

# *In vitro* Antioxidant & Antidiabetic Activity of *Indigofera prostrata*

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**Abstract** Numerous diseases, such as cancer, diabetes mellitus, aging, arthritis are linked to free radicals. Antioxidant therapy has become increasingly important in the management of several illnesses. Diabetes mellitus is an intricate metabolic ailment characterized by insistent hyperglycemia that interferes with protein, lipid and carbohydrate metabolism. Therefore, using a variety of conventional *in vitro* models, a modern investigation sought to investigate the antioxidant and antidiabetic properties of *Indigofera prostrata*. The methanol extracts of *Indigofera prostrata* were assessed in this endeavor for their ability to block radicals via the use of superoxide radical action, DPPH, lipid peroxidation assay, and the nitric oxide method. Numerous factors, including glucose diffusion, the inhibitory actions of alpha amylase, alpha glucosidase, and the ability of yeast cells to absorb glucose, were also used to measure the *in vitro* antidiabetic efficacy. When compared to conventional antioxidants, the revealed results of plant extracts demonstrated a higher capability for radical scavenging in relation to their antioxidant activity. Similarly, a variety of characteristics demonstrated that *in vitro* extracts of *Indigofera prostrata* had excellent antidiabetic action. In summary, *Indigofera prostrata* has strong anti-oxidant and antidiabetic qualities.

**Keywords** Methanol Extract, *In vitro* Antioxidant and Antidiabetic Properties, *Indigofera prostrata*

characterized by insistent hyperglycemia that impairs bodily processes. The cause of diabetes mellitus varies greatly but steadily with each release of insulin or tissue vibration [1]. Diabetes exhibits a considerable amount of oxidative stress. Hyperglycemia is caused by the auto-oxidation of glucose that generates free radicals. The result supports the advance of diabetes and its associated difficulties. Antioxidants can have incalculable effects such as preventing ROS from organizing, removing free radicals or enhancing the protective and catalytic properties of cells. Antioxidants like N-acetylcysteine and vitamin C are effective in reducing diabetic complications, suggesting that these could be beneficial when taken as a dietary supplement or when ingested with typical cell reinforcements. Antioxidants are usually used to lessen the complications associated with diabetes [2]. Antioxidants have a tendency to destroy harmful free radicals at the molecular and cell levels. Reactive oxygen species have been shown to play a significant role in the progression of several diseases. According to Aruoma, free radicals are created when oxidation events start chain reactions that destroy cells and cause a variety of degenerative illnesses, including cancer [3]. Antioxidants function as oxygen scavengers and eliminate free radicals to stop this chain reaction. A chemical that can lessen or prevent molecules from oxidizing is called an antioxidant. Natural antioxidants are receiving more and more attention from consumers; they are primarily found in fruits and vegetables [4]. Epidemiological research has demonstrated a link between a lower risk of disease and regular consumption of antioxidants from natural sources [5]. Estimating its antioxidant and antidiabetic effects using *in*

## 1. Introduction

Diabetes mellitus is an intricate metabolic ailment

*vitro* models was the main focus of the recent studies. The goal of the current study was to evaluate multiple parameters in an *in vitro* model to determine the antioxidant activity of *Indigofera prostrata*.

## 2. Materials & Methods

### 2.1. Authentication & Collection of Plants

In the Indian district of Chittoor, in the province of Andhra Pradesh, the full *Indigofera prostrata* plant was gathered and authenticated in July. Retired Associate Professor of Botany at Sri Venkateshwara University in Tirupathi, India, Dr. K. Madhava Chetty has verified the authenticity of the selected whole plant material of *Indigofera prostrata*. Following that, a sample was preserved for later use in a herbarium with the appropriate voucher numbers for *Indigofera prostrata* (1129).

### 2.2. Extraction

The entire plant material of *Indigofera prostrata* was shade-dried and then milled into a coarse powder. After being put through a sieve, the material was gathered onto a plate. The plant material was extracted by macerating it in solvents such as methanol, ethyl acetate, and chloroform. Once the solvents were eliminated using a rotating vacuum evaporator, the remaining extracts were dried, concentrated, and stored in a desiccator for further study. 500 grams of the plant are initially extracted, and 10% of the plant is produced as an extract.

### 2.3. Antioxidant Activity in Vitro

#### 2.3.1. DPPH Test

Methanol extracts were added to refined water to create compositions in a variety of concentrations, including 5, 10, 20, 40, and 80 µg/ml. Ethanol was added and DPPH 0.1 milli molar composition was created. Four milliliters of variously composed plant extracts were added to two milliliters of prepared standard, and the mixture was then vortexed to bring it to room temperature. The spectrophotometer was used to measure absorbance at 517 nm. Quercetin is used as a benchmark. [6]

#### 2.3.2. Assay for Nitric Oxide

Different extract compositions (ranging from 5 to 160 µg/ml) of *Indigofera prostrata* were still arranged separately. Through different compositions of plant methanol extract, 3 ml of sodium nitroprusside was coupled with 0.5 ml of saline phosphate buffer, and it was incubated at 300C for 3 hours. After the incubation period was over, ethylenediamine dihydrochloride, 2 milliliters of reagent (Griess) and phosphate buffer (pH-8.4) were poured into it. Then the composition was incubated for 40-60 minutes at 26°, and the outcome was measured.

Rutin was the standard reference [7].

#### 2.3.3. Assay for Lipid Peroxidation

To resolve the thiobarbituric acid responsive compounds in this investigation, separate methanol concentrates of *Indigofera prostrata* and a liver microsomal portion of rat varying constitution (10-160 µg/ml) are collected [8]. Extracts of 200 µl of plant, 400 µl microsomal part of liver and µl100 of FeCl<sub>3</sub> (1 mM) were mixed separately, and then 100 µl of vitamin C was added. Lipid peroxidation was assessed after the compositions were maintained at 39.1°C for an hour and the outcomes were applied through thiobarbituric acid. The response was measured at 532 nm. Every response is given again. Vitamin E was used as usual.

#### 2.3.4. The Ability of Superoxide Anion Radicals to Scavenge

This task was completed using the Nishimiki [9] model. Subsequent mixtures (5-160 µg/ml) were arranged in distinct groups. To each dilution, 1 milliliter of nitroblue tetrazolium and nicotinamide adenine dinucleotide were added. After adding a phenazine methosulphate mixture (100µl), the reaction initiated. Absorbance was measured at (560nm) Curcumin standard.

#### 2.3.5. Activity of Hydroxyl Group Radicals for Scavenging

*Indigofera prostrata* methanol extract was used to assemble a variety of compositions ranging from 10 to 160 µg/ml [10]. 400 microliters of variously composed methanol extracts were mixed with two hundred microliters of 2-deoxy 2-ribose and two hundred microliters of 1.04 mM EDTA. Afterwards, a hundred microliters of 2.0 mM H<sub>2</sub>O<sub>2</sub> & an equivalent amount of ferric chloride were added to it. Lastly, it was combined with 100 microliters of nutritional C. After the incubation period of one hour, 1 ml of each of trichloroacetic acid and thiobarbituric acid was added to the response composition and the mixtures were incubated for an additional half an hour. To assess it, 498nm absorbance was used for the vitamin E as standard.

### 2.4. Anti-Diabetic Effects Using an in Vitro Model

#### 2.4.1. Alpha Amylase Inhibitory Activity

4 mg of starch azure and 0.5M Tris-HCl (0.4 ml) containing CaCl<sub>2</sub> were added. The composition mixture-containing cylinders were first heated for a few minutes and then preincubated for a few minutes at 38 °C. *Indigofera prostrata* methanol extracts were separately added to DMSO to get compositions ranging from 5 to 100 µg/mL. Subsequently, separate mixes of 0.2 mL extracts of *Indigofera prostrata* with specific compositions were added to the cylinder containing the composition combination. Similarly, 0.2 mL of porcine pancreatic amylase was added to Tris-HCl in the tube containing the composition combination and the extracts of *Indigofera*

*prostrata*. It was centrifugated for seven minutes at 7 °C and rpm 3000. Absorbance was evaluated at 595 nm employing a spectrophotometer. As normal, acarbose was utilized. There were three repetitions of the experiments.  $\alpha$ -amylase's inhibitory effect was assessed using the following methodology:

The amount of acarbose and plant extract needed to inhibit  $\alpha$ -amylase activity under certain conditions was taken into consideration when determining the IC<sub>50</sub> value. In addition to its IC<sub>50</sub> values being determined, the inhibitory effect of  $\alpha$ -amylase on plant distillates and even acarbose is assessed [11].

#### 2.4.2. Inhibition of Alpha Glucosidase Activity

In the investigation,  $\alpha$ -glucosidase was broken down to a concentration of 0.3 U/ml in a phosphate buffer (100 mM) at pH 6.8. Additionally, sodium azide (0.3 g/liter) and serum albumin (bovine) (2 g/liter) were used as a source enzyme while paranitrophenyl- $\alpha$ -d-glucopyranoside was used as a substrate. Methyl alcohol concentrations of *Indigofera prostrata* were prepared in equal parts of distilled water and DMSO, followed by sequential dilutions ranging from 5 to 100  $\mu$ g/ml. For five minutes, microliters of 10 of dilutions of extract were incubated by a 50  $\mu$ l source enzyme. After incubation, 50  $\mu$ l substrate was combined, and later incubated at room temperature for a further five minutes. At 406 nm, absorbances were measured on a microplate. Every test was conducted repeatedly, and estimates were made using the average value. As a benchmark, acarbose is used [12].

#### 2.4.3. Study of Glucose Diffusion Inhibitors

In this experiment, 2 ml of 0.17 M sodium chloride containing glucose 19 mM and 3 ml of separate *Indigofera prostrata* methanol extract were added to 4 cm sections of the dialysis layer. They were knotted at both ends with a nylon string and put in a tumbler with 39 ml of 0.16 M sodium chloride and 9 ml of filtered water to change the strength of media. After that, the device was put in an orbital shaker and kept in a typical environment. The control sample was 1 milliliter of distilled water and NaCl containing glucose. Test samples were taken from every measuring cup, and a reagent was used to determine the glucose content. The study was conducted three times in 180 minutes [13].

### 2.5. Assessment of Glucose Absorption Capability by Yeast Cells

The Cirillo model was used to conduct the investigation [14]. Liquefied Baker's yeast in filtered water was kept at 25 °C to prepare 1% suspension. Later, the yeast cell suspension was subjected to centrifugation for five minutes at 4200 rpm. The sequence is adjusted by gradually adding distilled water until the top layer is formed. To make a suspension of yeast cells, small amounts of the clearest

liquids are combined with distilled water. A plant extract of about 6 mg was mixed with DMSO. Later, the mixture was enhanced by adding different amounts of 1 milliliter of glucose preparation and incubating it for a short while. A 100 L yeast suspension was added to the mixture, thoroughly mixed, and then incubated at 37°C for an additional 59 minutes in order to elicit a reaction. After incubation, the cylinders were centrifuged for six minutes at 3801 rpm, and the glucose was measured using a spectrophotometer at 521 nm. A comparable standard value was used to record absorbance. Here, the control consists solely of reagents; there is no test component. Metronidazole was the standard medication used. It was carried out independently for the methanol extract of *Indigofera prostrata* to ascertain the yeast cells' ability to absorb glucose.

## 3. Results

### 3.1. Antioxidant Activity *In vitro*

#### 3.1.1. DPPH Test

*Indigofera prostrata* for DPPH assay results was verified and shown in Figure 1. The IC<sub>50</sub> estimation for *Indigofera prostrata* was 13.76  $\mu$ g/ml, while for the quercetin standard, it was 9.54  $\mu$ g/ml.

#### 3.1.2. The Assay for Nitric Oxide (NO) Radical Inhibition

Based on IC<sub>50</sub> estimates, it was determined that the standard for rutin was 39.54  $\mu$ g/ml and the methanol extract of *Indigofera prostrata* was 49.12  $\mu$ g/ml. The results were verified and shown in Figure 2.

#### 3.1.3. Assay for Lipid Peroxidation

As shown in Figure 3, the methanol extracts of *Indigofera prostrata* and vitamin E standard demonstrated consistent hydroxyl group scavenging at various compositions. The methanol extract from *Indigofera prostrata* was shown to have an IC<sub>50</sub> of 76.49  $\mu$ g/ml, while the standard for vitamin E was found to be 74.28  $\mu$ g/ml.

#### 3.1.4. Activity of Superoxide Anion Radical Scavenging

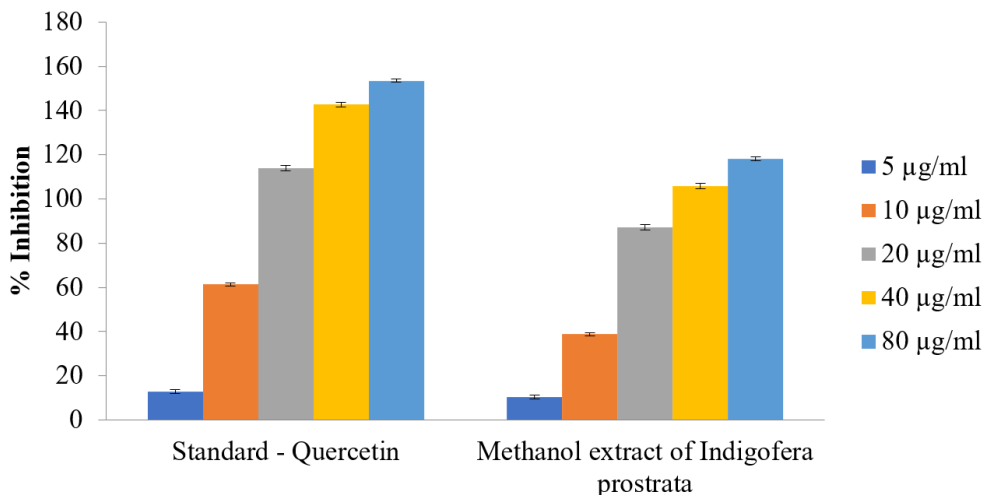
The assay's results show that the curcumin standard, with an IC<sub>50</sub> evaluation of 16.89  $\mu$ g/ml, and the methanol extract of *Indigofera prostrata*, with IC<sub>50</sub> estimations of 25.42  $\mu$ g/ml, are shown in Figure 4.

#### 3.1.5. Activity of Hydroxyl Radical Scavenger

The investigation evaluated employing the Fenton response and consequences shown below. The IC<sub>50</sub> values were found to be 38.91  $\mu$ g/ml for the methanol extract of *Indigofera prostrata* and 29.34  $\mu$ g/ml for the vitamin E standard.

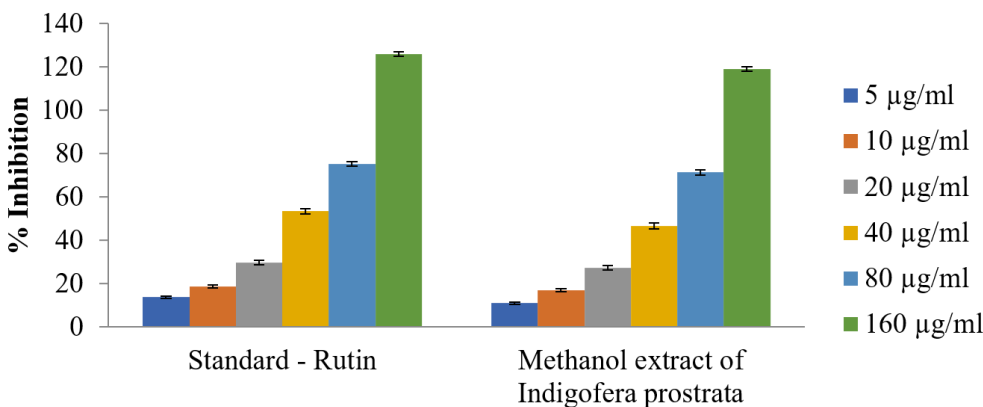
The results were indicated in Figure 5.

**Scavenging effect of methanol extracts of *Indigofera prostrata* and standard quercetin on DPPH radical**



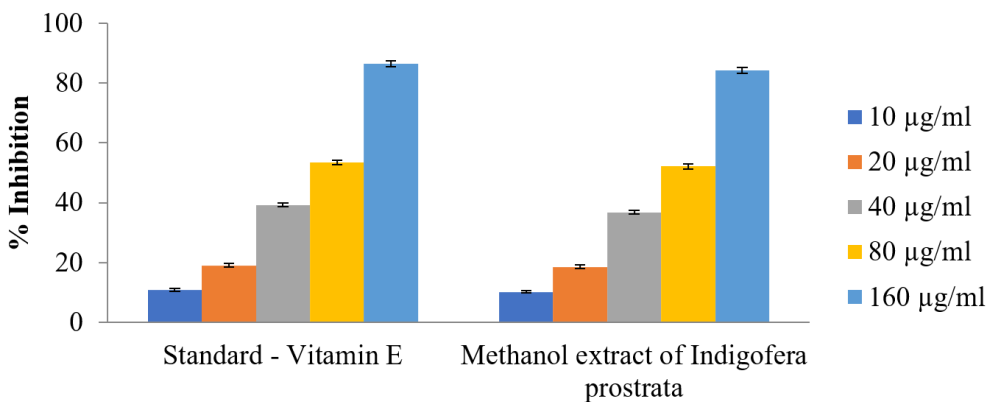
**Figure 1.** Assay for DPPH radical

**Scavenging effect of methanol extract of *Indigofera prostrata* and standard rutin on Nitric oxide radical**



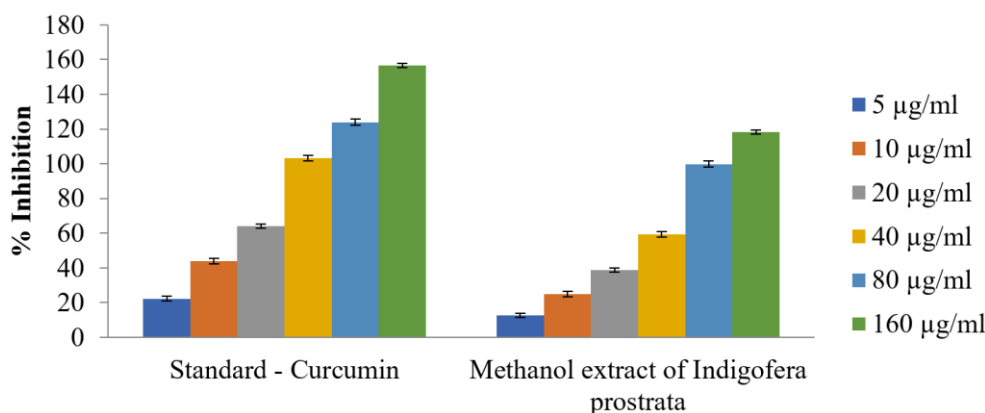
**Figure 2.** Assay for Nitric oxide radical

**Scavenging effect of methanol extracts of *Indigofera prostrata* and standard vitamin E on lipid peroxidation of liver microsome induced by ascorbate**



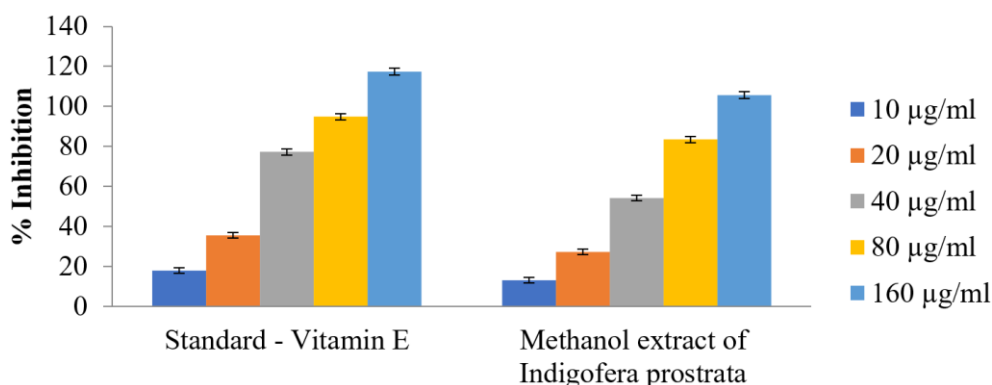
**Figure 3.** Assay for Lipid peroxidation

### Scavenging effect of methanol extract of *Indigofera prostrata* and standard curcumin on superoxide anion radical formation



**Figure 4.** The results of the methanol extract of Curcumin standard and *Indigofera prostrata* for scavenging

### Scavenging effect of methanol extract of *Indigofera prostrata* and standard vitamin E on hydroxyl radical



**Figure 5.** The scavenging effect on hydroxyl radical

## 3.2. Antidiabetic Action *In vitro*

### 3.2.1. The Ability of Alpha Amylase to Inhibit

Figure 6 shows the results of the inquiry into the inhibitory activity of *Indigofera prostrata* and alpha amylase inhibitory acarbose. The methanol extracts of *Indigofera prostrata* have IC<sub>50</sub> estimates of 8.49 µg/ml, which is higher than the standard of 6.36 µg/ml for acarbose.

### 3.2.2. Alpha Glucosidase Inhibitory Activity

Figure 7 shows the repressive action of alpha glucosidase in methanol extracts of *Indigofera prostrata* contrasted with standard-acarbose. Methanol extract of *Indigofera prostrata* had IC<sub>50</sub> estimates of 8.54 µg/ml, which is higher than the standard of 6.43 µg/ml for

acarbose.

### 3.2.3. Examination of Glucose Diffusion Inhibitors

Results of the research on *Indigofera prostrata* are shown in Figure 8. The glucose diffusion through the dialysis layer was interrupted by the plant's methanol extract; at 180 minutes, the relative movement with respect to the control was  $55.78 \pm 2.69$  and  $54.12 \pm 2.69$ , respectively.

### 3.2.4. Determination of the Ability of Yeast Cells to Absorb Glucose

The evaluation of the yeast cells' uptake of glucose in the methanol extract of *Indigofera prostrata* at diverse concentrations like 5, 10 and 25 mM is shown in Figures 9, 10, and 11.

**Alpha amylase inhibitory activity of methanol extract of *Indigofera prostrata* and standard acarbose**

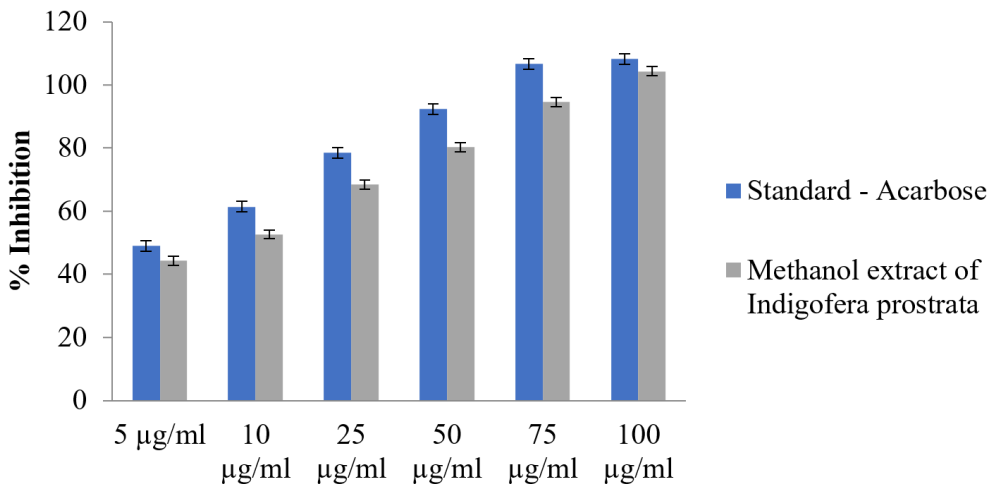


Figure 6. *Indigofera prostrata* and conventional acarbose methanol extract alpha amylase inhibitory effect

**Alpha glucosidase inhibitory activity of methanol extract of *Indigofera prostrata* and standard acarbose**

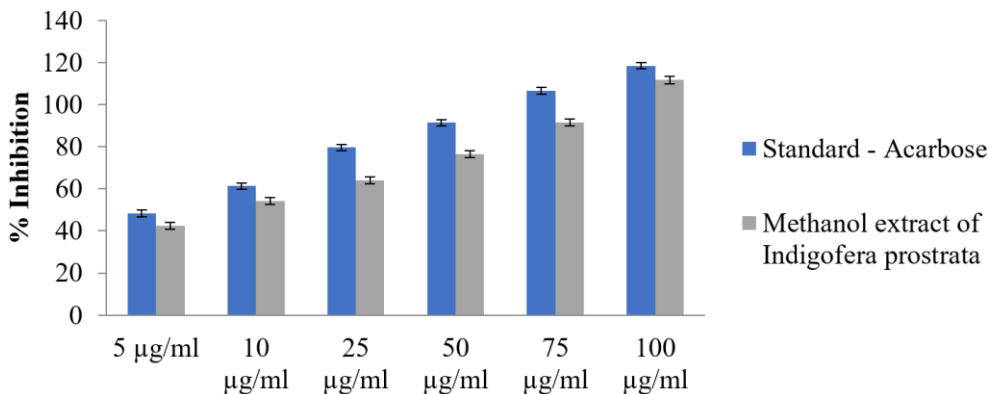


Figure 7. Standard acarbose and the alpha glucosidase extract of *Indigofera prostrata* exhibit inhibitory action

**Effect of methanol extract of *Indigofera prostrata* on diffusion of glucose out of a bio-membrane over 180 minutes**

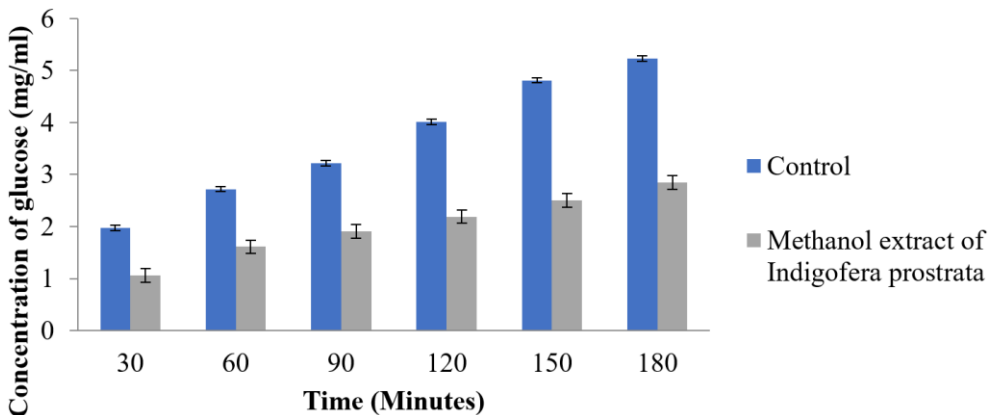
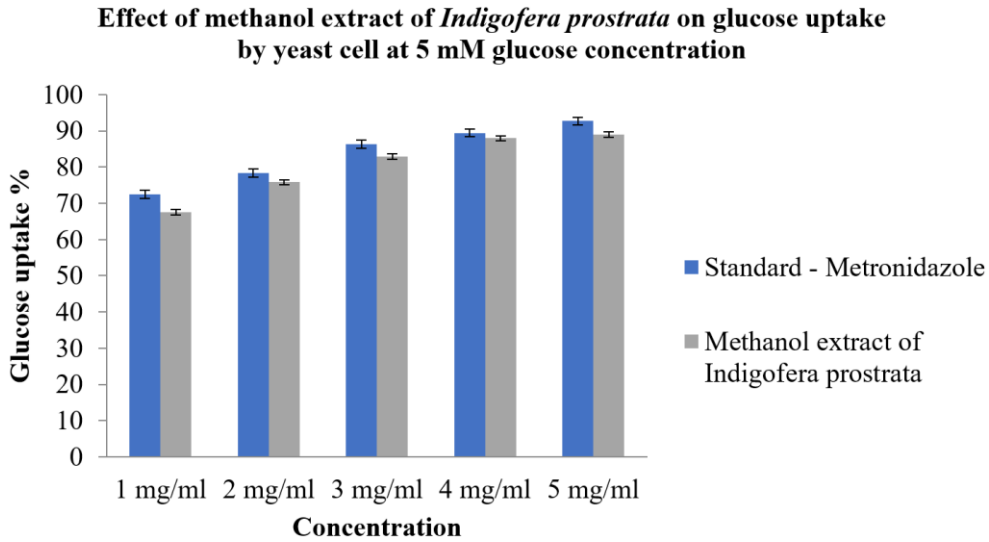
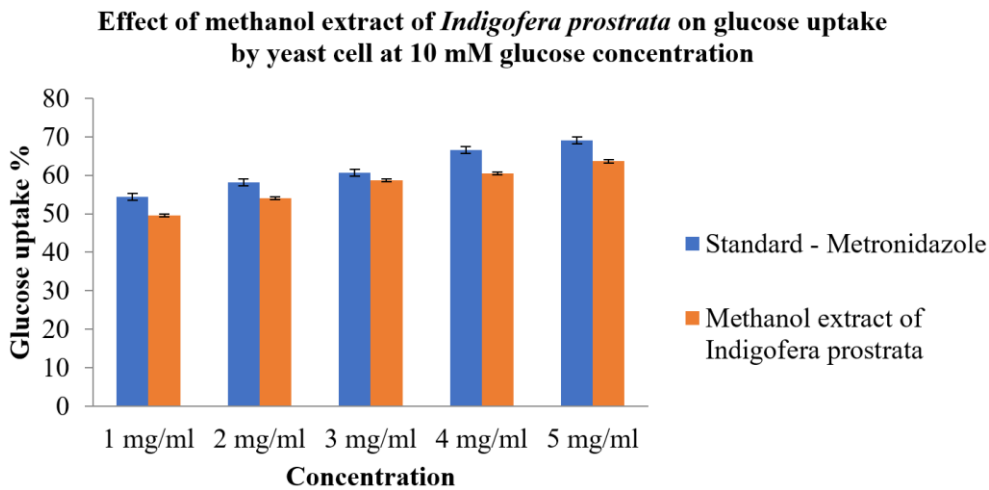


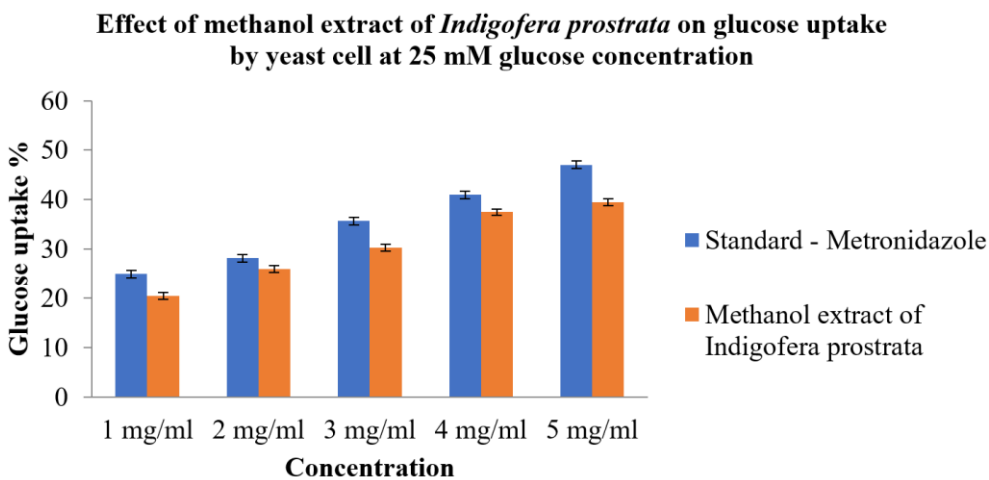
Figure 8. Study on the inhibitory effects of glucose diffusion



**Figure 9.** *Indigofera prostrata* methanol extract's effect on glucose absorption by yeast cells at a concentration of 5 mM



**Figure 10.** *Indigofera prostrata* methanol extract's effect on glucose absorption by yeast cells at a concentration of 10 mM



**Figure 11.** Effect of *Indigofera prostrata* methanol extract on glucose absorption by yeast cells at a 25 mM concentration of glucose

## 4. Discussion

### 4.1. Antioxidant Activity *In vitro*

Both plant extracts in methanol demonstrated efficient radical scavenging ability against the DPPH test. According to the Bors [15] model, the scavenging activity of *Indigofera prostrata* may be caused by the presence of flavonoids, as indicated by DPPH radical investigation. Conferring to Bors et al., the hydroxyl component of the flavonoid element that united with the B ring may operate as a diminishing specialist and designate a hydrogen molecule for the deactivation of free radicals. Consequently, a progressive decrease in absorbance was seen as nitrite content increased. The plant extract that competes with oxygen to cause nitric oxide to react and cause a decrease in absorbance through nitric oxide availability may be the cause of the reduction. It might be because the plant extract contains flavonoids, which function as nitric oxide scavengers to limit the generation of nitric oxide [16]. When compared to standard, the IC<sub>50</sub> evaluations of *Indigofera prostrata*'s methanol extract demonstrated a strong ability to scavenge radicals. Steroids and phenols are the cause of the dramatic decrease in lipid peroxidation seen in the *Indigofera prostrata* extract [17]. In contrast to the standard, plant extract prevented lipid peroxidation in a dose-dependent manner. Superoxide anion consumption by plant terpenes in response mixture is demonstrated by formazan staining, which shows a decrease in absorbance in the presence of plant extracts. The results of superoxide showed that in contrast to regular curcumin, methanol extract of *Indigofera prostrata* had additional beneficial effects. In biological systems, hydroxyl radicals are potent reactive oxygen species that react with the polyunsaturated fatty acid moieties of phospholipids in cell membranes and cause cell damage. The methanol extract of *Indigofera prostrata* has IC<sub>50</sub> values that are higher than the vitamin E standard.

### 4.2. *In vitro* Model of Antidiabetic Action

#### 4.2.1. The Inhibiting Activity of Alpha Amylase

It is in charge of breaking down carbs and producing a variety of glucose products that might lead to hyperglycemia in addition to accelerating the development of diabetes mellitus. The alpha amylase activity was inhibited and the increased blood glucose levels were decreased by the methanol extract of *Indigofera prostrata*. In contrast to the acarbose standard, the methanol extracts demonstrated remarkable efficacy. One helpful strategy for managing diabetes is to take advantage of the  $\alpha$ -amylase inhibitory effect that plants regulate.

#### 4.2.2. Alpha Glucosidase Inhibitory Activity

Similar action is shown by the enzyme alpha glucosidase and alpha amylase. When the conventional inhibitor acarbose was compared to the suppressive activity of the

alpha glucosidase of *Indigofera prostrata*, the plant extract showed the most effective action. One method for treating type-2 diabetes is to use synthetic medications to limit alpha-glucosidases' activity.

#### 4.2.3. Study of Glucose Diffusion Inhibitors

The results lead us to the conclusion that the methanol extract of *Indigofera prostrata* blocks the blood glucose's ability to diffuse over the dialysis membrane. The body has a variety of transporters that organize through diverse molecules to transfer glucose. In the contemporary investigation, glucose was set up in sodium chloride and this was accomplished by sodium particles. Atoms of glucose require a transporter element to move across all cells. The evidence from consequences suggests that one likely component of plants' antihyperglycemic activity is glucose diffusion.

#### 4.2.4. Assessment of Glucose Absorption Potential Using Yeast Cells

*Indigofera prostrata*'s absorption of glucose was nearly identical to that of ordinary metronidazole. When compared to methanol extract of *Indigofera prostrata*, metronidazole has a negligibly greater effect on the absorption of glucose by yeast cells. The yeast cells' glucose absorption limit was extended in response to an increase in the concentration of *Indigofera prostrata* extracts. On the other hand, increase in the methanol extract of *Indigofera prostrata* show the opposite relationship with regard to the moment of glucose by yeast cells in equivalent amounts. Yeast cells will often ingest glucose through facilitated diffusion, as opposed to other methods and the phosphotransferase enzyme system.

## 5. Conclusions

According to a recent analysis, *in vitro* antioxidant and antidiabetic properties may be present in methanol extracts of *Indigofera prostrata*. The plant material that was removed produced results that were comparable to the specific standard that was used. The highest levels of antioxidant and antidiabetic activity were demonstrated *in vitro* by *Indigofera prostrata* methanol extracts. Plants are therefore rich natural sources of antioxidants and can be exploited in the medicinal or nutritional industries to eradicate ailments caused by free radicals.

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