VER
Vascular & Endovascular Review

Physicochemical, Extraction and Phytochemical Screening of Leaves of Crotalaria hebecarpa (DC.) Rudd

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ABSTRACT

The genus Crotalaria is well known for its diverse phytochemical composition and broad therapeutic properties. This study focuses on evaluating the physicochemical parameters, performing solvent-based extraction, and carrying out preliminary phytochemical screening of leaves of Crotalaria hebecarpa (DC.) Rudd—a lesser-explored medicinal plant species. The investigation aimed to establish quality control parameters and identify bioactive phytoconstituents present in various solvent extracts of the leaves. The findings provide a foundation for future pharmacological and phytochemical research.

KEYWORDS: Crotalaria hebecarpa, Phytochemicals, Leaves.

How to Cite: Sushma Dwivedi*, Sudha Vengurlekar, (2025) Physicochemical, Extraction and Phytochemical Screening of Leaves of Crotalaria hebecarpa (DC.) Rudd, Vascular and Endovascular Review, Vol.8, No.8s, 31-34.

INTRODUCTION

Medicinal plants have been an integral part of human healthcare for centuries, forming the basis of traditional medicine systems such as Ayurveda, Traditional Chinese Medicine, and Unani. They contain a wide range of bioactive compounds, including alkaloids, flavonoids, terpenoids, tannins, glycosides, and phenolic compounds, which contribute to their therapeutic properties. [1-2] These plants have been historically used to treat various ailments, including infections, inflammation, digestive disorders, neurological conditions, and metabolic diseases. The growing interest in natural remedies and plant-based therapeutics has led to extensive research to validate their efficacy and safety through modern pharmacological and clinical studies [3]. *Crotalaria hebecarpa* (DC.) Rudd, belonging to the family Fabaceae, is a less-studied species known to grow in tropical and subtropical regions. While other *Crotalaria* species are reported to exhibit anti-inflammatory, antimicrobial, and antioxidant properties, *C. hebecarpa* remains relatively unexplored in terms of its phytochemical and pharmacognostic potential. [4-5]

Crotalaria hebecarpa (DC.) Rudd is a lesser-explored member of the Fabaceae family, known for its erect growth habit and characteristic yellow flowers. It is primarily found in certain parts of central India and is locally recognized for its traditional medicinal applications. Though limited scientific literature exists on this specific species, related species within the *Crotalaria* genus have been documented to exhibit diverse phytochemical profiles, including the presence of alkaloids (notably pyrrolizidine alkaloids), flavonoids, tannins, saponins, glycosides, and phenolic compounds. Preliminary phytochemical screenings of *C. hebecarpa* indicate the presence of similar classes of bioactive compounds, especially in polar solvent extracts, suggesting potential therapeutic relevance. Pharmacological studies, though sparse, hint at possible antioxidant, anti-inflammatory, antimicrobial, and cytotoxic activities, inferred through the phytochemical composition and comparative analysis with other *Crotalaria* species. [6] The plant holds promise for further investigation into its bioactive constituents and their potential pharmacological applications in modern herbal medicine. The present study was undertaken to assess the physicochemical characteristics, perform standardized extraction, and evaluate the presence of various secondary metabolites through preliminary phytochemical screening.

MATERIAL AND METHODS

Collection and Authentication of Plant Material

Fresh, mature leaves of *Crotalaria hebecarpa* were collected during the vegetative phase (Nov–Dec 2023) from the Malwa region of Madhya Pradesh. The plant was authenticated by Dr. Smruti Sohani, Professor, SAGE University, Indore, and confirmed with herbarium records; a voucher specimen (Pt.-CHL-016) was deposited for reference. The leaves were washed, shade-dried, and powdered for subsequent pharmacological and phytochemical studies.

Physicochemical Evaluation

The physicochemical evaluation of *Crotalaria hebecarpa* leaves was carried out using standard pharmacopoeial methods. Freshly collected and shade-dried leaves were powdered and passed through a 60-mesh sieve for uniformity. The powdered material was assessed for moisture content (loss on drying at 105 °C) and total ash, acid-insoluble ash, and water-soluble ash values were determined to evaluate inorganic matter. Extractive values were estimated using water and alcohol as solvents to determine the presence of soluble phytoconstituents. [7-8]

Successive Extraction of Plant Material

The shade-dried leaves of *Crotalaria hebecarpa* were coarsely powdered and subjected to successive solvent extraction using

Soxhlet apparatus. About 250 g of powdered material was first extracted with petroleum ether (40–60 °C) for 48 hours to remove fats, oils, and other non-polar constituents. The marc obtained was then air-dried and extracted successively with chloroform, followed by ethanol, and finally with distilled water, each for 48 hours or until the solvent in the siphon tube became colorless. After each extraction, the solvent was recovered under reduced pressure using a rotary evaporator, and the concentrated extracts were dried, weighed, and stored in airtight containers at 4 °C for further phytochemical and pharmacological evaluation. The percentage yield of each extract was calculated with respect to the starting material. [9]

PRELIMINARY PHYTOCHEMICAL SCREENING

Qualitative Test Screening

The petroleum ether, chloroform, ethanol, and aqueous extracts of *Crotalaria hebecarpa* leaves were subjected to preliminary phytochemical screening using standard qualitative tests to detect the presence of major secondary metabolites. Each extract was dissolved in its respective solvent and tested for alkaloids (Mayer's, Wagner's, and Dragendorff's reagents), flavonoids (Shinoda and alkaline reagent tests), tannins and phenolic compounds (Ferric chloride and lead acetate tests), saponins (froth test), glycosides (Keller–Killiani test for cardiac glycosides), terpenoids (Salkowski test), steroids (Liebermann–Burchard test), and carbohydrates (Molisch's and Benedict's tests). Proteins and amino acids were screened by Biuret and Ninhydrin tests, while fixed oils and fats were checked in the petroleum ether extract. The appearance of characteristic color changes or precipitates was noted as an indication of the presence of respective phytoconstituents. All tests were carried out in triplicate to ensure reproducibility and reliability of the results. [9]

Quantitative Test Screening

The total flavonoid, total phenolic and total alkaloidal contents of the petroleum ether, chloroform, ethanol and aqueous extracts of *Crotalaria hebecarpa* leaves were determined using standard spectrophotometric and gravimetric procedures. For total flavonoids, each extract was dissolved in methanol to prepare an appropriate concentration series; 1.0 mL of extract solution was mixed with 0.3 mL 5% sodium nitrite, after 5 min 0.3 mL 10% aluminum chloride was added, and after 6 min the reaction was stopped with 2.0 mL 1 M sodium hydroxide and made up to volume with methanol. Absorbance was measured at 415 nm against a reagent blank and flavonoid content was calculated from a quercetin calibration curve and expressed as mg quercetin equivalents (QE) per g extract. For total phenolics, the Folin–Ciocalteu method was employed: 0.5 mL of suitably diluted extract was mixed with 2.5 mL 10% Folin–Ciocalteu reagent, allowed to react for 5 min, then 2.0 mL of 7.5% sodium carbonate was added and the mixture incubated at room temperature for 30–45 min; absorbance was read at 765 nm and phenolic content was determined from a gallic acid standard curve and reported as mg gallic acid equivalents (GAE) per g extract. Total alkaloids were estimated by an acid–base extraction and gravimetric method: a known weight of extract was dissolved in 10% acetic acid in ethanol and allowed to stand for 4–6 h, filtered and concentrated to one-quarter of the original volume; concentrated ammonium hydroxide was then added dropwise to precipitate alkaloids until the solution became basic (pH ~9–10), the precipitate was collected by filtration, washed with dilute ammonium hydroxide, dried to constant weight in a hot-air oven and weighed to obtain total alkaloid content (expressed as % w/w of the extract). All assays were performed in triplicate and results were reported as mean ± SD; linear regression (calibration curve) parameters and limits of detection were recorded for the spectrophotometric assays. [10-12]

RESULTS AND DISCUSSION

The physicochemical parameters of *Crotalaria hebecarpa* leaves were evaluated, and the results are presented in Table 1. The percentage of foreign organic matter was found to be 0.45% w/w, indicating proper collection and minimal contamination with extraneous material. The moisture content was 6.72% w/w, which is within acceptable pharmacopoeial limits (<10%) and suggests lower susceptibility to microbial degradation and longer shelf life of the plant material. The total ash value was 8.34% w/w, reflecting the total amount of inorganic residues present, including physiological and non-physiological ash. The water-soluble ash value (3.65% w/w) and acid-insoluble ash value (1.12% w/w) were within normal ranges, indicating that the plant material contained a reasonable amount of water-soluble inorganic salts and a low content of siliceous matter such as sand and silica, respectively. These values provide useful markers for the purity and authenticity of the plant drug. The extractive values provide an estimate of the presence of active constituents soluble in different solvents. The ethanol-soluble extractive value (12.48% w/w) was highest, suggesting the predominance of polar and moderately polar phytoconstituents such as flavonoids, tannins, glycosides, and phenolics. The water-soluble extractive value was also appreciable (9.73% w/w), further supporting the presence of hydrophilic secondary metabolites. In contrast, the chloroform-soluble (4.26% w/w) and petroleum ether-soluble (2.19% w/w) extractive values were comparatively lower, indicating that fewer non-polar constituents such as fats, fixed oils, terpenes, and steroids are present.

Table 1: Physicochemical Parameters of C*rotalaria hebecarpa* leaves

S/No.	Parameters	Values obtained (% w/w)
1.	Foreign organic matter	0.45
2.	Moisture content	6.72
3.	Total ash value	8.34
4.	Water soluble ash value	3.65
5.	Acid insoluble ash value	1.12
6.	Water soluble extractive value	9.73
7.	Ethanol soluble extractive value	12.48
8.	Chloroform soluble extractive value	4.26
9.	Petroleum ether soluble extractive value	2.19

The successive solvent extraction of powdered leaves of *Crotalaria hebecarpa* was carried out using petroleum ether, chloroform, ethanol, and water. The percentage yield, physical nature, and color of each extract are presented in Table 2. Among the extracts, the highest yield was obtained with ethanol (11.57% w/w), producing a dark green solid powder, which indicates the presence of abundant polar and moderately polar phytoconstituents such as flavonoids, tannins, glycosides, and phenolics. The aqueous extract also showed a comparatively high yield (7.26% w/w) as a green solid powder, suggesting the extraction of hydrophilic compounds. The chloroform extract yielded 3.42% w/w as a pale green sticky semi-solid, while the petroleum ether extract gave the lowest yield (1.86% w/w) with a pale green sticky semi-solid consistency, indicating the presence of a smaller fraction of non-polar constituents like oils, fats, and terpenoids.

Table 2: Percent Extract of C*rotalaria hebecarpa* leaves

S/No.	Parameters	Values obtained (% w/w)	Nature of Extract	Color
1.	PEECHL	1.86	Sticky Semi Solid	Pale green
2.	CECHL	3.42	Sticky Semi Solid	Pale green
3.	EECHL	11.57	Solid Powder	Dark Green
4.	AECHL	7.26	Solid Powder	Green

The preliminary phytochemical screening of petroleum ether (PEECHL), chloroform (CECHL), ethanol (EECHL), and aqueous (AECHL) extracts of *Crotalaria hebecarpa* leaves revealed the presence of various classes of secondary metabolites (Table 3). Alkaloids were absent in the petroleum ether extract but present in chloroform, ethanol, and aqueous extracts, suggesting their predominance in medium to polar solvents. Carbohydrates and glycosides were detected only in ethanol and aqueous extracts, indicating their solubility in polar solvents. Proteins and amino acids also showed positive reactions in ethanol and aqueous extracts.

Saponins and triterpenoids were detected in petroleum ether, chloroform, and aqueous extracts, but absent in ethanol extract. Fixed oils and fats were observed exclusively in the petroleum ether extract, highlighting its ability to dissolve non-polar constituents. Flavonoids were present in chloroform, ethanol, and aqueous extracts, while tannins and phenolic compounds were strongly detected in ethanol and aqueous extracts, further confirming the affinity of these polar constituents towards hydroalcoholic and aqueous solvents. Gums and mucilages were absent in all extracts.

Overall, the results demonstrate that *Crotalaria hebecarpa* leaves are rich in alkaloids, flavonoids, tannins, phenolics, glycosides, and saponins, particularly in ethanol and aqueous extracts, suggesting these extracts may possess significant pharmacological potential. Non-polar extracts (petroleum ether) predominantly contained fats, fixed oils, and traces of saponins, while chloroform extract showed the presence of alkaloids, flavonoids, and triterpenoids in moderate quantities.

Table 3: Preliminary Phytochmeical Screening of Extract of C*rotalaria hebecarpa* leaves

S/No.	Test	PEECHL	CECHL	EECHL	AECHL
1.	Alkaloids	-	+	+	+
2.	Carbohydrates	-	-	+	+
3.	Glycosides	-	-	+	+
4.	Protein and Amino acid	-	-	+	+
5.	Sapnins & Triterpenoids	+	+	-	+
6.	Fixed oil	+	-	-	-
7.	Flavonoids	-	+	+	+
8.	Tannins and Phenolic compounds	-		+	+
9.	Gums & mucilages	-	-	-	-
10.	Fats	+	-	-	-

+ = Present; - = Absent

The quantitative estimation of major phytoconstituents in the petroleum ether (PEECHL), chloroform (CECHL), ethanol (EECHL), and aqueous (AECHL) extracts of *Crotalaria hebecarpa* leaves is presented in Table 4. Among the extracts, the ethanol extract exhibited the highest total flavonoid content (72.14 mg/g) and total phenolic content (84.65 mg/g), indicating a significant presence of polar bioactive compounds. The aqueous extract also showed appreciable levels of flavonoids (58.76 mg/g) and phenolics (69.88 mg/g), confirming the solubility of these constituents in polar solvents. In contrast, the chloroform extract contained moderate amounts of flavonoids (29.42 mg/g) and phenolics (35.26 mg/g), while the petroleum ether extract had the lowest content of flavonoids (14.37 mg/g) and phenolics (18.91 mg/g), consistent with the non-polar nature of the solvent.

Total alkaloid content followed a similar trend, with the highest concentration observed in the ethanol extract (5.84%), followed by the aqueous extract (4.52%), chloroform extract (2.17%), and petroleum ether extract (1.03%). These findings suggest that alkaloids in *Crotalaria hebecarpa* leaves are predominantly soluble in polar and moderately polar solvents.

Overall, the quantitative analysis confirms that ethanol and aqueous extracts are rich sources of flavonoids, phenolics, and alkaloids, which are likely to contribute to the pharmacological activities of the plant, while non-polar extracts contain comparatively lower levels of these bioactive compounds.

Table 4: Quantitative Estimation of Extract of C*rotalaria hebecarpa* leaves

S/No.	Test	PEECHL	CECHL	EECHL	AECHL
1.	Total flavonoid, content	14.37 mg	29.42 mg	72.14 mg	58.76 mg
2.	Total phenolic content	18.91 mg	35.26 mg	84.65 mg	69.88 mg
3.	Total alkaloidal content	1.03%	2.17%	5.84%	4.52%

CONCLUSION

The current investigation reveals significant physicochemical and phytochemical characteristics of *Crotalaria hebecarpa* leaves. High extractive values and rich presence of bioactive compounds in the ethanol and aqueous extracts suggest that the plant is a potential source of therapeutic agents. The study provides essential baseline data for future pharmacological and phytochemical investigations and supports its use in traditional medicine.

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