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EVALUATION OF ANTIOXIDANT ACTIVITY OF *TUBIFLORA ACAULIS*

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Abstract

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

INTRODUCTION

It is a perennial herb with a short creeping sometimes branched rootstock; apical part of root stock (where leaves attached) with long (up to 3 mm) hairs. Leaves in a basal rosette, sub-sessile; lamina elliptic to obovate, apex sub-acute to broadly rounded, base attenuate. Spikes 2–10 cm long, branched or un-branched; peduncle 6–20 cm long; sterile bracts imbricate, ovate, 4.5–7 mm long, acuminate, glabrous but for the finely ciliate margin. Corolla white, when fully developed with tube 5–7

mm long and lobes 2–3 mm long; cleistogamous flowers not seen in East African material, but common in southern Africa. [1]

Tubiflora acaulis is well known plant used all over the world in folk medicine for various purposes. In Rajasthan whole plant is used in Abscess of mammary glands, boils, burns, colic, diarrhoea, rickets, throat compliments, and tonsillitis. Decoction of root is mixed in equal amount in local liquor and one cup of this mixture is taken daily for 3–4 days in the morning for easy expulsion of guinea-worm. Half tea spoon root extract is given to children once a day for two days in Asthma. [2] The plant is used in aravali hills as insecticidal and wormicidal.[3] In Rajasthan Leaf powder with water is used for Kidney Stone.[4] Half tea spoon of root extract is used in asthma and migraine.[5]

Beside of various use of plant it is not yet well explored as there are only few literatures on that plant. The methanol extract contain two pyrazole alkaloids with asomnine (120 mg) and4'-hydroxywithasomnine (30 mg).[6] and 4H-1-Benzopyran-4-one,3-((6-O-(6-deoxy-beta-Lmannofuranosyl)- beta-O-galactofuranosyl)oxy)-7-((6-deoxy-beta-mannopyranosyl) oxy)-5-hydroxy-2-(4-hydroxyphenyl), having molecular formula C27 H30O15 and molecular weight is 595.518 g/mol.[7]

MATERIALS & METHODS

Collection of plant material

The plant Tubiflora acaulis was collected from Satpuda hills near Akkalkuwa, Dist: Nandurbar, Maharashtra, India, in September 2011, cleaned and dried at room temperature in shade and away from direct sunlight. The dried aerial part was coarsely powdered in grinder. Large difference in particle size of crude drug results in long extraction time as the coarse particles increases the extraction time and fine may form bed, so the powdered material was sieved through 60-120 mesh to remove fine and the powder was subjected for further study.

The plant authenticated by Dr. D. A. Patil, HOD, Department of Botany, S S V P S College, Dhule by comparing morphological features and a sample voucher specimen of plant was deposited for future reference.

Preparation of extract

The plant Tubiflora acaulis 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.[8]

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.[9]

Quantitative estimation of phytoconstituents

I. 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.[10]

II. 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. [11]

Evaluation of Antioxidant activity

I] Peroxide scavenging activity

One milliliter of extract prepared in phosphate buffer was incubated with 0.6 ml of 4mM H_2O_2 solution for 10 min. the absorbance of the solution was considered at 230nm against blank solution. [12]

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

II. 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. [13]

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

III. 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. [14]

RESULTS AND DISCUSSION

Quantitative estimation of phytoconstituent

I. Total Phenolic Content:

Equation Y=0.031X + 0.015 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 15.16%, 36.12% and 15.34% w/w respectively.

II. Total flavonoids content

Equation Y=0.0149X - 0.006 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 19.58%, 30 %, 5.40% w/w respectively.

Evaluation of Antioxidant activity of extracts:

I. Peroxide scavenging activity

Ic 50 Value of chloroform, methanolic and aqueous extract was found to be 128, 103 and 120 µg/ml (graph 3) respectively, by the comparison of standard curve of the Peroxide scavenging activity of ascorbic acid (graph 4).

II. Nitric oxide scavenging activity

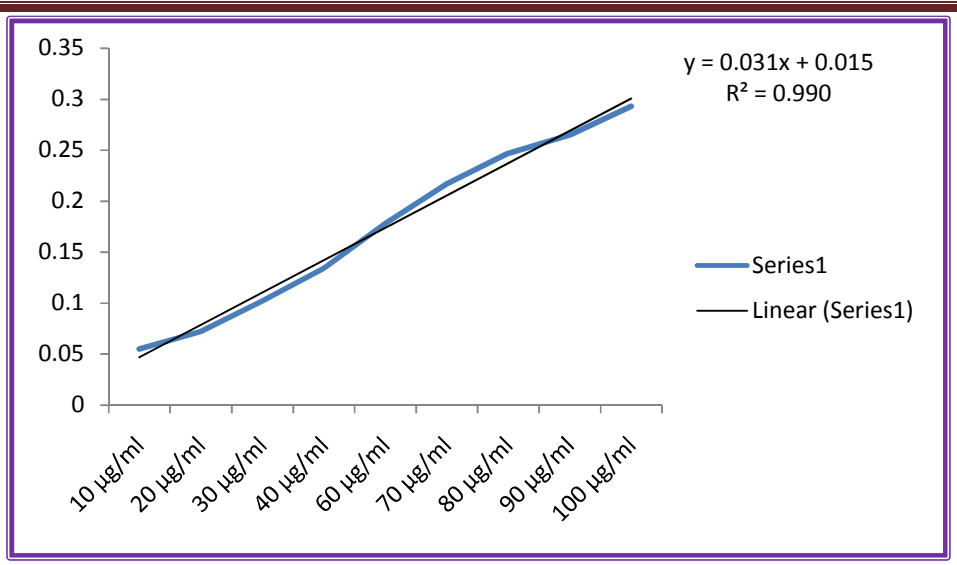
Ic 50 Value of chloroform, methanolic and aqueous extract was found to be 139, 109 and 125 µg/ml (graph 5) respectively, by the comparison of standard curve of the Nitric oxide scavenging activity of ascorbic acid (graph 6).

III. Reducing Power determination

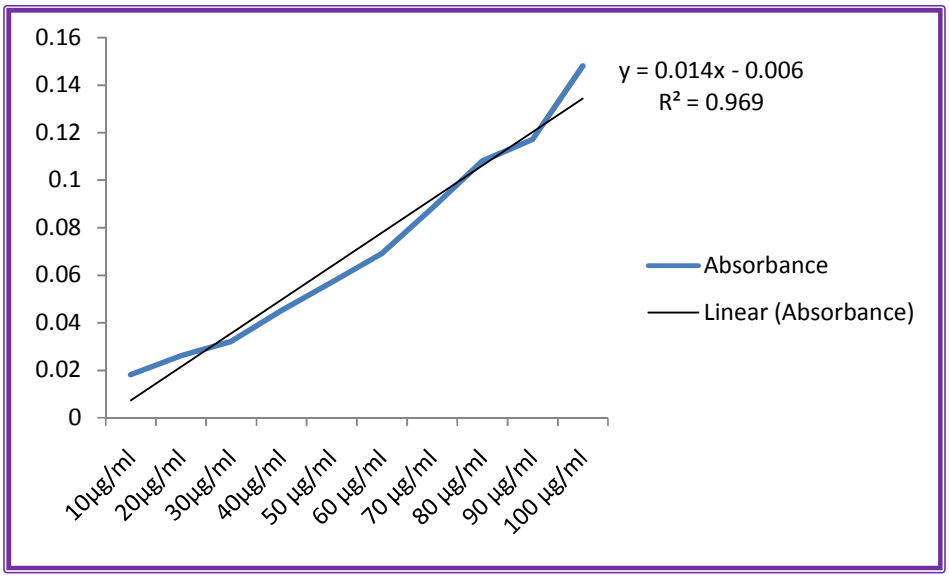
Absorbance Value of chloroform, methanolic and aqueous extract was found to be in increasing order (graph 7) respectively, by the comparison of standard curve of the absorbance of reducing power assay of ascorbic acid (graph 8). The methanolic extract shows highest absorbance as well as antioxidant activity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

CONCLUSION

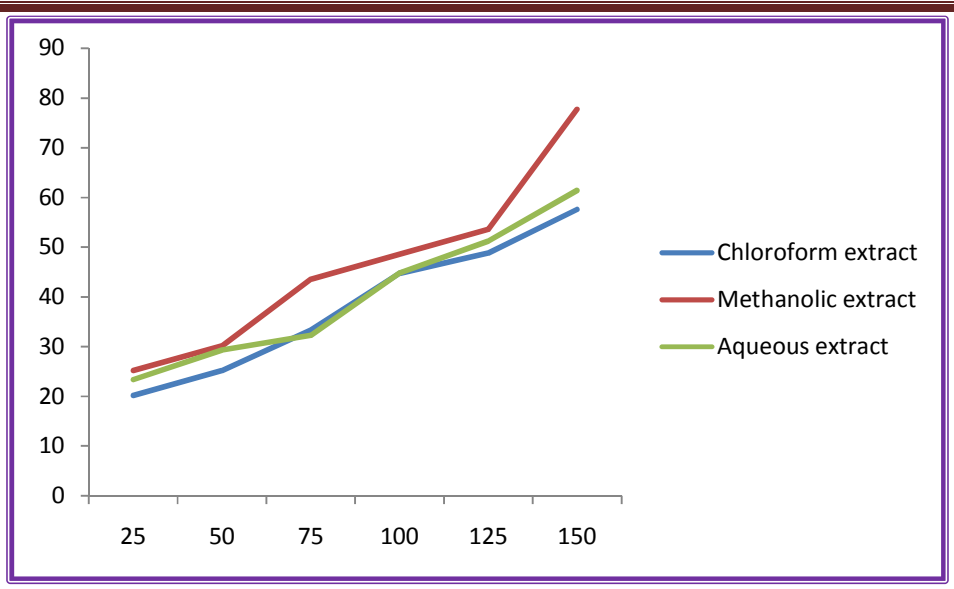
From the above discussion it was concluded that the methanolic extract of Tubiflora acaulis have significant anti oxidant activity than the chloroform and aqueous extract by comparing with ascorbic acid as standard.



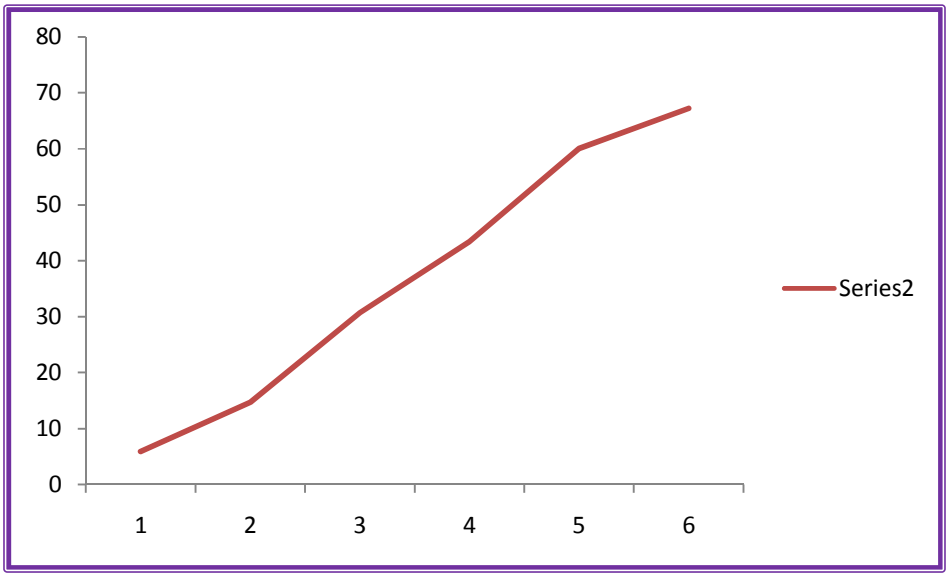
Graph 1.Concentration response curve for Gallic acid at different concentration



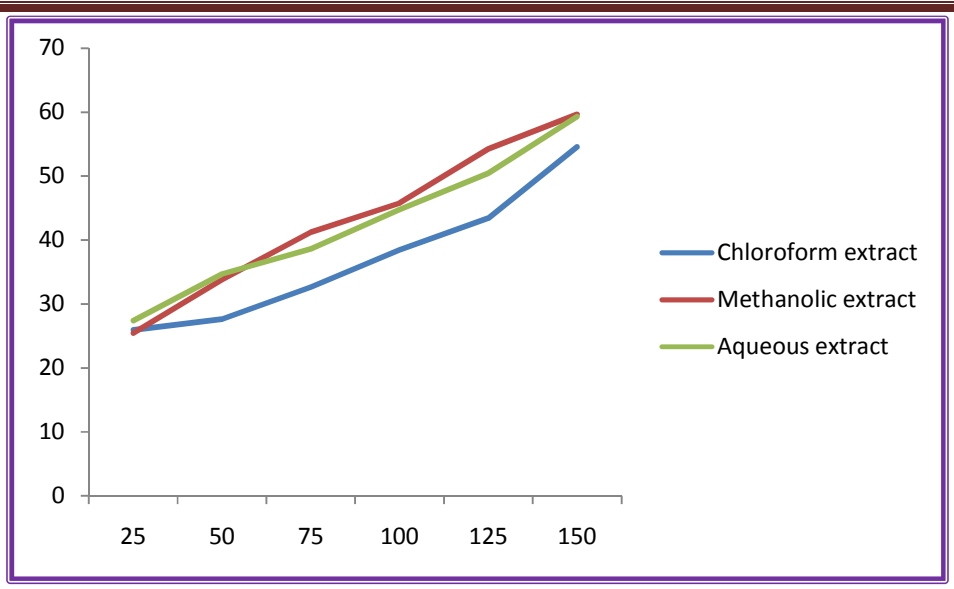
Graph 2. Concentration response curve for Quercetin at different concentration



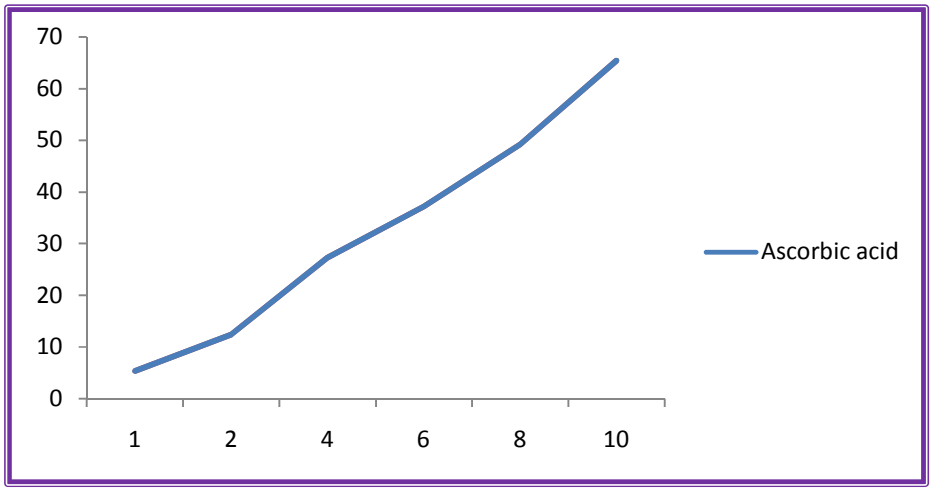
Graph 3. Results of Peroxide scavenging activity of various extract



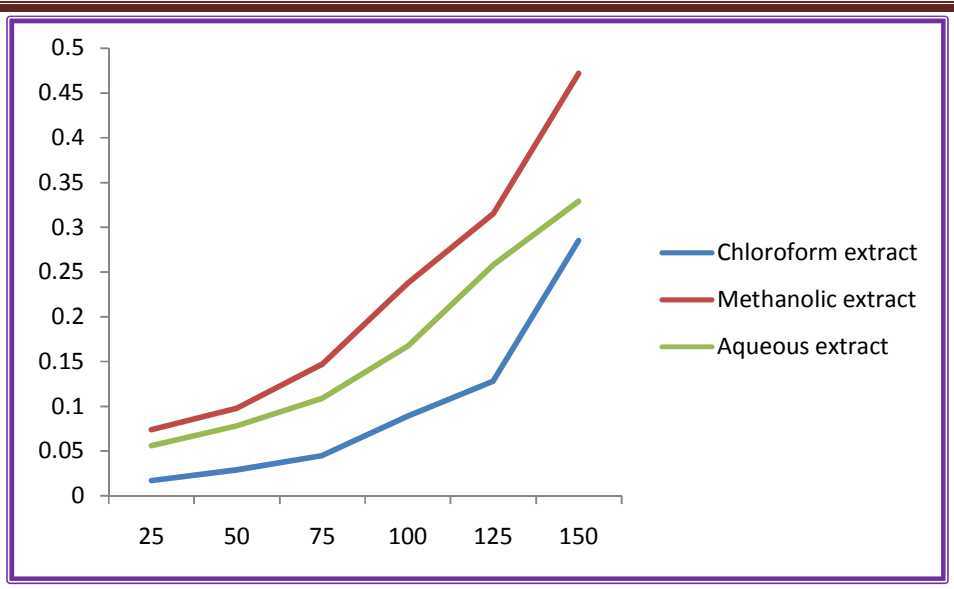
Graph 4. Results of Peroxide scavenging activity of ascorbic acid



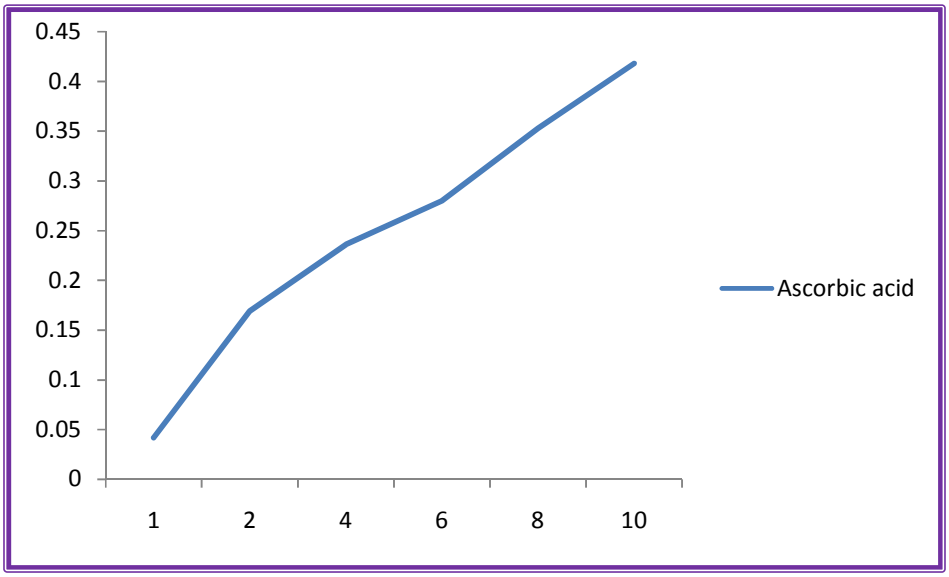
Graph 5. Results of Nitric oxide scavenging activity of various extract



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



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



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

Table 1

Absorbance for Total Phenolic content

Sr. No.	concentration	Absorbance
1	10 µg/ml	0.055
2	20 µg/ml	0.072
3	30 µg/ml	0.102
4	40 µg/ml	0.134
6	60 µg/ml	0.178
7	70 µg/ml	0.217
8	80 µg/ml	0.247
9	90 µg/ml	0.265
10	100 µg/ml	0.293

Table 2

Results of total phenolic content

Sr. no	Sample	Absorbance	Concentration% w/w
1	Chloroform extract	0.062	15.16
2	Methanolic extract	0.127	36.12
3	Aqueous extract	0.065	15.34

Table 3

Absorbance for Total Flavonoid content

Sr. No.	concentration	Absorbance
1	10µg/ml	0.018
2	20µg/ml	0.026
3	30µg/ml	0.032
4	40µg/ml	0.045
5	50 µg/ml	0.057
6	60 µg/ml	0.069
7	70 µg/ml	0.088
8	80 µg/ml	0.108
9	90 µg/ml	0.117
10	100 µg/ml	0.148

Table 4

Result of Total Flavonoid content

Sr. no	Sample	Absorbance	Concentration% w/w
1	Chloroform extract	0.025	19.58
2	Methanolic extract	0.032	30.00
3	Aqueous extract	0.009	5.40

Table 5

Results of Peroxide scavenging activity

	% Scavenging activity					
	Concentration(µg/ml)					
Herbal extract	25	50	75	100	125	150
Chloroform extract	20.17	25.18	33.3	44.72	48.78	57.56
Methanolic extract	25.17	30.13	43.55	48.54	53.58	77.67
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

Table 6

Results of Nitric oxide scavenging activity

	% Scavenging activity					
	Concentration(µg/ml)					
Herbal extract	25	50	75	100	125	150
Chloroform extract	25.92	27.61	32.66	38.45	43.45	54.6
Methanolic extract	25.46	33.85	41.27	45.78	54.26	59.67
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

Table 7

Observation of Reducing Power determination

	Absorbance					
	Concentration(µg/ml)					
Herbal extract	25	50	75	100	125	150
Chloroform extract	0.017	0.029	0.045	0.089	0.128	0.285
Methanolic extract	0.074	0.098	0.147	0.238	0.315	0.472
Aqueous extract	0.056	0.078	0.109	0.167	0.258	0.329
	1	2	4	6	8	10
Ascorbic acid	0.042	0.169	0.236	0.280	0.353	0.418

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