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Rohan Sharadanand Phatak
Directorate of Research, Krishna Institute of Medical Sciences University, Karad-415110, Maharashtra, India,

Anup Subhash Hendre
Department of Biochemistry, Krishna Institute of Medical Sciences Karad-415110, Maharashtra, India

Correspondence
Rohan Sharadanand Phatak
Directorate of Research, Krishna Institute of Medical Sciences University, Karad-415110, Maharashtra, India,
Email: phatak.rohan1983@gmail.com

Total antioxidant capacity (TAC) of fresh leaves of *Kalanchoe pinnata*

Rohan Sharadanand Phatak, Anup Subhash Hendre

ABSTRACT

Herb *Kalanchoe pinnata* is grown as weeds in the tropical countries like India. It has a wide range of active constituents which has potent medicinal properties. The main objective was to overview the total antioxidant capacity of the herb *Kalanchoe pinnata*. Antioxidant activities were evaluated in terms of total phenolics content, total antioxidant activity, and reducing power. Different studies were carried out by comparing *Kalanchoe pinnata* extract with antioxidant references such as gallic acid; ascorbic acid. All these antioxidant activities increased with increasing concentrations in a dose dependent manner. It was found to be significant and valuable.

Keywords: Antioxidant, *Kalanchoe pinnata*, total antioxidant capacity

1. Introduction

Kalanchoe pinnata is a perennial herb belonging to family Crassulaceae which grows as weeds in the tropical countries like India [1, 2]. It has two more Latin synonyms such as *Kalanchoe pinnata* Pers, *Bryophyllum calycinum* Salisb. It has different commons like miracle plant or air plant, panfuti (Hindi), life plant, love plant, air plant (Mexican), Good luck or resurrection plant, Zakhm-e-hayat, Canterbury bells, Cathedral bells, parnabija etc. [1, 3, 4]. It is astringent, sour in taste, sweet in the post digestive effect and has hot potency. The plant grows all over India in hot and moist areas, especially in Bengal.

Hydroalcoholic extract of *Kalanchoe pinnata* was optimized in ratio of methanol to water (1:1, v/v) which may be superior to its ethanolic extract to observe the exact antioxidant nature of the herb *Kalanchoe pinnata* [2, 5].

On revising the reviews and the studies of the herb *Kalanchoe pinnata* till the date, a majority of studies has been worked on its phytochemical profile of different active principles rich in alkaloids, triterpenes, glycosides, flavonoids, cardenolides, steroids, bufadienolides and lipids, pharmacognostic studies, pharmacological activities such as antileishmanial, hepatoprotective, nephroprotective, neuropharmacological, antimutagenic, anti-ulcer, antibacterial, antidiabetic, immunosuppressive, antihypertensive, analgesic, anti-inflammatory, wound healing, uterine contractility, insecticidal, fungitoxic, phytotoxic activities[1] and other *in-vitro* activities. However studies on *in-vitro* antioxidant activities in the different important antioxidant parameters have not been done in terms of total antioxidant capacity by using novel methods such as CUPRAC. Thus in the present study, an effort has been made to overview the total antioxidant capacity of the herb *Kalanchoe pinnata*.

2. Material and Methods

Chemicals: Folin & Ciocalteu's phenol reagent, Ammonium molybdate, Cupric chloride ($CuCl_2$), Neocuproine, Ammonium acetate, Potassium Ferricyanide, Trichloroacetic acid, Ferric chloride ($FeCl_3$) were purchased from Loba chemicals Ltd.

Collection of plant: Plant material was collected in September 2013 and authenticated from the Dept. of Botany, Yashwantrao Chavan College of Sciences, Karad on October 2013.

Extraction: Fresh leaves of *Kalanchoe pinnata* were chopped into small pieces by hand and put into a conical flask. Volume of methanol to water was in ratio of 80 ml: 80 ml was added to the conical flask and covered with a cotton plug on the mouth of conical flask. It was kept in maceration for 15 days at 4 °C in order to maximize the extraction. After 15 days it was filtered through Whatman filter paper and transferred to a suitable container and kept for analysis.

2.1 Folin-Ciocalteu reagent (FCR) assay

Phenolics content assay was performed in the method of Mello *et al*. [6].

Procedure

Hydroalcoholic extract of *Kalanchoe pinnata* in different concentration range from 100 µl to 500 µl were added to each test tube containing of 900 µl to 500 µl distilled water respectively; and 500 µl of Folin-Ciocalteu reagent solution. 500 µl of 100 mg/ml sodium carbonate was added after 5min. These tubes were kept aside for 2 hrs. Absorbance was measured at 765 nm. The concentrations of phenolic compounds in *Kalanchoe pinnata* extract were expressed as gallic acid equivalents (GAEs). All assays were conducted in triplicate and its mean was calculated.

2.2 Ferric ion reducing antioxidant power assay (FRAP)

Ferric ions reducing power was measured according to the method of Oyaizu with a slightest modification [7].

Procedure

Hydroalcoholic extract of *Kalanchoe pinnata* in different concentrations ranging from 100 µl to 500 µl were mixed with 2.5 ml of 20 mM phosphate buffer and 2.5 ml 1%, w/v potassium ferricyanide, and then the mixture was incubated at 50 °C for 30 min. Afterwards, 2.5 ml of 10%, w/v trichloroacetic acid and 0.5 ml 0.1%, w/v ferric chloride were added to the mixture, which was kept aside for 10 min. Finally, the absorbance was measured at 700 nm. Ascorbic acid was used as positive reference standard. All assays were run in triplicate way and averaged.

2.3 Cupric ion reducing capacity assay (CUPRAC)

Cupric ion reducing capacity was measured in accordance to the method of Apak [8].

Procedure

1 ml 10 mM cupric chloride, 1 ml 7.5 mM neocuproine and 1 ml 1 M ammonium acetate buffer of pH 7 solutions were added to test tubes containing 2 ml of distilled water. Hydroalcoholic extract of *Kalanchoe pinnata* in different concentration ranging from 100 µl to 500 µl were added to each test tube separately. These mixtures were incubated for half an hour at room temperature and measured against blank at 450 nm. Ascorbic acid was used as positive reference standard. All methods were repeated in triplicate in order to get mean value.

2.4 Phosphomolybdenum Assay (PM)

Total antioxidant activity was estimated by phosphomolybdenum assay [9].

Preparation of Molybdate Reagent Solution

1ml each of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate were added in 20 ml of distilled water and made up volume to 50 ml by adding distilled water.

Procedure

Hydroalcoholic extract of *Kalanchoe pinnata* in different concentration ranging from 100 µl to 500 µl were added to each test tube individually containing 3 ml of distilled water and 1 ml of Molybdate reagent solution. These tubes were kept incubated at 95 °C for 90 min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695 nm. Mean values from three independent samples were calculated for each extract. Ascorbic acid was used as positive reference standard.

3. Statistical analysis

Results were given as mean±standard deviation of 3 replicates. The results are expressed as mean values and standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test with α=0.05. This treatment was carried out using SPSS v.16.0 (Statistical Program for Social Sciences) software.

4. Results

The reducing capacity of antioxidant was coined in a single measure as "Total Antioxidant Capacity" (TAC) [10]. TAC of this hydroalcoholic extract was estimated based on its reducing capacity by different methods such as FRAP, CUPRAC, and PM assays. Absorbance reflects directly to the reducing power in the methods of FRAP, CUPRAC, PM.

4.1 FCR assay

As phenolics contribute to the reducing capacity so we have focused on the phenolics concentration determined by FCR method. FCR assay was used to quantify the reducing capacity of antioxidant. It was suggested to define as FCR method better rather than as total phenolics content of antioxidant [10].

Table 1: FCR Assay

	Absorbance at 765nm	
Volume(µl)	**KE**	**GA**
100	0.446±0.05	0.163±0.01
200	0.766±0.006	0.314±0.01
300	1.284±0.27	0.433±0.04
400	1.471±0.12	0.610±0.03
500	1.857±0.37	0.792±0.03

Values are Mean±SD (n=3); *Kalanchoe pinnata* Extract - KE; Gallic Acid – GA

4.2 FRAP assay

FRAP measures the reducing potency of extract and standard antioxidant. Higher absorbance indicates higher reducing potency.

Table 2: FRAP Assay

	Absorbance at 700nm	
Volume(µl)	**KE**	**AA**
100	0.411±0.06	0.316±0.01
200	0.430±0.06	0.357±0.02
300	0.436±0.07	0.401±0.003
400	0.517±0.13	0.436±0.02
500	0.684±0.16	0.450±0.01

Values are Mean±SD (n=3); *Kalanchoe pinnata* Extract - KE; Ascorbic Acid - AA

4.3 CUPRAC assay

CUPRAC assay is based on the utilization of the copper (II)-neocuproine [Cu (II)-Nc] reagent as the chromogenic oxidizing agent [8].

Table 3: CUPRAC Assay

	Absorbance at 450nm	
Volume(µl)	**KE**	**AA**
100	0.977±0.06	0.471±0.004
200	1.509±0.04	0.537±0.01
300	2.046±0.08	0.593±0.008
400	2.309±0.03	0.647±0.01
500	2.731±0.02	0.707±0.02

Values are Mean±SD (n=3); *Kalanchoe pinnata* Extract - KE; Ascorbic Acid - AA

4.4 PM assay

PM assay is based on the reduction of Phosphate-Mo (VI) to Phosphate Mo (V) by the sample and subsequent formation of a bluish green colored phosphate/Mo (V) complex at acid pH. The phosphomolybdenum method is routinely applied in the laboratory to evaluate the total antioxidant capacity of plant extracts [9].

Table 4: PM Assay

	Absorbance at 695nm	
Volume(µl)	**KE**	**AA**
100	0.362±0.02	0.143±0.01
200	0.753±0.05	0.333±0.04
300	1.124±0.03	0.529±0.02
400	1.451±0.03	0.871±0.02
500	1.764±0.07	1.135±0.01

Values are Mean±SD (n=3); *Kalanchoe pinnata* Extract - KE; Ascorbic Acid – AA

Figure 1 portrays the FCR along with different total antioxidant capacity methods which evaluates the increase in concentration of phenolics increases reducing capacity of antioxidant. CUPRAC assay being a novel method which shows superiority among assays. Studies in combination of different reducing power methods help to understand the exact nature of antioxidant.

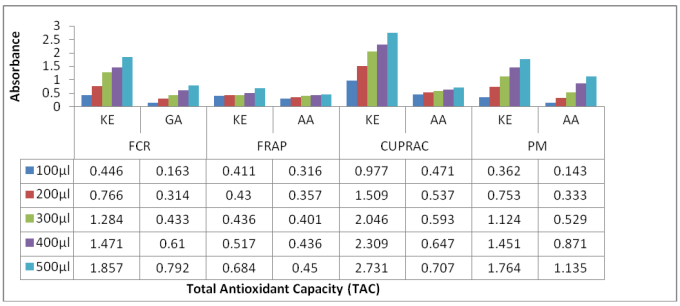

Fig 1: FCR and Total Antioxidant Capacity Assays *Kalanchoe pinnata* Extract – KE; Ascorbic Acid – AA, Gallic Acid – GA

5. Discussion

Antioxidant capacity assays may be broadly classified as single electron transfer (SET) and hydrogen atom transfer (HAT) based assays. Majorities of HAT assays are kinetics based and involve a competitive reaction scheme in which antioxidant and substrate compete for free radicals thermally generated through the decomposition of azo compounds. SET assays measure the capacity of an antioxidant in the reduction of an oxidant which changes colour when reduced. SET assays are easier than HAT assays. SET assays like PM, CUPRAC, FRAP were selected to analyze the reduction capacity. These methods are involving in the mechanism of single electron transfer system. In this system electron from oxidized antioxidant transferred to the substrate by inhibiting oxidation of oxidant.

FRAP, CUPRAC and PM methods are based on the redox antioxidant reaction. FRAP [8] and CUPRAC as a novel method [9] is to assess the reduced concentration of ferric and cupric ions respectively.

FRAP assay include the simultaneous use of ferricyanide and ferric ions as chromogenic oxidants supplied more favorable redox conditions for a greater variety of antioxidants.

1. FRAP assay gives an immediate result of a large range of individual antioxidants in dose-response manner.
2. Higher degree of color formation indicates the more reducing power of analyte.
3. Simple, reproducible analysis.

CUPRAC involves both of complexometric and redox reactions. It has specific features distinct from FRAP.

1. The redox reaction giving rise to a colored chelate of Cu (I)-Nc is relatively not affected by many parameters such as air, sunlight, solvent type, and pH.
2. The CUPRAC reagent is reasonably selective, stable, easily accessible, and sensitive comparing with the FRAP method.
3. The reaction is carried out at normal pH as opposed to acidic pH of FRAP.

PM assay measures the reduction degree of Mo (VI) to Mo (V).

1. PM assay is a quantitative method to investigate the reduction reaction rate among antioxidant, oxidant and molybdenum ligand. It involves in thermally generating auto-oxidation during prolonged incubation period at higher temperature.
2. It gives a direct estimation of reducing capacity of antioxidant.
3. It is distinctive from FRAP and CUPRAC assays as it remains intact irrespective of concentration of free metal ions.
4. Unlike CUPRAC and FRAP, it forms a green phosphomolybdenum complex without induction of free metal ions solution. So it shows uniqueness among *in-vitro* antioxidant assays.

Fenton reaction involves the creation of free radicals by transition-metal ions such as iron and copper that are present *in vivo* by donating or accepting free electrons via intracellular reactions. Although most intracellular iron is in ferrous (+2 ion) form, superoxide ions can convert it to the ferric (+3) form to take part in Fenton reaction. Metal-Catalyzed Oxidation (MCO) systems catalyze the reduction of intracellular iron which is in the form of Fe (III) to Fe (II) mostly through superoxide ions and of O_2 to H_2O_2. These products react at metal-binding sites on the protein to produce active oxygen species (viz; OH, ferryl ion) which alters the nature of proteins at the metal-binding site and cause DNA and protein damage [11,12].

6. Conclusion

On the basis of results obtained from different antioxidant capacity assays, the hydroalcoholic extract of *Kalanchoe pinnata* has shown a significant total antioxidant capacity. The reducing capacity and reduction capacity of free oxidative metallic ions such ferric and cupric ions by hydroalcoholic extract of *Kalanchoe pinnata* can be approximated through PM assay, through two methods namely FRAP and CUPRAC assays. The reducing capacity depends on phenolic contents which can be estimated with the help of FCR assay. Over viewing the reducing capacity, the use of *Kalanchoe pinnata* might contribute a certain level of health protection against oxidative damages. With the established antioxidant activity of this extract, the specific isolation of the active components in the hydroalcoholic extract of *Kalanchoe pinnata* and characterization should be further investigated.

7. References

1. Kamboj A, Saluja AK. *Bryophyllum pinnatum* (Lam.) Kurz.: Phytochemical and pharmacological profile. A review Phcog Rev 2009; 3:364-74.
2. Dassharma K, Bhatkar A, Pandey P, Shaikh N. *Bryophyllum pinnatum* (Lam): A potential source of antioxidants. Bionano Frontier 2013; 6:140-143.
3. Quazi MA, Tatiya AU, Khurshid M, Nazim S, Shaikh S. The miracle plant (*Kalanchoe pinnata*): A phytochemical and pharmacological review. IJRAP 2011; 2:1478-1482.
4. Prasad AK, Shankul K, Iyer SV, Sudani RJ, Vaidya SK. Pharmacognostical, Phytochemical and Pharmacological Review on *Bryophyllum pinnata*. IJPBA 2012; 3:423-433.
5. Bhatti M, Kamboj A, Saluja AK, Jain UK. *In vitro* evaluation and comparison of antioxidant activities of various extracts of leaves and stems of *Kalanchoe pinnatum*. Int J Green Pharm 2012; 6:340-347.
6. Andressa B, Gisely CL, João CPM. Application and Analysis of the Folin Ciocalteu Method for the Determination of the Total Phenolic Content from *Limonium Brasiliense* L. Molecules 2013; 18:6852-6865.
7. Oyaizu M. Studies on products of browning reaction: Antioxidant activities of products of browning reaction prepared from glucosamine. Jpn J Nutr 1986; 44:307-315.
8. Apak R, Güçlü K, Ozyürek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. J Agric Food Chem 2004; 52:7970-7981.
9. Prieto P, Pineda M, Aguilar M. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. Anal Biochem 1999; 269:337-341.
10. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem 2005; 53:1841-1856.
11. Robbins, Cotran. Pathologic Basis of Disease. Edn 7, Elsevier, 2008, 16.
12. Stadtman ER. Metal ion-catalyzed oxidation of proteins: Biochemical mechanism and biological consequences. Free Radical Biology and Medicine 1990; 9:315-325.