ORIGINAL ARTICLE

In vitro evaluation and comparison of antioxidant activities of various extracts of leaves and stems of *Kalanchoe pinnatum*

Manisha Bhatti, Anjoo Kamboj, Ajay Kumar Saluja[1], Upendra K. Jain
Department of Pharmaceutical Chemistry, Chandigarh College of Pharmacy, Landran, Mohali (Punjab), [1]Pharmacognosy, A. R. College of Pharmacy, V. V Nagar, Gujarat, India

Aim: Many diseases are associated with oxidative stress caused by free radicals. The present research was carried out to evaluate the total phenolic contents, flavonoid contents and *in vitro* evaluation of antioxidant potentials by five different assay method of various (benzene, chloroform, acetone, and ethanol) extracts of leaves and stems of *Kalanchoe pinnatum* as the plant is an ingredient of various traditional preparations used in the treatment of various diseases. **Materials and Methods:** Antioxidant and free radical scavenging activity was determined by using five different *in vitro* antioxidant assays including 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH), hydrogen peroxide, nitric oxide, ferric reducing antioxidant power and phosphomolybdenum assays. In the present investigation, quantitative estimation of flavonoid content and phenolic content was carried out by colorimetric methods using aluminium chloride and Folin-Ciocalteu reagent methods respectively to establish relationship between antioxidant activity and total phenolic and flavonoid contents. **Results and Conclusions:** The plant powder (100 mg) yielded 0.49, 0.64, 0.99, 1.17 %w/w total phenolic content in leaves and 0.18, 0.27, 0.48, 0.62 %w/w total phenolic content in the benzene, chloroform, acetone, ethanol extracts of stems respectively using gallic acid as standard and plant contains about 0.24, 0.37, 0.56, 0.75 %w/w of total flavonoids content in leaves and 0.15, 0.22, 0.42, 0.54 %w/w of total flavonoids content in the benzene, chloroform, acetone, ethanol extracts of stems respectively using quercetin as standard. The extracts showed significant antioxidant activity in dose dependent manner in all the assays. The result obtained in the present study indicated that leaves and stems of *K. pinnatum* could be a potential source of natural antioxidant and justified the traditional use of herb in preventing disease induced by oxidative stress.

Key words: DPPH scavenging activity, *Kalanchoe pinnatum*, no scavenging activity, phytochemical screening

INTRODUCTION

Oxygen radicals, the products of some biochemical and physiological reactions, initiate cell signaling pathways, damage cellular lipids, proteins, and nucleic acids. Reactive oxygen species are pivotal for the onset of various conditions such as hypertension, atherosclerosis, cancer, and alzheimer's disease. During normal aerobic metabolism, activated oxygen popularly known as superoxide is formed in a stepwise reduction of oxygen to water. When these activated oxygen molecules diffuse into cells, they become reactive to damage these cells which continue till antioxidants are introduced to scavenge all the free radicals available. Williams reported that the body handles this cell damage process by producing a number of antioxidant enzymes which can be found both in the human body and in plants. Thus, antioxidants protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite.[1]

Kalanchoe pinnatum (*Bryophyllum pinnatum*; Lamarck Crassulaceae) is an erect, succulent, perennial shrub that grows about 1.5 m tall and reproduces through seeds and also vegetatively from leaf bulbils.[2] It has a tall hollow stems, freshly dark green leaves that are distinctively scalloped and trimmed in red and dark bell-like pendulous flowers. *K. pinnatum* can easily be propagated through stems or leaf cutting. It is an introduced ornamental plant that is now growing as a weed around plantation crop.[2,3] *K. pinnatum* is used in ethno medicine for the treatment of earache, tooth ache, eye infections, burns, wounds, abscesses, ulcers, insect bites, vomiting, diarrhea, dysentry and lithiasis.[3] In Southeastern Nigeria, this herb is used to facilitate the dropping of the placenta of new born baby.[3] The lightly roasted leaves are used externally for skin fungus and inflammations. The leaf infusions are an internal remedy for fever.[4]

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.108255

Address for correspondence: Dr. Anjoo Kamboj, Chandigarh College of Pharmacy, Landran, Mohali (Punjab), India.
E-mail: anjookamboj@gmail.com
Received: 11-07-2012; **Accepted:** 26-09-2012

In ethno medicine, *K. pinnatum* is used to cure acute, as well as chronic bronchitis, pneumonia and other forms of respiratory tract infections. It is also employed for the treatment of kidney stones, gastric ulcers and edema of the leg. Other works have also shown that this plant possesses analgesic, anticonvulsant, anti-inflammatory, anti-arthritic and anti-spasmolytic properties.[4,5]

K. pinnatum leaves contain alkaloids, saponins, flavonoids, terpenoids and phenolic compounds.[2,6,7] Plant also contains syringic acid, caffeic acid, 4-hydroxy-3-methoxy-cinnamic acid, 4-hydroxybenzoic acid, astragalin, 3, 8-dimethoxy-4,5, 7-trihydroxyflavone, friedelin, epigallocatechin-3-o-syringate, luteolin, rutin, kaempferol, quercetin, quercetin-3L-rhamonsido-L-arabino furanoside.[8,9]

As the plant extracts are quite safe and their toxicity is not a problem of concern unlike those of synthetic antioxidants (BHT), they could be exploited as antioxidant additives or as nutritional supplements. So the present study was thought worthwhile to determine and compare antioxidant and free radical scavenging activity of various extracts of leaves and stems of *K. pinnatum* by *in vitro* studies by multi-mechanistic assays in view of their higher phenolic and flavonoids contents.

MATERIALS AND METHODS

Collection of Plant Material

Fresh plant of *K. pinnatum* were collected from Herbal Garden of Chandigarh College Of Pharmacy Landran, Mohali (Punjab), India in June 2011, cleaned and dried at room temperature in shade and away from direct sunlight. The plant was authenticated by Dr. H. B. Singh, Chief Scientist and Head, Raw Materials Herbarium and Museum (RHMF), NISCAIR, New Delhi, by comparing morphological features. A herbarium sample of this plant is preserved in the department. The plant parts were separated and dried under shade until a constant weight was obtained. Material was coarsely ground and used for extraction.

Chemicals and Instruments

1, 1-diphenyl-2-picryl hydrazyl (DPPH), trichloroacetic acid (TCA), potassium ferricyanide were purchased from sigma chemicals. Gallic acid, quercetin, ascorbic acid, Folin-Ciocalteu reagent and ferric chloride were purchased from Merck. All other reagents were of analytical grade. UV-spectra were recorded in Shimadzu 1800 UV-Visible spectrophotometer.

Preparation of Extracts

The powdered plant materials (100 g) (Leaves and stems) extracted in soxhlet apparatus with petroleum ether (60°-80°C), chloroform and ethanol solvent (95%v/v). The extracts were concentrated to dryness in rotary evaporator under controlled temperature (40°-50°C). The extract was preserved for subsequent use in study.

Quantitative Estimation of Phytoconstituents

Total phenolic content

Total phenolic compound was determined using Folin-Ciocalteu method and the results were expressed as gallic acid equivalents or %w/w. In brief, 0.005 g of dried extract dissolved in 10 ml of methanol-water mixture (4:6 v/v ratios). 0.2 mL of such solution was pipette onto a glass tube and 1 mL of Folin-Ciocalteu reagent 0.8 ml of sodium carbonate (7.5%) were added to it. The mixture was stored at room temperature for 30 min and then the absorbance was read at 765 nm. Total phenolic compounds were calculated using a standard curve prepared with dilutions of gallic acid.[10]

Total flavonoid content

The total flavonoid content was determined using the Dowd method. 5 mL of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the ethanolic extract solution (0.5 mg/mL). Absorption readings at 415 nm using Shimazdu UV-visible spectrophotometer were taken after 10 minutes against a blank sample consisting of a 5 mL extract solution with 5 mL methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with quercetin (0-100 µg/ml) as the standard. Total flavonoid content is expressed as mg/g or %w/w.[11]

Evaluation of Antioxidant and Free Radical Scavenging Activity

Scavenging of DPPH radical

DPPH scavenging activity or the hydrogen donating capacity was quantified in presence of stable DPPH radical on the basis of Blois method.[12] Briefly, to a methanolic solution of DPPH (100 mM, 2.95 ml), 0.05 ml of test extracts dissolved in methanol was added at different concentration (20-100 µg/ml). Reaction mixture was shaken and absorbance was measured at 517 nm. Ascorbic acid was used as standard.[13] The degree of discoloration indicates the scavenging efficacy of the extracts. The experiment was performed in triplicate and percentage of scavenging activity was calculated using the following equation:

$$\% \text{ DDPH Scavenging Activity} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Scavenging of nitric oxide

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction according to the method of Marcocci. The chemical source NO was sodium nitroprusside (5 mM) in 0.5 M phosphate buffer, PH 7.4, spontaneously generates nitric oxide in aqueous solution. Nitric oxide interacts with oxygen to produce stable

products, leading to the production of nitrites. About 1 ml sodium nitroprusside (5 mM) in 0.5 M phosphate buffer was mixed with 3.0 ml of different concentrations (20-100 µg/ml) of the drugs dissolved in the suitable solvent systems and incubated at 25°C for 150 min. Absorbance was measured at 517 nm using ascorbic acid as standard.[13,14] The experiment was performed in triplicate and the capability to scavenge the NO radical was calculated using the following equation

$$\% \text{ No Scavenging Activity} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Scavenging of hydrogen peroxide

The ability of the extracts to scavenge hydrogen peroxide was determined using hydrogen peroxide. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Extracts (20-100 µg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The experiment was performed in triplicate and the capability to scavenge the hydrogen peroxide scavenging by the extracts and standard compounds were calculated using following equation:

$$\% \text{ Scavenging Activity} = \frac{A_0 - A_1}{A_0} \times 100$$

Where Ao was the absorbance of the control and A1 was the absorbance in the presence of the sample of extract and standard.[15]

Reducing power determination

The Reducing power of extracts was determined according to the method of Yen and Chen.[16] The extracts (20-100 µg/ml) in water were mixed with phosphate buffer (2.5 ml, 0.2 M, ph 6.6) potassium ferricyanide (2.5 ml, 1%). Then the mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) adds to the mixture, to stop the reaction and then centrifuge at 1000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%) and then absorbance was measured at 700 nm. Ascorbic acid was taken as standard, phosphate buffer used as blank solution.[17] The experiment was performed in triplicate.

Phosphomolybdenum method

The assay is based on the reduction of Mo(VI)-Mo(V) by the extract and subsequent formation of green phosphate/MO(V) complex at acidic pH. 0.3 ml extract was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mm sodium phosphate and 4 mm ammonium molybdate). Then the tubes containing the reaction solution was incubated at 95°C for 90 min. Then, the absorbance of solution measured at 695 nm using a spectrophotometer against blank cooling to room temperature. Use methanol (0.3 ml) in the place of extract as the blank. The antioxidant activity is express as the number of equivalent of ascorbic acid.[18]

RESULTS

Total phenolic and flavonoid contents

Total phenolic compounds, as determined by Folin-Ciocalteu method, are reported as gallic acid equivalents by reference to standard curve (y = 0.008x-0.001, r^2 = 0.999). The total flavonoid contents are reported as %w/w of extract powder by reference (y = 0.007x + 0.017, r^2 = 0.996). It was noted that leaves had significant higher total phenolic and flavonoid content than stems. Comparing the phenolic and flavonoid content in the various extracts of plant found the following order ethanol> acetone>chloroform>benzene. The high amount of phenols and flavonoids in extract may explain their high antioxidative activity [Table 1, Figures 1 and 2].

Determination of Antioxidant Activity

DPPH scavenging activity

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples.[16] DPPH is a stable nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers.[19] It was found that the radical-scavenging activities of all the extracts increased with increasing concentration. Usually, higher total phenol and flavonoids contents lead to better DPPH-scavenging activity.[16,19,20] Leaves with high level of phenolic contents and highest amount of flavonoids showed the best activity. The antioxidant activities of *K. pinnatum* increase with increase in concentration from 15.2% to 64.4% in case of leaves and from 8.4% to 49.1% in case stems in ethanolic extracts. The antioxidant activity of other extracts also increases with increase in concentration. The IC_{50} values of *K. pinnatum* of BE, CE, AE, EE are 160, 140, 120, 94 µg/ml respectively in leaves and IC_{50} values of *K. pinnatum* of

Table 1: Total phenolic and flavonoids content in benzene, chloroform, acetone and ethanolic extracts of leaves and stems of *K. pinnatum*

Extract	Total phenolic content (%w/w)		Total flavonoid content (%w/w)	
	Leaves	Stems	Leaves	Stems
Benzene	0.49±0.010	0.18±0.004	0.24±0.009	0.15±0.008
Chloroform	0.64±0.014	0.27±0.011	0.37±0.014	0.22±0.012
Acetone	0.99±0.017	0.48±0.008	0.56±0.016	0.42±0.004
Ethanol	1.17±0.008	0.62±0.012	0.75±0.012	0.54±0.016

All values are expressed as mean±standard deviation of three replications. (*n*=3)

BE, CE, AE, EE are 185, 164, 130, 108 µg/ml respectively in stems [Table 2, Figures 3 and 4].

Hydrogen peroxide method

Scavenging of H_2O_2 by extracts may be attributed to their phenolic, which can donate electrons to H_2O_2, thus neutralizing it to water.[16,21] The ability of the extracts to effectively scavenge hydrogen peroxide, determined according to the method of Ruch,[20] where they are compared with that of ascorbic acid as standard. The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. Scavenging capacity increases with increase in concentration and leaves shows maximum scavenging effects then stems. Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important. The antioxidant activities of leaves and stems of *K. pinnatum* increases with increase in concentration from 19.8% to 68.1% in case of leaves and from 15.4% to 50.0% in case stems in EE. The antioxidant activities of other extracts also increase with increase in concentration. The IC_{50} values of *K. pinnatum* of BE, CE, AE, EE are 172 145, 120, 71 µg/ml respectively in leaves and IC_{50} values of *K. pinnatum* of BE, CE, AE, EE are 200, 160, 150, 100 µg/ml respectively in stems [Table 3, Figures 5 and 6].

Nitric oxide scavenging activity

The extracts showed good nitric oxide-scavenging activity and % inhibition was increased with increasing

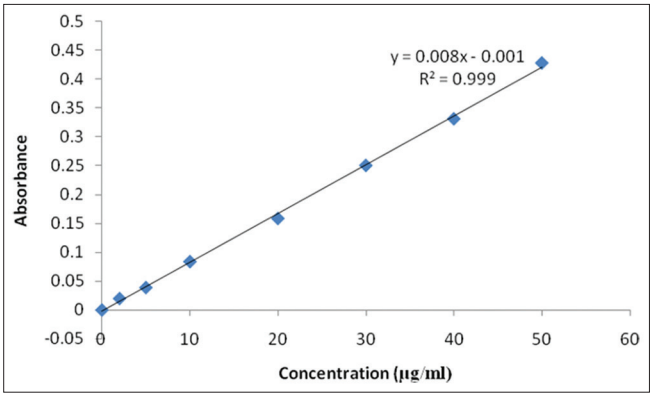

Figure 1: Standard curve of gallic acid

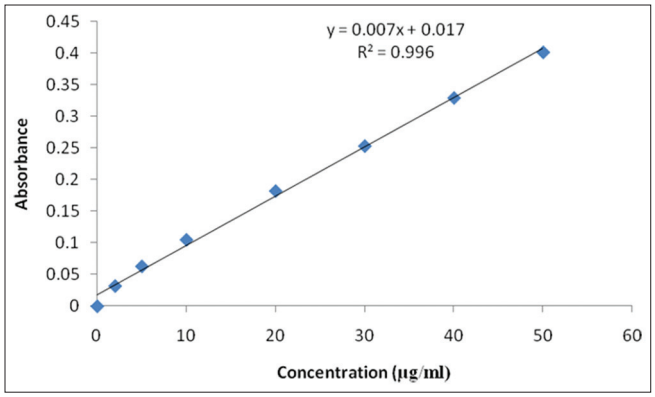

Figure 2: Standard curve of quercetin

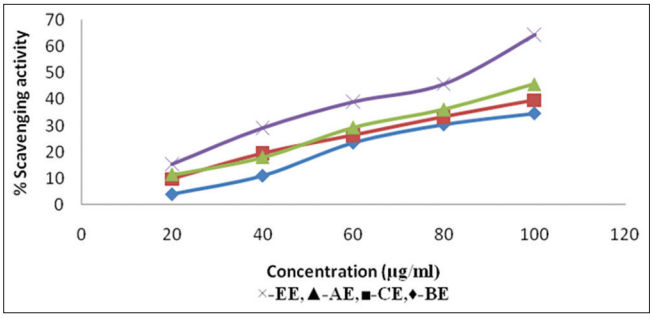

Figure 3: *In vitro* free radical scavenging activity of benzene, chloroform, acetone and ethanol extracts of leaves of *K. pinnatum* by DPPH method. EE- Ethanol extract; AE- Acetone extract; CE- Chloroform extract; BE- Benzene extract

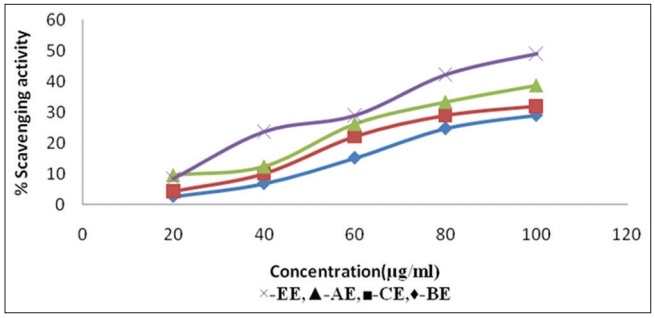

Figure 4: *In vitro* free radical scavenging activity of benzene, chloroform, acetone and ethanol extracts of stems of *K. pinnatum* by DPPH method. EE- Ethanol extract; AE- Acetone extract; CE- Chloroform extract; BE- Benzene extract

Table 2: *In vitro* free radical scavenging activity of benzene, chloroform, acetone and ethanol extracts of leaves and stems of *K. pinnatum* by DPPH method

Conc. (µg/ml)	% Scavenging activity (mean±standard deviation, *n*=3)							
	Leaves				Stems			
	BE	CE	AE	EE	BE	CE	AE	EE
20	4.1±0.16	9.7±0.23	11.4±0.16	15.2±0.12	2.7±0.11	3.9±0.16	8.9±0.10	8.4±0.12
40	11.1±0.12	19.4±0.14	18±0.11	29±0.14	6.9±0.12	10±0.18	12.5±0.12	23.7±0.24
60	23.6±0.32	26.3±0.12	29.1±0.14	38.9±0.12	15.2±0.19	22.2±0.24	26.3±0.18	29±0.16
80	30.5±0.19	33.3±0.22	36.2±0.19	45.7±0.18	24.8±0.16	29.1±0.28	33.4±0.21	42.3±0.19
100	34.7±0.14	39.6±0.18	45.8±0.21	64.4±0.23	29.1±0.14	32.1±0.19	38.8±0.14	49.1±0.23
IC_{50}	160	140	120	94	185	164	130	108

EE – Ethanol extract; AE – Acetone extract; CE – Chloroform extract; BE – Benzene extract

concentration of the extract. The leaves extract had shown better reducing power than stem extracts. However, activity of ascorbic acid was very more pronounced than that of our extracts. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions.[20] The antioxidant activities of leaves and stems of *K. pinnatum* increase with increase in concentration from 21.3% to 65.6% in case of leaves and from 12.5% to 48.4% in case stems in EE. The antioxidant activities of other extracts also increase with increase in concentration.[20] The IC_{50} values of *K. pinnatum* of BE, CE, AE, EE are 140, 118, 100, 60 µg/ml respectively in leaves and IC_{50} values of *K. pinnatum* of BE, CE, AE, EE are 162, 144, 122, 102 µg/ml respectively in stems [Table 4, Figures 7 and 8].

Reducing power determination

Fe(III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action.[20] In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive power. Figure 6 shows dose-response curves for the reducing powers of the extract. It was found that the reducing powers of all the extracts also increased with the increase of their concentrations. All extracts had shown good reducing power that was comparable with ascorbic acid. The

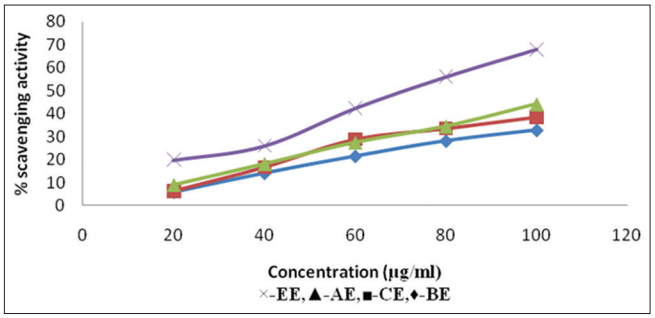

Figure 5: *In vitro* free radical scavenging activity of benzene, chloroform, acetone and ethanol extracts of leaves of *K. pinnatum* by hydrogen peroxide method. EE- Ethanol extract; AE- Acetone extract; CE- Chloroform extract; BE- Benzene extract

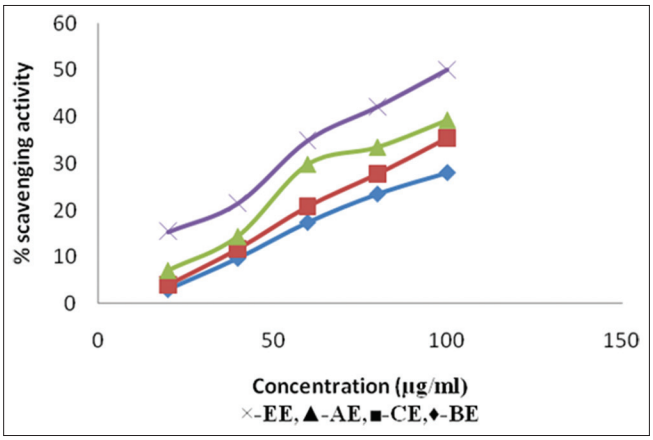

Figure 6: *In vitro* free radical scavenging activity of benzene, chloroform, acetone and ethanol extracts of stems of *K. pinnatum* by hydrogen peroxide method. EE- Ethanol extract; AE- Acetone extract; CE- Chloroform extract; BE- Benzene extract

Table 3: *In vitro* free radical scavenging activity of benzene, chloroform, acetone and ethanol extracts of leaves and stems of *K. pinnatum* by Hydrogen peroxide method

Conc. (µg/ml)	% Scavenging activity (mean±standard deviation, *n*=3)							
	Leaves				Stems			
	BE	CE	AE	EE	BE	CE	AE	EE
20	5.7±0.16	6.3±0.22	9.1±0.12	19.8±0.33	3±0.09	4±0.12	7.1±0.21	15.4±0.32
40	14±0.18	16.7±0.29	18.2±0.18	26±0.16	9.7±0.12	11.7±0.26	14.4±0.19	21.5±0.22
60	21.4±0.19	28.8±0.16	27.5±0.24	42.4±0.23	17.4±0.19	20.8±0.16	29.8±0.12	34.9±0.16
80	28.1±0.32	33.5±0.14	34.5±0.34	56.1±0.12	23.5±0.21	27.8±0.23	33.5±0.18	42.1±0.18
100	32.8±0.14	38.5±0.19	44.2±0.18	68.1±0.18	28.1±0.23	35.5±0.21	39.3±0.32	50±0.29
IC_{50}	172	145	120	71	200	160	150	100

EE – Ethanol extract; AE – Acetone extract; CE – Chloroform extract; BE – Benzene extract

Table 4: *In vitro* free radical scavenging activity of benzene, chloroform, acetone and ethanol extracts of leaves and stems of *K. pinnatum* by nitric oxide method

Conc. (µg/ml)	% Scavenging activity (mean±standard deviation, *n*=3)							
	Leaves				Stems			
	BE	CE	AE	EE	BE	CE	AE	EE
20	3.6±0.14	5.1±0.19	18.7±0.12	21.3±0.23	2.5±0.16	3.3±0.18	9.5±0.12	12.5±0.16
40	11±0.24	16.2±0.16	26.1±0.16	28.6±0.19	9.1±0.19	15.4±0.24	16.9±0.15	20.6±0.24
60	21.3±0.23	27.9±0.21	34.5±0.26	48.2±0.36	19.4±0.21	24.6±0.18	23.8±0.21	25.1±0.21
80	28.4±0.32	39.7±0.23	42.5±0.29	61±0.28	28.3±0.24	31.6±0.29	37.5±0.18	37.4±0.23
100	37.1±0.19	42.6±0.34	49.6±0.23	65.6±0.29	32.7±0.32	38.2±0.34	42.3±0.19	48.4±0.19
IC_{50}	140	118	100	60	162	144	122	102

EE – Ethanol extract; AE – Acetone extract; CE – Chloroform extract; BE – Benzene extract

antioxidant activity of *K. pinnatum* increases with increase in concentration from 0.032±0.014 to 0.173±0.019 µg/ml in leaves and from 0.022±0.016 to 0.152±0.029 µg/ml in case stems in ethanolic extract [Table 5, Figures 9 and 10].

Phosphomolybdenum reducing method

Total antiox idant capacity by phosphomolybdenum method assay is based on the reduction of Mo(VI) to Mo(V) by the sample analyte and the subsequent formation of green phosphate/Mo(V) complex at acidic pH. The phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid. The antioxidant activities of leaves and stems of *K. pinnatum* increase with increase in concentration from 0.042 to 0.134 in case of leaves and from 0.038 to 0.128 in case stems in EE. The antioxidant activities of all other extracts also increases with increase in concentration [Table 6, Figures 11 and 12].[21]

DISCUSSION

In Indian system of medicine, certain herbs are claimed to cure various pathological conditions. Reactive oxygen species (ROS) generated endogenously or exogenously are associated with pathogenesis of various diseases. Thus antioxidants which can scavenge ROS are expected to improve these disorders.

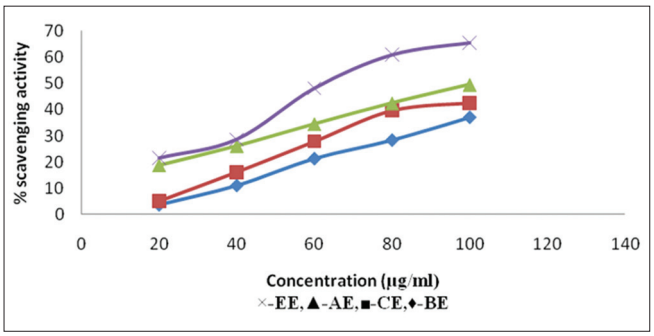

Figure 7: *In vitro* free radical scavenging activity of benzene, chloroform, acetone and ethanol extracts of leaves of *K. pinnatum* by nitric oxide method. EE- Ethanol extract; AE- Acetone extract; CE- Chloroform extract; BE- Benzene extract

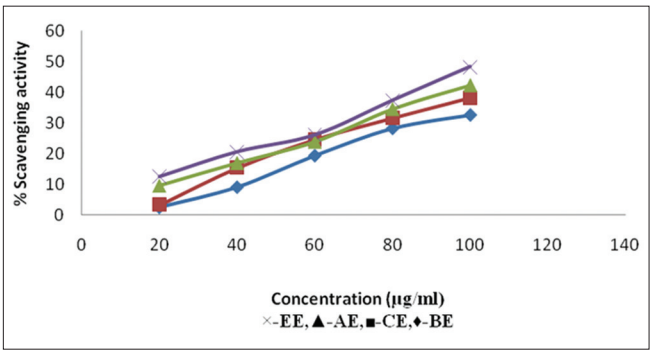

Figure 8: *In vitro* free radical scavenging activity of benzene, chloroform, acetone and ethanol extracts of stems of *K. pinnatum* by nitric oxide method. EE- Ethanol extract; AE- Acetone extract; CE- Chloroform extract; BE- Benzene extract

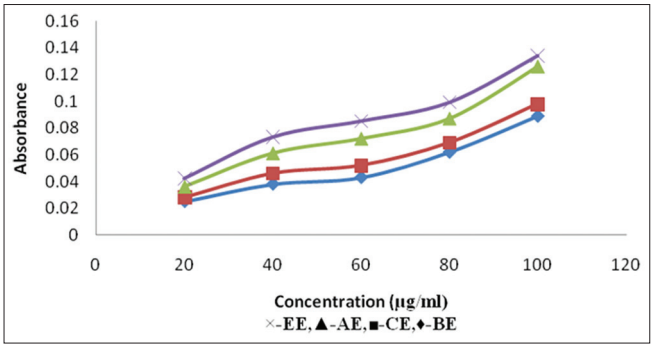

Figure 9: *In vitro* reducing power determination of benzene, chloroform, acetone and ethanol extracts of leaves of *K. pinnatum* by iron reduction method. EE- Ethanol extract; AE- Acetone extract; CE- Chloroform extract; BE- Benzene extract

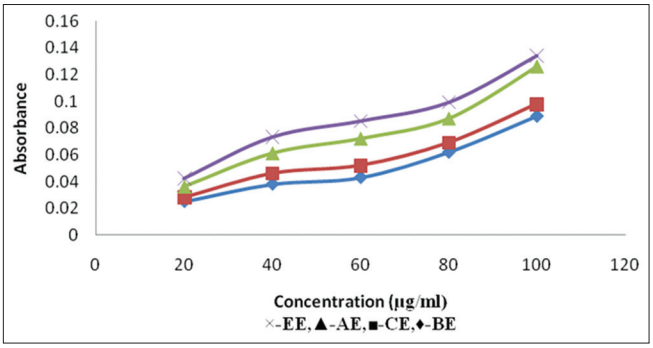

Figure 10: *In vitro* reducing power determination of benzene, chloroform, acetone and ethanol extracts of stems of *K. pinnatum* by iron reduction method. EE- Ethanol extract; AE- Acetone extract; CE- Chloroform extract; BE- Benzene extract

Table 5: *In vitro* reducing power determination of benzene, chloroform, acetone and ethanol extracts of leaves and stems of *K. pinnatum* by iron reduction method

Conc. (µg/ml)	Absorbance (mean±standard deviation, *n*=3)							
	Leaves				Stems			
	BE	CE	AE	EE	BE	CE	AE	EE
20	0.019±0.006	0.024±0.014	0.029±0.008	0.032±0.014	0.009±0.004	0.013±0.009	0.017±0.008	0.022±0.006
40	0.032±0.012	0.038±0.018	0.048±0.012	0.054±0.019	0.019±0.006	0.023±0.016	0.028±0.006	0.047±0.012
60	0.059±0.008	0.062±0.027	0.072±0.015	0.097±0.032	0.048±0.021	0.058±0.024	0.069±0.019	0.077±0.023
80	0.118±0.023	0.130±0.013	0.144±0.008	0.152±0.023	0.073±0.029	0.099±0.023	0.108±0.023	0.126±0.029
100	0.134±0.019	0146±0.029	0.158±0.023	0.173±0.019	0.109±0.013	0.122±0.024	0.134±0.036	0.152±0.033

EE – Ethanol extract; AE – Acetone extract; CE – Chloroform extract; BE – Benzene extract

Table 6: *In vitro* antioxidant capacity of benzene, chloroform, acetone and ethanol extracts of leaves and stems of *K. pinnatum* by phosphomolybdenum method

Conc. (µg/ml)	Absorbance (mean±standard deviation, *n*=3)							
	Leaves				Stems			
	BE	CE	AE	EE	BE	CE	AE	EE
20	0.025±0.016	0.028±0.012	0.036±0.006	0.042±0.016	0.016±0.009	0.023±0.009	0.032±0.023	0.038±0.006
40	0.038±0.008	0.046±0.014	0.061±0.014	0.073±0.024	0.028±0.013	0.039±0.004	0.054±0.016	0.062±0.018
60	0.043±0.023	0.052±0.012	0.072±0.023	0.086±0.036	0.048±0.027	0.053±0.023	0.068±0.019	0.078±0.019
80	0.062±0.019	0.069±0.019	0.087±0.018	0.099±0.012	0.069±0.019	0.074±0.019	0.082±0.015	0.094±0.014
100	0.089±0.021	0.098±0.032	0.126±0.022	0.134±0.029	0.082±0.018	0.096±0.014	0.119±0.032	0.128±0.023

EE–Ethanol extract; AE–Acetone extract; CE–Chloroform extract; BE–Benzene extract

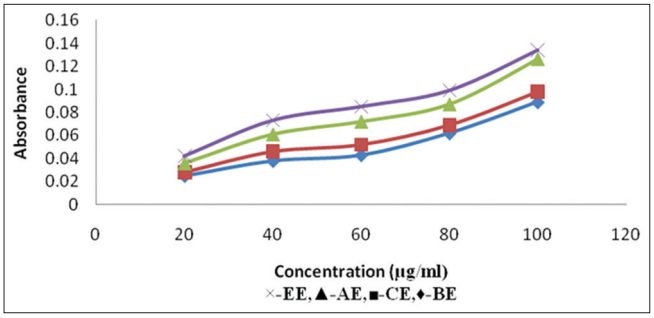

Figure 11: *In vitro* antioxidant capacity of benzene, chloroform, acetone and ethanol extracts of leaves of *K. pinnatum* by phosphomolybdenum method. EE- Ethanol extract; AE- Acetone extract; CE- Chloroform extract; BE- Benzene extract

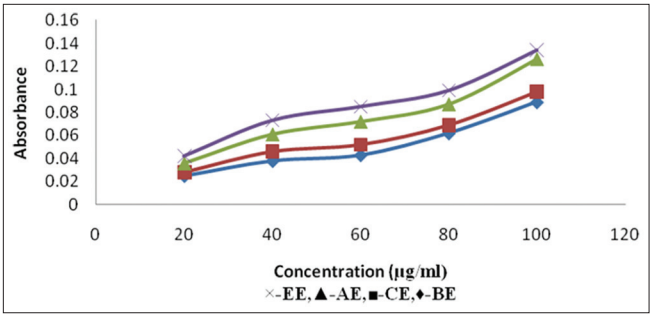

Figure 12: *In vitro* antioxidant capacity of benzene, chloroform, acetone and ethanol extracts of stems of *K. pinnatum* by Phosphomolybdenum method EE- Ethanol extract; AE- Acetone extract; CE- Chloroform extract; BE- Benzene extract

The free radical scavenging activity of the extracts was evaluated based on the ability to scavenge the synthetic DPPH. This assay provided useful information on the reactivity of compounds with stable free radicals, because of odd number of electrons. DPPH shows strong absorption band at 517nm in visible spectrum (deep violet color). As the electron became paired off in the presence of free radical scavenging, the absorption vanishes and the resulting discoloration stoichiometrically coincides with respect to number of electrons taken up. The bleaching of DPPH absorption is representative of the capacity of the extracts to scavenge free radicals independently.

The reducing properties are generally associated with the presence of reductones, which have been shown to exert anti-oxidant action by breaking the free radical chain by donating a hydrogen atom or by donating electron. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. Similarly reduction of Fe^{3+} to Fe^{2+} and Mo^{6+} to Mo^{5+} by donating electron. Increase in the absorbance of the extracts indicated the antioxidant activity.

Nitric oxide is an essential gas required for several normal physiological processes like neural signal transmission, immune response and control of blood pressure. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions. However the elevation of NO level was found in several pathological conditions including cardiovascular disease, diabetes etc. sodium nitroprusside serves as chief source of free radicals. The plant extracts which scavenge the NO formed from the sodium nitroprusside and hence the absorbance decreases as the concentration of the extracts concentration increases in a dose dependent manner.

It can be concluded that the extracts have constituents which were capable of showing antioxidant activity and the said *in-vitro* antioxidant activity may also be due to the presence of antioxidant principles present in the extracts like flavonoids and phenolic compounds.

CONCLUSION

The result of the present study suggested that the total phenolic and flavonoid content in leaves was found to be higher than stems in all the extracts and in order: Ethanol > acetone > chloroform > benzene, which is further proved by *in vitro* antioxidant studies. Potential antioxidant activity has good correlations with the therapeutic use in the treatment of cardiovascular disorders. Extracts contains the significant amount of phenolic and flavonoids and exhibits the significant antioxidant activity through the scavenging of free radicals which participate in various pathophysiology of diseases including ageing. Further research to isolate individual compounds, their *in-vivo* antioxidant activities with different mechanism is needed.

REFERENCES

1. Gyekyel IJ, Antwi DA, Bugyei KA, Awortwe C. Comparative study of two Kalanchoe species: Total flavonoid, phenolic contents and antioxidant properties. Afr J Pure Appl Chem 2012;6:65-73.
2. Okwu DE, Nnamdi FU. A novel antimicrobial phenanthrene alkaloid from Bryopyllum *pinnatum*. J Chem Pharm Res 2011;3:27-33.
3. Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigerian medicinal plants. Afr J Biotech 2006;5:357-61.
4. Egereonu UU, Mokwe NR. Characterization of the extract from Bryophyllum *pinnatum* Leaves. J Chem Soc Nig 2005;30:192-6.
5. Ojewole JA. Antinociceptive, anti-inflammatory and antidiabetic effects of ryophyllum *pinnatum* (Crassulaceae) leaf aqueous extract. J Ethnopharmacol 2005;99:13-9.
6. Adenike K, Eretan OB. Purification and partial characterization of a lectin from the fresh leaves of Kalanchoe crenata (Andr.) Haw. J Biochem Mol Biol 2004;37:229-33.
7. Gaind KN, Gupta RL. Flavonoid glycosides from Kalanchoe pinnata. Planta Med 1971;20:368-73.
8. Kamboj A, Saluja AK. Bryophyllum *pinnatum* (Lam.) Kurz: Phytochemical and Pharmacological Profile: A review. Pharmacogn Rev 2009;3:364-74.
9. Yamagishi T, Haruna M, Yan XZ, Chang JJ, Lee KH. Antitumor Agents, 110, Bryophyllin B, A Novel Potent cytotoxic Bufadienolide from Bryophyllum *Pinnatum*. J Nat Prod 1989;52:1071-9.
10. Hosein HK, Zinab D. Phenolic Compounds and antioxidant Activity of Henna Leaves Extacts (Lawsonia Inermis). World J Dairy Food Sci 2007;2:38-41.
11. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in burkina fasan honey, as well as their radical scavenging activity. Food Chem 2005;91:571-7.
12. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity, Songklanakarin. J Sci Technol 2004;26:211-9.
13. Mani RP, Pandey A, Goswami S, Tripathi P, Kumudhavalli V, Singh AP. Phytochemical Screening and In- vitro Evaluation of Antioxidant Activity and Antimicrobial activity of the Leaves of Sesbania sesban (L) Merr. Free Radic Antioxid 2011;1:412-4.
14. Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide scavenging properties of Ginkgo biloba extract EGb 761. Biochem Biophys Res Commun 1994;201:748-55.
15. Sridharamurthy NB, Yogananda R, Sriniwas U. In-vitro Antioxidant and Antilipidemic Activities of Xanthium strumarium L. Curr Trends Biotechnol Pharm 2011;5:1362-71.
16. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J Agri Food Chem 1995; 43:27-32.
17. Li XM, Li XL, Zhou AG. Evaluation of antioxidant activity of polysaccharides extracted from Lycium barbarum fruits *in vitro*. Eur Polym J 2007;43:488-97.
18. Ebrahimzadeh MA, Pourmorad F, Bekhradnia AR. Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran. Afr J Biotechnol 2008;32:43-9.
19. 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;269:337-41.
20. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A, Bekhradnia AR. Determination of antioxidant activity, phenol and flavonoids content of Parrotia persica Mey. Pharmacol online 2008;2:560-7.
21. Singleton VL, Rossi JA. Colorimetry of total phenolic with Phosphomolybdic acid-phosphot tungistic acid reagents. Am J Enol Viticult 1965;16:144-58.

How to cite this article: 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;6:340-7.

Source of Support: Nil, **Conflict of Interest:** None declared.