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Research Article

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EVALUATION OF ANTIOXIDANT ACTIVITY OF *KALANCHOE PINNATA* ROOTS

Quazi Majaz*, Molvi Khurshid, Sayyed Nazim, Khan Rahil, Shikh Siraj
Ali Allana College of Pharmacy, Akkalkuwa, Dist Nandurbar, Maharastra, India

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***Corresponding author**
Email: quazimajaz@gmail.com

ABSTRACT
The plant *Kalanchoe pinnata* is widely used in ayurvedic system of medicine as astringent, analgesic, carminative and also useful in diarrhea and vomiting. Naturalized throughout the hot and moist parts of India. In this first roots are subjected to pet.ether, chloroform, methanol and aqueous solvent respectively for extraction. And evaluation of antioxidant activity was done by DPPH scavenging, Nitric oxide scavenging and reducing power assay. Methanolic extract of roots of *K. pinnata* was found to be most effective as antioxidant as compare to other.

Keyword: *Kalanchoe pinnata*, DPPH, Nitric oxide, ascorbic acid.

INTRODUCTION

Various species of Kalanchoe Adams are used medicinally in Indo-China and Philippines Islands, whereas *Kalanchoe pinnata* Pers. (synonyms: *Bryophyllum calycinum* Salisb. Parad. Lond., *B. pinnatum* Kurz.) (Family Crassulaceae) is naturalized throughout the hot and moist parts of India. The leaves and bark is bitter tonic, astringent to the bowels, analgesic, carminative, useful in diarrhoea and vomiting[1]. Antiulcer[2], anti-inflammatory[3&4] and antimicrobial activity[5] of leaf extract was reported. Oral treatment with leaf extract significantly delayed onset of disease in BALB/c mice infected with *Leishmania amazonensis* as compared to untreated mice or mice receiving *K. pinnata* by the intravenous or topical routes[6]. Potent cytotoxic compounds bersaldegenin-1,3,5-orthoacetate[7] and bufadienolide-bryophyllin B[8] were isolated. Other chemical constituents from this plant are bryophyllol, bryophollone, bryophollenone, bryophynol and two homologous phenanthrene derivatives 2(9-decenyl)-phenanthrene (I) and 2-(undecenyl)-phenanthrene (II) from leaves; 18α-oleanane, ψ-taraxasterol, α- and β-amyrins and their acetates also isolated[9]. Isolation and structure elucidation of 24-epiclerosterol [24(*R*)-stigmasta-5, 25-dien-3β-ol], 24(*R*)-5α-stigmasta-7, 25-dien-3β-ol, 5α-stigmast-24-en-3β-ol and 25-methyl-5α-ergost-24 (28)-en-3β-ol from aerial parts was done[10]. This species is also included in the plants species, which are used by the tribes of Kerala for treating cancer symptoms[11]. Juice of the fresh leaves is used very effectively for the treatment of jaundice in folk medicines of Bundelkhand region of India, but no systemic study to assess this activity has been carried out. As the aerial parts of plant have many pharmacological activity but roots of this plant was not focused yet hence the present investigations were carried out to evaluate the root of kalanchoe pinnata for its antimicrobial activity.

MATERIALS AND METHODS

Collection of plant material

The roots of *Kalanchoe pinnata* was collected from Satpuda hills near Akkalkuwa, Dist: Nandurbar, Maharashtra, India, in June 2010, cleaned and dried at room temperature in shade and away from direct sunlight. The plant authenticated by T. Chakraborthy, Deputy Director Botanical Survey of India, Koregaon Road Pune, by comparing morphological features and a sample voucher specimen of plant was deposited for future reference (Voucher specimen number QMAKP1).

Preparation of extract

The root of *Kalanchoe pinnata* was collected and dried in the shade and then pulverized in a grinder. The powdered drug was utilized for extraction. Material was passed through 120 meshes to remove fine powders and coarse powder was used for extraction. A method described in Mukherjee was used for extraction of powdered plant. Extraction was done by Pet. Ether, Chloroform, Methanol and Aqueous.[12]

Preliminary Phytochemical screening

The extracts were then subjected to preliminary phytochemical screening to detect the presence of various phytoconstituent. The results shows presence that petroleum ether extract contain steroids, the chloroform extract contain steroids and alkaloids, the methanolic extract contain Steroids, Saponins, Alkaloids, Glycosides, Flavonoids, Tannins, Carbohydrates, Proteins and aqueous extract contain Saponins, Glycosides, Flavonoids, Tannins, Carbohydrates, Amino acids.[13]

Quantitative estimation of phytoconstituents

Total phenolic content

Total soluble phenolics in the extracts were determined with Folin–Ciocalteau reagent according to the method using gallic acid as a standard phenolic compound; 1.0 ml of extract solution containing 1.0 g extract in a volumetric flask was diluted with 46 ml of distilled water. 1.0 ml of Folin–Ciocalteau reagent was added and mixed thoroughly. Three minutes later 3.0 ml of 2% sodium carbonate was added and the mixture was allowed to stand for 3 h with intermittent shaking. The absorbance of the blue color that developed was read at 760 nm. The concentration of total phenols was expressed as mg/g of dry extract. The concentration of total phenolic compounds in the extract was determined as µg of gallic acid equivalent using an equation obtained from the standard gallic acid graph.[14]

Total flavonoid content

A known volume of extract was placed in a 10 ml volumetric flask. Distilled water was added to make 5 ml, and 0.3 ml $NaNO_2$ (1:20) were added. 3 ml $AlCl_3$ (1:10) were added 5 min later. After 6 min, 2 ml 1 mol litre^{-1} NaOH was added and the total was made up to 10 ml with distilled water. The solution was mixed well again and the absorbance was measured against a blank at 510 nm with a UV-VISIBLE spectrophotometer. Quercetin was used as the standard for a calibration curve. The flavonoid content was calculated using the following linear equation based on the calibration curve.[15]

Evaluation of Antioxidant activity

DPPH scavenging activity

This assay based on the measurement of the scavenging ability of antioxidant test extract towards the stable radical. The free radical scavenging activity of the plant extracts were examined *in vitro* using DPPH [1,1-Diphenyl,2-picryl-hydrazyl] radical. The test

extract treated with different concentration (5µg/ml-200µg/ml). The reaction mixture consists of 2ml of 100 µM DPPH in ethanol, 2ml of extract solution, after 30min at room temperature, and the absorbance was recorded at 517nm. The experiment was repeated for three times. Ascorbic acid was used as standard controls. IC50 value was calculated from %inhibition which was calculated by following formula.[16]

$$\% \text{ Inhibition} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

Nitric oxide scavenging activity

Sodium nitroprusside (5mM) in standard phosphate buffer solution was incubated with different concentration of extracts dissolved in standard phosphate buffer (pH 7.4) and the tubes were incubated at 25º C for 5 hours. After 5 hours, 0.5ml incubated solution was removed and diluted with 0.5ml of Griese reagent. The absorbance of chromophore formed was read at 546nm.[17]

$$\% \text{ inhibition} = \frac{\text{O.D of standard} - \text{O.D of test} \times 100}{\text{O.D of standard}}$$

Reducing power assay

The reductive potential of plant extracts were determined according to the method of The reaction mixture containing varying concentrations of the plant extract (5–200 µg/ml) and standard Ascorbic acid (0.1-1.0 µg/ml) in 1 ml of distilled water, phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1% w/v) was incubated at 50 °C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10% w/v) was added to the mixture, which was then centrifuged for 10 min at 1000*g*. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1% w/v), and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reductive potential.[18]

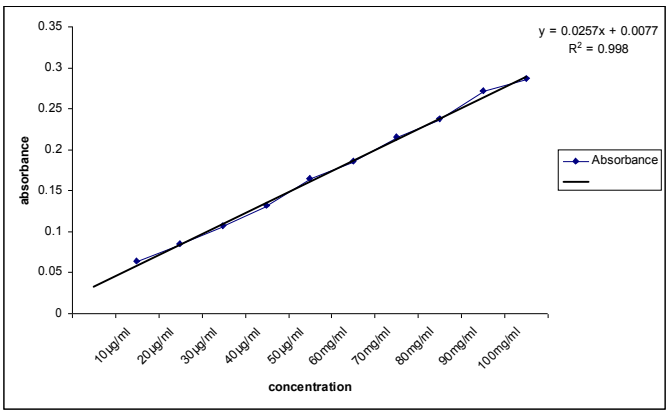
RESULTS AND DISCUSSION

Quantitative estimation of phytoconstituent

Total Phenolic Content

Table 1: Absorbance for Total Phenolic content

Sr. No.	concentration	Absorbance
1	10µg/ml	0.063
2	20µg/ml	0.085
3	30µg/ml	0.107
4	40µg/ml	0.132
6	60mg/ml	0.186
7	70mg/ml	0.215
8	80mg/ml	0.237
9	90mg/ml	0.271
10	100mg/ml	0.287



Graph 1: Concentration response curve for Gallic acid at different concentration

Table 2: Results of total phenolic content

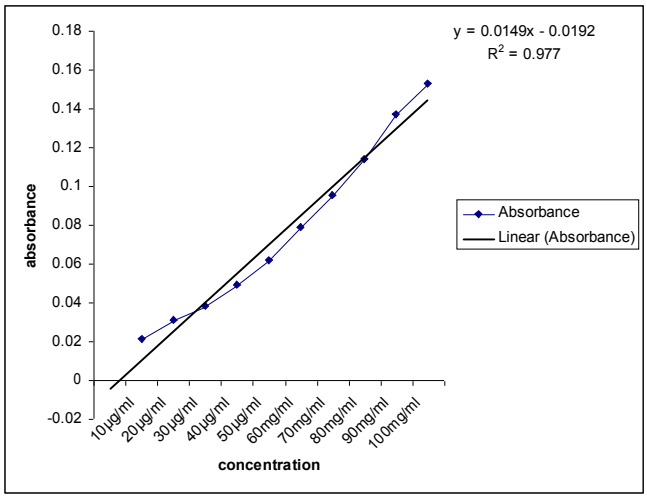
Sr. no	Sample	Absorbance	Concentration% w/w
1	Chloroform extract	0.072	17.00
2	Methanolic extract	0.149	43.28
3	Aqueous extract	0.065	10.12

Equation Y=0.0257X + 0.0077 was obtained from graph 1. From this equation concentration of extract was determine. The total Phenolic content of Chloroform, Methanol and Aqueous extract was found to be 17%, 43.28% and 10.12% w/w respectively.

Total flavonoids content

Table 3: Absorbance for Total Flavonoid content

Sr. No.	concentration	Absorbance
1	10µg/ml	0.021
2	20µg/ml	0.031
3	30µg/ml	0.038
4	40µg/ml	0.049
5	50 µg/ml	0.062
6	60 µg/ml	0.079
7	70 µg/ml	0.095
8	80 µg/ml	0.114
9	90 µg/ml	0.137
10	100 µg/ml	0.153



Graph 2: Concentration response curve for Quercetin at different concentration

Table 4: Result of Total Flavonoid content

Sr. no	Sample	Absorbance	Concentration% w/w
1	Chloroform extract	0.023	10.20
2	Methanolic extract	0.034	25.38
3	Aqueous extract	0.015	7.40

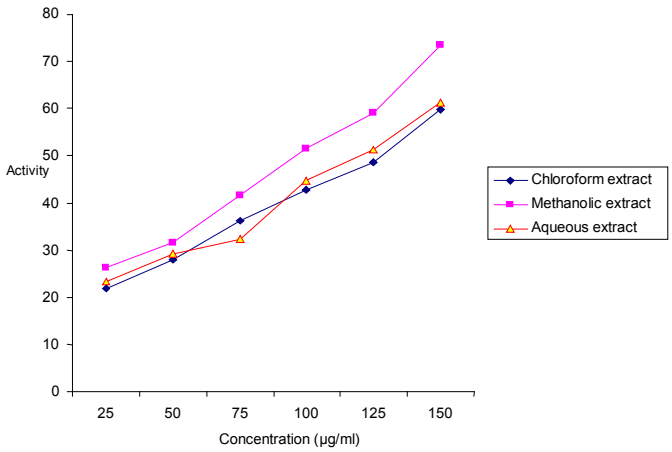
Equation Y=0.0149X - 0.0192 was obtained from graph 2. From this equation concentration of extract was determine. The total Flavonoid content of Chloroform, Methanol and Aqueous extract was found to be 10.20%, 25.38, 7.40% w/w respectively.

Evaluation of Antioxidant activity of extracts

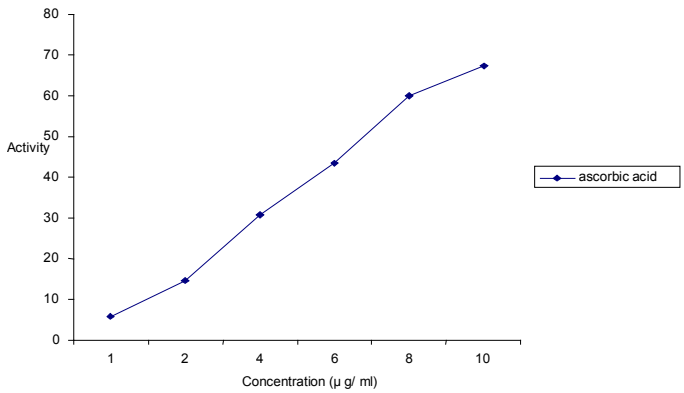
DPPH scavenging activity

Table 5: Results of DPPH scavenging activity

	% Scavenging activity					
	Conentraction(µg/ml)					
Herbal extract	25	50	75	100	125	150
Chloroform extract	21.97	28.08	36.3	42.75	48.71	59.75
Methanolic extract	26.18	31.73	41.56	51.56	58.98	73.37
Aqueous extract	23.38	29.3	32.28	44.78	51.19	61.39
Ascorbic acid	1	2	4	6	8	10
	5.92	14.7	30.73	43.43	60.04	67.24



Graph 3: Results of DPPH scavenging activity of various extract



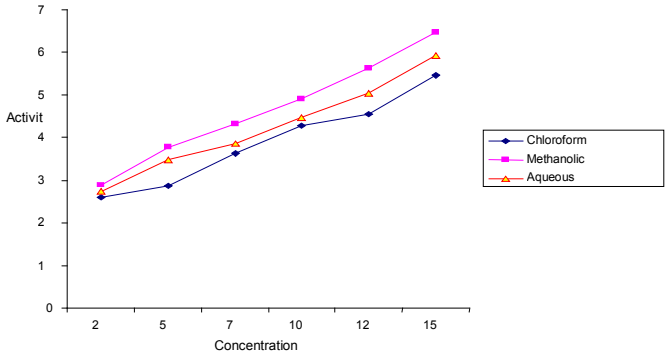
Graph 4: Results of DPPH scavenging activity of ascorbic acid

Ic 50 Value of chloroform, methanolic and aqueous extract was found to be 130, 92 and 117 µg/ml (graph 3) respectively, by the comparison of standard curve of the DPPH scavenging activity of ascorbic acid (graph 4).

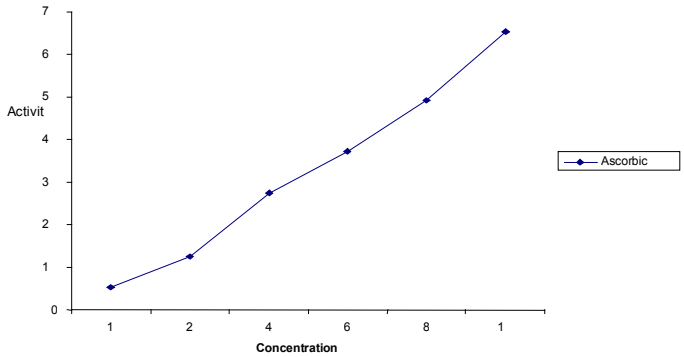
Nitric oxide scavenging activity

Table 6: Results of Nitric oxide scavenging activity

	% Scavenging activity					
	Conentraction(µg/ml)					
Herbal extract	25	50	75	100	125	150
Chloroform extract	25.92	28.71	36.16	42.77	45.5	54.6
Methanolic extract	28.96	37.8	43.29	49.18	56.28	64.63
Aqueous extract	27.41	34.74	38.68	44.74	50.47	59.33
Ascorbic acid	1	2	4	6	8	10
	5.4	12.46	27.35	37.23	49.17	65.39



Graph 5: Results of Nitric oxide scavenging activity of various extract



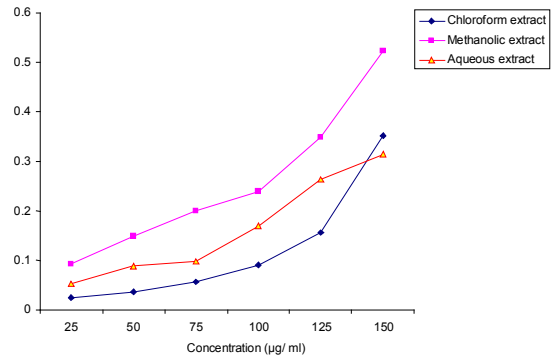
Graph 6: Results of Nitric oxide scavenging activity of ascorbic acid

Ic 50 Value of chloroform, methanolic and aqueous extract was found to be 132, 100 and 125 µg/ml (graph 5) respectively, by the comparison of standard curve of the Nitric oxide scavenging activity of ascorbic acid (graph 6).

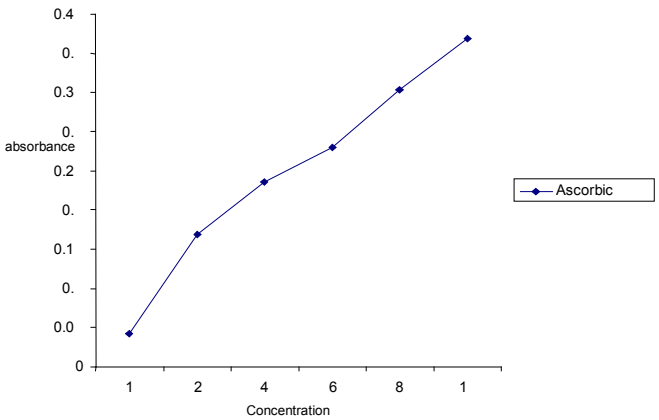
Reducing Power determination

Table 7: Observation of Reducing Power determination

	Absorbance					
	Concentration(µg/ml)					
Herbal extract	25	50	75	100	125	150
Chloroform extract	0.025	0.036	0.056	0.091	0.156	0.351
Methanolic extract	0.093	0.148	0.199	0.238	0.348	0.522
Aqueous extract	0.052	0.088	0.098	0.170	0.264	0.315
Ascorbic acid	1	2	4	6	8	10
	0.042	0.169	0.236	0.280	0.353	0.418



Graph 7: Concentration response curve of Reducing Power determination for various extract



Graph 8: Concentration response curve of Reducing Power determination for ascorbic acid

Absorbance Value of chloroform, methanolic and aqueous extract was found to be in increasing order (graph 7) respectively, by the comparison of standard curve of the absorbance of reducing power assay of ascorbic acid (graph 8). The methanolic extract shows highest absorbance as well as antioxidant activity as compare to other extract like chloroform and aqueous extract (Table 8).

CONCLUSION

From the above discussion it was concluded that the methanolic extract have significant anti oxidant activity than the chloroform and aqueous extract by comparing with ascorbic acid as standard.

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