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Physicochemical, phytochemical and spectroscopic profile of ethanolic leaf extract of *Cardiospermum halicacabum* L. by ultrasound-assisted extraction

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ABSTRACT

Cardiospermum halicacabum L. (Sapindaceae), often referred to as balloon vine, is a medicinal plant recognized for its historical applications and rich phytochemical composition. The present study investigated selected physicochemical characteristics of the powdered leaves and the qualitative phytochemical and spectroscopic profile of a 70% ethanolic leaf extract obtained using ultrasound-assisted extraction (UAE). Loss on drying, ash values, and solvent-extractable values were determined for the powdered material. Preliminary phytochemical screening was performed using qualitative chemical reactions, while the evaluation of total phenolic and flavonoid contents was performed using colorimetric assays. FT-IR and UV–Visible spectroscopy were used to obtain preliminary spectral fingerprints. The 70% ethanolic extract tested positive for glycosides, terpenoids, saponins, tannins, flavonoids, alkaloids, proteins/amino acids, phenolic compounds, and carbohydrates. Total phenolic and flavonoid contents were reported; the basis should be stated according to the calibration standards actually used. These findings provide a preliminary quality and spectroscopic profile of the leaf extract. The results should be interpreted as characterization data rather than direct proof of superior extraction efficiency or individual compound identity.

Keywords: *Cardiospermum halicacabum L. ultrasound-assisted, phytochemical screening, FT-IR, UV–Visible spectroscopy.*

Introduction

Medicinal plants continue to serve as significant sources of structurally varied natural products and continue to receive attention for phytochemical characterization, quality control and discovery of potentially useful constituents. *Cardiospermum halicacabum L. (Sapindaceae*) is herbaceous climbing plant found in tropical and subtropical areas and possesses a historical background of traditional use for inflammatory and rheumatic complaints, skin disorders and other ailments [1–5]. Experimental investigations have described antioxidant, anti-inflammatory, analgesic, antihyperglycaemic and other biological activities, although reported effects vary according to plant part, extraction procedure, dose and experimental model [2–9,21,25]. Previous investigations of *C. halicacabum* have reported flavonoids and phenolic constituents together with saponins, tannins, glycosides, alkaloids and terpenoid or steroid-related metabolites [1,3,10–12]. Rutin has been reported among the leaf constituents and has been investigated for antioxidant and anti-inflammatory activity [3]. Differences in plant part, geographical origin, solvent composition and extraction conditions can influence the chemical profile of an extract; therefore, reproducible sample preparation and basic quality-control measurements are important before biological or pharmaceutical interpretation. [21,24-26,28]. Extraction conditions affect both the recovery and composition of plant-derived extracts. Ultrasound-assisted extraction relies largely on acoustic cavitation, which can promote solvent penetration and mass transfer and can facilitate disruption of plant tissues. These effects may reduce processing time and solvent requirements under appropriate conditions [13–17]. Hydroethanolic systems are commonly used when recovery of a broad range of moderately polar constituents, including many phenolic and flavonoid compounds, is desired [14–16,25].

Characterization of a crude botanical extract commonly combines physicochemical evaluation, qualitative phytochemical screening, colorimetric estimation of broad compound classes, and spectroscopic fingerprinting. The Folin–Ciocalteu procedure is widely used for estimating total phenolic content [18], while aluminium-chloride assays are frequently applied to estimate total flavonoid content [19]. Such colorimetric assays are operational measures rather than compound-specific analyses, and their responses can vary among different chemical constituents [20]. FT-IR and UV–Visible spectroscopy likewise provide useful fingerprints but cannot, by themselves, establish the identity or concentration of individual phytochemicals [21,24,25,28]. The aim of the current research was to characterize specific physicochemical properties of C. halicacabum leaves and to examine the qualitative phytochemical and spectroscopic profile of a 70% ethanolic leaf extract prepared by UAE. The resulting data are intended as a preliminary reference profile for subsequent chromatographic, quantitative, and biological investigations [25].

Materials and Methods

Collection and preparation of plant material

Leaves from the plant *Cardiospermum halicacabum* L. were harvested during January–March 2026 from the rural region of Ambad, Jalna District, Maharashtra, India. The collected material was rinsed with tap water to remove adhering dust and particulate matter and then dried in the shade at room temperature for a duration of 6 to 7 days. The dried leaves were pulverized to obtain a relatively uniform powder and stored under appropriate conditions until analysis.

Ultrasound-assisted extraction

The dried leaf powder was combined with 70% ethanol in a 1:5 ratio of sample to solvent and then subjected to ultrasound treatment in an ultrasonic bath. The extraction cycle consisted of 15 min sonication, 15 min resting, and a further 15 min sonication, with the bath maintained at approximately 40 °C. The resulting mixture was processed to separate the extract from the plant residue, and the solvent was removed under the conditions used in the original experiment. The resulting extract was used for qualitative phytochemical and spectroscopic analyses.

Physicochemical evaluation

Selected physicochemical parameters of the powdered leaves were determined using established pharmacopoeial or standard pharmacognostic procedures. The measurements included loss on drying, total ash, acid-insoluble ash, water-soluble ash, water-soluble extractive and alcohol-soluble extractive values [10].

Loss on drying

Approximately 4 g of powdered leaf material was precisely weighed and subsequently dried in a hot-air oven at a temperature of 105 °C. The sample was cooled and weighed at successive intervals until a constant mass was obtained. The loss on drying process was determined by calculating the difference between the initial and final weights, and it was expressed as a percentage of the original sample mass.

Total ash

About 2–3 g of air-dried powder was introduced into a silica crucible that had been weighed beforehand and was then incinerated slowly at a temperature not exceeding 450 °C until a residue free of carbon was achieved. The crucible was subsequently cooled in a desiccator and weighed. The total ash content was determined as a percentage of the mass of the air-dried sample.

Water-soluble ash

A total of 25 mL of water was added to the total ash and heated to boiling. The insoluble portion was isolated using an ashless filter medium, subsequently washed with hot water, and then ignited. The mass of the water-soluble ash fraction was determined by subtracting the mass of the water-insoluble residue from the total ash.

Acid-insoluble ash

The ash underwent treatment with 25 mL of dilute hydrochloric acid and was heated gently. The insoluble material was separated by filtration, subjected to washing with water and subsequently ignited until a stable mass was achieved. The acid-insoluble ash was expressed as a percentage of the original air-dried sample.

Water-soluble extractive

A quantity of one gram of powdered substance was subjected to maceration with 20 mL of the designated chloroform-water solvent mixture in a sealed flask for a duration of 24 hours, during which intermittent shaking was performed. Following this, the mixture underwent filtration, and a specific volume of the filtrate was evaporated to dryness in a pre-weighed dish. The resulting residue was then weighed, and the water-soluble extractive value was determined in accordance with the established protocol.

Alcohol-soluble extractive

A powdered sample weighing one gram was combined with 20 mL of alcohol of the designated strength and allowed to macerate for 24 hours, with regular shaking during the initial phase, followed by a period of standing. The resulting mixture was then filtered, and a specific volume of the filtrate was evaporated to dryness in a pre-weighed shallow dish. The remaining residue was dried until a constant mass was achieved, and the alcohol-soluble extractive value was subsequently determined.

Preliminary phytochemical screening

The 70% ethanolic extract was subjected to qualitative chemical tests for glycosides, terpenoids, saponins, tannins, flavonoids, alkaloids, proteins/amino acids, phenolic compounds and carbohydrates. The tests were interpreted from the characteristic color changes, precipitate formation, or frothing responses associated with the respective reactions. The procedures were based on established phytochemical screening protocols [1,10–12].

Phytochemicals	Test/reagent	Positive indication
Alkaloids	Wagners reagent	Brown/reddish precipitate
Saponins	Froth test	Persistent foam
Glycosides	Keller Killiani test	Interfacial coloration
Tannins	Ferric chloride reaction	Green/brown coloration
Flavonoids	Alkaline reagent test	Yellow coloration
Carbohydrate	Benedict test	Orange /Red precipitate
Protein/Amino acids	Ninhydrin reaction	Blue coloration
Phenolics	Iodine reaction	Transient red color

Total phenolic content

The total phenolic content was determined using a Folin–Ciocalteu colorimetric method. An aliquot of the extract was combined with Folin–Ciocalteu reagent and sodium carbonate, following the experimental conditions established, followed by colour development and measurement at 650 nm against the corresponding solvent blank. Catechol was stated as the calibration standard in the experimental method. Accordingly, the final unit should be reported as catechol equivalents unless the experiment was actually calibrated with gallic acid. The numerical result is retained below pending confirmation of the standard used.

Total flavonoid content

The total flavonoid content was determined through an aluminium-chloride colorimetric method, utilizing rutin as the calibration standard. The extract underwent a sequential reaction with sodium nitrite, aluminium chloride, and sodium hydroxide, followed by measuring the absorbance at 510 nm.

The concentration was derived from the rutin calibration curve and expressed based on the equivalence defined by that calibration.

FT-IR spectroscopy

FT-IR spectroscopy was used to obtain a vibrational fingerprint of the dried leaf extract and to support tentative assignment of major functional groups. The dried extract was analyzed using a Shimadzu FT-IR spectrometer over the 400–4000 cm^{-1} range. The observed bands were interpreted by comparison with commonly reported vibrational regions for plant-derived functional groups. These assignments are considered tentative because FT-IR spectra of complex extracts contain overlapping contributions from multiple constituents.

UV–Visible spectroscopy

The UV–Visible absorption spectrum was recorded using UV-Vis (Shimadzu UV-1800) spectrophotometer over the wavelength range of 200-800nm. The extract was appropriately diluted with the corresponding solvent, and the solvent blank was used for baseline correction. The absorption spectrum was measured in a quartz cuvette that has a path length of 1 cm, conducted at room temperature. The obtained spectrum was used to identify characteristic absorption maxima (λmax) and to provide a preliminary spectral fingerprint of the extract. The observed absorption bands were correlated with the presence of different classes of phytoconstituents, particularly compounds containing conjugated chromophores and aromatic systems. UV–Visible spectroscopy was used as a preliminary characterization technique and was not considered sufficient for definitive identification of individual phytochemical constituents.

Results and Discussion

Physicochemical characteristics

The powdered leaves showed 12.02% loss on drying, 9.82% total ash, 1.78% acid-insoluble ash and 4.14% water-soluble ash. The alcohol-soluble and water-soluble extractive values were 13.55% and 16.07%, respectively. These measurements provide preliminary information on moisture, inorganic residue and solvent-soluble matter in the powdered plant material. The relatively low acid-insoluble ash value is compatible with a limited proportion of acid-insoluble mineral material in the tested sample. Extractive values should be interpreted in relation to solvent composition and experimental conditions rather than as direct measures of pharmacological activity.

Table 1: Physicochemical parameters of C. halicacabum L. leaves

Parameter	Value (%)
Loss on drying	12.02
Total ash	9.82
Acid-insoluble ash	1.78
Water-soluble ash	4.14
Alcohol-soluble extractive	13.55
Water-soluble extractive	16.07

Preliminary phytochemical screening

The 70% ethanolic extract gave positive qualitative reactions for glycosides, terpenoids, saponins, tannins, flavonoids, alkaloids, proteins/amino acids, phenolic compounds and carbohydrates. The pattern is broadly consistent with previous phytochemical reports for C. halicacabum [1,3,10–12]. These reactions indicate the presence of broad classes of constituents but do not establish their individual molecular identities, concentrations or biological activities. [23,24,25,28,29]

Table 2: Preliminary phytochemical screening of the 70% ethanolic extract of C. halicacabum L. leaves

Constituent class	Result
Glycosides	+
Terpenoids	+
Saponins	+
Tannins	+
Flavonoids	+
Alkaloids	+
Proteins and amino acids	+
Phenolic compounds	+
Carbohydrates	+

Total phenolic and flavonoid contents

The ultrasound-assisted 70% ethanolic extract contained a total phenolic content (TPC) of 14.02 mg catechol equivalents/g dry extract and a total flavonoid content (TFC) of 11.00 mg rutin equivalents/g dry extract under the assay conditions used in this study. The phenolic value is expressed as catechol equivalents because catechol was the calibration standard used in the experimental method, whereas rutin was used as the calibration standard for the flavonoid assay. These values indicate measurable phenolic- and flavonoid-reactive constituents in the extract; however, colorimetric assays provide group-level estimates and should not be interpreted as compound-specific measurements [18–20,24,25,28]

Table 3: Quantitative phytochemical measurements of the 70% ethanolic extract of C. halicacabum L. leaves

Parameter	Result
Total flavonoid content	11.00 mg rutin equivalents/g dry extract
Total phenolic content	14.02 mg equivalent/g dry extract

FT-IR spectral profile

The FT-IR spectrum of *Cardiospermum. halicacabum L.* leaves displayed prominent absorptions near 3350, 2126, 1643, 1404, 1340, 1209, 1170 and 1064 cm^{-1} (as shown in fig.1). The broad band near 3350 cm^{-1} is consistent with O–H stretching and may arise from hydroxyl-containing constituents such as phenolics, flavonoids and other polyoxygenated compounds. The band near 1643 cm^{-1} may include contributions from C=C or carbonyl-containing structures and, in aqueous or hydrated samples, H–O–H bending. Bands around 1404 and 1340 cm^{-1} can arise from different C–H, O–H, or C–N-related vibrational modes depending on the constituent matrix. The absorptions in the 1209–1064 cm^{-1} region are compatible with C–O and C–O–C vibrations associated with alcohols, ethers, glycosides and carbohydrate-containing compounds. Because the spectrum represents a complex mixture, these assignments should be regarded as tentative rather than definitive identification of individual phytochemicals. [11,24,25]

Table 4: FT-IR interpretation of compounds of ethanolic (70%) extract of Cardiospermum. halicacabum L. leaves.

Frequency Range (cm^{-1})	Functional Group	Phytochemicals/Biomolecules
3350	O–H stretching	Phenolics, flavonoids, tannins and other hydroxyl-containing constituents
2126	Weak band; assignment requires confirmation	Complex/overlapping extract matrix
1643	C=C/C=O-related vibration and/or H–O–H bending	Aromatic/conjugated compounds, carbonyl-containing constituents, hydrated sample
1404	C–H/O–H-related bending	Organic acids, phenolic and amino-containing constituents
1340	C–N/O–H-related vibration	Alkaloids, proteins and other nitrogen/oxygen-containing constituents
1209	C–O-related vibration	Polyphenols, flavonoids and glycosidic constituents
1170	C–O–C stretching	Carbohydrates, glycosides and polysaccharides
1064	C–O stretching	Sugars, carbohydrates and other oxygenated constituents

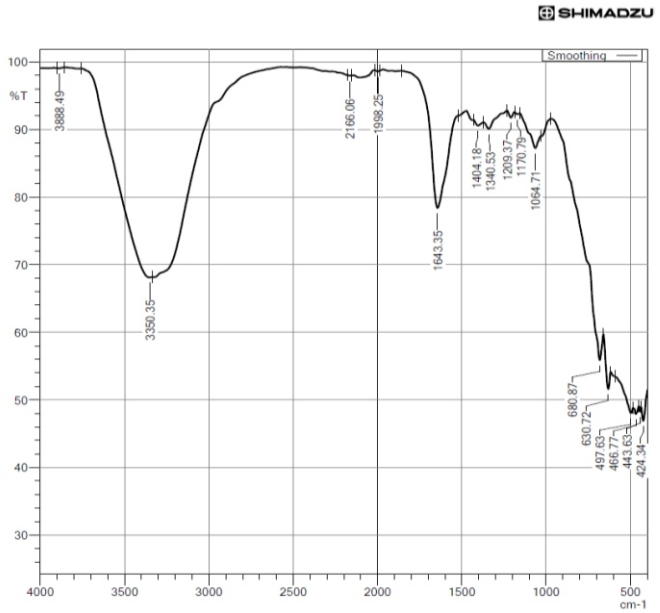

Fig. 1: FT-IR spectrum of Cardiospermum. halicacabum L. leaves

UV–Visible spectral profile

The UV–Visible spectrum showed absorption maxima near 218 and 278 nm, with weaker bands around 430 and 660 nm (as shown in fig.2). The lower-wavelength region may reflect π→π* transitions associated with aromatic or conjugated chromophores, while the band near 278 nm is compatible with phenolic or flavonoid-type chromophores. Absorptions near 430 and 660 nm may arise from plant pigments, including chlorophyll-related species, although the exact contribution of individual pigments cannot be established from the present spectrum alone. The spectral pattern therefore provides a useful fingerprint for the extract but should not be used as definitive evidence for specific compounds. [24,25,28]

Table 5: Characteristics UV-Vis absorption band of ethanolic (70%) extract of Cardiospermum. halicacabum L. leaves.

λmax (nm)	Possible phytochemicals/bioactive compounds
218	Aromatic/conjugated organic chromophores
278	Phenolic or flavonoid-type chromophores
430	Plant pigments and other conjugated constituents
660	Chlorophyll-related/photosynthetic pigments

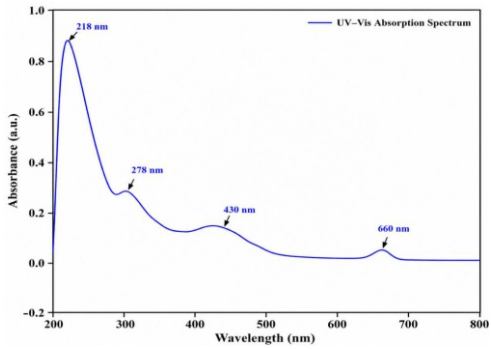

Fig. 2: UV–Visible spectrum of Cardiospermum. halicacabum L. leaves

Conclusion

The present study provides a preliminary physicochemical, phytochemical and spectroscopic characterization of a 70% ethanolic leaf extract of Cardiospermum halicacabum prepared using ultrasound-assisted extraction. The powdered leaves showed measurable ash, moisture and extractive characteristics, while qualitative screening indicated the occurrence of several broad classes of secondary metabolites. The extract also exhibited measurable phenolic- and flavonoid-reactive constituents and characteristic FT-IR and UV–Visible spectral features. The findings are useful as an initial analytical profile; however, the study does not establish the molecular identity of individual constituents or demonstrate that UAE is superior to conventional extraction because an appropriate comparator was not included. Future work should incorporate chromatographic and mass-spectrometric profiling, validated quantitative marker assays, extraction optimization with suitable controls, and biological evaluation to establish the relevance of the detected constituents.

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