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RESEARCH

Pharmacological Evaluation of *Crassula ovata* of Antinociceptive Activity in Albino Rat

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ABSTRACT

The current research was based on the pharmacological evaluation of *crassula ovata* of anti-nociceptive activity in albino rat. The fresh leaves of *Crassula ovata* were collected from Uttar Pradesh region and authenticated from a botanist with ref. no. CIMAP/Bot-Pharm/2021/20. The leaves were washed making dust-free and dried at room temperature or shade. The dried leaves were rendered into coarse powders and then finally into fine ones. The powder is weighed and soaked into ethanol solvent for 15 days with gradual stirrings (maceration process). The herbal extract was evaluated for their anti-nociceptive activity using hot-plate and tail-flick methods. Upon calculation, the percentage yield of ethanolic leaves extract of *Crassula ovata* was found as 63.58%. It showed phenols, flavonoids and terpenoids in moderate amount. However, phytosterol, steroid, carbohydrates, and proteins were found positive in *C. ovata* leaves extract. Saponins, While, saponins, tannins, glycoside, and plobatanin and starch were found absent. In results, ethanolic leaves extract of *Crassula ovata* were shown anti-nociceptive or analgesic potential when tested at 200mg/kg &400mg/kg. They exhibited dose-dependent activity and markedly increased the basal reaction time at various time intervals. In conclusion, inhibition of inflammatory mediators such as interleukins, thromboxane, cytokines, etc. may be the cause of analgesic activity. It refers to determine the mode of action that how ethanolic leaves extract of *Crassula ovata* subside pain whether its internal or external one.

Keywords: *Crassula ovata,* ethanolic extract, analgesic activity, hot plate method, tail-flick method.

INTRODUCTION

An illness is defined as "an impairment of the normal state of the living animal or plant body or one of its parts that interrupts or modifies the performance of the vital functions." [1]. Disease is described as a response to environmental stimuli, infectious agents, innate flaws, or a mix of these. It is characterized by characteristic signs and symptoms [2]. IASP defines pain as a fearful experience linked to potential tissue damage. Nociception, pain perception, suffering, and pain behaviors are the four primary categories that account for the variety of pain types [3].

NSAIDs act on the central nervous system (CNS) or peripheral pain mechanisms to selectively reduce pain without significantly altering consciousness. On one extreme of the scale are general anesthetics, while mild OTC drugs include acetaminophen and aspirin [4]. Oral opioid analgesics, known as "Tincture of laudanum," were initially introduced in Britain at the end of the 17th century. These are the drugs, synthetic or natural, that affect the body in the same way as morphine.

The capsule of the poppy (Papaver somniferous) contains a dark brown, sticky material. It belongs to two distinct alkaloid classes [5].

Plant profile: *Crassula ovata*

Often referred to as the money tree or jade plant, Crassula ovata is a member of the Orpine family, or Crassulaceae. These are a group of succulent, dicotyledonous plants that have tiny white or pink flowers. Their succulent leaves serve as their primary water storage unit [6].

Evergreen and reaching heights of one to three metres, the jade plant has thick branches and smooth, spherical, fleshy leaves that grow on opposite sides of the branches, which are likewise short, stubby, and proportionate. Rich jade green in hue, the leaves are elliptic to egg-shaped, measuring 30 to 90 mm in length and 18 to 40 mm in width. When a stem grows, it initially has the same feel and colour as the leaves, but as it ages, it turns brown and woody [7].

Taxonomy

Kingdom: Plantae

Order: Saxifragales

Family: Crassulaceae

Genus: *Crassula*

Species: *ovata*

It was discovered that there were several phytochemicals present in both the water and methanol extracts of Crassula ovata leaves. Money plant showed itself as a rich source of saponin, phenol, steroids, terpenoids, flavonoids, carbohydrate and protein when tested in the methanolic and aqueous leaves extract of *Crassula ovata* [8]. It has been reported for different pharmacological properties including antimicrobial, antioxidant and antidiabetic potentials.

MATERIALS AND METHODS

Experimental Requirements

Crassula ovata leaves, ethanol, distilled water, diclofenac and NaOH.

Digital balance, beaker, hot plate, laboratory thermometer and pH meter.

Collection, authentication, and extraction of the plant

Fresh Crassula ovata leaves were gathered from the Uttar Pradesh area and verified by a botanist using reference number CIMAP/Bot-Pharm/2021/20. The leaves are cleansed to get rid of any dust and then allowed to dry at room temperature or in the shade. The dried leaves are first processed into a coarse powder and then into a fine powder. After weighing the powder, it is gradually mixed and immersed in an ethanol solvent for 15 days (maceration procedure). The resultant slurry of the mixture is dried under partial vacuum using a rotary evaporator. The % yield of the Crassula ovata extract was calculated using the formula below [9].

Formula-

$$\text{percent yield} = \frac{\text{actual yield}}{\text{theoretical yield}} \times 100\%$$

Phytochemical screening

Alkaloids

Each extract was separately dissolved in diluted HCl before being filtered.

Mayer's Test: Potassium mercuric iodide, or Mayer's reagent, was applied to the filtrates. Alkaloids are present when a precipitate with a yellow hue forms.

Wagner's Test: Wagner's reagent, which is iodine in potassium iodide, was applied to the filtrates. Alkaloids are present when a brown or reddish precipitate forms.

Hager's Test: Hagers Reagent was used to treat the filtrates. The appearance of yellow precipitation suggests the presence of alkaloids.

Glycosides

With distilled water dilution, Fehling's solutions A and B were heated for one minute. There were 8 drops of plant extract added to this transparent blue solution. It was then combined with 1 ml of Fehling's solution and heated for 5 minutes in a water bath. Brick red precipitation is an indication of glycoside content.

Saponins

For a stable, long-lasting froth, about 2g of the plant extract was combined with 10ml of distilled water and vigorously shaken. Saponins are indicated by the appearance of foam [10].

Tannins

Ferric chloride test: In a test tube, 0.5 grams of the dried powdered material was cooked in 20 milliliters of water before being filtered. After adding a few drops of 0.1% FeCl3, the coloration was checked for brownish green-black or blue-black.

Lead acetate test 2 ml of distilled water and 2 ml of plant extract were mixed together. After adding 0.01g of lead acetate to the mixture, give it a good shake. Tannins are present when white turbidity and precipitate develop [11].

Flavonoids

NaOH test: After treating a little amount of extract with aqueous NaOH and HCl, the production of a yellow-orange color was noticed.

H_2SO_4 test: Conc.H2SO4 was applied to a portion of the extract, and the production of orange color was monitored.

Terpenoids

After mixing 2.0 ml of chloroform with 5 ml of the aqueous plant extract, the mixture was added, allowed to evaporate on the water route, and then heated to a boil using 3 ml of concentrated H2SO4. A grey coloration developed as terpenoids took shape.

Steroids

5 ml of aqueous plant crude extract was combined with 2 ml of chloroform and concentrated H2SO4. The presence of steroids was detected by the appearance of red hue in the lower chloroform layer.

Tests for sugars and carbohydrates

Molisch's test

Add a few drops of α-naphthol solution in alcohol to 2-3 ml of extract of each solvent, agitate, and then add concentrate H2SO4 from the test tube's sides. a violet ring where two liquids converge.

Fehling's test

It is utilised to find decreasing sugars. Make a volume of 500 mL by dissolving 34.66 grammes of copper sulphate in distilled water (solution A). 50 grammes of sodium hydroxide and 17.3 grammes of potassium sodium tartrate should be dissolved in distilled water to a volume of up to 50 millilitres (Solution B). Prior to usage, combine two solutions in an equal volume. Fehling's A and B solution in a 1 mL mixture should be boiled for one minute. Equal parts of the test solution should be added. Heat for five to ten minutes in a kettle of boiling water. There came a flash of brick red, followed by yellow.

Animal preparation

Rats (any sex) weighing 120–150g were submitted to Animal House, Department of Pharmacology, HIPER, Lucknow. A 12-hour light and dark cycle and a room temperature of 25°C were used to keep the rats healthy. The relative humidity was kept at 50±2%, and the mice were given a regular diet and unlimited access to

water. Up until one hour before to the start of the trial, the rats were allowed unlimited access to water while maintaining their fast.[12].

Experimental design

Rodents were divided into 4 groups (n=6) as below-

Group 1: administered saline water daily up to 21 days.

Group 2: administered diclofenac (50mg/kg, i. p.) for 21 days.

Group 3: administered ethanolic leaves extract of *Crassula ovata* (ELCO) (200mg/kg, i. p.) for 21 days.

Group 4: administered ethanolic leaves extract of *Crassula ovata* (ELCO) (400mg/kg, i. p.) for 21 days.

Evaluation parameters

i. Hot plate method

The animal's baseline reaction time was assessed by placing them on a hot plate that was maintained at a consistent temperature of 55°C. This was done by keeping an eye out for the first evident behavior, like jumping or licking the rear paws. It took 6–8 seconds to notice this response. A 10-second time limit was used as the cutoff point in order to protect the paws. At least three to five basal reaction times were recorded for each rat every five minutes to make sure they were behaving normally. Reaction times at 15, 30, 45, 60, and 90 minutes after the drug was administered were noted. The animals were removed from the hot plate as soon as the reaction time reached 10 seconds, which was considered to be maximum analgesia, to avoid damaging the paws. Every time, the test compound's data were compared to those of the standard drug, and the percentage increase in reaction time (analgesia index) was computed [13].

ii. Tail-flick method

The basal reaction time to radiant heat was assessed by placing the tail tip—the last one to two centimeters—on the radiant heat source and immersing it in hot water maintained at 58°C. The rat's tail-withdrawal from the heat (flicking response), which usually happens in three to five seconds, indicates the end point. To prevent harming the tail, a cutoff time of 10 to 12 seconds is observed. At least three to five basal reaction times were recorded for each rat every five minutes to make sure they were behaving normally. Reaction times at 15, 30, 45, 60, and 90 minutes after the drug was administered were noted. As soon as the reaction time reached 10 seconds, which was considered maximum analgesia, the tail was removed from the heat source to prevent tissue damage. Every time, the test compound's data were compared to those of the reference drug, and the percentage increase in reaction time (analgesia index) was computed [14].

RESULTS AND DISCUSSION

Percentage yield

Upon calculation, the percentage yield of ethanolic leaves extract of *Crassula ovata* was found as 63.58%.

Determination of phytochemicals screening

It showed phenols, flavonoids and terpenoids in moderate amount. However, phytosterol, steroid, carbohydrates, and proteins were found positive in *C. ovata* leaves extract. Saponins, While, saponins, tannins, glycoside, and plobatanin and starch were found absent. Therefore, *C. ovata* shown a rich source of numerous phytochemicals as mentioned in below table:

Table 1. Determination of Phytochemicals

S.N.	Phytochemicals	Ethanolic *Crassula ovata* extract
1.	Saponins	–
2.	Phenols	++
3.	Phytosterol	+

4.	Steroid	+
5.	Tannins	_
6.	Terpenoids	++
7.	Glycoside	_
8.	Plobatanin	_
9.	Flavonoid	++
10.	Carbohydrate	+
11.	Protein	+

Where, (+)= Positive, (++)= Moderated Positive, (-)=Negative

Pharmacological evaluation

➢ Hot plate method

The ethanolic leaves extract of *Crassula ovata* exhibited the basal reaction time as 3.73±0.34 sec and 4.19±0.19 sec at the dose of 200mg/kg and 400mg/kg, respectively. However, after standard group, the maximum basal reaction time was seen at 90 min, as 4.67±0.34 sec and 5.11±0.48 sec in ethanolic leaves extract of *Crassula ovata* treated rats at dose of 200mg/kg and 400mg/kg, respectively. So that it suggested that biological response was increasing with the time. The herbal extract exhibited dose-dependent pharmacological effects. It indicates that Crassula ovata has a good ability to reduce pain.

Table 2. Basal reaction time of ethanolic leaves extract of *Crassula ovata* in hot plate method

Treatment	Basal reaction time (sec)				
	0 min	30 min	45 min	60 min	90 min
Normal Saline	2.34±0.30	2.39±0.47	2.36±0.20	2.38±0.24	2.42±0.35
Diclofenac (50mg/kg)	2.39±0.31	3.47±0.38	3.69±0.29	4.31±0.11	5.35±0.13
ELCO (200mg/kg)	2.36±0.29	2.41±0.55	3.24±0.18	3.73±0.34	4.67±0.34
ELCO (400mg/kg)	2.37±0.10	2.45±0.27	3.58±0.37	4.19±0.19	5.11±0.48

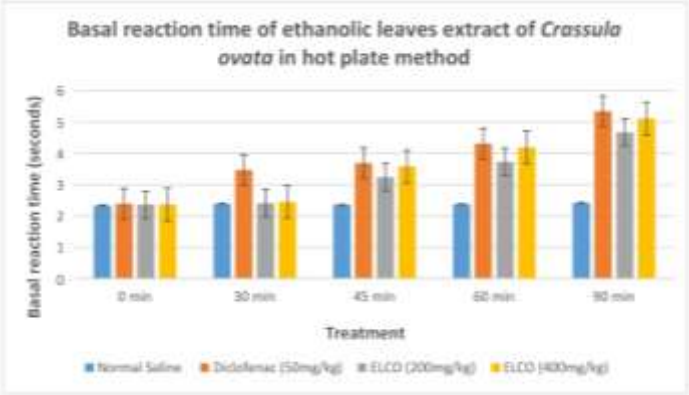

Fig 1. Basal reaction time of ethanolic leaves extract of *Crassula ovata* in hot plate method

The ethanolic leaves extract of *Crassula ovata* was shown pharmacological effect in dose-dependent manner. It represents that the herbal extract is effective in pain-relieving thorough blocking the synthesis and release of inflammatory medicators like COX-2, PGs and Cytokines etc.

- **Tail-flick method**

Diclofenac (50mg/kg) treated rats demonstrated the basal reaction time as 4.84±0.20 sec, 5.34±0.17 sec, and 6.20±0.11 sec after 45 min, 60 min and 90 min, respectively.

Moreover, the *Crassula ovata* extract demonstrated the analgesic potential almost similar to diclofenac sodium. At 90 min, the highest basal reaction time was observed as 5.43±0.34 sec, and 5.94±0.64 sec in *Crassula ovata* extract treated rats at 200mg/kg and 400mg/kg, respectively. The herbal extract shown pharmacological effect in dose-dependent manner. It represents that the *Crassula ovata* is effective in pain management.

Table 3. Basal reaction time of ethanolic leaves extract of *Crassula ovata* in Tail-Flick method

Treatment	Basal reaction time (sec)				
	0 min	30 min	45 min	60 min	90 min
Normal Saline	3.45±0.27	3.42±0.20	3.47±0.24	3.44±0.45	3.48±0.33
Diclofenac (50mg/kg)	3.42±0.14	4.69±0.27	4.84±0.20	5.34±0.17	6.20±0.11
ELCO (200mg/kg)	3.46±0.27	4.16±0.30	4.56±0.39	4.87±0.16	5.43±0.34
ELCO (400mg/kg)	3.43±0.35	4.32±0.26	4.64±0.57	5.06±0.24	5.94±0.64

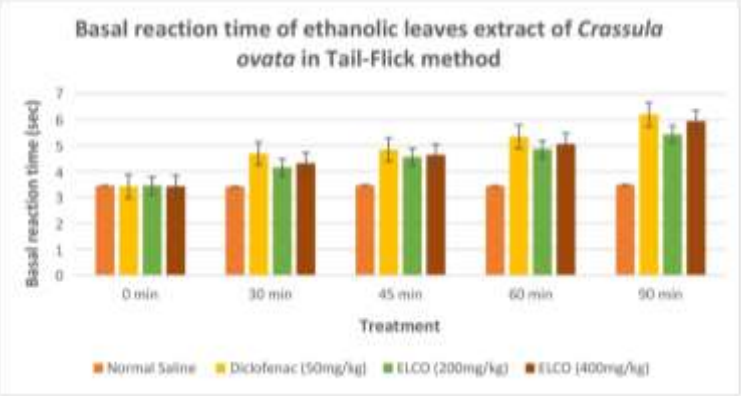

Fig 2. Basal reaction time of ethanolic leaves extract of *Crassula ovata* in Tail-Flick method

Analogous findings have been reported in earlier studies on peripheral analgesic activity. Comparing Swertia chirata and Moringa stenopetalaBak to the negative control revealed statistically significant effects. This study's models, dose ranges, and extraction solvent were comparable to those of a previous investigation that found that using the writhing approach increased analgesic activity in a dose-dependent manner. As previously mentioned, the extract's anti-nociceptive properties may be mediated by secondary metabolites such alkaloids. When it comes to glycosides, it has been found that the glycosidic metabolite morphine-6-glucuronide binds to mouse brain 1 and 2 receptors with affinities comparable to those of morphine.

To completely understand the mechanism of action of anti-inflammatory, analgesic, and anti-pyretic agents, more research into the mechanisms behind these actions is important. By altering this structure to improve the pharmacokinetic profile and solve the issues related to the adverse effects of NSAIDs and traditional analgesics, pharmaceutical researchers may be able to optimize the effectiveness and caliber of their drug discovery endeavors. Nearly 50 million adults in America experience chronic or severe pain, according to a 2012 study.

The extract was found to have more potent effects than typical medications when applied to paw edema models generated by formalin, carrageenan, and acetic acid. This notion is consistent with the well-established assumption that different components of medicinal plants have different modes of action, which combine to produce synergistic effects. Because the extract had both analgesic and anti-inflammatory effects, it may have behaved like an NSAID. On the other hand, because NSAIDs reduce the formation of PG, they have been connected to stomach discomfort. Terpenoids and saponins are two phytoconstituents that have been shown to have a gastroprotective effect. Further research is necessary to determine whether these phytoconstituents can reduce stomach irritation caused by blocking PG production and thereby elicit the extract's anti-ulcer effect.

In results, ethanolic leaves extract of *Crassula ovata* were shown anti-nociceptive potential when tested at 200mg/kg & 400mg/kg. At various time intervals, they markedly accelerated the basal reaction time, and their activity was dose-dependent. The inhibition of inflammatory mediators such as thromboxane, cytokines, and interleukins may be the cause of the analgesic effect.

CONCLUSION

The results clearly show that the Crassula ovata plant's methanolic and aqueous plant extracts from the leaves and stems can only suppress the Escherichia coli bacterium. Pseudomonas aeruginosa is another gram-negative bacterium that was unaffected by the plant extracts. This indicates that the plant extracts had an active ingredient that targeted the E. coli bacterium. The null hypothesis was also rejected as a result of this data, which showed that the Crassula ovata plant's active chemicals and microbial growth differed significantly. The Crassula ovata plant is ineffective against all other microorganisms tested, with the exception of Escherichia coli. The general and specific goals of this investigation were both accomplished. The plant Crassula ovata contained components that were active phytochemicals. Alkaloids, steroids, carbohydrates, and saponins were among them. Escherichia coli bacteria were shown to be susceptible to the plant's antibacterial properties.

It may work well to alleviate central or peripheral pain, which is a typical sign of a number of illnesses or injuries. It would be easily accessible in society and cost-effective. To determine which fraction is the most active, further research on fractionation is necessary. To completely understand the mechanisms unique to Crassula ovata that are linked to its anti-pain and anti-inflammatory actions. The goal is to ascertain the exact mechanism via which the ethanolic leaf extract of Crassula ovata reduces pain, whether it be external or internal.

CONFLICT OF INTEREST

Authors declare for none conflict of interest.

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