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Research Article

Pharmacognostic and *In vitro* Anti diabetic Activity on Aerial Parts of *Leucas aspera* (Willd). Link

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ABSTRACT

The study was designed to identify the Phytochemical work, microscopical characters and *Invitro* antidiabetic activity of *Leucas aspera.* Plants are being used from more than 1000 years to treat many diseases. *Leucas aspera* commonly known as "Thumbai" or Gumma is found all over India. The preliminary phytochemical screening of *Leucas aspera* was done by using Ethyl acetate, Acetone and Hydro alcoholic extracts. Ethyl acetate extract showed more intense on carbohydrates, proteins, glycosides, terpenoids. Acetone extract showed the presence of carbohydrates, phenols. Hydro alcoholic extract showed positive results on alkaloids, carbohydrates, tannins, glycosides, flavanoids, terpenoids and saponins. On Microscopic study involves the T.S. of leaf and stem, Powder microscopy was carried out and it showed the presence of dominating diacytic stomatas when compared to anisocytic stomata. Starch grains, glandular trichomes, lignified phloem fibres, lignified xylem vessels, prismatic calcium oxalate crystals are present. Diabetes mellitus is a metabolic disorder characterized by high blood glucose level resulting from defects in insulin secretion, insulin action or both. The incidence of diabetes increasing day by day and this indicates the increasing need for the treatment of diabetes. In the present study *L.aspera* were screened for antidiabetic activity by using assays such as glycosylation of hemoglobin assay, glucose uptake by yeast cells. Glycosylation of hemoglobin assay was done by taking the standard drug (Metformin) 5mg/ml and the plant extracts 2,4,6,8,10 mg/ml. However, 10mg/ml of plant extract showed equal inhibition of glycosylation when compared with standard drug. Glucose uptake by yeast cells was carried out by using 1, 2,3,4,5 mg/ml of plant extracts and the result shows the % increase in glucose uptake by yeast cells at different glucose concentrations, the hydro alcoholic extract of 3 mg/ml has showed significant activity when compared to the standard drug.

Keywords: *Leucas aspera*, preliminary phytochemical, microscopical and *invitro* antidiabetic activity

INTRODUCTION

Medicinal plants are the only source for the treatment of diseases in ancient days and since then numerous herbs and plants have been recognized as a medicinal plants because of their potency to cure ailments[1]. The newly discovered and the existing medicinal plants are being screened for many diseases and to identify significant therapeutic importance[2]. *Leucas aspera*, is a herb is widely distributed in Tropical Asia, Africa and grows in highland crop fields, homesteads, fallow lands and roadsides[3]. *Leucas aspera* commonly known as Thumbai is widely distributed throughout India from the Himalayas down to Ceylon. The plant is used traditionally as an antipyretic and insecticide. Flowers are used as stimulant, expectorant and diaphoretic. Leaves are considered useful in chronic rheumatism, psoriasis and other chronic skin eruptions. Bruised leaves are applied locally in snake bites[4]. Many phyto chemicals belong to the class of terpenoids, fatty acids, glycosides, flavanoids, lignans and alkaloids were identified and isolated by using different extraction methods[5-6], various microscopic characters were identified. Diabetes Mellitus is an established non communicable disease and often described as fourth or fifth leading cause of mortality in high income countries[7]. According to World Health Organization, the global prevalence of diabetes is estimated to increase from 4% in 1995 to 5.4% by the year 2025 majorly in the developing countries. India presently has the largest number of diabetic patients in the world and has been infamously known as the diabetic capital of the world [8]. Sulfonylureas, biguanide, thiazolidinedione, and glycosidase inhibitors are widely used to control the hyperglycemia, hyperlipidemia and insulin resistance of type 2 diabetes, but these drugs fail to significantly alter the course of diabetic complications and have limited use because of undesirable side effects and high rates of secondary failure. Moreover, they are not safe for use during pregnancy[9]. Thus, the management of diabetes without any side effects is still a challenge. There is continuous search for alternative drugs[10]. As a result of the global epidemic of diabetes, the limited potency and many side effects of medications currently in use, the need for new diabetes therapies is expected to grow dramatically during the next decade. An intense research has been conducted to identify new therapeutic targets and pharmacologic compounds that might correct the impaired glucose tolerance. During the recent years many investigators have shown that natural products are a potential source for new drug candidates for many diseases

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in general, and diabetes in particular[11]. Some recent studies showed the medicinal value of mangroves, and associated plants persist to provide invaluable treatment modalities, both in modern and traditional systems of medicine[12,13]. The medicinal properties of mangrove trees provide a wide domain for medical uses including diabetes, cancer, etc. Recently, there has been a growing interest in the identification of biomolecules from plant sources to reduce the hyperglycemic conditions [14]. Several mechanisms have been proposed for the hypoglycemic effect of primary and secondary phytochemicals, such as manipulation of glucose transporters, β-cell regeneration and enhancing insulin-releasing activity [15,16], glucose adsorption, retarded diffusion and inhibition of carbohydrate metabolizing enzymes at gut level, stimulating effect on glucose utilization, food adjuvants for diabetic patients, and uptake of glucose by cells through facilitated diffusion in yeast cell model system[15,17,18]. The recent advances in understanding the activity of intestinal enzymes (α amylase and α-glucosidase) have led to the development of newer pharmacological agents [19].

MATERIALS AND METHODS

The freshly collected aerial parts of *L.aspera* were shade dried. The plant parts were powdered mechanically and stored in an air tight container. The extraction was carried out by hot percolation method by using soxhlet apparatus. The solvent used was hydroalchol. About 40gms of powder was extracted with 200ml of solvent. The extract was concentrated to dryness under controlled temperature 40-50^0 c . The extract was preserved in refrigerator till further use.

Phytochemical Analysis

The prepared extract was tested for the type of chemical constituents present by known qualitative tests. [20, 21,22,23,24,25,26]

The following tests were carried out on the extracts to detect various phyto constituents present in them.

1. Test for Alkaloids

About 50mg of solvent-free extract was stirred with little quantity of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents as follows.

a) Mayer's test

To a few ml of filtrate, two drops of Mayer's reagent was added along with the sides of test tube. If the test is positive, it gives white or creamy precipitate.

b) Wagner's test

To a few ml of the filtrate, few drops of Wagner's reagent were added along with the sides of the test tube. Formation of reddish brown precipitate confirms the test as positive.

c) Hager's test

To a few ml of filtrate 1or 2 ml of Hager's reagent was added. A prominent yellow precipitate indicates positive test.

d) Dragendroff's test

To a few ml of filtrate, 1 or 2 ml of Dragendroff's reagent was added. A prominent reddish brown precipitate indicates positive test.

2. Test for Carbohydrates

About 100mg of the extract was dissolved in 5ml of distilled water and filtered. The filtrate was subjected to the following tests.

a) Molisch's test

To 2 ml of filtrate, two drops of alcoholic solution of α-napthol was added. The mixture was shaken well and 1 ml of concentrated sulphuric acid was added slowly along the sides of the test tube, the test tube was cooled in ice water and allowed to stand. A violet ring at the junction of two liquids indicates the presence of carbohydrates.

b) Fehling's test

1 ml of filtrate was boiled on a water bath with 1 ml each of Fehling's solution A and B. Formation of red precipitate indicates the presence of sugar.

c) Barfoed's test

To 1 ml of the filtrate, 1 ml of Barfoed's reagent was added and heated on a boiling water bath for 2 minutes. Red precipitate indicates the presence of sugars

d) Benedict's test

To 0.5 ml of filtrate 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes. A characteristic brick red precipitate indicates the presence of sugar.

3. Test for Glycoside

For the detection of glycosides, about 50 mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and filtrate was subjected to following tests.

a) Borntrager's test

To 2 ml of filtrate hydrolysate, 3 ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it. Formation of pink color indicates the presence of anthraquinone glycosides.

b) Legal's test

About 50 mg of the extract was dissolved in pyridine. Sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide solution. Presence of glycoside is indicated by a characteristic pink color.

4) Test for Saponins

a) Froth test

A small quantity of the extract was diluted with distilled water to 20 ml. The suspension was shaken in graduated cylinder for 15 minutes. A two centimeter layer of foam or froth which is stable for 10 minutes indicates the presence of saponins.

5) Test for Phytosterols and Triterpenoids

a) Liebermann- Burchard 's test

The extract was dissolved in acetic anhydride, heated to boiling cooled and then 1 ml of concentrated sulphuric acid was added along the side of the test tube. Red, pink or violet color at the junction of the liquids indicates the presence of steroidal triterpenoids and their glycosides.

b) Salkowski test

Few drops of concentrated sulphuric acid was added the chloroform extract, shaken on standing, red color in the lower layer indicates the presence of steroids and golden yellow color indicates the presence of triterpenoids.

6) Test for Phenols and Tannins

a) Ferric chloride test

About 50 mg of extract was dissolved in distilled water and to this few drops of neutral 5% ferric chloride solution was added. Formation of blue, green and violet color indicates the presence of phenolic compounds.

b) Gelatin test

A little quantity of extract was dissolved in distilled water and 2 ml of 1% solution of gelatin containing 10% sodium chloride was added to it. Development of white precipitate indicates the presence of phenolic compounds.

c) Lead acetate test

A small quantity of extract was dissolved in distilled water and to this; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicates the presence of phenolic compounds.

7) Test for Flavanoids

a) Alkaline reagent test

An aqueous solution of extract was treated with 10% ammonium hydroxide solution- yellow fluorescence indicates the presence of flavanoids.

b) Shinoda test

A little quantity of extract was dissolved in alcohol and few fragments of magnesium turnings and conc. Hydrochloric acid (drop wise) were added. If any pink or crimson- red color develops, presence of flavonol glycoside is inferred.

c) Zinc- hydrochloric acid reduction test

The alcoholic solution is treated with pinch of zinc dust and few drops of conc. Hydrochloric

Pharmacognostic Study

Pharmacognostic study includes parameters which help in identifying adulteration in dry powder form also. This is again necessary because once the plant is dried and made into powder form, it loses its morphological identity and easily prone to adulteration. Pharmacognostic studies ensure plant identity, lays down standardization parameters which will help and prevents adulterations. Such studies will help in authentication of the plants and ensures reproducible quality of herbal products which will lead to safety and efficacy of natural products. The pharmacognostic standardization parameters which are generally done are described below[30,31]

Macroscopic Study

Macroscopic identity of medicinal plant materials is based on shape, size, color, surface characteristics, texture, fracture characteristics and appearance of the cut surface. However, since these characteristics are judged subjectively and substitutes or adulterants may closely resemble the genuine material, it is often necessary to substantiate the findings by microscopy and/or physicochemical analysis.

Microscopic study

The microscopic study is the anatomical study which is done by taking appropriate section of the plant parts under study. Each distinguishing character can be noted down, some of which are retained in the powder study also. Some of the chemicals which are used in obtaining clear sections are phloroglucinol, chloral hydrate, safranine, methyl orange, etc.

Microscopy

The parts of the plant were dipped in a test tube containing sufficient water and were boiled for few minutes. Then it was transversally sliced into fine sections which were subjected to staining reagent 0.1%w/v phloroglucinol followed by concentrated conc. hydrochloric acid. The stained sections were observed under microscope. Different layers of cells and identifying characters were observed then photomicrography was done. [6,30,31,32,33]

The leaf of Midrib shows epidermis on either side with uni to tricellular trichomes, followed by 1-2 layers collenchymas towards the lower surface, 3-4 layers towards upper surface, followed by round to oval parenchyma, 4-7 layered; vascular bundle are shaped, present in center.

The stem shows Squarish outline with four ridges and furrows, consists of a single layered epidermis, composed of oval to rectangular, thin walled cells having a number of uni to tricellular trichomes; secondary cortex 5-9 layered, oval or irregular collenchymatous cells at the ridges covered with hairs. Phloem is very narrow consisting of usual elements; xylem consists of vessels, tracheids, fibres and xylem parenchyma.

Powder Microscopy

The dried plant was powdered and studied under microscope. Different staining reagents (such as iodine for detection of starch grains and phloroglucinol for detection of lignified components) were used. To a little quantity of stem bark powder taken over a microscopic slide, 1-2drops of 0.1%w/v phloroglucinol solution and a drop of concentrated hydrochloric acid were added and covered with a cover slip. The slide preparation was mounted in glycerol and examined under microscope. The characteristic structures and cell components were observed.

In-Vitro Anti-Diabetic Activity

Non-Enzymatic Glycosylation Of Hemoglobin Assay[34,35] :

Antidiabetic activity of *Leucas aspera* aerial parts was investigated by estimating the degree of non enzymatic haemoglobin glycosylation, measured colorimetrically at 520 nm Glucose (2%), haemoglobin (0.06%) and Gentamycin (0.02%) solution were prepared in phosphate buffer 0.01M, pH 7.4. 1 ml each of above solution was mixed. 2mg/ml 4mg/ml, 8mg/ml, 10mg/ml extract was added to above mixture. Mixture was kept in dark at room temperature for incubation for 72 hrs. At 520nm haemoglobin glycosylation was measured colorimetrically .The standard drug used for assay was Metformin. %inhibition was calculated.

b) Glucose Uptake In Yeast Cells Method

The 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 both plant extracts (1–5 mg) were added to 1ml of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100µl 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. Metformin was taken as standard drug. [39,40]

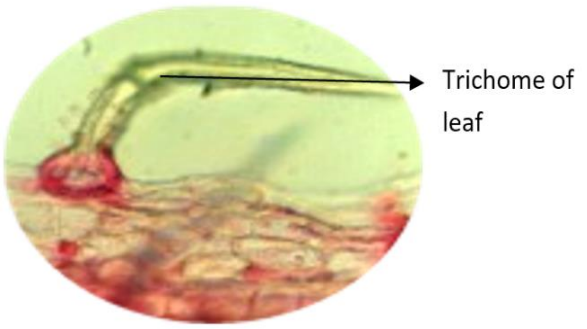
RESULTS AND DISCUSSION

Table 1: Preliminary Phytochemical tests of the extract of *L.aspera*

Phytochemical analysis	Ethyl acetate extract	Acetone extract	Hydroalcoholic extract
Alkaloids	_	+	++
Carbohydrates	++	++	++
Tannins	_	+	++
Phenols	+	++	++
Glycosides	++	+	+
Flavonoids	_	_	+
Steroids	_	_	_
Proteins	++	_	_
Saponins	_	+	+
Terpenoids	++	+	++

"-" indicates Absent, "+" indicates Presence , "++" indicates more intense

The preliminary phytochemical screening *Leucas aspera* was done by using Ethyl acetate, Acetone and Hydro alcoholic extracts. Ethyl acetate extract showed the presence of carbohydrates, proteins, glycosides, terpenoids. Acetone extract showed the presence of carbohydrates, phenols. Hydro alcoholic extract showed the presence of Alkaloids, Carbohydrates, Tannins, Glycosides, Flavanoids, terpenoids, Saponins. The microscopic study of *L.aspera* midrib of leaf showed epidermis on both sides with uni to tricellular trichomes, followed by collenchymatous and vascular bundles.The microscopic study of stem showed quadrangular shaped with four ridges and furrows, uni to tricellular trichomes, lignified phloem, Xylem vessels. Powder microscopy showed stomata, starch grains, glandular trichomes, lignified phloem fibres, lignified vessels, prismatic calcium oxalate crystals. Human being minimizes the production of reactive oxygen species by enzymatic and non enzymatic anti-oxidant mechanism, which plays a key role in many degenerative diseases including diabetes. High glucose levels in body leads to its binding to haemoglobin which may result in the production of reactive oxygen species. End products of glycosylation can be inhibited by plant extracts[36]. Upon incubation of haemoglobin with different concentration of glucose over a period of 72 hour will increase glycosylation. However, upon increasing the concentration of haemoglobin the plant extracts inhibited haemoglobin glycosylation. *Leucas aspera* exhibited higher inhibition of glycosylation when compared with the standard drug(5mg/ml). Over the period of 72 hour the plant extracts decreases haemoglobin glycosylation by decreasing the formation of the glucose-haemoglobin complex and amount of free haemoglobin increases [38]. Control of blood sugar levels 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. The present study indicates that the hydro alcoholic extract possess good anti diabetic activity. Glucose transport takes place through facilitated diffusion in yeast. Type 2 Diabetes is characterized by the deficiency of insulin causing increased amount of glucose in blood. After the



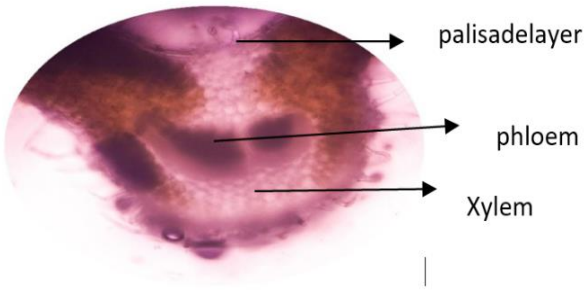
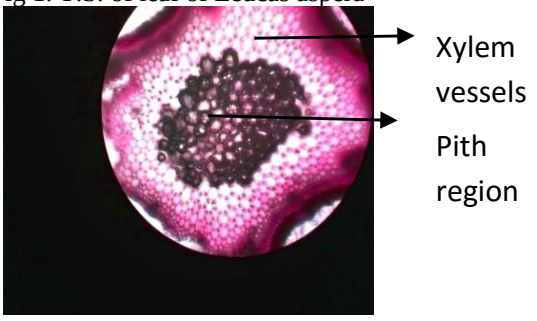


Fig 1. T.S. of leaf of Leucas aspera



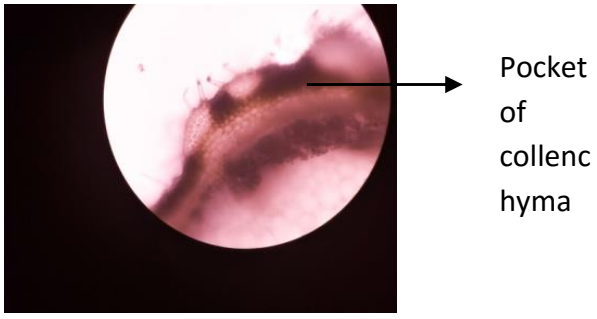


Fig 2: T. S. of Stem of *L.aspera*

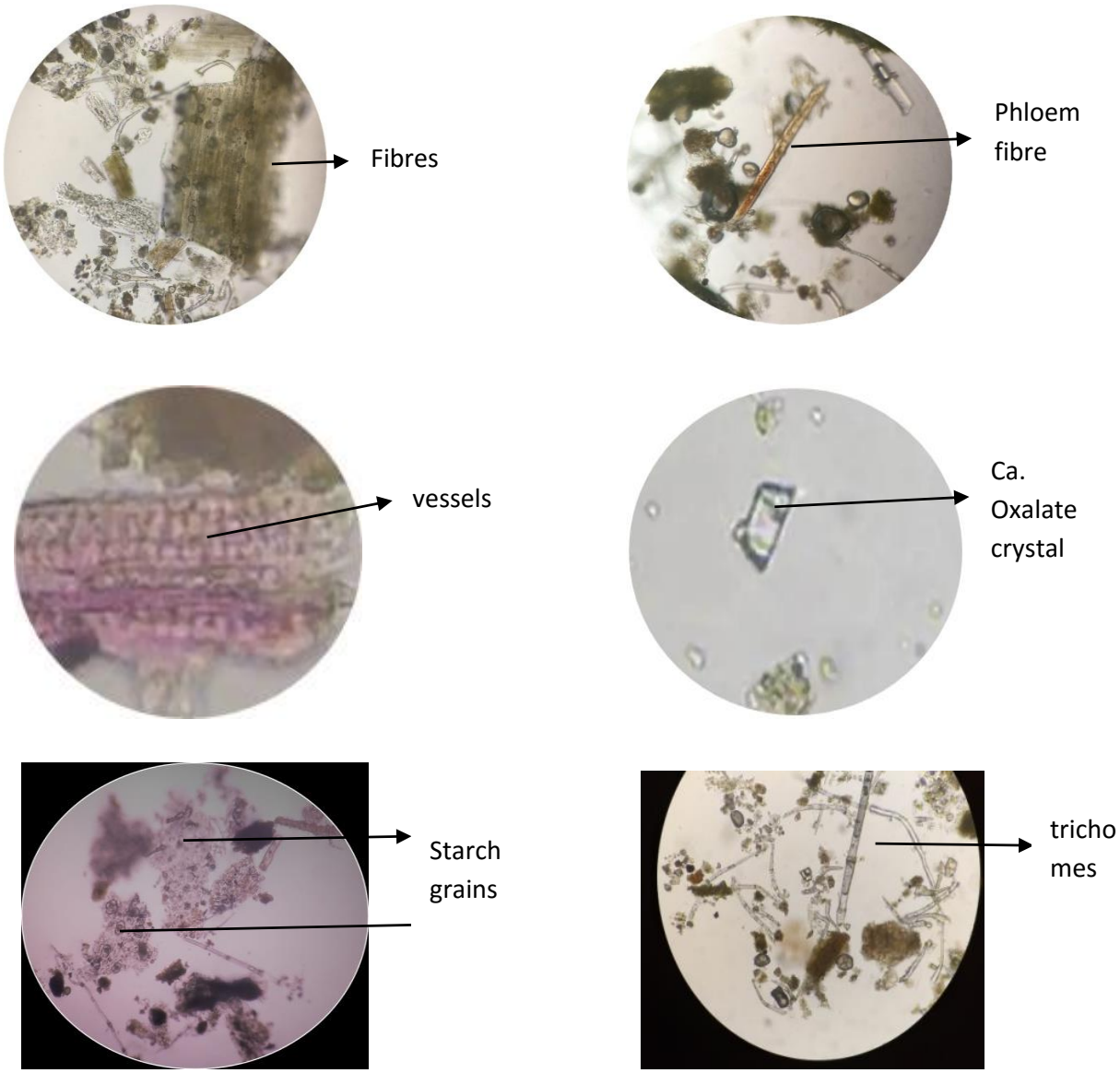


Fig 3 Powder microscopy of *L.aspera*

treating yeast cells by these plant extract, the glucose uptake was found to increase as per dose. The results shows the % increase in glucose uptake by yeast cells at different glucose concentrations, the hydro alcoholic extract of 3 mg/ml has showed significant activity when compared to the standard drug.

CONCLUSION

In conclusion of our findings showed by *L.aspera* showed to possess anti diabetic components. *L.aspera* and its quantification of individual phytoconstituents as well as pharmacological profile based on in-vitro and in-vivo studies and on clinical trails should be investigated further.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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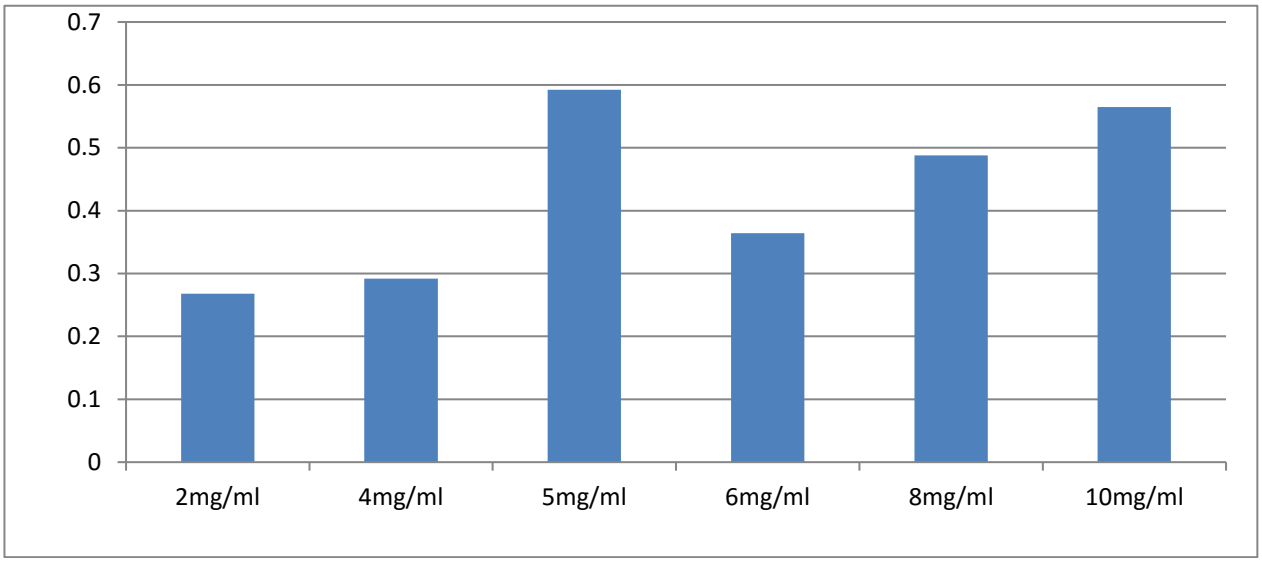
Table 2: Non-Enzymatic Glycosylation Of Hemoglobin Assay:

Blank	Standard			Hydro alcoholic extract		
Absorbance	concentration	Absorbance	%inhibition	concentration	Absorbance	%inhibition
0.129	5	0.592	77%	2	0.268	52%
				4	0.292	56%
				6	0.364	65%
				8	0.488	74%
				10	0.565	77%

Table 3: Glucose Uptake In Yeast Cells Method

Conc. (mg/ml)	blank	Standard Absorbance	%inhibition	Extract Absorbance	%inhibition
1.0		0.121	16%	0.124	20%
2.0		0.152	32%	0.135	24%
3.0		0.169	39%	0.166	40%
4.0	0.102	0.157	35%	0.149	32%
5.0		0.162	37%	0.152	33%



Graph 1: Non- enzymatic Glycosylation of haemoglobin assay
Standard(5mg/ml), Extracts (2, 4, 6, 8, 10 mg/ml)

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