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Research Article

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# IN VITRO ANTI-DIABETIC AND ANTI-INFLAMMATORY ACTIVITY OF STEM BARK OF BAUHINIA PURPUREA

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**ABSTRACT**

The nature has provided abundant plant wealth for all the living creatures, which possess medicinal virtues. Therefore, there is a necessity to explore their uses and to ascertain their therapeutic properties. Hence, the present study aims to open new avenues for the improvement of medicinal uses of *Bauhinia purpurea* (Leguminosae) stem bark for the selected area for anti-diabetic and anti-inflammatory activity. Dried (crude) petroleum ether and hexane extracts of stem bark of *Bauhinia purpurea were* subjected for *in-vitro* anti-diabetic activity and petroleum ether and methanol extracts were subjected for *in-vitro* anti-inflammatory activity. The results obtained indicate that the extracts possessed significant level of activity; the highest concentration of extract was high effective as an anti-diabetic and anti-inflammatory agent. However, these effects need to be confirmed using *in vivo* models and clinical trials for its effective utilization as therapeutic agents.

**Keywords:** *Bauhinia purpurea*, *In vitro* anti-diabetic activity, Haemoglobin glycosylation, α-amylase, *In vitro* anti-inflammatory activity, Albumin denaturation, Membrane stabilization

## INTRODUCTION

Medicinal plants play an appreciable role in the development of modern herbal medicines as many diseases like cancer, liver diseases and arthritis find no complete cure in allopathy. The bioactive compounds of medicinal plants are used as anti diabetic, chemotherapeutic, anti inflammatory, anti arthritic agents where no satisfactory cure is present in modern medicines. Medicinal plants have been used as dietary adjunct and in the treatment of numerous diseases without proper knowledge of their function. Although physiotherapy continues to be used in several countries, few plants have received scientific or medical scrutiny [1]. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the World. Among these 2,500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as Botanical garden of the World [2]. It has a great biodiversity due to its geographical and climatic conditions. In 2002 WHO launched its first comprehensive traditional medicine strategy to assist efforts to promote affordable, effective and safe use of traditional medicine and complimentary alternative medicine [3]. In Indian systems of medicine most practitioners formulate and dispense their own recipes. Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments [4]. Prior epidemiological studies have shown that the intake of natural antioxidants is allied with reduced risks of several diseases like diabetes and anti inflammatory disease. Recently, there is a growing interest in finding natural antioxidants to replace synthetic ones

[5]. Treatment to diabetes by western classical medicines is costly [6]. Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by increased fasting and post prandial blood sugar levels. The global prevalence of diabetes is estimated to increase from 4% in 1995 to 5.4% by the year 2025. WHO has predicted that the major burden will occur in developing countries [7]. Inflammation is a bodily response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions. It is triggered by the release of chemical mediators from injured tissue and migrating cells [8]. The commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs, which have several adverse effects especially gastric irritation leading to formation of gastric ulcers [9]. Natural products have contributed significantly towards the development of modern medicine. Previous researchers have reported some pharmacological properties on *Bauhinia purpurea* [10, 11]. There is no distinction made between the uses of *Bauhinia purpurea* for medicinal purposes. The review of literature reveals that *Bauhinia purpurea* is belonging to the family Leguminosae. The genus *Bauhinia*, consisting of 300 species. Native to India and this plant is among the most multi-purpose and conditions that have been treated with this plant include ulcer, cancer (prevention and treatment), antimicrobial, anti-oxidant, anti-diabetic and anti-inflammatory activity were reported and treatment of skin diseases (leucoderma and leprosy), wounds, cough, dysentery, snakebite, tumours, flatulence, indigestion, piles and also lots of other ailments [12]. It has been reported that the pharmacological significance was noted due to the presence of various bioactive compounds in the *Bauhinia* species such as flavonoids, steroidal glycosides, saponins, and tannins, flavanoids glycosides which are secondary metabolites [13, 14].

## MATERIAL AND METHODS

### Collection and identification and Plant materials

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 [15].

### 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 Methanol for 24 hrs and then at last the marc was subjected to hexane 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 [16, 17].

### 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 [18].

### *In-vitro* anti-diabetic activity

### Non-enzymatic glycosylation of haemoglobin assay

Antidiabetic activity of stem bark of *Bauhinia purpurea* were investigated by estimating degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically at 520nm. Glucose (2%), haemoglobin (0.06%) and Gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4. 1 ml each of above solution was mixed. 1 ml of each concentration was added to above mixture. Mixture was incubated in dark at room temperature for 72 hrs. The degree of glycosylation of haemoglobin was measured colorimetrically at 520nm. Alpha-Tocopherol (Trolax) was used as a standard drug for assay. % inhibition was calculated as previously published protocol [18, 19]. All the tests were performed in triplicate.

### Glucose uptake in Yeast cells

Yeast cells were prepared according to the method of Yeast cells [19] briefly, commercial baker's yeast was washed by repeated centrifugation (3,000×g; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (1–5 mg) were added to 1 mL of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µl of yeast suspension, vortex and further incubated at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant. Metronidazole was taken as standard drug.

The percentage increase in glucose uptake by yeast cells was calculated using previously published protocol. All the tests were performed in triplicate [20].

Table : 1 *In-vitro* Non-enzymatic glycosylation of haemoglobin method

| Blank | Conc$^{n}$ | STD | | PE | | HE | |
|---|---|---|---|---|---|---|---|
| | µg/ml | Abs | % inh | Abs | % inh | Abs | % inh |
| Abs (0.153 ± 0.002) | 20 | 0.191±0.04 | 19.8 | 0.209±0.02 | 26.7 | 0.193±0.03 | 26.1 |
| | 40 | 0.298±0.06 | 48.6 | 0.345±0.04 | 55.1 | 0.309±0.02 | 50.4 |
| | 60 | 0.358±0.07 | 57.2 | 0.498±0.05 | 68.9 | 0.396±0.06 | 61.3 |
| | 80 | 1.432±0.05 | 645 | 0.568±0.04 | 72.8 | 0.479±0.03 | 68.0 |
| | 100 | 1.511±0.03 | 70.0 | 0.635±0.02 | 75.7 | 0.582±0.02 | 73.7 |

STD- Standard, PE- Petroleum Ether extract, HE- Hexan extract, Abs- Absorbance, % inh - % inhibition, Conc.$^{n}$- Concentration. Values are expressed as mean ± SEM.

Table : 2 *In-vitro* Non-enzymatic glycosylation of haemoglobin method

| Sr. No. | STD | PE | HE |
|---|---|---|---|
| STDEV | 19.72 | 20.13 | 18.77 |
| $IC_{50}$ | 43 | 47 | 40 |

STDEV- standard deviation, $IC_{50}$- Inhibitory concentration at 50 % .

Table 3: % inhibition of Glucose uptake in 20mM glucose concentrations

| Glucose 20mM | | | | | | | |
|---|---|---|---|---|---|---|---|
| Blank | Conc$^{n}$ | STD | | PE | | HE | |
| | µg/ml | Abs | % inh | Abs | % inh | Abs | % inh |
| Abs (0.004 ± 0.001) | 20 | 0.005±0.03 | 20 | 0.006±0.02 | 33.3 | 0.005±0.03 | 28.5 |
| | 40 | 0.110±0.09 | 60 | 0.024±0.05 | 79.1 | 0.013±0.01 | 61.5 |
| | 60 | 0.119±0.04 | 96.6 | 0.190±0.07 | 97.3 | 0.123±0.09 | 95.9 |
| | 80 | 0.197±0.05 | 97.9 | 0.220±0.03 | 97.7 | 0.203±0.04 | 97.5 |
| | 100 | 0.213±0.02 | 98.1 | 0.297±0.09 | 98.3 | 0.311±0.06 | 98.3 |

STD- Standard, PE- Petroleum Ether extract, HE- Hexan extract, Abs- Absorbance, % inh- % inhibition, Conc.$^{n}$- Concentration. Values are expressed as mean ± SEM.

Table 4 : % inhibition of Glucose uptake in 10mM glucose concentrations

| Glucose 10 mM | | | | | | | |
|---|---|---|---|---|---|---|---|
| Blank | Conc$^{n}$ | STD | | PE | | HE | |
| | µg/ml | Abs | % inh | Abs | % inh | Abs | % inh |
| Abs (0.004 ± 0.001) | 20 | 0.020±0.01 | 50 | 0.123±0.04 | 91.8 | 0.111±0.04 | 90.9 |
| | 40 | 0.122±0.06 | 91.1 | 0.259±0.02 | 96.1 | 0.232±0.01 | 95.6 |
| | 60 | 0.197±0.04 | 94.9 | 0.358±0.05 | 97.2 | 0.329±0.07 | 96.9 |
| | 80 | 0.239±0.02 | 95.8 | 0.619±0.03 | 98.3 | 0.533±0.05 | 98.1 |
| | 100 | 0.302±0.05 | 96.6 | 0.987±0.02 | 98.9 | 0.898±0.09 | 98.8 |

STD- Standard, PE- Petroleum Ether extract, HE- Hexan extract, Abs- Absorbance, % in % ±inhibition, Conc.$^{n}$- Concentration. Values are expressed as mean ± SEM.

Table :5 % inhibition of Glucose uptake in 5mM glucose concentrations

| Glucose 5 mM | | | | | | | |
|---|---|---|---|---|---|---|---|
| Blank | Conc$^{n}$ | STD | | PE | | HE | |
| | µg/ml | Abs | % inh | Abs | % inh | Abs | % inh |
| Abs (0.004 ± 0.001) | 20 | 0.091±0.03 | 45.1 | 0.182±0.02 | 72.5 | 1.231±0.0 | 78.3 |
| | 40 | 0.145±0.02 | 65.5 | 0.212±0.06 | 76.4 | 0.260±0.02 | 80.7 |
| | 60 | 0.212±0.05 | 76.4 | 0.394±0.04 | 87.3 | 0.389±0.09 | 87.1 |
| | 80 | 0.334±0.08 | 85.0 | 0.619±0.07 | 91.9 | 0.487±0.05 | 89.7 |
| | 100 | 0.417±0.03 | 88.0 | 1.112±0.01 | 95.5 | 0.641±0.04 | 92.1 |

STD- Standard, PE- Petroleum Ether extract, HE- hexan extract, Abs- Absorbance, % inh- % inhibition, Conc.$^{n}$- Concentration. Values are expressed as mean ± SEM.

Table : 6 % inhibition of Glucose uptake in different glucose concentrations

| Sr.No. | 5mM | | | 10mM | | | 20mM | | |
|---|---|---|---|---|---|---|---|---|---|
| | STD | PE | HE | STD | PE | HE | STD | PE | HE |
| STDEV | 17.39 | 17.33 | 13.69 | 20.05 | 17.84 | 16.34 | 34.54 | 27.94 | 30.90 |
| $IC_{50\ Value}$ | 30.8 | 24.3 | 23.1 | 50.5 | 20.5 | 21.4 | 35.6 | 27.5 | 30.6 |

STDEV- standard deviation, $IC_{50}$- Inhibitory concentration at 50 % .

Table 7: Alpha Amylase inhibition

| Blank | Conc$^{n}$ | STD | | PE | | HE | |
|---|---|---|---|---|---|---|---|
| | µg/ml | Abs | % inh | Abs | % inh | Abs | % inh |
| Abs (0.09 ± 0.002) | 20 | 0.098±0.06 | 8.1 | 0.104±0.06 | 13.4 | 0.115±0.01 | 21.7 |
| | 40 | 0.158±0.04 | 43.0 | 0.321±0.04 | 71.9 | 0.389±0.02 | 76.8 |
| | 60 | 0.293±0.05 | 69.2 | 0.878±0.05 | 89.7 | 0.947±0.01 | 90.4 |
| | 80 | 0.375±0.08 | 76.0 | 1.070±0.08 | 91.5 | 1.177±0.08 | 92.3 |
| | 100 | 0.553±0.02 | 83.7 | 1.293±0.03 | 93.0 | 1.391±0.03 | 93.5 |

STD- Standard, PE- Petroleum Ether extract, HE- Hexan extract, Abs- Absorbance, % inh- % inhibition, Conc.$^{n}$- Concentration. Values are expressed as mean ± SEM.

Table : 8 *In-vitro* Alpha amylase inhibition method

| Sr. No. | STD | PE | HE |
|---|---|---|---|
| STDEV | 30.84 | 33.79 | 30.50 |
| $IC_{50\ Value}$ | 46.3 | 32.4 | 29.7 |

STDEV- standard deviation, $IC_{50}$- Inhibitory concentration at 50 % .

Table :9 Albumial denaturation assay

| Sr. No | Conc.$^{n}$ (µg/ml ) | % of activity | | |
|---|---|---|---|---|
| | | STD | PE | ME |
| 1 | 50 | 53.04±0.04 | 34.32±0.02 | 37.09±0.03 |
| 2 | 100 | 69.43±0.07 | 45.09±0.02 | 40.69±0.02 |
| 3 | 150 | 80.12±0.02 | 49.65±0.06 | 50.89±0.05 |
| 4 | 200 | 89.56±0.09 | 62.90±0.05 | 66.73±0.01 |
| 5 | 250 | 96.04±0.01 | 81.36±0.01 | 79.90±0.08 |
| 6 | SDEV | 17.02 | 18.10 | 18.02 |
| 7 | $IC_{50}$ value (µg/ml ) | 51.1 | 125.1 | 129.2 |

STD- Standard, PE- Petroleum Ether extract, ME- Methanol extract, % inh- % inhibition, Conc.$^{n}$- Concentration. Values are expressed as mean ± SEM, STDEV- standard deviation, $IC_{50}$- Inhibitory concentration at 50 %

Table : 10 Membrane stabilization assay

| Sr. No | Conc.$^{n}$ (µg/ml ) | % of activity | | |
|---|---|---|---|---|
| | | STD | PE | ME |
| 1 | 50 | 53.04±0.04 | 39.46±0.21 | 40.28±0.41 |
| 2 | 100 | 69.43±0.07 | 52.89±0.02 | 49.80±0.63 |
| 3 | 150 | 80.12±0.02 | 67.91±0.91 | 55.73±0.75 |
| 4 | 200 | 89.56±0.09 | 66.91±0.24 | 53.23±0.21 |
| 5 | 250 | 96.04±0.01 | 64.60±0.43 | 53.99±0.12 |
| 6 | SDEV | 17.02 | 15.58 | 9.88 |
| 7 | $IC_{50}$ value (µg/ml ) | 51.1 | 75.5 | 75 |

STD- Standard, PE- Petroleum Ether extract, ME- Methanol extract, % inh- % inhibition, Conc.$^{n}$- Concentration. Values are expressed as mean ± SEM, STDEV- standard deviation, $IC_{50}$- Inhibitory concentration at 50 %

Table :11 Proteinase inhibition assay

| Sr. No | Conc.$^{n}$ (µg/ml ) | % of activity | | |
|---|---|---|---|---|
| | | STD | PE | ME |
| 1 | 50 | 53.04±0.04 | 29.07±0.32 | 30.45±0.75 |
| 2 | 100 | 69.43±0.07 | 36.76±0.54 | 47.89±0.01 |
| 3 | 150 | 80.12±0.02 | 54.72±0.62 | 61.92±0.32 |
| 4 | 200 | 89.56±0.09 | 71.90±0.21 | 57.29±0.75 |
| 5 | 250 | 96.04±0.01 | 70.68±0.90 | 55.25±0.43 |
| 6 | SDEV | 17.02 | 19.41±0.83 | 12.32±0.19 |
| 7 | $IC_{50}$ value (µg/ml ) | 51.1 | 140.5 | 115.8 |

STD- Standard, PE- Petroleum Ether extract, ME- Methanol extract, % inh- % inhibition, Conc.$^{n}$- Concentration. Values are expressed as mean ± SEM, STDEV- standard deviation, $IC_{50}$- Inhibitory concentration at 50 % .

**Alpha- Amylase inhibition assay**

Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitory activity was based on the starch iodine method that was originally developed by [21] In alpha amylase inhibition method 1ml substrate- potato starch (1% w/v), 1 ml of drug solution (Acarbose std drug/ petroleum ether extract/ aqueous extract) of four different concentration such as 40, 60, 80 and 100 µg/ml, 1ml of alpha amylase enzyme (1% w/v) and 2ml of acetate buffer (0.1 M, 7.2 pH) was added. NOTE- Potato starch solution, alpha amylase solution and drug solution was prepared in acetate buffer.

The above mixture was incubated for 1 hr. Then 0.1 ml Iodine-iodide indicator (635mg Iodine and 1gm potassium iodide in 250 ml distilled water) was added in the mixture. Absorbance was taken at 565 nm in UV-Visible spectroscopy. % inhibition was calculated as previously published protocol. All the tests were performed in triplicate.

***IN-VITRO* ANTI-INFLAMMATORY ACTIVITY**

**Inhibition of albumin denaturation**

The protein denaturation bioassay was selected for *in vitro* assessment of anti-inflammatory property of petroleum ether and methanol extracts of *Bauhinia purpurea.* Methods of [22, 23]. The reaction mixture was consisting of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount at 37°C HCl. The sample extracts were incubated at 37°C for 20 min and then heated to 51°C for 20 min after cooling the samples. The colorimetric assay of albumin denaturation was performed according to previously publish protocol. The absorbance was measured at 660 nm and all the tests were performed in triplicate.

**Membrane stabilization test**

**Preparation of red blood cells (RBCs) suspension**

Fresh whole human blood (10 ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The

volume of blood was measured and re constituted as 10% v/v suspension with normal saline [24, 25].

**Heat induced hemolytic assay**

The reaction mixture (2 ml) consisted of 1 ml of test sample solution and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30 min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. Percent of membrane stabilization activity was calculated by the formula mentioned above [26].

**Protein inhibitory action**

The test was performed according to the modified method of [27,28] The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml of 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations. The reaction mixture was incubated at 37°C for 5 min and then 1ml of 0.8% (W/V) casein was added. The mixture was inhibited for an additional 20 min, 2 ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank.The experiment was performed according to previously publish protocol. The absorbance of the supernatants was taken at 210 nm against buffer as blank. The experiment was performed in triplicates. The percentage of inhibition of proteinase inhibitory activity was calculated from the formula mentioned above protocol.

**RESULT AND DISCUSSION**

***In-vitro* Non-enzymatic glycosylation of hemoglobin assay for *Bauhinia purpurea***

Human bodies possess enzymatic and non- enzymatic antioxidative mechanisms which minimize the generation of reactive oxygen species, responsible for many degenerative diseases including diabetes. Increased concentration of glucose in the blood leads to its binding to hemoglobin which may result in the formation of the reactive oxygen species [29]. Plant extracts play an important role the inhibition of the glycosylation end products. An increase in the glycosylation was observed on incubation of hemoglobin with the increasing concentration of the glucose over a period of 72hrs (Figure 1). However, the plant extracts significantly inhibited the haemoglobin glycosylation which is indicated by the presence of increasing concentration of haemoglobin. *Bauhinia purpurea* exhibited higher inhibition of glycosylation as compared with the standard druge. The plant extracts also displayed the inhibition of haemoglobin glycosylation at different physiological concentrations of the glucose over the period of 72 hrs, indicating that the plant extracts decreases the formation of the glucose-haemoglobin complex and thus amount of free haemoglobin increases.

*In-vitro* Non-enzymatic glycosylation of hemoglobin assay of *Bauhinia purpurea* evaluated previously worked.

***In-vitro* Glucose uptake in Yeast cells**

A study of ancient literature indicates that diabetes (Madhumeha/Prameha) was fairly well known and well conceived as an entity in India. The rate of glucose transport across cell membrane in yeast cells system is presented in Figure. 2, 3 and 4. Regulation of glucose level in the blood of the diabetic patient can prevent the various complications associated with the disease. The maintenance of plasma glucose concentration for a long term under a variety of dietary conditions is one of the most important and closely regulated processes observed in the mammalian species [30]. The i*n vitro* assays of the present study indicated that petroleum ether extract and hexane extract possess good anti diabetic activity. In Yeast (Saccharomyces cerevisiae) glucose transport takes place through facilitated diffusion. Type 2 Diabetes is characterised by the deficiency of insulin causing increased amount of glucose in blood. After the treatment of the yeast cells with these plant extracts, the glucose uptake was found to increase in a dose dependent manner. Figures 1, 2 and 3 depict the % increase in glucose uptake by the yeast cell at different glucose concentrations i.e. 25mM, 10mM and 5mM respectively. The petroleum ether extract of *Bauhinia purpurea* exhibited significantly higher activity than hexane extract at all glucose concentrations showing the maximum increase in 10mM Glucose concentration.Results also indicated that *Bauhinia purpurea* had greater efficiency in increasing the glucose uptake by yeast cells as compared to standard drug metronidazole.

***In vitro* study using alpha-amylase inhibition assay**

The intestinal digestive enzymes alpha-amylase plays a vital role in the carbohydrate digestion. One antidiabetic therapeutic approach reduces the post prandial glucose level in blood by the inhibition of alpha-amylase enzyme. These can be an important strategy in management of

blood glucose [31]. The in-vitro **α**-amylase inhibitory studies demonstrated that *Bauhinia purpurea* has well anti diabetic activity. The percentage inhibition at 100, 80,60, 40, 20 µg/ml concentration of crude plant extracts shown concentration dependent reduction in percentage inhibition. At a concentration of 20 µg/ml of *B. purpurea* extracts petroleum ether and hexane showed a percentage inhibition 13.4 % and 21.7% and for 100 µg/ml extracts showed inhibition of 93.0% and 93.5% as per Table 7.

As the result shows petroleum ether and hexane extract of *Bauhinia purpurea* shows significant activity as compared to Acarbose standard drug, and 80 and 100 µg/ml concentration of petroleum ether extract shows greater activity than Acarbose shown in fig 5.

**Inhibition of albumin denaturation by *Bauhinia purpurea***

Inflammation is a bodily response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti inflammation activity, ability of extract protein denaturation was studied [32]. It was effective in inhibiting heat induced albumin denaturation as per Table 9 and figure 6. Maximum inhibition 81.36 % was observed from petroleum ether extract and hexane extract shows 79.90% at the concentration of 250 µg/ml. Aspirin, a standard anti-inflammation drug showed the maximum inhibition 96.04% at the concentration of 250 µg/ml. The IC50 value of both extract in higher than standard (Aspirin) value 51.1 µg/ml as per Table 10.

**Membrane stabilization test by *Bauhinia purpurea***

The HRBC membrane stabilization has been used as a method to study the invitro anti inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may well stabilize lysosomal membranes. Stabilization of lysosomal is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular release. The lysosomal enzymes released during inflammation produce a various disorders. The extra cellular activity of these enzymes are said to be related to acute or chronic inflammation. The non steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane .Stabilization of RBCs membrane was studied [33] for further establishes the mechanism of anti-inflammatory action of different extracts of Bauhinia purpurea. Both the extracts were shown inhibiting the heat induced hemolysis compare with standard Aspirin. These results provide evidence for membrane stabilization as an additional mechanism of their anti inflammatory effect.

The extracts inhibited the heat induced hemolysis of RBCs to varying degree as per Table 11 and figure 7. The maximum inhibitions 75.5% was observed from petroleum ether extract followed by methanol 75% and the aspirin standard drug showed the maximum inhibition 51.1%. This result is well supported with reported study of inflammatory activity reported.

**Proteinase inhibitory activity by *Bauhinia purpurea***

Neutrophils are known to be a rich source of serine proteinase and are localized at lysosomes. It was previously reported that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitorsThe Bauhinia purpurea crude extract exhibited significant antiproteinase activity from different extracts. The maximum inhibition was observed from petroleum ether extract 140.5%, in decreasing order was methanol 115.8%. The standard aspirin 51.1% drug showed the maximum proteinase inhibitory action as per Table 12 and figure 8 shows the activity with compare to standard drug Aspirin.

Bauhinia purpurea extract had reported for anti-inflammatory and antipyretic properties.

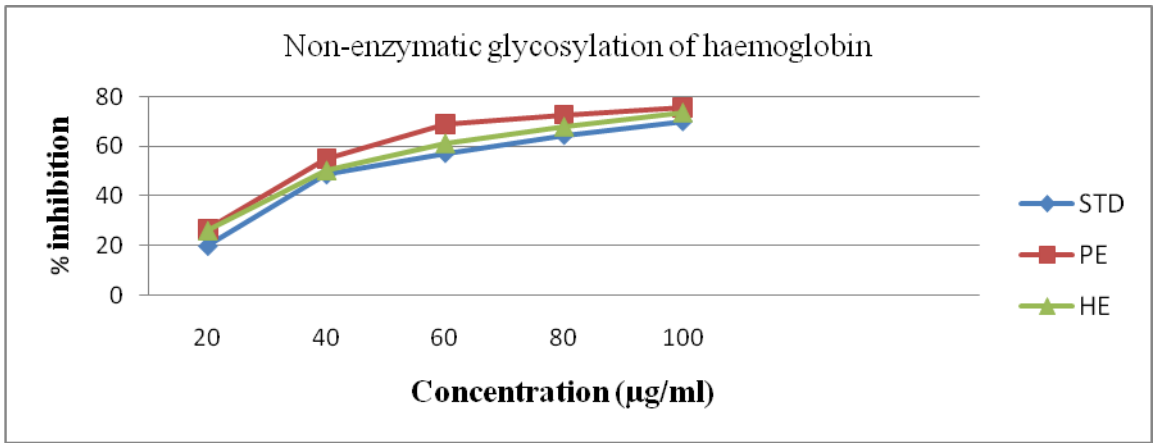


Figure 1. Non-enzymatic glycosylation assay.

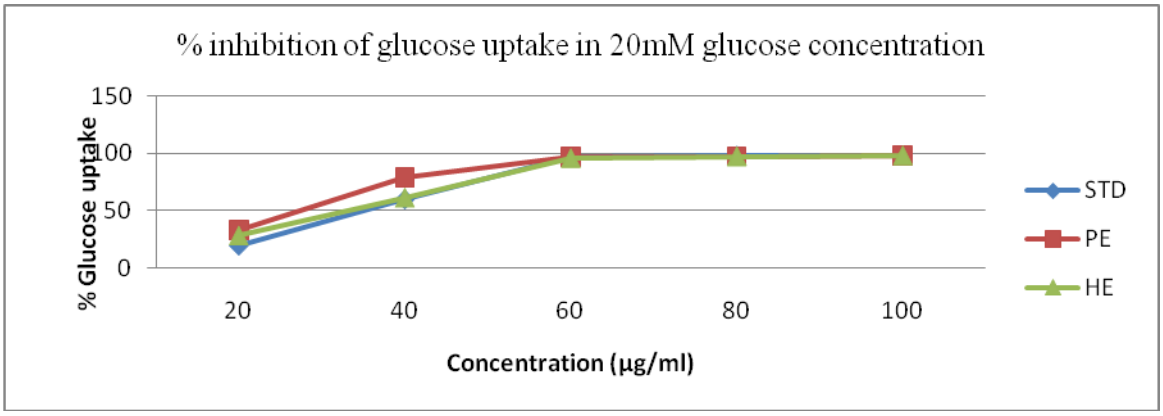


Figure : 2 % Glucose uptake in 20mM glucose concentrations

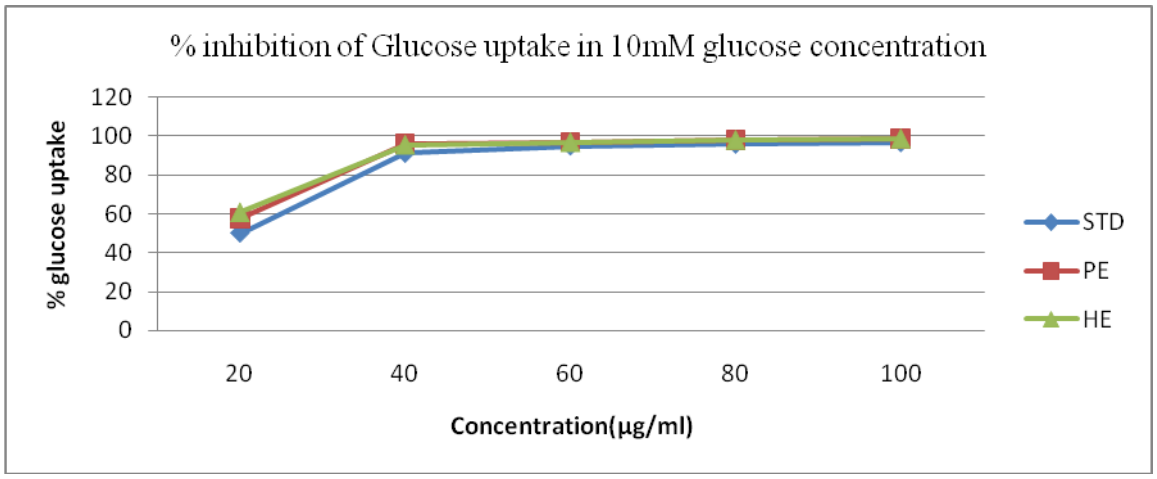


Figure :3 % Glucose uptake in 10mM glucose concentrations

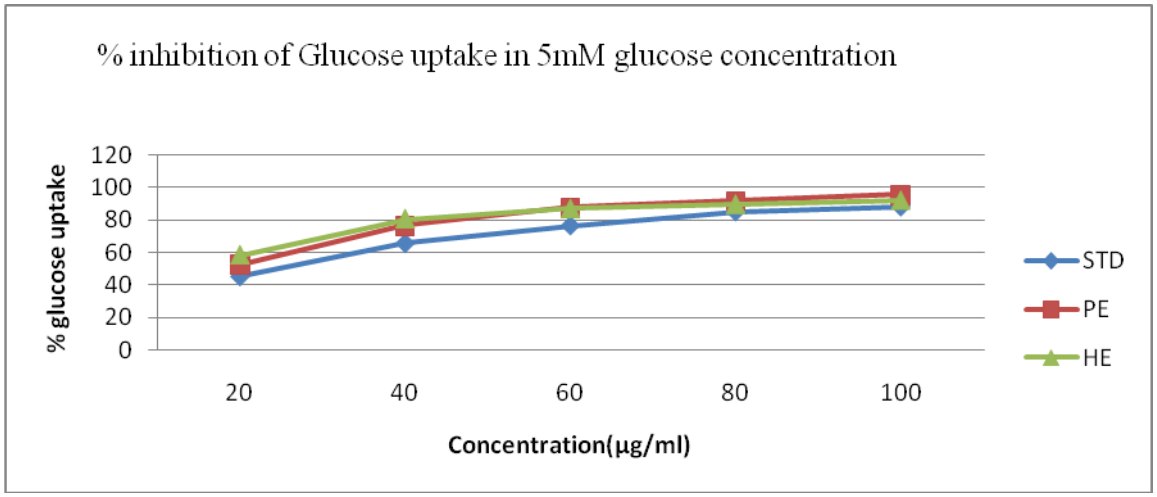


Figure : 4 % Glucose uptake in 5mM glucose concentrations

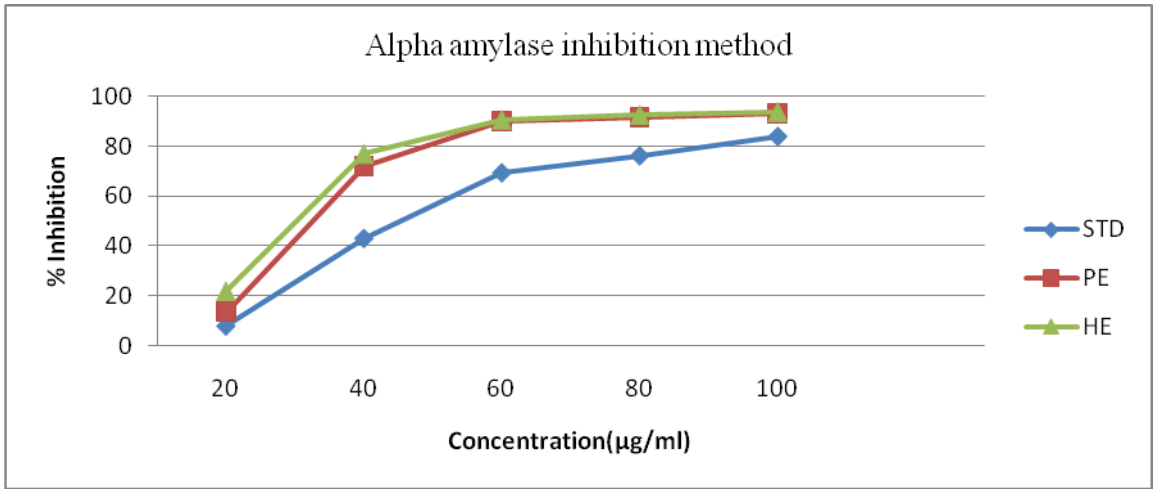


Figure : 5 Alpha amylase inhibition assay of *B. purpurea*

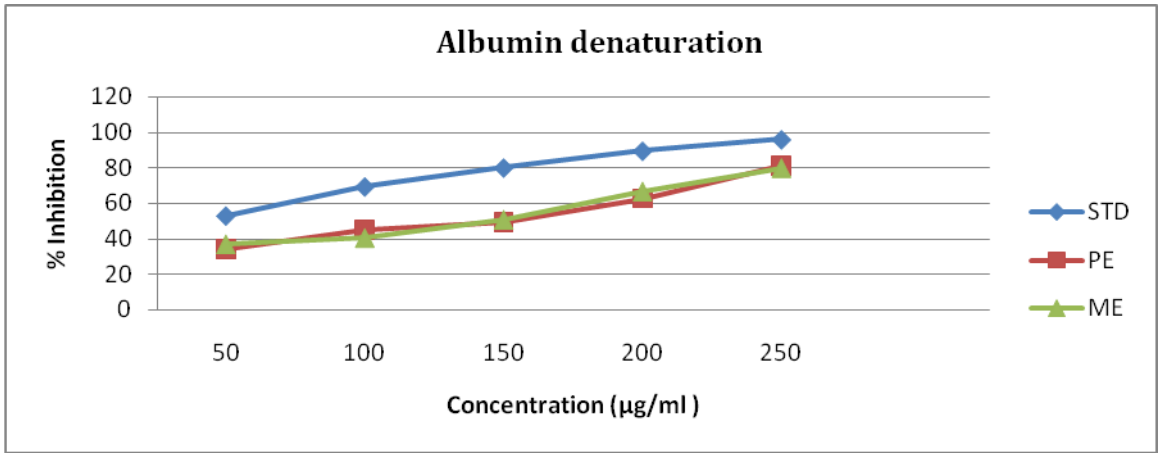


Figure : 6 Albumin denaturation activity of *B. purpurea*

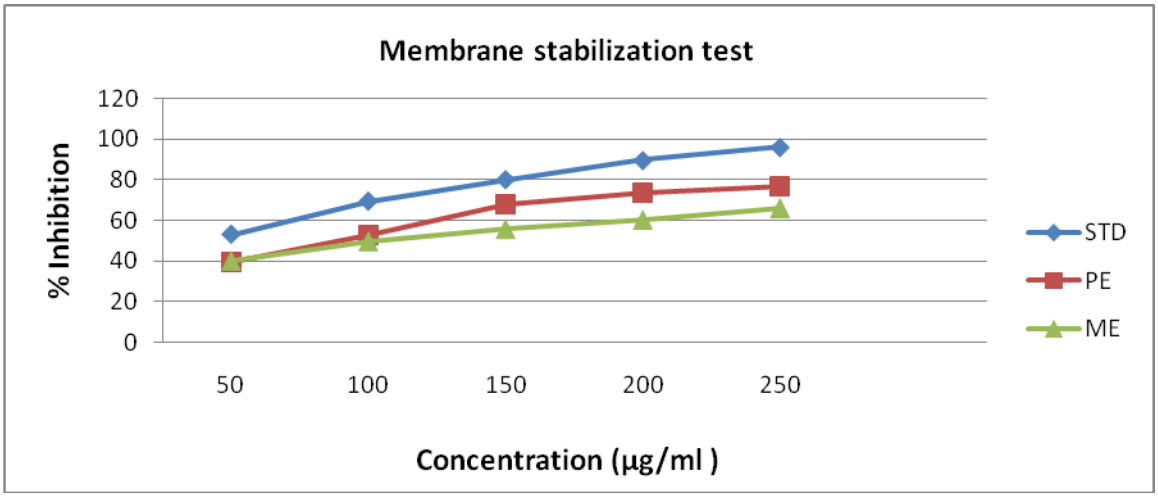


Figure : 7 Membrane stabilization activity of *B. purpurea*

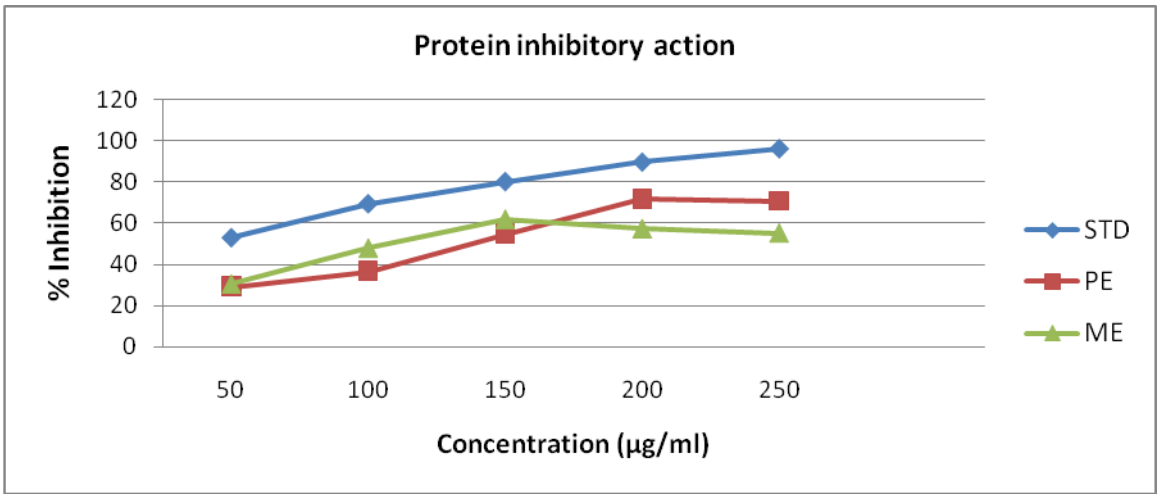


Figure : 8 Protein inhibitory action of *B. purpurea*

**CONCLUSION**

In conclusion, our findings showed Leguminosae plant, *Bauhinia purpurea* have the potential to be explored further to identify the anti diabetic and anti inflammatory compounds in this plant. This *Bauhinia purpurea* and its quantification of individual phytoconstituents as well as pharmacological profile based on *in-vitro* and *in-vivo* studies and on clinical trial should be further investigated.

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