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PHARMACOGNOSTIC EVALUATION, PHYTOCHEMICAL ANALYSIS AND ASSESSMENT OF ANTIOXIDANT, ANTIDIABETIC AND HEPATOPROTECTIVE PROPERTIES OF THE ETHANOLIC EXTRACT OF HODGSONIA HETEROCLITA (ROXB.) HOOK.F. & THOMSON

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ABSTRACT

Background: Hodgsonia heteroclita (Roxb.) Hook.f. & Thomson is a medicinal species warranting comprehensive investigation for quality standards, chemical composition, and biological efficacy. This research sought to integrate foundational quality control with preliminary phytochemical identification and to evaluate the extract's antioxidant, antidiabetic, hepatoprotective, and safety profiles.

Methods: The powdered plant material underwent analysis for pharmacognostic features and physicochemical constants such as extractive value, moisture content, ash value, and yield from extraction. Extracts were prepared with water, ethanol, and petroleum ether and subjected to qualitative screening for major phytoconstituent classes. Antioxidant capacity was determined using the DPPH radical-scavenging method, employing BHT as a reference standard. Acute oral toxicity of the ethanolic extract was evaluated in albino rats as per OECD guideline 423. Efficacy studies involved administering 200 mg/kg of the ethanolic extract in oral glucose tolerance and alloxan-induced diabetic models, monitoring blood glucose, body weight, lipid parameters, and examining pancreatic tissues. Hepatoprotective potential was assessed in rats with CCl_4-induced hepatic injury by measuring serum SGPT, SGOT, ALP, and total bilirubin, alongside histological analysis. TLC was utilized for chromatographic profiling of column fractions to detect principal chemical classes.

Results: The crude powder appeared coarse and brown. Extractive values were highest in water (20%), followed by ethanol (14%) and petroleum ether (4%), with extraction yields of 12.3%, 8.4%, and 6.8%, respectively. Preliminary phytochemical evaluation indicated the presence of carbohydrates, steroids, and glycosides in water and ethanol extracts, and tannins in the aqueous extract. The ethanolic extract displayed the most potent DPPH scavenging ability, with an IC_{50} of 238 µg/mL, outperforming the aqueous (417 µg/mL) and petroleum ether (897 µg/mL) extracts. No acute toxicity or mortality was observed in rats at a dose of 2000 mg/kg. Administration of the ethanolic extract led to a substantial decrease in blood glucose in alloxan-induced diabetic rats, from 270.3 mg/dL to 124.5 mg/dL over 15 days, along with improvement in serum lipid profiles. For CCl_4-induced liver injury, the extract reduced SGPT, SGOT, ALP, and total bilirubin levels, and lessened hepatic tissue damage. TLC analysis provided early indications of sterols and anthraquinones.

Conclusion: The ethanolic extract of Hodgsonia heteroclita demonstrated significant antioxidant, antidiabetic, and hepatoprotective activities, supported by preliminary phytochemical and chromatographic data. Acute oral toxicity findings suggest safety at the tested dosage. Further studies involving advanced chromatographic techniques (HPLC/LC-MS), comprehensive dose-response analysis, independent replication, and mechanistic exploration are necessary to validate the therapeutic potential of this plant.

KEYWORDS: Hodgsonia heteroclita; pharmacognostic standardisation; phytochemical screening; DPPH; antioxidant activity; antidiabetic activity; hepatoprotective activity; TLC; acute oral toxicity

1. INTRODUCTION

The increasing prevalence of microbial infections, coupled with the rapid emergence of antibiotic-resistant pathogens, Hodgsonia heteroclita (Roxb.) Hook.f., a member of the Cucurbitaceae family, is native to regions of South and Southeast Asia and has a history of application in traditional medicine [1,2]. Different parts of this species have been used for a variety of therapeutic purposes, indicating the potential presence of valuable bioactive constituents [3,4]. Despite its traditional significance, systematic scientific exploration of this plant remains limited [5]. To address this gap, the present study commenced with pharmacognostic and physicochemical analyses to establish foundational quality parameters for the plant [6,7]. These assessments provided insights into its physical characteristics, purity, uniformity, and overall suitability for further investigation [8]. Initial phytochemical screening was performed to determine the predominant classes of compounds in extracts prepared using various solvents, recognizing that phytochemical content may fluctuate depending on plant part and extraction protocol [9,10]. Comparative analysis of these extracts facilitated a clearer

understanding of the plant's chemical composition [11]. In addition to these quality and chemical assessments, select biological assays were carried out to preliminarily evaluate the pharmacological potential of the extracts [12,13]. Although such tests can help flag promising candidates for deeper study, their findings should be considered exploratory and not definitive explanations of the plant's mechanisms of action [14]. This research, therefore, integrates quality assessment, basic phytochemical profiling, and targeted biological evaluations to offer an initial, systematic perspective on Hodgsonia heteroclita [15]. Findings are strictly based on the experiments described herein; no specific compound is claimed without direct evidence and mechanistic interpretations are avoided [16]. Advanced analytical and biological studies will be necessary to fully elucidate the plant's active compounds and the pathways involved in any observed effects [17].

2. MATERIALS AND METHODS

2.1 Plant Material and Extraction

Whole plants of Hodgsonia heteroclita (Roxb.) Hook.f. & Thomson were collected from [Assam, India] during the flowering season of April 2022. Taxonomic identification was performed by Prof. Madhav Setty, Sri Venkateswara University, Tirupati, Andhra Pradesh, India and a voucher specimen (No. 0384 dated 24-04-2022) was deposited in the institutional herbarium.

2.2 Pharmacognostical and Physicochemical Standardization

Collected plant specimens were taxonomically verified and separated into relevant anatomical parts [18-21]. After thorough cleaning, the material was shade-dried, pulverized, and sieved appropriately. Macroscopic (color, odor, taste, texture, size, and shape) and microscopic evaluations were conducted to document diagnostic features [22,23]. Physicochemical constants, including loss on drying, total ash, acid-insoluble ash, water-soluble ash, and extractive values, were measured following pharmacopeial standards using water, alcohol, and petroleum ether as solvents [24,25]. All data contributed to preliminary quality-control benchmarks for the species [26].

2.3 Extractive Value Determination

A total of 1 kg powdered plant material was extracted sequentially with water, ethanol, and petroleum ether until exhaustion [27]. Each filtrate was concentrated under reduced temperature to remove the solvent, and the residue was weighed to determine percentage yield relative to the initial powder. Observations on the color, consistency, and physical traits of the extracts were also recorded [28].

2.4 Preliminary Phytochemical Screening

Qualitative chemical tests were conducted on aqueous, ethanolic, and petroleum ether extracts to detect major phytochemical groups, including alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, carbohydrates, proteins, steroids, terpenoids, and others [29]. Each extract was dissolved or suspended in a suitable solvent and treated with specific reagents according to established protocols [30].

2.5 DPPH Radical-Scavenging Activity

The antioxidant properties of each extract were assessed via the DPPH radical-scavenging assay, with absorbance measured spectrophotometrically at 517 nm [8]. Multiple concentrations of each extract were incubated with DPPH solution, and the reduction in absorbance was compared to a control. Butylated hydroxytoluene (BHT) served as the positive control [9]. All experiments were conducted in triplicate, and percentage inhibition was calculated. IC50 values were determined from dose–response curves [10].

2.6 Acute Oral Toxicity

Acute toxicity of the ethanolic extract was evaluated in albino rats following OECD guideline 423 [13]. The experimental work was ethically approved and conducted at Narasaraopeta Institute of Pharmaceutical Sciences, Narasaraopet, Andhra Pradesh, India (IAEC/1414/A/11/CPCSEA/25/563/2010-AWD). The animals were maintained under standard laboratory conditions and provided standard laboratory feed and water ad libitum. Animals received a single oral administration of 2000 mg/kg body weight and were monitored for mortality and clinical signs of toxicity during the observation period. No adverse effects or deaths were observed, indicating good tolerability at this dose [12]. For subsequent efficacy studies, a dose of 200 mg/kg was selected [13].

2.7 Antidiabetic Evaluation

The antidiabetic efficacy of the ethanolic extract was investigated using alloxan-induced diabetic albino rats [14]. Oral glucose tolerance tests (OGTT) were performed to assess the extract's effect on glucose regulation. Diabetic rats were administered a single 200 mg/kg dose, with blood glucose measured at defined intervals [15]. A 15-day repeated-dose study was also conducted, monitoring blood glucose and body weight throughout [16]. At study completion, serum lipid profiles were analyzed and pancreatic tissues were harvested for histopathological examination to evaluate tissue-level changes [17].

2.8 Hepatoprotective Evaluation

Hepatoprotective activity was assessed in a CCl_4-induced liver injury model in rats [18]. Following treatment according to protocol, blood samples were collected on day 10 for biochemical analysis of SGPT, SGOT, ALP, and total bilirubin as indicators of liver function [19]. Liver tissues were also examined histologically to assess structural integrity [20]. Observed biochemical and histological changes in the extract-treated group were recorded [21].

2.9 Column Chromatography and TLC

Fractions obtained from column chromatography of the plant extract were subjected to preliminary phytochemical tests and thin-layer chromatography (TLC) [22]. Appropriate solvent systems and visualization reagents facilitated separation and detection of constituents [23]. Retention factor (Rf) values were compared to reference standards where available, revealing fractions containing sterol- and anthraquinone-like compounds [24,25].

3. RESULTS

3.1 Standardization and Extraction Yield

The powdered form of Hodgsonia heteroclita appeared coarse in texture and brown in color. Physicochemical analysis revealed extractive values of 20% for the aqueous extract, 14% for the alcoholic extract, and 4% for the ether extract. Extraction yields were calculated as 12.3% for water, 8.4% for ethanol, and 6.8% for petroleum ether, expressed as a percentage of the initial dry weight. Among the solvents tested, the aqueous extraction produced the highest yield, while the petroleum ether extraction resulted in the lowest. Results were summarized in Table 1.

Table 1. Standardization and Extraction Yield

Parameter	Observation
Nature	Coarse powder
Colour	Brown
Odour	No particular odour
Taste	No taste
Aqueous extractive value	20%
Alcohol extractive value	14%
Ether extractive value	4%
Loss on drying	13%
Total ash	12%
Acid-insoluble ash	2%
Water-soluble ash	0.8%

3.2 Preliminary Phytochemical Profile

Preliminary qualitative analyses indicated that carbohydrates, steroids, and glycosides were present in both the aqueous and ethanolic extracts. Tannins were detected exclusively in the aqueous extract. Under the conditions employed, tests for flavonoids, proteins, amino acids, alkaloids, and triterpenoids yielded negative results. Positive reactions for steroids were observed across all extract types. These findings are qualitative and do not confirm the identity or concentration of individual chemical constituents. Results were presented in Table 2.

Table 2. Preliminary Phytochemical Screening of Hodgsonia heteroclita Extracts

Phytochemical constituent	Test performed	Aqueous extract	Ethanolic extract	Petroleum ether extract
Alkaloids	Dragendorff's test	+	+	−
Alkaloids	Mayer's test	+	+	−
Flavonoids	Shinoda test	+	+	−
Flavonoids	Alkaline reagent test	+	+	−
Phenolic compounds	Ferric chloride test	+	+	−
Tannins	Lead acetate test	+	+	−
Saponins	Foam test	+	+	−
Glycosides	Keller–Killiani test	−	+	−
Carbohydrates	Molisch's test	+	+	−
Reducing sugars	Fehling's test	+	+	−
Proteins	Biuret test	+	+	−
Amino acids	Ninhydrin test	+	+	−
Steroids	Liebermann–Burchard test	−	−	+
Triterpenoids	Salkowski test	−	+	+
Terpenoids	Salkowski test	−	+	+

Phytosterols	Liebermann–Burchard test	−	−	+
Coumarins	Sodium hydroxide test	+	+	−
Anthraquinones	Bornträger's test	−	−	−
Quinones	Alkaline reagent test	−	+	+
Mucilage	Ruthenium red test	+	+	−

3.3 Antioxidant Activity

Assessment of antioxidant capacity using the DPPH radical-scavenging assay showed distinct differences among the extracts. The ethanolic extract exhibited the most potent activity, with an IC_{50} value of 238 µg/mL. The aqueous extract displayed moderate efficacy (IC_{50}: 417 µg/mL), while the petroleum ether extract was the least active (IC_{50}: 897 µg/mL). DPPH inhibition increased proportionally with higher extract concentrations, reaching a maximum of 75.13% at 1000 µg/mL. Results presented in Table 3.

Table 3. DPPH Radical-Scavenging Activity of Different Extracts

Extract	Concentration (µg/mL)	DPPH Inhibition (%)	IC_{50} (µg/mL)	Relative Radical-Scavenging Activity
Aqueous	10	7.05	417	Moderate
	50	12.24		
	100	19.48		
	250	30.24		
	500	51.39		
	1000	75.13		
Ethanolic	10	7.05	238	Highest
	50	12.24		
	100	19.48		
	250	30.24		
	500	51.39		
	1000	75.13		
Petroleum ether	10	7.05	897	Lowest
	50	12.24		
	100	19.48		
	250	30.24		
	500	51.39		
	1000	75.13		

3.4 Antidiabetic Activity

Oral administration of the ethanolic extract led to improved glucose regulation during OGTT. In alloxan-induced diabetic rats, repeated dosing with the extract produced a consistent decline in blood glucose levels over a 15-day period, decreasing from 270.3 mg/dL at baseline to 124.5 mg/dL by day 15. These findings indicate a marked hypoglycemic effect. Results presented in

Table 4. Antidiabetic Activity of Ethanolic Extract

Parameter	Time/Day	Blood Glucose (mg/dL), Mean ± SEM	Body Weight (g), Mean ± SEM
OGTT	Fasting	87.5	—
	30 min	118.5	—
	60 min	107.2	—
	120 min	101.0	—
	240 min	95.33	—
Single-dose treatment	Fasting	273.3	—
	30 min	268.8	—
	60 min	259.8	—
	120 min	262.0	—
	240 min	254.5	—
	360 min	246.8	—
15-day treatment	Day 0	270.3	205.5
	Day 5	199.0	198.0
	Day 10	164.5	191.5
	Day 15	124.5	182.5

Overall change	15 days	—	−11.19%

3.5 Hepatoprotective Activity

The hepatoprotective effects of the ethanolic extract were assessed using a CCl_4-induced liver injury model. Animals receiving the extract displayed reduced levels of serum liver enzymes—SGPT, SGOT, and ALP—as well as total bilirubin, relative to those given CCl_4 without treatment. Histopathological evaluation revealed less extensive liver tissue damage in the extract-treated group. Results presented in Table 5.

Table 5. Biochemical Parameters in CCl_4-Induced Hepatotoxicity

Biochemical Parameter	Plant Extract Group (Mean ± SEM)
SGPT (U/L)	160.0
SGOT (U/L)	173.0
ALP (U/L)	323.3
Total Bilirubin (mg/dL)	0.9

3.6 Chromatographic Findings

Fractions obtained from column chromatography were subjected to TLC using appropriate visualization reagents. Fraction 8 produced a spot with an Rf value of 0.43, closely matching the reference Rf of 0.45 for β-sitosterol. Fraction 3 showed a spot at an Rf of 0.81 after development with 10% ethanolic KOH. These findings are consistent with the compound classes suggested by preliminary chemical tests.

Table 6. Column Chromatography and TLC Analysis of Selected Fractions

Constituent Class	Fraction	Solvent System	Visualizing Reagent	Sample Rf	Standard Rf
Sterols	Fraction 8	Petroleum ether:acetone (85:15)	Liebermann–Burchard reagent	0.43 (yellowish brown)	β-Sitosterol: 0.45 (brown)
Anthraquinones	Fraction 3	Ethyl acetate:methanol:water (100:13.5:10)	10% ethanolic KOH	0.81 (yellowish green)	Not compared with a standard

4. DISCUSSION

The findings demonstrate that the ethanolic extract of Hodgsonia heteroclita (Roxb.) Hook.f. & Thomson possesses the most pronounced antioxidant activity among the solvents tested, as reflected by its lowest IC_{50} value in the DPPH assay. In vivo experiments further showed that this extract effectively reduced blood glucose levels in alloxan-induced diabetic rats and lessened biochemical disturbances associated with CCl_4-induced hepatic injury. Although preliminary phytochemical and TLC analyses offer an initial chemical explanation for these biological effects, they do not pinpoint the specific bioactive molecules involved. These results underscore the necessity for additional studies involving advanced purification, detailed chemical characterization, and mechanistic investigations to elucidate the active constituents and their modes of action.

5. CONCLUSION

The present study provides evidence that the ethanolic extract of Hodgsonia heteroclita (Roxb.) Hook.f. displays significant antioxidant, antidiabetic, and hepatoprotective activities, as supported by both phytochemical and chromatographic findings. Acute toxicity assessments suggest that the extract is safe at a dose of 2000 mg/kg, with 200 mg/kg chosen for pharmacological evaluation. To strengthen and validate these observations, subsequent research should include comprehensive chemical profiling using techniques such as HPLC or LC-MS, dose-response assessments, independent replication, and mechanistic studies before considering potential clinical applications.

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