

# Phytochemical Insights and In Vitro Antidiabetic Screening of *Cryptostegia madagascariensis* Leaf Extract

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**Abstract:** Rich in bioactive phytoconstituents, *Cryptostegia madagascariensis* is a medical plant that has garnered interest for its potential therapeutic uses. Elevated blood glucose levels are the feature of diabetes mellitus, a chronic metabolic disease that continues to be a major global health concern. Due to their potential effectiveness, fewer side effects, and affordability, the hunt for plant-based antidiabetic drugs has increased. The current study used conventional screening techniques to evaluate the in-vitro antidiabetic potential of *Cryptostegia madagascariensis* extract. The plant material was obtained, confirmed, dried, ground into a powder, and extracted using proper solvents using standard methods of extraction. To find secondary metabolites like alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, and terpenoids, a preliminary phytochemical screening of the extract was completed. The biological function and possibly antidiabetic advantages of these phytoconstituents are popular. Using in vitro tests including the glucose adsorption assay and the  $\alpha$ -amylase inhibition assay, the antidiabetic efficacy was evaluated. The extract showed glucose-binding ability and concentration-dependent inhibitory activity against  $\alpha$ -amylase, suggesting that it may lessen the absorption of glucose and the digestion of starches. The presence of active phytochemical components in the plant extract may be linked to the reported activity. According to the results, *Cryptostegia madagascariensis* extract may be a natural source for the creation of herbal antidiabetic formulations and has promising in-vitro antidiabetic potential. To verify its effectiveness and mode of action, more research involving the isolation of active molecules and in vivo assessment is required. The use of medicinal plants as alternative therapies in the management of diabetes has been backed by this study.

**Keywords:** *Cryptostegia madagascariensis*, Phytochemical insights, In vitro antidiabetic activity, Leaf extract, Hypoglycemic potential

## I. INTRODUCTION

Native to Madagascar (Africa), *Cryptostegia madagascariensis* Bojer ex Decne. (Apocynaceae) is a scandent shrub that occurs as a shrub or someone who climbs plant in dry forests, disturbed areas, and riverbanks. It has become an invasive species in northeastern Brazil, as it has mostly established dense communities close to rivers. This species' ability to produce a large number of seeds during a single reproduction event that are distributed by the wind and germinate in large quantities is one of its traits. One of the regions where C most frequently invades. Madagascariensis in Brazil are the riverine "carnaubais", locations marked by high water availability for a portion of the year and the prominent presence of the endemic carnauba palm, *Copernicia prunifera* (Mill.) H. E. Moore. Therefore, it may be useful to understand the traits that allow C. madagascariensis to invade Brazilian semiarid regions by knowing how abiotic factors (such as temperatures, light, water, and saline stresses) affect germination and how water availability, the most limiting factor for the establishment of seedlings in dry regions, interferes with its initial growth. This will also assist in determining the region most vulnerable to invasion and tracing control tactics according to the elements that



encourage their establishment. I through C are our hypotheses. Madagascariensis has great germination rates and can withstand a wide range of temperatures; II. Because it mostly invades damaged regions, light availability promotes germination; III. Because increased water availability conditions concentrate the invaded areas, reduced water availability has a detrimental impact on germination and its capacity for invasion. Therefore, our goals were to assess the effects of a water deficit on the early growth of seedlings of the invasive *C. madagascariensis* and how abiotic variables interfere with germination.

## **II. MATERIALS AND METHOD:**

### **Physicochemical Investigation**

- Collection and Authentication
- Standardization Parameters.
- Extraction of leaves of *Cryptostegia madagascariensis* Bojer ex Decne

### **Physicochemical Investigation**

- Phytochemical Screening

### **Pharmacological Investigation**

- In-Vitro Study

### **Physicochemical Investigation**

#### **1. Collection and Authentication of *Cryptostegia madagascariensis* Bojer ex Decne. Plant (Leaves)**

The plant, *Cryptostegia madagascariensis* Bojer ex Decne. Was collected in the drier area in Akluj. Identified at Department of Botany, Shankarrao Mohite Mahavidyalaya, Akluj. A voucher specimen has been maintained in the museum of our department for further reference.

#### **1. Collection of plant materials**

Fresh and healthy leaves of, *Cryptostegia madagascariensis* Bojer ex Decne. Were collected from local areas of Akluj during the morning hrs to avoid moisture loss due to high temperature.

The collected plant materials were carefully inspected to remove:

- Diseased leaves
- Damaged leaves
- Dust and solid particles
- Foreign plant materials

The plant was authenticated by an expert from the department of pharmacognosy/ Botany to confirm its botanical identity.

- Purpose

Authentication ensures that the correct medicinal plant is used for extraction and analysis.

#### **2. Cleaning and Drying of plant materials**

##### **Washing**

The collected leaves were washed under running tap water to remove dirt and debris.

After this, the leaves were rinsed 2-3 times with distilled water to remove any remaining contamination.

##### **Drying**

The washed leaves were spread on clean blotting paper and dried under shade at room temperature (25-30°C) for 7-10 days.

The leaves were turned daily for uniform drying.

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Direct sunlight was avoided because it can:  
Destory heat-sensitive phytochemicals  
Reduce phenolic content  
After medicinal activity

- **Purpose:**

Shade drying preserves active constituents.

### 3. Pulverization (Powder Preparation)

After complete drying, the leaves became brittle. The dried leaves were crushed using:

- Mechanical grinder

#### **Purpose:**

Fine powder increases surface area, allowing better solvent penetration during extraction.

### 2. Standardization of *Cryptostegia madagascariensis* Bojer ex Decne.

Botanical evaluation of *Cryptostegia madagascariensis* Bojer ex Decne.



Fig1. *Cryptostegia madagascariensis*

**Synonym:** Purple rubber vine, Madagascar rubber vine, Rubber vine .

**Family :** Apocynaceae.

#### **Description**

*Cryptostegia madagascariensis*, commonly known as Madagascar rubber vine or purple rubber vine, is a fast-growing, woody perennial climber belonging to the Apocynaceae family. Native to Madagascar, it is widely distributed in tropical and subtropical regions. The plant may grow as a shrub about 2–4 meters tall or climb up to 10–15 meters when support is available, often forming dense thickets. Its stems are woody, grey to brown, and contain a toxic milky latex. The leaves are arranged oppositely, are ovate to elliptic in shape, and have a thick, leathery, glossy dark green surface. The plant produces large, attractive, funnel-shaped flowers that are pink to purple in color and usually appear in clusters. The fruits are paired pod-like structures (follicles) that split open when mature to release numerous seeds, each having silky hairs that aid in wind dispersal. This plant thrives in tropical climates, commonly found along roadsides, riverbanks, and open lands, but it is also considered highly invasive due to its rapid growth and ability to spread easily.

#### **Chemical constituents:**

- Cardiac glycosides (cardenolides) – major toxic principles responsible for cardiotoxic activity
- Flavonoids – antioxidant compounds

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- Phenolic compounds (polyphenols) – contribute to antioxidant and pharmacological effects
- Steroids – present as secondary metabolites Triterpenoids – important for anti-inflammatory activity
- Proteins and primary metabolites
- Latex constituents (cis-polyisoprene type hydrocarbons) – rubber-like compounds
- Fatty acids (in related species studies) such as oleic, linoleic, and linolenic acids

#### Uses

##### 1. Medicinal Uses

Antidiabetic activity-extract are studied for reducing blood glucose level.

Antiovidant- due to flavonoids and phenolic compounds.

Anti-inflammatory- helps reduce inflammation in experimental studies.

Antimicrobial-shows activity against some bacteria and fungi.

Cardiotonic (traditional use) – due to cardiac glycosides (use with caution due to toxicity)

##### 2. Industrial Uses

Natural rubber source –latex contains rubber-like compounds(cis-polyisoprene)

Fiber production- stem fibers can be used for making ropes and cords

##### 3. Traditional / Folk Uses

Latex sometimes applied externally for skin diseases

Used in small quantities in traditional remedies (not widely recommended due to toxicity)

##### 4. Ornamental Use

Grown as an ornamental climber because of its attractive purple flowers. Traditional / Folk Uses

### III. METHODOLOGY

#### 3. Extraction of leaves of *Cryptostegia madagascariensis* Bojer ex Decne .

Fresh leaves of *Cryptostegia madagascariensis* were collected and authenticated. The collected leaves were washed thoroughly with distilled water to remove dust and impurities and shade-dried at room temperature for 10–15 days. After complete drying, the leaves were coarsely powdered using a mechanical grinder and stored in an airtight container.

#### Soxhlet extraction method:

About 100 g of powdered leaf material was packed in a thimble and placed in a Soxhlet apparatus. Extraction was carried out using 500–700 mL of ethanol (70% or 95%) as the solvent. The extraction process was continued for 6–8 hours or until the solvent in the siphon tube became colorless, indicating complete extraction.

The obtained extract was filtered and concentrated using a rotary evaporator or evaporated on a water bath at 40–45°C to remove the solvent. The concentrated extract was dried to obtain a semisolid mass and stored in an airtight container at 4°C for further phytochemical and antidiabetic studies.

Percentage yield formula:

$$\text{Percentage Yield} = \frac{\text{Weight of dried extract}}{\text{Weight of powdered drug}} \times 100$$





Fig. no.2 Soxhlet extraction apparatus

**Physicochemical Investigation**

1. Phytochemical Screening

**A. Alkaloid test:**

TEST	OBSERVATION
1. Mayer's Test: Take 2-3 ml of plant extract in a test tube. Add few drops of dil.HCL and warm gently. Add Mayer's reagent.	Pale yellow precipitate.
2. Dragendroff's Test: Take 2-3 ml of plant extract in test tube. Add few drops of dil.HCL and warm gently. Add Dragendroff's reagent.	Reddish-brown precipitate.
3. Wagner's Test: Take 2-3 ml of plant extract in a test tube . Add few drops of dil.HCL and warm gently. Add Wagner's reagent.	Reddish-brown precipitate.

Table no.1

**B. Flavonoids Test:**

1. Shinoda Test: Take 2-3 ml of plant extract in a test tube. Add small Zinc Dustm Add few drops of dil.HCL.	Reddish-brown precipitate.
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Table no. 2

**C. Saponin Test:**

1. Foam Test (Forth test): Take 1ml plant extract Add 5-10ml distilled water. Shake vigorously for 30 sec. Let it stand for 10-15 min.	Persistent Foam ( $\geq 1$ cm height)
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Table no. 3



**D. Tannin Test & Phenol Test:**

1.Ferric Chloride Test: Take 2ml of plant extract. Add 2-3 drops of $FeCl_3$ solution.	Black colour observed.
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Table no. 4

**E. Terpenoids & Steroids Test:**

1.Salkowski's Test: Take 2ml plant extract. Add 2ml choloform. Carefully add 2-3 ml conc. $H_2SO_4$ . Along the side of the test tube.	Brown ring at junction.
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Table no. 5

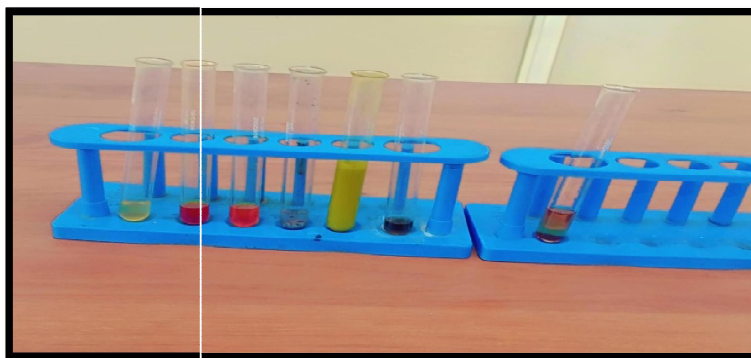


Fig.no.3 Preliminary Phytochemical screening Tests

**2. ASH VALUE:**

The ash value of *Cryptostegia madagascariensis* is determined by the incineration (heating) method used in pharmacognosy to measure the inorganic residue left after burning the plant material.

**Principle:**

When the powdered drug is heated at high temperature, all organic matter burns off and the remaining residue is called ash. It indicates the presence of:

- Natural mineral content
- Adhering dirt, sand or impurities
- Adulteration
- Heating / incineration method for Ash Value Determination

**Procedure:**

1. Weigh about 2-3 g of air-dried powdered *Cryptostegia madagascariensis*.
2. Place it in a previously ignited and weighed silica crucible.
3. Heat gently at first to avoid loss due to smoke. become
4. Then incinerate in a muffle furnace at about 450-600°C until the sample become white, indicating absence of carbon.
5. cool in a desiccator and weight the ash.



**Formula:**

$$\text{Ash Value (\%w/w)} = \frac{\text{Weight of Ash}}{\text{Weight of air dried crude drug}} \times 100$$

**3. LOSS OF DRYING**

**Principle:**

The powdered drug is heated at 105°C until constant weight is obtained. The weight loss indicates moisture and volatile components removed during drying.

**Procedure :**

1. Weigh about 2–5 g of powdered drug.
2. Place in a previously dried and weighed evaporating dish.
3. Dry in hot air oven at 105°C.
4. Cool in desiccator and weigh.
5. Repeat until constant weight is obtained.

Formula:

$$\text{Loss of Drying (\%w / w)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

**IV. PHARMACOLOGICAL ACTIVITY:**

**1. ANTI-DIABETIC ASSAY BY TITRATION METHOD:**

**Principle-**

Diabetic reaction leaves free reducing sugars in solution. These are estimated by titration. The lower the titration volume, the higher the anti-Diabetic activity.

Key Idea-Instead of control flask:

Use 0 µg/mL (blank mixture) as reference titre value

Then compare all test samples against it.

**Materials-**

Fructose (1% or 0.5%), Gelatin or egg albumin, Plant extract (20–100 µg/mL), Fehling's solution A & B, Methylene blue indicator, Distilled water.

**Procedure-**

1. Prepare Reaction Mixture

For each tube:

- 2 ml fructose solution
- 2 ml protine solution
- 1 ml extract (or water for blank)

**2. Set of Tubes**

You prepare :

TUBE	EXTRACT
Blank (V <sub>0</sub> )	0 µg / mL
T1	20 µg / mL
T2	40 µg / mL
T3	60 µg / mL



T4	80 µg / mL
T5	100 µg / mL

Table no.6

Incubation-

60°C water bath for 1 hour.

Titration:

For each Tube :

1. Take Fehling's A + B (5 mL each)
2. Boil gently
3. Titrate with incubated sample
4. Add methylene blue near endpoint
5. Record volume

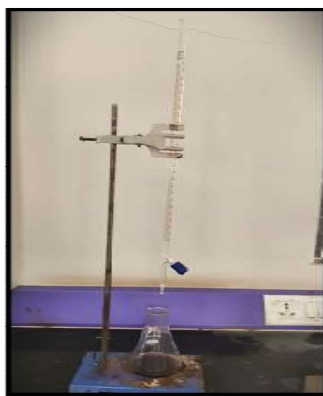


Fig no. 4 Anti-Diabetic Assay By Titration Method

#### V. RESULTS AND DISCUSSION :

Preliminary Phytochemical screening Tests:

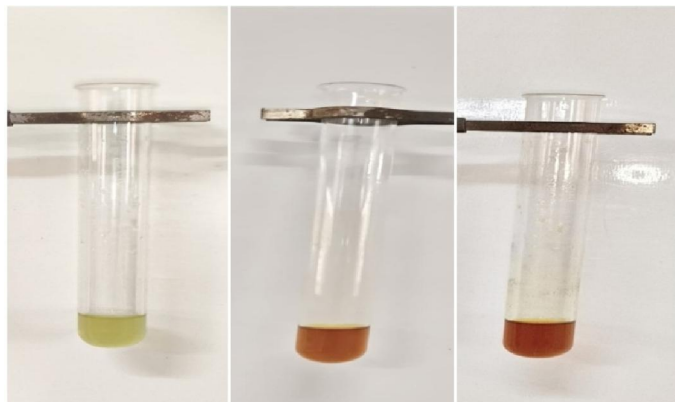
Alkaloid Test:

TEST	OBSERVATION	INFERENCE
1. Mayer's Test: Take 2-3 ml of plant extract in a test tube. Add few drops of dil. HCL and warm gently. Add Mayer's reagent.	Pale yellow precipitate.	Alkaloid is present.
2. Dragendroff's Test: Take 2-3 ml of plant extract in test tube. Add few drops of dil.HCL and warm gently. Add Dragendroff's reagent.	Reddish-brown precipitate.	Alkaloid is present.
3. Wagner's Test: Take 2-3 ml of plant extract in a test tube .	Reddish-brown precipitate.	Alkaloid is present.



Add few drops of dil.HCL and warm gently. Add Wagner's reagent.		
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**Table no.7 Result Alkaloid Test**



**Table no.5 Result Alkaloid Test**

**Flavonoids Test**

1. Shinoda Test: Take 2-3 ml of plant extract in a test tube. Add small Zinc Dust.Add few drops of dil.HCL.	Reddish-brown precipitate.	Flavonoid is present.
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**Table no. 8 Result Flavonoids Test**



**Fig no. 6 Result Flavonoids Test**

**Saponin Test**

1. Foam Test ( Forth test): Take 1ml plant extract Add 5-10ml distilled water. Shake vigorously for 30 sec. Let it stand for 10-15 min.	Persistent Foam ( $\geq 1$ cm height)	Saponin is present.
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**Table no.9 Result Saponin Test**



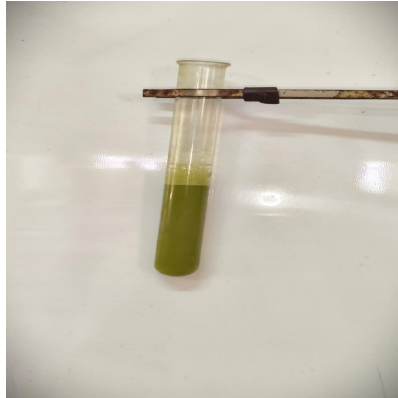


Fig no. 7 Result Saponin Test

**Tannin Test & Phenol Test:**

1. Ferric Chloride Test: Take 2ml of plant extract. Add 2-3 drops of $FeCl_3$ solution.	Black colour observed.	Tannin is present.
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Table no.10 Result Tannin Test

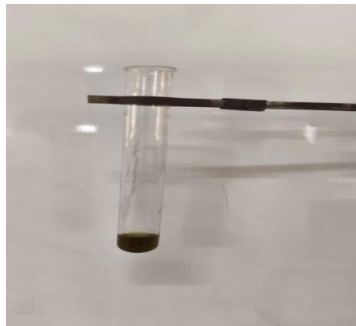


Fig no. 8 Result Tannin Test

**Terpenoids Test**

1. Salkowski's Test: Take 2ml plant extract. Add 2ml chloroform. Carefully add 2-3 ml conc. $H_2SO_4$ . Along the side of the test tube.	Brown ring at junction.	Terpenoid is present.
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Table no.11 Result Terpenoids Test

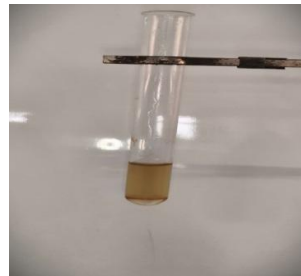


Fig no.9 Result Terpenoids Test



**Ash value:**

Ash Value (% w/w) = 5 % w/w

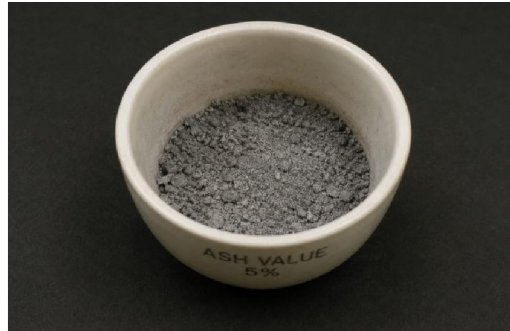


Fig no.10 Ash Value

**Loss of Drying**

Loss of Drying (% w/w) = 10% w/w



Fig no.11 Loss of Drying

**Anti-Diabetic Assay By Titration Method:**

Observation Table:

Concentration (µg/mL)	Initial reading	Final reading	Titre value	Percentage Inhibition
0 (Blank)	0.0	10.0	10.0	-
20	0.0	9.5	9.5	5%
40	0.0	8.7	8.7	13%
60	0.0	6.5	6.5	35%
80	0.0	6	6	40%
100	0.0	5	5	50%

Table no. 12 Observation Table of Anti-Diabetic Assay



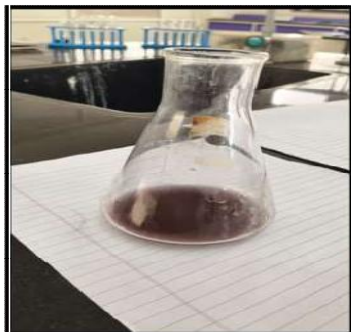


Fig no. 12 Result of Anti-Diabetic Assay By Tiration Method

## VI. CONCLUSION

The present study was conducted to investigate the phytochemical constituents, physicochemical parameters, and antidiabetic potential of *Cryptostegia madagascariensis* leaf extract. The plant leaves were collected, authenticated, shade dried, powdered, and extracted by Soxhlet extraction using ethanol as the solvent. The extraction method successfully yielded a concentrated extract containing biologically active compounds.

Preliminary phytochemical screening revealed the presence of important secondary metabolites such as alkaloids, flavonoids, saponins, tannins, phenolic compounds, and terpenoids. These phytoconstituents are known for their therapeutic significance and may contribute to various pharmacological activities including antioxidant, anti-inflammatory, antimicrobial, and antidiabetic effects. The presence of flavonoids and phenolic compounds particularly indicates strong antioxidant potential.

The physicochemical evaluation of the crude drug showed an ash value of 5% w/w and loss on drying of 10% w/w. These values indicate acceptable purity, low inorganic contamination, and suitable moisture content, thereby supporting the quality and standardization of the plant material.

The in-vitro antidiabetic assay demonstrated that the ethanolic extract exhibited concentration-dependent inhibitory activity. The percentage inhibition increased progressively with increasing concentration of the extract, reaching maximum inhibition of 50% at 100 µg/mL. This suggests significant antidiabetic potential of the plant extract, which may be attributed to the synergistic action of phenolic and flavonoid compounds involved in glucose metabolism and antioxidant defense mechanisms.

Overall, the findings of the study support the medicinal importance of *Cryptostegia madagascariensis* as a promising natural source of bioactive compounds with antidiabetic activity. However, further detailed pharmacological, toxicological, and clinical investigations are required to isolate the active constituents, determine the exact mechanism of action, and establish its safety and therapeutic efficacy for future herbal drug development.

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