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In-vitro Analysis of Antioxidant and Anti-inflammatory potential of the leaf extract of *Kalanchoe laciniata* (L.).

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Abstract: Free radicals cause a wide range of illnesses and chronic diseases by damaging biomolecules such as nucleic acids, proteins and lipids. Medicinal plants and plant extracts have potent antioxidant activity to scavenge these free radicals and protect from diseases. *Kalanchoe laciniata* (L.) is one of the most extensively used plants in folklore, grown as native to Brazil, Africa, Yemen and India also distributed in Burma, Ceylon, Tropical Asia and other African countries. This plant has a potential to aid a cure in diabetes and it is also used as anti-dysenteric, astringents, anti-inflammatory, antiseptic, antiulcer, anti-irritant etc. The present study was aimed to evaluate the total phenol content, and antioxidant potential toward free radical propagation in methanol, hexane and water leaf extracts of *Kalanchoe laciniata*(L.). The extracts were prepared by using water, methanol and hexane solvents. The Antioxidant potential of the extract was determined by a set of assays which included DPPH assay, Total reducing power assay and total phenol content (the Folin- Ciocalteu method). The in-vitro analysis of anti- inflammatory property of the extract was determined by Human RBC membrane st1abilization assay. All the experiments were carried out using standard protocol. These phytochemical screening experiments are used in the following study. The underlying study suggested that *Kalanchoe laciniata* (L.) leaf extract serves as a good source of natural antioxidant agent and phytochemicals.

Keywords: *Kalanchoe laciniata*(L.), *antioxidant potential, DPPH, phenol content*

INTRODUCTION

Kalanchoe laciniata (L.) also known as the Christmas tree plant belongs to the family Crassulaceae. This family of plants usually presents xenomorphic characteristics and hence is referred to as succulents. These plants tend to adapt easily to bright light and water scarcity. It has been a part of various cultures and has been used as a medicinal plant for its anti-inflammatory properties. The plant is native to Brazil but also has found to be used in the folk-medicine in China and the Indian subcontinent. (Tantary S et al, 2016) (Herrera, 2008). Its sister plant, *Kalanchoe pinnata* is quite famous in the field of medicine and many studies have shown that the leaf extract of the plant holds antimicrobial, antioxidant, anti-inflammatory and antihypertensive properties. (Y. Obregón-Díaz et al, 2019). *Kalanchoe laciniata (L.)* is studies before for the presence of Flavanoids (Costa et al., 1994), polysaccharides (Bhatti et al., 2013) from different extracts. The plant has been used extensively in traditional medicine (J. M. Fernandes et al., 2019). Plant extracts into different solvents has been documented for their use as antibacterial (Silva et al., 2009), Larvicidal activity (Trevisan et al., 2006) etc. Our study focuses on the in-vitro analysis of antioxidant and anti-

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inflammatory properties of *Kalanchoe laciniata* from West Bengal.

Fig 1 - *K. lacinata* (Pic from -http://tropical.theferns.info/viewtropical)

METHODS

Collection of plant material

The fully matured leaves of *Kalanchoe laciniata*were collected from The Agri-Horticultural Society of India, West Bengal, Kolkata, India. The authenticity of the plant was confirmed in Botanical Survey of India, Kolkata, West Bengal by referring to the deposited specimen. The collected disease free leaves were washed to make free from dust and other plant materials, shade-dried, and then homogenized to fine powder and stored in air tight bottles.

Preparation of plant extract

The air-dried leaves (10g) were coarsely powdered and extracted with methanol (100 ml) in cold condition at room temperature for 24 h. The extract was filtered and stored at 4^{o}C for further analysis.

DPPH assay

The stable DPPH radical was used for determination of free radical scavenging activity of test samples (Lee et al., 2003). A solution of DPPH (0.1 mM) in methanol was freshly prepared. Ascorbic acid with concentration of 100 ug to 500ug was prepared in methanol and used as control. Five ml of above solution was added to 5 ml of DPPH solution and kept in dark for 30 min at room temperature. After 30 min, the absorbance was recorded at 517nm using UV/Vis spectrophotometer against methanol as blank.

DPPH scavenging effect (%) or Percent inhibition = A_0 - A_1 / A_0 × 100.

Where A_0 was the Absorbance of control reaction and A_1 was the Absorbance in presence of test or standard sample.

The DPPH antioxidant activity for the two extracts was determined using 100ug to 500ug of the extract. The percentage inhibition of scavenging activity in terms of IC50 values were calculated.

Table 1: DPPH assay for different plant extract.

Concentration of extract	Water extract % inhibition	IC50	Methanol extract % inhibition	IC50	Hexane extract % inhibition	IC50
100	36.67		3.34		43.34	
200	46.67	322.164 µg/ml	13.25	535.9 µg/ml	46.66	312.5 µg/ml
300	50		26.67		56.67	
400	60		42		60	
500	66.67		50		66.67	

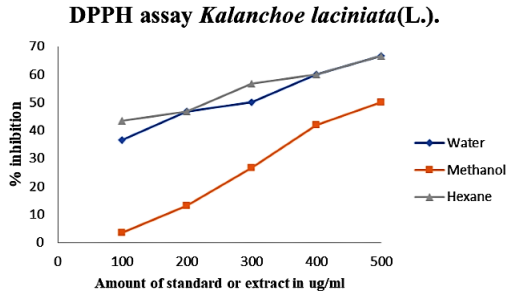

Fig. 2. Showing antioxidant potential of plant extracts.

Total reducing power

Total reducing power was done according to the method described by Oyaizu (1986). Phosphate buffer 0.2 M (pH 6.6) 2.5 mL and 2.5 mL of $K_3Fe(CN)_6$ (1% w/v) were added to 1.0 mL of extract. The resulting mixture was incubated at 50 ^{o}C for 20 min, followed by the addition of 2.5 mL of trichloro acetic acid (10% w/v). The mixture was centrifuged at 3000 rpm for 10 min, 2.5ml of the solution was mixed with distilled water (2.5 mL) and 0.5 mL of $FeCl_3$

(0.1%, w/v). The absorbance is then measured at 700 nm against blank sample.

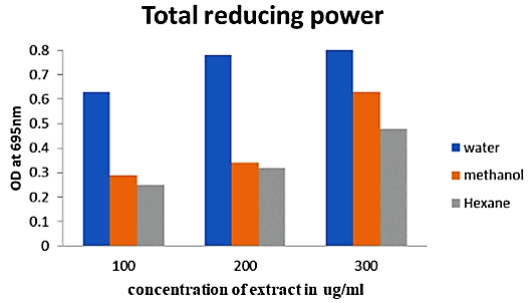

Fig. 3. Showing total reducing power of plant extracts.

Estimation of total Phenolics

Ten mg of gallic acid was dissolved in 100 ml of 50% methanol (100 µg/ml) and then further diluted to 6.25, 12.5, 25 or 50 µg/ml (Lin and Tang, 2007). One ml aliquot of each dilution was taken in a test tube and diluted with 10 ml of distilled water. Then 1.5 ml Folin-Ciocalteu's reagent was added and allowed to incubate at room temperature for 5 min. Four ml of 20% (w/w) Na_2CO_3 was added in each test tube, adjusted with distilled water up to the mark of 25 ml, agitated and left to stand for 30 min at room temperature. Absorbance of the standard was measured at 765 nm using UV/VIS spectrophotometer against blank, i.e., distilled water.

One ml aliquot of 1:10 diluted sample was taken in a test tube and diluted with 10 ml of distilled water. Then 1.5 ml Folin-Ciocalteu's reagent was added and allowed to incubate at room temperature for 5 min. Four ml of 20% (w/w) Na_2CO_3 was added, adjusted with distilled water up to the mark of 25 ml, agitated and left to stand for 30 min at room temperature. Absorbance of the sample was measured at 765 nm using UV/VIS spectrophotometer nm against distilled water blank. Quantification was done on the basis of a standard curve of gallic acid. A standard curve of extinction against gallic acid concentration was prepared (Figure 4). The concentration of the phenolics in water and methanol extract was expressed in terms of Gallic acid equivalents per gram of tissue.

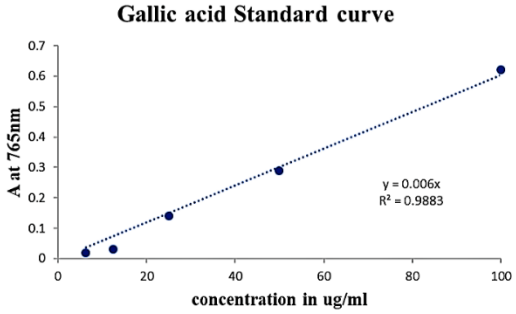

Fig. 4. Showing Standard graph of Gallic acid

Human RBC (HRBC) Stabilization assay

Fresh whole blood (2ml) collected from healthy volunteers in tubes containing heparin was centrifuged at 3000 rpm for 10 min. Pellet contained RBC. Equal volume of normal saline was used to dissolve the red blood pellets. The volume of the dissolved red blood pellets obtained was measured and reconstituted as a 40% v/v suspension with isotonic buffer solution (10 mM sodium phosphate buffer, with NaCl, pH 7.4).

The anti-inflammatory activity of methanol and hexane extracts of leaves of *Kalanchoe laciniata* was assessed by the in vitro HRBC (human red blood cell) membrane stabilization assay (Varadarasu et al., 2007) with minor modifications. The reconstituted blood was directly used. To 0.5 mL of HRBC suspension, equal volume of test drug (phenylhydrazine) in four different concentrations, 20, 40, 60 and 80 ng/mL, were added. All the assay mixtures were incubated at 37°C for 60 minutes and centrifuged. The haemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560 nm (James et al 2009). The percentage of hemolysis was calculated then by the formula as given below:

Percent of hemolysis = OD of test/OD of control × 100

The percentage of protection can be hence calculated from the equation as given below:

Percent of Protection = OD of Test/ OD of control ×100

Here "OD of test" is optical density or the test sample's absorbance and "OD of control" is optical density or absorbance of the negative control. Here, the negative control used was saline solution with blood in it and it contained no Phenyl hydrazine or plant extract in it.

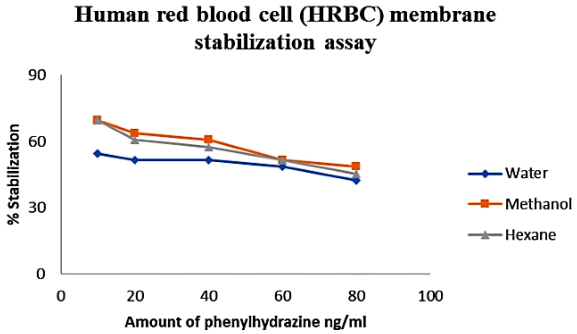

Fig. 5. Showing Membrane stabilization property of plant extracts.

STATISTICAL ANALYSIS

All the determinations were conducted at least three times (n=3); the statistical mean was calculated with ±SD using Excel 2013.

RESULTS AND DISCUSSION

In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals was monitored. The leaves, water and methanol extract showed antioxidant potential (Fig-2). Different concentrations of the two extracts were analyzed and aqueous extract showed best IC50 value than the other extracts. DPPH assay showed IC50 of 322.164 µg/ml, 535.9 µg/ml and 312.5 µg/ml for water, methanol and hexane extract respectively.

The antioxidant potential of the extracts was supported by performing total reducing power assay. Increase in the absorbance indicates the reducing power of the samples. In this method, antioxidant compound forms a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, which was measured at 700 nm. Both the water and methanol extracts showed an increase in reducing power as increase in concentration of extracts (Fig-3). Total Reducing power assay showed an increase indicating the antioxidant potential of the plant extracts. Total phenol content was found to be 203.78±1.82 (mg GAE/g of dry mass) and 86.367±0.07 (mg GAE/g of dry mass) for water and methanol extracts respectively.

Total phenolics was assayed according to the method of Singleton et al., 1999 method.

In this method there is chemical reduction of the reagent containing a mixture of tungsten and molybdenum oxides. The molybdenum centre is reduced from Mo (VI) to Mo (V) with an electron donated by an antioxidant to produce a blue colour. The product is blue colour that exhibits a broad light absorption with a maximum at 765 nm (750-770nm). The intensity of light absorption at that wavelength is proportional to the concentration of phenols and results are expressed in Gallic acid equivalents (GAE). This method is sensitive, quantitative and relatively independent of the degree of polymerization.

RBC membrane is more susceptible to oxidative stress due to the presence of both high concentration of polyunsaturated fatty acids (PUFA) in the membrane and the oxygen transport associated redox active haemoglobin (Hb), which are the targets of ROS. PHZ generate ROS and leads to hemolysis by triggering oxidation of Hb into hemichromes and to form free radicals (Winterboun 1985, Vilsen and Nielsen 1984). In our study the PHZ induced damage is tried to overcome by addition of methanol or hexane extracts.

The protective action of the extracts in terms of free radical quenching is monitored. The study shows that the leaf extract of *Kalanchoe laciniata* exhibits a considerable amount of

free radical scavenging and hence can be considered as a potent antioxidant which supports its application in ayurvedic medicine. The methanolic and water extracts showed comparatively higher presence of phenolics. Also the membrane stabilization assay shows a good percentage indicating a sign of promising anti-inflammatory properties. This work will provide an understructure for further works and possible applications of *Kalanchoe laciniata* in ayurvedic medicine.

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ABBREVIATIONS

ROS- Reactive Oxygen Species,
DPPH- 2,2-diphenyl-1-picrylhydrazyl,
HRBC- Human Red Blood Cell,
OD- Optical Density,
IC50- Inhibitory Concentration 50

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