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Pharmacological Screening of Dikamali Resin Extract

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Abstract – In the present study, dikamali resin (obtained from the leaf buds and the young shoots of *Gardenia gummifera* Linn.) was extracted with diethyl ether and the extract was vacuum dried. Qualitative tests confirmed the presence of flavonoids and free phenolic compounds in the extract. The antioxidant property (qualitative) of the extract was performed by TLC method (β-carotene-linoleate method). The LD_{50} of the extract was found to be 2227 mg/kg by Karber's arithmetic method. The extract was screened for analgesic, anti-inflammatory, antipyretic (100, 200 and 400 mg/kg) and anthelmintic (0.1, 0.2 and 0.5 %w/v) activities by standard methods. The extract exhibited antioxidant property and prevented oxidation of β-carotene. The extract exhibited significant graded dose response for analgesic, anti-inflammatory, antipyretic and anthelmintic activities. The extract caused the death of earthworms in all experimental concentration whereas the standard drug (piperazine) only effected paralysis. The present study proved the claims of dikamali resin mentioned in the Indian system of medicine.

Key words – dikamali, *Gardenia gummifera*, analgesic, anti-inflammatory, antipyretic, anthelmintic, antioxidant

Introduction

The leaf buds and the young shoots of *Gardenia gummifera* Linn (Kumar *et al.*, 1984) yield a resinous exudation known in commerce as dikamali or cumbi resin. In the Indian system of medicine, dikamali resin (Anonymous, 1956) has been claimed to possess antipyretic, antihelmintic, antispasmodic, expectorant and antidiarrhoeal activities. The Dikamali resin was reported to contain 2 flavonoids namely gardenin-A and 5-demethyl-tangeritin (Purushothaman *et al.*, 1973). In the present study, diethyl ether extract of dikamali resin was studied for its antioxidant property by thin layer chromatographic (TLC) method (β-carotene-linoleate method). Qualitative chemical tests were performed to detect the presence of flavonoids and free phenolic compounds. The extract was screened for analgesic, anti-inflammatory, antipyretic and anthelmintic activities by standard methods.

Materials and Methods

Plant material and extraction – Dikamali resin was acquired form Country drugs dealer, Chennai, India and the sample was authenticated by Dr. P. Brinda, Botanist, Captain Srinivasamurthi Drug Research Institute for Ayurveda, Chennai, India. The coarsely powdered dikamali resin was extracted with diethyl extract for 48 hours by cold maceration. The extract was filtered and vacuum dried (yield = 0.2% w/w).

Preliminary identification of chemical constituents – Preliminary chemical tests (Geissman, 1995) were performed to detect the presence of flavonoids and free phenolic compounds. The qualitative chemical tests like shinoda test, ammonia fuming test, lead acetate test, ferric chloride test, chalcones test, boric acid test, zirconium oxychloride test, gibbs test, p-benzoquinone test and o-dinitro benzene test confirmed the presence of flavonoids and free phenolic compounds.

Antioxidant property by TLC method – The extract was solubilized in methanol (Onyencho, 1992; Kanner *et al.*, 1994) and subjected to TLC on 20×20 cm glass plates precoated with silica gel-G. The developing solvents used was chloroform: methanol (9:1) for flavonoids and chloroform: ethyl acetate: formic acid (5:4:1) for free phenolic compounds. The locations of the spots were marked under UV light. β-Carotene-linoleate (a mixture of β-carotene in 30 ml of chloroform and 2 ml of purified linoleic acid in 60 ml of 95% ethanol) was sprayed uniformly on the plates and exposed to daylight

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for about 4 hours. The background was bleached and the spots that contained the flavonoids and phenolic compound retained the yellow color that is indicative of antioxidant activity.

Animals – Inbred wistar albino rats and wistar albino mice 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 (0600 to 1800 h-light and 1800 to 0600 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 dikamali resin extract was evaluated for analgesic, anti-inflammatory, antipyretic and anthelmintic activities. Acute toxicity study was performed for the extract to ascertain the LD_{50} values by Karber's arithmetical method (Ghosh, 1984). The test compounds and the standard drugs were administered in the form of a suspension using 0.1% carboxymethylcellulose as vehicle. Earthworms (*Lampito mauritii*) were obtained from Bell Foundations, Chennai, India for screening anthelmintic activity.

Analgesic activity – The analgesic activity (Ghosh, 1984) was determined by acetic acid induced writhing method using wistar albino mice (25-30 g) of either sex selected by random sampling technique. Paracetamol at a dose level of 100 mg/kg was administered as a standard drug. The extract at 3 dose levels (100, 200 and 400 mg/kg) was administered orally by gavage 15 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 30 min and percentage protection was calculated for analgesic activity. The results are presented in Table 1.

Table 1. Analgesic activity of dikamali resin extract

Group	Dose (mg/kg)	Writhings	% Protection
Extract	100	25.6 ± 1.24*	42.34
	200	19.4 ± 0.89*	56.3
	400	14.4 ± 0.93*	67.5
Paracetamol	100	7.3 ± 0.41*	84.23
Control	–	44.4 ± 1.63	–

Significance levels: *p<0.05.

Acute anti-inflammatory activity – The acute anti-inflammatory activity (Laurence and Bacharach, 1964) was determined by formalin induced paw edema method in wistar albino rat (150-200 g) of either sex by using plethysmograph. Diclofenac sodium (25 mg/kg) was administered as standard drug. The extract at 3 dose levels (100, 200 and 400 mg/kg) was administered orally by gavage 30 minutes prior to administration of formalin (0.1 ml of 1% w/v) in the plantar region of the paw. The paw volumes were measured at 15, 30, 45, 60 and 120 min after formalin administration and the percentage reduction of edema was calculated. The results are presented in Table 2.

Antipyretic activity – The antipyretic activity (Loux, 1972) was determined by experimentally induced pyrexia in wistar albino rat (150-200 g) of either sex. Paracetamol (100 mg/kg) was administered as standard drug. The extract at 3 dose levels (100, 200 and 400 mg/kg) was administered orally by gavage prior to administration of 20% w/v of suspended brewer's yeast (1 ml/100 g) subcutaneously. After administration of yeast, the rectal temperature of the rats was recorded by using telethermometer at 30, 60, 90, 120, 150 and 180 min intervals. The results are presented in Table 3.

Anthelmintic activity – Dikamali resin extract was tested for anthelmintic activity (Nargundi, 1999) using earthworms (8±1 cm length). Earthworms (n=6) were placed in petridishes containing 0.1%, 0.2%, and 0.5% w/v of the extract in 5% propylene glycol at room temperature (28±1°C). Piperazine citrate (0.1, 0.2 and 0.5% w/v in normal saline) was used as standard. 5% propylene glycol and normal saline were used as control. The time taken for complete paralysis (motionless) and death (absence of movement when induced by external

Table 2. Acute anti-inflammatory activity of dikamali resin extract

Group	Dose (mg/kg)	% Reduction of edema				
		15 min	30 min	45 min	60 min	120 min
Extract	100	11	14.4	26.9	27.2	27.6
	200	20.1	25.6	35.5	36.3	36.7
	400	32	42.8	59.9	63.6	63.8
Diclofenac	50	40.1	52.8	64.9	67.3	67.4

Table 3. Antipyretic activity of dikamali resin extract

Group	Dose (mg/kg)	Rise in body temperature					
		30 min	60 min	90 min	120 min	150 min	180 min
Extract	100	0.48 ± 0.1**	0.83 ± 0.19*	1.63 ± 0.16*	2.15 ± 0.12*	2.42 ± 0.19*	2.8 ± 0.2**
	200	0.45 ± 0.16*	0.77 ± 0.08*	1.45 ± 0.14*	2.03 ± 0.19*	2.05 ± 0.18*	2.18 ± 0.14**
	400	0.3 ± 0.06***	0.77 ± 0.13***	1.02 ± 0.09***	1.22 ± 0.08***	1.33 ± 0.11***	1.46 ± 0.14**
Paracetamol	100	0.15 ± 0.05***	0.28 ± 0.06***	0.42 ± 0.09***	0.63 ± 0.06***	0.9 ± 0.08***	1.36 ± 0.05***
Control	–	0.98 ± 0.12	2.01 ± 0.11	2.32 ± 0.26	2.88 ± 0.38	3.1 ± 0.15	3.96 ± 0.27

Significance levels: *p< 0.1, **p< 0.05, ***p< 0.001 compared to control .

Table 4. Anthelmintic activity of dikamali resin extract

Group	Concentration (% w/v)	Time (min)	
		Paralysis	Death
Extract	0.1	195.33 ± 0.49*	320.66 ± 0.88*
	0.2	180.16 ± 0.98*	235.5 ± 0.42*
	0.5	35.16 ± 0.54*	100.16 ± 0.79*
Piperazine citrate	0.1	71.8 ± 0.54*	–
	0.2	62.5 ± 0.76*	–
	0.5	52.16 ± 0.60*	–

Significance levels: * $p < 0.05$ compared to control.

physical stimuli) were recorded. The data are presented in Table 4.

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

Results and Discussion

The LD_{50} of the extract was found to be 2227 mg/kg by Karber's arithmetic method. Qualitative tests confirmed the presence of flavonoids and free phenolic compounds in the extracts. The flavonoids and free phenolic compounds present in the extract exhibited antioxidant property, which was evident from the non-bleaching of β-Carotene-linoleate reagent from thin layer chromatographic studies. The extract exhibited significant analgesic, anti-inflammatory and antipyretic at the experimental dose levels. The extract also exhibited graded dose response. The extract caused the death of earthworms in all experimental concentration whereas the standard drug (piperazine) only effected paralysis. The present study proved the claims of dikamali gum mentioned in the Indian system of medicine.

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