

Analgesic Activity of Bark of *Hibiscus mutabilis*

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Hibiscus mutabilis (Malvaceae) is a large bushy shrub or small tree, about 8 ft in height. It is cultivated in Indians gardens as an ornamental plant for its beautiful flowers, which may be single or double. Leaves are 10-23 cm in length, hairy, cordate, long petioled, suborbicular, 5-7 lobed or angled, irregularly crenate-dentate, often entire near the base, more or less softy pubescent or tomentose, stipules linear lanceolate. Flowers are 7-12 cm in diameter, white or pink in the morning turning red by night. The Plant material is used in traditional medicines for their emollient in pectoral and pulmonary complaints. It is prescribed as a stimulant and leaves are applied to the swellings.^{1,2} A flavonone glycoside naringenin, eriodictyol, ilicyanin and chrysanthemine have been isolated from the plant.^{3,4}

The bark of *H. mutabilis* was collected from Ahmednagar district of Maharashtra in August 2005 and authenticated by Botanical Survey of India, Pune (Voucher specimen No. PBG1). The bark was shade dried, reduced to coarse powder and subjected to successive solvent extraction using solvents as petroleum ether (60-80°C), ethyl acetate and methanol in Soxhlet extractor. Extracts were vacuum dried.

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Healthy wistar albino mice of either sex and of approximately the same age, weighing about 20-25 g were used for study. They were housed in polypropylene cages maintained under standard condition (12 hour light/12 hour dark cycle; 30 ± 4°C, 36-60% humidity).

The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethical Committee and was cleared by the same before starting.

Central analgesic activity of petroleum ether, ethyl acetate, and methanol extract was evaluated using hot plate method.⁵ The mice of either sex were divided into five groups of four animals each. The first group served as control and received only vehicle (2% DMF), second group was administered standard drug pentazocine lactate (50 mg/kg, i.p.) dissolved in 2% DMF in water for injection. The animals of third to fifth group were treated with petroleum ether, ethyl acetate, and methanol extracts (50 mg/kg, i.p.) suspended in 2% DMF in saline water. Mice were placed individually on the hot plate maintained at 55 ± 1°C and the latency to lick paws was noted. The basal reaction time was noted before and 30, 60, 90, 120, 150, 180 min after the administration of treatment.

The experiment was terminated 20 sec after their placement on the hot plate to avoid damage to

the paws. 0 min readings are the predrug reaction time.

Peripheral analgesic activity was evaluated using acetic acid-induced writhing test.^{6,7} Mice of either sex were prescreened 48 hrs before the actual experiment and those sensitive to acetic acid-induced writhing were divided into five groups, of six animals each. The animals received petroleum ether or ethyl acetate or methanol of *H. Mutabilis* bark (100 mg/kg, i.p.) in 2% DMF or standard drug paracetamol (50 mg/kg, i.p.) or vehicle as 2% DMF, 30 min before intraperitoneal injection of 0.1 ml of 0.6% solution of acetic acid. Mice were placed individually into glass beakers after administration of acetic acid and five minutes were allowed to elapse. The mice were then

observed for the period of 30 minutes and then number of writhes recorded for each animal.

All the extracts of *H. mutabilis* showed significant analgesic activity at 50 mg/kg, i.p. dose (Table 1). Analgesic activity was comparable with standard drug pentazocine. Among all the extracts, petroleum ether extract of *H. mutabilis* showed highest increase in reaction time. In case of analgesia, prostaglandins and bradykinins were suggested to play an important role in the pain process.⁸ Some sterols and triterpenes are responsible for analgesic activity.⁹ As phytochemical tests showed presence of these constituents in petroleum ether extracts, they may be responsible for the activity.

Table 1. Effect of Various Extracts of *H. mutabilis* Bark on Thermic Stimulus-induced Pain in Mice (Hot plate test)

Treatment	Latency to lick the paw (Sec)±SEM						
	Predrug reaction time	30 min	60 min	90 min	120 min	150 min	180 min
Vehicle	12.45 ± 0.64	13.8 ± 0.79	14.0 ± 1.23	13.8 ± 0.79	12.8 ± 0.69	11.57 ± 0.59	11.78 ± 0.60
PE	13.5 ± 0.71#	18.30 ± 2.51*	20.00 ± 2.92	19.52 ± 0.72#	17.38 ± 2.3*	10.71 ± 0.41	8.57 ± 0.29
CH	10.96 ± 0.53	16.50 ± 1.82	19.92 ± 2.56#	14.18 ± 3.49	20.00 ± 2.95	9.74 ± 0.31#	13.67 ± 0.78*
EA	13.62 ± 0.76*	15.03 ± 1.4#	10.49 ± 0.36#	12.76 ± 0.68	19.81 ± 2.53*	12.55 ± 0.67	14.84 ± 1.24
Pentazocine	14.71 ± 1.1	12.30 ± 0.66#	13.58 ± 0.74*	20.01 ± 2.93#	16.30 ± 1.79	8.19 ± 0.21	11.79 ± 0.61*

All the values are expressed as mean ± SEM; n = 6, # P < 0.05, *P < 0.0001 significant compared to control. All the extracts and pentazocine were given intraperitoneally at 50 mg/kg dose. PE, CH, EA are petroleum ether, chloroform, ethyl acetate extract of bark of *H. mutabilis*, respectively.

All the extracts of *H. mutabilis* at dose of 100 mg/kg, i.p., significantly attenuated the number of writhing and stretching induced by intraperitoneal 0.1ml 0.6% acetic acid (Table 2). Methanol extract of bark of *H. mutabilis* showed more inhibitory effect on writhing induced by acetic acid as compared to other extracts as well as standard drug paracetamol. Result shows that peripheral analgesic activity is in descending order like methanol, ethyl acetate and petroleum ether. Tannins, flavonoids and sterols were detected in above extract respectively. These compounds are having good analgesic activity by inhibiting Prostaglandin synthesis.¹⁰ Thus this supports peripheral analgesic activity of above extracts and the activity is may be because of PG synthesis inhibition

Table 2. Effect of various extracts of bark of *H. mutabilis* on acetic acid-induced writhing in mice

Treatment	Number of writhing	% inhibition of writhing
Vehicle	63.66 ± 0.729*	100.00
Paracetamol	42.06 ± 0.726	66.07
PE	28.06 ± 0.621*	44.08
EA	39.00 ± 0.722*	61.26
ME	17.3 ± 0.711*	27.08

All values are expressed as mean ± SEM (n = 6), *P < 0.05 significant as compared to control. PE, CH, EA are petroleum ether, chloroform, ethyl acetate extract of bark of *H. mutabilis*, respectively.

The results were analyzed for statistical significance using student's 't' test. P < 0.05 was considered significant.

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