Phytochemical Screening and *In-Vitro* Evaluation of Antioxidant Activities of Various Extracts of Leaves and Stems of *Kalanchoe crenata*

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Abstract: Many diseases are associated with oxidative stress caused by free radicals. Current research is directed towards finding naturally occurring antioxidants of plant origin. *Kalanchoe crenata* Andr. (Crassulaceae), commonly known as "never die" or "Dog's liver," has been traditionally used for the treatment of ailments, such as, earache, smallpox, headache, inflammation, pain, asthma, palpitations, convulsion, and general debility. The aim of present research deals with phytochemical screening and in-vitro evaluation of antioxidant activities of the leaves & stems of *K.crenata*.

Method: Successive extracts of leaves & stems was subjected for phytochemical screening. The preliminary screening reports the presence of saponins, phytosterols, flavanoids, phenols and alkaloids in the extracts. Various extracts of *K.crenata* leaves & stems was studied in-vitro for total antioxidant activity, for scavenging of nitric oxide, hydrogen peroxide, the antioxidant capacity by phosphomolybdenum, reducing power determination and determination of phenolic and flavonoid content in the extracts. 1,1-Diphenyl-2-picrylhydryzyl (DPPH) scavenging activity or the hydrogen donating capacity was quantified in presence of stable DPPH radical on the basis of Blois method. Nitric Oxide (NO) radical scavenging method was performed in the presence of nitric oxide generated from sodium nitroprusside using ascorbic acid as standard in both methods. The phenolic content was determined by using Folin-Ciocalteu reagent and flavonoid content was determined by aluminum chloride.

Result: The radical scavenging activity was found to dose dependent. Thus extract has been established as an antioxidant. The reducing capacity serves as significant indicator of antioxidant activity. The reducing power was found to increase with the increasing concentration of extract. The 100mg plant powder yielded 0.34, 0.49, 0.72, 0.98%w/wand 0.15, 0.23, 0.39, 0.56%w/w phenolic content in the benzene, chloroform, acetone, ethanol extracts of leaves and stems respectively using gallic acid as standard. Plant contains about 0.19, 0.29, 0.48, 0.64%w/w and 0.11, 0.17, 0.32, 0.47 %w/w of flavonoid content in the benzene, chloroform, acetone, ethanol extracts of leaves and stems respectively using quercetin as standard.

Conclusion: The present study provides evidence that different extracts of *K.crenata* leaves and stems is potential source of antioxidant activity. The extracts were found to contain phenolic compounds which could be responsible for the antioxidant properties. So *K. crenata* traditional use is justified in the present research work.

Keywords: *Kalanchoe crenata*, DPPH scavenging activity, 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 superoxides are 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 [7,22].

The external applications of *Kalanchoe crenata* are also very effective in treatment of skin diseases. The juice obtained by squeezing the leaves that have been passed over fire slightly, is most commonly used for the treatment of headache, general debility, dysentery, smallpox and convulsion. One or two drops of the leaf juice are dropped into the ear for earache. A poultice of the leaves is applied over wounds and sores. The leaves can be boiled in water and the extract is given as a sedative for asthma and palpitation. Also the leave juice mixed with salt and honey is a remedy for chronic cough. The extract of dried leaves is applied to septic wound [17].

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In East Africa, the slightly heated leaves (heated over fire) are rubbed over the body as treatment for stiff joint and rheumatism [6]. Other parts of the plant especially the root is prescribed for gonorrhoea, vermifuge and abortion [17]. Alkaloids and saponins are present in the aqueous and alcoholic extracts of leaves and lectins in the juice from the fresh leaves. The green callus of the plant contains malic acid, quinones and tocopherol [17]. Other works have also shown that this plant possesses analgesic, anticonvulsion, antiinflammatory, antiarthritic and antispasmolytic properties [19]. *Kalanchoe* is reported to contain considerable amounts of flavonoid and phenolic compounds [1,5]. 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 [2,20].

The present study deals with the determination of antioxidant and free radical scavenging activity of various extracts of leaves and stems of *Kalanchoe crenata* by in-vitro studies, by multi-mechanistic assays and determination of the total phenolic and flavonoid content.

MATERIALS AND METHODS

Collection of Plant Material

The leaves and stems of *Kalanchoe crenata* 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. The herbarium of the plant is preserved in the department. The plant parts were collected and dried in the shade and then pulverized in a grinder. Material was passed through 120 meshes to remove fine powders and coarse powder was used for extraction.

Chemicals

1, 1-diphenyl-2-picryl hydrazyl (DPPH), trichloroacetic acid (TCA), potassium ferrocyanide were purchased from Sigma Chemicals. Gallic acid, quercetin, ascorbic acid, Folin-Ciocalteu reagent and ferric chloride were purchased from Merck. All chemicals were of AR grade.

Preparation of Extracts

The powdered plant material was extracted successively in soxhlet apparatus with various solvents like petroleum ether (60^0-80^0), benzene, chloroform, acetone, ethanol and water. All the extracts were subjected to qualitative tests for the identification of various active constituents by different chemical tests.

About 100g of the plant parts (Leaves, & stems) extracted in soxhlet apparatus with benzene, chloroform, acetone ethanol as solvents. The extract was concentrated to dryness in rotary evaporator under controlled temperature (40^0 -50^0C). The extract was preserved for subsequent use in study.

QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS

Total Phenolic Content

Total phenolic compound was determined using Folin-ciocalteu methodand the results were expressed as gallic acid equivalents. In brief, 5mg 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 1ml of Folin-coicalteu 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 765nm.Total phenolic compounds were calculated using a standard curve prepared with dilutions of gallic acid [8].

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 (0.5mg/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 of extract [12].

DETERMINATION OF ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITY

DPPH Radical Scavenging Capacity

DPPH scavenging activity or the Hydrogen donating capacity was quantified in presence of stable DPPH radical on the basis of Blois method by (Molyneux, 2004). Briefly, to a methanolic solution of DPPH (100

mM, 2.95 ml), 0.05 ml of test compounds dissolved in methanol was added at different concentration (20-100 µg/ml). Reaction mixture was shaken and absorbance was measured at 517nm using ascorbic acid as standard [10]. The degree of discoloration indicates the scavenging efficacy of the extracts. The capability to scavenge the DPPH radical was calculated using the following equation:

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

Nitric Oxide Radical Scavenging Activity

Nitric oxide was generated from sodium nitroprusside and measured by the Griess reaction according to the method of [11]. The chemical source of NO was sodium nitroprusside (5 mM) in 0.5M 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 extracts dissolved in the suitable solvent system and incubated at 25°C for 150min. Ascorbic acid was used as standard [10,11,13]. The capability to scavenge the NO radical was calculated using the following equation:

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

Scavenging of Hydrogen Peroxide

The ability of the extracts to scavenge hydrogen peroxide was determined. 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 (20-100µg/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 percentage of hydrogen peroxide scavenging by the extracts and standard compounds were calculated as follows:

$$\% \text{ Scavenging Activity} = \frac{A0 - A1}{A0} \times 100$$

Where Ao was the absorbance of the control and A1 was the absorbance in the presence of the sample of extract and standard [18].

Reducing Power Determination

The Reducing power of extracts was determined according to the method of Yen and Chen [21]. The extracts (20-100µg/ml) in water were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) potassium ferricyanide (2.5ml, 1%). Then the mixture was incubated at 50^0 C for 20 minute. A portion (2.5 ml) of trichloroacetic acid (10%) added to the mixture, to stop the reaction and then centrifuge at 3000 rpm for 10 min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) & ferric chloride (0.5ml, 0.1%) & then absorbance was measured at 700nm. Ascorbic acid was taken as standard, phosphate buffer used as blank solution [9].

Phosphomolybdenum Method

The assay is based on the reduction of Mo(IV)-Mo(V) by the extract and subsequent formation of green phosphate/MO(V) complex at acidic pH. 0.3ml extract was mixed with 3ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate & 4mM ammonium molybdate). Then the tubes containing the reaction solution was incubated at 95^0c for 90min. Then, the absorbance of solution was measured at 695nm 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 [15].

RESULT AND DISCUSSION

Phytochemical Screening

The following active constituents have been identified from the tests in the various successive extracts are sterols, glycosides, alkaloids, flavonoids, carbohydrates, and phenols The phytochemical constituents present in successive extracts of leaves & stems are presented in Table **1-2**.

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 mg quercetin equivalent/g of extract powder by reference (y=0.007x+0.017, r^2=0.996). It was noted that leaves of *K. crenata* had significant higher total phenolic and flavonoid content than stems. Phenols and polyphenolic compounds, such as

Table 1: Phytochemical Screening of Various Extracts of Leaves of *K. crenata*

S. No.	Type of constituents	Pet. Ether	Benzene	Chloroform	Acetone	Ethanol	Water
1	Alkaloid	-	-	-	-	+	-
2	Flavonoid	-	-	-	+	+	-
3	Saponin	-	-	-	+	+	+
4	Carbohydrate	+	+	+	+	+	+
5	Phytosterol	+	+	+	+	+	+
6	Tannin	-	-	-	+	+	+
7	Phenolic	-	-	+	+	+	-
8	Coumarin	-	-	-	-	-	-
9	Cardiac glycoside	-	-	+	+	+	+
10	Anthraquinone	-	-	+	+	-	-
11	Essential oil	+	-	-	-	-	-

+ = Present; - = Absent.

Table 2: Phytochemical Screening of Various Extracts of Stems of *K. crenata*

S. No.	Type of constituents	Pet. Ether	Benzene	Chloroform	Acetone	Ethanol	Water
1	Alkaloid	-	-	-	-	+	-
2	Flavonoid	-	-	-	+	+	-
3	Saponin	-	-	-	+	+	+
4	Carbohydrate	+	+	+	+	+	+
5	Phytosterol	+	+	+	+	+	+
6	Tannin	-	-	-	+	+	-
7	Phenolic	-	-	-	+	+	
8	Coumarin	-	-	-	-	-	-
9	Cardiac glycoside	-	-	-	+	+	-
10	Anthraquinone	-	-	-	-	-	-
11	Essential oil	-	-	-	-	-	-

+ = Present; - = Absent.

flavonoid are widely found in plant products, and they have been shown to possess significant antioxidant activities. The high amount of phenols and flavonoid in extract may explain their high antioxidative activity.

The standard curve of Gallic acid and Quercetin has shown in Figure **1** and Table **3** indicates the Total Phenolic and Flavonoid contents in the different extracts of leaves & stems of *K.crenata.*

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 [3]. 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 [13]. 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 [3,4,14]. Leaves with high level of phenolic contents and highest amount of flavonoids showed the best activity. The antioxidant activityof ethanolic extract of *K.crenata* increases with increase in concentration from 13.5 to 59.1% in leaves and from 7.9% to 45.7% in stems. The antioxidant activities of other extracts also increase with increase in concentration. The IC_{50} values of *K.crenata* of BE, CE, AE, EE are 90, 120, 156, 178µg/ml respectively in leaves and IC_{50} values of *K.crenata* of BE, AE, CE, EE

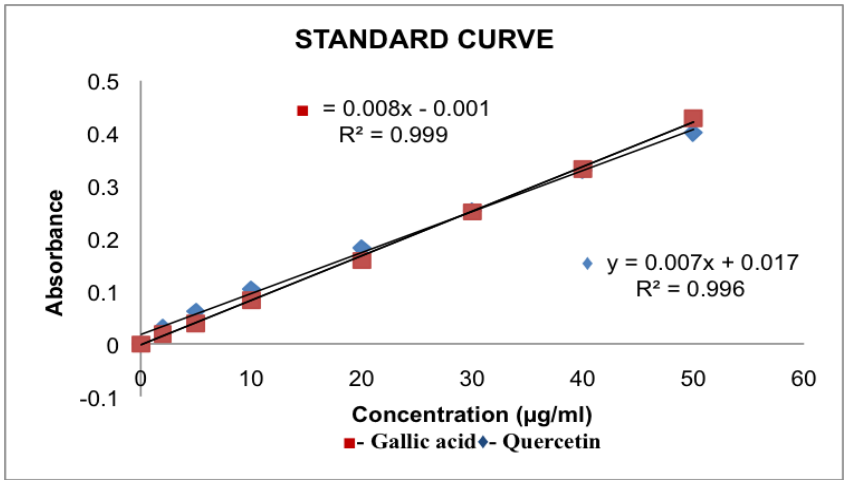

Figure 1: Standard curve of Gallic acid and Quercetin.

Table 3: Total Phenolic and Flavonoid Contents of Leaves & Stems in Benzene, Chloroform, Acetone and Ethanolic Extracts of *K. crenata*

Extract	Total Phenolic content(%w/w)		Total Flavonoid content(%w/w)	
	Leaves	Stems	Leaves	Stems
Benzene	0.34± 0.016	0.15± 0.016	0.19± 0.008	0.11± 0.012
Chloroform	0.49± 0.008	0.23± 0.006	0.29± 0.011	0.17± 0.008
Acetone	0.72± 0.012	0.39± 0.013	0.48± 0.014	0.32± 0.016
Ethanol	0.98± 0.013	0.56± 0.012	0.64± 0.016	0.47± 0.009

All values are expressed as mean ± standard deviation of three replications (n=3).
[a]µg of gallic acid equivalent per 5mg of extract.
[b]µg of quercetin equivalent per 10mg of extract.

are 192, 180, 160, 116µg/ml respectively in stems (Table **4**; Figures **2**, **3**).

Hydrogen Peroxide Method

Scavenging of H_2O_2 by extracts may be attributed to their phenolic compounds, which can donate electrons to H_2O_2, thus neutralizing it to water [3,14]. The ability of the extracts to effectively scavenge hydrogen peroxide, determined according to the method of Ruch [14]. In which results 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 throughout food systems. The antioxidant

Table 4: DPPH Radical Scavenging Activity of Benzene, Chloroform, Acetone & Ethanol Extracts of Leaves & Stems of *K. crenata*

Concentration (µg/ml)	% Scavenging activity							
	Leaves				Stems			
	BE	CE	AE	EE	BE	CE	AE	EE
20	5.5± 0.23	8.3± 0.16	12.5± 0.18	13.5±0.23	4.1± 0.14	6.9± 0.21	8.3± 0.24	7.9± 0.12
40	9.7± 0.12	13.8± 0.21	17.8± 0.26	25.4± 0.17	8.3± 0.19	11.1± 0.27	16.4± 0.29	18.6± 0.18
60	16.6± 0.19	25± 0.39	22.2± 0.37	35.5± 0.36	13.8± 0.26	19.4± 0.19	25± 0.36	27.1±0.25
80	26.3± 0.34	31.9± 0.17	34.7± 0.23	40.6± 0.28	20.8± 0.32	23.6± 0.17	29.1± 0.17	37.8±0.13
100	31.9± 0.29	37.5± 0.28	43± 0.19	59.3± 0.19	27.7± 0.21	29.4± 0.14	34.7± 0.16	45.7±0.29
IC_{50}	90	120	156	178	192	180	160	116

Results expressed as mean ± standard deviation of three replications.

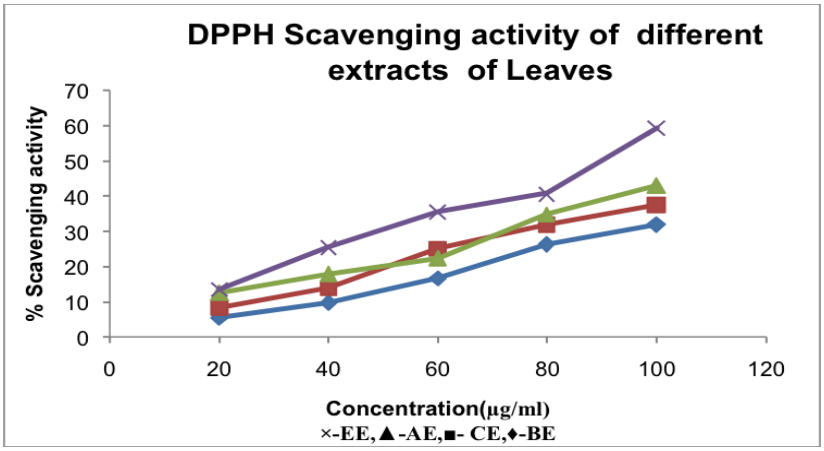

Figure 2: DPPH radical scavenging activity of Benzene, Chloroform, Acetone & Ethanol extracts of leaves *K. crenata.*
EE- Ethanol extract; **AE**- Acetone extract; **CE**- Chloroform extract; **BE**- Benzene extract.

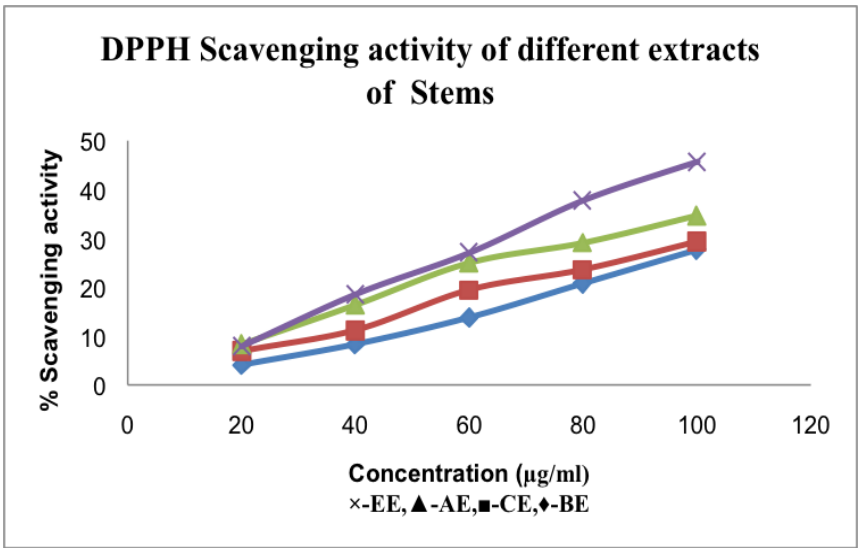

Figure 3: DPPH radical scavenging activity of stems of Benzene, Chloroform, Acetone & Ethanol extracts of *K. crenata.*
EE- Ethanol extract; **AE**- Acetone extract; **CE**- Chloroform extract; **BE**- Benzene extract.

activities of ethanolic extract of *K.crenata* increases with increase in concentration from 18.8 % to 60.6 % in leaves and from 13.7 % to 47.2 % in stems. The antioxidant activities of other extracts also increase with increase in concentration. The IC_{50} values of *K.crenata* of BE, CE, AE, EE are 164, 154, 114, 78µg/ml respectively in leaves and IC_{50} values of *K.crenata* of BE, CE, AE, EE are 200, 179, 148, 112 µg/ml respectively in stems (Table **5**; Figures **4**, **5**).

Nitric Oxide-Scavenging Activity

In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions [14]. The extracts showed good nitric oxide-scavenging activity and percentage inhibition was increased with increasing concentration of the extract. The ethanolic extracts of leaves and stems were found to have maximum nitric oxide scavenging activity i.e. better reducing power than other. The antioxidant activities ethanolic extract of *K.crenata* increases with increase in concentration from 17.7 % to 61.0 % in leaves and from 11.8 % to 46.5 % in stems. The antioxidant activities of other extracts also increase with increase in concentration [11]. The IC_{50} values of *K. crenata* of BE, CE, AE, EE are 158, 138, 110, 78 µg/ml respectively in leaves and IC_{50} values of *K. crenata* of BE, CE, AE, EE are 164, 158, 138, 102µg/ml respectively in stems (Table **6**; Figures **6**, **7**).

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 [14]. In the

Table 5: Hydrogen Peroxide Scavenging Activity of Benzene, Chloroform, Acetone & Ethanol Extracts of Leaves & Stems of *K. crenata*

Concentration (µg/ml)	% Scavenging activity							
	Leaves				Stems			
	BE	CE	AE	EE	BE	CE	AE	EE
20	4.6± 0.12	5.4± 0.11	8± 0.10	18.8± 0.29	2.0± 0.09	3.4± 0.15	6.7± 0.16	13.7± 0.18
40	13.4± 0.23	14.7± 0.27	15.4± 0.23	23.9± 0.34	9.1± 0.16	14± 0.11	18.2± 0.21	17.5±0.28
60	24.1± 0.18	27.1± 0.31	28.1± 0.12	37.3± 0.12	19.3±0.23	24.8± 0.23	29.8± 0.14	31.8±0.12
80	29.8± 0.16	32.2± 0.19	33.2± 0.28	51.7± 0.16	24.3±0.12	29.5± 0.16	34.5± 0.29	39.7± 0.19
100	35.5± 0.21	37.9± 0.15	43.9±0.12	60.6± 0.21	27.8±0.14	32.8± 0.19	37.5± 0.23	47.2± 0.23
IC_{50}	164	154	114	78	200	179	148	112

Results expressed as mean ± standard deviation of three replications.

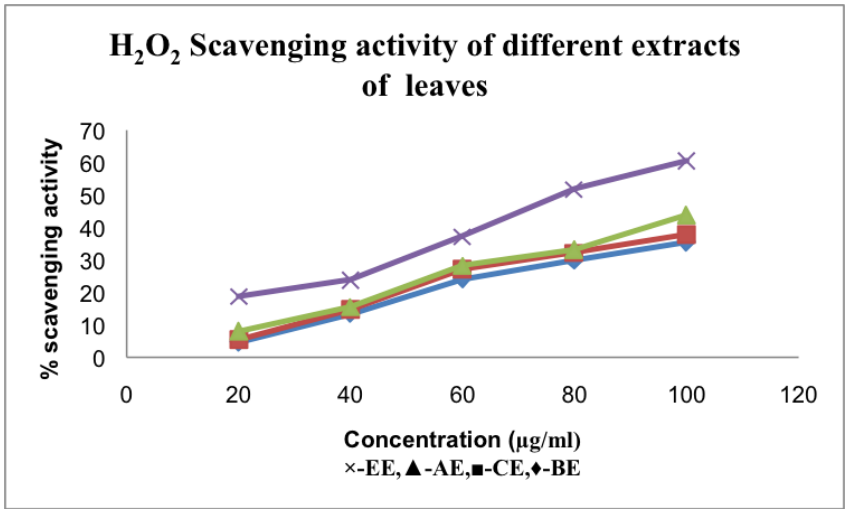

Figure 4: Hydrogen peroxide Scavenging activity of Benzene, Chloroform, Acetone & Ethanol extracts of leaves of *K. crenata.* **EE**- Ethanol extract; **AE**- Acetone extract; **CE**- Chloroform extract; **BE**- Benzene extract.

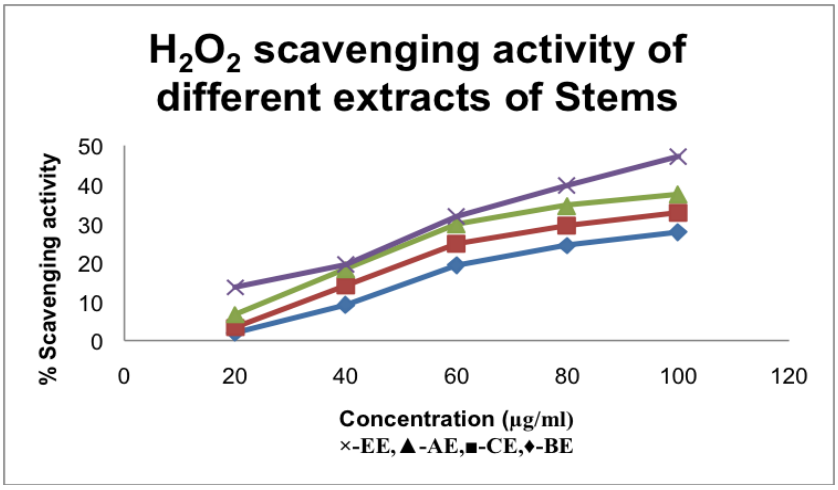

Figure 5: Hydrogen peroxide scavenging activity of Benzene, Chloroform, Acetone & Ethanol extracts of stems of *K. crenata.* **EE**- Ethanol extract; **AE**- Acetone extract; **CE**- Chloroform extract; **BE**- Benzene extract.

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 ability.

Table 6: Nitric Oxide Scavenging Activity of Benzene, Chloroform, Acetone & Ethanol Extracts of Leaves of *K. crenata*

Concentration (µg/ml)	% Scavenging activity							
	Leaves				Stems			
	BE	CE	AE	EE	BE	CE	AE	EE
20	2.9±0.12	4.4± 0.18	16.1± 0.12	17.7± 0.10	1.8±0.10	2.5± 0.13	8.0± 0.16	11.8± 0.23
40	8.8±0.18	13.9±0.23	22± 0.16	21.3± 0.23	9.5± 0.19	12.5± 0.19	17.6± 0.19	19.8± 0.16
60	17.2±0.23	23.5± 0.26	33.2± 0.24	34.3± 0.34	14.3±0.23	20.2± 0.26	24.2± 0.23	28.2± 0.21
80	26.8±0.19	37.1± 0.34	40.8± 0.22	50.7± 0.31	21.6± 0.16	27.2± 0.31	34.9± 0.18	32.7± 0.12
100	33.2±0.21	40.4± 0.21	47.4± 0.14	61± 0.12	29.4±0.24	33.8± 0.29	39.7± 0.12	46.5± 0.29
IC_{50}	158	138	110	78	164	158	138	102

Results expressed as mean ± standard deviation of three replications.

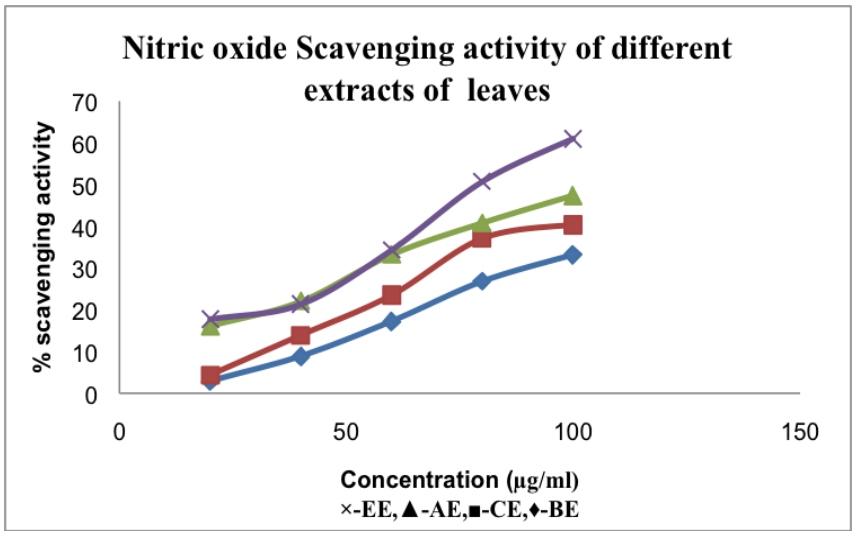

Figure 6: Nitric oxide scavenging activity ofBenzene, Chloroform, Acetone & Ethanol extracts of leaves of *K. crenata*.
EE- Ethanol extract; **AE**- Acetone extract; **CE**- Chloroform extract; **BE**- Benzene extract.

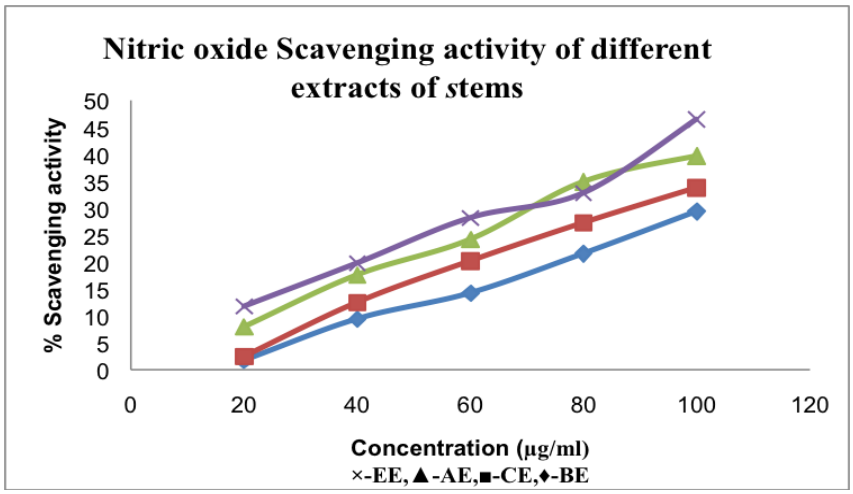

Figure 7: Nitric oxide scavenging activity of Benzene, Chloroform, Acetone & Ethanol extracts of stems of *K. crenata*.
EE- Ethanol extract; **AE**- Acetone extract; **CE**- Chloroform extract; **BE**- Benzene extract.

Figure 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 (Table **7**; Figures **8**, **9**).

Table 7: Reducing Power Determination of Benzene, Chloroform, Acetone & Ethanol Extracts of Leaves & Stems of *K. crenata*

Conc. (µg/ml)	Absorbance							
	Leaves				Stems			
	BE	CE	AE	EE	BE	CE	AE	EE
20	0.015±0.008	0.019±0.006	0.026±0.006	0.029±0.007	0.005±0.002	0.008±0.002	0.012±0.002	0.018±0.002
40	0.027±0.002	0.031±0.013	0.040±0.002	0.051±0.008	0.015±0.006	0.017±0.008	0.023±0.004	0.039±0.009
60	0.048±0.006	0.056±0.018	0.069±0.008	0.091±0.016	0.028±0.003	0.049±0.009	0.059±0.007	0.056±0.006
80	0.104±0.016	0.119±0.019	0.128±0.011	0.148±0.018	0.053±0.007	0.078±0.012	0.098±0.012	0.119±0.014
100	0.126±0.019	0.138±0.008	0.151±0.014	0.168±0.027	0.083±0.011	0.099±0.016	0.121±0.023	0.144±0.021

Results expressed as mean ± standard deviation of three replications.

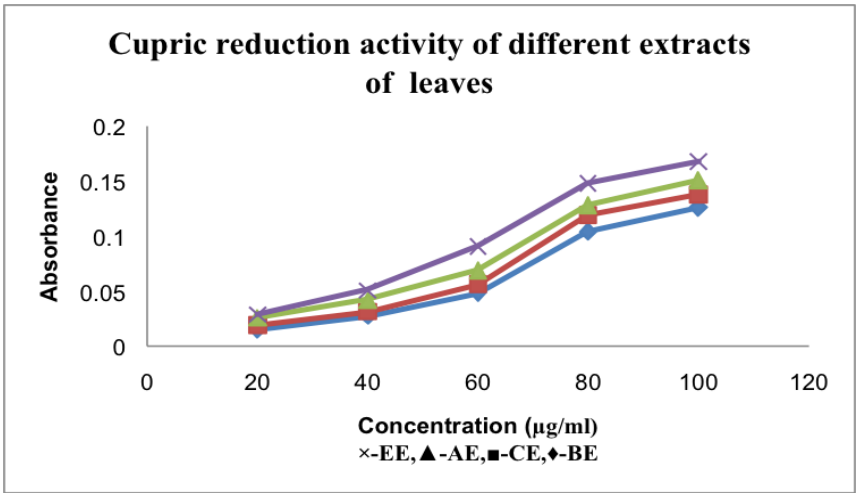

Figure 8: Reducing power determination of Benzene, Chloroform, Acetone & Ethanol extracts of leaves of *K. crenata*. **EE**- Ethanol extract; **AE**- Acetone extract; **CE**- Chloroform extract; **BE**- Benzene extract.

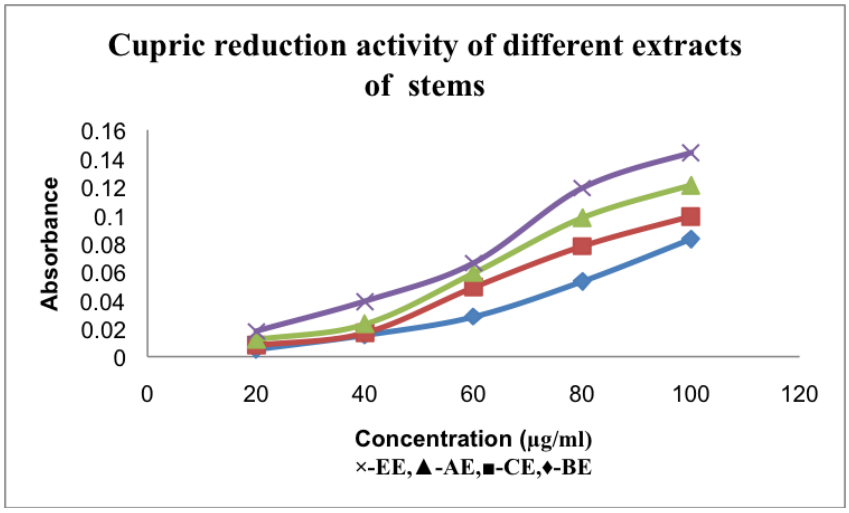

Figure 9: Reducing power determination of Benzene, Chloroform, Acetone & Ethanol extracts of stems of *K. crenata*. **EE**- Ethanol extract; **AE**- Acetone extract; **CE**- Chloroform extract; **BE**- Benzene extract.

Phosphomolybdenum Method

Total antioxidant 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 reducing

Table 8: Antioxidant Capacity by Phosphomolybdenum Method of Benzene, Chloroform, Acetone & Ethanol Extracts of Leaves & Stems of *K. crenata*

Conc. (µg/ml)	Absorbance							
	Leaves				Stems			
	BE	CE	AE	EE	BE	CE	AE	EE
20	0.020±0.006	0.024±0.008	0.032±0.004	0.039±0.004	0.012±0.004	0.018±0.002	0.024±0.006	0.026±0.004
40	0.034±0.007	0.038±0.06	0.056±0.012	0.068±0.009	0.023±0.009	0.027±0.008	0.049±0.004	0.048±0.009
60	0.039±0.004	0.046±0.006	0.064±0.014	0.083±0.012	0.038±0.017	0.046±0.009	0.053±0.011	0.057±0.012
80	0.056±0.012	0.060±0.010	0.075±0.021	0.089±0.016	0.053±0.019	0.062±0.013	0.076±0.019	0.079±0.017
100	0.078±0.016	0.084±0.019	0.118±0.029	0.128±0.021	0.057±0.023	0.069±0.017	0.088±0.023	0.112±0.024

Results expressed as mean ± standard deviation of three replications.

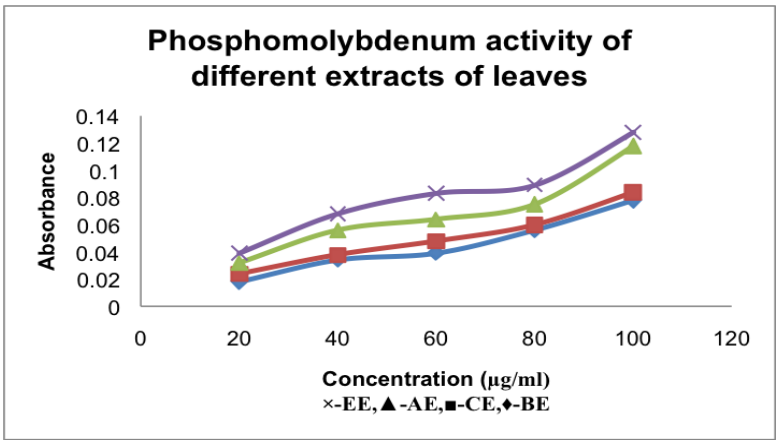

Figure 10: Antioxidant capacity by Phosphomolybdenum method of Benzene, Chloroform, Acetone & Ethanol extracts of leaves of *K. crenata.*
EE- Ethanol extract; **AE**- Acetone extract; **CE**- Chloroform extract; **BE**- Benzene extract.

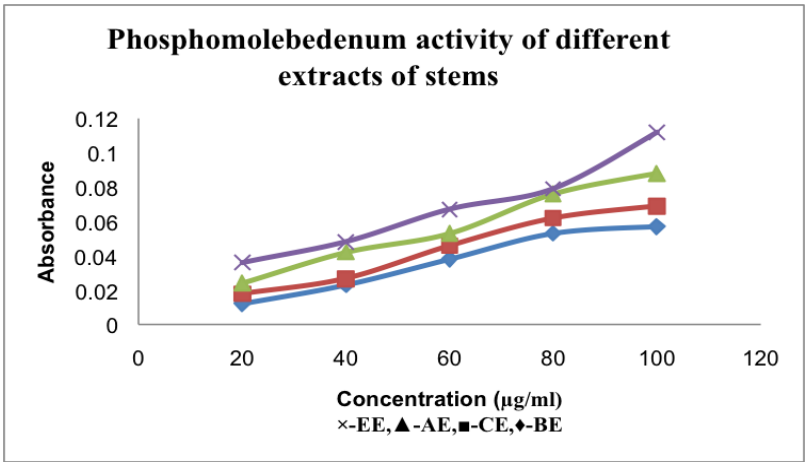

Figure 11: Antioxidant capacity by Phosphomolebedenum method of Benzene, Chloroform, Acetone & Ethanol extracts of stems of *K. crenata.*
EE- Ethanol extract; **AE**- Acetone extract; **CE**- Chloroform extract; **BE**- Benzene extract.

capacity of all the extracts was found to increase with increase in concentration [15] (Table **8**; Figures **11**, **12**).

CONCLUSION

The present study provides evidence that different extracts of *K. crenata* leaves and stems is potential source of antioxidant activity. The extracts were found to contain phenolic compounds which could be responsible for the antioxidant properties. So *K. crenata* traditional use is justified in the present research work.

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