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RESEARCH

# Phytochemical Screening and Evaluation of Antioxidant and Anti-Arthritic Potential of *Crassula ovata* Leaves Extract

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## ABSTRACT

The study was based on Phytochemical screening and evaluation of antioxidant and anti-arthritic potential of *Crassula ovata* leaves extract. The fresh leaves of *Crassula ovata* were collected from the Unnao region in Uttar Pradesh. The plant herbarium will be authenticated by a botanist. The leaves are washed for making dust-free dried under shade, sieved, and dried at room temperature or shade. A 50g leaves powder of *Crassula ovata* was weighed and extracted through cold maceration process i.e., soaked into beaker (mounted with aluminium foil) of water: ethanol (1:) for 15 days with gradual stirrings. DPPH radical scavenging activity was performed. Evaluation of the anti-arthritic potential of herbal extract was done through models i.e., Inhibition of protein denaturation using bovine serum albumin and Inhibition of protein denaturation using egg albumin. In results, the hydroalcoholic *C. ovata* leaves extract demonstrated the % inhibition of protein denaturation using BSA as 57.3±0.4 % and 93.6±0.2 % at the conc. of 12.5 μg/ml and 800 μg/ml respectively. Nonetheless, the *C. ovata* leaves extract demonstrated the % inhibition of protein denaturation using egg albumin as 42.4±0.1 % and 92.6±0.2 % at the conc. of 12.5 μg/ml and 800 μg/ml respectively. In conclusion, *C. ovata* leaves possess the potent antioxidant and anti-arthritic activity. On the other hand, a dose-dependent pharmacological response was observed.

**Keywords:** *Crassula ovata,* DPPH, anti-arthritic, BSA.

## INTRODUCTION

It can impact places other than the joints and is an inflammatory kind of arthritis [1]. It is mostly brought on by environmental causes, including smoking cigarettes, and genetic predisposition [2]. Typically, it affects small peripheral joints first, but if addressed, it can spread to proximal joints and become symmetric [3]. When inflammation erodes cartilage and pushes bone into the joint socket, degeneration of the joints results. While the symptoms of established RA usually manifest after the disease has been present for more than six months, those of early RA frequently manifest within the first six months of the disease's inception [4]. Increased mortality is a result of untreated RA as well as impairment [5].

As per Global Burden of Disease 2010, the prevalence of RA is approximately 0.24% worldwide [6]. Based on epidemiological data, women are more likely than men to develop RA [7]. Africa has very little data, and what little is available varies widely by country; in sub-Saharan Africa, RA is less frequent than in North Africa. In East Asia, RA is significantly less common than it is in the rest of Asia. Between 1980 and 2019, there was a 9.75% increase in the global period prevalence [8].

## 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. 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 [9][10].

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 [11][12].

### Taxonomy

Kingdom: Plantae

Order: Saxifragales

Family: Crassulaceae

Genus: *Crassula*

Species: *ovata*

Chiefly five compounds were isolated and identified from *Crassula ovata* [14] including:

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

The secondary metabolites that are typically present in plant components are responsible for their biological activity and therapeutic advantages. One of the most prevalent is phenol [15]. Secondary metabolites, such as phenolic compounds, exhibit notable cytotoxic effects on a range of cell lines due to their antioxidant qualities, ability to scavenge free radicals, and impact on many pathways, including apoptosis, cell proliferation, metastasis, and angiogenesis [16].

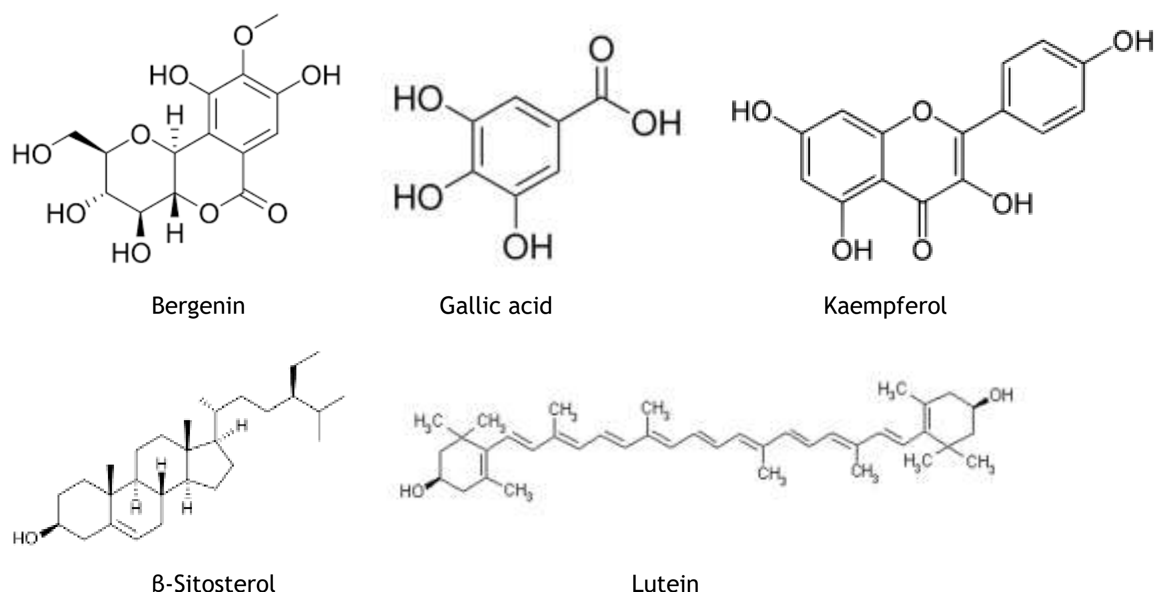


**Fig 1. Isolated compounds from *Crassula ovata***

## MATERIALS AND METHODS

### Experimental requirements

*Crassula ovata* leaves, DPPH, egg albumin, bovine serum albumin, ethanol, distilled water, digital balance, rotating evaporator, etc.

### Collection, Identification & Authentication of plant

The fresh leaves of *Crassula ovata* were collected from the Unnao region in Uttar Pradesh. The plant herbarium will be authenticated by a botanist. The leaves are washed for making dust-free dried under shade, sieved, and dried at room temperature or shade.

### Extraction of plant

A 50g leaves powder of *Crassula ovata* was weighed and extracted through cold maceration process i.e., soaked into beaker (mounted with aluminium foil) of water: ethanol (1:) for 15 days with gradual stirrings. The mixture was filtered with filter paper and finally with whatman filter paper. The obtained extract residue was kept on the water bath at the temperature of ≤40ºC to obtain the herbal extract in dried form [17].

### Preliminary phytochemical screening

The methanolic herbal extract was screened for different phytoconstituents to check their presence [18][19].

#### Detection of Alkaloids

Extracts are dissolved individually in dilute HCl and filtered.

**Mayer's Test:** Filtrates are treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow-colored precipitate indicates the presence of alkaloids.

**Wagner's Test:** Filtrates are treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

**Hager's Test:** Filtrates are treated with Hager's Reagent. Formation of yellow ppt indicates the presence of alkaloids.

#### Detection of Glycosides

**Fehling's test:** With distilled water dilution, Fehling's solutions A and B are heated for one minute. There were 8 drops of plant extract added to this transparent blue solution. It is 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.

#### Detection of Saponins

**Foam test**: About 2g of the plant extract was mixed with 10ml of distilled water and shaken vigorously for a stable persistent froth. Appearance of froth indicates the presence of saponins.

#### Detection of Tannins

**Ferric chloride test**: 0.5g of the dried powdered sample is boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% $FeCl_3$ is added and observed for brownish green-black or a blue-black coloration.

**Lead acetate test:** 2ml of plant extract is combined with 2ml of distilled water. 0.01g lead acetate is added to this combined solution and shaken well. Development of white turbidity and precipitate indicates the presence of tannins.

#### Detection of Flavonoids

**NaOH test:** A small amount of extract is treated with aqueous NaOH and HCl, and observed for the formation of yellow orange color.

**$H_2SO_4$ test**: A fraction of the extract is treated with Conc.$H_2SO_4$ and observed for the formation of orange color.

## Detection of terpenoids

5 ml of the aqueous plant extract is combined with 2.0 ml of chloroform, which is then added, evaporated on the water path, and boiled with 3 ml of concentrated H2SO4. As terpenoids took shape, a grey colour emerged.

## Detection of Steroids

2 ml of chloroform and concentrated $H_2SO_4$ are added with the 5 ml aqueous plant crude extract. In the lower chloroform layer red color appeared that indicates the presence of steroids.

## Test for Reducing Sugars and Carbohydrates

#### Molisch test

To 2-3ml extract of individual solvents add few drops of α-naphthol solution in alcohol, shake and add concentrate $H_2SO_4$ from sides of test tube. Violet ring at the junction of two liquids.

#### Fehling's test

It is used to determine decreasing sugars. 34.66 grams of copper sulphate should be dissolved in 500 millilitres of distilled water (solution A). Distilled water should be used to dissolve 50 grams of sodium hydroxide and 17.3 grams of potassium sodium tartrate to a maximum volume of 50 millilitres (Solution B). Mix two solutions in the same volume before using. Boil a 1 mL combination of Fehling's A and B solution for one minute. Equal amounts of the test solution should be added. Heat for five to ten minutes in a kettle of boiling water. The colour changed from yellow to brick red.

# Evaluation of pharmacological potential

## Antioxidant activity

### Estimation of DPPH Radical Scavenging Assay

The free radical scavenging potential of hydro-ethanolic leaves extract of *C. ovata* is measured by the 1,1′-diphenyl-1-picrylhydrazyl (DPPH) according to the method by Tariq et al. [20]. The assay experimented by reacting 1.6 mL of 0.135 mM DPPH dissolved in 100% v/v methanol with 0.4ml of various concentrations of synthesized derivatives. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture measured at 517 nm after 2 min. Thus, the concentration of the derivatives needed to decrease the absorbance of DPPH radical by 50% was calculated.

## Anti-arthritic activity

### Inhibition of protein denaturation using bovine serum albumin (BSA)

The reaction mixture (0.5 ml) contains 0.45 ml of BSA (5% aqueous solution) and 0.05 ml of various concentrations of fractions, hydro-ethanolic leaves extract of *C. ovata*, and indomethacin (reference medication) (12.5, 25, 50, 100, 200, 400, and 800µg/ml), respectively. Using 1 N HCl, each solution was adjusted to pH 6.3. The samples were heated to 57°C for 30 minutes after being incubated for 20 minutes at 37°C. Next, 2.5 ml of phosphate buffer was added, and a spectrophotometer was used to detect absorbance at 660 nm. The hydro-ethanolic leaves extract of *C. ovata* were replaced with 0.05 ml of distilled water for the test control, whereas BSA was absent from the product control [21].
Formula:

% inhibition = [Absorption control–Absorption test/Absorption Control] × 100

### Inhibition of protein denaturation using egg albumin

Diclofenac sodium was added to the reaction mixture (5 ml) at different concentrations (12.5, 25, 50, 100, 200, 400, and 800µg/ml), egg albumin (0.2 ml), phosphate buffered saline, 2.8 ml (pH 6.4), and 2 ml of hydro-ethanolic leaves extract of *C. ovata*, respectively. A control group of the same volume of double-distilled water was used. The mixtures were heated to 70°C for five minutes after being incubated for fifteen minutes at 37 ± 2 °C in a biochemical oxygen demand (BOD) incubator. At 660 nm, their absorbance was measured [21].
Using the formula below, the percentage inhibition of protein denaturation was calculated:

% inhibition = [Absorption control–Absorption test/Absorption Control] × 100

## RESULTS AND DISCUSSION

### Percentage yield

The percentage yield of hydroalcoholic leaves extract of *Crassula ovata* was obtained as 24.68 %.

### Phytochemical screening

**Table 1. Phytochemicals of hydroalcoholic leaves extract of *Crassula ovata***

| Phytochemical | Hydroalcoholic *Crassula ovata* leaves extract |
|---|---|
| Flavonoid | ++ |
| Protein | + |
| Saponins | _ |
| Phytosterol | ++ |
| Steroid | + |
| Tannins | _ |
| Terpenoids | + |
| Glycoside | _ |
| Carbohydrate | + |

Absent (-), Moderate (++), Abundance (+++)

### Pharmacological evaluation

#### DPPH Scavenging capacity Assay

At concentration 800µg/ml, the % inhibition i.e., DPPH Scavenging capacity was estimated as 96.29±0.18 %, and 62.54±0.19 % in the ascorbic acid and *C. ovata* leaves extract, respectively. Therefore, *C. ovata* leaves extract showed significant antioxidant potential in contrast to water. However, ascorbic acid recorded for highest percent inhibition. Its antioxidant action might be due to the blockage or inhibition of free radical generation in the cells.
The DPPH Scavenging assay was observed as follow-

**Table 2. DPPH Scavenging capacity assay**

| Concentration (µg/ml) | DPPH Scavenging capacity assay (% Inhibition) | | |
|---|---|---|---|
| | Water | Ascorbic acid | Leaves extract |
| 200µg/ml | 19.34±0.20 | 36.11±0.27 | 31.19±0.34 |
| 400µg/ml | 23.18±0.45 | 63.20±.31 | 43.27±0.16 |
| 800µg/ml | 21.39±0.15 | 96.29±0.18 | 62.54±0.19 |

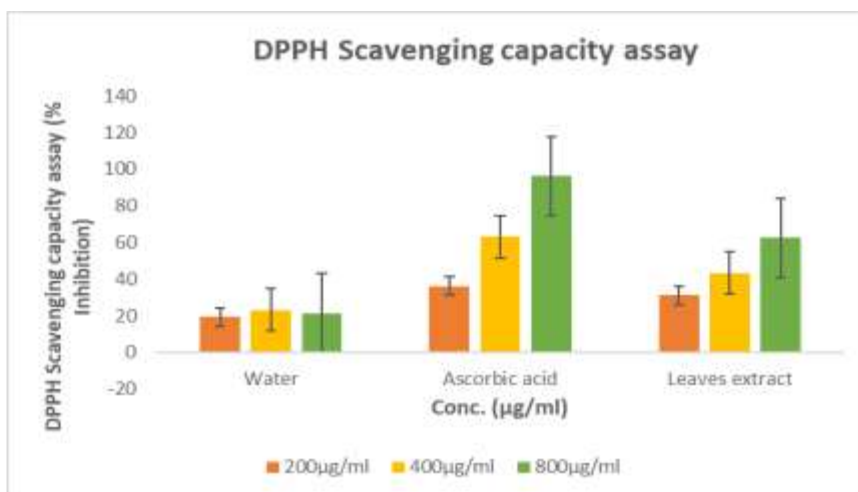


**Fig 2. DPPH Scavenging capacity of *Crassula ovata***

## Evaluation of anti-arthritic activity

### Inhibition (%) of protein denaturation through bovine serum albumin

The % inhibition of protein denaturation through bovine serum albumin was estimated at different concentrations i.e., 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml, 400 µg/ml, and 800 µg/ml, respectively.

Hydroalcoholic *C. ovata* leaves extract of demonstrated the % inhibition of protein denaturation using BSA as 57.3±0.4 % and 93.6±0.2 % at the conc. of 12.5 µg/ml and 800 µg/ml respectively.

**Table 3. Determination of inhibition (%) of protein denaturation through bovine serum albumin of *Crassula ovata* leaves extract**

| Treatment | Inhibition (%) of protein denaturation through bovine serum albumin [Conc. (µg/ml)] | | | | | | |
|---|---|---|---|---|---|---|---|
| | 12.5 | 25 | 50 | 100 | 200 | 400 | 800 |
| ***Crassula ovata* leaves extract** | 57.3±0.4 | 73.4±0.6 | 76.3±0.5 | 80.4±0.2 | 83.2±0.5 | 88.4±0.2 | 93.6±0.2 |

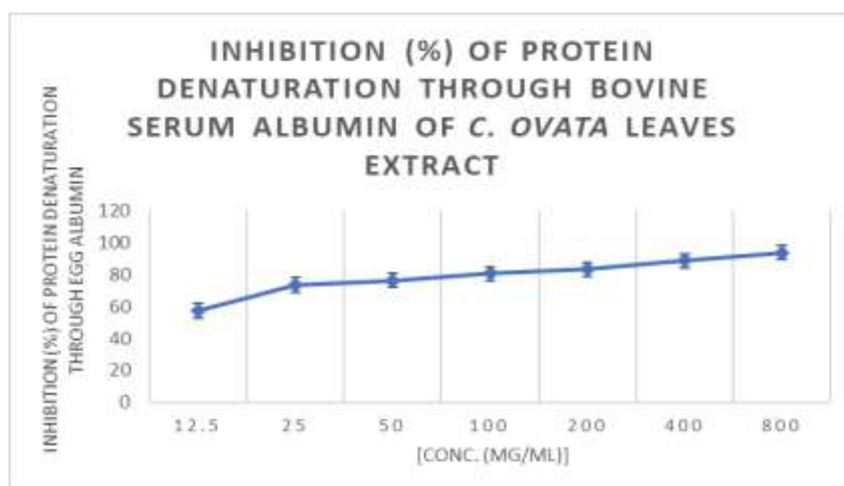


**Fig 3. Determination of inhibition (%) of protein denaturation through bovine serum albumin of *Crassula ovata* leaves extract**

### The % Inhibition of protein denaturation through egg albumin

The % Inhibition of protein denaturation using egg albumin was estimated at different concentrations i.e., 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml, 400 µg/ml, and 800 µg/ml respectively.

The hydroalcoholic C. ovata leaves extract demonstrated the % inhibition of protein denaturation as 42.4±0.1 % and 92.6±0.2 % at the conc. of 12.5 µg/ml and 800 µg/ml respectively.

**Table 4. Inhibition (%) of protein denaturation through egg albumin of *Crassula ovata* leaves extract**

| Treatment | Inhibition (%) of protein denaturation through egg albumin [Conc. (µg/ml)] | | | | | | |
|---|---|---|---|---|---|---|---|
| | 12.5 | 25 | 50 | 100 | 200 | 400 | 800 |
| *Crassula ovata* leaves extract | 42.4±0.1 | 63.2±0.6 | 64.1±0.3 | 71.4±0.6 | 75.2±0.1 | 84.1±0.3 | 92.6±0.2 |

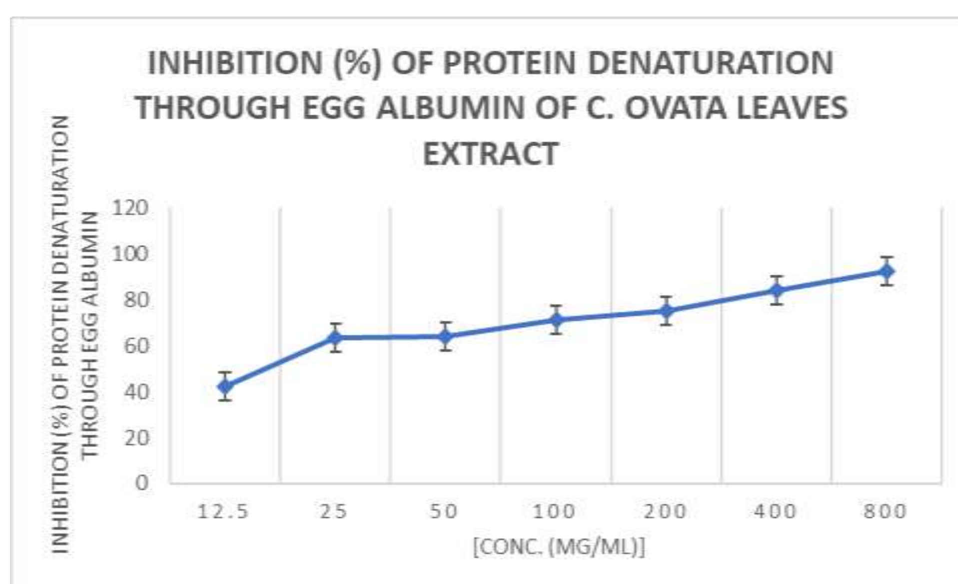


**Fig 4. Inhibition (%) of protein denaturation through egg albumin of *Crassula ovata* leaves extract**

By scavenging ROS, natural antioxidants shield humans against a range of illnesses [22]. It is advised to use reducing power assays in conjunction with assays measuring free radical scavenging activity to determine the potential of natural antioxidants. In order to ascertain the antioxidant efficiency of a few commonly occurring grass species, six distinct antioxidant assays were carried out, namely the phosphomolybdate, TRP, CUPRAC, SOR, DPPH, and ABTS assays. Nonetheless, several plants were shown to exhibit moderate antioxidant activity. Positive outcomes in every antioxidant activity test employed in this investigation suggest that the different components in the crude extracts have the ability to reduce power in the Fe3+, Cu2+, and Mo (IV) complex or function as scavengers of free radicals, such as DPPH, ABTS, and SOR [23]. As a result, health is improved. Additionally, hydroxyl radicals have the potential to disrupt DNA strands, which can lead to mutagenesis, carcinogenesis, and cytotoxicity [24]. Using genomic DNA, the DNA protective activity of certain plant extracts was evaluated in response to DNA strand breaking. DNA bands were examined after plant extracts at various concentrations were treated with Fenton's reagent. In order to assess the efficacy of plants, band intensity was also examined [25].

Because they are more successful at treating infectious infections while also reducing many of the negative effects connected to synthetic antibiotics, antimicrobials derived from plants are now typically advised [26]. The majority of plant extracts were more effective against L, according to the results. monocytogenes, W. unusual, A. Mucor specie and flavus, whereas S. aureus, F. oxysporum together with A. niger were discovered to be extremely resistant bacteria. Generally, against the majority of the studied diseases, all plant extracts

shown moderate to weak efficacy. These plants' crude extracts have potential applications as an adjuvant for treating resistant microbe-caused infectious illnesses such as food poisoning and preserving food [27].

In results, the hydroalcoholic *C. ovata* leaves extract demonstrated the % inhibition of protein denaturation using BSA as 57.3±0.4 % and 93.6±0.2 % at the conc. of 12.5 µg/ml and 800 µg/ml respectively.

## CONCLUSION

In conclusion, *C. ovata* leaves possess the potent antioxidant and anti-arthritic activity. On the other hand, a dose-dependent pharmacological response was observed. Furthermore, in both models, the herbal extract demonstrated a higher percent suppression of protein denaturation as well as % inhibition in DPPH radical scavenging assay.

It suggests, fellow researchers to determine the mode of action that how hydro-ethanolic leaves extract of *Crassula ovata* treat and prevent the progression of cellular oxidant and rheumatoid arthritis.

## CONFLICT OF INTEREST

Authors declare for none conflict of interest.

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