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Anticonvulsant, Anthelmintic and Antibacterial activity of *Lawsonia inermis*

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ABSTRACT

Lawsonia inermis L. is a branched glabrous shrub or small tree, cultivated for its leaves although stem bark, roots, flowers and seeds have also been used in traditional medicine. In the present study anticonvulsant, anthelmintic and antibacterial activity of chloroform, ethanol and water extract of *Lawsonia inermis* has been carried out. Anticonvulsant activity was performed using electroshock method, anthelmintic assay using adult earthworm *Eicinia fetida* and antibacterial activity was determined by cup-plate agar diffusion method. The phytochemical study of extracts shows the presence of flavonoids, tannins and coumarin. The activity may be due to these compounds. This study shows the anticonvulsant activity, anthelmintic activity and antibacterial activity of henna leaves.

Keywords: Henna, Antibacterial activity, Anthelmintic activity, Anticonvulsant activity.

INTRODUCTION

Lawsonia is monotypic genus, represented by *Lawsonia inermis*, native of North Africa and south-west Asia, widely cultivated as an ornamental and dye plant throughout India [1]. *Lawsonia inermis* L. is a much branched glabrous shrub [2] or small tree, cultivated for its leaves although stem bark, roots, flowers and seeds have also been used in traditional medicine. It is a natural red colouring agent, commonly named "Henna", which is used to dye skin and hair [3] and as tattooing agent in many civilizations and cultures. Henna leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat rheumatoid arthritis, headache, ulcers, diarrheoa, leprosy, fever, leucorrhoea, diabetes, cardiac disease, hepatoprotective and colouring agent [4-6].

Henna is an important source of phytochemicals such as naphthoquinone derivatives, aliphatic components, triterpenes, sterols, phenolic derivatives, coumarins, xanthones, flavonoids, gallic acid, hennotannic acid and mannitol which are effective as immunomodulators and other allied agents [7]. In the present study anticonvulsant, anthelmintic and antibacterial activity of various extracts of *Lawsonia inermis* is carried out.

MATERIAL AND METHODS

Plant material

The leaves of *Lawsonia inermis* Linn. were collected in November 2014 from Newasa town situated in Ahmednagar district, Maharashtra. The plant and plant material were identified and authenticated. The plant material was dried, powdered, and used for further extraction.

Extraction of crude drug

The powdered leaves of *Lawsonia inermis* were extracted with chloroform, ethanol and water using soxhlet extractor. Extract obtained subjected to preliminary pharmacological investigation.

Anticonvulsant activity [8]

Adult, healthy, overnight fasted, male albino mice, weighing between 20-25 gm were used for the evaluation of anticonvulsant activity. The mice were divided in a group of six. All animals had free access to water and standard pelletized laboratory animal diet *ad libitum*.

The animals were divided with each group consisting of six animals. After 30 minutes of administration, animals were stimulated through corneal electrodes with 50 mA current at a pulse of 60 Hz alternating current for 0.2 sec. The abolition of hind limb tonic extensor spasm was recorded as a measure of anticonvulsant activity. The above procedure was repeated after 60 and 90 minutes of administration.

The results for anticonvulsant activity are given in Table 1.

Statistical analysis

Data obtained from pharmacological experiments are expressed as mean ± S.E.M (Standard Error Mean). At the end of experiment, test groups were compared with control and the data was analysed by ANOVA followed by Dunnett's test. Values of $P < 0.05$ or lower were regarded as significant.

Anthelmintic activity [9]

All the extracts were used for anthelmintic assay using adult earthworm *Eicinia fetida*. Worms were collected and washed with normal saline solution. Test samples of all extracts were prepared at the concentrations, 10, 20, 50 and 100mg/ml by using Tween 80 as emulsifying agent and diluted to 10 ml with saline solution. Three worms of approximately equal size (same type) were placed in nine cm Petri dish containing above solution of extracts. Albendazole (10, 20, 50 and 100 mg/ml) was used as reference standard and normal saline as control. Time for paralysis and time for death of worms was noted are given in Table 2.

Antibacterial activity [10]

Antibacterial activity was determined by cup-plate agar diffusion method. The plates were inoculated by microorganisms such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Proteus vulgaris*, bores were made in the solidified agar plate by using a sterile borer. The test solutions and standards at 100, 200, 400 and 800µg/ml concentration was added in the bore and the plates were kept in freeze for 1hour and then incubated. After 24 hours the plates were examined and zone of inhibition were recorded. The zone of inhibition obtained was compared to the standard streptomycin and ampicillin as given in Table 3.

RESULTS AND DISCUSSION

Anticonvulsant activity

All the Henna extracts were subjected to anticonvulsant activity at 20 mg/kg, b.w. dose using electroshock method in mice. Many of the synthesized compounds showed significant anticonvulsant activity as compared to diazepam as standard. The results of the anticonvulsant activity are presented below at 30, 60, 90 minutes. Chloroform and ethanol extracts of henna exhibited anticonvulsant activity but significant activity is shown by chloroform extract against maximal electroshock induced convulsions in mice.

Anthelmintic activity

Results obtained indicate that the higher concentration of *Lawsonia innermis* extracts produced paralytic effect much earlier and the time to death was shorter (Table. 2). *Lawsonia innermis* extracts have shown significant anthelmintic activity as compared to standard at all concentrations. Among all the extracts aqueous extract shows a good anthelmintic activity.

Antibacterial activity

In the current study, various extracts of *Lawsonia innermis* were compared for their antibacterial activity with the standard antibacterial drugs. The result shows that ethanol extract does not show activity against *S. aureus* and *B. subtilis* while others shows activity. Among all the extracts aqueous extract shows good activity.

Table 1: Duration of hind limb extensor of the Henna extracts

Group	Treatment	Dose (mg/kg)	Duration of hind limb extensor in seconds (mean ± S.E.M)		
			30 minutes	60 minutes	90 minutes
I	Control	0.1ml/10gm	63±0.577	67.66±0.333	68±0.577
II	Standard (Diazepam)	5 mg	21.33±0.666**	28.33±0.333**	28.33±1.202**
III	Water	20mg/kg, b.w.	66.66 ±0.666*	62.33±1.202**	63.33±0.8819**
IV	Ethanol	20mg/kg, b.w.	46±1.155**	44.66±0.8819NS	54.33±0.801NS
V	Chloroform	20mg/kg, b.w.	42.66±0.333**	34.33±0.881**	43.66±1.202**

N=6, * = P<0.05, ** = P<0.01, when compared with control group

Table 2: Anthelmintic activity of various extracts against *Eicinia fetida*

Groups/Extracts	Concentration (mg/ml)	Time taken for paralysis (P) in min. (Mean ± S.E.M)	Time taken for death (D) in min. (Mean ± S.E.M)
Albendazole (Standard)	10	8.425±0.005	14.34±0.250
	20	5.315±0.015	11.615±0.395
	50	3.295±0.015	8.21±0.180
	100	2.5±0.010	5.80±0.220
Ethanol	10	16.575±0.425	31.55±0.450
	20	14.42±0.060	25.81±0.510
	50	12.55±0.445	18.66±0.000
	100	8.295±0.295	11.79±0.770
Water	10	13.50±0.500	31.5±1.500
	20	11.15±0.850	26.56±0.560
	50	8.85±0.550	24.19±0.195
	100	5.75±0.450	21.01±1.010
Chloroform	10	15.51±0.510	48.79±0.770
	20	14.27±0.115	42.75±0.635
	50	11.79±0.595	32.51±0.510
	100	8.795±0.505	28.74±0.255

Each value represents mean ± SEM (N=6).

Table 3: Antibacterial activity of various extracts

Extracts	Zone of Inhibition (mm)															
	S. aureus				*B. Subtilis.*				*E. coli*				*P. vulgaris*			
Concentration (µg /ml)	100	200	400	800	100	200	400	800	100	200	400	800	100	200	400	800
Ethanol	-	-	-	-	-	-	-	-	10	15	18	20	12	14	15	17
Chloroform	-	11	14	17	14	15	16	17	15	18	19	20	10	12	13	15
Water	16	18	20	21	12	15	17	19	12	15	18	21	13	16	18	19
Streptomycin	17	19	22	23	16	17	18	20	17	19	21	22	15	18	20	21
Ampicillin	17	20	23	22	17	18	19	21	17	20	21	22	16	19	21	23

CONCLUSION

The phytochemical study of extracts shows the presence of flavonoids, tannins and coumarin. The activity may be due to these compounds. This study shows that the leaves of this plant showed anticonvulsant activity, anthelmintic activity and antibacterial activity.

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