RESEARCH PAPER

Macroscopic Features, Phytochemical Analysis and, Standardization *Cryptostagia Grandiflora Roxb* Leaves

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ABSTRACT

Cryptostegia grandiflora Linn. Rubber vine is commonly referred to as Roxb. and serves as a medicinal herb, popular throughout tropical and subtropical countries, historically used in medicine. The existing work was conducted to discover phytochemical profile and standardization parameters of *Cryptostegia grandiflora* leaves. Using Soxhlet equipment, freshly picked leaves were dried in the shade, ground into a powder, and extracted with methanol. The resulting extract lacks tannins, alkaloids, carbohydrate content, including terpenoids, but it does include flavonoids, cardiac glycosides, and saponins, according to the initial phytochemical investigation. The extract was found to be soluble in ethanol, methanol and chloroform and insoluble in water and acetone, according to solubility studies. In order to assess the plant material's integrity along with pureness, standardized parameters such as extractive values, moisture level, cumulative ash, acid insoluble ash, as well as water-based ash were also applied. The macroscopic examination revealed that the leaves are thick, dark green, shiny and bitter. The results corroborate the medicinal value of *Cryptostegia grandiflora* and gives helpful information for extra pharmacological and herbal formulation research. The study confirms that the plant possesses bioactive constituents with pharmaceutical applications. These results may contribute to investigations on antimicrobial, antioxidant, and therapeutic activities, as well as the development of herbal medicines from *Cryptostegia grandiflora* leaves.

Keywords: *Cryptostegia grandiflora*, Phytochemical screening, Methanolic extract, Herbal standardization, Medicinal plant.

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INTRODUCTION

In the last 5000 years, people have used medicinal plants. Written by Emperor Shen-Nung approximately 3000 years ago, Pen-t'sao is the oldest known herbal. There are 365 medications in it, one for every day of the year. The Indians sorted and inspected the herbs with great care. Ten herbs in each of the fifty categories that Charaka created would be more than a regular physician could require. Known to most as "Vilaytivakundi," *Cryptostegia grandiflora Linn.* Roxb. (Apocynaceae) is prevalent throughout India's arid regions. To prepare various extracts for detailed investigation, the study employs sequential solvent extraction. Different sequential extracts and powders were subjected to fluorescence examination under both ulta violet and regular light, indicating their respective features. Glycosides, flavonoids, fixed oils & lipids, phenolic substances & proteins were determined by preliminary qualitative chemical tests conducted on a variety of extracts. The existence of proteins, tannins, flavonoids, glycosides, and cardiac glycosides was further confirmed.

Native to southwest Madagascar, *Cryptostegia grandiflora* is a woody-perennial vine also referred to as rubber vine. Thanks to its eye-catching blossoms and the commercial quality rubber it carries in its latex (thus the name), it has also been spread by humans to most other tropical and subtropical locations [1]. It resembles the native Madagascar purple rubber vine (C. madagascariensis) quite closely [2-3].

The woody vine *Cryptostegia grandiflora* is sturdy. The leaves are 6-10 centimetres long, arched towards base level as well as pointy towards the tips. They are oblong-ovate to elliptic-ovate in shape. Cymes are brief. The green, eight-millimetre-long sepals are green. The pale purple, 4-centimeter-long corolla is frequently broader. It has escaped cultivation and turned invasive in several nations, where it can choke out trees, lower biodiversity, and destroy habitat. Dense, impenetrable thickets that obstruct stock movement and water availability are one of its possible formations [4].

Two cardenolides, ole and rigenin, and gitoxigen were obtained from phytochemical analyses of flowers, in conjunction with the aglycones of two flavonoid glycosides, kaempferol and hyperoside, as well as astragalin as well [5].The portion of oil produces saturated as well as unsaturated fatty acid groups, for such acids like arachidic:22.28%, linoleic:24.76%: myristic:5.24%, and stearic:3.8%. Lauric acid is present in amounts as well. (Refer to the study below) [6]. Proteins, tannins, flavonoids, cardiac and saponin glycosides, and flavonoids were extracted from leaves using methanol [7].

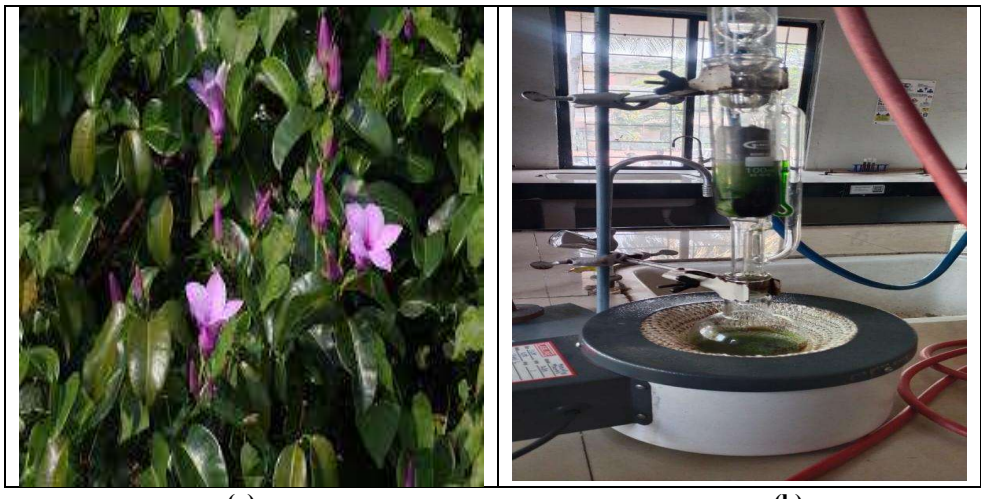
The objective of the research is to examine and assess the phytochemical profiles and medicinal potential of *Cryptostegia grandiflora Linn. Roxb*., a plant used traditionally. The research seeks to systematically identify the plant's biologically active components leaves and to provide standardization parameters.

The objectives include: Collecting and authenticating *Cryptostegia grandiflora* leaves. Preparing various extracts using sequential solvent extraction. Perform qualitative phytochemical screening for the presence of flavonoids, glycosides, saponins and other chemicals. Determination of solubility of extracts in solvents such as water, ethanol and methanol. Setting up standardization parameters (extractive values, moisture content, etc.) to guarantee the quality and uniformity of the plant material.

MATERIALS AND METHODS

Plant Collecting and Confirmation

An entire *Cryptostegia grandiflora* plant (Figure 1 a) was assembled in Kolhapur area and confirmed as authentic at Y.C. Warana Mahavidyalaya, Warananagar. Leaves were carefully washed, powdered, sun-dried, and stored within airtight vessels over further utilization in research.



(a) **(b)**

Figure 1: a)***Cryptostegia grandiflora*** **Plant**

b) **Soxhlet extracting of leaf from *Cryptostegia grandiflora* Linn Roxb**

Extraction of the Plant Material
Methanolic extraction of leaf powder was made with the help of Soxhlet apparatus (Figure 1 b). The extract was concentrated, dried into viscous extract and stored at 4°C for analysis[8].

Chemicals
The reagents as well as chemicals utilized in this investigation are purchased from Research Lab Finechem, Mumbai: methanol, ethanol, orange oil, trichloroacetic acid and glacial acetic acid. Granulated sugar, n-Butanol, nitric acid, acetic acid, purified water and citric acid were purchased from Loba Chemie Pvt. Ltd. and Beetroot powder was purchased from Sakshi Herbals, Amritsar. Both of the compounds were utilized regardless of purification along with all of them were of analytical standard.

Instruments Used
The analytical weighing balance (Wensar) was used for accurate weighing of the samples and a cooling incubator (Remi) was used to maintain the controlled temperature condition, electric water bath (Technico) was used for heating and a hot air oven (Narang Scientific Works) was used for drying and sterilization. The pH determine (Eutech) and a muffle furnace (Labline) were used for various high-temperature studies and pH values of different liquids. Samples were continuously extracted by Soxhlet continuous extraction apparatus (Labline).

EVALUATION
Macroscopic Features of *Cryptostegia grandiflora Linn. Roxb.* Leaves
Fresh, mature *Cryptostegia grandiflora Linn* leaf. Roxb. have been collected, the attached dust removed and observed by naked eye under daylight. The organoleptic and morphological characteristic of the leaves (shade, colour, scent, flavour, dimension, structure and surface) were observed and systematically recorded.

Solubility of extracts in different solvents
Solubility of extracts studied in water, ethanol, methanol, chloroform and acetone shown in Figure 2. [9].

Figure 2: Solubility of extract in different solvents

Qualitative Phytochemical Analysis of Metabolic Corticosterea Glycoside (MCG)
Following qualitative phytochemical testing, a tiny amount of the extracts was diluted in five milliliters of distilled water, filtrated, as well as the pure filtrate were utilized and presented in Table 1 as below.

Table 1: Qualitative Phytochemical Analysis of MCG

Test	Description	Inference
Tests for Carbohydrates		
Molisch's	Add several drops of Molisch's reagent +concentrated H_2SO_4 around the test tube's edges to two millilitres of extracts.	Carbohydrates are shown by the development of violet rings near the interaction point.
Fehling's	Fehling's solution with equivalent quantities A& B +extract, then heated.	A brick-red precipitate confirms the existence of reducing glucose.
Benedict's	Benedict's reagent +extract, + then a bath of water was used to warm up the blend.	Reducing sugars occur when red-brown precipitate appears.
Tests for Alkaloids		
Dragendorff's	The extract +Dragendorff's reagent.	Alkaloids are indicated by the development of an orange-red-colored precipitate.
Mayer's	A few drops of Mayer's reagent + extract.	The alkaloids are confirmed by a creamy precipitate.
Wagner's	Wagner's reagent + extract.	Typically, alkaloids can be recognized by the formation of a reddish-brown

		precipitate.
Hager's	The extract + Hager's reagent (picric acid).	Formation of a yellow precipitate confirms alkaloids.
Tests for Terpenoids		
Liebermann–Burchard	The extract + acetic anhydride, +concentrated H_2SO_4	The development of green or red coloration indicates terpenoids.
Salkowski	The extract + chloroform, +concentrated H_2SO_4 was added carefully.	A yellow coloration in the lower layer confirms terpenoids.
Tests for Glycosides		
Keller–Killiani	The extract + glacial acetic acid +ferric chloride, + sulfuric acid.	Formation of a reddish-brown ring indicates cardiac glycosides.
Baljet	Baljet reagent + extract.	A yellow to orange colouration confirms the presence of glycosides.
Tests for Tannins		
Lead Acetate	The lead acetate solutions in a couple of drops+ extract.	Development comprising a yellow or red precipitate indicates tannins.
Ferric Chloride	Ferric chloride solution + extract.	The appearance of a blackish-blue colour confirms tannins.
Tests for Saponins		
Foam	The extract was shaken vigorously with distilled water and allowed to stand.	Formation of stable foam (about 1 cm after 10 minutes) confirms saponins
Liebermann–Burchard	The extract + acetic anhydride + concentrated sulfuric acid.	The development of a red coloration indicates saponins.
Tests for Flavonoids		
Alkaline Reagent	Sodium hydroxide + extract, producing a yellow color which disappeared upon addition of hydrochloric acid.	This confirms flavonoids.
Shinoda's	Magnesium turnings + concentrated HCl + extract.	Formation of crimson or green-blue color indicates flavonoids
Lead Acetate	Lead acetate solution + extract.	Appearance of a yellow precipitate confirms flavonoids.
Tests for Proteins		
Biuret	The extract + sodium hydroxide + a few drops of $CuSO_4$ solution.	Formation of a violet colour indicates proteins.
Xanthoproteic	Concentrated HNO_3 + extract followed by NaOH.	The development of a yellow colour confirms proteins.
Millon's	Millon's reagent + extract was gently heated.	Formation of a white precipitate turning red indicates proteins [10-12].

***Cryptostegia grandiflora Linn.* leaf standardization.**

Roxb: Physicochemical Characteristics

Values Extracted (% w/w)

The air-dried powdered leaves were macerated separately with water and methanol for a specified period with occasional shaking. The filtrates were evaporated to dryness, and the extractive values were calculated as % w/w.

Water-Soluble Extractive

A known quantity of leaf powder was macerated filtrated and thoroughly evaporated using distilled water. The water-based extract indices were calculated by weighing the residue[13].

Methanol-Soluble Extractive

After filtering and macerating the powdered component using methanol, the resulting solvent had been evaporated until the mixture was completely dry. The methanol-solving extraction value was determined using the weight for the dry residue.

Moisture Content (% w/w)

A weighed quantity of powdered leaves dried out at 105 °C using a hot air oven till a consistent weight had been reached. Weight lessening was computed based on the moisture content[12].

Ash Value (% w/w)

A known weight of powdered drug was incinerated in a muffle furnace at about 450–600 °C eventually free of carbon ashes had been produced. The quantity of ash was chosen to calculate ash values.

Total Ash

The sample was completely incinerated to remove organic matter. The residue obtained represented the total ash content.

Acid-Insoluble Ash

After filtering and boiling total ash with diluted HCl, the remainder was burned. The remaining ash was weighed as acid-insoluble ash.

Water-Soluble Ash

After filtering along with boiling total ash within distilled water, the non-soluble material was burned. Overall water-based ash value was determined by calculating the weight differential[14-15].

RESULTS AND DISCUSSION

Macroscopic Features of *Cryptostegua grandiflora Linn Roxb* Leaves: Fresh, mature leaves of *Cryptostegia grandiflora Linn. Roxb*. were cleaned and examined visually under daylight to assess macroscopic characters. The leaves are dark green above and lighter green below, odourless or faintly scented, and slightly bitter in taste. They are large (8–15 cm × 4–8 cm), simple, opposite, thick, and leathery with an entire margin and prominent midrib. The surface is smooth, glossy, glabrous, and waxy on both sides.

Solubility of Extracts

Solubility of extracts shown in different solvents.Summaried in Table 2. as below as.

Table 2: Solubility of extract in different solvents

Solubility Solvent	Solubility
Water	Insoluble
Ethanol	Soluble
Methanol	Soluble
Chloroform	Soluble
Acetone	Insoluble

Analysis of Phytochemicals & Analysis of Qualitative Chemistry

The procedure section demonstrates the phytochemical analysis of the CG-leaf extract. A qualitative chemical investigation of *Cryptostegia grandiflora* leaf extracts yielded the following results shown in Table 3. Have suggested the existence of the following compounds-substances include are: carbohydrates, flavonoids, and saponins.

Table 3: Qualitative analytical study of extract derived from *Cryptostegia grandiflora Linn Roxb*

Test Number	Phytochemical component	Inference
1	Flavonoids	Present
2	Tannins	Absent
3	Cardiac Glycoside	Present
4	Alkaloids	Absent
5	Saponin	Present
6	Carbohydrate	Absent
7	Terpenoids	Absent

***Cryptostegia grandiflora Linn.* leaf standards. Roxb.: Physical Characteristics**

Cryptostegia grandiflora Linn. leaf standards. Roxb.: Physical Characteristics summarized in Table 4.as below as

Table 4: *Cryptostegia grandiflora Linn Roxb* leaf standardizing

	Physical Contents	Result
1	Extracted Values (% w/w)	
	Water base extractive	14.4
	Methanol base extractive	10.4
2	Moisture content (% w/w)	10
3	Ash Values (% w/w)	
	Total Ash	4.75
	Acid insoluble based ash	1.5
	Water Soluble based Ash	3.5

CONCLUSION

The study on *Cryptostegia grandiflora Linn. Roxb*. concludes that the plant possesses significant bioactive compounds that could have medicinal value. Through sequential solvent extraction and qualitative phytochemical tests, the study identified the presence of flavonoids, glycosides, saponins, and cardiac glycosides. Promising therapeutic advantages have already been discovered for them such as antioxidant and cardioprotective activities. The study also found that the leaves of *Cryptostegia grandiflora* contain high protein content with no presence of carbohydrates and alkaloids. The extract was found to dissolve within methanol, ethanol, as well as chloroform but insoluble in water and acetone in the solubility profile of the

extract. Further, the plant material was standardized with the help of physical factors such as ash indices, extractive indices, as well as moisture contents. Overall, *Cryptostegia grandiflora* is a potential source of bioactive compounds for medicinal use and herbal products in the future. The pharmacological properties and safety of its uses should be explored further.

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