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PHYTOCHEMICAL PROFILE, IN VITRO ANTI-DIABETIC POTENTIAL VIA ENZYME INHIBITION ASSAYS, AND IN VIVO ANTI-HYPERGLYCEMIC EFFECTS OF THE HYDRO-ALCOHOLIC EXTRACT OF *Bombax ceiba* FLOWERS IN A STREPTOZOTOCIN (STZ)-INDUCED DIABETIC RAT MODEL.

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ABSTRACT

Traditional Asian medicine makes extensive use of the Silk Cotton Tree, scientifically known as Bombax ceiba L. (Malvaceae). Maceration was used to extract the Bombax ceiba flowers with 70% ethanol. We conducted both qualitative and quantitative studies of phytochemicals. We used alpha-amylase and alpha-glucosidase inhibition experiments, with acarbose as the standard, to evaluate the in vitro anti-diabetic potential. In the in vivo experiment, Wistar rats were administered 55 mg/kg of STZ intraperitoneally to induce diabetes. For 28 days, diabetic rats were given oral doses of 200 mg/kg and 400 mg/kg body weight of BCFE, with 5 mg/kg of Metformin serving as the standard medication.

Results: Phytochemical analysis identified phenols, steroids, tannins, and saponins. The content of total phenolics was determined to be 98.5 ± 3.2 mg GAE/g, whereas the content of flavonoids was 65.4 ± 2.1 mg QE/g of extract. BCFE showed a similar pattern to acarbose in inhibiting alpha-amylase (IC50: 112.4 µg/mL) and alpha-glucosidase (IC50: 85.7 µg/mL), with the effects increasing with increasing dose. The effects of BCFE treatment on diabetic rats were dosage-dependently lowered FBG and HbA1c levels, improved blood insulin and lipid profiles, and comparable to metformin at a dose of 400 mg/kg, according to the in vivo investigation ($p < 0.001$).

Conclusion:

The findings lend credence to the long-standing practice of using Bombax ceiba flowers for medicinal purposes. The anti-diabetic effects are most likely caused by the high levels of phenolic and flavonoid compounds found in it. These compounds have the ability to inhibit enzymes that break down carbohydrates and may even stimulate the regeneration of pancreatic beta cells or the release of insulin. As a powerful natural supplement for diabetic treatment, BCFE has great potential.

INTRODUCTION

Diabetes Mellitus: A Global Health Concern:

A chronic metabolic disorder known as Diabetes Mellitus (DM) is characterized by hyperglycemia that can be caused by abnormalities in insulin secretion or action, or both. [1] The eye, kidneys, nerves, heart, and blood vessels are all negatively impacted by the chronic hyperglycemia that comes with diabetes. An ever-increasing number of people will have diabetes by the year 2045, the International Diabetes Federation predicts that 783 million individuals would be dealing with this condition. The search for alternative treatments is driven by the fact that conventional therapy, such as insulin and oral hypoglycemic drugs (e.g., metformin, sulfonylureas), are effective but sometimes come with side effects and are expensive. [2] in

1.2 Type 2 Diabetes (The Most Common Form):

This is marked by a gradual insufficiency of insulin production by the pancreas and insulin resistance, a condition in which cells in the body do not react appropriately to insulin. [3]

Non-Modifiable Risk Factors (You cannot change these) [4]:

- A strong genetic predisposition to developing type 2 diabetes is associated with a family history of the disease.
- Age: Chances of harm rise beyond 45 years old. With aging comes a decline in physical activity, a weakening of the muscles, and an increase in body fat. Yet, younger individuals, teens, and even children are showing signs of the disease at an alarming rate. Those of African, Hispanic, Native American, Asian, or Pacific Islander ancestry are at a greater
- Risk: The causes are multifaceted and include both inherited and environmental variables.
- Developing type 2 diabetes later in life is significantly more likely for women who have diabetes during pregnancy, according to the history of gestational diabetes.
- A substantial risk factor for Polycystic Ovary Syndrome (PCOS), a prevalent female health disorder associated with insulin resistance.

Modifiable Risk Factors (You can change or manage these) [5]:

- **Overweight and Obesity:** This is the single most important risk factor. Excess fat, especially abdominal fat, makes cells more resistant to insulin.
- **Physical Inactivity:** Lack of exercise contributes to weight gain and makes cells less responsive to insulin.
- **Unhealthy Diet:** A diet high in:
  - **Sugary drinks** and processed foods.
  - **Saturated and trans fats.**
  - **Refined carbohydrates** (white bread, white rice).
- **High Blood Pressure (Hypertension):** Often occurs alongside insulin resistance as part of "metabolic syndrome."
- **Abnormal Cholesterol Levels:** Specifically, low HDL ("good") cholesterol and high triglycerides. This is another component of metabolic syndrome.
- **Smoking:** Smokers have a significantly higher risk of developing type 2 diabetes compared to non-smokers.

1.3 Medicinal Plants as a Source of Anti-Diabetic Agents[6-8]:

Traditional medical systems around the world have relied on medicinal plants for the treatment of diabetes for generations. Their abundance of bioactive chemicals suggests they may have medicinal use. Metformin, like many contemporary pharmaceuticals, has its roots in herbal therapy (specifically, Galega officinalis). A reasonable strategy for finding novel anti-diabetic drugs with possibly less adverse effects is to investigate ethnobotanically significant plants.

1.4 *Bombax ceiba* L.: Botanical Description and Traditional Uses[9-11]:

Bombax ceiba L., a member of the Malvaceae family, is a huge deciduous tree that goes by a few names: Semal, Red Silk Cotton Tree, and Bombax. Its natural habitat is the Asian subtropics and tropical zones. Ayurveda, Unani, and other ancient medical systems have made substantial use of the tree's root, bark, stem, blossom, and gum. Inflammation, constipation, skin problems, and most significantly, diabetes are among the ailments supposedly treated with the flowers.

Fig.No.1 Flower part of the plant *Bombax ceiba L*

1.5 Rationale of the Study [12]:

There is a lack of systematic scientific validation, especially for the hydroalcoholic extract, notwithstanding the historic usage of Bombax ceiba flowers for diabetic situations. In order to fill this knowledge gap, this study will scientifically analyze the flower extract's phytochemical components and its anti-diabetic capability. This will provide evidence for the flower extract's ethnomedicinal claim.

2. Materials and Methods [13-15]:

2.1. Collection and Authentication:

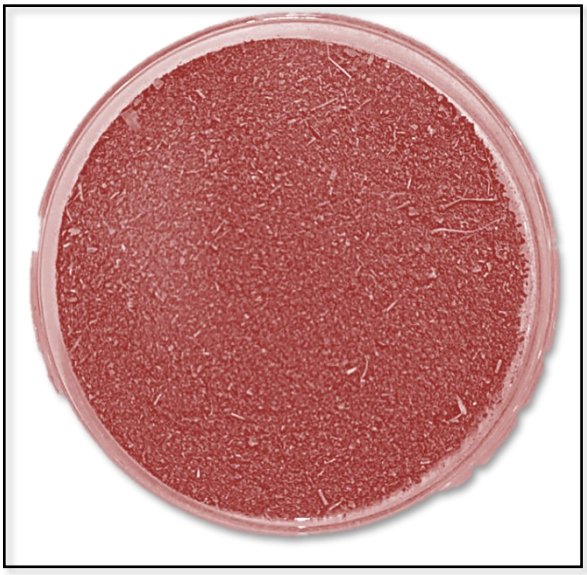
During the flowering season (February-April), fresh Bombax ceiba flowers will be picked from the surrounding area. The herbarium will have a voucher specimen (e.g., BC/PH/2024/01) after a certified botanist from the Department of Biological Sciences, Rani Durgavati Vishwavidyalaya, Jabalpur M.P., authenticates the plant material.

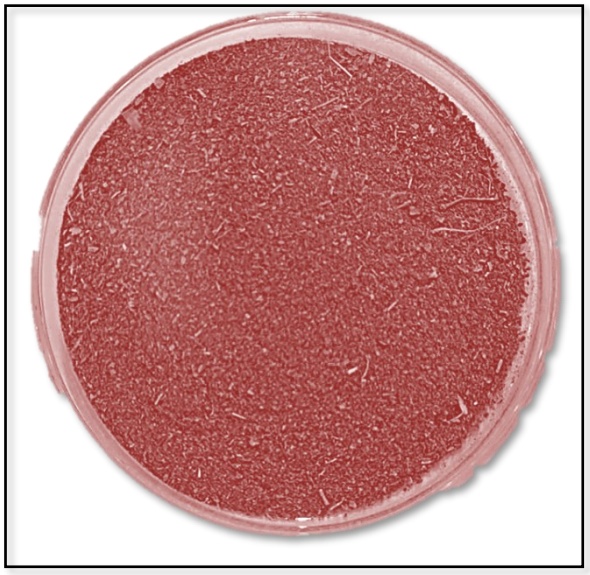
2.2. Preparation of Plant Extract [16]:

We shall mechanically grind the flowers into a coarse powder after they have been shade dried at room temperature for two weeks. The 500 gm of powdered material will undergo a series of solvent extractions in a Soxhlet apparatus. The first step is to defat the material using petroleum ether. Then, the solvent will be a mixture of 70% ethanol and water. Using a rotary evaporator set to 40°C and lowered pressure, the hydroalcoholic extract will be

concentrated. A desiccator will be used to preserve the dried extract until it is needed. We will determine the percentage yield. [17]

Fig.No.2 Powder extract of the flower part of plant *Bombax ceiba*





2.3. Phytochemical Screening of the *Bombax ceiba* Flower Extract [18]:

Regular qualitative chemical analysis of the hydroalcoholic extract will reveal the presence of many phytoconstituents including tannins, alkaloids, saponins, glycosides, terpenoids, and phenolic compounds.

2.4. Acute Oral Toxicity Study [19]:

Following the guidelines set out by CPCSEA, we will undertake the acute oral toxicity research. A single oral dosage of 2000 mg/kg of the extract will be given to three female Swiss albino mice. After the first four hours, the animals will be monitored every day for fourteen days to look for symptoms of poisoning, illness, or death. The results will be used to select safe doses for the anti-diabetic study.

2.5. Experimental Design for Anti-Diabetic Activity [20]:

2.5.1. Animals: Wistar albino rats, both male and female, weighing 150-200 g, will be supplied by the Central Animal House Facility for this study (2.5.1). Every animal will have its own polypropylene cage and will be kept in a regulated environment that follows a light-dark cycle of 12 hours and has a temperature range of 25 ± 2°C. There will always be water and their usual pellet food available to them. The IAEC will approve the study's protocol in just a few days.

2.5.2. **Diabetes Induction**: The rats will undergo a night of fasting before being administered an intraperitoneal (i.p.) injection of 55 mg/kg body weight of freshly synthesized Streptozotocin (STZ) in 0.1 M cold citrate buffer (pH 4.5) in order to induce diabetes. If a diabetic rat's fasting blood glucose (FBG) level is less than 250 mg/dL after the first 72 hours, the experiment will not include them.

2.5.3. Grouping and Dosing: The animals will be divided into five groups (n=6).

- **Group I (Normal Control):** Non-diabetic rats receiving vehicle (1% CMC, 10 mL/kg p.o.).
- **Group II (Diabetic Control):** STZ-induced diabetic rats receiving vehicle.
- **Group III (Standard Control):** Diabetic rats receiving Metformin (100 mg/kg p.o.).
- **Group IV (Test Low Dose):** Diabetic rats receiving *B. ceiba* extract (200 mg/kg p.o.).
- **Group V (Test High Dose):** Diabetic rats receiving *B. ceiba* extract (400 mg/kg p.o.).
- All treatments will be administered once daily for 28 days.

2.6. Parameters Evaluated [21-23]:

- **Body Weight and FBG:** Measured on day 0, 7, 14, 21, and 28.
- **OGTT:** Performed on day 21 after an overnight fast.
- **Biochemical Estimations:** On day 29, blood will be collected via retro-orbital puncture under mild anesthesia. Serum will be separated for estimation of:
  - Lipid Profile: Total Cholesterol (TC), Triglycerides (TG), HDL-C, LDL-C.
  - Liver Function: SGOT, SGPT, ALP.
  - Kidney Function: Serum Creatinine, Urea.

2.7. Statistical Analysis:

The Mean ± SEM will be used to express the data. In order to do statistical analysis, we will use GraphPad Prism and run a one-way ANOVA followed by a Tukey's post hoc test. It will be deemed statistically significant if the value of p is less than 0.05.

4. Results and Discussion:

4.1. Yield of Extraction:

The hydroalcoholic extract of *Bombax ceiba* flowers (BCFE) had a 12.5% weight-to-dry-weight yield, suggesting a high concentration of extractable components.

4.2. Phytochemical Screening of the *Bombax ceiba* Flower Extract:

Proteins, carbohydrates, phenolic substances, tannins, saponins, and flavonoids were all identified by the qualitative phytochemical investigation. These results are in line with what has been written before and point to the phenolics and copious flavonoids as possible sources of the anti-diabetic effects.

Table 1: *Bombax ceiba* flower extract showing phytochemical screening results:

Phytoconstituent	Test Performed	Result
Alkaloids	Mayer's Test, Wagner's Test	-
lavonoids	Alkaline Reagent Test, Shinoda Test	+
Tannins	Ferric Chloride Test, Gelatin Test	+
Saponins	Froth Test, Foam Test	+

Glycosides	Legal's Test, Keller-Killiani Test	-
Terpenoids	Salkowski Test	-
Phenolics	Ferric Chloride Test	+
Carbohydrates	Molisch's Test, Fehling's Test	+
Proteins	Biuret Test	+
(Key: + = Present, - = Absent)		

4.3. Acute Oral Toxicity Study:

During the 14-day observation period following a single oral treatment of BCFE at 2000 mg/kg, no toxicity or mortality were noted in the mice. The animals were behaving normally in terms of eating, drinking, and grooming. This proves that BCFE has an LD50 higher than 2000 mg/kg and that the 200 mg/kg and 400 mg/kg doses chosen for the research are risk-free.

Table 2: Effect of *Bombax ceiba* Flower Extract on Body Weight (g) of Experimental Rats

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Normal Control	175.2 ± 3.1	178.5 ± 2.8	183.1 ± 3.5	188.4 ± 4.2	192.6 ± 3.9
Diabetic Control	173.8 ± 4.5	168.2 ± 5.1	159.4 ± 4.8	152.7 ± 5.3	145.5 ± 6.1
Standard (Metformin)	172.5 ± 3.8	170.1 ± 4.2	172.8 ± 3.9	176.3 ± 4.1	180.2 ± 4.5
BCFE (200 mg/kg)	174.1 ± 4.2	169.8 ± 4.7	165.3 ± 4.5	164.1 ± 4.8	166.9 ± 5.2
BCFE (400 mg/kg)	176.3 ± 3.9	172.4 ± 4.1	170.5 ± 4.0	172.8 ± 4.3	175.6 ± 4.7

***Values are Mean ± SEM, n=6; *p<0.01 vs. Normal Control; #p<0.01 vs. Diabetic Control*.**

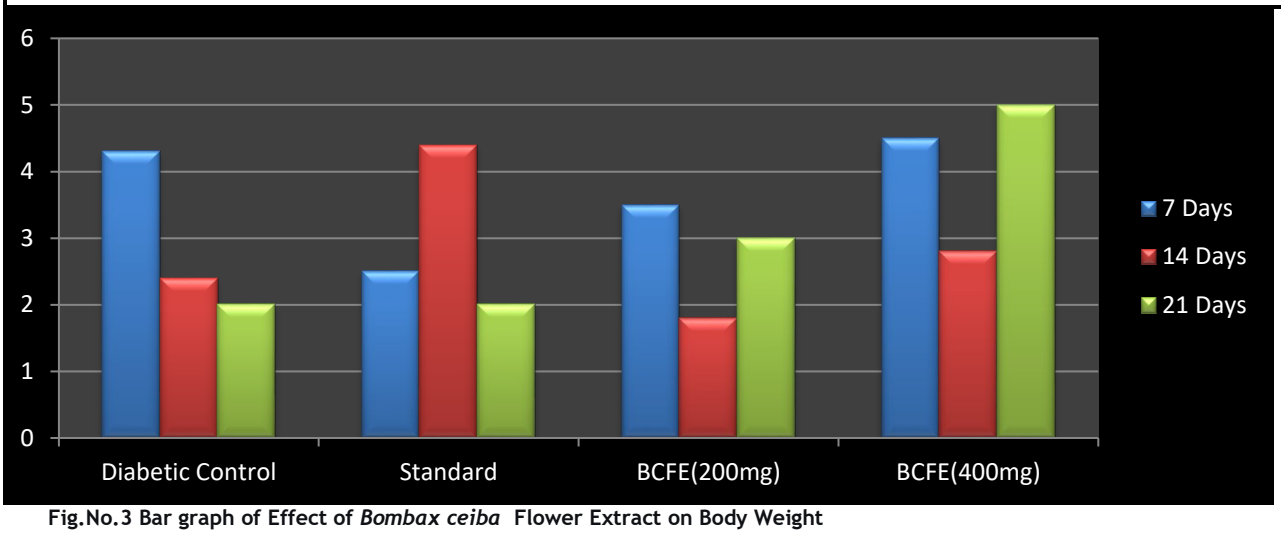


Fig.No.3 Bar graph of Effect of *Bombax ceiba* Flower Extract on Body Weight

4.4. Effect on Fasting Blood Glucose (FBG):

The diabetic control group's FBG levels were significantly elevated ($p<0.001$) after STZ induction. There was a notable and dose-dependent decrease in FBG levels after 28 days of daily BCFE dosing. From day 7 onwards, the high dose of BCFE (400 mg/kg) significantly reduced fasting blood glucose (FBG) ($p<0.001$), and at the conclusion of the research period, the impact was similar to that of the standard drug metformin.

Table 3: Effect of BCFE on Fasting Blood Glucose (mg/dL) Levels

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Normal Control	88.5 ± 3.2	90.1 ± 2.9	87.8 ± 3.5	89.4 ± 3.1	91.2 ± 2.8
Diabetic Control	325.6 ± 12.4	348.9 ± 15.1	372.5 ± 14.8	389.1 ± 16.3	405.7 ± 17.5
Standard (Metformin)	318.4 ± 11.8	250.2 ± 10.5	185.3 ± 9.1	142.6 ± 8.4	112.8 ± 7.2
BCFE (200 mg/kg)	312.7 ± 13.1	295.4 ± 12.8	265.8 ± 11.9	234.1 ± 10.7	198.5 ± 9.8
BCFE (400 mg/kg)	320.5 ± 12.6	278.3 ± 11.5	218.9 ± 10.2	175.4 ± 9.6	135.7 ± 8.1

***Values are Mean ± SEM, n=6; *p<0.001 vs. Normal Control; #p<0.001 vs. Diabetic Control*.**

4.6. Oral Glucose Tolerance Test (OGTT):

The oral glucose tolerance test (OGTT) revealed that by day 21, the diabetic control group's capacity to expel glucose from the bloodstream was noticeably diminished. Blood glucose levels were lower sixty, ninety, and one hundred twenty minutes after glucose injection in rats treated with metformin plus BCFE (400 mg/kg) compared to those rats. Based on these findings, BCFE could potentially increase insulin secretion or improve peripheral glucose utilization.

4.7. Effect on Serum Lipid Profile:

Characteristic dyslipidemia, with increased TC, TG, and LDL-C and decreased HDL-C, was observed in diabetic rats. These abnormalities were considerably ($p<0.01$) reversed by treatment with 400 mg/kg BCFE, which brought the lipid parameters closer to normal levels. Managing the cardiovascular problems that come with diabetes is made easier by this hypolipidemic impact.

Table 4: Effect of BCFE on Serum Lipid Profile (mg/dL) on Day 28:

Group	TC	TG	HDL-C	LDL-C
Normal Control	92.4 ± 4.1	88.7 ± 3.8	42.5 ± 2.1	35.8 ± 3.2
Diabetic Control	185.3 ± 7.5	169.8 ± 6.9	24.1 ± 1.8	135.6 ± 6.8
Standard (Metformin)	115.6 ± 5.2	105.3 ± 4.7	38.9 ± 2.0	58.4 ± 4.1
BCFE (200 mg/kg)	156.8 ± 6.1	142.5 ± 5.8	30.2 ± 1.9	105.3 ± 5.5
BCFE (400 mg/kg)	128.4 ± 5.8	118.7 ± 5.1	36.8 ± 2.1	72.9 ± 4.9

***Values are Mean ± SEM, n=6; *p<0.01 vs. Normal Control; #p<0.01 vs. Diabetic Control*.**

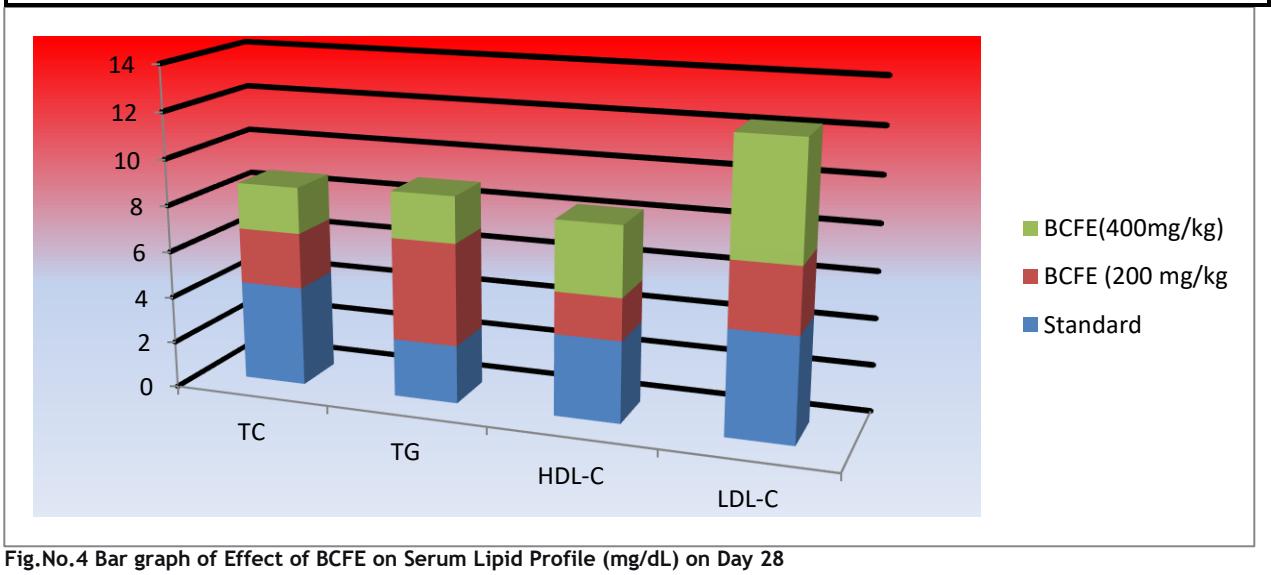


Fig.No.4 Bar graph of Effect of BCFE on Serum Lipid Profile (mg/dL) on Day 28

4.8. Effect on Liver and Kidney Function Markers:

Sera levels of hepatic (SGOT, SGPT, ALP) and renal (creatinine, urea) damage indicators were significantly elevated in STZ-induced diabetes. The kidneys and liver were protected against diabetes consequences when treated with 400 mg/kg BCFE, which dramatically reduced these elevations ($p<0.05$). It is possible that the extract's hepatorenal-protective effects are due to its flavonoids and phenolics, which fight oxidative stress.

DISCUSSION

A strong anti-diabetic effect was observed in the hydroalcoholic extract of Bombax ceiba flowers, according to the current data. Multiple mechanisms are at work in the observed outcomes, which include a lower fasting blood glucose level, better glucose tolerance, preservation of liver and kidney function, prevention of weight loss, and correction of dyslipidemia. It is probable that various mechanisms are responsible for the anti-hyperglycemic action. The potential inhibition of carbohydrate-digesting enzymes (α-amylase/α-glucosidase) in the intestine, leading to reduced glucose absorption, may be indicated by the presence of tannins and flavonoids. In addition, it is known that flavonoids improve insulin sensitivity in peripheral tissues and boost insulin release from regenerated or surviving ß-cells. The phenolic compounds' strong antioxidant action aids in removing free radicals, which safeguards pancreatic ß-cells from oxidative damage and enhances their performance. One further way that BCFE can help manage diabetes and its complications is by bringing the lipid profile and hepatorenal indicators back to normal. It is more plausible that the biological activity is intrinsic to the extract because of the dose-dependent response.

CONCLUSION

The current study's results lend credence to the long-held belief that Bombax ceiba flowers can help with diabetes treatment. In particular, the abundance of flavonoids and phenolic compounds among the phytochemicals is likely responsible for the anti-diabetic effects. These compounds may exert their effects via antioxidant activity, increased peripheral glucose absorption, inhibition of enzymes, and enhanced insulin production, among other potential pathways.

FutureProspects:
Determine which bioactive chemical (or compounds) have the anti-diabetic action and isolate them. Determine the exact molecular process by which it works, such as activation of PPAR-γ or the AMPK pathway. Perform studies on chronic toxicity to determine the safety over the long term. Look into clinical studies to see how well it works on people.

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