

Evaluation of Antidiabetic and Antioxidant Potential of Methanolic Extract of *Bixa orellana* Seeds

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Diabetes is considered a serious health problem in Bangladesh and worldwide. The disease is infesting all over the mother earth possessing the most damage associated with physical, mental and financial situation of the population. In this context, it's truly essential to find an effective, safe yet economically suitable alternative medicine compared to commercially available noxious chemical drugs. This study aims to find the potential of an alternative natural extract produced from *Bixa orellana* seeds which is safer and more affordable comparable to the commercially available synthetic agents to reduce Hyperglycemia. It also makes effort to find the seed extracts' ability to reduce oxidation in comparison to a standard drug, Ascorbic acid. This is a Pharmacological study using animal model utilising rat as subjects. The rats were induced as diabetic by injecting alloxan intraperitoneally and as treatment *Bixa Orellana* seed extracts were given to them at 500mg/kg and 1000mg/kg doses and compared with the hypoglycemic effect of standard anti-diabetic drug, Metformin Hydrochloride at the dose of 100mg/kg. For analyzing the anti-oxidant activity, the DPPH free radical scavenging assay method has been used, where the IC₅₀ values of both the *Bixa Orellana* seed extract and the reference drug, Ascorbic acid was collated. It was found that the seed extracts used at 500 mg/kg and 1000 mg/kg evidently reduced the blood glucose level as opposed to the diabetic control group, more importantly with the rats treated with standard, Metformin Hydrochloride. Therefore, the study revealed that the seed extract can be effectively used to treat diabetic patients without any side effects. This experimentation, also by assessing the IC₅₀ values, disclosed that the *Bixa Orellana* seed extract has moderate to substantial anti-oxidant potential in contrary to the reference drug.

Keywords: Alloxan-induced Diabetic rat model; Anti-diabetic activity; Anti-oxidant activity; Animal model; *Bixa Orellana*; Diabetes; DPPH free radical Scavenging Assay.

Natural plants carry therapeutically active molecules thereby can supply precedent structures which can be used to emerge comparatively more safe and effective medicines as an alternative to

commercially available toxic and synthetic agents to alleviate different ailments. ¹ Since over 80% of people on earth rely mostly on herbal remedies for their necessary health management, it may

be claimed that the world's health care system is founded on this conventional, nature-based medicine system.² *Bixa Orellana*, belonging to the Bixaceae family and the Genus *Bixa*, is an indigenous plant species native to Central America. The plant in concern is purportedly native to Mexico, Panama, Ecuador, Brazil, Bolivia, and New Granada. In her work published in 1960, Julia F. Morton discusses, the plant referred to as the Lipstick tree is widely farmed across the globe due to its production of orange-yellow pigments known as Bixin and Norbixin, which are extracted from its seeds.³ Certain indigenous populations utilize it for cosmetic applications.⁴ The aforementioned pigment serves as a dye for the purpose of coloring dairy dishes, rice, soup, and textile materials. Annatto oil is commonly referred to as "the inexpensive alternative to saffron."⁵ In Trinidad, a decoction made from roots is utilized for the treatment of several ailments such as Diabetes, influenza, Jaundice, Venereal illness, and as a diuretic.⁶

The Bixaceae, a family containing dicotyledonous plants are commonly known as achiote family, a small family comprising only one genus, *Bixa* consisting of only five species which are named as: *Bixa arborea*, *Bixa excelsa*, *Bixa Orellana*, *Bixa platycarpa* and *Bixa urucurana*. Among them *Bixa Orellana* possess the most therapeutic significance.⁷ Ayurveda and traditional Indian practices incorporate its usage. The fruit of this plant exhibits anti-dysenteric qualities, whereas the seed pulp possesses both anti-dysenteric and homeostatic properties.⁸ Historically, the utilization of seed pulp has been documented for its therapeutic properties in addressing conditions such as constipation, burns, gonorrhoea, and bleeding. The utilization of leaves, bark, and root extract as an antidote for poisoning, particularly in the context of snakebite, has been found to be beneficial. This practice is particularly prevalent among Columbian healers, who employ these extracts as antivenom agents. The Brazilian population utilizes it as a moderate diuretic and for the treatment of heartburn. The seeds are utilized for their expectorant properties, while the roots are considered to possess properties that aid in relieving cough symptoms. Peruvian herbal medicine utilizes this particular remedy for addressing internal

inflammation, hypercholesterolemia, obesity, arterial hypertension, and renal insufficiency.⁹

Bixa may also have effects that include diaphoretic, antiemetic, antispasmodic, analgesic, adaptogenic, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardioprotective, and antiemetic.¹⁰

Diabetes Mellitus is a prominent contributor to morbidity, and its related complications impose a substantial economic burden on the healthcare system. In 2019, the global impact of this phenomenon was observed in a population of 463 million individuals. The prevalence of diabetes among individuals in Bangladesh witnessed a substantial rise, escalating from 4.5% in 1994 to 35% in 2013. The projected figure for 2045 is approximately 13.7 million.¹¹ The condition is characterized by a metabolic dysfunction resulting from inadequate insulin synthesis or reduced sensitivity to insulin. The primary factors contributing to this phenomenon are inflammation, the ageing process, a sedentary lifestyle, obesity, and the consumption of highly processed foods. Insulin resistance is known to contribute to the development of both macrovascular and microvascular problems, including retinopathy, neuropathy, nephropathy, and cardiovascular issues.¹² Therefore, it encompasses a diverse range of diseases. Only the desired blood glucose level can be maintained with insulin therapy; additional complications including retinopathy, nephropathy, and cardiovascular illnesses are not diminished. Diabetes patients have a shorter life expectancy than people without the disease due to these serious complications.¹³

The main objective of managing diabetes mellitus is to preserve life and mitigate symptoms. The overarching objective is to mitigate problems and enhance life expectancy by reducing the risk factors associated with it. Diabetes can be classified into two distinct types: Type 1 and Type 2. Type 2 diabetes mellitus can be managed with the administration of different classes of oral hypoglycemic medications, including sulphonylureas, alpha glucosidase inhibitors, thiazolidenediones, biguanides, and meglitinide analogues. In order to get best outcomes, it is imperative to incorporate lifestyle management practices such as regular exercise

and a balanced diet. Type 1 diabetes necessitates the implementation of insulin therapy in order to address insufficient insulin production and insulin resistance. Cardiovascular problems account for approximately 70% to 80% of morbidity in patients diagnosed with diabetes. The timely identification and subsequent medical intervention have been shown to significantly decrease mortality rates, as well as enhance both the duration and quality of life for individuals. The management of diabetes can be achieved through non-pharmacological interventions such as diet and exercise, as well as through the use of oral hypoglycemic medications or insulin in specific cases.¹⁴

The chemical compounds derived from the seeds of *Bixa orellana* include bixin, norbixin, isobixin, beta-carotene, cryptoxanthin, lutein, zeaxanthin, orellin, bixein, bixol, crocetin, ishwarane, ellagic acid, salicylic acid, threonine, tomentosic acid, tryptophan, and phenylalanine. Additionally, it is comprised of apocarotenoids and volatile oil. The anti-oxidant and hypoglycemic action of this substance can be attributed to its significant concentration of carotenoids.¹⁵

Antioxidants have the ability to mitigate the process of oxidation, even when present in minimal quantities. This substance should exhibit the ability to eliminate free radicals and bind to heavy metals, all while maintaining stability and solubility within a physiological condition.¹⁶ Oxidative stress induces damage to biological components, including proteins, lipids, and nucleic acids. This phenomenon has the potential to result in cellular demise ultimately. Glycation has the potential to induce modifications in protein structure, resulting in the formation of advanced glycation end products. Both glycation and oxidative stress have the potential to induce alterations in the physiological milieu, hence serving as catalysts for the development of degenerative diseases such as Diabetes.¹⁷

The primary objective of this study was to investigate the possibility of anti-diabetic and anti-oxidant characteristics of *Bixa orellana* seed extract. This research was motivated by the low number of existing data pertaining to the aforementioned capabilities of the seed extract in Bangladesh, despite the extensive study of the leaf extract.

MATERIALS AND METHODS

Instruments

Test tube, volumetric flask (5 L), beakers (500 mL), funnels, measuring cylinder (1000 mL), glass rod, gavage (oral feeding syringe), 3 mL Syringe, feeding needle (JMI, Bangladesh), heater, refrigerator, digital electronic balance, pH meter, glucometer (On Call EZ II, Acon Laboratories, inc. San Diego, USA), strip, water bath, Rota evaporator, Soxhlet extractor.

Reagents and Drugs

Methanol and Alloxan Monohydrate were purchased from Lova, India. The active pharmaceutical ingredient (API) metformin hydrochloride was collected from Square Pharmaceuticals Limited in Bangladesh. Acarbose was purchased from a local drug store in Savar area, Dhaka, Bangladesh.

Plant Collection

Seeds of *Bixa Orellana* (Bengali name – Rongdana/ Doi gota) were collected from karwanbazar, Dhaka. Specialist oversight was provided for the collecting.



Fig. 1. Glucometer (On Call EZ II, Acon Laboratories, inc. San Deigo, USA) that was used to check glucose level

Table 1. Preparation of extract solution for experimental rats

| Group | Extract (g) | Distilled Water (mL) |
|------------|-------------|----------------------|
| seeds 500 | 1 g | 10 mL |
| Seeds 1000 | 2 g | 20 mL |

Preparation of the Extract

The seeds were washed carefully with water to remove any adhering dirt. The seeds were sun-dried for seven days and then dried at 65°C in the oven. The seeds were crushed into a fine powder after being dried by grinding machine. By cold maceration for 48 hours in a Soxhlet apparatus, approximately 1 kg of the powder was extracted with 2.5 L of methanol. ^[19] Rotary Evaporators were then used under reduced pressure at 50°C temperature to effectively and gently remove any remaining solvents from the samples obtained from Soxhlet apparatus, and the leftover extract was then refrigerated at 4 °C for storage. ²⁰

Experimental Animals

From the Pharmacy Department of Jahangirnagar University in Savar, Dhaka, laboratory female Sprague Dawley rats (weighing 100–180g) were purchased.

Induction of diabetes mellitus

Alloxan monohydrate was used to induce diabetes at a single intraperitoneal dose of 150 mg/kg BW. In this experiment, rats with blood glucose levels greater than 7 mmol/L (126 mg/dl) had been used. ^[21]

Preparation of standard drug solution

20 mL of distilled water was used to dissolve 200 mg of metformin HCl and this solution was administered orally to MET100 group rats (Standard drug group) at 1X dose once daily for 21 days. A fresh medication solution was made per day.

Preparation of Extract solution

The following method was used to prepare the extract solutions for the various groups: –

Experimental design

Thirty Sprague Dawley rats were divided into five groups. Twenty four of them were induced diabetes by IP (intraperitoneal) administration of Alloxan monohydrate at a single dose of 150 mg/

kg BW. These 24 diabetic rats were divided into 4 classes for clinical experiment. Group III and IV were given methanol extract of *Bixa orellana* seeds at different doses.

Group I: Normal control group and were given only 0.5ml water daily

Group II: (Diabetic Control): Alloxan induced diabetic Rat that served as Diabetic Control and were given 0.5ml water only.

Group III: Alloxan induced diabetic rat treated with 500 mg/kg of *Bixa Orellana* seeds extracts orally once daily.

Group IV: Alloxan induced diabetic rat treated with 1000 mg/kg of *Bixa Orellana* seeds extracts orally once daily.

Group V: Alloxan induced diabetic rat treated with 100 mg/kg of body weight of Metformin Hydrochloride orally once daily. ²⁰

Bixa Orellana seeds extracts and Metformin Hydrochloride were administered orally for 21 days after induction of diabetes.

Fasting Blood Glucose Level

The rats' tail veins were used to collect blood samples, and fasting blood glucose was measured with a glucometer (On Call EZ II, Acon Laboratories, Inc. San Diego, USA) after 6 hours of fasting on the first, seventh, fourteenth, and twenty-first days.

DPPH Free Radical Scavenging Assay

A reactive free radical called DPPH works as an electron acceptor (oxidant/oxidizing agent) and oxidises other compounds. Antioxidants, however, function as electron donors (reductant/reducing agent). Antioxidants counteract DPPH by becoming oxidised. In its solid state, DPPH is a deep violet-colored crystalline powder made of stable free-radical molecules. The transformation of the deep violet colour into pale yellow or colourless (neutralisation) is an indication that the DPPH free radical has been scavenged. ²²

| Reagent | Source |
|---|-----------------------------------|
| Absolute Ethanol/Methanol | Merck, Germany |
| 1, 1-diphenyl-2-picrylhydrazyl (DPPH) | Sigma Chemicals, USA |
| Ascorbic acid (Analytical or Reagent grade) | SD Fine Chem. Ltd., Biosar, India |

DPPH Solution: 0.004gm (4mg) DPPH is dissolved in 100 ml of solvent to make 0.004% solution.

Preparation of Standard/ Extract solution

10 ml of methanol was used to dissolve 8 milligrammes of ascorbic acid or extract. Ascorbic

acid/extract concentration in the solution was 0.8 mg/ml. The stock solution was used to create the experimental concentrations in the following ways

| Concentration (µg/ml) | Solution taken from stock solution | Solution taken from others | Adjust the volume by Absolute methanol | Final volume |
|-----------------------|------------------------------------|----------------------------|--|--------------|
| 400 | | 1 ml(800µg/ml) | 1 ml | 2.0 ml |
| 200 | – | 1 ml (400µg/ml) | 1 ml | 2.0 ml |
| 100 | – | 1 ml (200µg/ml) | 1 ml | 2.0 ml |
| 50 | – | 1 ml (100µg/ml) | 1 ml | 2.0 ml |
| 25 | – | 1 ml (50µg/ml) | 1 ml | 2.0 ml |

Procedure

To achieve the concentrations of 400 g/ml, 200 g/ml, 100 g/ml, 50 g/ml, and 25 g/ml, the stock solution is serially diluted.

Each test tube has been correctly marked and contains 1ml of each concentration.

To make the final volume of each test tube 3 ml, 2 ml of a 0.004% DPPH solution in the solvent are added (caution: DPPH is light-sensitive, thus preparing the solution and applying it to the test tubes should be done with the least amount of light exposure).

Mixture should be kept at room temperature and kept in a dark area for 30 minutes.

The absorbance is then measured in the solvent at 517 nm against a diluted extract solution.

Statistical analysis

The results of the antidiabetic activity of seed extracts in the alloxan induction rat models were provided as mean ±SEM (n=4) and statistical analysis was done using one-way ANOVA,

followed by the Dunnett test. While p 0.05 and P 0.01 were regarded as statistically significant values.²³

RESULTS AND DISCUSSION

Diabetes mellitus is a metabolic illness that is currently one of the biggest health concerns in the entire world. Moreover, it is thought to be an irreversible metabolic condition affecting 2.8% of the world's population.²⁴ The last few decades have seen a growing awareness of the importance of medicinal plants as a fundamental substance for maintaining health and treating ailments. This is mostly caused by the usage of synthetic medications, which induce undesirable side effects that are comparatively insignificant in pharmaceuticals with plant origins.²⁵ The leaf extract of *Bixa Orellana* shows potent anti-diabetic activity.^{26,27} Hence, the anti-diabetic activity of *Bixa Orellana* seeds were evaluated against a

Table 2. The effect of *Bixa orellana* seeds extracts (500 mg and 1000) on Fasting Blood Glucose Level

| Group No. | Day-1 mmol/L | Day-7 mmol/L | Day-14 mmol/L | Day-21 mmol/L |
|------------------------------|--------------|--------------|---------------|---------------|
| 1. D+Seeds 500 mg | 10± 2.75 | 7.03±1.09 | 7 ±0.82 | 6.5 ±0.605 |
| 2. D+seeds 1000 mg | 8.84±4.12 | 5.88±2.81 | 5.82±2.32 | 5.54±2.06 |
| 3. D+Metformin Hydrochloride | 15.68±1.39 | 15.13±1.99 | 14.45±1.58 | 13.98±1.83 |
| 4. Diabetic Control | 13.36±6.89 | 13.73±8.11 | 14.63±8.59 | 14.98±7.77 |
| 5. Normal Control | 6.05±0.5 | 6.11±0.18 | 6.1±0.22 | 6.05±0.16 |

Resultis shown as Mean ± SEM. *p<0.01: Significant compared to diabetic control group. D= Diabetic

popular synthetic drug Metformin Hydrochloride and found to be more effective than the standard treatment. Moreover, in this experiment, while comparing the anti-oxidant potential the seed extracts proved to be more or less equally potent to the conventional drug, Ascorbic acid.

In-vivo anti-diabetic activity Assay
The Effect of *Bixa orellana* seeds extracts (500mg) on fasting blood glucose level:

Table 2 displays the anti-diabetic effects of 500 mg of *Bixa Orellana* seed extract on the FBG

levels of Alloxan-induced diabetic rats. The result shows that the blood glucose level was lower in the extract group compared to the diabetic control group throughout the study period (Table 2). On the 21st day, the blood glucose level in group II was 14.98 ± 7.77 mmol/L whereas in groups I and III it was 6.050.16 mmol/L and 6.5 ± 0.605 mmol/L, respectively (Table 2). Therefore, treatments with the *Bixa orellana* seed extract (500 mg), FBG levels in diabetic rats were significantly ($p < 0.01$) reduced and were nearly identical to those in the normal

Table 3. IC₅₀ values of the Leaves extract in DPPH scavenging assay

| Concentration | Absorbance | % SCV | IC50 |
|---------------|------------|----------|---------|
| 400 | 0.202 | 66.72158 | |
| 200 | 0.362 | 40.36244 | |
| 100 | 0.577 | 4.942339 | 296.717 |
| 50 | 0.55 | 9.390445 | |
| 25 | 0.587 | 3.294893 | |
| Blank | 0.607 | | |

Table 4. IC₅₀ values of the Standard (Ascorbic Acid) in DPPH scavenging assay

| Concentration | Absorbance | %SCV | IC50 |
|---------------|------------|----------|--------|
| 400 | 0.155 | 87.52013 | |
| 200 | 0.342 | 72.46377 | |
| 100 | 0.467 | 62.39936 | |
| 50 | 0.501 | 59.66184 | 39.556 |
| 25 | 0.685 | 44.84702 | |
| 12.5 | 0.791 | 36.3124 | |
| Blank | 1.242 | | |

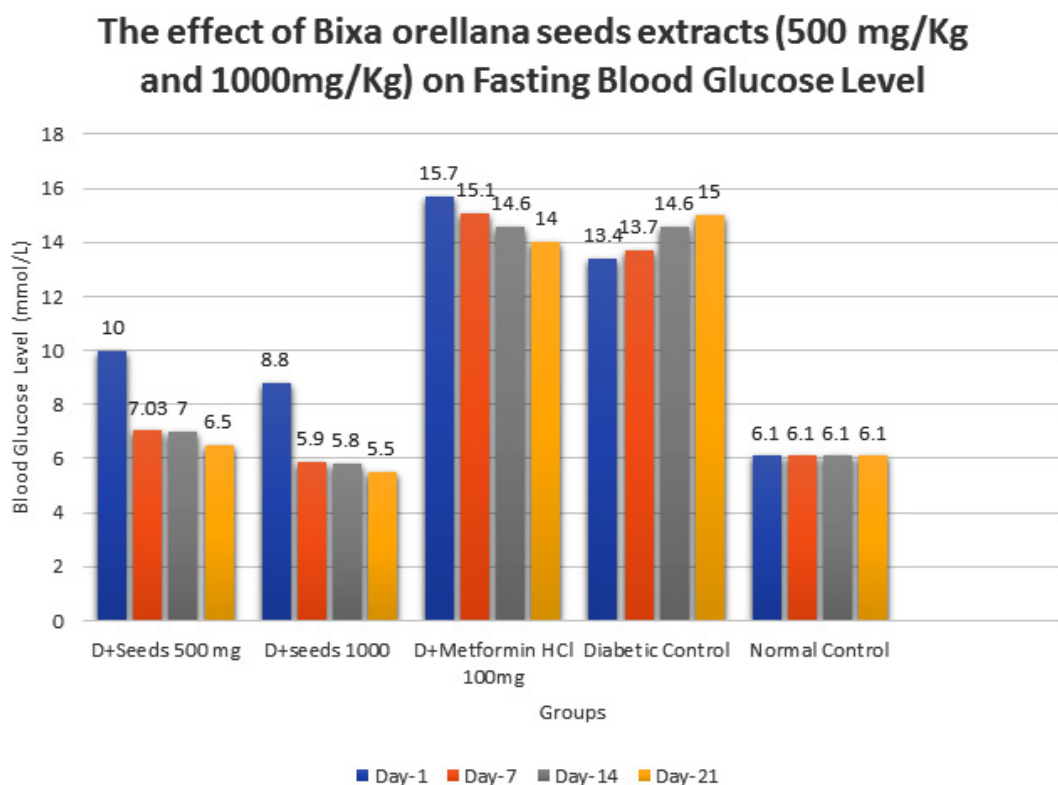


Fig. 2. The effect of *Bixa orellana* seeds extract (500 mg) on fasting blood glucose level

control group after the 7th, 14th and 21st days (Table 2). Similar to the treatment with the Metformin Hydrochloride group, which has been employed as the standard anti-diabetic reference medicine to compare the positive effects of *Bixa orellana* seeds extracts, there is no appreciable decrease in FBG levels on the 7th, 14th and 21st days (Table 2, figure 2).

So, the study's results (Figure 2) showed that *Bixa orellana* seeds extracts (500 mg) had a great blood sugar-lowering impact after 21 days of treatment. Values represented as Mean \pm SEM. * $p < 0.01$: Significant compared to diabetic control group. D= Diabetic

The anti-diabetic effect of the *Bixa orellana* seed extracts (1000 mg) on the FBG

DPPH Free Radical Scavenging Assay of Standard (Ascorbic Acid)

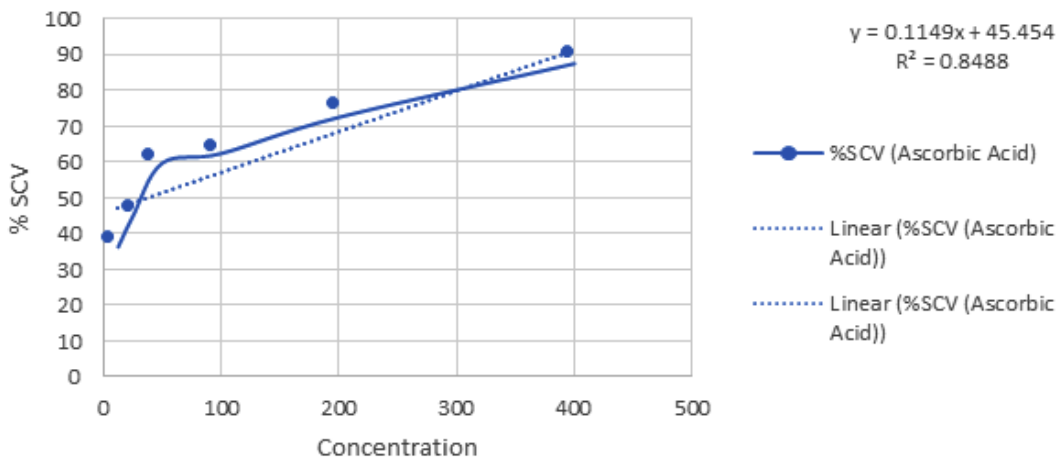


Fig. 3. DPPH Free Radical Scavenging Assay of seeds extract of Standard (Ascorbic Acid)

DPPH Free Radical Scavenging Assay of *B. Orellana* seed extract

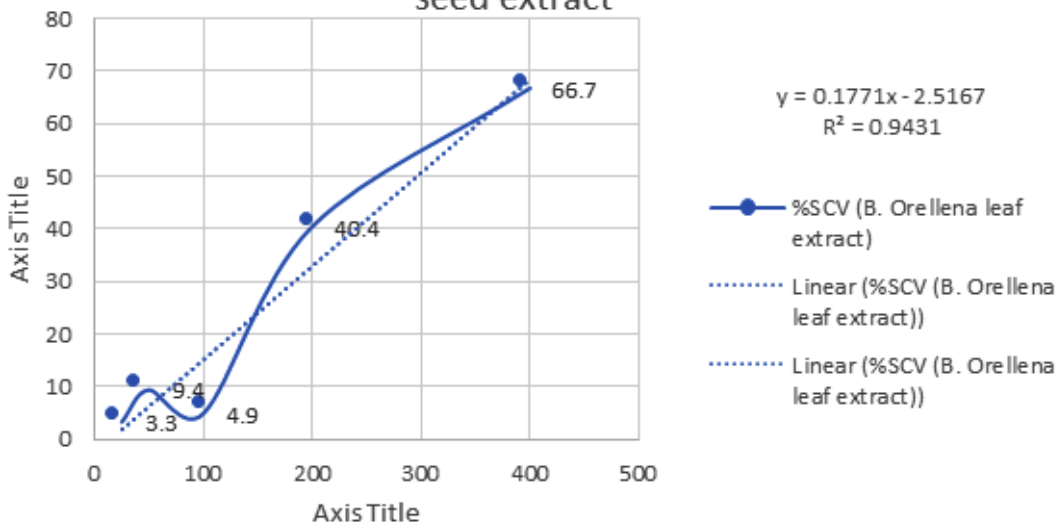


Fig. 4. DPPH Free Radical Scavenging Assay of seeds extract of *Bixa Orellana*

levels of Alloxan-induced diabetic rats is shown in figure 2. Throughout the study, the FBG levels of the diabetic control group were considerably ($p < 0.001$) higher than those of the normal control group. Nevertheless, diabetic rat treatments with the seed extracts of *Bixa orellana* (1000 mg) resulted in significantly ($p < 0.01$) decreased FBG levels that were nearly identical to those of the normal control group on days 7, 14, and 21 (Table 2). The reduction is more significant than the 500 mg extract group. So, it could be said that the decrease was dose-dependent (Table 2). However, therapy with the Metformin Hydrochloride group, which has been employed as a common anti-diabetic reference medication to assess the positive effects of *Bixa orellana* seed extracts, resulted in lower FBG levels but not significantly lower FBG levels than *Bixa orellana* seed extracts on 7th, 14th and 21st days (Table 2, Figure 2).

Values represented as Mean \pm SEM. * $p < 0.01$: Significant compared to diabetic control group. D= Diabetic

Therefore, we may conclude that the anti-diabetic activity of *Bixa orellana* seed extracts (1000 mg/kg) in diabetic rats treated with Alloxan was more evident.

DPPH Free Radical Scavenging Assay

Table 3 lists the IC₅₀ values for the *Bixa Orellana* seed extract. The extract of *Bixa orellana* had an IC₅₀ value of 296.717.

Bixa orellana extracts' ability to scavenge DPPH radicals may be mostly due to its Bixin. The concentration of hydrogen peroxide in water can also happen depending on phenolic and flavonoid chemicals. Since the phenolic chemicals in the extract are effective electron donors, they might quicken the process of converting H₂O₂ to H₂O.

The antioxidant activity of the *Bixa Orellana* extracts ranges from moderate to substantial, with an IC₅₀ value of 296.717 compared to 39.556 for ascorbic acid as a reference.

CONCLUSION

The alloxan induced diabetes model, is the most well-known, dependable and easily repeatable drug-induced model to cause diabetes mellitus in laboratory animals. In this study, while using this model the *Bixa Orellana* seed extracts has showed significant anti-hyperglycemic potential

particularly at the 1000 mg/kg dose when compared to the widely used synthetic drug Metformin. So, the seed extracts can safely and economically be used as the alternative to the commercial drug to reduce Hyperglycemia in diabetic patients. Additionally, the *Bixa Orellana* seed extracts have moderate to significant anti-oxidant property when compared to the standard drug, Ascorbic acid and can be used as natural anti-oxidant drug to boost immunity in patients, particularly to the young and elderly. Further, studies can be done to prove the safety of the extracts using human volunteers to ensure its beneficial uses for mankind.

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Conflict of Interest

There is no conflict of interest.

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