

PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON LEAVES OF HIPTAGE BENGALENSIS (L) KURZZ

M.V.Kumudhavalli*, B.Jayakar, R.Margret Chandira, M.Kumar, C.Saravanan

Vinayaka Mission's College of Pharmacy,

Vinayaka Missions University, Salem-636008, Tamil Nadu, India.

ABSTRACT: The main aim of this research is to find out new drugs from indigenous plant which are potent drugs. Present study deals with the phytochemical [26-28] and pharmacological [20-25] studies of leaves of hiptage Benghalensis(L) kurzz. The phytoconstituents were extracted by using various chemical tests. The ethanolic and aqueous extracts shows major phytoconstituents, hence it was selected for pharmacological screening. Further the ethanolic extract was chooses for compound isolation.

INTRODUCTION¹⁻¹¹

'Phytochemistry' or the 'chemistry of Natural Products' may be strategically placed somewhere in between natural product organic chemistry and plant biochemistry. Phytochemistry [26-28] or plant chemistry has developed in recent years as a distinct discipline. It is concerned with enormous variety of organic substances, their biosynthesis, turnover and metabolism, their norm distribution and their biological function. Development in the field of phytochemistry has increases the remedies for chronic diseases. This had created a new enthusiasm in researchers to develop herbal drugs.

OBJECTIVE

The main object as Phytochemistry is to isolate [26-28] the active phytochemicals, analyze their pharmacological activity [20-25] and characterize the isolated compounds.

EXPERIMENTAL WORK

The phytoconstituents were extracted by hot percolation process by using soxhlet apparatus with different solvents of increasing polarity like petroleum ether, chloroform, acetone, ethanol, and water and the phytoconstituents were identified by using various chemical tests (preliminary phytochemical screening studies).

Acute Toxicity studies¹²⁻¹⁹

Overnight Fasted swiss Albino mice with weight ranging (20-25gm female) were taken for the

experiment. The animals were made into a group or 3 each, dose of ethanol and aqueous extracts were given according to the body weight (mg/kg) starting dose of 5mg/kg was given to the first individual animal, no death was accrued higher doses were given to next group of animals dosed ranging from 50mg/kg, 300mg/kg, 2000mg/kg and the mortality due to these doses were observed. The Data showing acute toxicity study of Ethanolic and aqueous extracts of *Hiptage benghalensis* were given in Table No. 1.

Anti-inflammatory activity²⁰⁻²³

Anti-inflammatory activity of extracts was studied by carrageenan-induced rat paw oedema method. The animals were weighed and numbered. A mark was made on both the hind paws (right & left) just beyond tibiotarsal junction, so that a every time the paw was dipped in mercury column up to the fixed mark to ensure constant paw volume.

Four groups of rats were pre-treated with ethanol and aqueous extracts in dose of 200 mg/kg body weight orally. One group received only carboxymethyl cellulose which served as control, the other group received Diclofenac Sodium as standard drug for comparison in the dose of 10 mg/kg body weight under similar conditions. After 30min, 0.1 ml of carrageenan (1%) was injected into planter region of hind paw of rats. Measurement of paw volume (ml) was made by mercury displacement technique using plethysmometer in a time interval of 15, 30, 60, and

120 minutes, after carrageenan injection. The results were shown in the table no. 2.

Anthelmintic activity²⁴⁻²⁵

Ten groups each containing three earthworms was taken. Piperazine citrate was diluted with normal saline to obtain 5, 10 and 25 mg/ml served as standard and poured into Petri dishes. The effect of alcoholic and aqueous extracts was studied at the doses of 5, 10, and 25 mg/ml, the extracts were diluted with normal saline. Normal saline served as solvent control. All these extracts were poured into the Petri dishes. Three earthworms nearly equal size about 8 cm long were placed in each petridish at room temperature. The time taken to complete paralysis and death were recorded. The mean paralysis time and mean lethal time for each sample was recorded (Each reading was taken in triplicate). Paralysis occurred when the worms lost their motility. Time of death was the time when the earthworm did not respond to external stimuli and did not revive when placed in normal saline at 40. The result of anthelmintic activity was shown in table no. 3.

Isolation²⁶⁻²⁸

Ethanol extract was selected for the isolation of the available active constituents, because ethanol being a bipolar solvent, which can dissolve a wide range of phytoconstituents, whereas the aqueous extract contains polar compounds. Hence ethanol extract was selected for the isolation of available active constituents by column chromatography. The isolated compound was characterized by HPTLC, IR and Mass spectroscopy and interpreted the structure of Compound.

RESULTS AND DISCUSSION

The preliminary phytochemical studies shows the presence of Carbohydrate, proteins, amino acids, Saponins, tannins phenolic compounds and flavonoids in acetone extracts, Carbohydrate, proteins, amino acids, saponins, tannins, glycosides, phenolic compounds and flavonoids in Ethanol extract and Carbohydrate, proteins, amino acids, saponins, tannins glycosides, phenolic compounds and flavonoids in Aqueous extract.

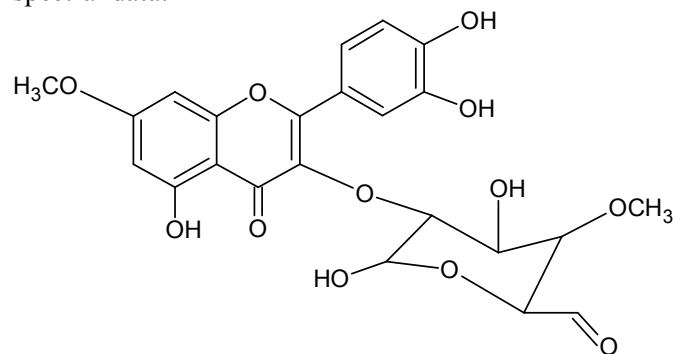
The LD₅₀ determination was done in mice by OECD guidelines and LD₅₀ [12-19] value of the Ethanol and aqueous extract was found to be 2000 mg/kg. Therefore its ED₅₀ was calculated as 200 mg/kg. The anti-inflammatory activity [20-23] of the Ethanol and aqueous extracts (200mg/kg) was

evaluated against the carrageenan induced paw oedma of rats were compared with the standard drug Diclofenac sodium. The effect of ethanol and aqueous extract of *Hiptage benghalensis* on carrageenan induced edema in rats was shown in Table.2 Edema suppressant effect of ethanol extract was found to be 66.66% whereas aqueous extract was found to be 60.00% which was nearly equivalent to that of 10mg/kg of Diclofenac sodium. The edema suppressant effect was significant ($P \leq 0.001$) in the dose of 200mg/kg ethanol and aqueous extracts, when compared with control. Between these two extracts, Ethanol extract shows maximum anti-inflammatory activity.

Helminthiasis [24,25] causes one of the major problems in world population about 2 billion affected worldwide helminthes like Pork worm, Beef worm, Round worm and Hook worm causes GIT as well as systemic complications. Ethanol and aqueous extracts showed paralysis and death of worms on concentration dependant manner.

Among two extracts of leaves of *Hiptage benghalensis*; aqueous extracts showed more potent anthelmintic activity.

Compound-2(3,4-dihydroxyphenyl)-3-(4,6-dihydroxy-3-methoxytetrahydro-2H-pyran-2-carbaldehyde)-5-hydroxy, 7-methoxy-4H-chromen-4-one was isolated and its structure was characterized by interpreting spectral data.



CONCLUSION

Ethanol and aqueous extracts show the presence of majority of phytoconstituents. Hence it was selected for the pharmacological studies. The ethanol extract which has the polarity in between the acetone and aqueous has been selected for isolation of the available active constituents. Ethanol and aqueous extracts of *Hiptage benghalensis* show significant effect on pharmacological studies.

Table No. 1: Data showing acute toxicity [12-19] study of Ethanolic and Aqueous extracts of *Hiptage benghalensis*.

Groups	No. of animals	Dose (mg/kg)	Results
1	3 animals	5	No Death
2	3 animals	50	No Death
3	3 animals	300	1 Death
4	3 animals	2000	2 Death

Table No. 2: Effect of Ethanol and Aqueous extracts of *Hiptage benghalensis* (L) on carrageenan induced rat paw edema[20-23]

Sr. No	Treatment	Dose (mg/kg)	Increase in paw edema										% inhibition
			0 min		15min		30min		60min		120min		
			R	L	R	L	R	L	R	L	R	L	
1	Control	-	0.45 ±0.05	0.45 ±0.05	0.45 ±0.05	0.8 ±0.0	0.45 ±0.05	0.85 +0.0	0.45 ±0.05	0.95 ±0.05	0.45 ±0.05	1.05 ±0.05	-
2	Diclofenac sodium	10	0.45 ±0.05	0.45 ±0.05	0.45 ±0.05	0.6 ±0.0*	0.45 ±0.05	0.55 ±0.05**	0.45 ±0.05	0.45 ±0.05***	0.45 ±0.05	0.25 ±0.05***	76.19%
3	Aqueous extract	200	0.45 ±0.05	0.45 ±0.05	0.45 ±0.05	0.7 ±0.0*	0.45 ±0.05	0.65 ±0.05**	0.45 ±0.05	0.55 ±0.05***	0.45 ±0.05	0.42 ±0.05***	60.00%
4	Ethanolic Extract	200	0.45 ±0.05	0.45 ±0.05	0.45 ±0.05	0.6 ±0.0*	0.45 ±0.05	0.55 ±0.05**	0.45 ±0.05	0.55 ±0.05***	0.45 ±0.05	0.35 ±0.05***	66.66%

* indicates significant $p < 0.1$, ** indicates significant $P < 0.01$, *** indicates significant $P < 0.001$, Values were expressed as mean \pm SEM.

Table No. 3: Anthelmintic Activity [24-25] of Ethanolic and Aqueous Extracts of *Hiptage benghalensis*

S.No	Treatment groups	Concentration (mg/ml)	Time taken for Paralysis (min)	Time taken for Death (min)
1.	Control	0.9 Nacl	-	-
2.	Piperazine Citrate (Std)	5	65	72.
		10	52	61
		25	33	40
3.	Aqueous extract	5	78	93
		10	61	81
		25	44	63
4.	Ethanolic extract	5	92	115
		10	76	99
		25	58	84

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