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IN VITRO ANTIOXIDANT ACTIVITY OF VARIOUS EXTRACTS/FRACTIONS OF *IXORA PAVETTA*

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ABSTRACT

Free radicals play an important role in the development of tissue damage and pathological events in living organism. There is a compelling evidence indicates that increased consumption of antioxidants from fruits and vegetables reduces the risk of diseases associated with oxidative stress. In our present study aqueous and ethanolic extract of leaves of *Ixora pavetta* have been evaluated for *in vitro* antioxidant activity. Among both extract, ethanolic extract showed potent antioxidant activity. Hence this ethanolic extract was further fractionated using various solvents systems (Pet ether – Chloroform - Ethyl acetate – n Butenol) and evaluated for antioxidant potential. DPPH assay, reducing power assay and H_2O_2 scavenging assay have been used to evaluate antioxidant potential. Ascorbic acid was used as reference compound for all the assays. All the analysis was made with the use of UV-Visible spectrophotometer. The results of all assays showed that extracts / fractions of *Ixora pavetta* leaves possess significant free radical scavenging and reducing power properties. n- butenol fraction separated from crude ethanolic extract was found to posses more superior antioxidant potential among all tested fractions in *in vitro* assays. Hence, it can be concluded that the *Ixora pavetta* leaves could be pharmacologically exploited for oxidative stress related disorders.

Key words: Antioxidant, DPPH, H_2O_2 Assay, *Ixora pavetta*, Reducing power assay.

INTRODUCTION

Oxidation is the chemical process by which an atom, molecules or ion robs another of one or more of its electrons. Chemicals exhibiting this tendency for stealing electron are referred to as oxidizing agents. The most familiar oxidizing agents are oxygen itself. Oxidation reactions may involve highly reactive molecules called free radicals. In simple words, free radicals are molecules that have lost an electron and try to replace it by reacting with other molecules. It act as electron acceptors and essentially "steal" electrons from other molecules. Free radicals referred to as oxidizing agents and they tend to cause other molecules to donate their electrons [1].

The most common cellular free radicals are superoxide radical (O_2-*), hydroxyl radical (OH*), and nitric oxide (NO*) [2, 3]. Other molecules, such as hydrogen peroxide (H_2O_2) and peroxynitrate (ONOO*) are not free radicals but can lead to their generation through various chemical reactions. Free radicals and related molecules are often classified together as reactive oxygen species (ROS) and reactive nitrogen species (RNS) to signify their ability to promote oxidative changes within the cell [3]. The phenomenon of oxidative modification is called as oxidative stress.

Oxidative stress has been reported to be linked with several diseases like diabetes, atherosclerosis, cancer, and tissue damage in rheumatoid arthritis [4, 5]. Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress [6]. In simple term, Antioxidants are exogenous (natural or synthetic) or endogenous compounds acting in several ways including removal of O_2, scavenging reactive oxygen/nitrogen species or their precursors, inhibiting ROS formation and binding metal ions needed for catalysis of ROS generation and up-regulation of endogenous antioxidant defenses. The protective efficacy of antioxidants depends on the type of ROS that is generated, the place of generation and the severity of the damage [1, 7].

At present most of the antioxidants are manufactured synthetically. They belong to the class of

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synthetic antioxidants. The main disadvantage with the synthetic antioxidants is the side effects when taken in vivo [8]. A strict governmental rule regarding the safety of the food and drug has necessitated the search for alternatives natural antioxidants [9]. The growing interest in the antioxidant properties of the natural phytochemical from plant source derives from their strong activity and low toxicity compared with those of synthetic phenolics antioxidant such as BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisole), and propyl gallate [10].

Recently, there have been great efforts to find safe and potent natural antioxidants from various plant sources. Plants are the potential source of natural antioxidants. Natural antioxidants or phytochemical antioxidants are the secondary metabolites of plants [11]. Carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, tocotrienols etc, are some of the antioxidants produced by the plant for their sustenance. Beta-carotene, ascorbic acid and alpha tocopherol are the widely used antioxidants [12].

The plant, *Ixora pavetta* Andr is reported for presence of various phytochemicals like essential oil, flavanoids, saponins, resins, phytosterols, alkaloids, tannins. Due to presence of these phytochemicals, various parts of plants like flowers, root and leaves are claimed to have medicinal benefits in various disorders like such as dysentery, leucorrhoea, dysmenorrhea, haemoptysis bitter tonic, aperients, purgative, urinary disorders and is frequently prescribed in visceral obstructions [13, 14] .

However, effect of *Ixora pavetta* or its phytoconstituents have not reported for antioxidant effect. . Therefore, the objectives of this study were to evaluate *Ixora pavetta* as a source of natural antioxidants using different extracting solvents to determine their antioxidant capacities in various *in vitro* models. Three methods namely DPPH radical assay, reducing power assay and H_2O_2 scavenging assay were used to find out and compaire antioxidant potential of various extract and fractions of *Ixora pavetta* .

MATERIALS AND METHODS

Collection of Plant material

Fresh leaves of *Ixora pavetta* was obtained from local area of Kadapa & authentified by Botanist; S.V University, Tirupathi, (A.P). The specimen voucher of same is kept in department of pharmacology; PRRM College of Pharmacy; Kadapa.

Extraction and fractionation of Plant material

The collected plant material of *Ixora pavetta* was washed thoroughly in water, and air dried for two weeks at 35-40° C temperature. The 500 gm of air dried and coarsely powdered material of plants were extracted with two solvent i.e water and 95% of ethanol by cold maceration method for 24 and 72 hrs respectively. Then these extract was filtered with muslin cloth and filtrate was evaporated under reduced pressure and vacuum dried. This yielded a brownish and greenish residue of 38 % and 25% W/W extract respectively with reference to dry starting material. Further alcoholic extract was fractioned with non polar solvents to polar solvents system (Pet ether – Chloroform - Ethyl acetate – n Butenol).

Aqueous extract (Aq IP), Alcoholic / Ethanolic extract (Et IP), Pet ether fraction (PetE IP), Chloroform fraction (Chl IP), Ethyl acetate fraction (Etace IP) and n-Butenol fraction (nBut IP) were subjected for *in vitro* antioxidant assay.

Chemicals

1'-Diphenyl-2-picryl-hydrazyl (DPPH) was procured from Sigma-Aldrich. Ascorbic acid, Potassium Ferricyanide, Trichloro acetic acid, Ferric chloride and hydrogen peroxide were obtained from either Fluka Chemicals or Merck Chemicals. All other materials used were analytical grade

Evaluation of Antioxidant activity by *in vitro* techniques

DPPH assay: (1, 1-diphenyl-2-picrylhydrazyl)

Principle

The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as:

$$(DPPH) + (H\text{-}A) \longrightarrow DPPH\text{-}H + (A)$$

(Purple) (Yellow)

Antioxidants react with DPPH, which is a stable free radical and is reduced to the DPPHH and as consequence the absorbance's decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Procedure

The effect of extracts on DPPH radical was estimated by Elusiyan et al. [15] with minor modification. In brief, 1 mL of DPPH in methanol (0.3 Mm) was added to 2.5 ml of various concentrations of extracts and fraction (5 µg/ml – 500 µg/ml). The mixture was vortexed for 15 sec and left to stand at 37^0C for 30 min. Thirty minutes later, the absorbance was measured at 517 nm. All determination was performed in triplicate. Ascorbic acid at various concentrations (5 µg/ml – 500 µg/ml) was used as standard. MeOH (2.5ml) plus DPPH solution was used as control. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated in terms of % inhibition using the following equation.

$$\% \text{ Inhibition} = [Abs_{Control} - Abs_{Sample}]/Abs\ Control \times 100$$

IC50 value for reference and all test compounds were determined by using Graph pad prism analysing tool.

Reducing power assay

Principle

Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

$$\text{Potassium ferricyanide + Ferric chloride} \xrightarrow{\text{Antioxidant}} \text{Potassium ferrocyanide + ferrous chloride}$$

Procedure

Reducing power assay was performed as per Arulpriya et.al [16]. Various concentrations (5 µg/ml – 500 µg/ml) of the plant extracts and fractions in 1.0 ml of de-ionized water were mixed with phosphate buffer (0.2 M, pH 6.6) (2.5 ml) and 1% w/v potassium ferricyanide (2.5 ml) and incubated at 50^0C for 20 min. 10% Trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min whenever necessary. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared 0.1% ferric chloride solution **(**0.5 ml). The absorbance was measured at 700 nm. A control was prepared without adding extract. Ascorbic acid at various concentrations (5 µg/ml – 500 µg/ml) was used as standard. All determination was performed in triplicate. Increased absorbance of the reaction mixture indicates increase in reducing power.

$$\% \text{ increase in Reducing Power} = \frac{A_{\text{sample}} - 1}{A_{\text{Control}}} \times 100$$

Where A_{sample} is absorbance of test solution; $A_{control}$ is absorbance of Control.

Hydrogen Peroxide Scavenging Capacity

Principle

The principle of this method is that there is a decrease in absorbance of H_2O_2 upon oxidation of H_2O_2.

$$H_2O_2 + \text{Antioxidant} \xrightarrow{\text{Oxidation}} \text{Absorbance of } H_2O_2$$

Procedure

The ability of the *Ixora pavetta* extracts and fractions to scavenge hydrogen peroxide was determined according to the method of Reddy et al [17]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Various concentrations (5 µg/ml – 500µg/ml) of the plant extracts in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. Ascorbic acid at various concentrations (5 µg/ml – 500 µg/ml) was used as standard. All determination was performed in triplicate.

The percentage of H2O2 scavenging by samples and standard compound was calculated using the following formula:

% Scavenged [H2O2] = $[(A_C - A_S)/A_C] \times 100$

Where A_C is the absorbance of the control and A_S is the absorbance in the presence of the sample.

Statistical Analysis

All data are presented as means ± SD for at least three replications for each prepared sample. % Coefficient of variant (CV) for each assay is represented in order to express the precision and repeatability test results. % CV less than 15 are accepted. Statistical analysis, plots, linear regression fits were carried using Graph Pad Prism 5.01.

RESULTS

DPPH assay

DPPH has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances. The DPPH radical-scavenging capacity was reported after 30 min reaction time for all samples evaluated. The parameter used to measure the radical scavenging activity of extracts and fractions evaluated is IC50 value, defined as the concentration of antioxidant required for 50% scavenging of DPPH radicals in this specified time period. The smaller IC50 value represents the higher antioxidant activity of the plant extract/fraction. The IC50 value of various plant extracts/fractions and positive controls were shown in Table No. 1 and 2 as well as Figure no. 1 and 2.

Among both tested extract of *Ixora pavetta* , ethanolic extract was found to be more active. IC 50 value for DPPH scavenging for the aqueous and ethanolic extract was found to be 72.64 and 51.88 µg/ml, while IC50 for the reference compound was found to be 4.46 µg/ml.

When ethanolic extract of *Ixora pavetta* was further fractionated, only n- butenol fraction (IC 50 = 59.60 µg/ml) was found equally active as ethanolic extract. Other fractions like Pet ether fraction (IC_{50} = 370.9 µg/ml), chloroform fraction (IC_{50} =443.6 µg/ml) and ethyl acetate (IC_{50} = > 500 µg/ml) were found to have low DPPH scavenging activity.

Reducing Power assay

The reducing power assay was performed as per Arulpriya et.al (2010). This method is based on ability of the sample to reduce ferric cyanide to ferro cyanide. The formation of Fe 2+ ions is measured at 700 nm. A higher absorbance at 700nm indicates a higher reducing power. The results of the reducing power assay are given in Table 3, 4 and figure 3, 4. Both extract as well as various fraction of *Ixora pavetta* showed concentration dependant increase of reducing power. At highest tested concentration i.e 500µg/ml aqueous extract showed reducing power of 64.58 % while ethnanolic extract showed reducing power of 78.70 %. It clearly indicates high antioxidant potential of ethanolic extract. Among all fractions, reducing power of n-butenolic fraction and pet ether fraction of *Ixora pavetta* were found to be with strong reducing power than ethanolic extract. Other fraction like ethyl acetate fraction and chloroform fraction of *Ixora pavetta* were found with poor reducing power. At 500 µg/ml concentration, the relative reducing power of the fractions of *Ixora pavetta* was as follows nBut IP > PetE IP > Etace IP > Chl IP. Ascorbic acid was used as reference compound, showed reducing power (at 500 µg/ml) of 97 – 98 % in both set experiments.

Hydrogen Peroxide Scavenging Capacity

The ability of the *Ixora pavetta* extracts and fractions to scavenge hydrogen peroxide was determined according to the method of Reddy et al (2012). The

compound having antioxidant potential will cause the oxidation of hydrogen peroxide, which will result in to reduction of the absorbance of hydrogen peroxide at 230 nm. The results were represented in % H2O2 scavenging capacity and showed in Table 5, 6 and figure 5, 6.

Percentage H2O2 scavenging capacity of aqueous extract, ethanolic extract and ascorbic acid at 500 µg/ml was found to be 69.24 %, 77.25 % and 88.23 % respectively.

Ethanolic extract of *Ixora pavetta* was further fractionated and tested for H2O2 scavenging capacity. Only n- butenol fraction and Pet ether fraction was found with comparable scavenging activity with ethanolic extract. Other fractions like chloroform fraction and ethyl acetate fraction were found with low H2O2 scavenging activity. Percentage H2O2 scavenging activity of all tested fraction were as follows PetE IP = 71.40 %, Chl IP = 64.32 %, Etace IP = 67.36 % and nBut IP = 79.06 %.

Table 1. Scavenging activity on 2,2-diphenyl-1-picrylhidrazyl (DPPH) radical (Aqueous Extract (Aq IP) Vs Ethanolic extract (Et IP)

Extract/ Fraction	Concentration	Av OD ± SD	% CV	% Scavenging of DPPH
Aq IP	500 µg/ml	0.551 ± 0.0005	0.10	58.38
	250 µg/ml	0.553 ± 0.030	5.43	58.21
	125 µg/ml	0.613 ± 0.008	1.30	53.76
	50 µg/ml	0.675 ± 0.028	4.25	49.03
	25 µg/ml	0.666 ± 0.041	6.15	49.68
	10 µg/ml	0.840 ± 0.006	0.76	36.57
	5 µg/ml	0.914 ± 0.007	0.78	31.01
	2.5 µg/ml	0.962 ± 0.001	0.11	27.37
Et IP	500 µg/ml	0.441 ± 0.030	6.80	66.69
	250 µg/ml	0.55 ± 0.045	8.22	58.49
	125 µg/ml	0.613 ± 0.089	14.5	53.73
	50 µg/ml	0.695 ± 0.016	2.31	47.54
	25 µg/ml	0.736 ± 0.015	2.05	44.45
	10 µg/ml	0.796 ± 0.005	0.66	39.92
	5 µg/ml	0.788 ± 0.066	8.44	40.47
	2.5 µg/ml	0.909 ± 0.003	0.39	31.39
Ascorbic Acid	500 µg/ml	0.264 ± 0.0001	0.21	80.05
	250 µg/ml	0.266 ± 0.030	11.27	79.87
	125 µg/ml	0.325 ± 0.009	2.46	75.42
	50 µg/ml	0.388 ± 0.029	7.40	70.69
	25 µg/ml	0.379 ± 0.041	10.80	71.34
	10 µg/ml	0.553 ± 0.006	1.16	58.23
	5 µg/ml	0.627 ± 0.007	1.15	52.67
	2.5 µg/ml	0.675 ± 0.001	0.17	49.03

Table 2. Scavenging activity on 2,2-diphenyl-1-picrylhidrazyl (DPPH) radical (Various Fractions of Ethanolic extract and positive control)

Extract/ Fraction	Concentration	Av OD ± SD	% CV	% Scavenging of DPPH
PetE IP	500 µg/ml	0.574 ± 0.06	9.61	53.12
	250 µg/ml	0.648 ± 0.01	0.90	47.13
	125 µg/ml	0.799 ± 0.05	5.70	34.80
	50 µg/ml	0.889 ± 0.02	2.34	27.43
	25 µg/ml	0.913 ± 0.01	0.83	25.47
	10 µg/ml	0.979 ± 0.01	1.20	20.05
	5 µg/ml	1.033 ± 0.07	6.59	15.65
	2.5 µg/ml	1.217 ± 0.08	6.28	0.68
Chl IP	500 µg/ml	0.580 ± 0.02	2.89	52.63
	250 µg/ml	0.656 ± 0.02	3.37	46.42
	125 µg/ml	0.779 ± 0.09	11.48	36.41
	50 µg/ml	0.861 ± 0.02	1.87	29.71
	25 µg/ml	0.902 ± 0.02	1.67	26.37
	10 µg/ml	0.962 ± 0.01	0.55	21.47
	5 µg/ml	0.955 ± 0.07	6.97	22.07
	2.5 µg/ml	1.075 ± 0.00	0.34	12.24

Etace IP	500 µg/ml	0.704 ± 0.03	4.26	42.50
	250 µg/ml	0.813 ± 0.05	5.56	33.63
	125 µg/ml	0.876 ± 0.09	10.21	28.49
	50 µg/ml	0.958 ± 0.02	1.68	21.80
	25 µg/ml	0.999 ± 0.02	1.51	18.45
	10 µg/ml	1.059 ± 0.01	0.50	13.55
	5 µg/ml	1.052 ± 0.07	6.33	14.15
	2.5 µg/ml	1.217 ± 0.08	6.28	0.68
nBut IP	500 µg/ml	0.485 ± 0.00	0.12	60.38
	250 µg/ml	0.488 ± 0.03	6.17	60.19
	125 µg/ml	0.547 ± 0.01	1.47	55.37
	50 µg/ml	0.609 ± 0.03	4.72	50.26
	25 µg/ml	0.601 ± 0.04	6.83	50.97
	10 µg/ml	0.774 ± 0.01	0.83	36.79
	5 µg/ml	0.848 ± 0.01	0.85	30.78
	2.5 µg/ml	0.896 ± 0.00	0.13	26.83
Ascorbic Acid	500 µg/ml	0.246 ± 0.00	0.23	79.89
	250 µg/ml	0.249 ± 0.03	12.09	79.70
	125 µg/ml	0.308 ± 0.01	2.61	74.88
	50 µg/ml	0.370 ± 0.03	7.76	69.77
	25 µg/ml	0.362 ± 0.04	11.34	70.48
	10 µg/ml	0.535 ± 0.01	1.20	56.30
	5 µg/ml	0.621 ± 0.02	3.35	49.28
	2.5 µg/ml	0.737 ± 0.03	3.68	39.86

Table 3. Reducing power capacity of Aqueous Extract and Ethanolic extract of *Ixora pavetta*

Extract/ Fraction	Concentration	Av OD ± SD	% CV	% Increase in reducing power
Aq IP	500 µg/ml	0.412 ± 0.023	5.69	64.58
	250 µg/ml	0.390 ± 0.023	5.93	55.93
	125 µg/ml	0.354 ± 0.046	12.85	41.54
	50 µg/ml	0.307 ± 0.020	6.48	22.50
	25 µg/ml	0.277 ± 0.005	1.67	10.52
	10 µg/ml	0.263 ± 0.040	15.22	5.19
	5 µg/ml	0.220 ± 0.026	12.03	0.00
Et IP	500 µg/ml	0.447 ± 0.016	3.47	78.70
	250 µg/ml	0.411 ± 0.014	3.43	64.18
	125 µg/ml	0.384 ± 0.009	2.42	53.26
	50 µg/ml	0.346 ± 0.016	4.73	38.22
	25 µg/ml	0.315 ± 0.017	5.25	25.83
	10 µg/ml	0.283 ± 0.013	4.75	13.05
	5 µg/ml	0.269 ± 0.018	6.70	7.46
Ascorbic Acid	500 µg/ml	0.495 ± 0.019	3.85	97.74
	250 µg/ml	0.479 ± 0.017	3.52	91.48
	125 µg/ml	0.449 ± 0.018	3.90	79.36
	50 µg/ml	0.419 ± 0.017	4.06	67.51
	25 µg/ml	0.371 ± 0.041	11.07	48.07
	10 µg/ml	0.320 ± 0.032	9.84	27.96
	5 µg/ml	0.292 ± 0.016	5.41	16.78

Table 4. Reducing power capacity of Various Fractions of *Ixora pavetta* and positive control

Extract/ Fraction	Concentration	Av OD ± SD	% CV	% Increase in reducing power
PetE IP	500 µg/ml	0.392 ± 0.020	5.11	74.11
	250 µg/ml	0.362 ± 0.036	9.88	60.65
	125 µg/ml	0.336 ± 0.044	13.11	49.22
	50 µg/ml	0.299 ± 0.032	10.56	32.69
	25 µg/ml	0.266 ± 0.026	9.89	17.90
	10 µg/ml	0.248 ± 0.020	7.87	10.06

	5 µg/ml	0.211 ± 0.017	8.26	0.00
Chl IP	500 µg/ml	0.376 ± 0.016	4.27	66.72
	250 µg/ml	0.367 ± 0.019	5.21	62.86
	125 µg/ml	0.320 ± 0.020	6.20	42.16
	50 µg/ml	0.279 ± 0.021	7.58	23.67
	25 µg/ml	0.250 ± 0.017	6.81	10.80
	10 µg/ml	0.244 ± 0.021	8.78	8.14
	5 µg/ml	0.222 ± 0.021	9.62	0.00
Etace IP	500 µg/ml	0.382 ± 0.019	4.95	69.38
	250 µg/ml	0.369 ± 0.026	7.08	63.61
	125 µg/ml	0.348 ± 0.013	3.67	54.44
	50 µg/ml	0.307 ± 0.022	7.09	36.09
	25 µg/ml	0.279 ± 0.025	9.09	23.96
	10 µg/ml	0.240 ± 0.026	10.85	6.36
	5 µg/ml	0.221 ± 0.017	7.88	0.00
nBut IP	500 µg/ml	0.410 ± 0.016	4.01	81.80
	250 µg/ml	0.369 ± 0.015	4.17	63.61
	125 µg/ml	0.351 ± 0.045	12.87	55.77
	50 µg/ml	0.321 ± 0.020	6.24	42.31
	25 µg/ml	0.282 ± 0.021	7.57	25.30
	10 µg/ml	0.250 ± 0.023	9.32	10.95
	5 µg/ml	0.228 ± 0.027	11.75	1.33
Ascorbic Acid	500 µg/ml	0.445 ± 0.030	6.80	97.49
	250 µg/ml	0.430 ± 0.022	5.00	90.83
	125 µg/ml	0.409 ± 0.030	7.30	81.51
	50 µg/ml	0.390 ± 0.014	3.64	73.22
	25 µg/ml	0.355 ± 0.038	10.62	57.69
	10 µg/ml	0.302 ± 0.018	6.01	34.17
	5 µg/ml	0.262 ± 0.022	8.21	16.27

Table 5. % H2O2 scavenging capacity of Aqueous Extract and Ethanolic extract of *Ixora pavetta*

Extract/ Fraction	Concentration	Av OD ± SD	% CV	% H2O2 scavenging
Aq IP	500 µg/ml	0.300 ± 0.014	4.53	69.24
	250 µg/ml	0.389 ± 0.024	6.14	60.08
	125 µg/ml	0.498 ± 0.058	11.74	48.92
	50 µg/ml	0.571 ± 0.028	4.87	41.43
	25 µg/ml	0.678 ± 0.021	3.12	30.38
	10 µg/ml	0.701 ± 0.024	3.48	28.02
	5 µg/ml	0.819 ± 0.039	4.81	15.91
Et IP	500 µg/ml	0.222 ± 0.018	8.14	77.25
	250 µg/ml	0.322 ± 0.021	6.61	66.95
	125 µg/ml	0.379 ± 0.017	4.55	61.14
	50 µg/ml	0.470 ± 0.030	6.38	51.73
	25 µg/ml	0.565 ± 0.038	6.78	42.01
	10 µg/ml	0.637 ± 0.035	5.53	34.59
	5 µg/ml	0.783 ± 0.043	5.45	19.60
Ascorbic Acid	500 µg/ml	0.115 ± 0.005	3.93	88.23
	250 µg/ml	0.189 ± 0.022	11.64	80.60
	125 µg/ml	0.261 ± 0.022	8.34	73.25
	50 µg/ml	0.321 ± 0.025	7.65	67.09
	25 µg/ml	0.392 ± 0.016	4.01	59.77
	10 µg/ml	0.450 ± 0.033	7.32	53.78
	5 µg/ml	0.577 ± 0.019	3.25	40.78

Table 6. % H2O2 scavenging capacity of Various Fractions of *Ixora pavetta* and positive control

Extract/ Fraction	Concentration	Av OD ± SD	% CV	% H2O2 scavenging
PetE IP	500 µg/ml	0.279 ± 0.017	6.05	71.40
	250 µg/ml	0.313 ± 0.023	7.18	67.84
	125 µg/ml	0.390 ± 0.019	4.89	59.97
	50 µg/ml	0.443 ± 0.038	8.56	54.57
	25 µg/ml	0.522 ± 0.034	6.42	46.42
	10 µg/ml	0.692 ± 0.025	3.55	28.98
	5 µg/ml	0.818 ± 0.031	3.79	16.01
Chl IP	500 µg/ml	0.348 ± 0.022	6.18	64.32
	250 µg/ml	0.416 ± 0.038	9.08	57.27
	125 µg/ml	0.587 ± 0.037	6.22	39.79
	50 µg/ml	0.610 ± 0.026	4.18	37.36
	25 µg/ml	0.726 ± 0.037	5.06	25.49
	10 µg/ml	0.858 ± 0.027	3.17	11.94
	5 µg/ml	0.971 ± 0.024	2.52	0.00
Etace IP	500 µg/ml	0.318 ± 0.022	6.80	67.36
	250 µg/ml	0.392 ± 0.028	7.22	59.80
	125 µg/ml	0.450 ± 0.034	7.57	53.78
	50 µg/ml	0.580 ± 0.034	5.79	40.51
	25 µg/ml	0.629 ± 0.024	3.75	35.41
	10 µg/ml	0.716 ± 0.022	3.10	26.55
	5 µg/ml	0.827 ± 0.030	3.64	15.16
nBut IP	500 µg/ml	0.204 ± 0.023	11.42	79.06
	250 µg/ml	0.264 ± 0.033	12.59	72.87
	125 µg/ml	0.318 ± 0.018	5.59	67.33
	50 µg/ml	0.393 ± 0.019	4.73	59.70
	25 µg/ml	0.450 ± 0.025	5.46	53.81
	10 µg/ml	0.518 ± 0.024	4.65	46.80
	5 µg/ml	0.645 ± 0.030	4.67	33.84
Ascorbic Acid	500 µg/ml	0.098 ± 0.004	4.14	89.98
	250 µg/ml	0.146 ± 0.008	5.20	85.05
	125 µg/ml	0.243 ± 0.011	4.36	75.06
	50 µg/ml	0.306 ± 0.016	5.32	68.63
	25 µg/ml	0.407 ± 0.019	4.70	58.19
	10 µg/ml	0.464 ± 0.032	6.91	52.34
	5 µg/ml	0.588 ± 0.021	3.54	39.65

Figure 1. IC50 Determination of Extracts of *Ixora pavetta* for DPPH assay Aqueous Extract (Aq IP) Vs Ethanolic extract (Et IP)

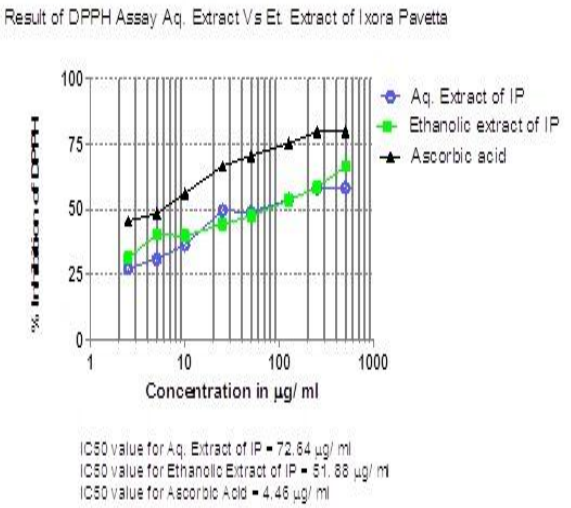


Figure 2. IC50 Determination of various fractions of *Ixora pavetta* for DPPH assay

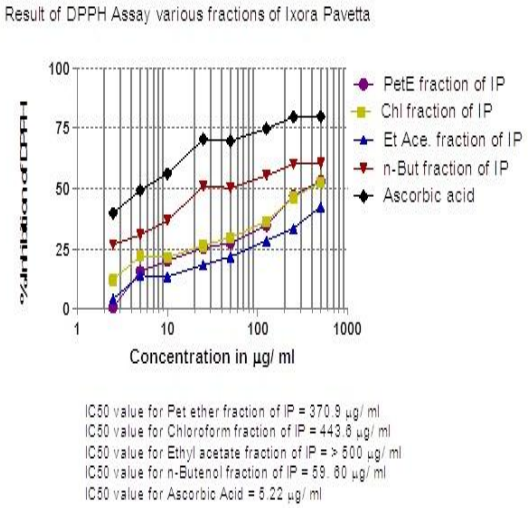


Figure 3. Reducing power capacity of Aqueous Extract and Ethanolic extract of *Ixora pavetta*

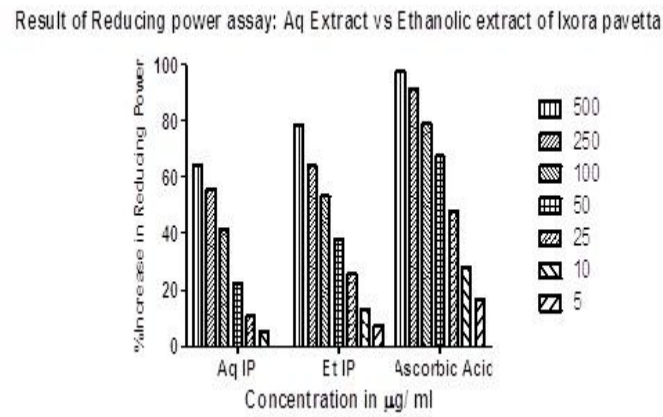


Figure 4. Reducing power capacity of Various Fractions of *Ixora pavetta* and positive control

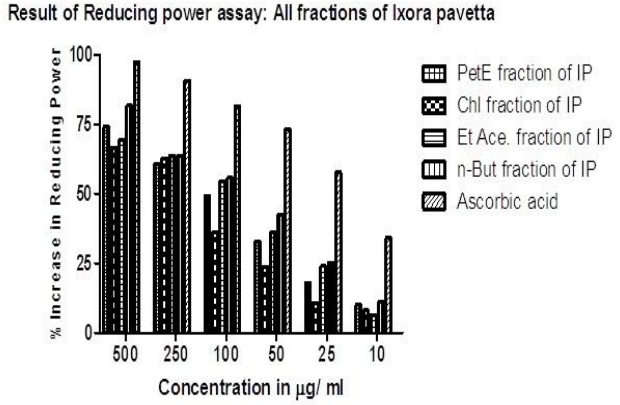


Figure 5. % H2O2 scavenging capacity of Aqueous Extract and Ethanolic extract of *Ixora pavetta*

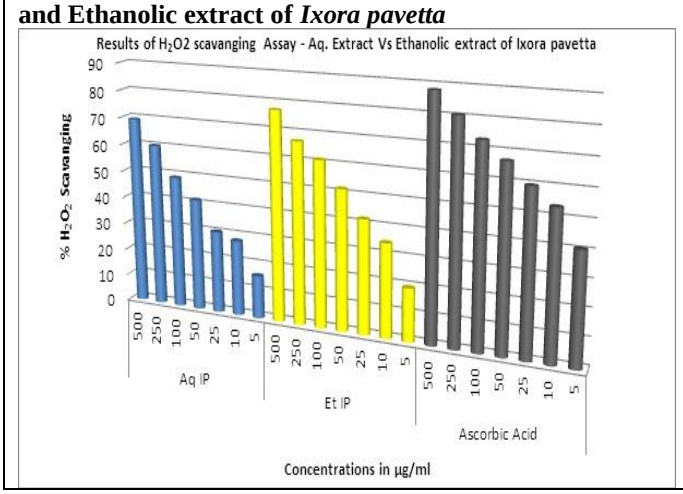
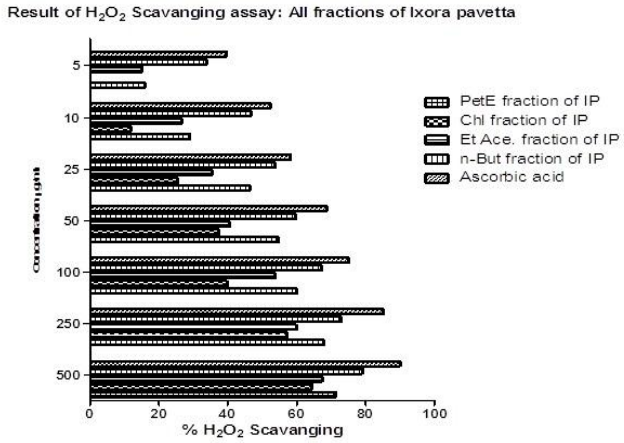


Figure 6. % H2O2 scavenging capacity of Various Fractions of *Ixora pavetta* and positive control



DISCUSSION

Free radicals play an important role in the development of tissue damage and pathological events in living organism. The free radicals are released due to the oxidative stress which causes the various diseases like CNS disorders, cardiovascular disease, diabetes and obesity. There is a compelling evidence indicates that increased consumption of antioxidants from fruits and vegetables reduces the risk of diseases associated with oxidative stress [18,19] and may contribute to improvement in quality of life by delaying the onset of various diseases. Ever growing research in herbal drugs from Indian system of medicine suggests that beneficial effect herbs or its phytoconstituents as antioxidants in *in vitro* and *in-vivo*. The present results demonstrate that crude extract and its various fractions separated from ethanolic extract of *Ixora pavetta* posses free radical scavenging and antioxidant capacity tested in *in vitro.* Free radical scavenging capacity of extracts and various fractions were investigated by DPPH, reducing power assay and H2O2 scavenging assay. The *in vitro* free radical scavenging capacity of the alcoholic and aqueous demonstrates effect polyvalent phytophore and its scavenging capacity in *in vitro* assays. Interestingly, ethanolic extract of *Ixora pavetta* showed high capacity to scavenge various radical than its aqueous extract. This finding direct us to fractionate alcoholic extract using various solvents and analyze the free radical capacity assays.

The results from reducing power assay and H2O2 scavenging assay reveals that fractions separated by butenol as well as pet ether form crude ethanolic extract have superior antioxidant potency and thus contain free radical quenching compounds, which act as primary radical scavengers that react with ferric cyanide in reducing power assay and also responsible for H_2O_2 scavenging by electron donating ability. This explains the capacity of the compounds present in various fractions towards various *in vitro* free radical assays [20]. The results of these assays have direct correlation with the results obtained from DPPH assay as reflected in the ability to show efficient quenching of DPPH*. Interestingly, In DPPH assay, only butenolic fraction was found with less IC 50 value indicates presence of more potent compounds with antioxidant property.

CONCLUSION

Results indicate the free radical scavenging potential of aqueous and ethanolic extract of *Ixora pavetta* . n- butenol fraction separated from crude ethanolic extract was found posses more superior antioxidant potential among all tested fraction in *in vitro* assays.

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