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SCREENING OF PHYTOCHEMICALS AND EVALUATION OF ANTI-MICROBIAL, ANTI-OXIDANT AND *IN-VITRO* ANTIDIABETIC ACTIVITY OF *CRASSULA OVATA* LEAVES

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Keywords:

Crassula ovate, Aqueous extract, Methanol Extract, Anti-diabetic, Anti-oxidant, Anti-microbial.

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ABSTRACT: The study involved both aqueous extraction and methanolic extraction of the powered leaves, which ensure all plant components are extracted for better results. Phytochemical analysis from the extracts was performed using the standard protocols. The antimicrobial susceptibility of the extracts was tested against *E-coli*, *Pseudomonas and Klebsilla sp* using agar disc diffusion method. Antioxidant property of the plant was evaluated using DPPH Method and Hydrogen Peroxide Scavenging activity method whereas antidiabetic property was evaluated using α-amylase inhibition assay. The plant extracts were used at varying concentrations to ensure which plant extract and concentration causes the most inhibition. Phytochemical analysis of the extract indicated the present of Saponin, phenol , phytosterol, steroid, terpenoid, flavanoid, carbohydrates and proteins while tannin, glycoside, plobatanin were absent. Anti-diabetic property of the plant was observed with a significant result of inhibiting a maximum of 83% of α-amylase activity (700µg/ml concentration of methanol extract) whereas antioxidant property with a maximum of 85% of (200µg/ml concentration of water extract) inhibition. Antimicrobial property of the extract shows effectiveness against *Escherichia coli*, *Pseudomonas* and *Klebsilla sp.* with a maximum inhibition zone of 6.53±0.35mm, 3.46±0.30 mm and 3.76±0.25 mm respectively.Not much work has been done on *Crassula ovata* to evaluate its efficacy in scientific way. Further large scale studies on animal models need to be carried out before using these extracts for drug designing.

INTRODUCTION: North east India is a hub to traditional herbal medicines and a large number of ethnic group use plant as a source to cure various diseases. Medicinal plants are currently in considerable significance view due to their special attributes as a large source of therapeutic phytochemicals that may lead to the development of novel drugs. Many people now take medicinal plant products on a daily basis, to maintain good health as much as to treat illness. The types of plants and methods of application vary from locality to locality.

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Crassula ovata commonly known as Jade plant is a medicinal herb, traditionally used to treat symptoms of diabetes. But till date, sporadic attempts have been made for the scientific and methodical validation of these traditional claims. Therefore, the present study was designed to investigate phytochemical screening, antioxidant, antimicrobial and antidiabetic activity of the leaf of *Crassula ovate.*

The plant *Crassula ovata* is native plant to South Africa. It is also common houseplant all over the world, but it is mostly located in the Northern Hemisphere particularly in cold and dry areas where water is scarce [1]. The *Crassula ovata* plant is able to remain minimum water loss while photosynthesizing efficiently through Crassulacean Acid Metabolism (CAM). The Plants succulent water-storing leaves, stems and roots give the

ability to survive droughts, being grazed on, trampled on or knocked over, as it is able to root from any piece of its stem, and even a single leaf. Any discarded leaves left around the foot of the plants send down roots and grow into new plants [2]. They can also be cut and placed in a water container until roots grow usually in about two weeks, then planted in soils. The flowers of *Crassula ovata* attract wasps, flies, bees, butterflies, and beetles. Wind helps disperse the fine dust-like seeds. The stems also make good bases for wasps to build their nests [3].

In some traditional practice, the leaf of *Crassula ovata* was used to treat warts where leaves were sliced in half and attached the moist inside to the wart for a few hours, or overnight. The unsightly growth would fall off with just three applications [4]. In Asian cultures particularly in China (700AD), jade plant is popular element. Medicine-men prescribed a tea of the jade plant to treat symptoms of diabetes. Because of its abundance and its softness in ancient times, it could easily be shaped into various forms thus it was used in the art of Bonsai. The plant was spread around as luxurious gift to royalties all over the Chinese empire. In many businesses, a jade plant is often placed near a cash register as in Chinese tradition as a way to attract prosperity [5].

From the traditional use of many communities from North East India (Especially in Manipur), it is revealed that *Crassula ovata* is mainly used in treatment of diabetes and to cure some infection. They used to drink the juice from the leaves of the plant to cure diabetes. But a very little work has been done to evaluate its efficacy in scientific way. Therefore this study was designed to evaluate the anti-microbial, anti-oxidant and *in-vitro* anti diabetic activity of *Crassula ovata* Leaves.

MATERIALS AND METHODS:

Study area: The study was conducted at Department of Biotechnology, Assam down town University and Central Instrumentation Facility Laboratory, Assam down town University, Assam.

Plant material:

Sample collection and identification: The healthy and disease free leaves of *Crassula ovata* was collected from Senapati district of Manipur in the month of January 2016 through random sampling. It was plugged with hand from the tree and kept in plastic bags. The plant was identified and confirmed by plant taxonomist using standard manual.

Preparation of crude extract: The collected leaves of *Crassula ovata* were transferred to Department of Biotechnology, Assam down town University. The leaves were washed thoroughly with distilled water, cut into thin slice with the help of knife and dried in shade condition at room temperature for 15 days. They were then powdered using wooden –made pestle and mortar. The powder of the leaves were sieved and stored in air-tight plastic container for further use.

40g of air-dried powder was measured by electronic balance (Afcoset ER-200A) and it was packed in the filter paper and placed in the thimble and was loaded in the main chamber of the Soxhlet extractor (Optics technology). Methanol was used as solvent for the extraction. 300 ml of methanol was filled in the round bottom flask. The apparatus was set up and extraction was performed for 15hrs. During extraction the non-volatile compounds dissolves in the solvent and gets concentrated in the round bottom flask. The phytochemical collected in the round bottom flask are separate from the solvent using rotator vaccum evaporator, yielding the extracted compounds. The advantage of this method of extraction is that instead of many portions of warn solvent running through the plant material, just one batch of the solvent is used and recycled. Therefore the quantity of the solvent required is less and also the solvent used for extraction can be recovered and it is reusable [6]. Water extraction of the plant was also carried out and standard procedures were followed for determining presence of phytochemical, anti-oxidant activity, anti-diabetic activity and anti-microbial activity.

Test for phytochemicals: Evaluation of different phytochemical from the extract was performed by previously described standard protocols. The result reveals the presence of medicinally active constituents like Saponin, phytosterol, steroid, terpenoid, flavanoid, carbohydrates and protein in the sample.

Anti-oxidant activity test: Two different method DPPH and H2O2 Scavenging assay were used to test the anti-oxidant activity.

DPPH Method: The antioxidant activity of methanol and aqueous extract of the plant material was assayed according to the method described before[7] with slight modification. The test extracts were prepared with the concentration of 1000µg/ml by dissolving 20mg of the methanol or water extract and the volume was made up to 20 ml by methanol or water separately. Different concentrations of the plant extracts (50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml) were taken and 2ml of methanolic DPPH solution was added followed by keeping it for incubation for a period of 30 minutes. The DPPH free radical scavenging was determined in a UV-Vis spectrophotometer (Systronic UV-Vis Spectrophotometre-117) by measuring absorbance at 517 nm against a blank solution by taking ascorbic acid as standard. . The percentage of hydrogen peroxide scavenging of *Crassula ovata was* calculated using

$$\%Inhibition = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

Hydrogen Peroxide (H_2O_2) Scavenging Activity: The ability of the *Crassula ovata* extracts to scavenge hydrogen peroxide was determined according to the method of Ruch et al 1989 also [8]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentration of *Crassula ovata* Extracts (50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml) in distilled water and methanol were prepared separately and later added to different test tube containing 600µL hydrogen peroxide solution. Absorbance of the test sample at 230 nm was determined 10 minutes later against a blank solution taking ascorbic acid as standard. The percentage of hydrogen peroxide scavenging of *Crassula ovata was* calculated using:

$$\textbf{Scavenging Activity (\%)} = \frac{A_{Blank} - A_{Sample}}{A_{Blank}} \times 100$$

Anti-diabetic activity test: A starch solution (0.1% w/v) was obtained by stirring 0.1g of potato starch in 100 ml of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha amylase in 100 ml of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartarate solution and 3, 5 dinitro salicylic acid solution 96 mM. Both control and plant extract were added with starch solution and left to react with alpha-amylase solution under alkaline conditions at 25ºC. The reaction was measured over 10 minutes. The generation of maltose was quantified by the reduction of 3, 5 dinitro salicylic acid to 3- amino-5- nitro salicylic acid. Absorbance was measured at 540 nm [9-10]. Percentage of inhibition was calculated using

% Inhibition =

$$\frac{\text{Absorbance of positive control} - \text{Absorbance of sample}}{\text{Absorbance of positive control}} \times 100\%$$

Anti –microbial test: The anti-microbial test was performed using Agar disc diffusion method [11] with different extract.

Test organisms and inocula preparation: Three different isolates of bacteria were selected based on their importance in heath, particularly based on their association with human diseases. The standard strains of *E. coli* (ATCC 25922) *Pseudomonas (*ATCC 27853*) and Klebsilla sp(*ATCC 700603*),* were collected from down town hospital, Guwahati, Assam, India. They were later transported into Biotechnology department by using nutrient agar slant and preserved at 4^0 C for further use. The test organisms from nutrient agar slant were then transferred to nutrient agar medium to get a pure colony at 37^0 C for 24h by using standard growth method [12-13]. 3-5 pure colonies were selected and transferred into a sterile test tube congaing 5 ml of bacterial culture broth and kept for overnight at 37^0C.

Agar disc diffusion method: Antibacterial test was performed by agar disc diffusion method using Muller-Hilton agar [14-15] followed by the procedures of CLSI [16]. The compounds with potential antimicrobial properties are deposited on a sterile filter paper disc which in turn shows the principle of diffusion through agar. For all the anti-microbial tests a concentration of 1mg/ml of extracts were used. The entire tests were repeated triplicate to get better result and after overnight incubation, Zone of inhibitions were measured in mm by measuring the radius using scale.

Statistical analysis: The zones of inhibition of all the bacteria were expressed as mean ± standard deviation (SD) of three replicates and were subjected to analysis of variance using the Minitab 17 free version.

RESULTS:

Presence of different Phytochemicals in both the water and methanol extract of *Crassula ovata* leaves are presented in **Table 1.**

TABLE 1: RESULT OF PHYTOCHEMICAL SCREENING OF THE LEAVE EXTRACT

Sl No.	Phytochemicals	Water Extract	Methanol Extract
1	Saponin	+ve	-ve
2	Phenol	+ve	+ve
3	Phytosterol	-ve	+ve
4	Steroid	+ve	+ve
5	Tannin	-ve	-ve
6	Terpenoid	+ve	+ve
7	Glycoside	-ve	-ve
8	Plobatanin	-ve	-ve
9	Flavanoid	+ve	+ve
10	carbohydrate	+ve	+ve
11	Protein	+ve	+ve

DPPH and Hydrogen Peroxide (H_2O_2) Scavenging Activity for Anti-oxidant activity of *Crassula ovata* leaves: DPPH method showed different percentage of inhibition based on the different concentration which was performed taking ascorbic acid as the standard (Positive Control: 0.856) and are presented in **Table 2.**

TABLE 2: PERCENTAGE OF INHIBITION FOR DPPH METHOD

Sl no.	Concentration of sample	Absorbance (Methanol extract)	% Inhibition	Absorbance (Water extract)	% Inhibition
1	50 µg/ml	0.442	48.36%	0.437	48.90%
2	100 µg/ml	0.428	50.00%	0.428	50.00%
3	150 µg/ml	0.400	53.27%	0.426	50.23%
4	200 µg/ml	0.399	53.38%	0.410	52.10%

Hydrogen Peroxide (H_2O_2) Scavenging Activity method also showed different percentage of inhibition based on the different concentration which was performed taking ascorbic acid as the standard **(Positive Control: 0.720)** and are presented in **Table3.**

TABLE 3: PERCENTAGE OF INHIBITION FOR H_2O_2 METHOD

Sl no.	Concentration of sample	Absorbance (Methanol extract)	% Inhibition	Absorbance (Water extract)	% Inhibition
1	50 µg/ml	0.558	22.5%	0.171	76.00%
2	100 µg/ml	0.478	33.6%	0.157	78.00 %
3	150 µg/ml	0.343	52.00%	0.146	79.00%
4	200 µg/ml	0.166	76.00%	0.101	85.00 %

Anti-diabetic activity: Different concentration of plant sample showed different inhibition of alpha-amylase for both the extract where ascorbic acid was taken as the standard (Positive control: 0.746) and are presented in **Table 4.**

TABLE 4: *IN VITRO* ANTIDIABETIC ACTIVITY OF ALPHA-AMYLASE METHOD

Sl no.	Concentration of sample	Absorbance (Methanol extract)	% Inhibition	Absorbance (Water extract)	% Inhibition
1	300µg/ml	0.203	72.00%	0.240	67.00%
2	400µg/ml	0.174	76.00%	0.211	71.00 %
3	600µg/ml	0.130	82.00%	0.207	72.00%
4	700µg/ml	0.124	83.00 %	0.195	73.80 %

Anti –microbial test: Agar disc diffusion method of both the extract showed significant inhibition zone with all the strains of bacteria and are presented in **Table 5.**

TABLE 5: ZONE OF INHIBITION AGAINST TEST ORGANISM OF LEAF EXTRACT OF *CRASSULA OVATA*

Sl No.	Strains	Zone of Inhibition (in mm) Values represent mean±SD, n=3	
		Methanol extract	Water extract
1	*E. Coli* (ATCC 25922)	4.13±0.15	6.53±0.35
2	*Pseudomonas(*ATCC 27853*)*	3.36±0.30	3.46±0.30
3	*Klebsilla sp(*ATCC 700603*)*	3.76±0.25	3.10±0.26

DISCUSSION: The entire study was carried out by using both aqueous and methanol extract of leaves of *Crassula ovata.* The preliminary phytochemical screening tests for the methanol and water extract of *Crassula ovata* leaves (**Table 1**) revealed the presence of several bioactive compounds which could be responsible for the diverse medicinal properties of this plant. Presence of Saponin, phytosterol, Phenol, Steroid, Terpenoid, Flavanoid, carbohydrate and Protein were seen in the extracts of the plant. Presence of any of the secondary metabolites, singly or in combination with others could be responsible for the anti-diabetic activity of the plant.

The plant showed a significant anti-oxidant activity with both the methods. Out of the two extracts, water extract showed the maximum anti-oxidant activity (85% inhibition in 200µg/ml) with the increase in the concentration (**Table 3**). The methanol extract showed a maximum of 76% inhibition in 200µg/ml of concentration which reveals a dose-dependent increase in percentage inhibitory activity. The present study suggests that both the extracts could be a potential source of natural antioxidant which could be of great importance for the treatment of radical related diseases and age associated diseases.

The aqueous and methanol extracts of *Crassula ovata* leaves showed significant inhibitory effect on alpha-amylase activity in vitro in a dose dependent manner (**Table 4**). A maximum of 83.00 % inhibition in 700µg/ml concentration in case of methanol extract and a maximum of 73.80 % inhibition in 700µg/ml concentration in case of water extract were seen. Further studies are required to elucidate whether *Crassula ovata* leaves have antidiabetic potential by in vivo for validating the traditional claim of the plant used by Manipuri tribes where they used only water extract. As the plant showed significant inhibition activity, so further the compound isolation, purification and characterization which is responsible for inhibiting activity, has to be done for the usage of the plant as antidiabetic agent.

In this study leave extract of *Crassula ovata* was administrated against the three standard strains (**Table 5**). The water extract demonstrated the maximum zone of inhibition against *E coli* (6.53±0.35mm), followed by *Pseudomonas* (3.46±0.30 mm) and *Klebsilla sp* (3.10±0.26 mm). In case of methanol extract the maximum zone of inhibition against *E coli* (4.13±0.15 mm), followed by *Klebsilla sp*(3.76±0.25 mm) and *Pseudomonas* (3.36±0.30 mm) were seen resectively. From the study standard *E coli* strain was found to be more susceptible to water extract of *Crassula ovata* leaves.

CONCLUSION: Not much work has been done on the plant *Crassula ovata* to evaluate its efficacy in scientific way which is traditionally being used by Manipuri people (Manipur, India) for a long time to cure diabetes. The findings of this work would help in future large scale studies.

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