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Free radical scavenging activities of different fractions of *Kalanchoe pinnata*

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Abstract: Aim and Objectives: In the present study, we have been successively extracted from methanolic extract of *Kalanchoe pinnata* into three successive different fractions like flavonoid rich fraction, saponin rich fraction and phenolics rich fraction to evaluate *in-vitro* models of antioxidant and anti-radical activities. Till date the correlation analysis between various parameters of *Kalanchoe pinnata* has been not reported. To our knowledge it may be first reported in the current research work.
Material and Methods: Different fractions of *Kalanchoe pinnata* were extracted successively and were evaluated for their total phenolic and flavonoid contents. Antioxidative abilities of these fractions individually were analyzed by Phosphomolybdenum Assay (PMA), Cupric ions Reducing Antioxidant Capacity (CUPRAC) and Ferric ions Reducing Ability Power (FRAP) methods. The free radical scavenging activities such as hydroxyl, DPPH, and anti-peroxidation like Thiobarbituric Acid Reactive Substance (TBARS), Metal Chelation Capacity (MCC) were assayed through *in-vitro* models.
Results: Antioxidant and antiradical effects of different fractions of *Kalanchoe pinnata* were ascertained through different *in-vitro* models. Results of individual tests demonstrated good correlation among themselves. In the correlation analysis, total flavonoids content has found to be negative correlation with all assays. Apart from total flavonoid contents, TBARS and DPPH assays have well correlated positively with other assays.
Conclusion: Methanolic extract of *Kalanchoe pinnata* and its successive fractions were performed at various concentrations using in vitro models of antioxidant assays studied. From these results, revealed *Kalanchoe pinnata* exhibited significant antioxidant activity and free radical-scavenging activity. However, further evaluation of their isolated bioactive compounds, antioxidant activities should be investigated.
Keywords: Antioxidant, Free radical, *Kalanchoe pinnata.*

Introduction

Oxidative stress is defined as an imbalance between reactive oxygen species (ROS) production and ability to readily detoxify the generated free radicals in the body[1]. It is involved in the mechanism of reactive oxygen species which lead to the formation of toxic compounds within organisms. Antioxidants are acting via in either inhibiting oxidative reaction or scavenging excess free radicals to reduce oxidative stress in the body[2]. *Kalanchoe pinnata, (*family Crassulaceae), is grown as a weed in tropical countries like India, Bangladesh. The leaves of *Kalanchoe pinnata* comprise complex chemical composition including triterpenoids, steroids, polyphenols; flavonoids [3] possess prominent ability to scavenge free radicals generated in the body. Saponins isolated from plant sources have a number of pharmacological effects like anti-inflammatory, antimicrobial, antispasmodic, antidiabetic, anthelmintic, and cytotoxic activities[4]. It is an astringent, sour in taste, sweet in the post digestive effect. It has different Latin synonyms such as *Bryophyllum Calycinum Salisb, Bryophyllum*

pinnatum Linn. There are diverse common folk names for this plant like miracle plant or air plant, panfuti (Hindi), life plant, love plant, air plant (Mexican), Good luck or resurrection plant, Zakham-e-hyat, Canterbury bells, Cathedral bells, popularly known as Katakataka for presence of astonishing taste.

In the present study, we have been successively extracted from methanolic extract of *Kalanchoe pinnata* into three successive different fractions like flavonoid rich fraction, saponin rich fraction and phenolics rich fraction to evaluate using *in-vitro* models of antioxidant and free radical scavenging activities. Till date the correlation analysis between various parameters using different fractions of *Kalanchoe pinnata* has been not reported. To our knowledge it may be first reported in the current research work.

Material and Methods

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

Chemicals and Reagents:

Ammonium persulphate, Thiobarbituric acid, Folin & Ciocateu's phenol reagent, Aluminum chloride, Gallic acid, Neocuproine, Cupric chloride, Ferric chloride, Nitro B.T., Ammonium molybdate, Trichloroacetic acid, Ferrous chloride, Ferrous Sulphate, Potassium phosphate, Sodium phosphate, Potassium ferricyanide, Sodium carbonate, Sodium nitroprusside, Sodium acetate, Sodium salicylate, Butan-1-ol were purchased from Loba chemicals. Griess reagent, Diphenyl-1-picrylhydrazyl (DPPH), Potassium persulfate was purchased from Sigma Aldrich. Riboflavin was given as a gift sample from Nes Ltd, Mumbai. Ferrozine SP was purchased from Hi-Media.

Preparation of different extracts

Methanolic extraction

250gm of chopped fresh leaves of *Kalanchoe pinnata* was percolated in 875ml methanol by method of maceration. It was filtrated and remaining organic solvent present in the residue was dried out. Methanolic extract was concentrated to 100ml volume and was labeled as methanolic fraction of *Kalanchoe pinnata* (MEKP).

Isolation of flavonoid-rich fraction from methanolic extract of *Kalanchoe pinnata*

Concentrated methanolic extract was subjected to n-butanol partition in a separating funnel. After shaking, methanolic fraction was separated as flavonoid rich fraction. It was evaporated till a thick slurry mass is formed. 10ml of methanol was added to thick slurry mass of dried flavonoid and kept in a suitable container and labeled as flavonoid-rich fraction of *Kalanchoe pinnata* (FRKP).

Isolation of saponin-rich fraction from methanolic extract of *Kalanchoe pinnata*

Separated n-butanol was subjected to addition of diethyl acetate for maximizing extraction yield of saponin. Sufficient amount of distilled water was added to ensure removal of water soluble constituents from the ethyl acetate-n-butanol mixture fraction. Aqueous fraction was poured out; the ethyl acetate-n-butanol mixture fraction was placed in a porcelain dish for evaporation till thick slurry mass was formed. Thick slurry mass was dissolved in 10ml of methanol and subjected to 40ml of diethyl ether in separating funnel. Crude saponin was precipitated in ether fraction. 10ml of methanol was added to thick slurry mass of crude saponin and placed in a suitable container and labeled as saponin-rich fraction of *Kalanchoe pinnata* (SRKP).

Isolation of phenolics-rich fraction from aqueous extract of *Kalanchoe pinnata* dried leaves

Dried leaves of *Kalanchoe pinnata* was resuspended into a beaker containing 500ml distilled water and boiled for half hr at 80°C for interfering protein and enzymes. Hot aqueous extract was filtrated through filter paper and filtrate was cooled to room temperature. Filtrate fraction was poured slowly to another beaker consisting 50ml of acetone with vigorously stirring. White coloured phenolics were precipitated on stirring. A small amount of phenolics was dissolved in 10ml of distilled water for analysis and was labeled as phenolics-rich fraction of *Kalanchoe pinnata* (PLKP)

Phytochemical estimation assays and *in-vitro* antioxidant methods:

All dried extracts of MEKP, FRKP, SRKP and PRKP thus obtained were dissolved in the particular solvents at the concentration of 1 mg/1 ml and used for assessment of antioxidant and free radical scavenging activities through various chemical assays.

Phenolics Content Estimation

Folin-Ciocalteu method was used to determine the total phenolics content of MEKP, FRKP, SRKP and PRKP extracts[5]. MEKP, FRKP, SRKP and PRKP extracts of *Kalanchoe pinnata* were added to each test tube containing of 3ml distilled water and 500µl Folin-Ciocalteu reagent solution. 500µl of 100mg/ml sodium carbonate was added after 5min. These tubes were kept aside for 2hrs. Absorbance was measured at 765nm. 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.

Flavonoids Content Estimation

Aluminum chloride colorimetric method was used for flavonoids determination with slight modification in MEKP, FRKP, SRKP and PRKP extracts[6]. MEKP, FRKP, SRKP and PRKP extracts of *Kalanchoe pinnata* were added to each test tube containing of 3ml distilled water and 100µl of 20% aluminum chloride in ethanol. After 5 minutes, 100µl of 5% sodium acetate and 800µl of distilled water. These tubes were kept aside for 30min. Absorbance was measured at 415nm. The concentrations of phenolic compounds in *Kalanchoe pinnata* extract were expressed as rutin equivalents (RTs). All assays were conducted in triplicate and its mean was calculated.

Phosphomolybdenum assay

Total antioxidant activity was estimated by phosphomolybdenum assay in MEKP, FRKP, SRKP and PRKP extracts as described by method[7]. 1ml each of 0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate were added in 20ml of distilled water and made up volume to 50ml by adding distilled water. MEKP, FRKP, SRKP, PRKP extracts of *Kalanchoe pinnata* and ascorbic acid were mixed with 1ml of Molybdate reagent solution and incubated at 95°C for 90min. After cooling the absorbance of the reaction mixture was measured against at 695nm.

Cupric ion reducing antioxidant capacity assay

Cupric ion reducing capacity was measured in MEKP, FRKP, SRKP and PRKP extracts in accordance to the method of CUPRAC[8]. 1ml 10mM cupric chloride ($CuCl_2$), 1ml 7.5mM neocuprione and 1ml 1M ammonium acetate buffer (pH 7) solutions were added to test tubes. MEKP, FRKP, SRKP and PRKP extracts and ascorbic acid were mixed with reaction mixture independently. These reaction mixtures were incubated for half hour at room temperature and measured against blank at 450nm.

Ferric reducing ability power

Ferric ions reducing power was measured according to the method of FRAP in MEKP, FRKP, SRKP and PRKP extracts [9] with a slight modification. MEKP, FRKP, SRKP and PRKP extracts and ascorbic acid were mixed with 1ml of 20mM phosphate buffer and 1ml potassium ferricyanide (1%, w/v) and incubated at 50°C for 30 min. 1ml of TCA (10%, w/v) and 0.5ml ferric chloride (0.1%, w/v) were added to the reaction mixture and absorbance was measured at 700nm.

Hydroxyl free radical scavenging activity

The scavenging ability of the extracts on hydroxyl radicals was determined in MEKP, FRKP, SRKP and PRKP extracts according to the method[10]. 0.041gm of $FeSO_4$ and 0.32gm of sodium salicylate was mixed to 100ml of distilled water. 4µl of $H_2O_2$was dropped to it, vortexed for uniform mixing and labeled as "Smirnoff Reagent". MEKP, FRKP, SRKP and PRKP extracts and ascorbic acid were mixed with 1ml of Smirnoff reagent and incubated about 30min at 37°C. Absorbance of the reaction mixtures was read at 562nm. The scavenging ability on hydroxyl radicals was calculated by use of given equation. The percentage of scavenged OH^- of extract was calculated using the following formula: Scavenged OH^- % = $[(A_c-A_e)/A_c \times 100]$ where, A_c = absorbance of control and A_e = absorbance of extract.

DPPH free radical scavenging activity

The capacity of extracts to scavenge the stable DPPH [2, 2'-diphenyl-2-picrylhydrazyl] free radical was measured in MEKP, FRKP, SRKP and PRKP extracts by the method[11]. MEKP, FRKP, SRKP and PRKP extracts and ascorbic acid were mixed with 1ml of 0.1mM DPPH and kept incubated in dark room at normal temperature for 30min. After incubation, optical density of these incubated tubes was measured at 517nm. Control was prepared by mixing 10µl of ethanol in place of extract with 3ml of ethanol and 1ml of 0.1mM DPPH and absorbance was determined immediately. The percentage of scavenged DPPH of extract was calculated using the following formula: Scavenged DPPH % = $[(A_c-A_e)/A_c \times 100]$ where, A_c = absorbance of control and A_e = absorbance of extract.

Thiobarbituric acid reactive substance assay

Lipid peroxidation assay was performed according to modified protocol of TBARS in MEKP, FRKP, SRKP and PRKP extracts[12] to measure the lipid peroxide formed using egg yolk homogenates as lipid-rich media. In the assay of lipid peroxidation, malondialdehyde (MDA) was detected by presence of pink color.

Egg Yolk Homogenate Preparation:

In accordance of Vasudewa *et al*[13] egg yolk was separated from the albumen and the yolk membrane was removed. 10ml of egg yolk solution was added in 1.15 gm of KCl or NaCl in 100ml of distilled water. The solution was homogenized for 30 seconds and ultrasonicated for 5 min. In (A+TBA) set, each test tube containing 1ml of MEKP, FRKP, SRKP and PRKP extracts and ascorbic acid, 100µl of diluted egg homogenate was transferred. To induce lipid peroxidation, 50µl of 0.07M $FeSO_4$ was added. These mixture tubes were kept for 30min for incubation. To stop lipid peroxidation, 50µl of 1.2M trichloroacetic acid (TCA) was added and following 0.8% thiobarbituric acid (TBA) and 3.5M acetic acid in amount of 0.5ml each were added to it and vortexed well. These resultant tubes were placed in the incubator at 95°C for 60min. To eliminate this non-MDA interference, another (B–TBA) set of extracts was treated in the same way as above mentioned set (A+TBA) by excluding TBA. The absorbance of (B–TBA) was subtracted to the absorbance of (A+TBA) for yielding the absorbance for extract (E). After cooling it, 5ml of butan-1-ol was added to each tube and vortexed for 5min. The absorbance of upper organic layer was measured at 532nm.

Percentage of lipid peroxidation inhibition was calculated by following formula. Antioxidant index (AI) was calculated using the following equation: $AI = (1-E/C) \times 100$ where, E = absorbance of extract [E= (A + TBA) – (B – TBA)], C = absorbance of fully oxidized control. All values are based on the anti-oxidant index whereby the control is completely peroxidized and each extract providing a degree of improvement, indicated as % protection.

Metal ion chelating capacity assay

The chelating ability of the extracts on ferrous ions was determined in MEKP, FRKP, SRKP and PRKP extracts according to the method of MICC[14]. MEKP, FRKP, SRKP and PRKP extracts and ascorbic acid were mixed with 50µl of solution of 2mM $FeCl_2.4H_2O$ and incubated about 30min at 37°C. The reaction was initiated by the addition of 200µl of 5mM ferrozine. The mixture was shaken vigorously and left standing at room temperature for 10min. The chelating activity measured by measuring the disappearance of purple color in absorbance of solution at 562 nm. Different concentration range of EDTA as standard was prepared in correspondence to the sample. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the following formula: Chelating % = $[(A_c-A_e)/A_c \times 100]$ where, A_c = absorbance of control and A_e = absorbance of extract.

Statistical Analysis:

All experiments were performed in triplicates and the results were expressed as mean ± standard deviation. Data was analyzed using student't' test for two sets while one-way analysis of variance (ANOVA) for more than two sets. Significant differences were considered when means of compared sets differed at $P < 0.05$. Data was carried out using SPSS v.16.0 (Statistical Program for Social Sciences) software.

Correlation Analysis:

Pearson's correlation method was used to analyze correlation between the results of different assays against phenolics along with flavonoids. All results of assays were correlated in the same method.

Results and Discussion

Phenolics Content Estimation

Different concentration range of gallic acid was assayed in the Folin Ciocalteu Reagent (FCR) assay for estimating phenolics content as shown in Table 1. The gallic acid standard line has equation, y=0.155x-0.003, (R^2=0.994). TPC was calculated from the following equation: TPC=C x V/M where, T=Total Phenolic Content (mg/g) of extract as GAE, C=Concentration of GA established from the calibration curve in (mg/ml), V=Volume of the extract solution in ml {0.1-0.5ml} & M= weight of extract in g {0.2-1.0g}. It was used x=(y + 0.003)/0.155 as a reversed formula.

Flavonoids Content Estimation

Different concentration range of rutin was assayed in the aluminum chloride method for estimating total flavonoid contents as shown in Table 1. The rutin standard line has equation, y=0.014x, (R^2=0.982). Total Flavonoid Content was calculated from the following equation: TFC=C x V/M where, T= Total Flavonoid Content (mg/g) of extract as RT, C= Concentration of RT established from the calibration curve in (mg/ml), V= Volume of the extract solution in ml {0.1-0.5ml} & M= weight of extract in g {0.2-1.0g}. It was used x=y/0.014 as a reversed formula.

Table 1 depicts the extraction yield, total phenolics content and total flavonoids content of different extracts of *Kalanchoe pinnata*

Extracts	Extraction Yield(EY)	Total Phenolics Content (TPC)	Total Flavonoids Content (TFC)
MEKP	1.0%	1.164 ± 0.56	0.102 ± 0.04
FRKP	0.24%	0.407 ± 0.59	0.565 ± 0.71
SRKP	0.36%	0.502 ± 0.76	0.880 ± 0.97
PRKP	0.16%	0.375 ± 0.59	0.529 ± 0.67

Values were taken in Mean ± S. D. MEKP- Methanolic extract of Kalanchoe pinnata, FRKP- Flavonoid rich fraction of Kalanchoe pinnata, SRKP- Saponin rich fraction of Kalanchoe pinnata, PRKP- Phenolic rich fraction of Kalanchoe pinnata

***In-vitro* antioxidant and free scavenging assays of extracts:**

Phosphomolybdenum assay

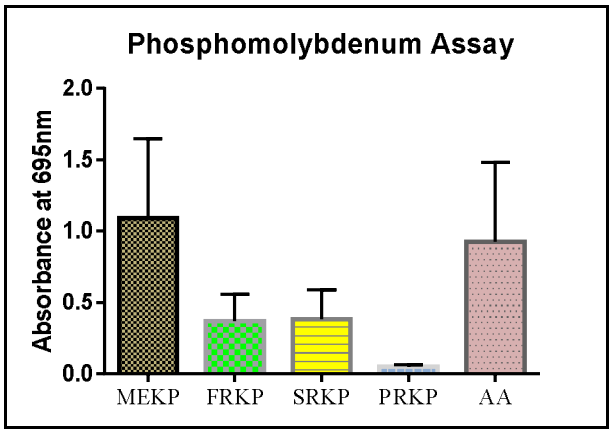

Fig.1: Phosphomolybdenum assay of *Kalanchoe pinnata* leaves. Values are mean of three replicate determinations (n = 3) ± standard deviation. MEKP – methanolic extract of *Kalanchoe pinnata* leaves; FRKP – flavonoid rich fraction of *Kalanchoe pinnata* leaves; SRKP – saponin rich fraction of *Kalanchoe pinnata* leaves; PRKP – phenolic rich fraction of *Kalanchoe pinnata* leaves; AA – Ascorbic acid.

Phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and subsequent formation of a bluish green colored phosphate/Mo (V) complex with absorbance at 695 nm[7]. It provides reduction capacity quantitatively through the reduction reaction rate among antioxidant,

oxidant and molybdenum ligand by thermally generating auto-oxidation during prolonged incubation period at higher temperature. The order of phosphomolybdenum activity was found to as follows: MEKP > AA > SRKP > FRKP > PRKP as shown in Fig. 1.

Cupric ion reducing antioxidant capacity assay

CUPRAC assay is based on the complexometric and redox reaction between copper (II)-neocuproine [Cu (II)-Nc] reagent as the chromogenic oxidizing agent[8]. CUPRAC evaluation of different extracts was found in the order of: MEKP > AA > FRKP > SRKP > PRKP as shown in Fig. 2.

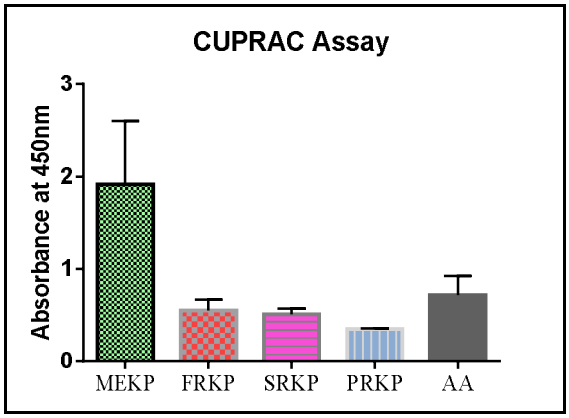

Fig.2: CUPRAC assay of *Kalanchoe pinnata* leaves. Values are mean of three replicate determinations (n = 3) ± standard deviation. MEKP – methanolic extract of *Kalanchoe pinnata* leaves; FRKP – flavonoid rich fraction of *Kalanchoe pinnata* leaves; SRKP – saponin rich fraction of *Kalanchoe pinnata* leaves; PRKP – phenolic rich fraction of *Kalanchoe pinnata* leaves; AA – Ascorbic acid.

Ferric reducing ability power

FRAP assay includes the simultaneous use of ferricyanide and ferric ions as chromogenic oxidants[9]. High absorbance indicates the more reducing power of different extracts. Ferric reducing ability power descended in the order of: PRKP > AA > MEKP > SRKP > FRKP as shown in Fig. 3.

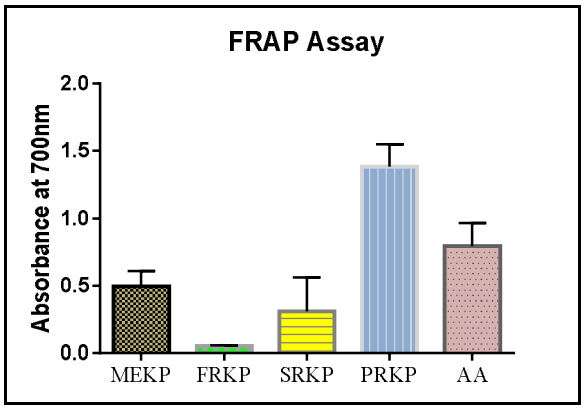

Fig. 3: FRAP activity of *Kalanchoe pinnata* leaves. Values are mean of three replicate determinations (n = 3) ± standard deviation. MEKP – methanolic extract of *Kalanchoe pinnata* leaves; FRKP – flavonoid rich fraction of *Kalanchoe pinnata* leaves; SRKP – saponin rich fraction of *Kalanchoe pinnata* leaves; PRKP – phenolic rich fraction of *Kalanchoe pinnata* leaves; AA – Ascorbic acid.

Hydroxyl free radical scavenging activity

Hydroxyl radical is the most reactive among the oxygen radicals inducing severe damage to proteins, DNA and lipids by crossing cell membranes and leads to lipid peroxidation[15]. Hydroxyl free radical scavenging activity of different extracts was found in the order: PRKP > AA > MEKP > SRKP > FRKP as shown in Fig. 4.

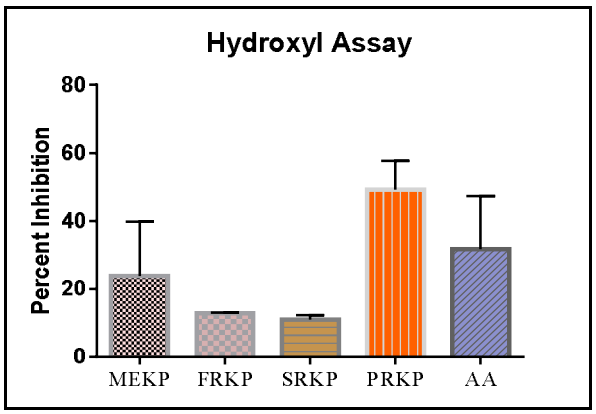

Fig. 4: Hydroxyl free radical scavenging activity of *Kalanchoe pinnata* leaves. Values are mean of three replicate determinations (n = 3) ± standard deviation. MEKP – methanolic extract of *Kalanchoe pinnata* leaves; FRKP – flavonoid rich fraction of *Kalanchoe pinnata* leaves; SRKP – saponin rich fraction of *Kalanchoe pinnata* leaves; PRKP – phenolic rich fraction of *Kalanchoe pinnata* leaves; AA – Ascorbic acid.

DPPH free radical scavenging activity

DPPH is a stable free radical which has been excessively used for assessment of scavenging activity of natural products. The ability of the investigated extracts to act as donors of hydrogen atoms or electrons in transformation of DPPH* radicals into its reduced form DPPH-H was measured[12]. DPPH free scavenging activity of different extracts was found to follow order of: MEKP > SRKP, PRKP > FRKP > AA as shown in Fig. 5.

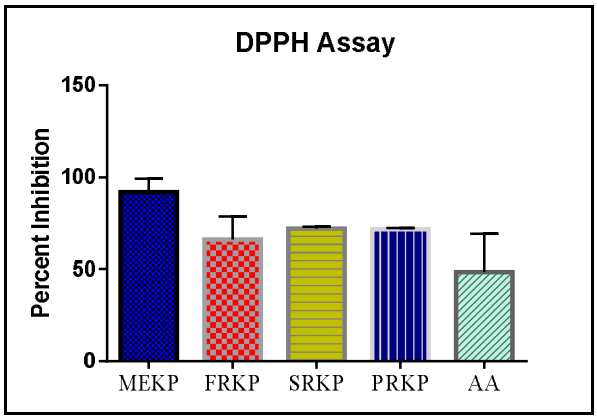

Fig. 5: DPPH• radical scavenging activity of *Kalanchoe pinnata* leaves. Values are mean of three replicate determinations (n = 3) ± standard deviation. MEKP – methanolic extract of *Kalanchoe pinnata* leaves; FRKP – flavonoid rich fraction of *Kalanchoe pinnata* leaves; SRKP – saponin rich fraction of *Kalanchoe pinnata* leaves; PRKP – phenolic rich fraction of *Kalanchoe pinnata* leaves; AA – Ascorbic acid.

Thiobarbituric acid reactive substance assay

Egg yolk lipids undergo rapid non-enzymatic peroxidation when hatched in the presence of ferrous sulfate. Malondialdehyde (MDA) is the end product in the egg-lipid peroxidation process. During oxidative degeneration by free oxygen free radicals which give pink colour as indicator in the presence of thiobarbituric acid[13]. Lipid peroxides inhibited by different extracts were arranged in the order: MEKP > AA > FRKP > PRKP > SRKP as shown in Fig. 6.

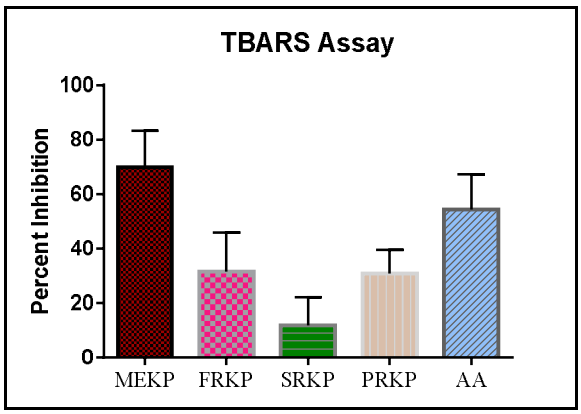

Fig. 6: TBARS assay of *Kalanchoe pinnata* leaves. Values are mean of three replicate determinations (n = 3) ± standard deviation. MEKP – methanolic extract of *Kalanchoe pinnata* leaves; FRKP – flavonoid rich fraction of *Kalanchoe pinnata* leaves; SRKP – saponin rich fraction of *Kalanchoe pinnata* leaves; PRKP – phenolic rich fraction of *Kalanchoe pinnata* leaves; AA – Ascorbic acid.

Metal ion chelating capacity assay

Metal ions catalyze the rate of free radicals formation. Ferrozine chelates with Fe^{+2}. In the presence of chelating properties of spices, the complex formation is disrupted, leading to a decrease in the red color of ferrous ion and ferrozine complex[14]. Ability of selected spices extracts and SME to chelate the free metal ions was found to be in the order of: EDTA > PRKP > SRKP > FRKP > MEKP as shown in Fig. 7.

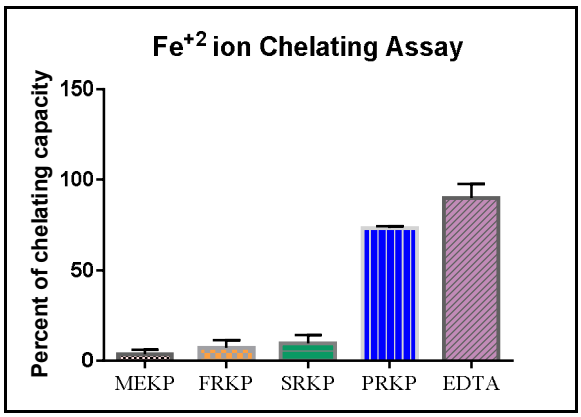

Fig. 7: Metal ion chelating capacity assay of *Kalanchoe pinnata* leaves. Values are mean of three replicate determinations (n = 3) ± standard deviation. MEKP – methanolic extract of *Kalanchoe pinnata* leaves; FRKP – flavonoid rich fraction of *Kalanchoe pinnata* leaves; SRKP – saponin rich fraction of *Kalanchoe pinnata* leaves; PRKP – phenolic rich fraction of *Kalanchoe pinnata* leaves; EDTA – Ascorbic acid.

Correlation analysis of all different assays

Pearson Correlation among the results of overall assays is specified in following Table 2.

Table 2 depicts the correlation analysis of all different assays

R2	TPC	TFC	PMA	CUPRAC	FRAP	OH	DPPH	TBARS	MCC
TPC	1								
TFC	-0.790	1							
PMA	0.963	-0.712	1						
CUPRAC	0.992	-0.838	0.971	1					
FRAP	-0.154	-0.140	-0.410	-0.194	1				
OH	-0.121	-0.287	-0.363	-0.133	0.973	1			
DPPH	0.973	-0.786	0.877	0.948	0.064	0.075	1		

TBARS	0.863	-0.99	0.804	0.9053	0.039	0.171	0.844	1	
MCC	-0.480	0.092	-0.689	-0.501	0.935	0.922	-0.285	-0.215	1

TPC- Total Phenolics Content, TFC-Total Flavonoids Content, PMA-Phosphomolybdenum Assay, CUPRAC- Cupric Ion Reducing Antioxidant Capacity, FRAP- Ferric Reducing Ability Power, OH-Hydroxyl Radical, TBARS-Thiobarbituric acid reactive substance assay, MCC-Metal ion chelating capacity

Correlation analysis between different assays

Antioxidant and free radical scavenging activities reflect upon the content of phenolics and flavonoids present in the natural products. Therefore it is significant to correlate their assays with antioxidant and antiradical assays for better understanding the role of each assay and combinations of the followed results.

In the correlation analysis, total flavonoids content has found to be negative correlation with all assays. Apart from total flavonoid contents, TBARS and DPPH assays have well correlated positively with other assays.

Correlation between TBARS and TPC indicate that non-phenolics may contribute to the lipid peroxidation reaction [16]. TBARS has no statistically significant relationship with flavonoids [17]. Correlation analysis helps to provide better understanding the actual relation between different activities and phenolics and flavonoids. Also it is useful to realize the comprehensive evaluation of different extracts in various parameters in which we have observed in our previous study[18, 19].

Not only depending on the phenolics and flavonoids contents for analysis of antioxidant or free radical scavenging activities, but also it should be studied bioactive components other than phenolics and flavonoids which could be accountable for antioxidant potentials.

Experimental difference in correlation analysis among different antioxidant and antiradical methods have indicated that only a single assay cannot taken as a whole to evaluate either total antioxidant activity or free radical scavenging activity [20]. Cross checking among different assays related with antioxidant and antiradical activities is more preferable rather than depending only two or three assays.

Conclusion:

Methanolic extract of *Kalanchoe pinnata* and its successive fractions were performed at various concentrations using in *vitro models* of antioxidant assays studied. From these results, revealed *Kalanchoe pinnata* exhibited significant antioxidant activity and free radical-scavenging activity. The major antioxidative components appeared to be to be phenolics and flavonoids. Out of all assays, *Kalanchoe pinnata* has proved hydroxyl radical scavenging as par excellence and used as a potential natural source of antioxidants for defense system in the body. However, further evaluation of their isolated bioactive compounds, antioxidant activities should be investigated.

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Conflict of Interest:

Authors declared that they have no interest in conflict.

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