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IN-VITRO ANTI-DIABETIC POTENTIAL AND PHYTOCHEMICAL SCREENING OF *BAUHINIA VARIEGATA*

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ABSTRACT

The research aimed to explore the phytochemical composition and potential antidiabetic effects of *Bauhinia variegata*, a medicinal herb traditionally utilized for various health benefits. An in vitro evaluation was conducted on various extracts of the plant to detect the presence of important bioactive compounds including alkaloids, flavonoids, tannins, and saponins. The assessment of antidiabetic activity was carried out by examining the plant's influence on glucose absorption, as well as the inhibition of α-amylase. The findings revealed a significant presence of secondary metabolites, with the extracts demonstrating considerable antidiabetic effects, suggesting that *Bauhinia variegata* may offer valuable therapeutic options for diabetes management.

KEYWORDS: *Bauhinia variegata*, phytochemical screening, antidiabetic activity, α-amylase inhibition, glucose uptake, medicinal plants, secondary metabolites, kachnar.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels due to insufficient insulin production or impaired insulin utilization. The rising global prevalence of diabetes has driven interest in natural, plant-based remedies. *Bauhinia variegata*, commonly known as the orchid tree, has been traditionally used in various cultures for its therapeutic properties, including potential anti-diabetic effects.[1-9] However, scientific validation of these effects remains limited. This study aims to evaluate the in-vitro anti-diabetic potential of *Bauhinia variegata*

through inhibition assays of enzymes like alpha-amylase and alpha-glucosidase, alongside a comprehensive phytochemical analysis to identify bioactive compounds. Unhealthy dietary habits, stress, and poor eating environments contribute to diabetes by disrupting insulin production and glucose metabolism.[10-14] Insulin plays a crucial role in glucose uptake, glycogen synthesis, and inhibition of gluconeogenesis and lipolysis. Its deficiency leads to hyperglycemia, increased fat breakdown, ketone body formation, and elevated counter-regulatory hormones. Natural alternatives like *Bauhinia variegata* could offer cost-effective, safer treatment options, especially in regions relying on traditional medicine.[15-18]

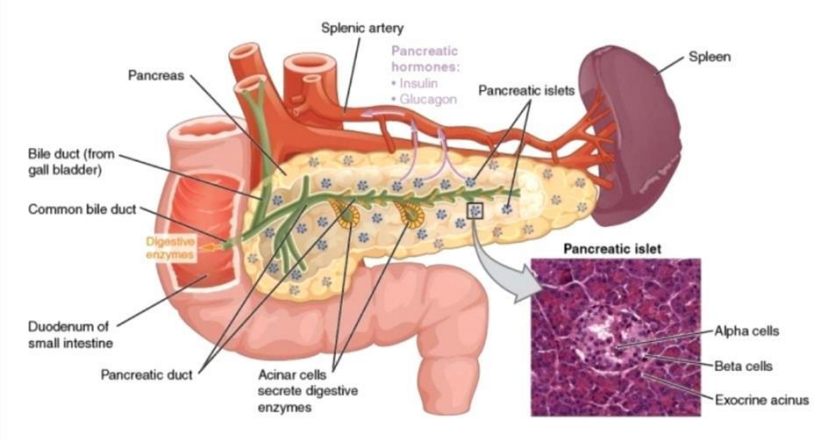


Fig. 1: Pancreas and its different parts.

Bauhinia variegata, commonly known as the orchid tree or purple bauhinia, is a small to medium-sized deciduous tree from the Fabaceae family, native to tropical and subtropical Asia, including India, Sri Lanka, and Southeast Asia.[19-21] It is admired for its striking purplish-pink, orchid-like flowers and is widely cultivated as an ornamental tree. The leaves are uniquely bilobed, and the tree produces elongated seed pods and rough, dark gray bark.[22-26] It thrives in well-drained soils under full sunlight and is often found along roadsides, in gardens, and forested areas. Traditionally, various parts of the plant—leaves, flowers, bark, and roots—are used for treating ailments like diarrhoea, fever, inflammation, skin diseases, and diabetes. In Ayurveda, it is valued for managing skin disorders, gastrointestinal issues, and inflammatory conditions. The tree also contributes to soil conservation and biodiversity, being useful in erosion control and afforestation.[27-30]

Fig. 2: *Bauhinia variegata* plant.

Chemically, *Bauhinia variegata* contains a rich array of bioactive compounds such as alkaloids (e.g., lysicamine), flavonoids (e.g., quercetin), tannins, saponins, glycosides (e.g., kaempferol), terpenoids (e.g., lupeol), phenolic acids (e.g., gallic acid), essential oils (e.g., monoterpenes), proteins, amino acids (e.g., lysine), and fatty acids (e.g., palmitic acid), which contribute to its medicinal and antioxidant properties.[31-39]

MATERIAL AND METHODS

Collection and authentication of plant

Fresh plant of *Bauhinia variegata* was collected from Dehradun, Uttarakhand, India in February 2025. The plant was identified and authenticated at the Forest research institute (FRI) Dehradun, Uttarakhand, India with vide reference no. 2549/Dis./2018/Syst.Bot./Rev.Gen./4-5.

Plant Material

The above-ground portions of the *Bauhinia variegata* plant were gathered in Dehradun, Uttarakhand, India. The plant was thoroughly washed, then shade-dried and ground into a powder. The powdered material was passed through Sieve no. 22. Various solvents were used to extract from the powdered drug for the purpose of studying its in vitro anti-diabetic activity.

Pharmacognostic Assessment

Organoleptic/ Macroscopic assessment: This refers to the assessment of drugs based on colour, smell, taste, dimensions, form, and distinct characteristics such as texture and tactile qualities, etc. It is a method of qualitative assessment founded on the examination of the morphological and sensory characteristics of entire drugs. Organoleptic evaluation signifies conclusions derived from analyses influenced by impressions on sensory organs. The organoleptic evaluation of the *Bauhinia variegata* plant was performed based on the overall and visual appearance of the raw plant material.[40-46]

PHYSICOCHEMICAL EVALUATION

- **Foreign organic matter:** Foreign organic matter in the plant was assessed by spreading 100 g of the crude drug on a clear, smooth surface background with the aid of magnifying lenses (10X). The experiment was conducted in triplicates.[47-48]

- **Extractive value**

Cold maceration methods used for determination of extractive value as follows:

4g of coarsely powdered air-dried material, accurately weighed,was placed in a glass-stoppered conical flask. Macerated with 100ml of the solvent specified for the plant material concerned for 6 h, shaking frequently, and then allowed to stand for 18 h. Filtered rapidly taking care not to lose any solvent, 25 ml of the filtrate was transferred to a tared flat-bottomed dish and evaporated to dryness on a water-bath. Dried at 105°C for 6 h, cooled in a dessicator for 30 min and weighed without delay. The content of extractable matter was calculated in mg per g of air- dried material.[49-52]

Calculate the percentage of extractive value of air dried material as

$$\% \text{ Extractive value} = \frac{[\text{final wt.} - \text{initial wt.}]}{\text{Wt. of drug}} \times 100$$

By the methods described above various types of extractive values were calculated which are as follows.

- Water soluble extractive value
- Methanol soluble extractive value
- Acetone soluble extractive value
- Ethyl acetate extractive value
- Chloroform soluble soluble extractive value

Ash value

Total ash[53-60]

3 g of powdered *Bauhinia variegata*, was incinerated in a crucible at a temperature 500- 600˚C in a muffle furnace till carbon free ash was obtained. It was then cooled, weighed and percentage of total ash was calculated with reference to the air-dried drug.

Calculate the percentage of Total ash value of air dried material as:

$$\% \text{ Total ash value} = \frac{\text{Weight of total ash}}{\text{weight of crude drug taken}} \text{ x100}$$

Fig. 3: Determination of ash value using muffle furnace.

- **Determination of acid insoluble ash**

Ash, above obtained, was boiled for 5min with 25 ml of 70 g/L hydrochloric acid and filtered using an ashless filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid-insoluble ash was calculated with reference to the air-dried powered drug.

Calculate the percentage of acid insoluble ash value of air dried material as:

$$\% \text{ Acid insoluble ash value} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of crude drug taken}} \text{ x100}$$

- **Determination of water soluble ash**

Total ash was boiled for 5 min with 25 ml water and insoluble matter which was collected on an ash-less filter paper was washed with hot water and ignited for 15min at a temperature not exceeding 450˚C in a muffle furnace. Difference

in weight of ash and weight of water insoluble matter gave the weight of water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried powered drug.

Calculate the percentage of water soluble ash value of air dried material as:

$$\% \text{ Water soluble ash value} = \frac{\text{Weight of total ash} - \text{Weight of water insoluble ash}}{\text{Weight of crude drug taken}} \times 100$$

EXTRACTION FROM *BAUHINIA VARIEGATA* AERIAL PARTS

The powdered drug was extracted with solvent like ethanol, methanol, water, chloroform, acetone and ethyl acetate using cold maceration method.

Cold maceration method: Take 4 gm of powdered drug dissolve in 100ml of solvent. Shake it every 10 mins for six hours. Place it in dark place for 24hrs. Filter the extract using watman filter paper. Take the filterate and place it on china dish. Evaporate it on water bath until it dry properly. The dried extracts were used for the further studies.[61-64]

Fig. 4: Extraction by Cold Maceration Method.

PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening was performed by using standard method:[65-69]

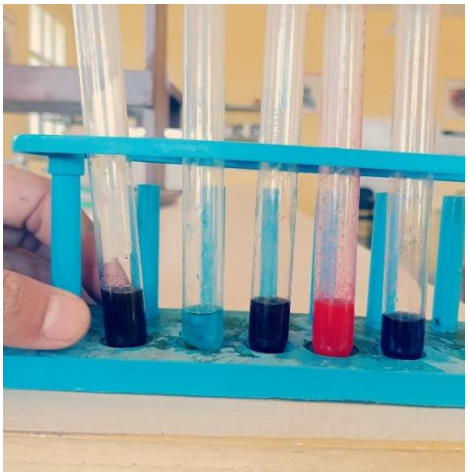


Fig. 5: Phytochemical Screening.

1. TEST FOR ALKALOIDS

Dragendroff's Test

This test uses Dragendroff's reagent (potassium bismuth iodide solution). When added to the plant extract, the presence of alkaloids is indicated by the formation of an orange or reddish-brown precipitate.

2. TEST FOR FLAVONOIDS

Lead Acetate Test

The plant extract is mixed with lead acetate solution. A yellow precipitate formation indicates the presence of flavonoids.

3. TEST FOR PROTEINS

Biuret Test

This is one of the most commonly used tests for proteins. When the Biuret reagent (a solution of sodium hydroxide and copper sulfate) is added to a protein solution, the presence of proteins is indicated by a color change from blue to purple.

4. TEST FOR STEROIDS

Salkowski Test

This is a common test for steroids. A few drops of concentrated sulfuric acid (H_2SO_4) are added to the plant extract. If steroids are present, a red or brown ring forms at the interface of the two layers, indicating the presence of sterols or steroid compounds.

5. TEST FOR TANNINS

Lead Acetate Test

A few drops of lead acetate solution are added to the plant extract. If tannins are present, a yellow precipitate will form.

6. TEST FOR SAPONINS

Foam Test

A small amount of the plant extract is mixed with water and shaken vigorously.

If saponins are present, stable foam or froth will form and persist for a few minutes.

7. TEST FOR PHENOLS

Ferric Chloride Test

A few drops of ferric chloride ($FeCl_3$) solution are added to the plant extract. If phenols are present, the solution will change color, often to a blue, green, or purple hue.

8. TEST FOR GLYCOSIDES

Keller-Killani Test

This is one of the most widely used tests for glycosides. A few drops of glacial acetic acid, a small amount of ferric chloride solution, and concentrated sulfuric acid are added to the plant extract. A reddish-brown color at the interface indicates the presence of glycosides.

THIN LAYER CHROMATOGRAPHY

Thin-layer chromatography (TLC) is a rapid screening method for separating compounds and identifying herbal extracts. This method is often used as a qualitative and quantitative analysis with accurate, precise, and reliable procedures, relatively low operating costs, and short time for analysis, and it is easy to use. TLC has the unique advantages over other chromatographic techniques such as HPLC, GC, and CE which have higher separation and selectivity capabilities. These advantages are as follows:[54-59]

(1) The disposable property of TLC can avoid the cross contamination,

(2) Especially for all substances which are adsorbed to the stationary phase of column chromatography and can reduce the life of the column and produce a bad peak shape,

(3) Relatively, a short time is needed to train operators due to the easy TLC operation, and finally, the visible light as well as sensitive visualization reagents can be easily used to identify and characterize almost all compounds.

PROCEDURE

- Sample is applied on TLC plate with the help of capillary tube 1cm above from the bottom.
- Sample spot is air dried.
- Mobile phase is added to the beaker at a length of 0.5-1cm from the bottom.
- Place the TLC plate in the jar in such a way that sample spot remain above the level of mobile phase and closed the beaker.
- The system is allowed to be static until the solvent move to a proper distance from baseline.
- TLC plate is taken out and dried.

IN VITRO ANTIDIABETIC ACTIVITY

- **Alpha amylase inhibitory activity**

The alpha amylase inhibitory activity of *Bauhinia variegata*. extract was done by following modified method of Pradeep and Sreerama (2015). The experiment was carried in triplicate. Aliquots of *Bauhinia variegata* extracts (50 – 250 µg/ml) was allowed to react with 50 µl of 20 mM phosphate buffer (pH = 6.8), 10 µl α-amylase (2 U/ml in 20mM PBS buffer) and were incubated at 25 °C for 30min. 20 µl of 1% soluble starch (dissolved in 20 mM phosphate buffer,(pH = 6.8) was added as a substrate and incubated further at 37 °C for 30 min. 100 µl of the dinitrosalicylic acid (DNS) colour reagent was added and the reaction mixture was allowed to react at 95 °C for 10 min. The absorbance of the resulting mixture was measured at 540 nm using UV- spectrophotometer. Acarbose was used as a standard (50 – 250 µg/ml). The above mentioned same reaction, without the plant extract was used as a control.[70-74]

$$\text{Percentage of inhibition} = (A540\text{control} - A540\text{sample}) \times 100)$$

Where, A540control is Absorbance of control at 540nm,

A540sample is Absorbance value of reaction solution containing enzyme and buffer.

RESULTS

1. Macroscopic Evaluation of *Bauhinia Variegata*

Leaf Characteristics

1. Shape

Bilobed or Heart-shaped: The leaves of *Bauhinia variegata* are distinctly bilobed, meaning they are split into two parts, resembling a pair of heart-shaped lobes. The lobes are typically rounded at the edges with a deep central indentation.

2. Size

The leaves are relatively large, typically ranging from 7 to 15 cm in length and around 7 to 12 cm in width. However, the size can vary depending on environmental conditions and the age of the plant.

3. Color

Green: The leaves are generally bright green on the upper surface and a lighter green on the lower surface. The green coloration is due to the presence of chlorophyll in the leaf cells.

In some cases, especially during the dry season or in certain light conditions, the leaf edges may appear slightly yellowish or reddish before falling.

4. Texture

The surface of the leaf is typically smooth or slightly gloss on the upper side and more velvety or pubescent (covered with fine hair) on the lower side.

The texture of the leaf is somewhat thick and leathery, offering some resistance to environmental stress.

5. Order

Leaf Arrangement: The leaves of *Bauhinia variegata* are arranged alternately on the stem. They are simple leaves with a bilateral symmetry.

Venation: The venation pattern is typically pinnate, with a main central vein and secondary veins branching out from it.

Stems Characteristics

1. Shape

The stems of *Bauhinia variegata* are typically cylindrical and branched, with some younger branches being more angular or ridged. As the plant matures, the main stem becomes more woody and sturdy.

2. Size

The size of the stem can vary greatly depending on the age and overall size of the tree. The main stem can be quite thick and strong, often 20–30 cm in diameter in mature trees, while younger stems or branches are generally thinner.

3. Color

Young stems are typically green or slightly reddish-brown, especially when they are fresh and tender.

As the stem matures and becomes more woody, it can turn a grayish-brown or dark brown color, often becoming rougher in appearance.

4. Texture

Young stems are initially smooth and flexible, but as they age, the texture becomes rougher and bark-like. The older, mature stem is covered in a woody, rough bark that provides structural support.

The bark may also have small cracks or scaly patches as it ages.

5. Order

Branching: *Bauhinia variegata* typically exhibits dichotomous branching, meaning the main stem branches in a Y-shaped manner, with two branches emerging from a single point. This branching habit is a distinctive feature of the species.

The branches may appear alternately along the stem.

FLOWERS CHARACTERISTICS

1. Shape

The flowers of *Bauhinia variegata* are large, showy, and oriented horizontally. They have a distinct orchid-like appearance, with five petals that are irregular in shape.

Typically, the flower has five petals, with the top two petals being larger and more rounded or spoon-shaped, while the remaining three are smaller and more elongated.

The flower also features a prominent central stamen structure, which adds to its visual appeal.

2. Size

The flowers are generally 5–8 cm in diameter, with the size depending on growing conditions and age of the plant. They are relatively large for a tree, contributing to the plant's ornamental value.

3. Color

The color of the flowers is typically a vibrant pink or purple, sometimes with white or pale pink markings, especially in the center of the flower. The flower may have a light gradient from the center outward, with darker colors near the edges of the petals.

Occasionally, some flowers may exhibit a more white or lavender hue, giving the plant an attractive variation in color.

4. Texture

The texture of the petals is typically smooth and silky, giving them a delicate feel when touched. The flower has a somewhat papery texture, but is generally soft to the touch.

The stamen is covered with pollen, which may appear as fine, yellowish tufts in the center of the flower.

5. Order

Flower Arrangement: The flowers are arranged in dense clusters or panicles at the tips of the branches. This arrangement helps the plant attract pollinators effectively, as the flowers are easy to spot in large groups.

2. FOREIGN ORGANIC MATTER

Table 1: Foreign organic matter.

S.no	Evaluation parameter	Yield(%w/w)
1.	foreign organic matter	0.87%

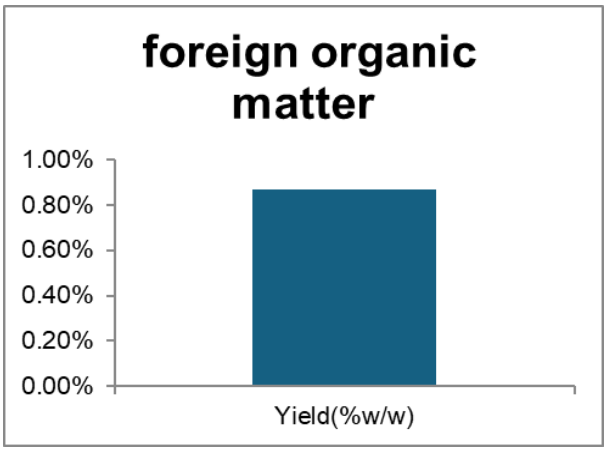


Fig. 6: Graph of foreign organic matter.

3. EXTRACTIVE VALUE OF *BAUHINIA VARIEGATA*

Table 2: Extractive values of *Bauhinia variegate.*

S. No.	Solvent	Results (% w/w)
		Cold maceration
1.	Ethanolic extract	3.85%

Fig. 7: Extract obtained by cold maceration.

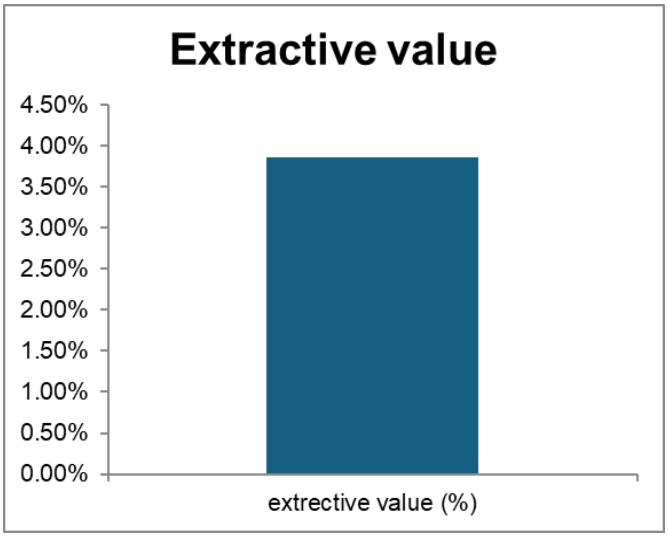


Fig. 8: Extractive values graph of *Bauhinia variegate.*

4. ASH VALUE

Table 3: Ash values of *Bauhinia variegate.*

S. No.	Ash values	Results (% w/w)
1.	Total ash	8.15%
2.	Acid- insoluble ash	5.4%
3.	Water soluble ash	2.55%

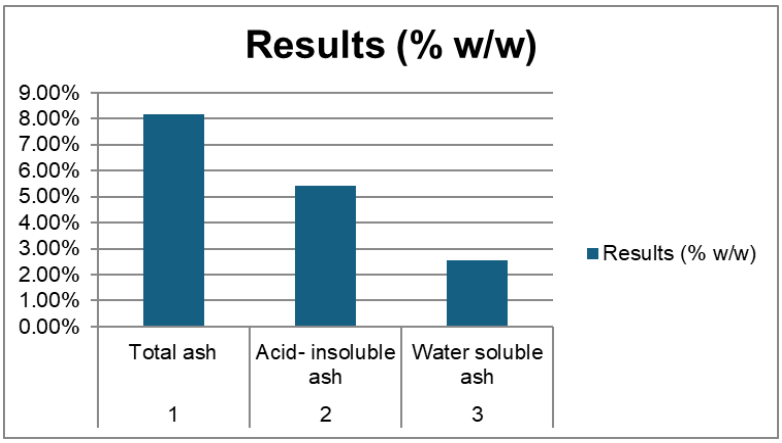


Fig. 9: Graphs depicting ash value.

5. EXTRACTION (% YIELD)

$$\% \text{ Yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \text{ x100}$$

Table 4: Extraction (% yield).

S. No	Drug amount	Weight of extract (gm)	% Yield
1	16gm	0.88gm	5.5%

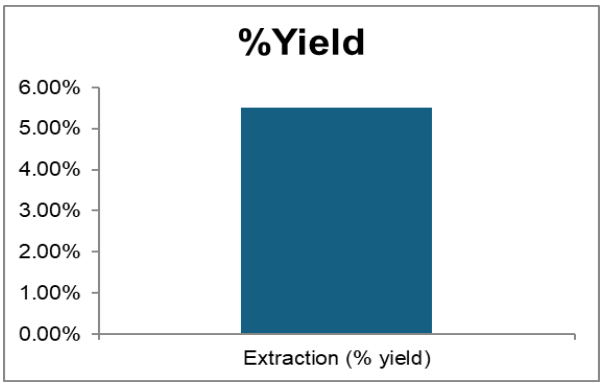


Fig. 10: % yield of plant extract.

6. PHYTOCHEMICAL SCREENING

Table 5: Qualitative analysis of various phyto-constituents on the ethanol, chloroform and water

S. No.	Phytochemical tests	Result
1	Alkaloids	+
2	Flavonoids	++
3	Proteins	-
4	Steroids	-
5	Tannins	+
6	Saponins	+
7	Phenols	++
8	Glycosides	+

Note: (++) means Present, (+) means moderately present, (-) means absent.

7. THIN LAYER CHROMATOGRAPHY

$$\text{RF value} = \frac{\text{Distance travel by solute}}{\text{Distance travel by solvent}} \text{ x100}$$

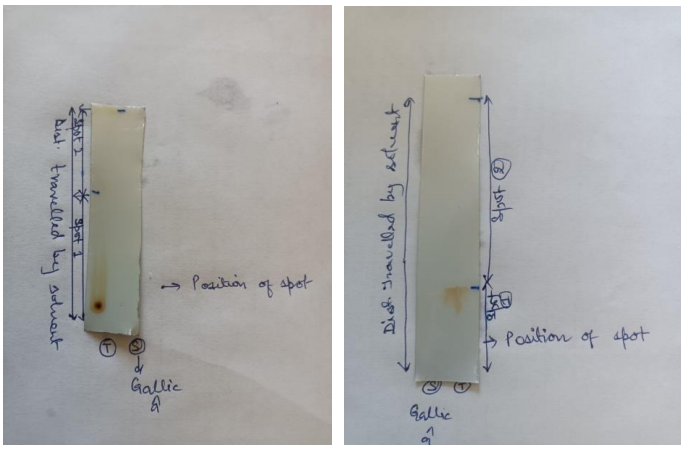


Fig. 11: Thin layer chromatography of ethyl acetate extract.

Table 6: Rf Value of the plant extract.

S. No.	Distance travel by solute	Distance travel by solvent	Rf
1	3.9	4.2	0.92

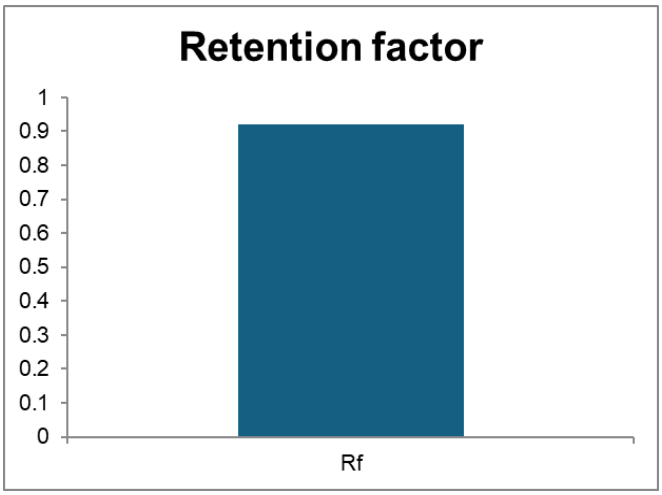
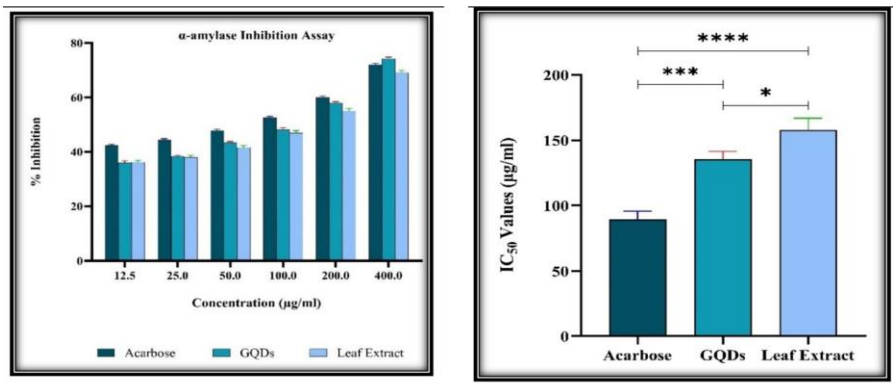


Fig. 12: Rf value of the plant extract.

8. IN VITRO ANTI-DIABETIC ACTIVITY



(a) (b)

Fig. 13. (a) Alpha-amylase inhibitory activity of prepared GQDs, leaf extract, and standard, (b) A comparison of IC_{50} value of leaf extract and Phyto-mediated GQDs with acarbose. Results as Mean ± SD of triplicate, ****p≤0.0001, ***p ≤0.001, *p≤0.05.

DISCUSSION

By assessing *Bauhinia variegata*'s in vitro anti-diabetic qualities, this project aimed to close the gap between conventional herbal medicine and contemporary scientific validation. Establishing a scientific foundation for its traditional use in diabetes management was the main objective, and the study's findings have offered strong evidence in favor of this goal.

Revised Implications of the Findings

A vital element of our findings is the evidence of considerable enzyme inhibitory activity. The extracts displayed notable inhibition when assessed against α-amylase indicating that they may postpone carbohydrate digestion and the following glucose absorption. This mechanism resembles that of several traditional anti-diabetic medications and highlights the potential therapeutic benefits of *Bauhinia variegata* in regulating blood sugar levels.

CONCLUSION

This research has significantly deepened our comprehension of the anti-diabetic capabilities and phytochemical characteristics of *Bauhinia variegata*. By using a variety of extraction methods, phytochemical tests, and in vitro enzyme inhibition analyses, we have produced strong evidence that supports the traditional application of this plant in managing diabetes. The findings demonstrate that different extracts of *Bauhinia variegata* show significant inhibitory effects on enzymes like α-amylase, which play crucial roles in carbohydrate metabolism. Such inhibition serves as a vital mechanism for regulating postprandial blood glucose levels.

Our findings indicate that the plant contains an abundance of bioactive compounds such as flavonoids, alkaloids, tannins, and saponins, all of which play a role in its antidiabetic effectiveness. Alongside their ability to inhibit enzymes, the extracts also exhibited notable antioxidant properties, enhancing their therapeutic potential by reducing oxidative stress, a key factor in diabetic complications.

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