RESEARCH ARTICLE

Physico-chemical Standardization and Phytochemical Screening of Potential Medicinal Herb: *Rubia cordifolia* (Roots)

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Abstract

Introduction: *Rubia cordifolia L.*, commonly known as Indian Madder, is a significant medicinal plant used in traditional Indian medicine to treat a variety of ailments.

Objective: This study aimed to analyze the biologically active compounds present in the root of *Rubia cordifolia* through ethanolic and aqueous extracts.

Material and Methods: Market-purchased and authenticated dried roots of *R. cordifolia* were used for carrying out physio-chemical (extractive value, moisture content, ash values, and phytochemical evaluations using standard methodologies.

Result and Discussion: Phytochemical screening revealed the presence of several beneficial compounds, including anthraquinones, glycosides, flavonoids, steroids, phenols, and saponins, while alkaloids, tannins, and quinones were absent in all solvent extracts. A thorough The results showed significant extractive values, indicating a rich presence of soluble compounds in the dried root powder.

Conclusion: The findings highlight the potential of *R. cordifolia* as a valuable source of medicinal compounds, supporting its use in traditional and modern therapeutic practices. The presence of these biologically active compounds confirms the plant's relevance in treating various health issues and suggests further exploration of its applications in contemporary medicine.

Keywords: *Rubia cordifolia*, Phytochemical screening, Traditional medicine, Anthraquinones.
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Introduction

Rubia cordifolia L., commonly known as Indian Madder or Manjistha, is a climbing herb belonging to the Rubiaceae family. It features a striking crimson rhizomatous base and roots, thriving in the hilly regions of India up to 3750 m. The mature roots are particularly prized in traditional herbal formulations, including Aswagandharistam, Gulguluthikthkarishtam, and Madhookasavam.[1]

This plant is recognized by various names across different languages: "manjit" in Hindi, "chit Ravalli" in Kannada, "ceevalli" in Tamil, "manchatti" and "manjatti" in Malayalam, and "manderti" in Telugu.[2] The roots and fruits are utilized internally for treating skin conditions and spleen-related issues, as noted in the ancient texts Charaka Samhita and Sushruta Samhita.[3]

Manjistha is known for its tonic and antitussive properties, providing relief for chronic low fevers. Root decoction is commonly prescribed for jaundice, urinary problems, and amenorrhea and is effective in regulating menstrual cycles. Additionally, the root extracts exhibit various medicinal effects, including astringent, thermogenic, febrifuge, antidysenteric, antihelmintic, galactopurifier, ophthalmic, and rejuvenating properties. It is also utilized in the treatment of tuberculosis and intestinal ulcers, underscoring its significance in traditional medicine.[4,5]

Materials and Methods

Collection of plant substances

Roots of *R. cordifolia Linn.* (2 kg) were collected from a local supplier in Delhi in October 2015.

Authentication of plant substances

The plant sample was authenticated at the Department of Botany, National Institute of Science Communication and Information Resources (NISCAIR), Delhi-110011, for identification and taxonomic verification.

Preparation of plant extract

The authenticated roots of *R. cordifolia* were subjected to shade drying. After drying, the roots were ground into a coarse powder and passed through an appropriate sieve to achieve a uniform particle size. The powdered root material was then extracted with ethanol and water using a soxhlet apparatus. The resulting extracts were filtered and collected,

then concentrated using a rotary flash evaporator. These extracts were prepared for further experimental analyses.[6]

Physicochemical evaluation

The crude dried plant material underwent a physicochemical evaluation, assessing various parameters such as extractive value, moisture content, and ash values, including acid-insoluble and water-soluble ash. The evaluations were conducted following standard procedures.[7-9] Key metrics like loss on drying (LOD), total ash, acid-insoluble ash, and water-soluble ash were calculated alongside water-soluble and alcohol-soluble extractives of the dried root powder, as summarized in Tables 1, 2 and 3. The values were determined using the appropriate formulas.

Procedure

$$\% \text{ Moisture content} = \frac{\text{Loss in weight of the sample}}{\text{Weight of sample}} \times 100$$

$$\% \text{ Total ash value} = \frac{\text{Weight of total ash}}{\text{Weight of crude drug taken}} \times 100$$

$$\% \text{ acid insoluble ash value} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of crude drug taken}} \times 100$$

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

Phytochemical Screening

The phytochemical examination of both ethanolic and aqueous extracts was conducted using standard methods.[10-15] The analysis revealed the presence of various phytochemical constituents, indicating the potential therapeutic properties of the extracts.

Preliminary Phytochemical Screening

The ethanolic and aqueous root extracts of *R. cordifolia* underwent systematic phytochemical screening to identify the presence of chemical constituents, specifically alkaloids. The results of phytochemical screening were tabulated in Table 4.

Detection of Alkaloids

The extracts were dissolved individually in dilute hydrochloric acid, shaken well, and filtered. The filtrates were then tested for alkaloids using the following methods:

Dragendorff's Test

The filtrate was treated with Dragendorff's reagent (potassium bismuth iodide). The formation of an orange-brown precipitate indicated the presence of alkaloids.

Mayer's Test

The filtrate was treated with Mayer's reagent (potassium mercuric iodide). A yellow cream precipitate suggested the presence of alkaloids.

Wagner's Test

The filtrate was treated with Wagner's reagent (iodine in potassium iodide). The formation of a brown or reddish-brown precipitate confirmed the presence of alkaloids.

Hager's Test

The filtrate was treated with Hager's reagent (saturated picric acid solution). The appearance of a yellow-colored precipitate indicated the presence of alkaloids.

The tests for detecting glycosides in *R. cordifolia* are well-structured. Here's a streamlined version that highlights the key points for clarity:

Detection of Glycosides in *R. cordifolia*

Hydrolysis

The extract is hydrolyzed with dilute hydrochloric acid to prepare for glycoside tests.

Modified Borntrager's Test

- *Procedure*

Treat the extract with ferric chloride solution, heat in a boiling water bath for 5 minutes, cool, and shake with an equal volume of benzene. Separate the benzene layer and add half its volume of ammonia solution.

- *Positive Result*

The formation of rose pink or cherry red color in the ammoniac layer indicates the presence of anthraquinone glycosides.

Baljet's Test

- *Procedure*

Treat the extract with sodium picrate.

- *Positive Result*

The formation of a yellowish-orange color confirms the presence of cardiac glycosides.

Legal's Test

- *Procedure*

Treat the extract with sodium nitroprusside in pyridine.

Table 1: Ash value of powdered roots of *R. cordifolia*

Name of the plant	*Ash values (% w/w)*		
	Total ash (% w/w)	*Acid insoluble ash*	*Water soluble ash*
Rubiacordifolia	6.52	2.1	1.6

Table 2: Extractive value of powdered roots of *R. cordifolia*

Name of the plant	*Extractive value (% w/w)*	
	Water soluble extract	*Alcohol soluble extract*
R. cordifolia	11.8	14.7

Table 3: Moisture content of powdered roots of *R. cordifolia*

Name of the plant	*Wt. of powder root (g)*	*% of moisture content*
R. cordifolia	380	0.39

- *Positive Result*

The formation of a pink-to-red color indicates the presence of cardiac glycosides.

Keller-Killiani Test

- *Procedure*

Treat the extract with glacial acetic acid, 5% ferric chloride, and concentrated sulfuric acid.

- *Positive Result*

The formation of a reddish-brown color at the junction of two liquid layers, with the upper layer appearing bluish-green, indicates the presence of cardiac glycosides.

Detection of Saponins in *R. cordifolia*

Foam Test (Froth's Test)

- *Procedure*

- Dilute the extract with 20 mL of distilled water in a graduated cylinder.
- Shake the mixture vigorously for about 15 minutes.

- *Positive Result*

- The formation of a stable layer of foam indicates the presence of saponins.
- This simple and effective test confirms the saponin content in the extract, which can contribute to various biological activities.

The results of phytochemical screening were tabulated in Table 4.

Detection of Phenolic Compounds and Tannins in *R. cordifolia*

Ferric Chloride Test

- *Procedure*

Add a few drops of neutral ferric chloride solution (5%) to the extract.

- *Positive Result*

The formation of a bluish-black color indicates the presence of phenolic compounds.

Gelatin Test

- *Procedure*

Add 1% gelatin solution containing sodium chloride to the extract.

- *Positive Result*

The formation of a white precipitate indicates the presence of tannins.

Lead Acetate Test

- *Procedure*

Treat the extract with a few drops of 10% lead acetate solution.

- *Positive Result*

The formation of a white precipitate confirms the presence of flavonoids.

Shinoda Test

- *Procedure*

Add a few fragments of magnesium metal to the extract, followed by a dropwise addition of concentrated hydrochloric acid.

- *Positive Result*

The formation of a magenta color indicates the presence of flavonoids.

These tests effectively identify phenolic compounds, tannins, and flavonoids in the extracts of *R. cordifolia*, which may contribute to its pharmacological properties.

Table 4: Phytochemical analysis of root extracts in *R. cordifolia* L.

S. No.	*Tests*	*Ethanolic extract*	*Aqueous extract*
1.	Test for alkaloids		
	Dragendorff's test	+	+
	Mayer's test	+	-
	Wagner's test	+	+
2.	Tannins and phenolic compounds test		
	Ferric chloride test	+	-
	Gelatin test	+	-
	Lead acetate test	+	+
	Acetic acid test	+	+
3.	Test for flavonoids		
	Shinoda test	+	+
4.	Test for glycosides		
	Keller-killianitest (Cardiac)	+	+
	Borntrager'stest (anthraquinone)	+	+
	Legal's test (cardiac)	+	+
	Foam test (saponin)	+	-
5.	Test for carbohydrates		
	Molish test	+	+
	Fehling's Test (Reducing sugars)	+	-
	Benedict Test (Reducing sugars)	-	+
6.	Test for proteins and amino acids		
	Biuret test	-	-
	Million's reagent test	-	-
7.	Test for steroids		
	Salkowski test	-	-
	Limberman-Buchard test	-	-

(+) – Signifies present, (-) – Signifies absent

Detection of Proteins and Amino Acids in *R. cordifolia*

Millon's Test:

- *Procedure*

Treat the extract with 2 mL of Millon's reagent.

- *Positive Result*

The formation of a white precipitate that turns red upon heating indicates the presence of proteins.

Biuret Test

- *Procedure*

Add 1-mL of 10% sodium hydroxide solution to the extract and heat the mixture. Then, add a drop of 0.7% copper sulfate solution.

- *Positive Result*

The formation of a purplish-violet color indicates the presence of proteins.

Ninhydrin Test

- *Procedure*

Add 0.25% ninhydrin reagent to the extract and boil for a few minutes.

- *Positive Result*

The formation of a blue color indicates the presence of amino acids.

These tests confirm the presence of proteins and amino acids in the extracts of *R. cordifolia*, which can be important for its biological activity.

Detection of Carbohydrates in *R. cordifolia*

Molisch's Test

- *Procedure*

Dissolve the extract in 5 mL of distilled water, filter, and treat the filtrate with 2 drops of alcoholic α-naphthol solution. Carefully add 2 mL of concentrated sulfuric acid along the sides of the test tube.

- *Positive Result*

The formation of a violet ring at the junction indicates the presence of carbohydrates.

Benedict's Test

- *Procedure*

Treat the filtrate with Benedict's reagent and heat in a water bath for 5 minutes.

- *Positive Result*

The formation of an orange-red precipitate indicates the presence of reducing sugars.

Fehling's Test

- *Procedure*

Hydrolyze the filtrate with dilute hydrochloric acid, neutralize with alkali, and then heat with Fehling's A and B solutions.

- *Positive Result*

The formation of a red precipitate indicates the presence of carbohydrates.

These tests effectively identify the presence of carbohydrates, including reducing sugars, in the extracts of *R. cordifolia*. The results of phytochemical screening were tabulated in Table 4.

Determination of Total Phenolic Content[16,17]

Preparation of Folin-Ciocalteu Reagent

Prepare 0.2 N Folin-Ciocalteu reagent by pipetting 1-mL of the reagent and diluting it to 10 mL with distilled water.

Preparation of Sodium Carbonate Solution

Dissolve 0.75 g of sodium carbonate in 10 mL of distilled water to prepare the sodium carbonate solution.

Preparation of Standard Gallic Acid

Prepare a standard stock solution by dissolving 5 mg of gallic acid in 10 mL of distilled water to achieve a final concentration of 500 µg/mL.

Preparation of Sample

Dissolve 100 mg of the root extract in 2 mL of distilled water.

These preparations set the stage for measuring the total phenolic content, which is an indicator of antioxidant activity in *R. cordifolia*.

Procedure for Determining Total Phenolic Content

Preparation of Test Tubes

Thoroughly wash the test tubes and dry them in a hot air oven at 100°C.

Assay Reaction Setup

Combine the following in a test tube:

- 0.5 mL of 50 mg/mL extract
- 0.25 mL of Folin-Ciocalteu's reagent
- 0.2 mL of sodium carbonate solution
- Add distilled water to make the total volume up to 10 mL.

Calibration Curve Preparation

Prepare a calibration curve for polyphenols using gallic acid as a standard in concentrations ranging from 10 to 50 µg/mL. Mix these standards with the same reagent mixture.

Absorbance Measurement

After 2 hours, measure the absorbance of the samples at 765 nm to determine the total phenolic content.

Replicates

Perform all determinations in triplicate to ensure accuracy.

Calculation of Total Phenolic Content

Express the total phenolic content in gallic acid equivalents (GAE) using the formula: T=C×VMT = \frac {C \times V} {M} T=MC×V

Where:

- T = Total phenolic content in mg/g plant extract (GAE)
- C = Concentration (mg/mL) of gallic acid from the calibration curve
- V = volume of extract (mL)
- M = Weight of plant extract (g)

The observations of total phenolic content analysis are presented in Table 5.

Results

The analysis of biologically active compounds in the root extracts of *R. cordifolia* was conducted using standard procedures. The results, presented in Tables 1 to 5, indicate the presence of bioactive compounds in all solvent extracts.

Interpretation

Ash Value

The total ash value of 6.52% indicates the inorganic residue left after the plant material is burned, which can be useful for assessing the purity and quality of the herbal material.

Extractive Value

The extractive values suggest that a higher percentage of the plant's constituents can be extracted with alcohol (14.7%) compared to water (11.8%), indicating a preference for non-polar solvents.

Moisture Content

The moisture content of 0.39% indicates that the powdered roots are relatively dry, which is essential for preserving the quality of herbal materials.

Determination of the total antioxidant capacity

Found to be 120 µg/mL.

Discussion

The significance of standardized procedures for crude drug extraction (medicinal plant parts) is to attain the therapeutically desired portions and to quit unwanted material by treatment with a selective solvent. These extracts, after standardization, are used as medicinal agents, such as in the form of tinctures, or further processed to be incorporated in any dosage form, such as capsules and tablets. These products contain a complex mixture of many secondary metabolites, such as alkaloids, glycosides, flavonoids, terpenoids and lignins. The screening of biologically active compounds from various solvent extracts of the root, stem and leaf of *R. cordifolia* revealed the presence of anthraquinones, glycosides saponins, steroids, phenols and flavonoids. Biologically active compounds are chemical in nature they have the potential to cure various diseases. *R. cordifolia* also revealed important phytochemical compounds and evidenced that this plant as important for curing various diseases in traditional medicine. Anthraquinones were mainly found in roots, stems and leaves, which have been shown to be antibacterial, antifungal and laxative and were also used as natural dyes. The plant extract has revealed the presence of saponins, which have anti-inflammatory,[18] anthelmintic, anti-cancer[19] and anti-diarrheal activities. The plant extract has shown the presence of steroids which are very important compounds with respect to antibacterial properties. Phenolic substances include flavonoids which are natural antioxidants and exhibit anti-inflammatory,[18] anticarcinogenic[19] and antiaging properties. The present studies on phytochemical screening of *R. cordifolia* revealed the presence of various phytochemicals present in various solvent extracts. Hence it can be utilized in the preparation of drugs used in the treatment of various ailments in traditional as well as in modern medicine.

Table 5: Total phenol content of roots extract of *R. cordifolia* plant

Sample	*Total phenol contents (mg/g)*
Aqueous extract of *R. cordifolia* root	380.2
Ethanolic extract of *R. cordifolia* root	413.6

Conclusion

Medicinal plants have curing properties of various diseases due to the presence of biologically active compounds which make the plant medicinally important. The huge diversity of phytochemicals present in various plant parts possesses a unique characteristic for curing diseases. The diversification of these phytochemicals and their properties attracts people to know the uses of such chemical compounds which are useful in treating different chronic diseases too. *R. cordifolia* revealed the presence of phytochemical compounds such as anthraquinones, flavonoids, steroids and glycosides, which have unique medicinal properties and are used in the treatment of various diseases. This is a preliminary work on screening of bioactive compounds in *R. cordifolia* which needs further investigation.

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