Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(1):197-202

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Metal ion chelating activity and hydrogen peroxide scavenging activity of medicinal plant *Kalanchoe pinnata*

S. Chandra Mohan[*1], V. Balamurugan[2], S. Thiripura Salini[1] and R. Rekha[1]

[1]*Department of Chemistry, PRIST University, Vallam, Thanjavur, Tamil Nadu, India*
[2]*Department of Chemistry, Saranathan College of Engineering, Thiruchirappalli, Tamil Nadu, India*

ABSTRACT

The phytochemical analysis of the medicinal plant Kalanchoe pinnata was carried out to evaluate the antioxidant potential. Alcoholic extracts of the leaves was subjected to `in vitro` *antioxidant activity screening models such as inhibition of metal ion chelating activity and hydrogen peroxide scavenging activity.EDTA is used as a standard for metal ion chelating activity and for hydrogen peroxide scavenging activity Ascorbic acid is used as a standard. In all the models studied , the extracts showed potent antioxidant activity, thereby augmenting it into the present day system of medicine.*

Key words: Phytochemicals, anti oxidant, *Kalanchoe pinnata*, scavenging activity.

INTRODUCTION

Oxygen is essential for the survival of all on this earth. During the process of oxygen utilization in normal physiological and metabolic processes approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals. Free radicals are types of Reactive Oxygen Species (ROS), which include all highly reactive, oxygen-containing molecules. Types of ROS include the hydroxyl radical, the super oxide anion radical,singlet oxygen, nitric oxide radical, hypochlorite radical and various lipid peroxides. All these are capable of reacting with membrane lipids, nuclecic acids, proteins and enzymes and other small molecules, resulting cellular damage. In living organisms various ROSs can be formed in different ways, including normal aerobic respiration, stimulates polymorphonuclear leckocytes and macrophages and peroxisomes. These appear to be main endogenous (originates within organism) sources of most of the oxidants produced by cells. Exogenous (coming from outside) sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents and pesticides. Free radicals may be defined as chemical species associated with an odd or unpaired electron. They are capable of attacking the healthy cells of the body, causing them to lose their structure and function, Cell damage caused by free radicals appears to be a major contribution to aging and degenerative diseases of aging such as caner, cardiovascular disease, cataracts, immune system decline, liver diseases, diabetes mellitus, inflammation, renal failure, brain dysfunction and stress among others. To protect the cells and organ system of the body against reactive oxygen species, human have evolved a highly sophisticated and complex antioxidant protection system, that functions interactively to neutralize free radicals. Thus, antioxidants are capable stabilizing or deactivating, free radicals before they attack cells[1]. Antioxidants are substances that are able to prevent or retard oxidation of lipids, proteins and DNA: and to protect the compounds or tissues from damage caused by oxygen or free radicals[2]. Naturally there is a

dynamic balance between the amount of free radicals produced the body and antioxidants to scavenge or quench them to protect the body against deleterious effects. The amount of antioxidant principles present under normal physiological conditions may be insufficient to neutralize free radicals generated. Therefore, it is obvious to enrich our diet with antioxidants to protect against harmful diseases. Hence there has been an increased interest in the food industry and in preventive medicine in the development of "Natural Antioxidants" from plant materials. Natural antioxidants occurs in all parts of plants[3]. These antioxidant include carotenoids, vitamins, phenols, flavanoids etc., play a significant role as physiological and dietary antioxidants, thereby augmenting the body's natural assistance to oxidative damage[4].

Kalanchoe pinnata (Lam) (syn. *Bryophyllum pinnatam*) is a succulent plant, commonly known as Master herb or a cure for all by a large community of Tribal and Herbal practioners[5] of various countries. There are many reports and studies an the biological activities of *Kalanchoe pinnata* like antiviral and antifungal, antiinsecticidal activity[6], antimicrobial activity[7], antidiabaetic activity[8], antiulcer activity[9], hepatoprotective activity[10], immumomodulatory effect[11], nephroprotective activity[12], wound healing activity[13]. *Bryophyllin* compounds have marked anticancer therapeutic values against cancer cells[14]. Leaf extract is taken in empty stomach is used in the treatment of urinary bladder stones[15]. Apart from these biological properties , the reports published on antioxidant properties of *Kalanchoe pinnata* were very few. Hence, in the present study is focused on *Kalanchoe pinnata* to determine its antioxidant and free radicals scavenging properties.

EXPERIMENTAL SECTION

Plant: ***K****alanchoe pinnata* collected from south part of India was taken for the study. The collected leaves were washed thoroughly with tap water followed with distilled water for the removal of dust and soil particles. The leaves were shade dried and used for extraction.

Figure 1: Photograph of medicinal plant *Kalanchoe pinnata*

Preparation of plant extract:

78g of *Kalanchoe pinnata* leaves were first defatted using petroleum ether and extracted with 700ml of 80% methanol using Soxhlet apparatus. The extraction was carried out for 8 hours and the extract was thereafter

concentrated by evaporation in a rota-vaccum, the work was carried out at the Department of Chemistry, PRIST University,Vallam, Thanjavur, Tamil Nadu.

Antioxidant Assays:
Determination of the antioxidant components:
Phenolics: Total phenolic compounds were determined according to Bray and Thorpe method [16] . To *Kalanchoe pinnata* extract, 20% Na_2CO_3 was added and shaken vigorously. After 2 minutes 0.2ml of 1:1 dilute Folin's reagent was added with constant shaking. The colour was allowed to develop for half an hour and absorbance at 750nm was taken and expressed as mg/g extract, by evaporation with calibration curve prepared from the reference solution and gallic acid.

Flavanoids: Aluminium chloride calorimetric method was used for flavanoids determination[17]. 0.5ml of different solvent extract of *Kalanchoe pinnata* was mixed with 95% methanol, 10% aluminium chloride, 1M potassium acetate distilled water. After incubation at room temperature for 30 minutes, the absorbance was measured at 415 nm along with standard quercetin and blank. The concentration obtained by comparing with the calibration curve prepared from a reference solution containing quercetin.

β –carotene & lycopene content: The dried extract was vigorously shaken with 10ml acetone-hexane mixture (4:6) for 1 minute and was filtered through Whatman No.4 filter paper. The absorbance was measured at 453nm, 505nm, 663nm. Contents of β-carotene and lycopene were calculated according to the following equations:

Lycopene (mg/100mg) = $-0.0458\ A_{663}+0.372A_{505}-0.0806\ A_{453}$

β-Carotene(mg/100mg) = $0.216\ A_{663}-0.304\ A_{505}+0.452A_{453}$

RESULTS AND DISCUSSION

Table 1 indicates the results of quantitative estimation of Phenolics, Flavanoids,Lycopenes, and Carotenoids

Table 1 : Results of quantitative estimation of Total phenolics, Flavanoids, Lycopenes and β – Carotenoids

Phytochemical	Result
Total Phenolics	58.95 gallic acid equivalents mg/g extract
Flavanoids	99.35 quercetin equivalents mg/g extract
Lycopenes	0.17 mg/100mg extract
β –Carotenes	0.89 mg/100mg extract

Antioxidant assays
Metal ion chelating activity:
The ferrous ion chelating potential of *kalanchoe pinnata* was measured according to the method of Yamaguchi et.al.[18] 1ml $FeSO_4$ solution was mixed with extract of different concentration. 1ml Tris HCl buffer (pH 7.4) and 2,2'-bipyridyl solution was added together with hydroxyl amine – HCl and ethanol respectively. The reaction mixture was adjusted to a final volume of 5ml with distilled water, shaken well and incubated for 10 minutes at room temperature. Absorbance was determined at 522nm and percent chelation was calculated using the following equation

Metal ion chelating activity scavenging effect = $[(A_0-A_1/A_0)X100]$, where A_0 was the absorbance of the control reaction and A_1 the absorbance in the presence of the sample. The extract concentration providing 50% inhibition (EC_{50}) was calculated was obtained by interpolation from linear regression analysis.

Hydrogen peroxide scavenging activity
The hydrogen peroxide scavenging ability of the *kalanchoe pinnata* was determined according to the method described by Ruch et. al.[19] Extract was dissolved in phosphate buffer (0.1nM, pH 7.4) at various concentrations and mixed with 600µl of hydrogen peroxide solution. Ascorbic acid was used as the reference compound. The concentration of the hydrogen peroxide was measured by reading the absorbance values of the reaction mixtures at 230nm after 10minutes. Hydrogen peroxide was determined using molar absorptivity for hydrogen peroxide.

***Invitro* antioxidant Assays**

Metal ion chelating activity: Transition metal ions, especially iron can stimulate lipid per oxidation by Fenton reaction (H_2O_2 + Fe^{2+} ---> Fe^{3+} OH^- +OH^-)and can also accelerate lipid per oxidation by decomposing lipid hydro peroxides into peroxyl and alkoxyl radicals that can perpetuate the chain reaction. Metal ion chelating capacity is significant since it reduces the concentration of the transition metal that catalyzes lipid per oxidation. According to the results, the plant extract is not good as the standard EDTA ; but the decrease in concentration – dependent colour formation in the presence of the extract indicates that it has iron chelating activity. Figure 2 shows the comparison of *Kalanchoe pinnata* with EDTA for its metal ion chelating activity.

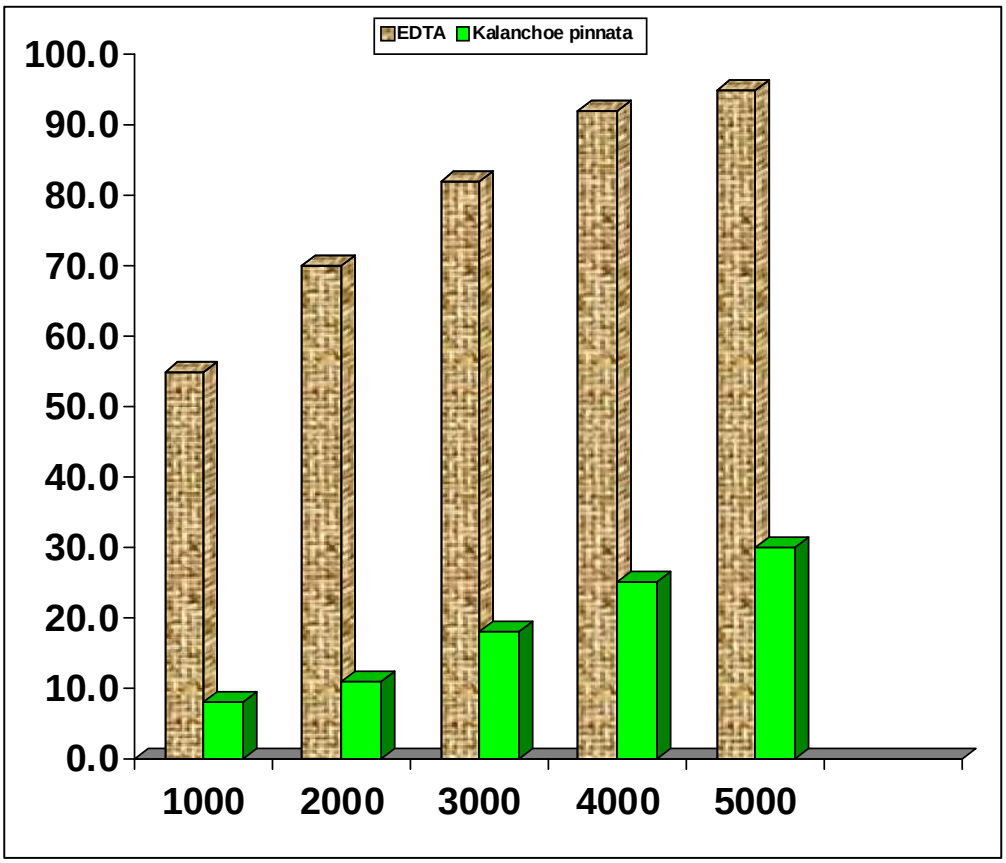

Figure 2: Assay of metal ion chelating activity of *Kalanchoe pinnata* and reference standard EDTA

Hydrogen peroxide scavenging activity:

Hydrogen peroxide is weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly; once inside the cell, it can probably react with and Fe2+possibly Cu2+ ions to form hydroxyl radicals and this may be the origin of many of its oxide effects. From the results, it appeared that H2O2 scavenging activity of the plant extract is significant compared to that of the standard ascorbic acid . Figure 3 shows the hydrogen peroxide scavenging ctivity of kalanchoe pinnata in comparison with Ascorbic acid standard.

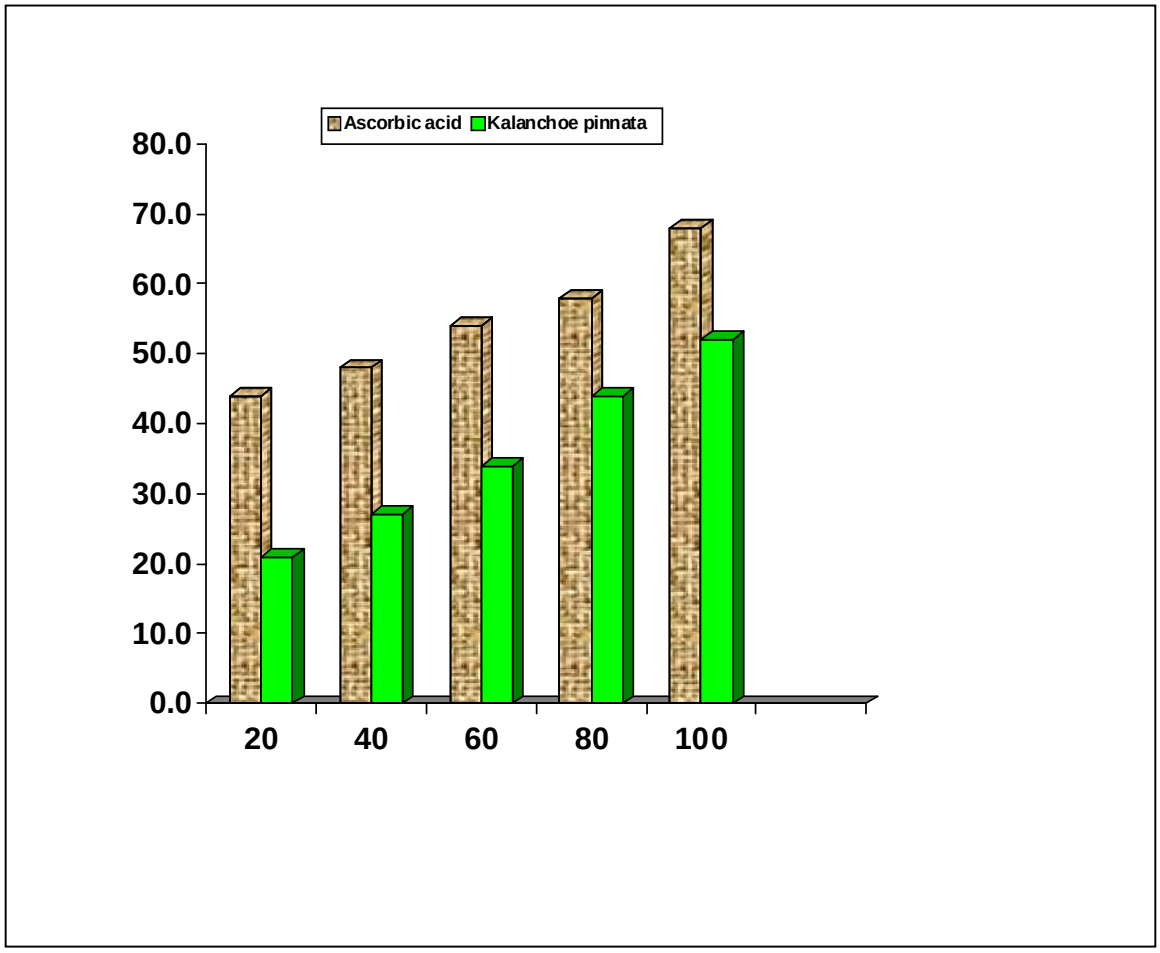

Figure 3: Hydroxyl radical scavenging activity of *Kalanchoe pinnata* and reference standard Ascorbic acid

Table 2 indicates IC_{50} values of *Kalanchoe pinnata* extract and reference standards for antioxidant activity

Tablele 2 : Comparison of values of IC_{50} of *Kalanchoe pinnata* reference standard used

Assay	IC_{50} of reference standard used	IC_{50} of Kalanchoe pinnata leaf extract
Metal ion chelation	50.70	909.91
Hydrogen peroxide cavenging	57.94	73.87

CONCLUSION

Alcoholic extract of *Kalanchoe pinnata* showed phytochemicals such as Total phenolics, Flavanoids, Lycophenes and β – Carotenes. The dried extract of *Kalanchoe pinnata* showed considerable inhibiting activity of hydrogen peroxide scavenging. Total antioxidant property of the extract was significant compared to controls. It would be appropriate to carryout HPLC analysis and chromatographic separation of active compounds in this medicinal plant and study their exact medicinal property.

REFERENCES

[1] H Beris, **1991**, *Drugs*,, 42, 569-605.
[2] Anu Kumar; PK Chauhan; VS Bharadwaj; Ramesh Kumar; Ankur Tyagi, *J.Chem.Pharm.Res*., **2011**, 3(4), 166-171.
[3] RK Nema; S Mandal; S Yadav, *J.Chem.Pharm.Res*., **2009**, 1(1), 102-104.
[4] R Kavitha; P Kamalakannan; T Deepa; R Elamathi; S Sridhar; J Suresh Kumar, *J.Chem.Pharm.Res*., **2011**, 3(6), 115-121.
[5] BS Nayak;JR Marshall; G Isitor. *Indian Journal of Experimental Biology*, **2010**, 48, 572-576.
[6] B Joseph; S Sridhar; Sankarganesh, Justin raj ; Biby T Edwin, *Research Journal of Microbiology*, **2011**, 6(4), 322-327.
[7] D A Akipelu, *Fitoterapia*, **2000**, 71(2), 193-194.
[8] O Ogbonnia Steve; I Odimegwu Joy; N Enwuru Veronica, *Aferican Journal of Biotechnology*, **2008**,7(5),2535-2539.
[9] C Andrea Parez; F Marilaine Correa ; R Silva Borges, *Arch. Vet. Science*, **1994**, 4(1), 111-112.
[10] NP Yadav; V K Dixit, *Journal of Ethanopharmacology*, **2003**, 86, 197-202.
[11] E A Cruz ; SAG Da-Silva; MF.Muzitano; PMR Silva; SS Costa; B Rossi Bergmann, International *immunopharmacology*, **2008**,8, 1616-1621.
[12] Gaurav Vijay Harlalka, Chandragauda Raosaheb Patil and Mahesh Ramu Patil, *Indian Journal of Pharmacology*, **2007**, 39(4), 201-205.
[13] K Mahmood; PK.Patil, *Indian Journal of Pharmacology*, **2002**, 34(2), 151.
[14] Unang supratman; Tomoyuki Fujita; Kohki Akiyama; Hideo Hayashi; Akira Murakami; Hirofumi Sakai et al., *Journal of Bioscience, Biotechnology and Biotechnology*, **2001**,65(4), 947-949.
[15] Prabal Sen,; Mihin Dollo; Manabendra Dutta Choudhury; Dhrupad Choudry, *Documentation and Traditional Herbal Knowledge of Khamptis of Arunachal Pradesh*, **2008**,7(3), 438-442.
[16] HG Bray; WV Thorpe, *Meth. Biochem. Anal.*, **1954**, 27-52.
[17] S Saboo; R Tapadiya; SS Khadabadi; UA Deokate, *J.Chem.Pharm.Res*., **2010,** 2(3), 417-423.
[18] K Yamaguchi; H Mori; M Nishimura, *Plant Cell Physiol*, **1995**, 36,1157–1162.
[19] RJ Ruch; SJ Cheng; JE Klaunig, *Carcinogenesis*, **1989**, 10, 1003.