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RESEARCH

Phytochemical Screening and Evaluation of Anti-Ulcer Activity of *Crassula ovata*

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ABSTRACT

The study was based on the Phytochemical Screening and Evaluation of Anti-Ulcer Activity of *Crassula ovata*. The leaves of *Crassula ovata* were obtained from the Unnao reason. These were identified and authenticated by a botanist at BSI, Prayagraj (Ref. no.2024-25/536. The leaves were washed making dust-free and dried at room temperature or shade. The leaves were weighed and soaked into hydroalcoholic solution: Ethanol + distilled water (1:1) for fifteen days with gradual stirrings. The obtained slurry of mixture was dried under partial vacuum using a rotary evaporator. Preliminary phytochemical screening of the herbal extract was done. It was evaluated the anti-ulcer (in-vitro) effect of the *Crassula ovata* extract i.e., Acid Neutralizing activity and H+/K+ - ATPase Inhibition Activity. The percentage yield of the hydroalcoholic leaves extract of *Crassula ovata* was calculated which found to be 58.24 %. When compared to the control group hydroalcoholic leaves extract of *Crassula ovata* considerably modulated the H+/K+ - ATPase Inhibition Activity. It also showed an excellent acid neutralizing activity when compared with the Al(OH)3+Mg(OH)2 (as standard antacid). In conclusion, *Crassula ovata* might be given in the management of gastric ulcer and other gastric related problems that was effective when compared with aluminum hydroxide and magnesium hydroxide (standard antacids). It demonstrated a significant inhibition in the H+/K+ - ATPase Inhibition Activity and neutralized the gastric acid volume effectively.

Keywords: *Crassula ovata,* Acid Neutralizing activity, ATPase Inhibition Activity, Maceration.

INTRODUCTION

Peptic ulcer involves acid-induced injury to the intestine that normally occurs in the stomach or upper part of the duodenum; described as having bared mucosa that extends into submucosa even. Peptic ulcer illness is estimated to affect five to ten percent of the population [1]. Mucosal disruption is thought to be caused by hypersecretory environment of HCl as well as dietary changes or stress, in individuals with corrosive peptic disease. Helicobactor pylori infection & NSAIDs use are the two main risk factors in the progression of gastric as well duodenal ulcers [1].

A total of 23 patients were investigated for UC, resulting in 44.3% prevalence rate. This incidence was assessed again post a year reported as 6.02 per 1 lac. UC is frequent in India, according to these studies. India has the highest disease burden. In Asia, the total incidence of IBD, Ulcerative Colitis & CD was 1.37, 0.76, & 0.54/ 100,000, consecutively, compared to 23.67, 7.33, and 14.00 in Australia [2].

Plant profile: *Crassula ovata*

Evergreen and reaching heights of one to three metres, the jade plant has thick branches; smooth, spherical, and fleshy leaves growing on opposite sides of the branches. Rich jade green in hue, the leaves are elliptic to egg-shaped, measuring 30 to 90mm in length and 18 to 40mm in width [3]

When a stem grows, it initially has the same feel and colour as the leaves, but as it ages, it turns into brown colour with woody characteristics. They may blossom in early spring with tiny, star-shaped white or pink blooms if the correct circumstances are met. Later, the blossoms turn into little capsules that contain several tiny seeds inside of them [4].

Taxonomy

Kingdom: Plantae

Order: Saxifragales

Family: Crassulaceae

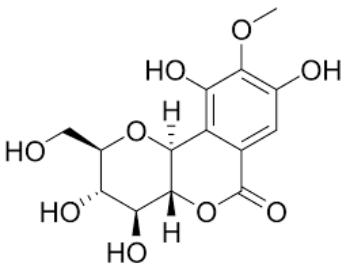
Genus: *Crassula*

Species: *Ovata*

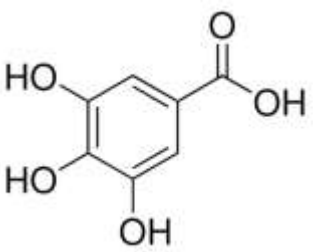
Fig 1. *Crassula ovata* plant (Gregory, 2004)

It was discovered that there were several phytochemicals present in water and methanolic extracts of *Crassula ovata* leaves. It showed itself as a rich source of saponin, phenol, sterols, steroids, tannins, terpenoids, anthraquinones, glycosides, flavonoids, carbohydrate, and protein when tested in the methanolic and aqueous leaves extract of *Crassula ovata*. Chiefly five compounds were isolated and identified from *Crassula ovata* [5] including:

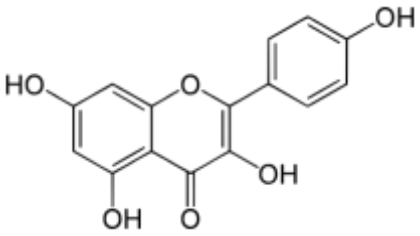
- Bergenin
- Gallic acid
- Kaempferol
- ß-Sitosterol
- Lutein



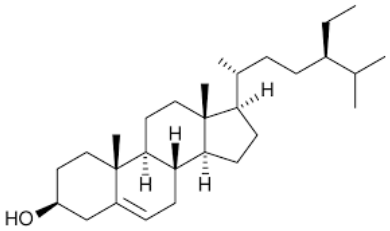
Bergenin



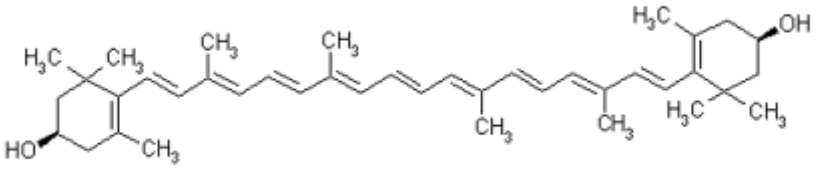
Gallic acid



Kaempferol



ß-Sitosterol



Lutein

Fig 2. Isolated compounds from *Crassula ovata*

MATERIALS AND METHODS

Experimental requirements

Fresh leaves of *Crassula ovata,* omeprazole, Water bath, distilled water, rotatory evaporator, weighing machine and ethanol.

Collection, Authentication and Extraction of plant

The Unnao reason is where the Crassula ovata leaves were acquired. A botanist at BSI, Prayagraj, recognized and verified these (Ref. no. 2024-25/536). After being cleaned to remove dust, the leaves were allowed to dry in the shade. After drying, the leaves were ground into coarse and then fine powders. After being weighed, the powder was steeped for fifteen days with slow stirring in a hydroalcoholic solution made of ethanol and distilled water (1:1). A rotary evaporator or water bath was used to partially vacuum-dry the resulting mixed slurry. The % yield of the *Crassula ovata* leaves extract was calculated formula as follows [6]-

$$\%\,\text{Yield} = \frac{\text{Actual Yield}}{\text{Theoretical Yield}} \times 100\%$$

Screening of phytochemical constituents [7][8]

Alkaloids

Before being filtered, each extract is dissolved in diluted HCl.

Mayer's Test: Filtrate treated with Mayer's reagent. The production of a yellow precipitate shows that there are alkaloids present.

Wagner's Test: The filtrates treated with Wagner's reagent. When a brown or reddish precipitate appears, alkaloids are present.

Hager's Test: We used Hager's Reagent to treat the filtrates. The appearance of yellow ppt shows that alkaloids are present.

Glycosides

Fehling's solutions A and B are heated for one minute with distilled water. Eight drops of plant extract were added to this clear blue solution. After that, it is cooked in a water bath for five minutes while mixed with one milliliter of Fehling's solution. The presence of glycosides is indicated by brick-red precipitation

Saponins

Herbal extract mixed with 10ml water and agitated quickly to make a stable, long-lasting foam. Foam shows that saponins are there.

Tannins

Ferric chloride test: To boil 0.5g of the dried powdered sample in a test tube, 20ml of water is needed. After that, the sample is filtered. You may test the color by adding a few drops of 0.1% FeCl3. It should be brownish green-black or blue-black.

Lead acetate test: Mix 2g extract with 2ml water. The mixture is shaken after 0.01g of lead acetate is added. When white turbidity and precipitate form, tannins are present.

Flavonoids

NaOH test: A tiny quantity of extract is treated with aqueous NaOH and HCl, and the development of a yellow-orange hue is monitored.

H_2SO_4 test: A part of the extract is treated with conc. H2SO4, and the color change to orange is watched.

Detection of terpenoids

Mix 2.0 ml of chloroform with 5 ml of the plant extract in water. Then, add the mixture to the water route, let it evaporate, and boil it with 3 ml of concentrated H2SO4. When the terpenoids grew, a gray color appeared.

Detection of Steroids

The 5g extract is mixed with 2ml of chloroform and conc. H2SO4. The red color that appeared in the bottom chloroform layer shows that steroids are present.

Estimation of reducing sugars and carbohydrates

Molisch's test

Put 2–3 ml of extract from different solvents into a test tube. Then, add a few drops of α-naphthol solution in alcohol. Then, shake the test tube and add H2SO4 from the sides. violet ring when two liquids meet.

Fehling's test

It is used to discover sugars that are going down. Put 34.66 grams of copper sulfate in 500 ml of distilled water (solution A). To create Solution B, combine 50 grams of sodium hydroxide and 17.3 grams of potassium sodium tartrate in 50 milliliters of distilled water. Mix two solutions in equal amounts before using. You should boil Fehling's A and B solutions together for a minute. You should add the same quantity of the test solution to each. Put in a saucepan of boiling water and let it cook for five to ten minutes. It started off yellow and then went brick red.

Evaluation of anti-ulcer activity

Acid Neutralizing activity

The aqueous extract can neutralize acids in amounts of 100 mg, 500 mg, 1000 mg, and 1500 mg. The benchmark has been compared to magnesium hydroxide (500 mg) and aluminum hydroxide. The entire amount was 70 ml after adding 5 ml of the mixture and enough water to make up the rest of the volume. Mix for 1 minute. After adding 30 milliliters of 1.0 N HCl and stirring for 15 minutes, phenolphthalein was added and mixed with the standard and test mixture. The extra HCl was quickly mixed with 0.5N sodium hydroxide until the right pink hue was reached [9].

Acid neutralizing capacity = moles of HCl neutralized/ extract (g)

H^+/K^+ ATPase Inhibition Activity

The reaction mixture of the sample, which had 0.1 ml of enzyme extract (300µg) and plant extract in different amounts (20µg, 40µg, 60µg, 80µg, and 100µg), was put in the incubator for 60 minutes at 37 °C. To initiate the reaction, substrate was added together with 2 mM ATP (200µL), 2 mM MgCl2 (200µL), and 10 mM KCl (200µL). After 30 minutes of incubation at 37°C, 4.5% ammonium molybdate was employed to stop the process. Next, 60% perchloric acid was added, and the mixture was spun at 2000 rpm for 10 minutes.

The Fiske-Subbarow technique was then used to find inorganic phosphate at 660 nm. After 10 minutes at room temperature, 1 milliliter of supernatant, 4 milliliters of Millipore water, 1 milliliter of 2.5% ammonium molybdate, and 0.4 milliliters of ANSA were added. Absorbance at 660 nm for inorganic phosphate has been tested at various extract doses, and enzyme activity has been calculated as micromoles of Pi released per hour [10].

Percentage of inhibition = [Activity (control) - Activity (test)/Activity (control)] × 100

Statistical Analysis

ANOVA and a two-tailed t-test were used to assess the statistical data. Mean± S.E.M. was used to express the values. Sigma Stat pro3.3 was used to conduct the statistical analysis. At level $p \leq 0.05$, the results observed were statistically significant.

RESULTS AND DISCUSSION

Percentage yield

The percentage yield of the hydroalcoholic leaves extract of *Crassula ovata* was calculated which found to be 58.24 %.

Screening of phytochemical constituents

Hydroalcoholic leaves extract of *Crassula ovata* showed a rich source of phytoconstituents. I showed the phenols, terpenoids, and carbohydrates in abundance. Moreover, phytosterol, steroid, flavonoids, and proteins were found in moderate amount. While, saponins, tannins, glycosides and Plobatanin were found absent in the herbal extract.

Table 1. Phytochemical screening of *C. ovata*

Phytochemicals	Hydroalcoholic leaves extract of *Crassula ovata*
Saponins	_
Phenols	+++
Phytosterol	++
Steroid	++
Tannins	_
Terpenoids	+++
Glycoside	_
Plobatanin	_
Flavonoid	++

Carbohydrate	+++
Protein	++

Where, (++)= Moderate; (+++)= Abundance; (-)=Negative

Evaluation of anti-ulcer activity

Acid Neutralizing activity

The volume of NaOH was consumed as 38.4ml, 32.6ml, 42.3ml and 35.6ml, in the *C. ovata* (100 mg), *C. ovata* (500 mg), *C. ovata* (1000 mg) and *C. ovata* (1500 mg) groups, respectively. Acid neutralizing capacity (ANC) was found as 108 in the *C. ovata* (100) treated group. *C. ovata* (1500 mg) exhibited the statistically significant ANC as 8.13. Moreover, *C. ovata* (500) + Al(OH)3+Mg(OH)2 group consumed 46.4 ml NaOH, and neutralized 6.8 moles of acid. It reported the ANC as 13.6.

Table 2. Acid neutralizing activity of *Crassula ovata*

Treatment (mg)	Vol. of NaOH consumed (ml)	Moles of acid neutralized	ANC/ g of antacid
C. ovata (100)	38.4	10.8	108
C. ovata (500)	32.6	13.7	27.4
C. ovata (1000)	42.3	8.85	8.85
C. ovata (1500)	35.6	12.2	8.13
C. ovata (500) + Al(OH)3+Mg(OH)2 (500)	46.4	6.8	13.6

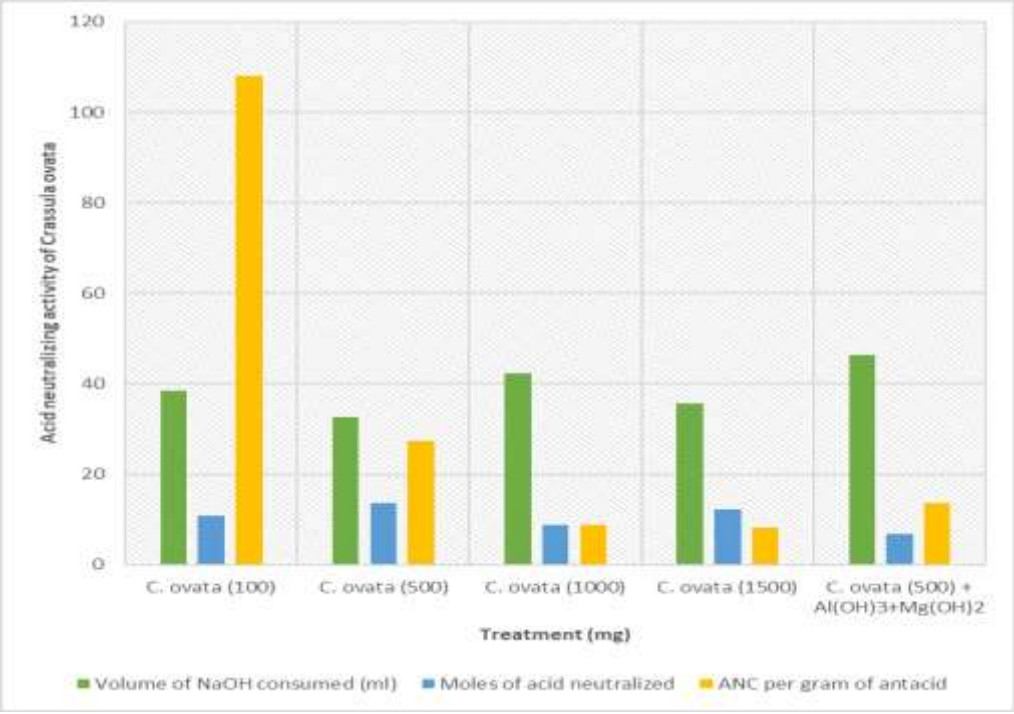


Fig 3. Graphical data of acid neutralizing activity of *Crassula ovata*

H^+/K^+ ATPase Inhibition Activity

Inhibition for Crassula ovata was estimated in the conc. of 20 µg, 40 µg, 60 µg, 80 µg and 100 µg. Percentage inhibition (%) was determined for the omeprazole (std. antacid) and *Crassula ovata* extract. In Omeprazole, the percentage inhibition was estimated as 34.68±0.17 %, 57.18±0.56 % and 67.32±0.21 %, respectively in conc. of 60 µg, 80 µg and 100 µg, respectively. *Crassula ovata* extract showed a significant inhibition when compared with the std. antacid. Herbal extract showed the percentage inhibition as 60.17±0.34 % at the conc. of 100 µg.

Table 3. H^+/K^+ ATPase Inhibition Activity of *Crassula ovata*

Treatment (µg)	Inhibition (%) (Mean ± SEM)	
	Omeprazole	*Crassula ovata* extract
C. ovata (20)	-49.26±0.54	-28.51±0.45
C. ovata (40)	-54.31±0.27	-18.62±0.65
C. ovata (60)	34.68±0.17	29.49±0.37
C. ovata (80)	57.18±0.56	54.21±0.68
C. ovata (100)	67.32±0.21	60.17±0.34

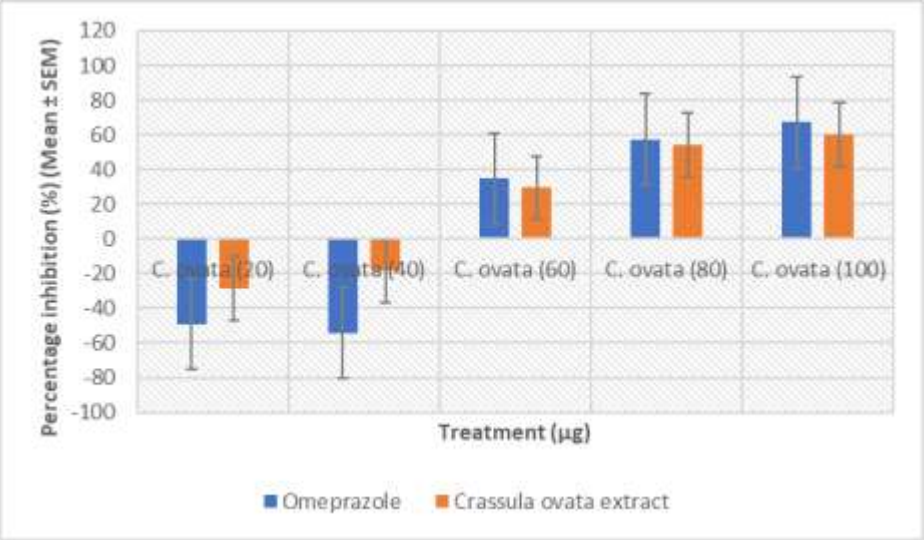


Fig 4. Graphical data of H^+/K^+ ATPase Inhibition Activity of *Crassula ovata*

The aqueous extract dramatically reduced ANC by 9.33 at a dose of 1500 mg. Uncontrolled hypersecretion of hydrochloric acid from the parietal cells of the stomach mucosa through the proton pump characterizes hyperchlorhydria. H+/K+-ATPase is an essential enzyme that makes things acidic. It is present on the apical secretory membrane of parietal cells. 21. The extract showed the greatest percentage inhibition of 62.18% in H+/K+-ATPase inhibitory activity at a dosage of 100µg. The compounds in the combination may make the aqueous extract work as an antacid, antisecretory, and antiulcer, based on the results shown below. To ascertain its specific mode of action and the active components responsible for its antiulcer efficacy, further investigation is required.

The quantity of stomach contents was found to be lower than in the control group. One possible explanation for group 4's success in reducing gastric content volume is its ability to block Histamine receptors in the stomach's lining. This, in turn, reduces production of gastrin and stomach acid. It could work to neutralize free acid, reducing the potentially devastating effects of free acid on the development of stomach ulcers and

perforations. Perhaps this is the case because pharmacological effect is induced and accommodated by the plant extract over time. No clear mechanism for this pharmacological effect has been identified.

In previous study, findings showed that Pleurotus florida's ethanolic extract had gastroprotective properties. Numerous factors can contribute to acidity, a common digestive problem associated with a functional impairment. The restored equilibrium is preserved when therapeutic medications are employed in place of suppressing the production of stomach acid. Back titration is a way to find out how much acid an antacid can neutralize. At a dose of 1500 mg, the ethanolic extract of Pleurotus florida had a substantially lower ANC of 10.7. The word "hyperchlorhydria" refers to the stomach mucosa's parietal cells making too much hydrochloric acid through the proton pump. H+/K+-ATPase is an enzyme found on the apical secretory membrane of parietal cells that is very important for producing acidity. The extract demonstrated a peak inhibition of 68% in H+/K+-ATPase activity at a dosage of 200µg. The results suggest that the ethanolic extract of Pleurotus florida may have antiulcer, antisecretory, and antacid qualities. The presence of bioactive compounds in the mushroom may be the cause of this [11].

Hyperchlorhydria is a condition in which the stomach mucosa's parietal cells secrete excessive amounts of hydrochloric acid through the proton pump. The apical secretory membrane of parietal cells contains the enzyme H+/K+ -ATPase, which is crucial for generating acidity. The extract showed a maximum percentage inhibition of 68.62±1.03% in H+/K+-ATPase activity at a concentration of 100µg. Therefore, the EESO decreased stomach ATPse's ability to hydrolyze ATP (IC50 of 49.46µg/ml). The results shown here suggest that the presence of specific compounds in the mixture may cause the ethanol extract to have antacid, antisecretory, and antiulcer characteristics. Its exact mode of action and the main components that give it its antiulcer properties, however, require further investigation [12].

The results show that hydroalcoholic leaves extract of *Crassula ovata* (500 mg, 1000 mg & 1500 mg) have statistically significant anti-ulcer potential. After successful clinical trials, it may be administered to humans. When compared to the control group hydroalcoholic leaves extract of *Crassula ovata* considerably modulated the H+/K+ - ATPase Inhibition Activity. It also showed an excellent acid neutralizing activity when compared with the Al(OH)3+Mg(OH)2 (as standard antacid).

CONCLUSION

In conclusion, *Crassula ovata* might be given in the management of gastric ulcer and other gastric related problems that was effective when compared with aluminum hydroxide and magnesium hydroxide (standard antacids). It demonstrated a significant inhibition in the H+/K+ - ATPase Inhibition Activity and neutralized the gastric acid volume effectively.

CONFLICT OF INTEREST

Authors declare for none conflict of interest.

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