

PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *Capparis decidua* (Forsk.) Edgew stem FOR CENTRAL NERVOUS SYSTEM DEPRESSANT ACTIVITY

PREETI GARG¹, KAMAL SACHDEVA², ISHA BHANDARI³

- 1. Department of Pharmacognosy, Suresh Gyan Vihar University, Jaipur, India**
- 2. Department of Pharmacology, Jaipur national university, Jaipur, India**
- 3. Department of Pharmaceutics, Rajendra college of pharmacy, Sirsa**

¹Corresponding Author-

Preeti Garg

Department of Pharmacognosy, Suresh Gyan Vihar University, Jaipur (India), 302025.

Mb +91-9660107668

Email id – preetimangla@ymail.com

Summary

The research work deals with the screening of ethanol and aqueous extracts of *Capparis decidua* stem for central nervous system (CNS) depressant activity. The plant possesses anthelmintic, hepatoprotective, antidiabetic, hypolipidemic activity and plant is known to contain alkaloids, flavanoids and glycosides as chemical components. Since stem of *Capparis decidua* is used as folk medicine in treatment of CNS disease, we made an attempt to study its phyto-constituent and CNS depressant effect. The different activities studied were test for locomotor activity, effect on muscle co-ordination and antianxiety activities. The result of the study reflected that ethanolic extract of the stem (100 mg/kg, p.o) decreased locomotor activity, produced muscle relaxation and showed antianxiety activity due to presence of alkaloids.

Key words: *Capparis decidua*, muscle relaxant, antianxiety, locomotor

Introduction

Advance in science and technology has contributed to an enormous improvement in the quality of life of humankind. However, modern life stress, associated trials and tribulation are responsible for the surge in incidence of variety of psychiatric disorders. Path breaking research in psychopharmacology has flooded the market place with drugs for specification. For instance, benzodiazepines (diazepam, nitrazipam lorazepam and alprazolam etc) are the most frequently prescribed synthetic drugs for variety of condition particularly anxiety, depression, epilepsy and insomnia. But these psychoneural drugs have very serious side effects like chronic use of benzodiazepines causes deterioration of cognitive function, physical dependence and tolerance. Besides addiction liabilities, benzodiazepines adversely affect the respiratory, digestive and immune system of body and the chronic treatment with benzodiazepines often prove more harmful in the longer run.¹

In this context, a resurgence of interest in medicine from natural sources (mainly plant products) is seen and there is tremendous hope that drugs of plant origin will have significantly lesser side effects than that observed with synthetic drugs while having comparable efficacy.

A variety of naturally occurring drugs such as *Thymus linearis*, *Lactuca seroila*, *Papaver somniferum* (opium) and *Atropa belladonna* were tested for psychopharmacological effects and were found to be effective in the treatment of psychiatric disorders.² The plant *Capparis decidua* (Forsk.) Edgew. belongs to family *Capparaceae*,³ a small much branched tree or shrub of arid regions in Africa, Middle East and Southern Asia, including the Thar Desert.⁴ It occurs in vegetative cover in dry, hot, sandy desert and arid regions where little else grows and is an extremely hardy species. It is a bushy shrub in dense tufts, 4-5 m high, or occasionally a small tree with many green vine-like apparently leafless branches, hanging in bundles. Bark turns whitish-grey colour with age, but most branches and twigs are a glossy dark green. Small, light brown caducous, linear 1-2cm long apex short, stiff, pale mucro-like prickles occur in pairs on the twigs at each node; flowers red or pink, rarely yellow in lateral corymbs, berries globose or ovoid 1-2cm in diameter, dull red; seed globose, 2-5mm in diameter.⁵ From alcoholic extract of root bark six oxygenated heterocyclic constituents capparisterpenolide (3-carboxy-6, 17-dihydroxy-7, 11, 15, 19 tetramethyleicos-13-ene-d-lactone) and deciduaterpenolides (d-lactone derivatives of 1, 3, 3-trimethyl- 1, 4-cyclohexadien-6-one) A, B, C, D and E were isolated and characterized.⁶ Stem, fruits and flowers⁷ contains n-triacontanol, water soluble stachydrine (2-Carboxy-1, 1-dimethyl Pyrrolidine), N-pentacosane, β -Sitosterol and β -Carotene and hydrocarbons Nonacosane and Triacontane. The stem possesses anthelmintic activity, hepatoprotective activity,⁸ antidiabetic activity, hypolipidemic activity.⁹ Flowers and fruits are sedative and anticonvulsant.¹⁰ Flowers are antiatherosclerotic,¹¹ anti-inflammatory, analgesic.¹² Based on the above information, we thought it is worthwhile to evaluate the *Capparis decidua* (Forsk.) Edgew. stem for CNS depressant activity.

Materials and methods

Plant materials and preparation of extract

The stem of *Capparis decidua* (*Capparaceae*) was collected in and around Jaipur and was identified in the Department of Botany, Rajasthan University Jaipur. A voucher specimen (No RUBL20861) was deposited in the department. The powder of dried stem was subjected to successively soxhlet extraction with various organic solvents such as petroleum ether (60-80°C), ethanol & water respectively.¹³

Phytochemical evaluation

Identification of phytoconstituents in the extracts was carried out using chemical test and thin layer chromatography method by different detecting reagents.¹⁴ Ethanolic extract was eluted through column chromatography and the samples collected from column were tested for thin layer chromatography to detect the presence of phyto-constituents.¹⁵ Sample which showed the presence of phyto-constituent was further carried out for IR analysis¹⁶ and the results are shown in table 1, 2, 3, 4.

Experimental animals

Swiss albino mice of either sex weighing between 12–35 g were used in the present study. The experimental protocol was approved by the Institutional Animal Ethics Committee. The animals were maintained under standard conditions in Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) approved Institutional Animal House.

Acute toxicity study

The acute oral toxicity study was performed according to the OPPTS (Office of Prevention, Pesticides and Toxic Substance) guidelines.¹⁷

Behavioral parameters used to test CNS depressant activity**Open field Model¹⁸**

The open field apparatus is made up of plywood consists of 56 x 56 (l x b) cm. In the entire apparatus 6 mm thick white lines divided the floor in to 16 square of identical dimension. 30 minutes after the treatment with control/diazepam (1 mg/kg, i. p.)/ Ethanol extract of *Capparis decidua* (EECD), (100 mg/kg, i. p.)/ Aqueous extract of *Capparis decidua* (AQECD), (100 mg/kg, i. p.), each animal were placed at one corner of the apparatus and the following behavioral aspects were noted in the next 5 min.

Ambulation: Number of square passed by animal

The percentage inhibition of locomotor activity was calculated by using following formula and the results are given in table no 5.

$$\% \text{ inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

Actophotometer Test¹⁹

The Locomotor activity was studied using actophotometer. The movement of the animal interrupts the beam of light falling on a photocell at which the count was recorded and displayed digitally. Each mouse was placed individually in the actophotometer for 5 min and the basal activity was obtained. Subsequently the animals were divided in to 4 groups each consists of six animals. Control, diazepam (1mg/kg, i. p.), EECD (100mg/kg, i. p.) and AQECD (100 mg/kg, i. p.), were administered and after 30 min of administration, the mice were placed in the actophotometer for recording the activity score. The percentage inhibition of locomotor activity was calculated by using following formula and the results are given in table no. 6.

$$\% \text{ inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

Rotarod Test²⁰

The apparatus consists of a horizontal wooden rod or metal rod coated with rubber with 3 cm diameter attached to a motor with the speed adjusted to 25 rotations per minute. The rod is divided into 3 sections by plastic discs, thereby allowing the simultaneous testing of 3 mice. The rod is in a height of about 50 cm above the table top in order to discourage the animals from jumping off the roller. Mice with a weight between 20 and 30 g undergo a test on the apparatus. Control/ diazepam (1 mg/kg, i. p.)/ Ethanol extract of *Capparis decidua* (EECD), (100 mg/kg, i. p.)/ Aqueous extract of *Capparis decidua* (AQECD), (100 mg/kg, i. p.), were administered intraperitoneally. Thirty minutes after intraperitoneal the mice are placed for 5 min on the rotating rod. Time spent in seconds by animals on the roller during this test is counted. The percentage inhibition of time spent on rotarod was calculated by using following formula and the results are given in table no. 7.

$$\% \text{ inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

Elevated plus-maze Model²¹

The plus-maze apparatus, consisting of two open arms (16 x 5 cm) and two closed arms (16 x 5 x 12 cm) having an open roof, was elevated (25cm) from the floor to observe anxiolytic behavior in mice. Mice were treated with control/diazepam (1 mg/kg, i. p.)/ Ethanol extract of *Capparis*

decidua (EECD), (100 mg/kg, i. p.)/ Aqueous extract of *Capparis decidua* (AQECD), (100 mg/kg, i. p.) 30 min before being placed individually at the center of the elevated plus maze with its head facing the open arm. During the 5 min experiment, following behavior of the mouse was recorded: Total time spent in open and closed arm. During the entire experiment, mice were allowed to socialize. Every precaution was taken to ensure that no external stimuli, other than the height of the plus-maze. The percentage protection was calculated using the following formula and the results are given in table no. 8.

$$\% \text{ protection} = \frac{V_t - V_c}{V_t} \times 100$$

Light-Dark Model²²

Light dark box is a rectangular box of 46 x 27 x 30 cm (l x b x h), which is divided in to 2 compartment 1/3 rd is for the dark compartment and 2/3 rd served as light compartment. Mice were treated with control/diazepam (1 mg/kg, i. p.)/ Ethanolic extract of *Capparis decidua* (EECD), (100 mg/kg, i. p.)/ aqueous extract of *Capparis decidua* (AQECD), (100 mg/kg, i. p.) 30 min before being placed individually on the light compartment and observe for a period of 5 min. Time spent in light and dark zones are observed during this observation period. The percentage protection was calculated using the following formula and the results are given in table no. 9.

$$\% \text{ protection} = \frac{V_t - V_c}{V_t} \times 100$$

Where, V_c = Control reading

V_t = Test reading

Statistical analysis

The data obtained by the various parameters was statistically evaluated by one way analysis of variance (ANOVA) followed by Dunnett's test. The mean values \pm SEM were calculated for each parameter.²³

Results

Phytochemical investigation

The Preliminary phytochemical investigation showed the presence of phytoconstituents and their results are given in table 1.

Table no. 1: Preliminary qualitative tests of powder drug & each extract

S. No.	Tests	Powder	Petroleum ether extract	Ethanolic extract	Aqueous extract
Alkaloids					
1.	Dragendroff's	+ve	-ve	+ve	+ve
2.	Wagner's	+ve	-ve	-ve	+ve
Glycosides					
1.	Legal test	+ve	+ve	+ve	+ve
2.	Keller-killiani test	+ve	+ve	+ve	-ve

Steroids					
1.	Salkowski test	-ve	-ve	-ve	-ve
Carbohydrates					
1.	Molisch test	+ve	+ve	-ve	-ve
2.	Fehling test	-ve	-ve	+ve	-ve
Flavanoids					
1.	Shinoda test	-ve	-ve	-ve	-ve
2.	Lead acetate test	-ve	-ve	-ve	-ve
Protein and amino acid					
1.	Ninhydrin test	+ve	-ve	+ve	+ve
2.	Biuret test	+ve	-ve	-ve	+ve
Tannin and Phenolic compound					
1.	Lead acetate test	+ve	+ve	+ve	+ve
2.	Ferric chloride test	+ve	-ve	-ve	+ve
Gum and Mucilage					
1.	Test with ruthenium red	+ve	+ve	+ve	+ve
Saponin					
1.	Foam test	+ve	-ve	+ve	-ve

+ve – Present, -ve – Absent

TLC study

Qualitative TLC studies confirm the presence of alkaloids, terpene glycoside and flavanoid in ethanol and alkaloid and flavanoid in aqueous extract by thin layer chromatography technique. The results are shown in table 2.


Table no. 2: TLC study of ethanol & aqueous extract

S. No.	Solvent System	For Phyto-constituents	R _f Observed		Remark	
			Ethanolic Extract	Aqueous Extract	Ethanolic Extract	Aqueous Extract
1.	Toluene: ethylacetate: diethylamine (70:20:10)	Alkaloids (opium alkaloid)	0.86	0.75	Spot targeted may be opium alkaloid as R _f compare to std. (0.0-0.85)	Spot targeted may be opium alkaloid as R _f compare to std. (0.0-0.85)
2.	Benzene: pyridine: formic acid (72:18:10)	Flavanoids (Rutin)	0.44	0.47	Spot targeted may be Rutin as R _f compare to std. (0.4)	Spot targeted may be Rutin as R _f compare to std. (0.4)
3.	Chloroform: methanol: water (64:50:10)	Terpene glycosides (Liquiritiae)	0.83	0.46	Spot targeted may be Liquiritiae as R _f compare to std. (0.6-0.8)	Spot targeted but not reci-propate with std. R _f (0.6-0.8), No specific observation

Column chromatography study

Solvent systems were eluted through column of ethanolic extract in the order of increasing polarities and fractions were collected. The isolated fractions were subjected to TLC study using suitable solvent system and the positive results are tabulated in table 3. On the basis of results we can say that there is possibility to the presence of alkaloid.

Table no. 3: TLC of collected fraction after column chromatography

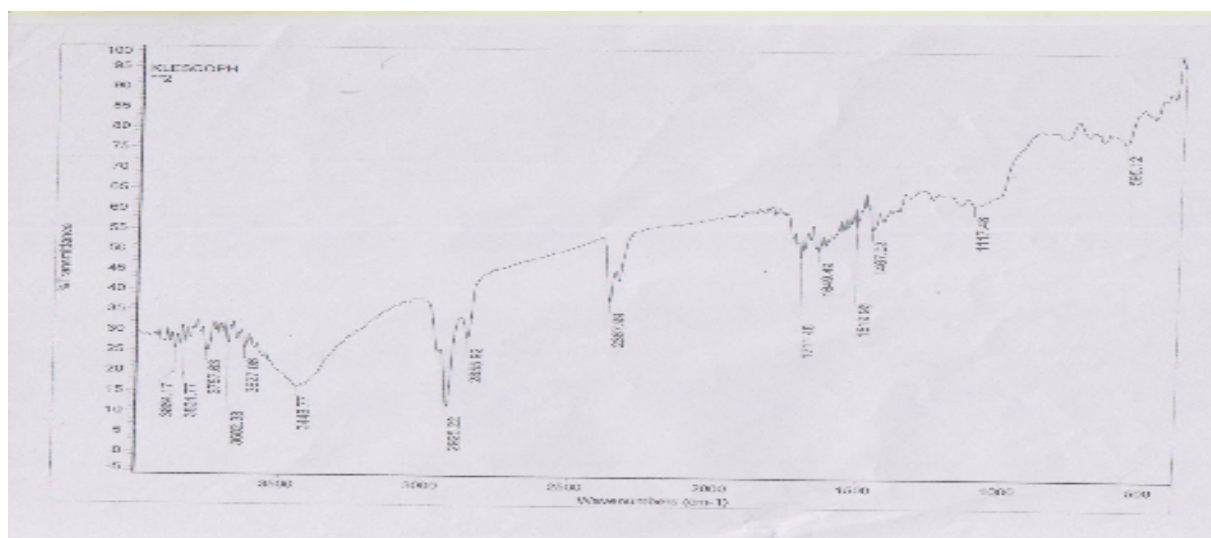
S. No.	Solvent System	R _f Observed	TLC Plate	Remark
1.	Toluene: ethylacetate: diethylamine (7:2:1)	0.20		R _f 0.20 of this fraction is compared to std.R _f of alkaloid

Spectroscopic study**IR spectroscopy**

The FTIR spectrum of isolated compound has shown characteristic peaks as listed in table 4. Spectra are given as:

Table no. 4: FTIR absorption bands of isolated compound

Sample No.	O-H stretching or N-H stretching	Aliphatic C-H stretching	C≡N stretching	C=O stretching	O-H stretching (Aromatic)	Aromatic C-H stretching
73	3448.77	2925.22, 2855.92	2367.99	1711.48	3864.17	1519.96



Hypothetically it may be revealed that these IR range fall under the category of alkaloids.

CNS depressant activity**Open field test**

It has been observed that AQECD (100 mg/kg i. p.), EECD (100 mg/kg I. p.) and diazepam (1 mg/kg i. p) decreased the locomotor activity significantly ($P < 0.01$) and the results are shown in table 5.

Table no.5: Effect on locomotor activity in Open field test

S. No.	Groups	Open field test	% inhibition
		No. of square cross in 5 min	
1.	Control	74.83±13.62	-
2.	Standard drug (1mg/kg, i. p.)	34.17±7.49**	54.33
3.	Ethanollic extract (100 mg/kg, i. p.)	35.00±7.00**	53.22
4.	Aqueous extract (100 mg/kg, i. p.)	37.50±7.38**	49.89

All value are expressed as Mean± SEM, n = 6, * $P < 0.05$, ** $P < 0.01$ when compared with control.

Actophotometer model

It has been observed that dose of EECD (100 mg/kg i. p.) and diazepam (1 mg/kg i. p) decreased the locomotor activity significantly ($P < 0.05$) whereas, dose of AQECD (100 mg/kg i. p.) did not show a significant reduction in the locomotor activity and the results are shown in table 6.

Table no. 6: Effect on locomotor activity in actophotometer

S. No.	Groups	Actophotometer	% inhibition
		Interruption with beam in 5 min.	
1.	Control	136.16±26.59	-
2.	Standard drug (1mg/kg, i. p.)	68.16±12.85*	49.44
3.	Ethanollic extract (100 mg/kg, i. p.)	82.63±14.35*	39.31
4.	Aqueous extract (100 mg/kg, i. p.)	84.16±13.07	38.19

All value are expressed as Mean± SEM, n = 6, * $P < 0.05$, ** $P < 0.01$ when compared with control.

Rotarod test

It has been observed that dose of AQECD (100 mg/kg i. p.), dose of EECD (100 mg/kg i. p.) and diazepam (1 mg/kg i. p) significantly reduced the time spent by the animals on revolving rod when compared to control ($P < 0.05$) and the results are shown in table 7.

Table no. 7: Effect on muscle coordination in rotated apparatus

S. No.	Groups	Time spent on revolving rod on rotarod apparatus (sec.)	% activity
1.	Control	106.17±22.36	-
2.	Standard drug (1mg/kg, i. p.)	45.17±11.53*	57.45
3.	Ethanollic extract (100 mg/kg, i. p.)	52.33±12.28*	50.71
4.	Aqueous extract (100 mg/kg, i. p.)	59.33±13.87*	44.12

All value are expressed as Mean± SEM, n = 6, *P<0.05, **P<0.01 when compared with control.

Elevated plus maze test

Dose of EECD (100 mg/kg i. p.), significantly increased the time spent in light (P<0.05) when compared with control. The standard drug (diazepam 1 mg/kg, i. p) showed a significant increased the time spent in light (P<0.01). Dose of AQECD (100 mg/kg, i. p.) did not show significantly increased in activity compared to control and the results are shown in table 8.

Table no. 8: Effect on anxiety in Elevated plus maze test

S. No.	Groups	Elevated plus maze test	
		Time spent in open arms in 5 min.(sec.)	% protection
1.	Control	41.67±6.44	-
2.	Standard drug (1mg/kg, i. p.)	85.00±11.29**	50.98
3.	Ethanollic extract (100 mg/kg, i. p.)	67.50±8.12*	31.12
4.	Aqueous extract (100 mg/kg, i. p.)	60.50±7.61	38.27

All value are expressed as Mean± SEM, n = 6, *P<0.05, **P<0.01 when compared with control.

Light-dark model

Dose of EECD (100 mg/kg i. p.), significantly increased the time spent in open arms (P<0.05) when compared with control. The standard drug (diazepam 1 mg/kg, i. p) showed a significant increased the time spent in open arms (P<0.01). Dose of AQECD (100 mg/kg, i. p.) did not show significantly increased in activity compared to control and the results are shown in table 9.

Table no. 9: Effect on anxiety in Light-dark model

S. No.	Groups	Light-dark model	
		Time spent in light in 5 min. (sec.)	% protection
1.	Control	68.50±5.21	-
2.	Standard drug (1mg/kg, i. p.)	89.00±5.19**	23.03
3.	Ethanollic extract (100 mg/kg, i. p.)	83.17±4.55*	7.64
4.	Aqueous extract (100 mg/kg, i. p.)	74.17±4.48	17.63

All value are expressed as Mean± SEM, n = 6, *P<0.05, **P<0.01 when compared with control.

Discussion

The phytochemical study showed the presence of alkaloid due to which the plant show the effect on CNS and the pharmacological study showed that EECD (100mg/kg, i. p.) possess sedative, anti-anxiety, muscle relaxant activity. The study on the locomotor activity by open field test and actophotometer test showed that EECD (100 mg/kg, i. p.) decreased the frequency and the amplitude of movements. The reduction of the spontaneous motor activity could be attributed to the sedative effect of the extract.

EECD (100 mg/kg, i. p.) also reduced the more time spent on the revolving rod by mice as comparison to AQECD (100mg/kg, i. p.) in the rotarod test, a test mainly used to screen centrally acting muscle relaxants. This represented that EECD may have muscle relaxant activity, which could be due to CNS depressant activity.

The Elevated plus maze test and Light-dark model was used for the assessment of anxiety and sedation. Greater number of time spent in open arm and light indicates anxiety like behavior and lesser number of steps ascended indicated increased sedation.²⁴ The present investigation successfully detected the anxiolytic-like effects of EECD and diazepam; both significantly decreased the number of time spent in open arm and light and number of steps ascended compared to control. This showed that EECD has both anxiolytic and sedative properties.

The sedative, muscle relaxant and anxiolytic effects of EECD could be due to the interaction of numerous alkaloids (chemical constituent of the plant) with the GABA/benzodiazepine receptor complex in brain²⁵ as well with drugs that stimulate glucocorticoid production and release in the adrenal cortex, after administration of 5-HT_{1B} receptor antagonists and 5-HT_{1A} agonists.²⁶ Therefore, with the present data, it is difficult to predict the precise mechanism for the anxiolytic activity of the *Capparis decidua* stem bark.

To conclude, the ethanolic extract of stem bark of *Capparis decidua* possess sedative, anti-anxiety and muscle relaxant properties.

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