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Phytochemical profiling and HPTLC studies on leaf of *Chrozophora rottleri* (Geiseler) Spreng

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ABSTRACT

For centuries, plants have been integral to traditional medicine, food preservation, and disease prevention. Chrozophora rottleri (Geiseler) A. Juss. ex Spreng., an annual herb of the Euphorbiaceae family, is widely used across India, Myanmar, Thailand, the Andaman Islands, and Malesia in various ethnomedicinal applications. This study aims to provide a comprehensive phytochemical profile and HPTLC analysis of Chrozophora rottleri leaves to highlight its medicinal potential. The leaves were subjected to hot continuous extraction using a Soxhlet apparatus with solvents of increasing polarity to maximize the yield of bioactive compounds. Phytochemical screening identified the presence of alkaloids, flavonoids, tannins, saponins, glycosides, triterpenoids, phenols, steroids, coumarins, cardiac glycosides, and phytosterols in different extracts. Quantitative analysis revealed that alkaloids were most abundant in chloroform and ethanol extracts (500 mg/g), while flavonoids were prominent in ethyl acetate and ethanol extracts (200 mg/g). Phenols and tannins were concentrated in ethyl acetate and aqueous extracts (200 mg/g). The HPTLC analysis displayed distinct separation of phytochemical bands at varying retention factor (Rf) values, using toluene: ethyl acetate (9:1 v/v) at 254 nm and a combination of ethyl acetate: water: formic acid: glacial acetic acid (100:26:11:11 v/v/v/v) at 366 nm and 540 nm. Chromatograms revealed multiple peaks, signifying a rich diversity of bioactive compounds. These findings support the traditional uses of Chrozophora rottleri and underscore its potential for further pharmacological research. The research concluded that the leaf extract of Chrozophora rottleri is abundant in various phytochemicals, potentially contributing to its therapeutic properties. Further investigation into its individual phytochemical profile is recommended for isolating novel compounds. High-performance thin-layer chromatography (HPTLC) fingerprint analysis of Chrozophora rottleri leaf extract could serve as a reliable method for precise plant identification, acting as both a phytochemical marker and a valuable indicator of genetic variability within plant populations.

Keywords: *Phytochemicals, HPTLC, Chrozophora rottleri, Euphorbiaceae and Leaf*

Introduction

Plants have played a pivotal role in human health, nutrition, and disease management for millennia. Their medicinal properties, attributed to bioactive compounds such as alkaloids, flavonoids, tannins, and saponins, have been used in traditional systems of medicine for treating a wide range of ailments [1-3]. *Chrozophora rottlerin* (Geiseler) A. Juss. Ex Spreng., a herbaceous annual plant belonging to the Euphorbiaceae family, is one such species with promising medicinal potential. It is widely distributed across India, Myanmar, Thailand, the Andaman Islands, and parts of Malesia. Traditionally, it has been employed in treating conditions such as fever, skin disorders, digestive issues, and inflammation in various regions of Asia [5-7]. Despite its extensive use in traditional medicine, limited scientific data is available on the phytochemical composition and medicinal potential of this plant, particularly its leaves [8-9]. Phytochemical profiling provides a comprehensive understanding of the chemical constituents of plants and is essential for correlating traditional uses with pharmacological properties. The bioactive compounds in plants such as *Chrozophora rottleri* are responsible for their therapeutic effects [10-12]. Identifying and quantifying these compounds can lead to a deeper understanding of the plant's potential applications in modern medicine. Previous studies on other species of the Euphorbiaceae family have reported diverse phytochemical profiles, including alkaloids, flavonoids, phenols, and terpenoids, all of which exhibit significant biological activity such as antioxidant, anti-inflammatory, and antimicrobial effects [13-16]. Hence, there is a pressing need to explore the chemical composition of *Chrozophora rottleri* particularly its leaves, to substantiate its medicinal applications. High-Performance Thin-Layer Chromatography (HPTLC) is a powerful analytical technique that is widely used for the identification and quantification of phytochemicals in medicinal plants [17-18]. This method allows for the simultaneous separation and detection of multiple compounds in complex mixtures, making it ideal for studying the phytochemical diversity in plant extracts. The technique offers advantages such as simplicity, accuracy, and the ability to handle large sample volumes with minimal preparation. HPTLC has proven effective in the analysis of bioactive compounds in various medicinal plants, and its application in the study of *Chrozophora rottleri* can provide valuable insights into its chemical makeup [19-25]. The present study aims to conduct a detailed phytochemical profiling and HPTLC analysis of the leaf extracts of *Chrozophora rottleri*. This investigation seeks to identify the key bioactive compounds and establish the foundation for further pharmacological studies on this promising medicinal plant.

Material and Methods

Collection and Authentication of Plant Material

In the rainy season months of July and August 2023, healthy, fresh plant materials were collected for research purposes, from many different of locations in the Udimilla Village, Amrabad Tiger Reserve (Nallamala Hills), Nagar Kurnool District, Telangana State., India, at latitude 16.3889° N and longitude

79.0214° E. The Nallamala region experiences a predominantly warm to hot climate year-round, with temperatures often rising during the summer months. The area receives an average annual rainfall of around 90 cm, primarily concentrated during the Southwest monsoon season from June to September. The Amrabad Tiger Reserve, nestled within the Nallamala forest range, boasts a rich biodiversity, supporting a variety of endemic plant and animal species. As the largest tiger habitat in Telangana, the reserve plays a crucial role in the conservation of this endangered species. Its rugged, hilly landscape, interspersed with deep valleys and gorges, forms a vital catchment area for the Krishna River, contributing to the region's ecological and hydrological balance. The herbarium specimen had been made and submitted to the Herbarium, Hyderabadensis Department of Botany, Osmania University, Hyderabad, Telangana, India. The plant species submitted for this study was carefully identified and authenticated by the Botanical Survey of India (BSI), Deccan Regional Centre, located in Hyderabad, Telangana. A comprehensive taxonomic examination was carried out to confirm its identity, and the species was assigned a unique herbarium voucher number (Voucher Specimen Number: BSI/DRC/2023-24/Identification/733). This authentication ensures the botanical accuracy of the species used in the study and provides a reliable reference for future research involving the same specimen.

Shade Drying of Plant Material

After being plucked, the leaves were washed and then finely chopped with sharp implements. The exact dimensions of these components were 0.5 x 1.5 x 1.2 x 3 cm. Over the course of the ten days that they were exposed to air, the blotting paper was stretched out in the shade. Before the anatomical investigations and extraction process could begin, the leaves were dried for one hour using hot air in an oven set at 40°C.

Successive Extraction Using Soxhlet Apparatus

The phytochemicals from the leaves of *C. rottleri* were extracted using a Soxhlet apparatus, which involves hot continuous repeated extractions. Twenty grammes of *C. rottleri* leaf powder, which had been finely crushed, was placed in the extraction chamber of the Soxhlet apparatus and sealed in porous bags or "thimbles" constructed of strong filter paper. After adding 200 ml of solvent to each round-bottom flask, the leaf powders were extracted using a series of solvents with increasing polarity: n-hexane at 70°C, chloroform at 61°C, ethyl acetate at 77°C, methanol at 65°C, and finally, aqueous at 100°C. In order to speed up the cycling of new solvents, the extraction temperatures were set to match the boiling points of the solvents. Every solvent was given six hours to extract, or until the solvent in the extractor's syphon tube lost its colour. After that, the extracts were chilled and filtered using Whatman No.1 filter paper. After that, they were tested for phytochemicals. A rotary evaporator, specifically a Heildolph 36000130 Hei-Vap Value Collegiate Rotary Evaporator with G5B Dry Ice Condenser, was used to concentrate the extracts. The next day, the crude remnants were stored in the refrigerator until they could be used again[43.44,45].

Calculation of Percentage Yield

The percentage yield of the plant extracts was calculated by weighing the dried extracts obtained from each solvent after the extraction process.

The yield was determined in relation to the air-dried weight of the initial plant material. The percentage yield was computed using the following formula:

$$\text{Percentage Yield} = \frac{\text{Weight of the crud(mg/g)}}{\text{Weight of the plant material}} \times 100$$

Screening of phytochemical analysis of *C. rottleri* (Qualitative methods)

The leaf extract was analyzed to determine if it contained any secondary metabolites. Standard procedures were used to carry out a range of tests to detect the presence or absence of different bioactive compounds such as alkaloids, flavonoids, saponins, steroids and terpenoids, phenolic compounds, tannins, glycosides, cardiac glycosides, coumarins, phytosterols, and quinones. The tests were conducted qualitatively to determine whether each compound was present or not [46,47,48,49,50,51,52,53,56,57].

Test for Alkaloids

To confirm the presence of alkaloids in the plant extract, the following procedure was followed:

1. Preparation of Extract

Fifty milligrams (50 mg) of the solvent-free extract was mixed with 5 ml of dilute hydrochloric acid. The mixture was stirred thoroughly and filtered. The clear filtrate was then subjected to various alkaloid tests using specific reagents.

2. Alkaloid Tests

a) Mayer's Test

To 3 ml of the filtrate, a few drops of Mayer's reagent (potassium mercuric iodide solution) were added along the side of the test tube. The appearance of a white or creamy precipitate indicated the presence of alkaloids.

b) Wagner's Test

A few drops of Wagner's reagent (iodine in potassium iodide solution) were added to a few milliliters of the filtrate. The formation of a reddish-brown precipitate confirmed the presence of alkaloids.

c) Hager's Test

To a few milliliters of the filtrate, 1-2 ml of Hager's reagent (saturated aqueous solution of picric acid) was added. The formation of a prominent yellow precipitate signified a positive result for alkaloids.

d) Dragendorff's Test

In this test, 1-2 ml of Dragendorff's reagent (potassium bismuth iodide solution) was added to the filtrate. A prominent yellow precipitate confirmed the presence of alkaloids in the extract.

Detection of Flavonoids

To confirm the presence of flavonoids, the following procedures were applied:

1. Initial Test for Flavonoids

One milliliter (1 ml) of the extract was mixed with 1 ml of chloroform, followed by the addition of 2-3 ml of acetic anhydride and 1-2 drops of concentrated sulfuric acid. The development of a dark green color indicates the presence of steroids, which can be differentiated later through specific flavonoid tests.

2. Specific Tests for Flavonoids

a) Alkaline Reagent Test

A few drops of sodium hydroxide solution were added to the extract. The formation of an intense yellow color, which becomes colorless upon the addition of a few drops of dilute acid, indicates the presence of flavonoids.

b) Lead Acetate Test

The extract was treated with a few drops of 10% lead acetate solution. The formation of a yellow precipitate indicates a positive result for flavonoids.

c) Shinoda's Test

To the test solution, a small amount of zinc dust and concentrated hydrochloric acid was added. After a few minutes, the solution developed a magenta or red color, confirming the presence of flavonoids.

Test for Saponins

To detect the presence of saponins, the following method was employed:

Approximately 0.5 g of the powdered plant material was gently boiled for 2 minutes with 20 ml of distilled water. The mixture was then filtered while still hot and allowed to cool. Five milliliters (5 ml) of the cooled filtrate was diluted with water and shaken vigorously. The formation of persistent froth or foam indicated the presence of saponins.

Detection of Steroids and Terpenoids

To detect the presence of steroids and terpenoids in the plant extract, the following test was performed:

a) Liebermann-Burchard's Test

The crude extract was treated with a few drops of acetic anhydride and heated gently before allowing it to cool. Concentrated sulfuric acid was then carefully added down the side of the test tube, forming two distinct layers. The appearance of a brown ring at the junction of the layers indicated a positive result for steroids. A green coloration in the upper layer confirmed the presence of steroids, while a deep red color in the lower layer suggested the presence of triterpenoids.

Detection of Phenols

a) Ferric Chloride Test

Fifty milligrams (50 mg) of the extract was dissolved in 5 ml of distilled water. To this solution, a few drops of a neutral 5% ferric chloride solution were added. The development of a dark green or blue color signified the presence of phenolic compounds.

b) Gelatin Test

The extract (50 mg) was dissolved in 5 ml of distilled water, and 2 ml of a 1% gelatin solution containing 10% sodium chloride was added. The formation of a white precipitate confirmed the presence of phenolic compounds in the extract.

c) Lead Acetate Test

Fifty milligrams (50 mg) of the extract was dissolved in distilled water, followed by the addition of 3 ml of a 10% lead acetate solution. The formation of a bulky white precipitate indicated the presence of phenolic compounds.

Detection of Tannins

The following tests were performed to detect the presence of tannins in the plant extract:

a) Ferric Chloride Test

The plant extract was treated with ferric chloride solution. The appearance of a blue or green coloration indicated the presence of tannins.

b) Potassium Ferricyanide Test

A small quantity of the test solution was mixed with 2% potassium ferricyanide solution and ammonia solution. The formation of a deep red color confirmed the presence of tannins.

c) Potassium Dichromate Test

To the test solution, a few drops of 2% potassium dichromate solution were added. A yellow precipitate indicated a positive test for tannins.

Detection of Glycosides

The test solution was prepared by dissolving the plant extract in alcohol or by boiling it in a hydro-alcoholic solution. The following tests were performed to confirm the presence of glycosides:

a) Baljet's Test

The test solution was treated with 2% sodium picrate. The appearance of a yellow to orange color confirmed the presence of glycosides.

b) Legal's Test

The test solution was treated with pyridine and then made alkaline. The addition of 2% sodium nitroprusside resulted in the formation of a pink to red color, indicating the presence of glycosides.

c) Keller-Killiani Test

Approximately 100 mg of the extract was dissolved in 1 ml of glacial acetic acid containing one drop of ferric chloride solution. This mixture was underlayered with 1 ml of concentrated sulfuric acid (H_2SO_4). The appearance of a brown ring at the interface confirmed the presence of glycosides. These tests provide a clear indication of the presence of tannins and glycosides through visual cues such as color changes and precipitate formation.

Detection of Cardiac Glycosides. The presence of cardiac glycosides was detected using the Keller-Killiani Test. In this qualitative analysis:

1. Two milliliters (2 mL) of the plant extract was mixed with 1 mL of acetic acid.
2. Two drops of ferric chloride solution were added to the mixture.
3. Afterward, 2 mL of concentrated sulfuric acid was added carefully.

The development of a reddish-brown color indicated a positive result for cardiac glycosides.

Test for Phytosterols

To detect the presence of phytosterols:
1. A few drops of concentrated sulfuric acid were added to the extract solution.

2. The mixture was shaken well and allowed to stand.
The appearance of a red color in the lower chloroform layer signified the presence of phytosterols.

Detection of Coumarins
The following procedure was used to detect coumarins in the extract:

1. One milliliter (1 mL) of the extract was placed in a test tube, and the opening was covered with filter paper moistened with dilute sodium hydroxide solution.
2. The test tube was then placed in a water bath for several minutes.
3. After removing the filter paper, it was exposed to ultraviolet (UV) light.
The appearance of green fluorescence on the filter paper indicated the presence of coumarins. These tests are commonly used methods to identify cardiac glycosides, phytosterols, and coumarins through observable color changes or fluorescence under UV light.

Detection of Anthraquinones
Borntrager 's test
The test solution was treated with 5ml of 10% sulphuric acid for 5min., filtered while hot, cooled, and the filtrate was shaken gently with an equal volume of benzene. The benzene layer was separated and treated with half of its volume with a solution of 10% ammonia allowed to separate it, the rose-pink color in the ammoniacal layer indicated the presence of anthraquinones.

Detection of Quinones
1 ml of extract was added to the 2 ml of dilute NaOH. The formation of blue-green or red coloration confirms the presence of quinones.

Detection of Resins
Take one gm of aqueous extract boiled the extract and then add a few drops of concentrated H_2SO_4 observed for reddish brown color indicates the presence of resins.

Test for Gums & Mucilage's
Dissolve 100mg extract in 10mL distilled water + 25mL absolute alcohol (constant stirring) , the appearance the white or cloudy precipitate showed presence of Gums & Mucilage's

Detection of Leuco-anthocyanins
2 ml of extract was treated with 2 ml of 2N hydrochloric acid and ammonia was added to it. The appearance of a pink-red color turning blue-violet indicates the presence of leuco-anthocyanins.

Detection of Anthocyanins :2mL plant extract + 2mL 2N HCl (+ Few mL ammonia)the pink-red sol. which turns blue-violet after addition of ammonia.

Detection of fixed oils: A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

Volatile Oils: 10 mL of extract, filtered till saturation, exposed to UV light Bright pinkish fluorescence appear presence of volatile oils.

Quantification of total content of alkaloids
1 mg of the plant extract was dissolved in dimethylsulphoxide and added 1ml of 2N HCl and filtered. This solution was transferred to a separating funnel, 5ml of bromocresol green solution then 5ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4ml of chloroform by vigorous shaking and collected in a 10ml volumetric flask and diluted to the volume with the chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100µg/ml) were prepared in the same manner as described already. The absorbance for standard solutions and test solutions were determined on the reagent blank at 470nm with an UV/Visible spectrophotometer. The content of alkaloids was expressed as mg of AE/g of plant extract [58].

Total content of flavonoids quantification
Colorimetric assay was used to determine the total content of flavonoid using aluminium chloride for the reaction, the plant extract of 1 ml and distilled water of 4 ml was taken in a 10 ml of flask. 0.30 ml of 5 % sodium nitrite and after 5minutes, 0.3ml of 10 % aluminium chloride was mixed in the flask. 5minutes later, 2 ml of 1M NaOH was treated and diluted using 10 ml distilled water. A set of standard solutions of quercetin (20, 40, 60, 80 and 100µg/ml) were prepared as mentioned earlier. The absorbance was measured for test and standard solutions using reagent blank at 510nm wavelength by UV-Visible spectrophotometer. The total content of flavonoid was denoted as mg of QE/g of extract [59].

Quantification of tannin total content
Folin-Ciocalteu method was used to quantify the tannin total content. About 0.1ml of plant extract was added in 10 ml of volumetric flask containing the distilled water of 7.5ml and Folin-Ciocalteu phenol reagent of 0.5ml, 35% Na2CO3 solution of 1 ml and diluted to 10ml using distilled water. The reagent mixture was well shaken and kept at 30°Ctemperature for 30min. A set of gallic acid solutions (20, 40, 60, 80 and 100µg/ml) were prepared as mentioned earlier. Absorbance of standard and test solutions was analyzed with blank at 725nm wavelength using UV-Visible spectrophotometer. The tannin total content of tannin was expressed as mg of GAE/g of extract [59].

Quantification of total content of phenolic compounds
The phenolic compounds concentration in extract was quantified by Spectrophotometry method. Folin-Ciocalteu method was employed for the quantification of total phenolic content. The reaction mixture contains 1 ml of plant extract and 9ml of distilled water. 1 ml of Folin-Ciocalteu phenol reagent was treated with the mixture and well shaken. After 5minutes, 10 ml of 7 % Na2CO3 solution was treated with the mixture. The volume was 25ml. A set of gallic acid standard solutions (20, 40, 40, 60, 80 and 100µg/ml) were prepared as earlier. Incubated for 90 min at 30°C and absorbance was analyzed for test and standard solutions with reagent blank at 550 nm with using UV-Visible spectrophotometer. The content of total phenolic compound was denoted as mg of GAE/gm of extract [59].

HPTLC Finger printing analysis of leaves of *Chrozophora rottleri*
Overview: Fingerprint analysis of, *Chrozophora rottleri* was performed using CAMAG HPTLC System in accordance with Anchrom's Inhouse method.

Sample Preparation:
Chrozophora rottleri (CR) powder was weighed to 100 mg and dissolved in 1 mL of methanol, sonicated for 10 minutes and centrifuged at 2500 RPM for 10 minutes and the supernatant was used for the analysis. Above prepared sample were diluted in the ratio of (1:1) and was used for the analysis. (50mg/mL of the sample was applied on the plate).

Diluent used: HPLC grade Methanol.

Chromatographic conditions
Stationary Phase Used: TLC Silica gel 60 F254 By Merck Product no. 1.05554.0007

- **Pre-conditioning (if any):** NA
- **Development Distance:** 70 mm
- **Mobile Phase Used:** Toluene: Ethyl acetate (9: 1 v/v) -254 nm

Ethyl Acetate: Water: Formic Acid: Glacial Acetic Acid
(100: 26: 11: 11 v/v/v/v) -366nm and 540 nm

- **Saturation Time:** 20 mins
- **Activation time (if any):** NA
- **Humidity maintained if any:** NA
- **Derivatization reagent:**

1. Natural Product Reagent A
Preparation: Dissolve 1 gm of 2-aminoethyl diphenylborinate in 200 mL of ethyl acetate
2.Anisaldehyde Sulphuric Acid reagent

Preparation: Place 170ml of methanol in 200ml glass bottle and cool it down in water-ice
cube bath. To the ice-cold methanol add slowly and carefully 1ml of anisaldehyde. 20ml of
acetic acid and 10ml of sulphuric acid and mix well. Allow the mixture to cool to room
temperature.

HPTLC Finger printing analysis of *Chrozophora rottleri (CR)*
The concentrated methanolic extract was spotted in the form of bands of length 5mm with 25µL syringe on pre-coated silica gel aluminum plate 60 F254, (5 x 10 cm with 0.25 mm thickness; Merck, Darmstadt, Germany) and the plates were washed with methanol before use. TLC development chamber was saturated with mobile phase using a filter paper. The sample and standard solutions were applied as bands of 6 mm wide and 10 mm apart using an Automatic TLC Sampler 4 applicator (CAMAG, Muttenz, Switzerland, supplied by Anchrom Technologists, Mumbai) fitted with a 25-µL Hamilton syringe supplied with nitrogen flow. A constant application rate of 15µL/sec was employed. The space between the bands was fixed as 20 mm/sec. The slit dimension was kept at 4 × 0.20 mm and a scanning speed was 20 mm/sec was employed. The mobile phase consisted of a saturated mixture of (254nm -Toluene: ethyl acetate -9: 1 v/v) and (366 and 540 nm- Ethyl Acetate: Water: Formic Acid: Glacial Acetic Acid -100: 26: 11: 11 v/v/v/v) were found to be a suitable mobile phase for separation of the phytoconstituents in the species studied, and chromatography was performed using 10 mL of mobile phase in a 10 × 10 cm twin-trough glass chamber (Camag, Muttenz, Switzerland) with linear ascending development (Sethi, 1996). The optimized chamber saturation time for the mobile phase was 20 min at room temperature with a chromatographic run length of 8.5 cm. After development, the TLC plates were dried in a current of air with the help of a hot air dryer in a wooden chamber with adequate ventilation., Densitometric scanning was performed with a (CAMAG TLC Scanner III) in the absorbance–reflectance mode at 366 nm and 540 nm with a slit dimension of 4 × 0.20 mm and a scanning speed 20 mm/sec was employed. All the instruments were operated by winCATS software (v. 1.4.3 CAMAG) resident in the system. The source of radiation utilized was a deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm, and the concentrations of the compounds chromatographed were determined from the intensity of diffusely reflected light. Further, for digital documentation, the Digi Store 2 documentation system (CAMAG) consisting of the illuminator, Reprostar 3, and digital camera power shot G2 (Canon, Tokyo, Japan) were used [60,61,62,63,64].

Observation and results
Morphological Description of *Chrozophora rottleri*
Chrozophora rottleri belongs to Euphorbiaceae family commonly known as Suryavarti. in Telugu "Erra miriyamu " or "Linga mirapa ". It is an erect herb with silvery hairs; lower part of stem is naked, upper part hairy and has slender tap root. It is an erect hairy annual, common waste lands, fulvous tomentose, monoecious herb, up to 75 cm tall with ascending branches trichotomously forked from base. Leaves alternate, broadly ovate, 2 6 ×1.5 7 cm, (sub) coriaceous, 3 nerved from
base; vein deeply impressed, petiole up to 8 cm, bi glandular at the base of lamina. Inflorescence terminal raceme, tomentose up to 3 cm; flowers (sub) sessile, crowded in upper axils, stamens yellow; flowers pedicellate below. Tepals 5 +5, inner petaloid. Stamens 12 15, in 2 series, connate, inner long. Ovary 3 locular; ovules 3, axile; styles 3, red, bifurcate. Disc glands 5. Capsule depressed, 3 lobed, stellate tomentose, 0.8 cm across; Fruit is a capsule & seeds globose[65], (Fig:1).

Flowering: February- August .

Table:1. Taxonomical features of Chrozophora rottleri (Geiseler) A.Juss. ex Spreng

Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Sub class	Magnoliidae
Order	Malpighiales
Family	Euphorbiaceae
Genus	*Chrozophora*
Species	*Chrozophora rottleri*

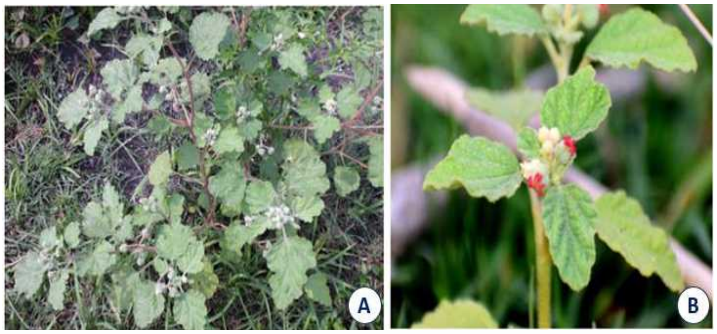


Fig.1A-F: C. rottleri habitat

Qualitative phytochemical analysis of *C.rottleri*

In recent years, there has been an increase in scholarly interest in the use of natural phytochemicals derived from herbal plants in a range of industries, such as food, medicine, and cosmetics.

The presence of secondary metabolites in the *C.rottleri* leaf extracts was assessed by phytochemical screening. A qualitative examination revealed that *C. rottleri* leaves contained an assortment of various phytochemicals. In order to compare the extraction solvents, the results of the analysis were displayed in Table 2 and Figures 2.

The alkaloids were detected in chloroform, ethanol, and aqueous extracts, while flavonoids were present in all extracts except petroleum ether. Saponins and anthocyanins were discovered just in ethanol and aqueous extracts. Steroids, terpenoids, phenols, tannins, coumarins, and leuco-anthocyanins were exclusively identified in ethyl acetate, ethanol, and aqueous extracts. Glycosides were solely found in chloroform and aqueous extracts, while cardiac glycosides and quinones were present in chloroform and ethanol extracts. Only the ethanol extract yielded positive results for anthraquinone. Resins were identified in petroleum ether, ethanol, and aqueous extracts, but were absent in chloroform and ethyl acetate extracts. Gums and mucilage's were exclusively found in the aqueous extract. Fixed oils and fats were identified in petroleum ether and chloroform extracts, while phytosterols were only detected in chloroform and ethyl acetate extracts. Finally, the volatile oils were exclusively identified in the petroleum ether extract.

There were more secondary metabolites with a high degree of precipitation (+++) in ethanolic leaf extracts than in any other solvent extract, but petroleum ether contained less phytoconstituents. The results differed based on the extraction procedure. Many phytochemicals that are physiologically active were found in the extraction using the method for extracts and screening tests. The yield percentages (%) of the crude extracts of petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts are 7.8%, 16.13%, 26.13%, 44.8%, and 40.15%, in that order.

Fig 2A-E: Phytochemical extractions of C. rottleri

Table 2: Phytochemical analysis of C. rottlerin

S. No	Phytochemicals	Pet. ether	Chloroform	Ethyl acetate	Ethanol	Aqueous
1.	Alkaloids	-	+++	-	+++	+++
2.	Flavonoids	-	++	++	++	++
3.	Saponins	-	-	-	++	+++
4.	Steroids &Terpenoids	-	-	+++	+++	+++
5.	Phenols	_	-	++	++	+++
6	Tannins	_	_	++	++	+++
7.	Glycosides	-	+++	-	-	+++
8.	Cardiac glycosides		+++	--	+++	--
9.	Coumarins	-	-	++	+++	+++
10.	Quinones	-	+++	-	+++	-
11.	Anthraquinones	-	-	-	+++	-
12.	Resins	+++	-	-	+++	+++
13.	Gums & Mucilage's	-	-	-	-	++
14.	Anthocyanins	-	-	-	+++	++
15.	Leuco-anthocyanins	-	-	+++	+++	+
16.	Fixed oils & fats	+++	+++	-	-	-
17.	Phytosterols	-	+++	+++	-	--
18.	Volatile Oils	+++	-	-	-	-

Quantitative phytochemical analysis of *C. rottleri*

The quantification of phytochemicals in *C. rottleri* across different solvent extracts provides a detailed insight into the distribution of bioactive compounds:

Alkaloids (Atropine equivalent, mg/g)

The alkaloid content was found to be the highest in the methanol extract at 502 mg/g, indicating that methanol is highly effective in extracting alkaloids from *C. rottleri*. The chloroform extract also showed a significant presence of alkaloids with a concentration of 310 mg/g. In contrast, no alkaloids were detected in the petroleum ether and ethyl acetate extracts, suggesting these solvents are less efficient for alkaloid extraction.

Flavonoids (Quercetin equivalent, mg/g)

Flavonoids were most abundant in the methanol extract, which had a concentration of 520 mg/g, followed by the ethyl acetate extract at 440 mg/g. The absence of flavonoids in both the petroleum ether and chloroform extracts highlights the specificity of solvent polarity in flavonoid extraction, with polar solvents like methanol and ethyl acetate proving more effective.

Phenols (Gallic acid equivalent, mg/g)

The methanol extract again exhibited the highest phenol content, with 390 mg/g, followed by the chloroform extract, which contained 250 mg/g of phenols. The petroleum ether and ethyl acetate extracts did not show any detectable levels of phenols, further emphasizing the selectivity of methanol and chloroform in extracting phenolic compounds.

Tannins (Gallic acid equivalent, mg/g)

Similar to the trends observed for other compounds, the methanol extract had the highest tannin content at 320 mg/g, followed by the chloroform extract at 180 mg/g. No tannins were found in the petroleum ether and ethyl acetate extracts, underscoring methanol's efficiency in extracting tannins, a class of polyphenolic compounds. Overall, methanol proved to be the most effective solvent for extracting all four classes of phytochemicals (alkaloids, flavonoids, phenols, and tannins), followed by chloroform for alkaloids, phenols, and tannins, and ethyl acetate specifically for flavonoids. The absence of these compounds in petroleum ether highlights its ineffectiveness as an extraction solvent for the phytochemicals studied. These findings provide a clear understanding of the solvent specificity in phytochemical extraction from *C. rottleri* (Fig 3 & table 3)

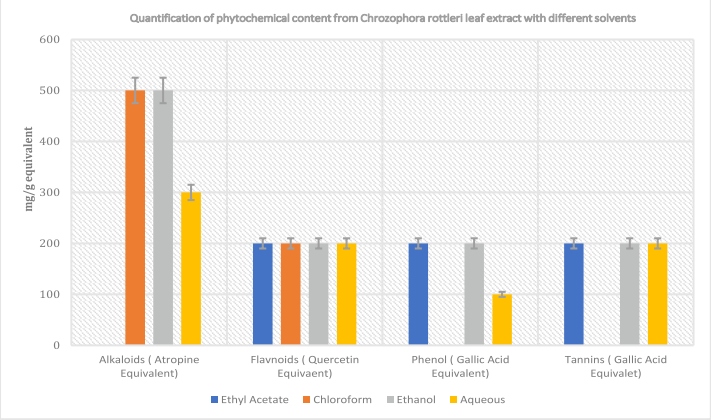


Fig 3. Graphical Representation on Quantification of Phytochemical analysis of C. rottleri

Table 3: Quantification of Phytochemical analysis of C. rottlerin

S.No	Alkaloids (Atropine equivalent) mg/g	Flavonoid (Quercetin equivalent) mg/g	Phenol (Gallic acid equivalent) mg/g	Tannin (Gallic acid equivalent) mg/g
Petroleum ether	0	0	0	0
Ethyl acetate	0	440	200	200
Chloroform	500	200	0	0
Ethanol	500	200	200	200
Aqueous	300	200	100	200

Profile of HPTLC finger printing of *Chrozophora rottleri (CR)* leaf

The chromatographic separation profile of leaf methanol extract of *C. rottleri* was analyzed at 254nm (Plate image after development) using track no 12 (10µL). The analysis revealed three spots with Rf values of 0.25 (17.12%), 0.37 (54.70%), and 0.83 (28.19%), accompanied by black colour spots (Fig 4&5). The plate image was processed under UV light at 366 nm. The analysis of track number 12 (10µL) shows two distinct spots (Fig 6&7), with spot 2 having the highest composition with Rf at 0.39 (22.85%) and Rf at 0.54 (77.15%), both highlighted in blue. Densitogram scanned at 366 nm using UV light with the derivatizing reagent natural product reagent A at track no 12 (10 µL) showed 3 spots with spot one (1) is showing the highest composition at Rf 0.35 (34.30%), the 2nd spot showing the highest composition at Rf 0.52 (41.95%), and the spot 3 is showing the highest composition at Rf 0.81 (23.75%) with orange, blue, and green colors respectively (Fig.8&9). Derivatized plate image under white light 540 nm using the derivatizing reagent Anisaldehyde Sulphuric Acid reagent. Track no 12 (10µL), revealed six spots, out of which spot 4 and 5 have the highest achievable percentage appear 0.44(50.45%) and 0.77(15.04%), with both the spots purple, and followed by the Rf values are 0.18(3.67% with blue colour), 0.28(12.10% with brown colour), 0.36(9.79% with yellow colour) and 0.85(8.96% with brown colour), (Fig10&11.), while at 366 nm derivatized plate, with Derivatizing reagent-Anisaldehyde Sulphuric Acid reagent, 4 spots appears at Rf, 0.36(44.48% with blue colour), 0.53(18.14% with blue colour), 0.63(6.25% with blue colour),, and 0.83(31.14% with blue colour), out of which spot one(1) is the highest concentration (Fig.12&13). For the purpose of verifying the dependability and uniformity of the health care sector, this fingerprint characteristic is necessary. These attributes enhance the identity and credibility of the plant medicine, as they are explicitly linked to the studied component based on the scientific evidence. Such products can be considered as valuable pharmacopeial standards.

Developed plate image under UV light (254 nm)

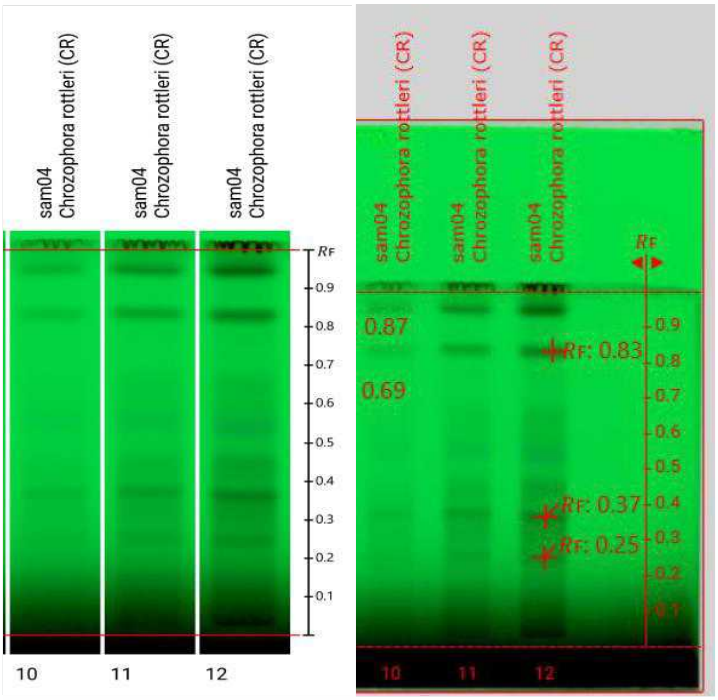
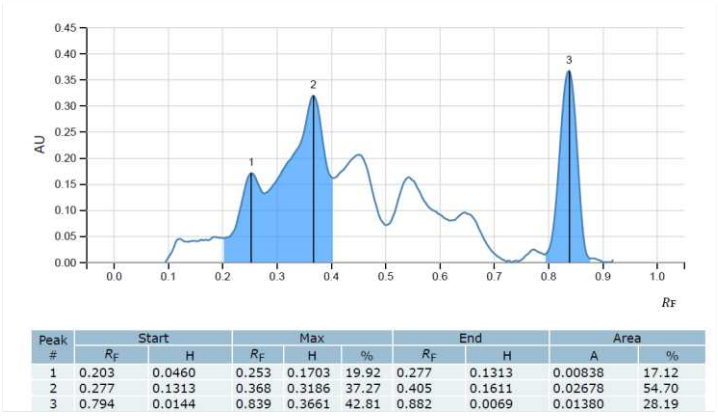


Fig:4. HPTLC chromatograms visualized of methanolic leaf extract of Chrozophora rottleri scanned at 254 nm by using Toluene: Ethyl acetate (9:1v/v).



Peak #	Start RF	Start H	Max RF	Max H	Max %	End RF	End H	Area A	Area %
1	0.203	0.0460	0.253	0.1703	19.92	0.277	0.1313	0.00838	17.12
2	0.277	0.1313	0.368	0.3186	37.27	0.405	0.1611	0.02678	54.70
3	0.794	0.0144	0.839	0.3661	42.81	0.882	0.0069	0.01380	28.19

Fig:5. HPTLC densitogram of methanolic extract of C. rottleri scanned at 254 nm by using Toluene: Ethyl acetate (9:1v/v).

Table 4 HPTLC of C. rottleri scanned at 254 nm with Rf values.

Sample	Track no.	Rf value
Chrozophora rottlerin (CR)	12	0.25 0.37 0.83

Developed plate image under UV light (366 nm)

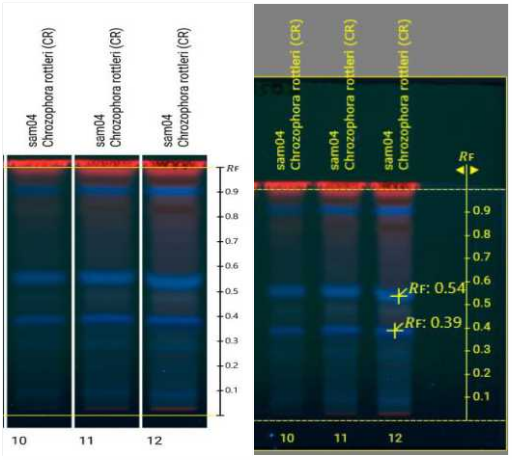
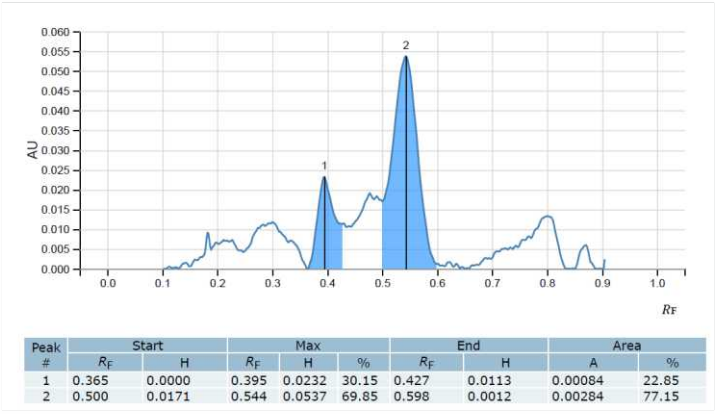


Fig: 6. HPTLC chromatograms visualized of methanolic leaf extract of Chrozophora rottleri scanned at 366 nm by using Ethyl Acetate: Water: Formic Acid: Glacial Acetic Acid -100: 26: 11: 11 v/v/v/v



Peak #	Start RF	Start H	Max RF	Max H	Max %	End RF	End H	Area A	Area %
1	0.365	0.0000	0.395	0.0232	30.15	0.427	0.0113	0.00084	22.85
2	0.500	0.0171	0.544	0.0537	69.85	0.598	0.0012	0.00284	77.15

Fig:7. HPTLC densitogram of methanolic leaf extract of Chrozophora rottleri scanned at 366 nm by using Ethyl Acetate: Water: Formic Acid: Glacial Acetic Acid -100: 26: 11: 11 v/v/v/v

Table 5: HPTLC of C. rottleri scanned at 366 nm with Rf values

Sample	Track no.	Rf value	Colour
Chrozophora rottlerin (CR)	12	0.39 0.54	Blue Blue

Derivatizing reagent: Natural Product Reagent A

Derivatized plate image under UV light (366 nm):

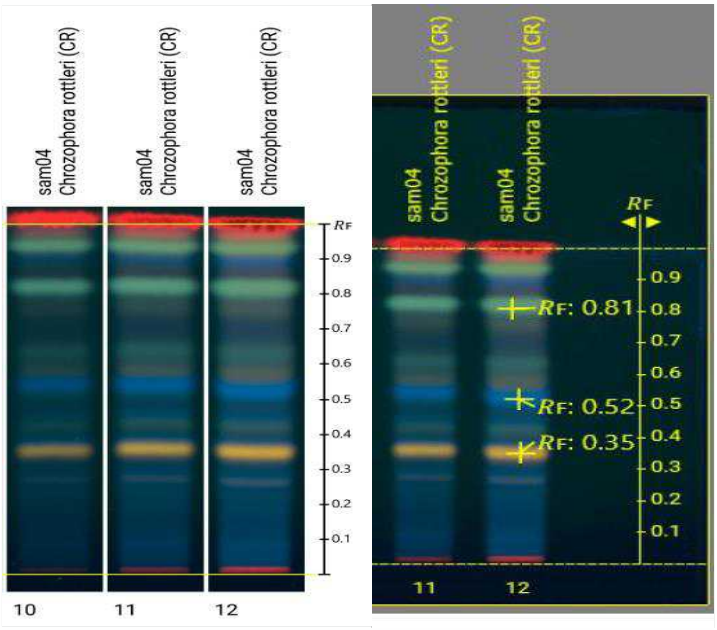
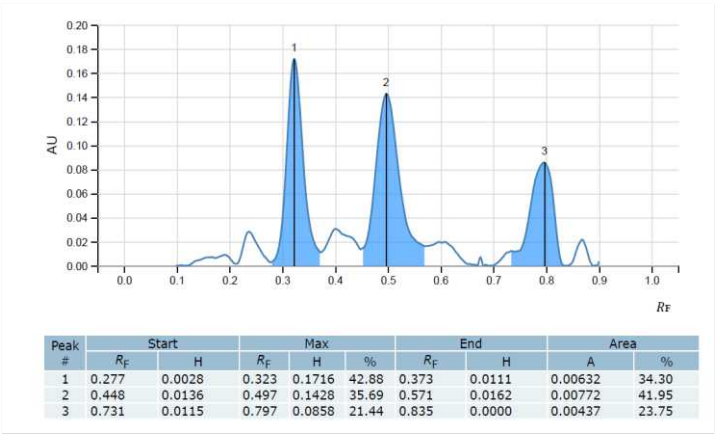


Fig: 8. HPTLC chromatograms visualized of methanolic leaf extract of C. rottleri scanned at 366 nm by using Ethyl Acetate: Water: Formic Acid: Glacial Acetic Acid -100: 26: 11: 11 v/v/v/v



Peak #	Start RF	Start H	Max RF	Max H	Max %	End RF	End H	Area A	Area %
1	0.277	0.0028	0.323	0.1716	42.88	0.373	0.0111	0.00632	34.30
2	0.448	0.0136	0.497	0.1428	35.69	0.571	0.0162	0.00772	41.95
3	0.731	0.0115	0.797	0.0858	21.44	0.835	0.0000	0.00437	23.75

Fig:9. HPTLC densitogram of methanolic leaf extract of C. rottleri scanned at 366 nm by using Ethyl Acetate: Water: Formic Acid: Glacial Acetic Acid -100: 26: 11: 11 v/v/v/v

Table 6 HPTLC of C. rottleri scanned at 366 nm with Rf values

Sample	Track no.	Rf value	Colour
Chrozophora rottlerin (CR)	12	0.35	Orange
		0.52	Blue
		0.81	Green

Derivatizing reagent: Anisaldehyde Sulphuric Acid reagent
Derivatized plate image under white light (540 nm):

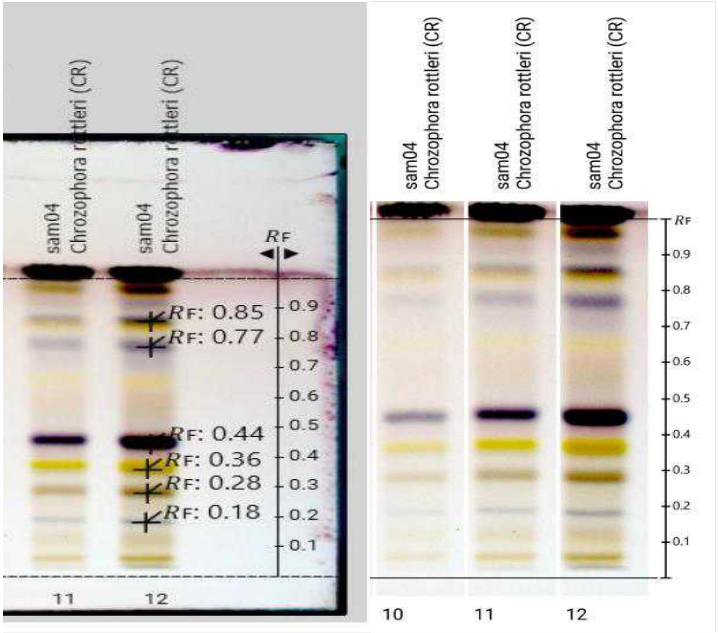
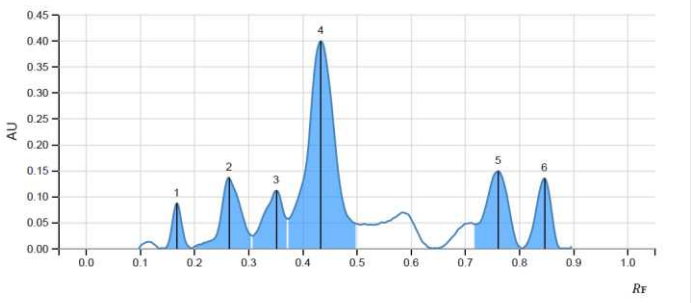


Fig: 10. HPTLC chromatograms visualized of methanolic leaf extract of C. rottleri scanned at 540nm by using Ethyl Acetate: Water: Formic Acid: Glacial Acetic Acid -100: 26: 11: 11 v/v/v/v



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.145	0.0000	0.168	0.0864	8.51	0.195	0.0000	0.00173	3.67
2	0.202	0.0039	0.265	0.1358	13.38	0.306	0.0243	0.00571	12.10
3	0.308	0.0242	0.352	0.1111	10.95	0.373	0.0570	0.00462	9.79
4	0.374	0.0569	0.434	0.3985	39.26	0.506	0.0454	0.02380	50.45
5	0.718	0.0463	0.761	0.1485	14.63	0.805	0.0001	0.00709	15.04
6	0.806	0.0000	0.847	0.1347	13.27	0.877	0.0000	0.00423	8.96

Fig:11. HPTLC densitogram of methanolic leaf extract of C. rottleri scanned at 540 nm by using Ethyl Acetate: Water: Formic Acid: Glacial Acetic Acid -100: 26: 11: 11 v/v/v/v

Table 7: HPTLC of C. rottleri scanned at 540 nm with Rf values.

Sample	Track no.	Rf value	Colour
Chrozophora rottlerin (CR)	12	0.18	Blue
		0.28	Brown
		0.36	Yellow
		0.44	Purple
		0.77	Purple
		0.85	Brown

Derivatizing reagent: Anisaldehyde Sulphuric Acid reagent
Derivatized plate image under UV light (366 nm):

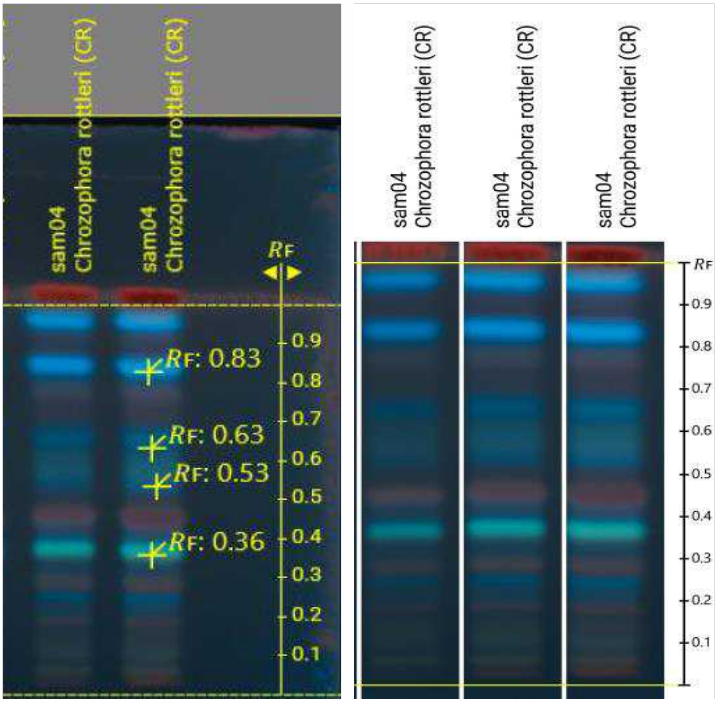
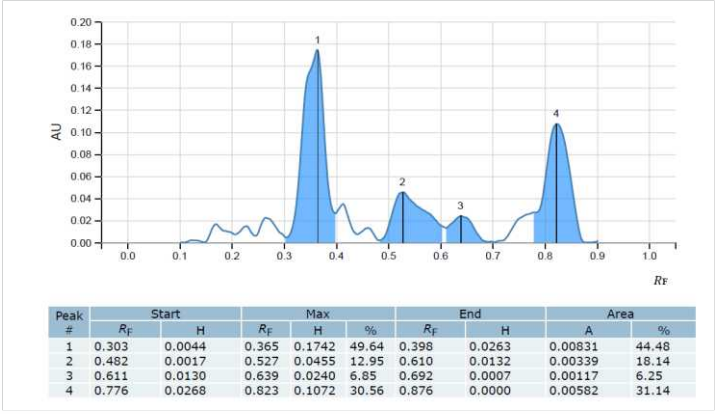


Fig: 12. HPTLC chromatograms visualized of methanolic leaf extract of C. rottleri scanned at 366 nm by using Ethyl Acetate: Water: Formic Acid: Glacial Acetic Acid -100: 26: 11: 11 v/v/v/v (Derivatizing reagent: Anisaldehyde Sulphuric Acid reagent)



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.303	0.0044	0.365	0.1742	49.64	0.398	0.0263	0.00831	44.48
2	0.482	0.0017	0.527	0.0455	12.95	0.610	0.0132	0.00339	18.14
3	0.611	0.0130	0.639	0.0240	6.85	0.692	0.0007	0.00117	6.25
4	0.776	0.0268	0.823	0.1072	30.56	0.876	0.0000	0.00582	31.14

Fig:13. HPTLC densitogram of methanolic leaf extract of C. rottleri scanned at 366 nm by using Ethyl Acetate: Water: Formic Acid: Glacial Acetic Acid -100: 26: 11: 11 v/v/v/v (Derivatizing reagent: Anisaldehyde Sulphuric Acid reagent).

Table 8: HPTLC of C. rottleri scanned at 366 nm with Rf values.

Sample	Track no.	Rf value	Colour
Chrozophora rottlerin (CR)	12	0.36	Blue
		0.53	Blue
		0.63	Blue
		0.83	Blue

Discussion

The *C. rottleri* healthy, fresh leaves were collected, from many different of locations in the Udimilla Village, Amrabad Tiger Reserve (Nallamala Hills), Nagar Kurnool District, Telangana State., India, at latitude 16.3889° N and longitude 79.0214° E. The leaves of *C. rottleri* are very much beneficial in treatment of skin diseases and are also used as depurative agent [66]. The major phytochemicals of *C. rottleri* include alkaloids, carbohydrate, glycosides, tannins, steroids, flavonoids and saponins, rutin, acacetin 7- orutinoside and apigenin 7-o-b-d-[6-(3,4- dihydroxybenzoyl)] –glucopyranoside [25-45-67]. Presently the qualitative phytochemicals reveled alkaloids, flavonoids, saponins, glycosides, phytosterols, anthocyanins, lecuco anthocyanins phenols, tannins steroids & terpenoids fixed oils and gums etc., The ethanolic leaf extracts had the highest concentration of secondary metabolites with a high precipitation degree (+++), whereas the petroleum ether extract had the lowest concentration of phytoconstituents. The crude extracts of petroleum ether, chloroform, ethyl acetate, ethanol, and aqueous extracts had yield percentages (%) of 7.8%, 16.13%, 26.13%, 44.8%, and 40.15%, respectively. The quantification of phytochemicals in Pluchea ovalis indicates that methanol is the most efficacious solvent, producing the largest amounts of alkaloids (502 mg/g), flavonoids (520 mg/g), phenols (390 mg/g), and tannins (320 mg/g). Chloroform extracted considerable quantities of alkaloids (310 mg/g), phenols (250 mg/g), and tannins (180 mg/g), but ethyl acetate was solely efficient for flavonoids (440 mg/g). Petroleum ether had no extraction efficacy for any of the substances. The results underscore the efficacy of methanol and chloroform in extracting bioactive components from *C. rottleri*.

The chromatographic separation of the methanol leaf extract of *C. rottleri* at 254 nm revealed three spots with Rf values of 0.25, 0.37, and 0.83, corresponding to 17.12%, 54.70%, and 28.19% composition, respectively. Under UV light at 366 nm, two spots were observed with Rf values of 0.39 (22.85%) and 0.54 (77.15%). A densitogram scanned at 366 nm showed three spots, with the highest compositions at Rf 0.35 (34.30%), 0.52 (41.95%), and 0.81 (23.75%). Using Anisaldehyde Sulphuric Acid reagent under white light at 540 nm, six spots appeared, with the most prominent at Rf 0.44 (50.45%) and 0.77 (15.04%). The fingerprint characteristics enhance the identification and credibility of the plant, supporting its potential as a pharmacopeial standard.

Conclusions

The phytochemical profile of *C. rottleri* extracts was generated during preliminary screening. These phytoconstituents may be associated with the plant's several established therapeutic applications. This may facilitate comprehensive investigation to identify and isolate compounds in *C. rottleri* that potentially possess medical value. We determine that HPTLC fingerprint analysis of *C. rottleri* leaf extract serves as a valuable phytochemical marker, an effective measure of genetic variability in plant populations, and a diagnostic instrument for precise plant identification.

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