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(RESEARCH ARTICLE)

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Phytochemical screening and evaluation of antioxidant activity of *Callisia repens* and *Crassula ovata*: An *in-vitro* study

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Abstract

Callisia repens (Turtle vine) and Crassula ovata (Jade) are ornamental plants that have been used pharmacologically as traditional medicine plants. This research aimed to investigate the phytochemistry and antioxidant activities of *Callisia repens* and Crassula ovata using in vitro models. The phytochemical analysis proved the presence of glycosides & terpenoids in *Callisia repens* and carbohydrates, proteins, steroids, and triterpenoids, flavonoids, and phenols in Crassula ovata. Further, in the DPPH radical scavenging activity with IC50 at 48.825 mg/ml and 470.314 mg/ml; 81.08 mg/ml and 181.51 mg/ml of hydrogen peroxide (H^2O_2) scavenging activity for turtle vine and Jade respectively. The potential antioxidant activity might be due to the active phytochemical constituents and hydrogen or electron-donating capacity of the selected ornamental plants.

Keywords: Jade; Turtle vine; DPPH; H_2O_2; Phytochemicals

1. Introduction

Ornamental plants are grown for decorative purposes in gardens and landscapes. These plants are used for design projects, house plants, cut flowers, and specimen displays, and they grab attention due to their attractive colors, beautiful designs, and mesmerizing looks [1]. They can be displayed both indoors and outdoors for decoration, as they have vibrant colors and add life to any setting. Ornamental plants are grown for the display of aesthetic features including flowers, leaves, scent, overall foliage texture, fruit, stem, and bark, and aesthetic form [2]. Because humans have a close relationship with nature, integrating the natural world into indoor spaces can effectively increase people's interaction with nature, which may contribute to health and comfort. Indoor environments are critical to health as people spend 80-90% of their time indoors [3]. Jade and Turtle vine are some of the most popular ornamental plants.

Callisia repens, commonly known as "creeping inch plant" or "turtle vine," is a plant species belonging to the *Commelinaceae* family a wonderful plant that lies in the succulent plant category [4]. The plants are widely grown in small hanging pots which gives your Garden a wonderful look. It is a popular indoor plant in India. The plant is generally native to South & Central America but they are also grown in India, Pakistan, Australia, Sri Lanka, and many parts of Africa. The plant requires little maintenance & care which makes it the most lovable [5]. The species is sometimes used as an ornamental plant and it is easily propagated by cuttings. The perennial plant spreads through creeping mats, with flowering shoots that ascend. Its delicate, fleshy leaves diminish in size towards the shoot's tip, oval to lanceolate, measuring 1-3.5 centimeters long and 0.6-1 centimeter wide. As the leaves reach the distal end, they become narrower than the open, spread leaf sheaths [6]. This plant proliferates rapidly, propagating easily through long, creeping shoots that root at each node upon contact with the ground. The plant can stand direct sunlight if there is much humidity. The optimum growth conditions are between 18- 22°C during the day and at least 12°C at night.

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Crassula ovata, commonly referred to as the Jade plant, belongs to *Crassulaceae* and is native to South Africa, is a widely cultivated houseplant found across the globe, particularly in the Northern Hemisphere, thriving in cold and arid regions where water is limited. This plant exhibits remarkable water conservation abilities, attributed to its efficient employment of Crassulacean Acid Metabolism (CAM) during photosynthesis [7]. Its succulent leaves and stems facilitate water storage, enabling it to minimize water loss effectively. Medicine practitioners recommended a jade plant tea to alleviate symptoms of diabetes. Its abundance and malleability in ancient times made it suitable for bonsai artistry [8] (Deepthi N., 2024). The plant became a favored gift among royalty across the Chinese empire. Following Chinese tradition, jade plants are often positioned near cash registers in businesses to attract prosperity. However, there has been limited scientific validation of these traditional uses. In North East India, particularly in Manipur, it has been discovered that *Crassula ovata* is primarily utilized for treating diabetes and combating certain infections. Locals traditionally consume the juice extracted from the leaves of this plant to manage diabetes. However, there has been limited scientific research conducted to assess its effectiveness. Therefore, due to the lack of scientific reports on the selected indoor ornamental plants such as *Callisia repens* and *Crassula ovata.* the present investigation was conducted to identify the potential phytochemicals and actions toward scavenging the free radicals using several in vitro methods.

2. Material and methods

2.1. Phytochemical analysis

2.1.1. Tests for carbohydrates

Molish'stest(general test)

To 2-3 ml aqueous extract, add a few drops of alpha naphthol solution in an alcohol shake and add con. Sulphuric acid from the sides of the test tube. A violet ring is formed at the junction of the two liquids.

Tests for reducing sugars

- Fehling's test: Mix 1 ml of Fehling‟ 's A and Fehling‟ 's B solution and boil for 1 min. Add an equal volume of test solution to the test tube. Heat in boiling water bath for 5-10 min. First yellow, then brick red ppt is observed.
- Benidict's test: Mix equal volumes of Benedict's reagent and test solution in the test tube. Heat in boiling water bath for 5 min. the solution appears green-yellow or red depending on the amount of reducing sugars present in the test solution.

Tests for monosaccharides

- Barfoed's test: M ix equal volume of Barfoed's reagent and test solution. Heat for 1-2 min boiling water bath and cool. Red ppt is observed.
- Tests for pentose sugars: Mix equal volume of the test solution and Hcl. Heat and add a crystal of phloroglucinol red color appears.
- Test for hexose sugars
    - Selwinoff'stest: Heat3mlselwinoff‟sreagentand1mltestsolutionin bearing water bath for 1-2 min. A red color is formed.
    - Tollen's phloro glucinol test for galactose: Mix 2-5 ml con Hcl and 4 ml 0.5% phloro glucinol. Add 1-2 ml test solution. Heat. Yellow to red color appears.

Test for Non-reducing polysaccharide(starch)

Iodine test: Mix 3 ml test solution and 3 drops of dilute iodine solution. A blue color appears, and it disappears on cooling.

2.1.2. Tests for proteins

- Biuret test (General test): To 3 ml test solution add 4% NaOH few drops of Cuso4 Solution, violet or pink color appears.
- Millions test: Mix 3 ml tests solution with 5 ml reagent white .ppt warm ppt turns into pink color
- Xantho protein test: Mix 3 ml test solution with 1 ml con. Sulphuric acid. White PPT is formed. Boil PPT turns yellow. Add ammonium hydroxide. PPT turns orange.

2.1.3. Tests for Amino Acids

- Ninhydrin test: Heat 3 ml test solution and 3 drops of 5% Ninhydrin solution in the boiling Water bath for 10 min. purple or bluish color appears.
- Test for tyrosine: Heat 3 ml of the test solution and add 3 drops of million‟s reagent. The solution shows a dark red color.
- Test for cysteine: To 5 ml test solution and a few drops of 40%NaoH and 10% lead Acetate solution. Boil. Black Ppt of lead sulfate is form

2.1.4. Tests for Glycosides

Tests for cardiac glycosides

- Baljet's test: A thick section shows a yellow to orange color with sodium picrate.
- Legal's test: To aqueous or alcoholic extract, add 1 ml pyridine and 1 ml sodium nitroprusside. Pink to red color appears.
- Keller Killiani test: To 2 ml extract add glacial acetic acid and one drop of 5% ferric chloride and con. Sulphuric acid. A reddish-brown color appears at the junction of the two liquid layers and the upper layer appears bluish-green.

Tests for Anthra quinone glycosides

- Borntrager's test: To 3 ml extract add dil. Sulphuric acid. Boil and filtrate. To cold filtrate add equal vol benzene or chloroform. Shake well. Separate the organic solvent and add ammonia. The ammonical layer turns pink or red.
- Modified Borntrager's test: To 5 ml extract add 5 ml 5% ferric chloride and 5 ml dilute Hcl. Heat for 5 min in a boiling water bath. Cool and add benzene or any other organic solvent. Shake well. Separate organic layer. Add equal volume dil. Ammonia. The ammonical layer turns a pinkish-red color.

Tests for saponin glycosides

- Foam test: Shake the drug extract or dry powder vigorously with water. Persistent foam observed.
- Hemolytic test: Add drug extract or dry powder to one drop of blood
- Baljet's test: A thick section shows a yellow to orange color with sodium picrate.
- Legal's test: To aqueous or alcoholic extract, add 1 ml pyridine and 1 ml sodium nitroprusside. Pink to red color appears.
- Keller Killiani test: To 2 ml extract add glacial acetic acid and one drop of 5% ferric chloride and con. Sulphuric acid. A reddish-brown color appears at the junction of the two liquid layers and the upper layer appears bluish green.
- Liebermann's test: mix 2 ml extract with chloroform. Add 1-2 ml acetic anhydride and 2 drops of con. sulphuric acid from the side of the test tube. First red, then blue and finally green color appears.
- Tests for Anthra quinone glycosides
- Borntrager's test: To 3 ml extract add dil. Sulphuric acid. Boil and filtrate. To cold filtrate add an equal volume of benzene or chloroform. Shake well. Separate the organic solvent and add ammonia. The ammonical layer turns pink or red.
- Modified Borntrager's test: To 5 ml extract add 5 ml 5% ferric chloride and 5 ml dilute Hcl. Heat for 5 min in a boiling water bath. Cool and add benzene or any other organic solvent. Shake well. Separate organic layer. Add equal vol diluted ammonia, Ammonical layer turns pinkish red color.

2.2. Tests for saponin glycosides

- Foam test: Shake the drug extract or dry powder vigorously with water. Persistent foam observed.
- Hemolytic test: Add drug extract or dry powder to one drop of blood on the glass slide. Hemolytic zone appears
- D. Tests 'for cyanogenetic glycosides: To dry drug powder or extract add 3% aqueous mercury nitrate solution. Metallic mercury forms.
- E. Tests for coumarin glycosides: Alcoholic extract when made alkaline, shows blue or green fluorescence.
- 5). Tests for Flavonoids
- Shinoda test: To dry powder or extract, add 5 ml 95% ethanol, a few drops con. Hcl and 0.5 g magnesium turnings. Pink colored observed. To a small amount of residue, add lead acetate solution. Yellow ppt is observed.

2.2.1. Tests For Steroids and Triterpenoids

- Salkowski reaction: To 2 ml extract, add 2 ml chloroform and 2 ml con. Sulphuric acid Shake well. The chloroform layer appears red and the acid layer shows greenish-yellow fluorescence.
- Liebermann Burchard reaction: Mix 2 ml extract with chloroform. Add 1-2 ml acetic anhydride and add 2 drops of con. Sulphuric acid from the side of the test tube. First red, then blue and finally green color appears.
- Sulphur powder test: Add a small amount of sulfur powder to the test solution, it sinks at the bottom.

2.2.2. Tests for Alkaloids

- Dragen Dorff's reagent: Alkaloids give reddish brown Ppt with this reagent. (mercuric iodide solution).
- Mayer's reagent: Alkaloids give cream color Ppt with Mayer" 's reagent. (Potassium mercuric iodide)..
- Wagner's reagent: Alkaloids give reddish brown Ppt. (lodine potassium iodide solution)
- Hager's reagent: Alkaloids give yellow Ppt (saturated solution of picric acid). Picrolonic acid Alkaloids give yellow Ppt.

2.2.3. tests for phenolic compound

Ferric chloride test: Treat the extract with ferric chloride solution, blue color appears if hydrolyzable tannins are present and green color appears if condensed tannins are present.

Test for chlorogenic acid: Treat the test solution with aq. Ammonia and exposure to air gradually green color is developed. Add Potassium dichromate: red Ppt. Add Bromine water. Discoloration of water

10) fats and fixed oils: Saponification test: Add a few drops of 0.5N alcoholic potassium hydroxide to a small qty of various extracts along with a drop of phenolphthalein separately and heat on a water bath for 1-2 hrs. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils.

3. In vitro antioxidant methods

3.1. Hydrogen Peroxide Scavenging Activity [9]

3.1.1. Hydrogen peroxide radical scavenging assay

The ability of extracts to scavenge hydrogen peroxide was determined by little modification here the solution of hydrogen peroxide (100mM) was prepared instead of 40mM in phosphate buffer saline of (PH 7.4), at various concentrations of extract (10 -50 µg/ml) were added to the hydrogen peroxide solution (2 ml). The absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. In the case of control takes the absorbance of hydrogen peroxide at 230 nm without sample extracts. Results are provided in the percentage inhibition activity was calculated from [(A0- A1)/A0] x 100, where A0 is the absorbance of the control and A1 the absorbance of extract/standard taken as Ascorbic acid (10 - 50 µg/ml)

3.2. DPPH Radical Scavenging Assay [10]

The antioxidant activity of the extracts is based on the scavenging activity of the stable 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radicals. Plant extract (0.1 ml) was added to 3 ml of a .004% MeOH solution of DPPH. Water (0.1 ml) in place of the plant extract was used as a control. Absorbance at 517 nm was determined after 30 min, and the percent inhibition activity was calculated as [(A0 _ A1)/A0] · 100, where A0 was the absorbance of the control, and A1 was the absorbance of the extract/standard

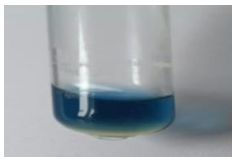
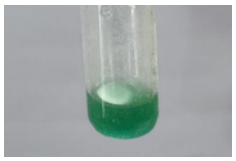
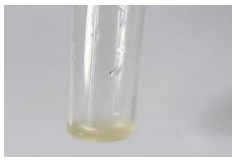
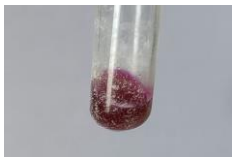
4. Results and discussion

Phytochemicals are bioactive compounds produced by plants for their protection. They can be derived from various sources such as whole grains, fruits, vegetables, nuts, and herbs, and more than a thousand phytochemicals have been discovered to date [11]. Some of the significant phytochemicals include carotenoids, polyphenols, isoprenoids, phytosterols, saponins, dietary fibers, and certain polysaccharides. These substances occur in low concentrations and typically have a pharmacological effect. Throughout history, people have utilized these effects in natural medicine through the use of medicinal herbs, spices, teas, and foods [12]. The advancement of highly sensitive analytical methods has allowed for the identification of a wide range of these substances. Present day, there has been a huge increase in

research in the field of natural products in finding natural antioxidants as an alternative to synthetic drugs. Due to its low adverse events compared to synthetic drugs. Natural antioxidants show good potential to act as therapeutic agents in their mechanism of eliminating radical chain reactions in biological systems. Growing experimental evidence suggests that their herbal products affect many cellular events in terms of their free radical scavenging activity [13]. The anti-oxidant vitamin C was found in animals and plants. That is not synthesized biologically and most of which can be obtained from food. Ascorbic acid is stored in a reduced manner in combination with glutathione and promotes the disulfide isomerase and glutaredoxin proteins. It acts as a reducing agent such as hydrogen peroxide to make neutralization of free radicals [14]. Free radicals are divided into oxygen and nitrogen free radicals based on performance. Excessive generation of free radicals may be due to uncontrolled stress caused by an imbalance between the body's natural defenses in the formation of stress-induced oxidative stress [15]. Radicals are involved in cellular components causing injury or death. The generation of all kinds of free radicals is stored in normal cells, and overproduction can now be seen as the leading cause of many diseases. Oxidative stress is considered a major factor in many chronic conditions such as diabetes, tumors, neuropathy, gastric ulcers, and others [16].

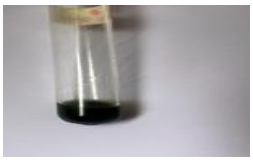
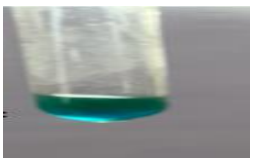
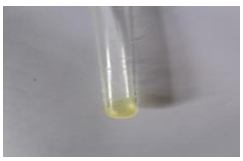
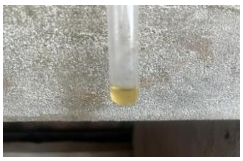
Hence, the present study was designed to evaluate the potency of selected leaf extract on stress using *in-vitro* scavenging activity of hydrogen peroxide and DPPH radicals.

Table 1 Preliminary Phytochemical Analysis of *Callisia repens*

Name of the chemical test	Observation	Present/absent	Result/interference
Test for proteins: Millions test	No change in the color	Absent	
Test for carbohydrates: Fehling test	A deep blue color observed	Absent	
Benedict's test	Green colour observed	Absent	
Test for saponin Glycosides: Foam test	Foam is observed	Present	
Test for amino acid: Ninhydrin test	Golden color appears	Absent	
Test for fats and fixed oil: Saponification test	Pink colour observed	Absent	
Test for steroids and triterpenoids: Salkowski test	Yellow colour observed	Present	

Test for anthraquinone glycoside: Brontragers test	The color does not appear	Absent	

Table 2 Phytochemical Analysis of *Crassula ovata* (Jade)

Name of the chemical test	**Observation**	**Present/absent**	**Result/interference**
Test for protein: Millions test	Brick red precipitate was not observed	Negative	
Test for carbohydrates: Fehling's test	A dark blue color was observed	Positive	
Test for carbohydrates: Benedict's test	The greenish-yellow colour was not observed	Absent	
Test for saponin glycosides: Foam test	Foam was observed	Positive	
Test for anthraquinone glycosides Borntrager's test:	The pinkish color does not appear	Absent	
Test for amino acids Ninhydrin test	The blue color is not observed	Absent	
Test for flavonoids Shinoda test	The pinkish color formed.	Present	
Test for steroids &triterpenoids Salkowski reaction	Layers are separated.	Present	

Test for fats and fixed oils: A. Saponification test	The formation of soap is not observed.	Absent	

The jade plant leaves contain carbohydrates, proteins, steroids and triterpenoids, flavonoids, and phenols (table1) and turtle vine contains glycosides and terpenoids (table 2).

The reaction of DPPH with an anti-oxidant or reducing complex produces the corresponding hydrazine DPPH, which can be followed by a change in color from purple to yellow [17]. The *Callisia repens* and *Crassula ovata* showed comparatively better action (figure 1 and 2) the effect of antioxidants on DPPH is thought to be due to their hydrogen-donating powder with IC50 values of 48.825 mg/ml and 470.314 mg/ml respectively.

Table 3 Evaluation of DPPH radical scavenging activity of *Callisia repens* and *Crassula ovata*

Concentration	***Callisia repens***	***Crassula ovata***
	%Inhibition (DPPH)	
100mg/ml	53.9615±1.24	24.425±1.35
200mg/ml	58.4582±1.45	39.528±1.48
300mg/ml	66.8808±1.34	55.792±1.52
400mg/ml	72.9479±1.54	68.762±1.64
500mg/ml	79.0864±1.83	81.423±1.92
IC50	48.825	470.314

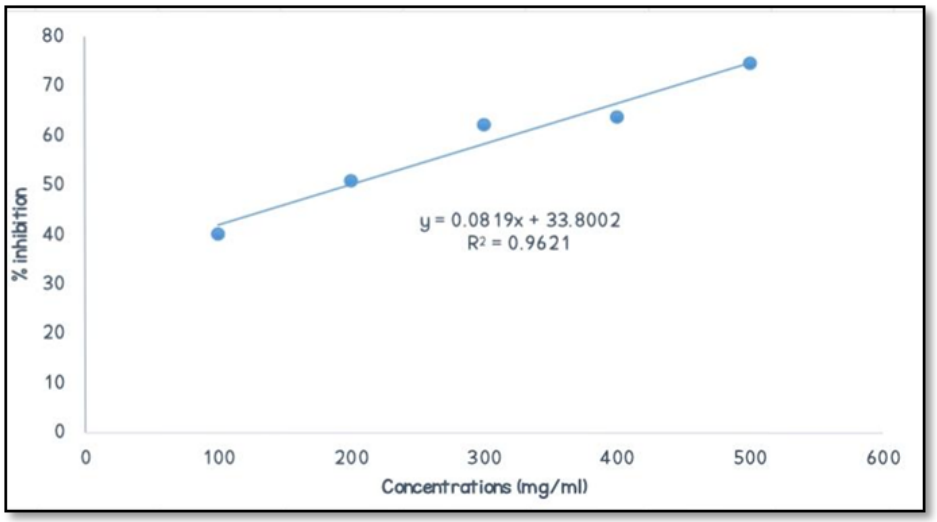


Figure 1 % Inhibition of DPPH radical activity of *Callisia repens*

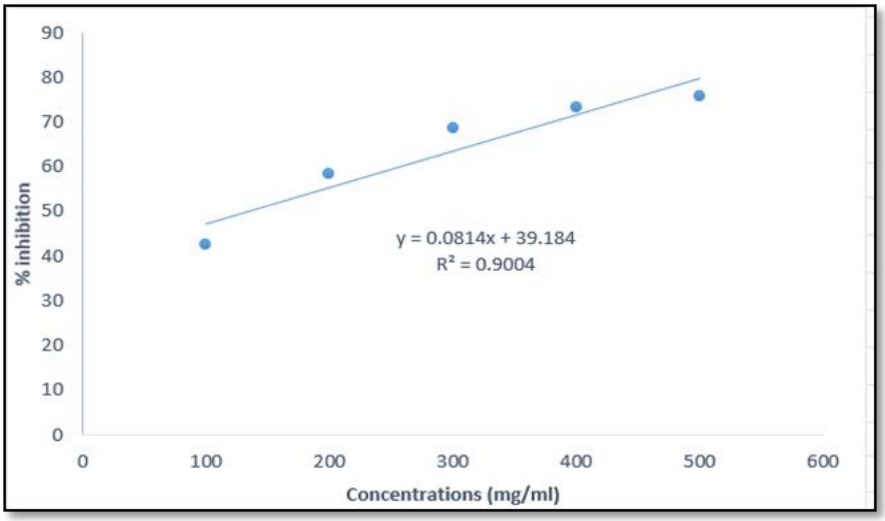


Figure 2 % Inhibition of DPPH Radical of Crassula Ovata

Table 4 Evaluation of hydrogen peroxide scavenging activity of *Callisia repens* and *Crassula ovata*

Concentration	***Callisia repens***	***Crassula ovata***
	%Inhibition (H_2O_2)	
100mg/ml	52.5761±1.32	21.254±1.42
200mg/ml	56.089± 1.46	34.921±1.55
300mg/ml	59.6019± 1.60	42.421±1.66
400mg/ml	68.3841± 1.72	53.619±1.83
500mg/ml	74.2389±1.34	64.538±1.45
IC50	81.082	181.51

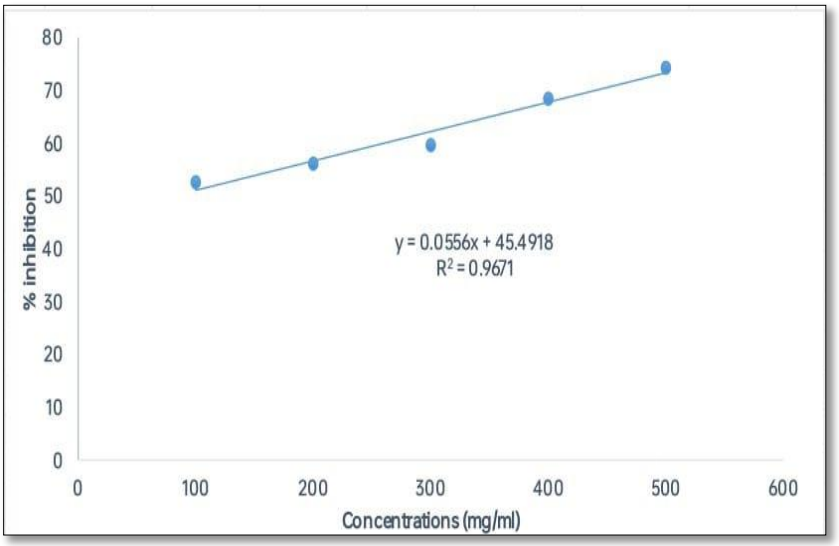


Figure 3 % Inhibition of H_2O_2 Radical of *Callisia repens*

Hydrogen peroxide is generated in vivo in a disrupted reaction catalyzed by the enzyme superoxide dismutase (SOD). It is not a free radical and even can cause damage to the cell at a low level, but at high levels, it inactivates cellular enzymes that produce enzymes such as glyceraldehyde -3- phosphate dehydrogenase [18]. It can easily penetrate the

membrane of biological systems but has no direct effect on DNA but can damage DNA by producing hydroxyl radical ions (OH-) in the presence of transitional metal ions [19]. The present study shows that *Callisia repens* (81.08 mg/ml) and *Crassula ovata* (181.51 mg/ml) exhibit greater action of hydrogen peroxide inhibition in a concentration-dependent manner (Figures 3 and 4).

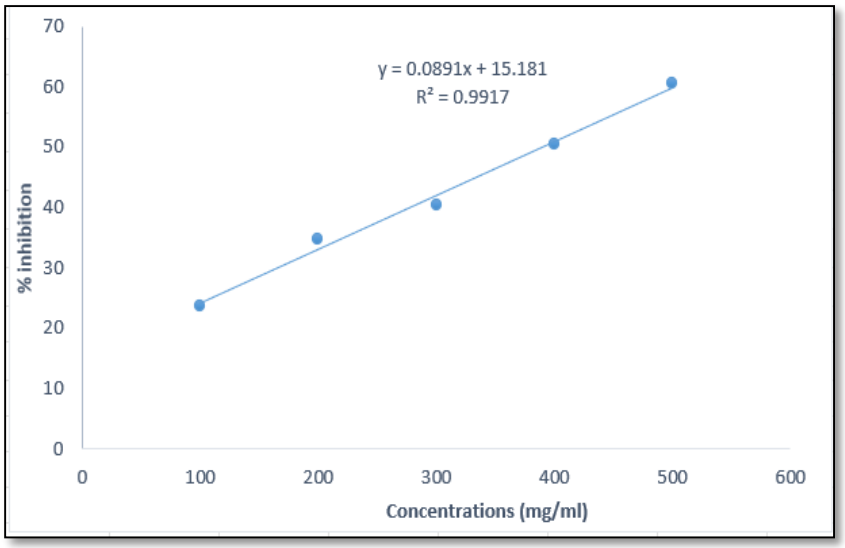


Figure 4 % Inhibition of H_2O_2 Radical of *Crassula ovata*

5. Conclusion

The *in-vitro* anti-oxidant activity of selected ornamental plants might be due to the hydrogen-donating capability or electron-donating capability. The phytochemicals such as carbohydrates, and reducing sugars present in *Callisia repens* and *Crassula ovata* must be responsible for scavenging hydrogen peroxide, and DPPH radical in a concentration-dependent manner.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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