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Phytochemical screening and *In-vitro* neuropharmacological activity of *Bauhinia racemosa* Lam Whole Plant

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Abstract

The aim of this article is to evaluate the phytochemical screening and *in-vitro* neuropharmacological activity of *Bauhinia racemosa* Lam Whole Plant by DPPH (2, 2-diphenyl-1-picryhydrazyl) radical scavenging assay**.** Extraction was carried out with petroleum ether, chloroform, ethanol and water by using maceration technique. The ethanolic extract of *Bauhinia racemosa* Lam Whole Plant exhibited maximum *in-vitro* neuropharmacological activity when compared with other extract and significant with the standard ascorbic acid.

Key words: *Bauhinia racemosa*, Radical scavenging assay, Extraction, Ethanol, Ascorbic acid

Introduction

Neuropharmacology is the study of how drugs affect cellular function in the nervous system. It is a very broad region of science that encompasses many aspects of the nervous system from single neuron manipulation to entire areas of the brain, spinal cord, and peripheral nerves. The DPPH assay is used to predict antioxidant activities by mechanism in which antioxidants act to inhibit lipid oxidation, so scavenging of DPPH radical and therefore determinate free radical scavenging capacity. Therefore discovering traditional herbal medicines which have been proved to be effective in control the neurological disorder then the

conventional medicine. Bauhinia racemosa, commonly known as Sonpatta tree is a member of the family Leguminosae. It is a small, crooked, bushy, deciduous tree with drooping branches, which can grow in poor and very harsh climatic conditions. This particular species racemosa is widely distributed throughout India. Traditional uses in the treatment of asthma, gastrointestinal pain, piles, urinary diseases, glandular inflammation, dysentery, diarrhea, malaria, pneumonia, epilepsy, dehydration, edema, constipation and gastric dyspepsia. [1-3]

Materials and Methods

Plant Collection and Authentification

The aerial part of the Bauhinia racemosa was collected during October 2020from near Bargur hills, Erode district, Tamilnadu, India. The plant material was authenticated by Prof.P.Jayaraman, Ph.D. director, Institute Plant Anatomy and Research Centre, West Thambaram Chennai – 45.

Preparation of Plant Extracts

For small scale extraction, maceration is generally consists of several steps. Firstly, grinding of plant materials into small particle it was used to increase the surface area for proper mixing with solvent. In this process, 100g of whole plant of Bauhinia racemosa coarsely powdered then it was placed in individual closed vessel with the solvents such as petroleum ether, chloroform, ethanol, water and allowed to stand for room temperature on atleast three days with frequent agitation until the soluble matter was dissolved. Then the mixture is strained, damp solid material is pressed and the combined liquids are clarified by filtration or decantation after standing.[4,5]

Phytochemical Analysis

Test for alkaloids

A small portion of the extract was stirred with few drops of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents such as **Mayer's reagent** (cream precipitate), **Dragendroff's reagent** (orange brown precipitate), **Hager's reagent (**yellow precipitate) and **Wagner's reagent**(reddish brown precipitate).

Test for flavonoids

NaOH test: A small amount of extract was treated with aqueous NaOH and HCl, observed for the formation of yellow orange colour indicated presence of flavonoids.

H2SO4 test: A fraction of extract was treated with concentrated H2SO4 and observed for the formation of orange colour indicated presence of flavonoids.

Lead acetate test: A small amount of extract was treated with lead acetate and observed for the formation of white precipitate indicated presence of flavonoids

Test for tannins

Few ml of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution indicated presence of tannins.

Test for Phenols

Ferric chloride test: The fraction of extract was treated with 5 % ferric chloride and observed for formation of deep blue or black colour indicated presence of phenols.

Test for terpenoids

Liebermann – Burchard test: Extract (1ml) was treated with chloroform, acetic anhydride and drops of H2SO4 was added and observed for the formation of dark green colour indicated presence of terpenoids.

Test for Anthraquinones

Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia; pink or deep red colourations of aqueous layer indicate the presence of anthraquinone.

Test for anthocyanin

NaOH test: A small amount of extract was treated with 2M NaOH and observed for the formation of blue green colour indicated presence of anthocyanin.

Test for Proteins

Biuret test: The extract is treated with an equal volume of 1% strong sodium hydroxide followed by a few drops of copper (II) sulphate, formation of purple colour indicated the presence of protein.

Million's test: To the extract million's reagent is added, a white precipitate is produced, while heating it turns brick red colour indicated the presence of protein.

Test for sterols

Liebermann-Burchard test: Extract (1ml) was treated with chloroform, acetic anhydride and drops of H2SO4 was added and observed for the formation of dark pink or red colour indicated presence of sterols.

Test for saponins

Foam test: The extract was diluted with 5ml distilled water. The suspension was shaken in graduated cyclinder for 15 min. A 2cm layer foam indicated the presence of saponins

Test for mucilage

The extract is treated with aqueous potassium hydroxide. Swelling indicated the presence of mucilage.

Test for carbohydrates

Molish's test: To the extract few drops of α-naphthol solution in alcohol, con.H2SO4 is added at the side of test tube, formation of violet ring at the junction of two liquids indicated the presence of carbohydrates.[6]

In Vitro Neuropharmacological Activity of *Bauhinia racemosa* Lam Whole Plant by DPPH (2, 2-Diphenyl-1-Picryhydrazyl) Radical Scavenging Assay

The ability of the plant extract to scavenge 1,1-diphenyl-2- picryhydrazyl (DPPH) free radicals was assessed by the standard method.. The stock solution of extracts were prepared in ethanol to achieve the concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 50, 100, 250, 500,1000 µg/ml. Diluted solutions (1 ml each) were mixed with 3 ml of ethanolic solution of DPPH (DPPH, 0.004%). After 30 min of incubation for dark at room temperature the reduction of the DPPH free radical was measured by reading the absorbance at 517nm using UV-visible spectrophotometer. The blank solution containing without extracts & with ethanolic solution of DPPH and control solution containing the ethanol. Ascorbic acid was used as standard. The experiment was carried out in triplicate.[7]

Percentage inhibition was calculated by using this formula

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

Results

Phytochemical Analysis

Petroleum ether, chloroform, aqueous and ethanolic extract of whole plant of *Bauhinia racemosa* Lam was subjected to various chemical tests for detection of phytoconstituents and results obtained are illustrated in **Table.1**.

Table.1: Phytochemical analysis of Petroleum ether, chloroform, aqueous and ethanolic extract of whole plant of *Bauhinia racemosa* Lam

S.No	Chemical Constituents	Petroleum ether extract	Chloroform extract	Ethanolic extract	Aqueous extract
1.	Carbohydrate	+	+	+	-
2.	Glycoside	-	-	+	-
3.	Alkaloid	-	+	+	+
4.	Phytosterols	-	-	-	+
5.	Saponins	-	-	+	+
6.	Phenol	+	-	-	+
7.	Tannin	+	-	+	+
8.	Flavonoids	-	-	+	+
9.	Protein & Amino acid	+	-	-	-

NOTE: (+) Present (-) Absent

Table.2: *In-Vitro* Neuropharmacological activity of *Bauhinia racemosa* Lam Whole plant by DPPH Radica Scavenging Assay

S.No	Concentration (µg/ml)	DPPH Radical Scavenging Activity (%)				
		Petroleum ether Extract	Chloroform Extract	Ethanolic Extract	Water Extract	Standard Ascorbic acid
1.	50	25.30±0.34	22.19±0.87	36.54±0.10	37.14±0.35	36.54±0.10
2.	100	29.78±0.67	25.74±0.44	50.80±0.06	50.07±0.25	51.39±0.07
3.	250	44.50±0.062	38.122±0.75	52.67±0.33	51.39±0.07	54.72±0.17
4.	500	54.75±0.63	46.30±0.50	64.77±0.23	63.12±0.06	68.88±0.22
5.	1000	61.77±0.53	63.01±15	81.1±0.55	76.08±0.31	82.96±0.26
6.	**IC_{50}**	**399**	**605**	**95**	**100**	**86**

Values are expressed as Mean±SEM (n=3)

Fig.1: Effect of *Bauhinia Racemosa* Lam Whole plant on DPPH Radicals

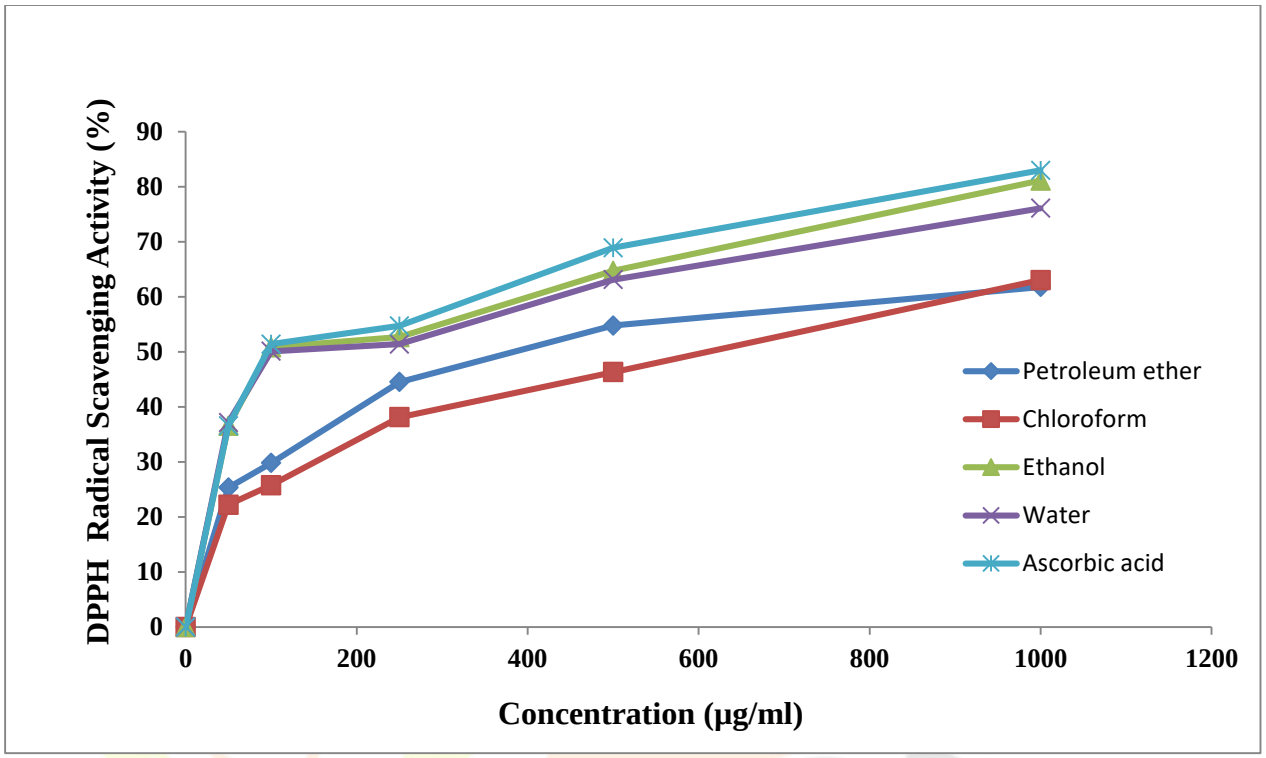


Fig.2: IC_{50} Value of Petroleum ether, Chloroform, Ethanol, Water extract of *Bauhinia racemosa* Lam Whole plant and standard ascorbic acid

Fig. 2.1: Petroleum ether extract

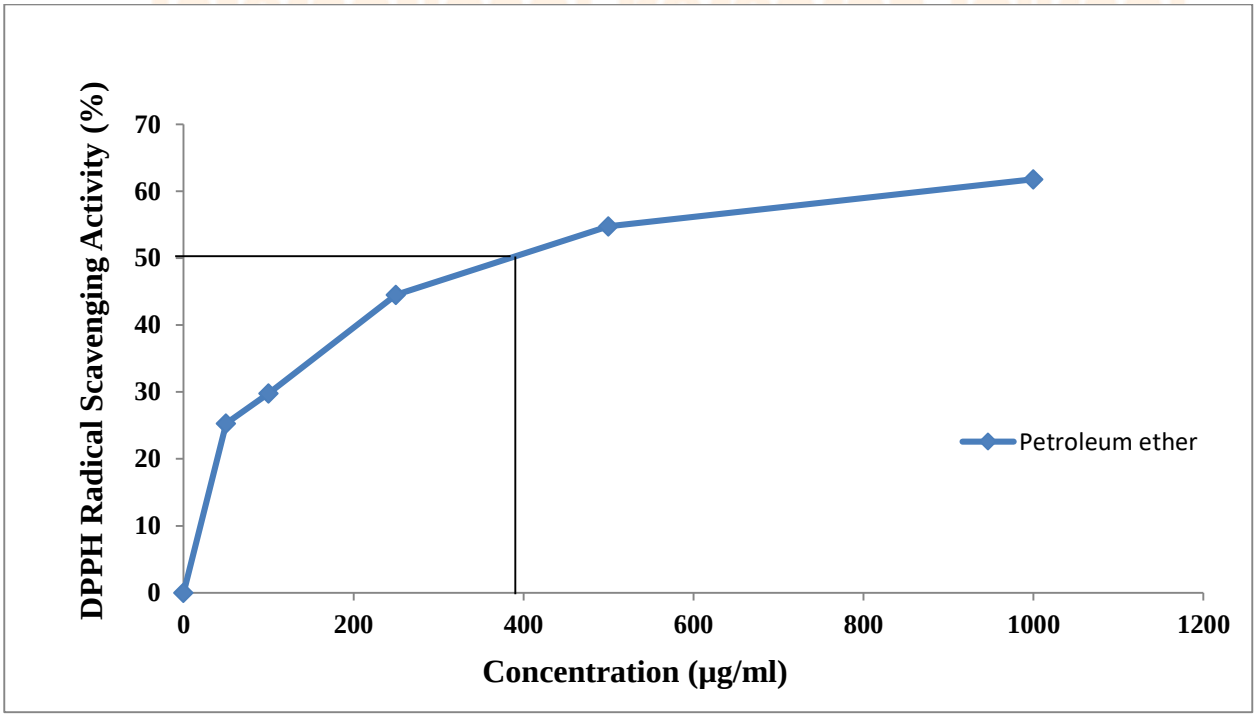


Fig. 2.2: Chloroform extract

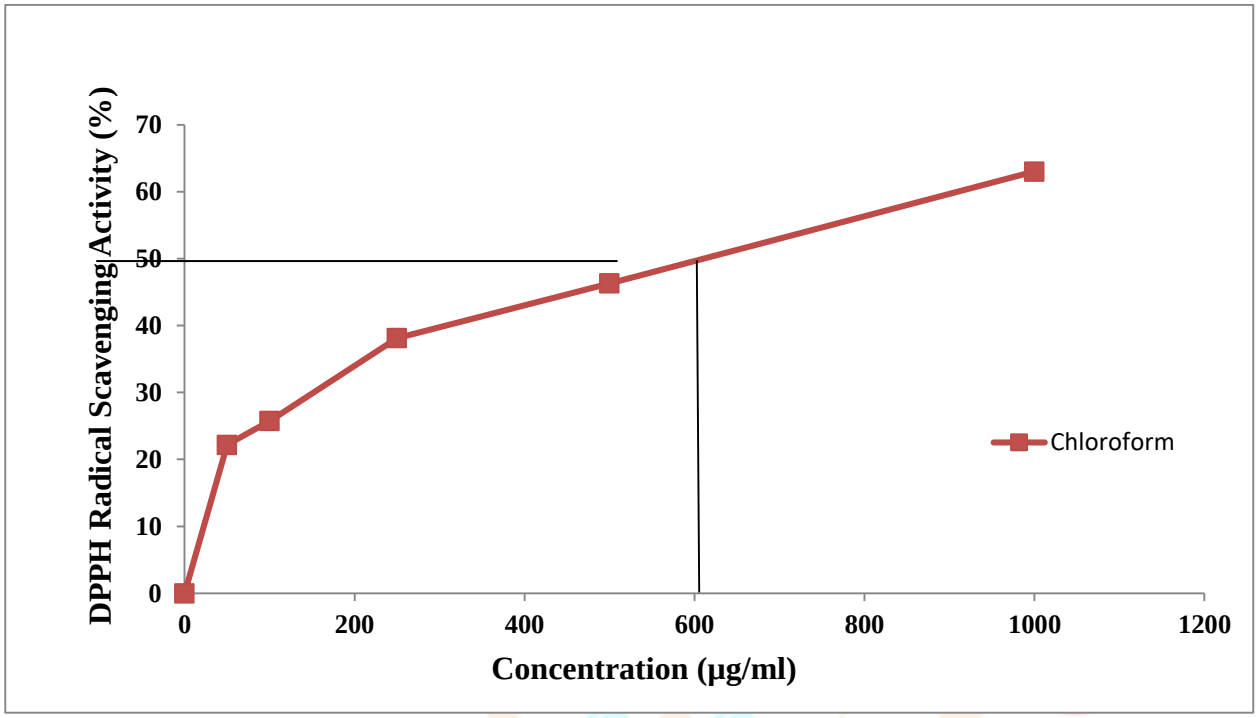


Fig. 2.3: Ethanolic extract

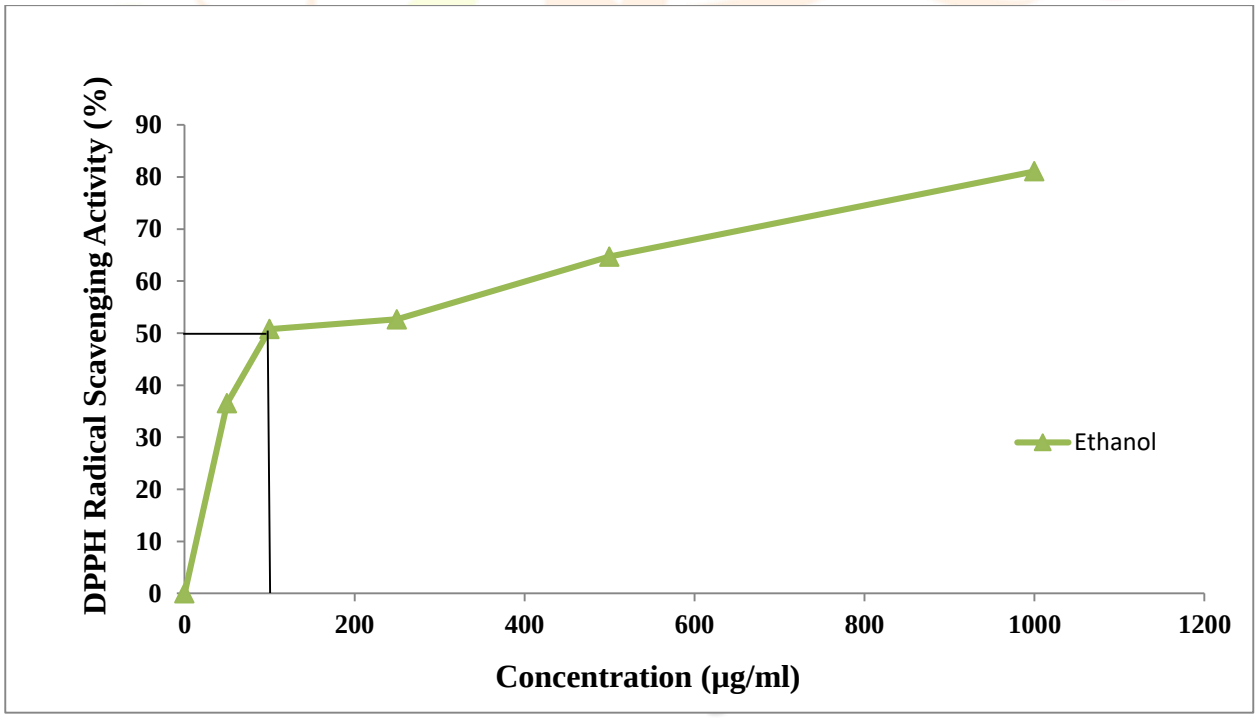


Fig.2.4: Water extract

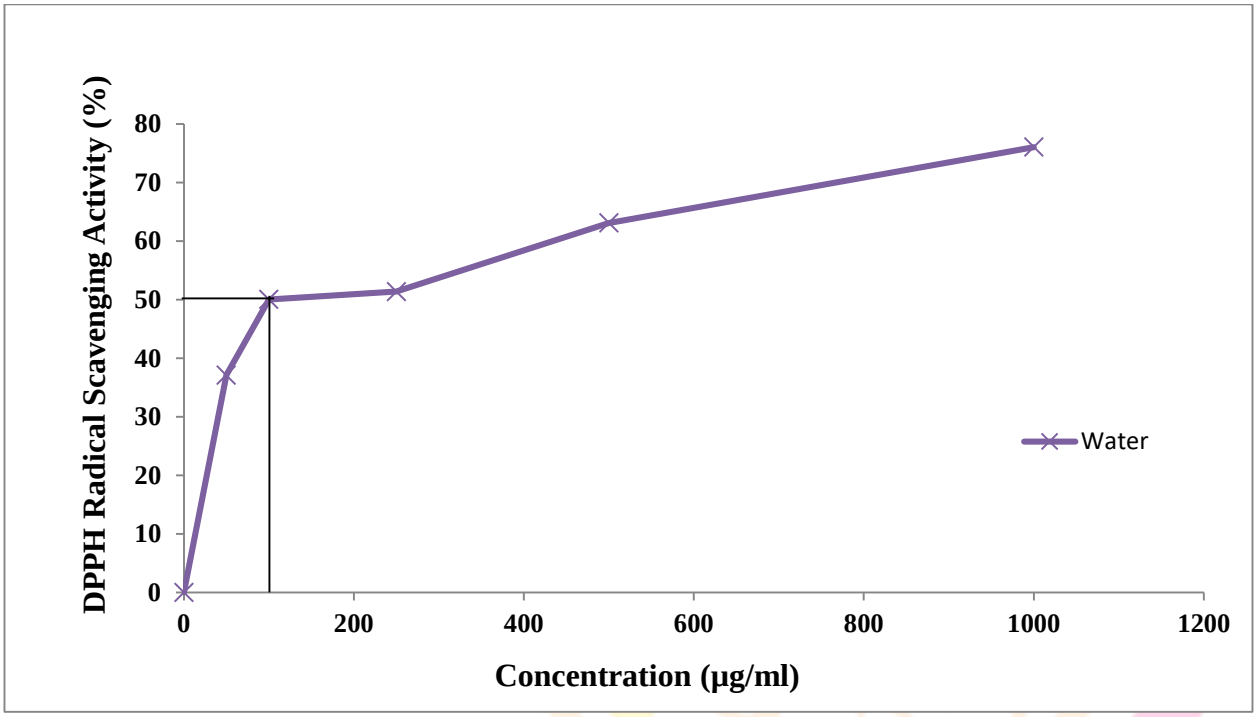
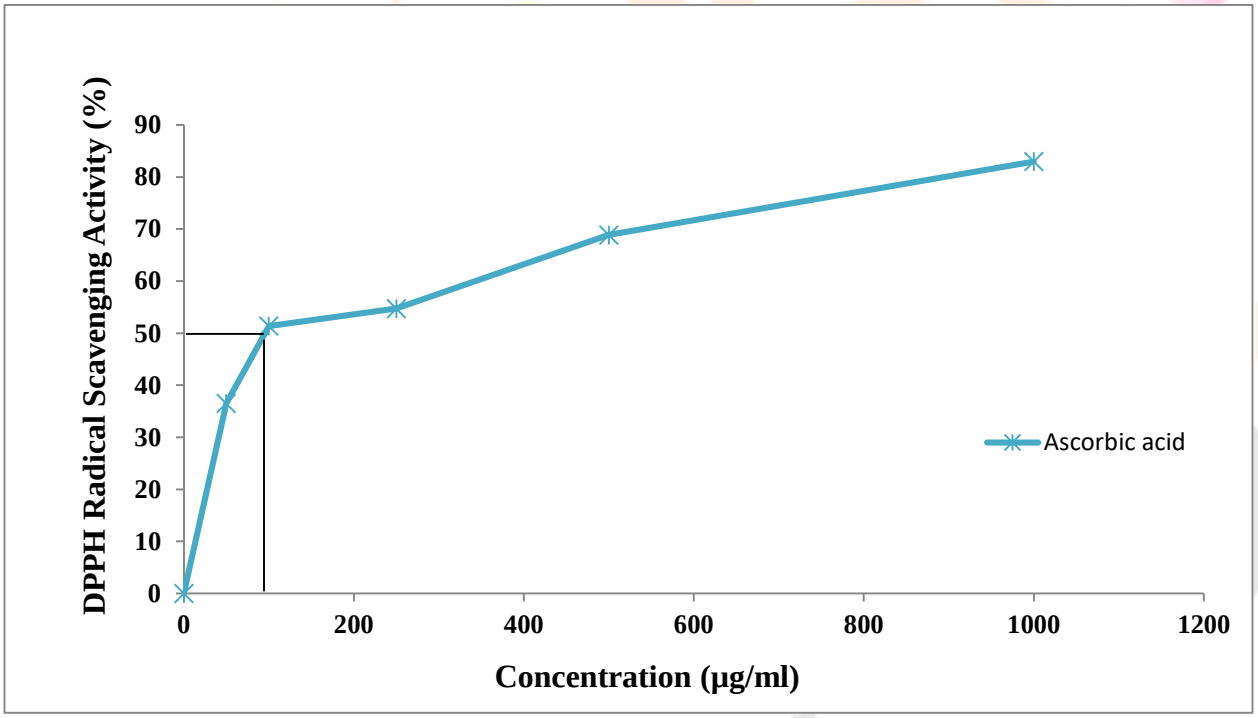


Fig. 2.5: Standard Ascorbic acid



Discussion

Active phytochemical such as flavonoids, tannin and saponins are known to responsible for the neuropharmacological Activity **(Table.1).**

The percentage inhibition of DPPH radical to various extracts was calculated by using formula. When compared these four extracts, present study clearly indicates the ethanolic extract of *Bauhinia racemosa* lam whole plant showed higher DPPH radical inhibition (**81.1%)** than the petroleum ether (**61.7%**), chloroform (**63.01%**) and water (**76.08%**) extract of *Bauhinia racemosa* lam whole plant for the anti-oxidant activity by DPPH radical scavenging assay. While ascorbic acid as a standard drug showed highest (**82.96%**) of DPPH radical scavenging activity **(Table.2**and**Fig.1)**.

The IC_{50} value of ethanolic extract of *Bauhinia racemosa* lam whole plant showed significant IC_{50} value (**95µg/ml**) than the petroleum ether (**399µg/ml**), chloroform (**605µg/ml**) and aqueous extract (**100µg/ml**) of *Bauhinia racemosa* lam whole plant. The IC_{50}values of ethanolic extract of *Bauhinia racemosa* lam whole plant were near to the ascorbic acid standard (**86µg/ml**) **(Table.2** and **Fig.2.)**

Conclusion

The ethanolic whole plant extract *Bauhinia racemosa* of possess maximum inhibitory activity on DPPH radical assay when compare to other extracts, Thus may be beneficial in the treatment of anxiety and depressant. Further evaluation of this plant with clinical trials may yield a solution for this anxiety and depressant problems.

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