrhoeal activity (Patil *et al.*, 1999) was performed by castor oil induced diarrhoea model in swiss albino mice of either sex selected by random sampling technique. Loperamide at the dose of 2 mg/kg was administered as standard drug for comparison. The test compounds at 2 dose levels (40 and 80 mg/kg) were administered orally 60 min prior to the administration of the irritant purgative (castor oil-10 ml/kg). The mice were then housed separately, the number and weight of diarrhoeal faeces was recorded at an interval of 30 min for a period of 4 h. The percentage protection was recorded for antidiarrhoeal activity. The results are presented in Table 2.

Table 2. Antidiarrhoeal activity of *Sesbania grandiflora* root extracts

Group	Dose (mg/kg)	Defecation	Weight of stools (g)	% Protection
PE	40	11.83±0.3*	0.56±0.04*	44.6
	80	8.01±0.44*	0.39±0.03*	62.13
CE	40	12.83±0.6*	0.63±0.04*	39.32
	80	10.83±0.68*	0.52±0.02*	50.48
ME	40	11.33±0.61*	0.57±0.03*	45.04
	80	8.16±0.4*	0.38±0.02*	63.1
AE	40	11.66±0.76*	0.58±0.04*	44.66
	80	8.66±0.49*	0.43±0.02*	57.96
Control	–	19.66±0.88	1.03±0.03	–
Loperamide	2	2±0.25*	0.11±0.18*	89.32

PE = Petroleum ether extract, EE = Ethyl acetate extract, CE = Chloroform extract, ME = Methanol extract, AE = Aqueous extract.
*$p<0.001$ compared to control.

Antimicrobial activity – The antibacterial activity (Gillespie, 1994; Hawkey and Lewis, 1994) of the test compounds was tested against *S. epidermidis, S. aureus, M. luteus, B. cereus* and *K. pneumonia* using tryptone soya agar medium. The antifungal activity of the compounds was tested against *C. albicans* and *A. niger* using sabourand dextrose agar medium.

The sterilized (autoclaved at 120°C for 30 min) medium (40-50°C) was innoculated (1 ml/100 ml of medium) with the suspension of the microorganism (matched to McFarland

Table 1. Analgesic activity of *Sesbania grandiflora* root extracts

Group	Dose (mg/kg)	Writhing reflex method		Tail immersion method -Pain reaction time (min)			
		Writhings	% Protection	0	60	120	180
PE	40	23.08±1.108*	46.51	2.4±0.24	9.6±0.21**	9.5±0.48**	5.5±0.19**
	80	16.93±0.542*	60.46	1.6±0.24	7.2±0.24**	10.5±0.48**	4.5±0.2**
CE	40	27.08±0.945*	37.2	2.2±0.21	6.8±0.24**	6.5±0.2**	5.2±0.24**
	80	22.75±1.147*	46.51	2±0.16	8.8±0.6**	9±0.24**	5±0.31**
ME	40	28.33±0.954*	33.72	1.6±0.24	6.8±0.67**	5.5±0.24**	4.5±0.21**
	80	23.75±1.176*	44.18	1.8±0.37	7.2±0.67**	8±0.58**	5.1±0.31**
AE	40	16.92±0.981*	60.46	2±0.33	10±0.7**	10.5±0.58**	8±0.2**
	80	13.92±0.792*	66.27	2.4±0.28	10.8±0.74**	11.5±0.4**	9.5±0.21**
Control		42.75±1.477	–	2.1±0.22	2.2±0.36	1.9±0.21	2±0.31
Rofecoxib	30	7.94±0.341*	72.09	2.4±0.4**	9.6±0.48**	9.5±0.4**	6±0.48**

PE = Petroleum ether extract, EE = Ethyl acetate extract, CE = Chloroform extract, ME = Methanol extract, AE = Aqueous extract. Pain reaction time in seconds.
*$p<0.001$ compared to control and **$p<0.001$ compared to 0 min reaction time (tail immersion method).

Table 3. Antimicrobial activity of *Sesbania grandiflora* root extracts

Group	Conc. (μg/ml)	Zone of inhibition (mm)						
		S. epidermidis	*S. aureus*	*B. cereus*	*M. luteus*	*K. pneumonia*	*C. albicans*	*A. niger*
PE	50	–	–	–	–	15	7.5	7
	100	9	10	8	7	21	14	15
CE	50	–	–	–	–	13	7	6.5
	100	10	9	10	9	18	13	14
ME	50	-	7	6.5	–	16	6.5	6.5
	100	11	9	13	7	22	17	14
AE	50	–	–	–	–	16	6.5	7
	100	8	–	9.5	6.5	20	9	12
Ciprofloxacin	100	24	26	23	24	25	–	–
Ketoconazole	100	–	–	–	–	–	20	18

PE = Petroleum ether extract, EE = Ethyl acetate extract, CE = Chloroform extract, ME = Methanol extract, AE = Aqueous extract.

barium sulphate standard) and poured into a petridish to give a depth of 3-4 mm. The paper impregnated with the test compounds (100 μg/ml in dimethyl formamide) was placed on the solidified medium. The plates were preincubated for 1 h at room temperature and incubated at 37°C for 24 h and 48 h for antibacterial and antifungal activity respectively. Ciprofloxacin (100 μg/disc) and ketoconazole (100 μg/disc) was used as standard for antibacterial and antifungal activity respectively. The observed zone of inhibition is presented in Table 3.

Statistical analysis – All data were expressed as mean ±SEM except antimicrobial activity (Table 2) and unpaired student-t-test (Spiegel and Meddis, 1980) was used for the statistical analysis.

Results and Discussion

All the extracts exhibited potent, dose dependant (40 and 80 mg/kg) and significant analgesic and antidiarrhoeal activity in the order of AE>PE>CE>ME and ME>PE>AE>CE respectively. AE at the experimental dose was found to exhibit more potent analgesic activity (tail immersion method) than the standard drug. All the extracts exhibited significant antibacterial and antifungal activity against all the microbial strains except AE against *S. aureus*. ME was found to exhibit the most potent antimicrobial activity against *S. epidermidis, M. luteus, K. pneumonia* and *C. albicans*. PE was found to exhibit the most potent antimicrobial activity against *S. aureus* and *A. niger*. CE was found to exhibit the most potent antimicrobial activity against *B. cereus*. PE, CE and AE did not possess antibacterial activity at 50 μg/ml. ME exhibited the most potent antibacterial activity. The extracts in general exhibited more effective antifungal than antibacterial activity.

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