Study of analgesic and anti-inflammatory activity from plant extracts of *Capparis decidua*

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Abstract

Four different types of extracts (Ethanolic extract, Petroleum ether soluble fraction, Chloroform soluble fraction and aqueous soluble fraction) of *Capparis decidua* plant were prepared and evaluated for Preliminary phytochemical constituents. Out of which EECD (Ethanolic extract of *Capparis decidua*) and AECD (Aqueous extract of *Capparis decidua*) were also used for the determination of analgesic and anti-inflammatory activity in graded doses of 200 and 400 mg/kg. From the results, it was concluded that extract of *Capparis decidua* have found to possess potent analgesic, anti-inflammatory activity and also ethanolic extract is more effective as compared to aqueous extract.

Keywords *Capparis decidua*, analgesic activity, anti-inflammatory activity, phytochemical evaluation

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Introduction

Currently, either narcotics, such as opioids, or non-narcotics, such as salicylates and corticosteroids, such as hydrocortisone, are used to treat pain and inflammation. All of these medications have well-known negative side effects and toxicities [1-5]. Additionally, the cost of developing synthetic pharmaceuticals is highly high since, in order to successfully introduce a new product, 3000–4000 molecules must be synthesised, screened, and tested, with development costs ranging from 0.5 to 5 million dollars [6-7]. On the other hand, numerous medications having a botanical origin have long been used without any negative side effects. Therefore, it is crucial that attempts be made to introduce new medicinal plants in order to provide less expensive medications. Plants still provide a significant untapped source of structurally unique chemicals that might be used as a starting point for the creation of new medications [8-10].

The International Association for the Study of Pain (IASP) defines pain as a distressing, sensory, and emotional experience connected to existing or potential tissue damage or expressed as such harm [11-15].

The body uses inflammation as a defence mechanism to get rid of noxious stimuli like germs, damaged cells, or irritants and start the healing process [16-17]. The body's initial reaction, which starts seconds to minutes after a tissue injury brought on by a damaging stimulus, is acute inflammation [18-19]. If harmful foreign chemicals persist for a longer period of time and cannot be eliminated by the body, acute inflammation will develop chronic [20-22].

Similar to how opioids, which are used as potent analgesics, are accompanied by side effects like addiction and dependence, nonsteroidal anti-inflammatory drugs are typically used to treat inflammation. However, these drugs are linked to harmful side effects like GI irritation, ulceration, bleeding, etc. As a result, researchers are becoming increasingly interested in herbal medications since they may be safer and more effective than NSAIDS and other analgesics [23-25]. Due to their extensive spectrum of pharmacological effects, natural compounds originating from plants, such as flavonoids, sterols, polyphenols, alkaloids, tannins, and terpenes, have gained relevance in recent years [26-27]. Researchers are now evaluating the biological properties these naturally occurring compounds generated from plants possess. Finding natural remedies with fewer adverse

effects and less risk for addiction, like opioids, might be crucial in the treatment of inflammation and pain conditions [28-30].

The WHO defined health as "a complete state of physical, mental, and social well-being and not merely the absence of disease or infirmity" [31]. Traditional medical practises have so grown in relevance during the past ten years on a global scale. According to current estimates, a significant section of the population in many poor nations relies significantly on traditional healers and medicinal herbs to address basic healthcare needs [32-33].

Although there may be access to modern medicine in some nations, herbal medicines, or phytomedicines, have frequently maintained their appeal due to historical and cultural factors. In parallel, a lot of individuals in wealthy nations have started using supplementary or alternative medicines, including herbal remedies [34-37].

For a long time, pickled capers have been used as a condiment. The Capparidaceae family includes the plant that is generally referred to as caper berries in English, Karira in Sanskrit, Karer or Kurrel in Hindi, Kerada in Gujarati, Nispatige in Kannad, Nepati in Marathi, Kair in Punjabi, Shengan in Tamil, and Enugadanta in Telegu. Natural habitats for herbal plants include wastelands, generally on slopes, that are dry and exposed. Its origins are mostly in the deserts of Punjab and Sind, particularly in Rajputana, and it spreads south to Karnataka and Tamil Nadu before becoming a wild plant in Rajasthan, Gujarat, and the Western Ghats. Both wild and developed caper plants are used to make caper buds; planted plants often lack spines. It was grown on sandy, well-drained soil in the sun. It is multiplied by mature woodcutting in summer at 19 to 24°C and seed sowed in fall or spring. According to Ayurveda, the bark is analgesic, diaphoretic, laxative, effective against dropsy, an anthelmintic, and effective against asthma, boils, ulcers, piles, vomiting, and other inflammatory disorders [38-45].

The paper reports on preliminary screening, analgesic and anti-inflammatory activities of *Capparis decidua.*

Experimental Work

Preliminary phytochemical evaluation of plant materials

The plant *Capparis decidua* was collected and cleaned to remove dust using tap water then dried in shade for a few weeks. The dried plant was then mechanically ground to fine powder. Four different types of extracts (Ethanolic extract, Petroleum ether soluble fraction, Chloroform soluble fraction and aqueous soluble fraction) were prepared. These four extracts were screened for the presence of phytochemical constituents such as carbohydrates, tannins, flavonoids, saponins, cardiac glycosides, alkaloids etc. and phytochemical constituents have been detected qualitatively by the characteristic colour changes and precipitation occurring upon treatment with specific reagents as mentioned below:

Test for Carbohydrate

- Molisch test- 2ml of extract was subjected to Molisch's reagent in a test tube. Observed that violet ring was formed.
- Fehling's test- 1ml of extract was treated with Fehling A and Fehling B solutions. The mixture was heated for 5 minutes observed that red precipitate was formed.
- Benedict's test- To 1ml of extract and 1ml of Benedict's reagent was added in a test tube. This mixture was heated at water bath for 7 minutes observed that red colour was formed.
- Barfoed's test- 2ml of extract was added to 1ml of Barfoed's reagent, observed that a red precipitate was formed.

Test for Protein and Amino Acids

- Biuret's test- 1ml of extract was mixed with 1ml of 10% sodium hydroxide solution in a test tube and heated. Further 0.7% copper sulphate solution was added to above solution. Observed that formation of violet colour, that indicated presence of protein.
- Millon's test-3ml of extract was treated with 5ml of Milion's reagent. A white precipitate was formed. On heating the solution, it was turned to brick red colour that indicated presence of protein.
- Ninhydrin test- 2ml of extract was treated with 4-5 drops of 5% ninhydrin solution and heated. Observed that formation of blue

colour. Concluded the presence of amino acid.

Test for Glycoside

- Borntrager's test- Dilute sulphuric acid was added to 3ml of extract, boiled for 5-6 minutes and filtered. Filtrate was cooled and 3ml of benzene was added and stirred the solution. Few ml of dilute ammonia solution was added. Observed that a pink to red coloured layer was formed indicated the presence of anthraquinone glycosides.
- Keller-Kiliani test- 2ml of extract was mixed with 3ml of glacial acetic acid and a drop of 5% ferric chloride in a test tube, followed by addition of 2-5 drops of concentrated sulphuric acid at the side of test tube, observed that blue colour was formed in acetic layers. Result that cardiac glycoside is present.

Test for Saponin

- Froth test- 1ml of extract diluted with distilled water and shake regularly in graduated cylinder for 20 minutes. No change observed in this extract. Result that forth tests is negative.

Test for Alkaloids

- Mayer's test- Few drops of Mayer's reagent were added to 2ml of extract. A white creamy precipitate formed.
- Dragendroff's test- Few drops of Dragendorff's reagent were added to 2ml of extract added in a test tube. Red precipitate formed.
- Hager's test- To 3ml of extract, few drops of Hager reagent were added in a test tube. Yellow precipitate was formed. Result that alkaloid is present.
- Wagner's test- Few drops of Wagner's reagent were added to 2ml of extract in a test tube. A reddish-brown precipitate was formed. Result that alkaloid is present.

Test for Flavonoids

- Shinoda's test- 1 ml of extract was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips were added to the filtrate followed by introducing few drops of conc. HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids.
- Alkaline reagent test- 2 ml of extract was treated with few drops of sodium hydroxide (NaOH) in test tube yellow colour is formed. In this mixture few drops of Conc. H_2SO_4 were added if the mixture becomes colourless. Results indicated that flavonoids are present.

Test for Triterpenoid and terpenoids

- Salkowski's test- 2ml of extract was treated with chloroform and filtered. To this filtrate, few drops of Conc. H_2SO_4 were added by shaking and allow to stand for few minutes. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids. In bottom yellow colour resulted that Triterpenoid are present.
- Liebermann-Burchard Test–2ml of extract was treated with chloroform and filtered. To this filtrate, few drops of acetic anhydride were added, boiled and cooled. Few drops of concentrated sulphuric acid were added through side of test tube. Observe that brown ring at junction of two layers was turned green indicating that a steroid is present. Another layer red colour showed resulted that Triterpenoid are present.

Test for Tannins and phenolic compounds

- Ferric chloride test- 2ml of extract was dissolved in distilled water. To this solution, 2ml of 5% ferric chloride was added. Violet colour was observed. Results revealed that phenolic compound is present.
- Lead acetate test- 2ml of extract was dissolved in distilled water. Then few drop of lead acetate solution were added. White precipitate was observed. Result that phenolic compound is present.
- Dilute iodine test- To 2ml of extract, dilute iodine solution was added. Red colour was observed. Result that phenolic compound is present.

Test for Coumarins: 1ml of extract was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

Test for Quinones: 1ml of the extract was treated with alcoholic potassium hydroxide solution. Quinines give coloration ranging from red to blue.

Test for Anthocyanins: 2 mL of the extract was treated with 2 ml of 2N HCl. The appearance of a pink-red color that turns purplish blue after addition of ammonia indicates the presence anthocyanins [46-55].

Extraction of *Capparis decidua*

Four different types of extracts were prepared using dried fine powder of *Capparis decidua* and different solvents viz: ethanol, petroleum ether, chloroform and water so as to get 250µg/ml.

***In vitro* Analgesic activity and Anti-inflammatory activity**

Experimental Animals

Wistar albino rats (220-250 g) of either sex/Swiss albino mice (22-25 g) were maintained in colony cages at 25±2°C, relative humidity 50–55% maintained under 12 h light and dark cycle (6–10 h light, 18–6 h dark). The animals were fed with Standard animal feed and water was applied *ad libitum*. All the animals were acclimatized to the laboratory conditions prior to experimentation [22/BNCP/IAEC/2018].

Acute toxicity study

The acute oral toxicity study was carried out according to Organization for Economic Cooperation and Development (OECD) guidelines 423. The limit test for acute toxicity of EECD was carried out in the dose of 2000 mg/kg, orally, in rats. The rats were observed for a period of 72 h and thereafter up to 14 days for behavioral, neurological, autonomic profiles and for any lethality, moribund state, or death [56].

Doses and treatments

Mice and rats were divided into six different groups and each group consist of six animals. EECD (Ethanolic extract of *Capparis decidua*) was suspended in 0.5% sodium carboxymethyl cellulose in normal saline while AECD (Aqueous extract of *Capparis decidua*) was solubilized as such for administration purpose. Both the extracts EECD (Ethanolic extract of *Capparis decidua*) and AECD (Aqueous extract of *Capparis decidua*) were administered in graded doses of 200 and 400 mg/kg, orally. The doses selected were based on acute toxicity study and previous reports. The extracts and standard drugs were given in volume of 10 ml/kg, orally and 5 ml/kg, intra-peritoneally (*i.p.*) All rodents were fasted for 12 h before starting the test.

Assessment of analgesic activity

Assessment of peripheral analgesic effect of EECD and AECD

The peripheral analgesic effect of EECD and AECD was evaluated using acetic acid-induced writhing test model. Writhing is defined as stretch, torsion to one side, retraction of the abdomen and opisthotonos so that the belly of the mouse touches to the floor. Abdominal writhing episodes were induced in mice by i.p. injection of acetic acid (10 ml/kg of 0.6%). Aspirin (25 mg/kg, orally) was used as standard drug. Mice were pre-treated with vehicle or EECD or AECD or Aspirin 1 h prior to acetic acid injection and thereafter the observations were made. The number of stretching's occurred immediately within 30 min after the acetic acid injection was recorded. The results were evaluated by calculating the mean number of stretching per group and represented as % inhibition of stretching movement compared with the control group as below:

$$\% \text{ Analgesic activity} = (n - n'/n) \times 100$$

where n is the average number of stretching in the control group and n' is the average number of stretching in the test group [57-58].

Assessment of central analgesic effect of EECD and AECD

The central analgesic effect of EECD and AECD was studied using Eddy's hot plate test. The rats were placed on the hot plate maintained at 55 ± 1°C and time in seconds for the paw licking or jumping was recorded as the reaction time. Rats, which have shown basal reaction time period below 6 sec, were selected and used for the screening. Pentazocine (5 mg/kg) was administered *i.p.* as standard drug. Rats were pre-treated with vehicle or EECD or AECD or pentazocine. The activity of the rats on the hot plate was closely observed and the reaction time (latency period) was recorded in seconds as the time taken for the animal to react to the thermal pain by licking its paw or attempting to jump out [59].

Assessment of anti-inflammatory activity of EECD and AECD carrageenan-induced paw oedema

The anti-inflammatory activity was assessed using carrageenan-induced paw oedema in the rat model [60]. Acute paw oedema (acute

inflammation) was induced by sub-plantar injection of carrageenan suspension (0.1 ml of 1% w/v freshly prepared suspension in normal saline) into the right hind paw of each rat. The left hind paw was injected with the same volume of 0.1 ml of normal saline. Indomethacin (10 mg/kg, orally) was used as standard drug [61]. Rats were pre-treated with vehicle or EECD or AECD or Indomethacin 1 h prior to carrageenan administration. The paw size was measured in mm using vernier caliper before (0 min) and at 1st and 3rd h after carrageenan administration. The percent inhibition was calculated by the following formula:

$$\text{Percentage inhibition} = (C_t - C_o)_{control} - (C_t - C_o)_{treated}/(C_t - C_o)control$$

Where C_t = Thickness of paw after carrageenan injection and C_o = Thickness of paw before carrageenan injection

Statistical analysis

Values were expressed as means±SEM. The comparisons within groups were evaluated utilizing one-way analysis of variance (ANOVA) with Dunnett's multiple comparisons test was used to compare the group means and $p<0.05$ was considered statistically significant. Prism 7.05, for Windows was used for the statistical analysis.

Results and Discussion

All the four extracts were evaluated for the presence of the phytochemical constituents. The results showed that EECD and AECD contains Saponins, Tannins, Steroids, Carbohydrates, Coumarines, Alkaloids, Proteins, Anthocyanins, PECD and CFCD contains Terpenoids, Coumarines, Alkaloids, Steroids and Anthraquinones. as shown in Table 1.

Table 1: Phytochemical test results of different extracts of *Capparis decidua*

Phytochemical tests	Ethanol Extract fraction	Petroleum ether soluble fraction	Chloroform soluble fraction	Aqueous soluble fraction
Saponins	++	-	-	+
Tannins	++	-	-	+++
Flavonoids	-	-	-	-
Terpenoids	-	+++	++	-
Steroids	++	+	+++	+++
Phlobatannins	-	-	-	-
Carbohydrates	+++	-	-	+++
Coumarines	++	+++	+	++
Alkaloids	++	+	+	+
Proteins	++	-	-	++
Emodins	-	-	-	-
Anthraquinones	-	+	++	-
Anthocyanins	++	-	-	+++

- = Absent, + = Mildly present, ++ = Moderately present, +++ = Highly present

Acute toxicity study

The limit test for acute toxicity showed that rats receiving EECD or AECD at 2000 mg/kg, orally, exhibited normal behavioural, neurological and autonomic profiles. No mortality or toxicity of any nature was observed during 72 h and thereafter up to 14 days observation. This indicated that approximate LD_{50} value of EECD or AECD is more than 2000 mg/kg, orally and it is relatively safe and non-toxic to rats as well as mice.

Assessment of analgesic activity

Analgesic activity of ethanolic and aqueous extracts of *Capparis decidua* were screened by following two methods:

- Peripheral analgesic effect by acetic acid-induced writhing test.
- Central analgesic effect by the hot plate test.

Peripheral analgesic effect of EECD and AECD on acetic acid-induced writhing test in mice

One-way ANOVA revealed a significant ($P< 0.01$) influence of EECD (400mg/kg) on acetic acid-induced writhing and showed the maximum protection (56.35%) comparable to standard (52.42%) and lower dose of the extract (200mg/kg) exhibit less inhibition of writhing as compared to standard and the % protection was found to be 28.7. Whereas AECD extract (400mg/kg) also exhibited potent peripheral analgesic activity (46.87%).

Table 2: Peripheral analgesic effect of EECD and AECD on acetic acid-induced writhing test

Group	Treatment	Number of Writhing (Mean±SEM)	% Protection
A	Normal Control	32.68±0.206	0.00
B	EECD (200 mg/kg)	23.30±0.115*	28.70
C	EECD (400 mg/kg)	14.26±0.117**	56.35
D	AECD (200 mg/kg)	24.61±0.200^{ns}	24.69
E	AECD (400 mg/kg)	17.36±0.306*	46.87
F	Aspirin (25 mg/kg)	15.55±0.150**	52.42

*Mice were pre-treated with vehicle or EECD or AECD (200 and 400 mg/kg, orally) or Aspirin (25mg/kg, orally). Results are expressed as mean±SEM (n=6), ^{ns}: not significant, **p<0.01, *p<0.05 compared to vehicle treated group (one-way repeat measure ANOVA followed by Dunnett's test). EECD: Ethanolic extract of Capparis decidua, AECD: Aqueous extract of Capparis decidua, ANOVA: Analysis of variance. SEM: Standard error of the mean*

Central analgesic effect of EECD and AECD on the hot plate test in mice

Central analgesic effect of EECD and AECD was accessed by the hot plate test method in mice. The results showed that EECD (400 mg/kg) significant increase in reaction time whereas EECD (200 mg/kg) also moderate increase but remaining all the extracts did not show any significant effect on reaction time compared with the control group after 30 min. EECD at 400 mg/kg produced maximum effect and increased reaction time from 8.94, 10.89 and 12.76 seconds at the end of 30 min, 90 min and 180 min respectively. The effect of EECD 400 mg/kg was comparable with standard drug pentazocine that showed significant increase in reaction at the end of 90 min and 180 min.

Table 3: Central analgesic effect of EECD and AECD in hot plate test

Group	Treatment	Hot plate reaction time (s)			
		0 min	30 min	90 min	180 min
A	Normal Control	2.65±0.021	3.12±0.027	3.42±0.033	2.89±0.018
B	EECD (200 mg/kg)	4.13±0.035**	6.54±0.053**	7.34±0.033**	6.68±0.036**
C	EECD (400 mg/kg)	4.35±0.044**	8.94±0.049**	10.89±0.057**	12.76±0.061**
D	AECD	3.24±0.044^{ns}	3.66±0.039^{ns}	3.76±0.071^{ns}	3.11±0.042^{ns}

	(200 mg/kg)				
E	AECD (400 mg/kg)	4.67±0.043*	3.93±0.042^{ns}	3.98±0.045*	3.21±0.039^{ns}
F	Pentazocine (5 mg/kg)	5.55±0.039**	9.11±0.044**	11.13±0.067**	13.66±0.055**

*Mice were pre-treated with vehicle or EECD or AECD (200 and 400 mg/kg, orally) or pentazocine (5 mg/kg intra-peritoneally). The reaction time (latency period) was recorded on hot plate (55±1°C) in seconds as the time taken for the animal to react to the thermal pain by licking paw or attempting to jump out. Results are expressed as mean±SEM (n=6). ^{ns}not significant, **p<0.01, *p<0.05 compared to vehicle treated group (one-way repeat measure ANOVA followed by Dunnett's test). EECD: Ethanolic extract of Capparis decidua, AECD: Aqueous extract of Capparis decidua, ANOVA: Analysis of variance. SEM: Standard error of the mean*

7.6 Anti-inflammatory effect of EECD and AECD on carrageenan-induced rat paw oedema

The anti-inflammatory activity was assessed using carrageenan-induced paw oedema in the rat model. Indomethacin (10 mg/kg, orally) was used as standard drug. Rats were pre-treated with vehicle or EECD or AECD or Indomethacin 1 h prior to carrageenan administration. The paw size was measured in mm using vernier caliper before (0 min) and at 1st and 3rd h after carrageenan administration.

EECD showed significant anti-inflammatory activity at 1st and 3rd hour, the % inhibition of EECD (200 mg/kg) was found to be 45.69 and 44.31% at 1st and 3rd hour respectively. The Anti-inflammatory activity % inhibition of EECD (400 mg/kg) was found to be 61.80 and 68.51% at 1st and 3rd hour respectively whereas standard drug indomethacin showed 65.17 and 69.97% inhibition at 1st and 3rd hour respectively.

Table 4: Assessment of anti-inflammatory activity of EECD and AECD in carrageenan-induced paw oedema

Group	Treatment	Volume of edema (mean) ± SEM (ml)		Anti-inflammatory activity (% inhibition)	
		1 hr	3hr	1 hr	3hr
A	Normal Control	2.67±0.023	3.43±0.031	-	-
B	EECD (200 mg/kg)	1.45±0.013*	1.91±0.017**	45.69	44.31
C	EECD (400 mg/kg)	1.02±0.018**	1.08±0.019**	61.80	68.51
D	AECD (200 mg/kg)	2.04±0.028^{ns}	2.56±0.036^{ns}	23.60	25.36
E	AECD (400 mg/kg)	1.82±0.019^{ns}	2.11±0.019*	31.84	38.48
F	Indomethacin (10 mg/kg, orally)	0.93±0.011**	1.03±0.016**	65.17	69.97

*Rats were pre-treated with vehicle or EECD or AECD (200 and 400 mg/kg, orally) or indomethacin (10 mg/kg, orally). 1 hr prior to carrageenan administration. The paw size was measured at 1st hr and 3rd hr after carrageenan administration and the percent inhibition was calculated. Results are expressed as mean±SEM (n=6). ^{ns}not significant, **p<0.01, *p<0.05 compared to vehicle treated group (one-way repeat measure ANOVA followed by Dunnett's test). EECD: Ethanolic extract of Capparis decidua, AECD: Aqueous extract of Capparis decidua, ANOVA: Analysis of variance. SEM: Standard error of the mean*

Conclusion

The limit test for acute toxicity showed that indicated that approximate LD_{50} value of EECD or AECD is more than 2000 mg/kg, orally and it is

relatively safe and non-toxic to rats as well as mice. Analgesic activity of ethanolic and aqueous extracts of *Capparis decidua* were screened by Peripheral analgesic effect by acetic acid-induced writhing test and Central analgesic effect by the hot plate test. A significant influence of EECD (400mg/kg) on acetic acid-induced writhing and showed the maximum protection comparable to standard and lower dose of the extract (200mg/kg) exhibit less inhibition. Central analgesic effect of EECD and AECD was accessed by the hot plate test method in mice. The results showed that higher dose of EECD significant increase in reaction time whereas lower dose of EECD also moderately increase but remaining all the extracts did not show any significant effect on reaction time compared with the control group but at the time EECD with 400 mg/kg dose was comparable with standard drug pentazocine that showed significant increase in reaction at the end of 90 min and 180 min. The anti-inflammatory activity of EECD and AECD was determined by carrageenan-induced rat paw oedema method. EECD showed significant anti-inflammatory activity at 1st and 3rd hour and the other extracts showed mild to moderate anti-inflammatory activity.

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