



In Vitro Screening of *Bauhinia Purpurea* Stem Bark with Reference Anti-Oxidant Activity

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Abstract:

The antioxidant capacity of Bauhinia purpurea stems bark extracts, obtained by sequential extraction with various polarities of solvents, by hot continuous percolation method. The antioxidant properties of different crude extracts were evaluated using different antioxidant tests such as free radical scavenging activity, metal chelating activity, superoxide anion scavenging assay, total antioxidant activity, and reducing power for comparison. In addition, total phenolic and flavonoid compounds in the extracts were determined as catechin equivalent. The various antioxidant activities were compared to standard antioxidant such as ascorbic acid. From the tested Bauhinia purpurea crude extracts showed potent different level of in vitro antioxidant activity. Results indicate the possible potential use of medicinal plant Bauhinia purpurea indicate that it may be a potential source of natural antioxidant.

Keywords: *Ascorbic acid, catechin, Bauhinia purpurea, In-vitro antioxidant activity*

1. Introduction

Nature has provided a complete storehouse of remedies to cure ailment of mankind. Herbal medicine, as the major remedy in traditional medical systems, have been used in medical practice for thousands of years and have made a great contribution to maintaining human health. A majority of the world's population in developing countries still relies on herbal medicines to meet its health needs (Kumar et al., 2007). *Bauhinia purpurea* is widely used in Ayurvedic and Yunani medical system. *Bauhinia purpurea* is belonging to the family Leguminosae and it is a small to medium-sized deciduous tree (Khare et al., 2004). The people use this plant in several ways for the treatment of skin diseases (leucoderma and leprosy), wounds, ulcers, cough, dysentery, snakebite, tumors, flatulence, indigestion, piles and also lots of other ailments (Gupta, et. al., 2012 & Santosh Sharma et al., 2012). Tribal people of sought gujarat eat as food of that plant leaves.

Bauhinia purpurea reported to exhibit various pharmacological activities such as, anti-oxidant activity, hepatoprotective activity, hypoglycaemic activity, antiproliferative activity, etc (C.D.Shajiselvin et al.,2011 & Z. A. Zakaria et al.,2009). The body uses oxygen and nutrients to make energy. Oxygen also helps the immune system fight disease and harmful substances. Oxidation is a process that uses by products formed from oxygen fighting disease to create molecular agents that react with body tissues. Unfortunately, this process can form "free radicals" that cause cell damage. Antioxidants help reduce the number of free radicals that form in the body, lower the energy levels of existing free radicals, and stop oxidation chain reactions to lower the amount of damage caused by free radicals. The antioxidants of food are thought to prevent diseases caused by oxidative stress (Frankel et al., 1993). Reactive oxygen species (ROS) play important roles in the mechanisms of inflammation/pain, oxidation and cancers. Inhibition of ROS has been claimed to be one of the mechanisms of, anticancer and antioxidant activities

(Polterait O et al.,1997). Based on these claims and our recent findings on the plant antioxidant activity of the various extracts of *Bauhinia purpurea* using the antioxidant activities like, DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay, superoxide anion scavenging assay, ABTS (2, 2-azino-bis 3-ethyl benz thiazoline-6-sulfonic acid) scavenging activity, Hydroxyl radical scavenging activity, Iron chelating activity, Total antioxidant activity and Reducing power assay and Total phenol and flavonoid contents of *Bauhinia purpurea* stem bark of different crude extracts. The present study of the current paper is to investigate the antioxidant properties of the plant part extracts that is traditionally used for medicinal purposes in India.

2. Material and Methods

2.1 Collection and identification of Plant material

The fresh part of the plant (stem bark) was collected from DMAPR (Directorate of Medicinal and Aromatic Plants Research) Boriavi, Anand. The collection was under specialist supervision. This plant *Bauhinia purpurea* was authenticated by a Botanist. The stem bark of *Bauhinia purpurea* was thoroughly washed and dried under oven (40°-50°C) for 5-7 days, segregated, pulverized by a mechanical grinder to fine powder prior to analysis (Megha Chaudhari et al.,2013 and Khandelwal et al.,2001).

2.2 Preparation of Extracts

This sample (1:20 w/v) was successively extracted with Petroleum ether (40°-60° C) by hot continuous percolation method in Soxhlet apparatus for 24 hrs. Then the marc was subjected to Ethyl acetate (76°-78°C) for 24 hrs and then chloroform at last the marc was subjected to Methanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained (Megha Chaudhari et al., 2013).

2.3 Preparation of *Bauhinia purpurea* stock solution

10 mg of *Bauhinia purpurea* extract was taken and dissolved in 1 ml of Dimethylsulphoxide (DMSO), which is used as stock solution with the concentration of 10,000 µg/ml. From this stock solution, different concentration viz, 10, 20, 30 mg/ml were prepared using DMSO solution (Patel Rakesh, 2004 and Kaur Aman Deep et al., 2012).

3. Antioxidant Assay

3.1 DPPH (1, 1-diphenyl-2-picrylhydrazyl) Method

The effect of extract on DPPH radical was assayed using the method of (Shajiselvin et al., 2011). A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity. The (%) Scavenging activity calculated as scavenging activity (%)

$$= \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

(Thambiraj J et al., 2012). All the tests were performed in triplicate. The extract concentration providing 50 % inhibition was calculated was obtained by interpolation from linear regression analysis.

3.2 Superoxide anion radical scavenging activity

Superoxide radical (O₂⁻) was generated from the photoreduction of riboflavin and was deducted by nitro blue tetrazolium dye (NBT) reduction method. Measurement of superoxide anion scavenging activity was performed based on the method described by (Shajiselvin et al.,2011).

The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbic acid was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

3.3 ABTS scavenging activity

The ability of *Bauhinia purpurea* to scavenge the free radical ABTS (2, 2-azino-bis 3-ethyl benz thiazoline-6-sulfonic acid) was studied using the method adopted by (Smith C et al. 1992). In this decolourisation assay, ABTS, the oxidant, is generated by persulphate oxidation of 2,2-azino-bis(3-ethylbenzoline-6-sulphonic acid), based on the inhibition of the absorbance of the radical cation $ABTS^+$, which has a characteristic long wavelength absorption spectrum. This can be measured spectrophotometrically at 745nm to analyze the ABTS scavenging ability of the plant extracts. For control, water was taken in place of extract. The absorbance was read at 745 nm and the per cent inhibition by the plant extracts was calculated as per (Shajiselvin et al.,2011).

3.4 Hydroxyl radical scavenging activity

The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+} -Ascorbate -EDTA - H_2O_2 system (Fenton reaction). The reaction mixture contained 0.1 ml deoxyribose (2.8mM), 0.1 ml EDTA (0.1 mM), 0.1 ml H_2O_2 (1mM), 0.1 ml Ascorbic acid (0.1mM), 0.1 ml KH_2PO_4 -KOH buffer, PH 7.4 (20mM) and various concentrations of plant extract in a final volume of 1 ml. The reaction mixture was incubated for 1 hour at 37 °C. Deoxyribose degradation was measured as TBARS and the percentage inhibition was calculated (Shajiselvin et al.,2011 & Thambiraj J et al.2012).

3.4 Iron chelating activity

The ferrous ion chelating potential of *Bauhinia purpurea* was measured according to the method of (S Chandra Mohan et al.,2012) 1ml $FeSO_4$ solution was mixed with extract of different concentration. 1ml Tris HCl buffer (pH 7.4) and 2,2'-bipyridyl solution was added together with hydroxyl amine - HCl and ethanol respectively. The reaction mixture was adjusted to a final volume of 5ml with distilled water, shaken well and incubated for 10 minutes at room temperature. Absorbance was determined at 522nm and percent chelation was calculated using the following equation. Metal ion chelating activity scavenging effect was calculated with reference to control. The extract concentration providing 50 % inhibition was calculated was obtained by interpolation from linear regression analysis.

3.5 Total antioxidant activity

The antioxidant activity of the sample was assayed according to the method of transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex (C.D.Shajiselvin et al.,2012). The experiment performed using protocol and the percentage inhibition was calculated. The absorbance of the mixture was measured at 695 nm against a blank in a spectrophotometer a. Ascorbic acid was used as the reference compound. All the tests were performed in triplicate and results averaged

3.6 Reducing power assay

Like the free radical scavenging and metal chelating activities, reductive potential was increased with increasing concentration. There are a number of assays designed to measure the overall antioxidant activity or reducing potential, as an indication of host's total capacity to withstand the

free radical stress. The reducing power was determined in accordance with the procedure of (P. Avinash et al.,2011). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as a standard. All the tests were performed in triplicate and results averaged.

3.7 Determination of total phenol

This method is used routinely in our lab to measure total phenol. The procedure is also used for analysis of total phenol in various plants and fruits. Phenolic is one of the major groups of phytochemical that can be found ubiquitously in certain plants. Phenolic compounds are potent antioxidants and free radical scavenger which can act as hydrogen donors, reducing agents, metal chelators and singlet oxygen quenchers. Studies have shown that phenolic compounds such as catechin and quercetin were very efficient in stabilizing phospholipid bilayers against peroxidation induced by reactive oxygen species (ROS) (Gulcin I et al.,2010). The colorimetric assay of total phenol was performed according to previously published protocol. The absorbance was measured at 650 nm and all the tests were performed in triplicate and results averaged (Yu-Ling HO et al.,2012).

3.8 Determination of total flavonoid contents

Flavonoids present in food of plant origin are also potential antioxidants. Most beneficial effect of flavonoids are attributed to their antioxidant and chelating abilities. The assay was performed with reference protocol (Shajiselvin et al.,2011 and Yu-Ling HO et al.,2012). The absorbance was read at 360 nm. A standard was run by using catechin. The percentage inhibition was calculated by comparing the result of control and test samples. The experiment was performed in triplicates

4. Result

4.1 DPPH Free radical scavenging activity

The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen or electron donating abilities. DPPH is a stable free radical. To become a stable diamagnetic molecule, it accepts an electron or hydrogen radical (Yu-Ling HO et al., 2012). Figure 1 illustrates a significant decrease in the concentration of DPPH due to the scavenging ability of extracts and standards. All amounts of different extracts showed higher activities than those of control and these differences were statistically very significant. The percentage of DPPH radical scavenging activity was present in Table 1. The different extracts Ethyl acetate extract, Petroleum ether extract and Methanol extract of *Bauhinia purpurea* and Ascorbic acid (standard) showed DPPH free radical scavenging activity in concentration ranged from 23-89 %, 18-82 %, 21-86% and 31-93% respectively. STDEV- standard deviation of different extracts showed in table. The IC₅₀ values of the Ethyl acetate extract, Petroleum ether extract and Methanol extract and Ascorbic acid (standard) were found to be 46µg/ml, 58µg/ml, 60µg/ml, 61µg/ml and 46µg/ml respectively.

From the above result clearly indicated that the methanol extract of *Bauhinia Purpurea* exhibits significant DPPH scavenging activity when compared with standard Ascorbic acid. Similar result was not found in other extracts. The IC₅₀ value of methanol and Ascorbic acid were observed at 61µg/ml and 46µg/ml respectively.

In its radical form, DPPH has been disappeared on reduction by an antioxidant compound or a radical species to become a stable diamagnetic molecule resulting the color changes from violate to yellow, which could be taken as an indication of the hydrogen donating ability of the tested sample (Kaur Amaan Deep et al., 2012).

4.2 Superoxide anion radical scavenging activity

Superoxide is a highly reactive molecule that reacts with various substances produced through Metabolic processes. Superoxide dismutase enzymes present in aerobic and anaerobic organisms catalyses the breakdown of superoxide radical. Superoxide scavenging ability of plant extract might primarily be due to the presence of flavanoids (C.D.Shajiselvin et al., 2011). The percentage of superoxide anion scavenging activity of Ethyl acetate extract of *Bauhinia purpurea* was presented in Table 2. Maximum scavenging activity of ethyl acetate extract and ascorbic acid at 100µg/ml was found to be 92.29% and 93.21% respectively. The IC₅₀ value of Ethyl acetate extract and was recorded as 64µg/ml and 46µg/ml respectively. The percentage of superoxide anion scavenging activity of Petroleum extract and methanol extract of *Bauhinia purpurea* was presented in Table 3. Maximum scavenging activity of Petroleum ether extract and methanol extract and at 100 ascorbic acid µg/ml was found to be 93.0% and 8423% and 93.21% respectively. The IC₅₀ value of Petroleum ether extract and methanol extract and was recorded ascorbic acid as 60µg/ml and 54µg/ml and 46µg/ml respectively. (Figure 2) presents the superoxide anion radical scavenging activity of the extracts and is compared with the same dose of known antioxidants Ascorbic acid. All of the extracts had strong superoxide anion radical scavenging activity and showed higher superoxide anion radical scavenging activity than the standard antioxidants. These results indicated that all extracts have a conspicuous effect on the scavenging superoxide anion radical. *Bauhinia purpurea* may have biological significance in the elimination of reactive free radicals. The active components of this plant can regulate some antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase in scavenging reactive free radicals. Rheumatoid arthritis, reperfusion injury, cardiovascular disease, immune injury, and cancer are related to antioxidant enzymes, which have an important role in retaining the physiological levels of superoxide, hydroxyl, alkoxyl and peroxy radicals (S Chandra Mohan et al.,2012).

4.3 ABTS scavenging activity

The antioxidant activity of the extract by ABTS radical assay implies that the action may be either inhibiting or scavenging radicals since both inhibition and scavenging properties of antioxidant towards this radical have been reported in earlier studies (Roy H et al., 1994). In this experiment the maximum scavenging activity was found at 100 µg/ml concentration with 29.31% Scavenging in ethyl acetate extract and minimum was found at 50 µg/ml concentrations with 28.09 % scavenging in methanol extract. IC₅₀ value was found to be 56.2 µg/ml, 63.3 µg/ml and 54.2 µg/ml for ethyl acetate, petroleum ether and methanol extract, where as that of standard was found to be 45.6 µg/ml I shows in table 3.

4.4 OH⁻ radical scavenging activity

The effect of *Bauhinia purpurea* extracts on inhibition of hydroxyl radical production was assessed by the iron (II)- dependent deoxyribose damage assay. The Fenton reaction generates hydroxyl radicals (OH⁻) that degrade deoxyribose using Fe⁺⁺ salts as an important catalytic component (S Chandra Mohan et al.,2012). Oxygen radicals may attack the sugar, which leads to sugar fragmentation. Addition of transition metal ions such as iron at low concentrations to deoxyribose causes degradation of the sugar into malondialdehyde and other related compounds which form a chromogen with thiobarbituric acid (TBA). Antioxidant activity of the extracts was compared with the standard drug ascorbic acid Table 4.

The results of the effects of the examined *Bauhinia purpurea* Ethyl acetate extract Petroleum ether extract and methanol extract as well as control solutions on OH⁻ radical production. They show that all extracts of *Bauhinia purpurea* and control solutions inhibited the production of OH⁻ radicals. The % of free radical scavenging activity of different extract of *Bauhinia purpurea* presented in Table 8. Have reducing power, the free radical OH⁻ scavenging activity of the extract

increases with increasing the concentration. The IC₅₀ value of *Bauhinia purpurea* Ethyl acetate extract, Petroleum ether extract and methanol extract and standard were shown the highest inhibitory activity with IC₅₀ 62µg/ml, 57µg/ml, 60µg/ml, and 46µg/ml respectively. When compared to the reference substances, the bark of *Bauhinia purpurea* extracts were found to be less efficient in radical scavenging. The scavenging effects of the examined extracts could be due to the flavonoids, but could also be a result of the activity of other secondary bimolecular present in the extracts. This indicates that the concentration of flavonoids is not the only factor related to the antioxidant activity. The possible synergism of flavonoids with other components present in the extracts may be responsible for this observation. Plant extract exhibited antioxidative potential and increased concentration of plant extract has shown increased antioxidative potential.

4.5 Iron chelating activity

Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases (Roy et al., 1994). They are also involved in autoimmune disorders like rheumatoid arthritis etc (Rao et al., 2004).

Iron is essential for life because it is required for oxygen transport, respiration and activity of many enzymes. However, iron is an extremely reactive metal and catalyzes oxidative changes in lipids, proteins and other cellular components (Smith et al., 1992). Iron antioxidant activity was expresses relative to that of ascorbic acid.

Binding capacity of the petroleum ether extract of *Bauhinia purpurea* and the metal chelator Ascorbic acid at various concentrations (20, 40, 60, 80, 100, µg/ml) were examined and the values were presented in table 5. Maximum chelating of metal ions at 100µg/ml for petroleum ether extract and Ascorbic acid was found to be 89.07% and 93.21% respectively. The IC₅₀ value of petroleum ether extract and Ascorbic acid was recorded as 67µg/ml and 45µg/ml respectively. Maximum chelating of metal ions at 100µg/ml for Ethyl acetate extract and Ascorbic acid was found to be 88.03 % and 93.21% respectively. The IC₅₀ value of ethyl acetate extract and Ascorbic acid was recorded as 44µg/ml and 45µg/ml respectively.

Maximum chelating of metal ions at 100µg/ml for methanolic extract and standard ascorbic acid was found to be 90.81% and 93.21% respectively. The IC₅₀ value of methanolic extract and ascorbic was recorded as 47µg/ml and 45µg/ml respectively.

Based on the above results clearly indicated that the petroleum ether extract of *Bauhinia purpurea* were found to most effective antioxidant activity than that of ethyl acetate extract and methanol when compared with standard ascorbic acid.

4.6 Total antioxidant activity (Phosphomolybdic acid method)

Table 6 has shown the percentage of total antioxidant activity of different extracts of *Bauhinia purpurea*. The ethyl acetate extract of *Bauhinia purpurea* exhibited a maximum total antioxidant activity of 90.74 % at 100µg/ml whereas for ascorbic acid (standard) was found to be 93.21 % at 100 µg/ml. The IC₅₀ values of the ethyl acetate to be 48µg/ml and 46µg/ml respectively. The petroleum ether extract of *Bauhinia purpurea* exhibited a maximum total antioxidant activity of 79.51% at 100 µg/ml whereas for ascorbic acid (standard) was found to be 93.21 % at 100µg/ml. The IC₅₀ values of the petroleum ether extract of *Bauhinia purpurea* and ascorbic acid were found to be 56µg/ml and 46µg/ml respectively.

The methanolic extract of *Bauhinia purpurea* exhibited a maximum total antioxidant activity of 87.90% at 100µg/ml whereas for ascorbic acid (standard) was found to be 93.21 % at 100µg/ml.

The IC₅₀ of the methanolic extract of *Bauhinia purpurea* and ascorbic acid were found to be 50µg/ml and 46µg/ml respectively. Based on the result clearly indicated, the methanolic extract of *Bauhinia purpurea* was found to be more effective than that other two extracts. But when compare all the extracts with standard the methanolic extract of *Bauhinia purpurea* was found strong antioxidant activity. The IC₅₀ of the methanolic extract of *Bauhinia purpurea* and ascorbic acid were found to be 50µg/ml and 46µg/ml respectively.

5. Reducing power

Different extracts of *Bauhinia purpurea* shows percentage of inhibition and STDEV- standard deviation and IC₅₀ value in Table 7. Figure 7 shows the reductive capabilities of extracts compared to ascorbic acid. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Periyar Rangasamy Rathi Sre et al.,2012). Reducing power of extracts exhibited the following order: E.AE > PE > ME. Like the free radical scavenging and metal chelating activities, reductive potential was increased with increasing concentration. There are a number of assays designed to measure the overall antioxidant activity or reducing potential, as an indication of host's total capacity to withstand the free radical stress. In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of antioxidant samples. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

6. Total phenolic contents

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups (C.D.Shajiselvin et al.,2011). The phenolic compounds may contribute directly to antioxidative action. The total phenolic content of various extract of whole plant of *Bauhinia purpurea* was depicted in Table 8.

Phenolic compounds have antioxidant properties due to their ability of scavenging free radicals and active oxygen species such as singlet oxygen, free radicals and hydroxyl radicals. Table shows total phenols as catechin equivalent in the extracts of plant. Methanol extract has the lowest phenolic contents among the extracts given in Table 8 while they demonstrated influential antioxidant activity. Based on these results, there was no relationship between the total phenols and total antioxidant activity in extracts. The high antioxidant activity was not correlated with the phenol content owing to other factors playing major roles as antioxidants (M. Hajimahmoodi et al.,2008).

7. Total flavonoid contents

The total amount of flavonoids content of various extract of whole plant of *Bauhinia purpurea* was summarized in Table 9. Flavonoids present in food of plant origin are also potential antioxidants (Kumar PS et al.,2008). Most beneficial effects of flavonoids are attributed to their antioxidant and chelating abilities. Recent studies showed that many flavonoids & related polyphenols contribute significantly to the total antioxidant activity of many plants. The higher content of flavonoids was found in ethyl acetate extract of *Bauhinia purpurea* than that of other two extracts. Flavonoids are very important plant constituents due to their active hydroxyl groups and antioxidant activity (M. Hajimahmoodi et al., 2008).

8. Conclusion

Bauhinia purpurea stem bark extract showed strong antioxidant activity, reducing power, superoxide anion radical scavenging, free radical scavenging, and metal chelating activities when compared with natural and synthetic standard antioxidants such as ascorbic acid. Moreover, the obtained results of the study showed that antioxidants are efficient protective agents against the degenerative diseases and the revealed features of *Bauhinia purpurea* may be promotive for

further medicinal investigations. Therefore, it is suggested that this plant could be used as an additive in the food industry providing good protection against oxidative damage.

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Table 1. DPPH radical scavenging activity of different bark extract of *Bauhinia purpurea*

Sr. No	Conc ⁿ (µg/ml)	% of activity			
		Std	E.AE	PE	ME
1	20	31.56±0.007	23.76±0.003	18.23±0.003	21.02±0.002
2	40	45.32±0.005	30.12±0.002	29.78±0.009	24.87±0.002
3	60	62.42±0.003	53.42±0.005	50.02±0.007	49.48±0.005
4	80	85.56±0.004	71.56±0.006	65.36±0.004	70.32±0.007
5	100	93.21±0.003	89.34±0.002	82.90±0.002	86.76±0.001
6	STDEV	26.08	27.57	26.14	18.43
7	IC ₅₀ value (µg/ml)	46	58	60	61

STD- Standard, E.AE- Ethyl acetate Extract, PE- Petroleum Ether extract, ME- Methanol extract, % inh-% inhibition, Conc.ⁿ- Concentration. Values are expressed as mean ± SEM, STDEV- standard deviation, IC₅₀- Inhibitory concentration at 50 % .

Table 2. Superoxide anion radical scavenging activity of different bark extract of *B. purpurea*

Sr. No	Conc ⁿ (µg/ml)	% of activity			
		Std	E.AE	PE	ME
1	20	31.56±0.007	15.34±0.005	28.07±0.003	26.09±0.005
2	40	45.32±0.005	23.12±0.003	25.87±0.003	31.76±0.009
3	60	62.42±0.003	45.09±0.007	50.67±0.006	57.98±0.001
4	80	85.56±0.004	70.46±0.009	74.32±0.008	74.90±0.001
5	100	93.21±0.003	92.29±0.002	93.00±0.007	84.23±0.004
6	STDEV	26.08	32.21	29.18	25.66
7	IC ₅₀ value(µg/ml)	46	64	60	54

STD- Standard, E.AE- Ethyl acetate Extract, PE- Petroleum Ether extract, ME- Methanol extract, % inh- % inhibition, Conc.ⁿ- Concentration. Values are expressed as mean ± SEM, STDEV- standard deviation, IC₅₀ - Inhibitory concentration at 50 % value.

Table :3 ABTS radical scavenging activity of different bark extract of *Bauhinia purpurea*

Sr. No	Conc ⁿ (µg/ml)	% of activity			
		Std	E.AE	PE	ME
1	20	31.56±0.007	25.76±0.003	20.24±0.003	31.02±0.002
2	40	45.32±0.005	32.42±0.002	39.88±0.009	29.87±0.002
3	60	62.42±0.003	59.92±0.005	49.02±0.007	59.48±0.005
4	80	85.56±0.004	75.56±0.006	75.26±0.004	80.32±0.007
5	100	93.21±0.003	95.84±0.002	92.40±0.002	91.76±0.001
6	STDEV	26.08	29.31	28.64	28.09
7	IC ₅₀ value (µg/ml)	46	56.3	63.3	54.2

STD- Standard, E.AE- Ethyl acetate Extract, PE- Petroleum Ether extract, ME- Methanol extract, % inh- % inhibition, Conc.ⁿ- Concentration. Values are expressed as mean ± SEM, STDEV- standard deviation, IC₅₀- Inhibitory concentration at 50 % .

Table 4. Hydroxyl radical scavenging activity of different bark extract of *Bauhinia purpurea*

Sr. No	Conc ⁿ (µg/ml)	% of activity			
		Std	E.AE	PE	ME
1	20	31.56±0.007	15.21±0.004	26.19±0.004	19.43±0.007
2	40	45.32±0.005	26.45±0.006	38.23±0.004	31.32±0.005
3	60	62.42±0.003	47.87±0.008	53.98±0.00	49.89±0.003
4	80	85.56±0.004	69.32±0.002	76.23±0.007	71.65±0.002
5	100	93.21±0.003	81.27±0.001	87.90±0.001	90.92±0.002
6	STDEV	26.08	28.78	25.66	29.11
7	IC ₅₀ value(µg/ml)	46	62	57	60

STD- Standard, E.AE- Ethyl acetate Extract, PE- Petroleum Ether extract, ME- Methanol extract, % inh- % inhibition, Conc.ⁿ- Concentration. Values are expressed as mean ± SEM, STDEV- standard deviation, IC₅₀- Inhibitory concentration at 50 % value.

Table 5. Iron-chelating method of different bark extract of *Bauhinia purpurea*

Sr. No	Conc ⁿ (µg/ml)	% of activity			
		Std	E.AE	PE	ME
1	20	31.56±0.007	30.54±0.004	18.27±0.005	20.45±0.001
2	40	45.32±0.005	43.05±0.004	38.58±0.009	41.67±0.001
3	60	62.42±0.003	57.87±0.008	46.42±0.006	54.06±0.003
4	80	85.56±0.004	79.34±0.005	60.87±0.002	72.84±0.007
5	100	93.21±0.003	88.03±0.002	89.07±0.002	90.81±0.005
6	STDEV	26.08	24.07	26.42	27.24
7	IC ₅₀ value µg/ml	45	44	67	47

STD- Standard, E.AE- Ethyl acetate Extract, PE- Petroleum Ether extract, ME- Methanol extract, % inh- % inhibition, Conc.ⁿ- Concentration. Values are expressed as mean ± SEM, STDEV- standard deviation, IC₅₀- Inhibitory concentration at 50 % value.

Table 6. Total antioxidant activity of different bark extract of *Bauhinia purpurea*

Sr. No	Conc ⁿ (µg/ml)	% of activity			
		Std	E.AE	PE	ME
1	20	31.56±0.007	32.30±0.006	20.42±0.004	29.28±0.001
2	40	45.32±0.005	44.07±0.009	36.90±0.007	40.43±0.006
3	60	62.42±0.003	59.98±0.004	54.43±0.004	58.68±0.003
4	80	85.56±0.004	83.53±0.004	68.27±0.002	65.87±0.006
5	100	93.21±0.003	90.74±0.003	79.51±0.002	87.90±0.005
6	STDEV	26.08	24.98	23.73	22.79
7	IC ₅₀ value µg/ml	46	48	56	50

STD- Standard, E.AE- Ethyl acetate Extract, PE- Petroleum Ether extract, ME- Methanol extract, % inh - % inhibition, Conc.ⁿ- Concentration. Values are expressed as mean ± SEM, STDEV- standard deviation, IC₅₀- Inhibitory concentration at 50 % value.

Table 7. Reducing power assay of different bark extract of *Bauhinia purpurea*

Sr. No	Conc ⁿ (µg/ml)	% of activity			
		STD	E.AE	PE	ME
1	20	31.56±0.007	31.10±0.003	27.07±0.005	30.04±0.008
2	40	45.32±0.005	40.32±0.003	42.98±0.004	45.23±0.005
3	60	62.42±0.003	57.90±0.009	59.12±0.008	53.89±0.004
4	80	85.56±0.004	83.61±0.007	80.18±0.001	76.53±0.006
5	100	93.21±0.003	93.05±0.006	91.63±0.003	80.19±0.002
6	STDEV	26.08	26.07	26.37	21.17
7	IC ₅₀ value µg/ml	46	52	49	45

STD- Standard, E.AE- Ethyl acetate Extract, PE- Petroleum Ether extract, ME- Methanol extract, % inh- % inhibition, Conc.ⁿ- Concentration. Values are expressed as mean ± SEM, STDEV- standard deviation, IC₅₀- Inhibitory concentration at 50 % value.

Table 8. The total Phenolic content of various extract of *Bauhinia purpurea*

Sr. No	Extracts	Total phenol content (µg/g of Catechin)
1	Petroleum ether extract of <i>B. purpurea</i>	2.21±0.04
2	Ethyl acetate extract of <i>B. purpurea</i>	3.98±0.03
3	Methanol extract of <i>B. purpurea</i>	1.73±0.01

All values are expressed as mean ± SEM.

Table 9. The total flavnoids content of various extract of *Bauhinia purpurea*

Sr. No	Extracts	Total Flavnoida content (µg/g Catechin)
1	Petroleum ether extract of <i>B. purpurea</i>	2.99±0.02
2	Ethyl acetate extract of <i>B. purpurea</i>	4.18±0.07
3	Methanol extract of <i>B. purpurea</i>	3.75±0.03

All values are expressed as mean ± SEM.

Tables

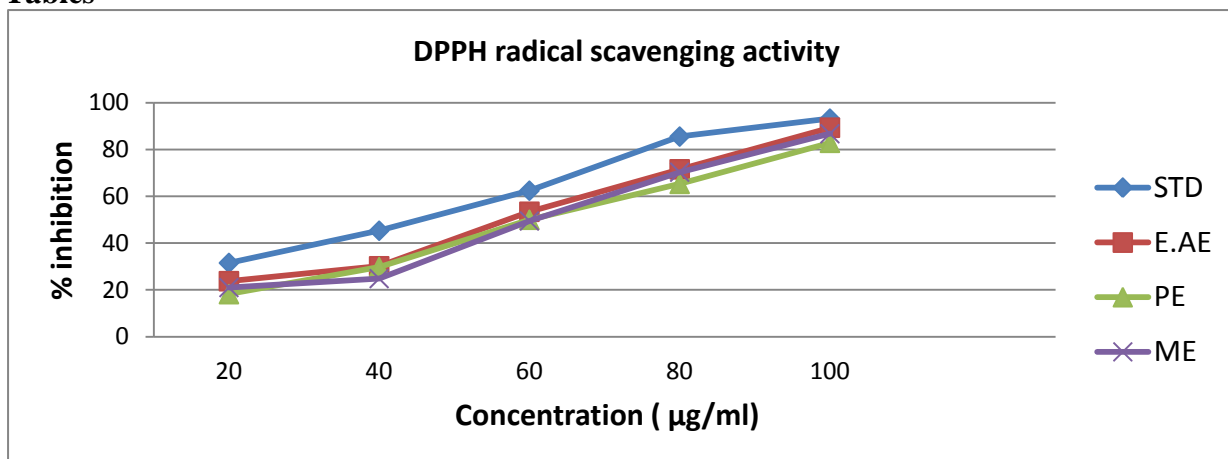


Fig. 1. DPPH radical scavenging activity of different bark extract of *B. purpurea*

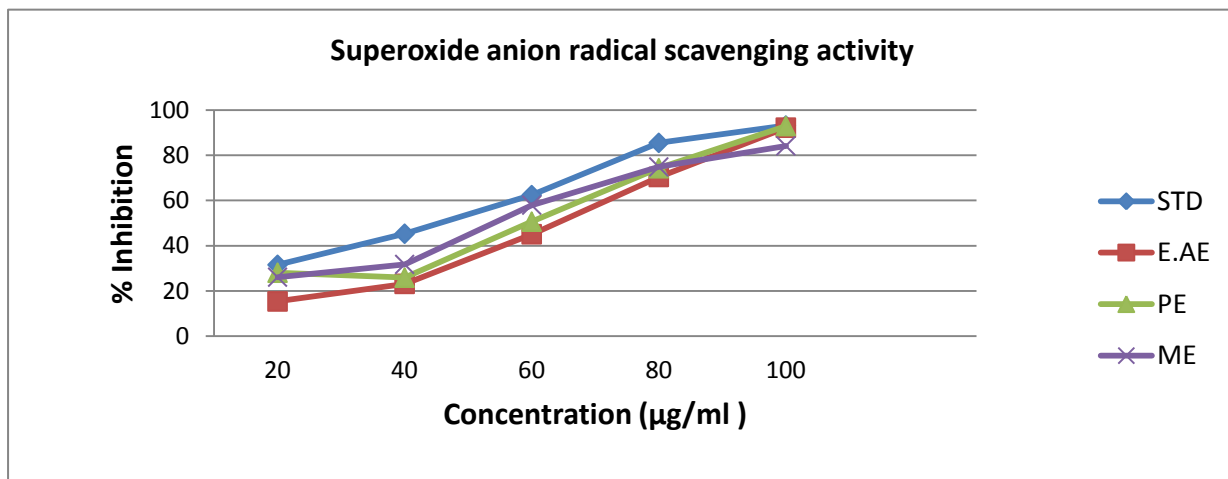


Fig. 2. Superoxide anion radical scavenging activity of different bark extract of *B. purpurea*

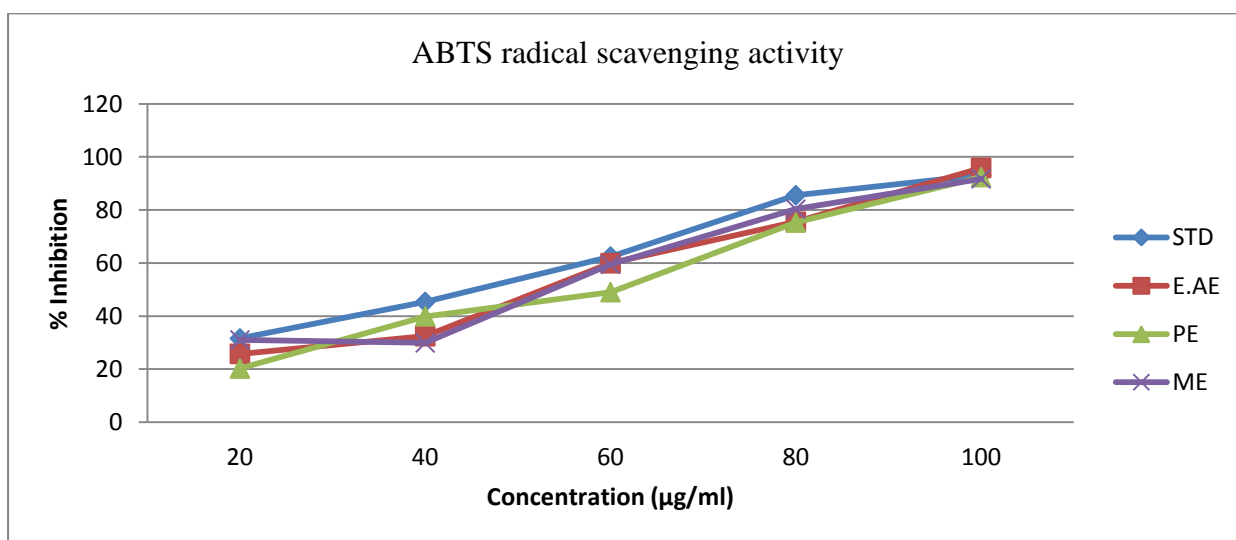


Fig. 3. ABTS scavenging activity of different bark extract of *B. purpurea*

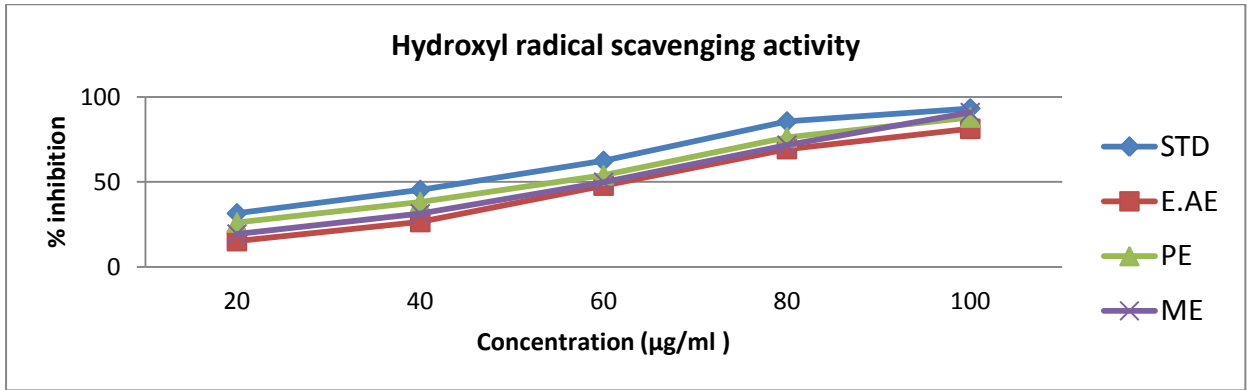


Fig. 4. OH[•] ion scavenging activity of different bark extract of *B. purpurea*

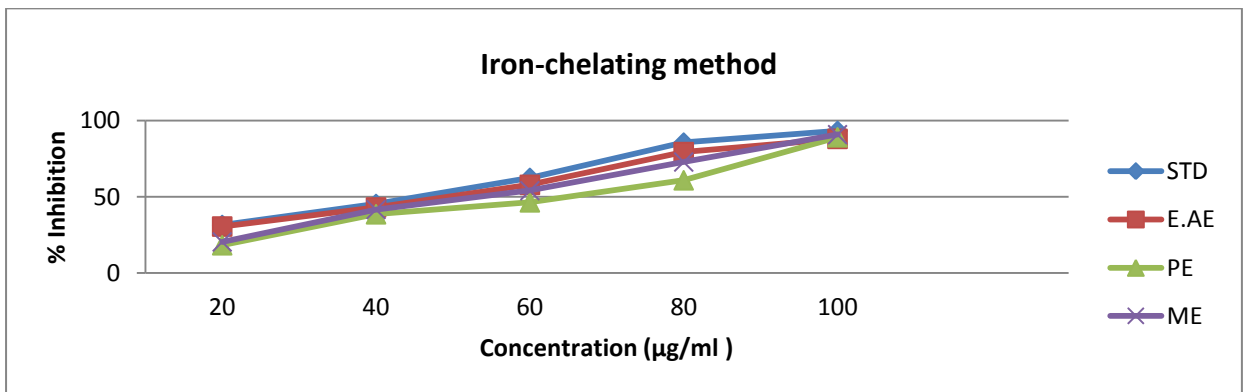


Fig. 5. Iron-chelating activity of different bark extract of *B. purpurea*

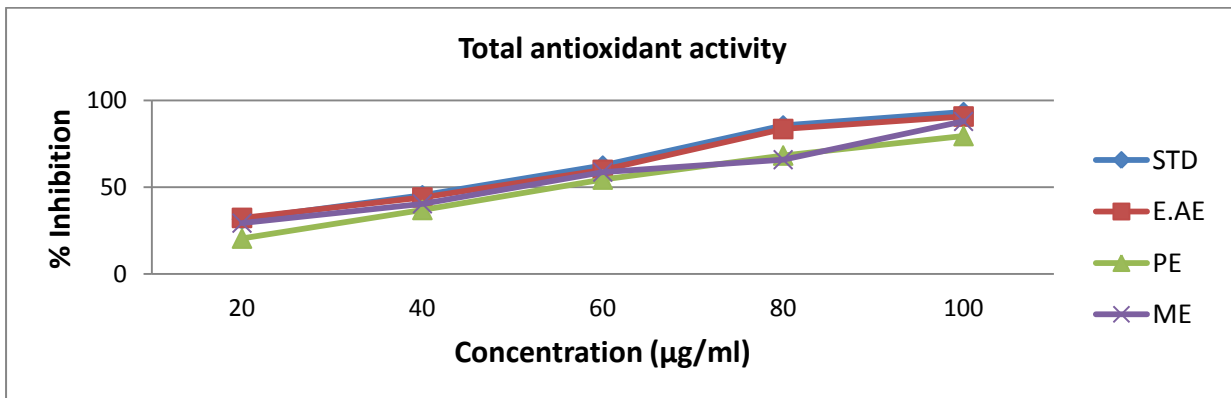


Fig. 6. Total antioxidant activity of different bark extract of *B. purpurea*

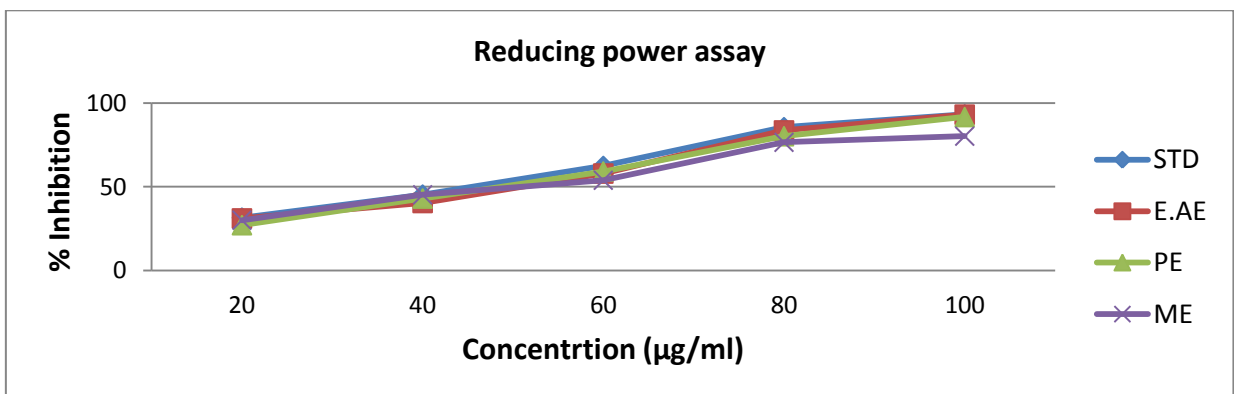


Fig. 7. Reducing power assay of different bark extract of *B. purpurea*

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