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RESEARCH ARTICLE

Isolation and Characterisation of Chemical Constituents From Aerial Parts of *Cardiospermum halicacabum*

K Reshma[1], RM Rohini[1,*]

[1] *Department of Pharmaceutical Chemistry, Krupanidhi College of Pharmacy, Bangalore, Karnataka, India*

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[*] *Corresponding author.*
RM Rohini

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ABSTRACT

Cardiospermum halicacabum or the "balloon vine" is a medicinal plant that belongs to the Sapindaceae family. The aim of this study was to isolate and identify its chemical constituents and derive insights into its pharmacological prospects. The aerial parts of *Cardiospermum halicacabum* were collected, washed, and dried at room temperature. The dried plant material was powdered and extracted using solvents such as petroleum ether, acetone, chloroform, and ethanol. The extractive values were calculated for each solvent. The extracts were analyzed by preliminary phytochemical screening to identify bioactive secondary metabolites. The active compounds were isolated by column chromatography and recrystallization methods. Thin-layer chromatography, high-performance thin-layer chromatography, high-performance liquid chromatography, and other spectroscopic methods have been used for compound identification. Phytochemical analysis confirmed the presence of saponins, phytosterols, triterpenoids, alkaloids, carbohydrates, proteins, and free amino acids in the extract. The isolated compound had a molecular formula of $C_{39}H_{56}O_2$, a molecular weight of 557 g/mol, and a melting point of 145°C. Structural elucidation using IR, NMR, and MS confirmed that the compound was [(3S,4aR,6aR,6bS,8aR,11R,12S,12aR,14aR,14bR)-4,4,6a,6b,8a,11,12,14b-octamethyl-2,3,4a,5,6,7,8,9,10,11,12,12a,14,14a-tetradecahydro-1H-picen-3-yl] (E) 3-phenylprop-2-enoate. This research was able to isolate and identify a bioactive compound from *Cardiospermum halicacabum*, validating its chemical profile. The diverse array of chemical constituents suggests a significant pharmacological potential, supporting further exploration of this plant for medicinal applications.

Keywords: Cardiospermum halicacabum; HPLC; IR Spectra

INTRODUCTION

Cardiospermum halicacabum (*C. halicacabum*) is an ornamental climber belonging to a small genus of herbaceous or shrub-like woody vines. C. halicacabum may be either an annual or perennial climber. The plant grows mostly as a weed. C. halicacabum may also be referred to as the balloon plant or love in a puff. It is also known by other names, such as balloon vine, heart vine, heart pea, love-in-a-puff, or heart seed. The morphological characteristics of a plant form the basis of its scientific name. "Cardiospermum" is derived from Latin and means "heart-shaped seed", whereas "halicacabum" refers to "salt container"[1]. The plant is found extensively in tropical and subtropical parts of the world. It is grown on the African and American plains, Bangladesh, India, and Pakistan. The roots of plants, leaves, seeds, and flowers are used based on their medicinally valuable content. Medicines made from medicinal plants have become an indispensable part of medical care programs in developing countries. Approximately 80% of the world's population depends on medicinally useful plants for their essential healthcare needs[2]. India's rich tradition in herbal medicine makes the study of plants such as C. halicacabum highly relevant in clinical research[3]. Medicinal plants play an important role in maintaining one's health and treating various ailments. Such medicinal activity comes from bioactive compounds known as phytochemicals, whose specific physiological actions on the human body are evident. Compounds, such as alkaloids, tannins, flavonoids, and phenolics, are prime examples of phytochemicals found in medicinal plants[4].

C. halicacabum has been used to treat a variety of ailments, such as dermatological conditions (rashes, itchiness,

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inflammation of the skin), dandruff, rheumatoid arthritis, gastrointestinal disorders, respiratory tract infections, and urogenital disorders[5]. Also possesses antibacterial, antifungal, antidiarrheal, antioxidant, anticancer, analgesic, antiparasitic, antimalarial, antipyretic, and antidiabetic properties[6–15]. In the current research, we intend to purify the significant phytochemical constituents of C. halicacabum leaves to perform structural characterisation by analytical techniques for the purified phytochemical constituents.

MATERIALS AND METHODS

The aerial parts of *C. halicacabum* were harvested, cleaned to remove soil and sticking debris, and subsequently dried at room temperature for 5-6 days. The dried plant material was powdered to a mesh size of #60 for subsequent experiments. A sample of the chemicals and solvents used during the course of the work was procured from authentic sources such as Merck-Schuchardt, Mumbai, and Loba Chemie Pvt. Ltd., Mumbai. Some of the chemicals used were ethanol (95%), petroleum ether (60-80°C), hexane, benzene, ethyl acetate, chloroform, methanol, and others were used for extraction and characterization work.

Collection of Plant Material: Authenticated dried C. halicacabum leaves were purchased from Green Chem Pvt Limited, Bangalore, and voucher specimens were deposited for reference.

Extractive Values: The extraction method was used to measure the number of active constituents that could be extracted from the plant material using various solvents. This is ideal for plants that are yet to undergo appropriate chemical or biological assays. The plant material was successively extracted with petroleum ether, cold acetone, chloroform, and ethanol on both the small and pilot scales to determine the yield.

Lab-scale Extraction: In lab-scale extraction, 500 g of air-dried C. halicacabum aerial parts were macerated in 600 mL of petroleum ether at 60-80°C for 8h. This process was repeated four times to obtain defatted marc, which was then extracted three times with cold acetone (1 Litre). The acetone extract was evaporated to obtain 28 g of crude material. Subsequently, using the same method, the defatted material was extracted thrice with chloroform (1 Litre) and ethanol (1 Litre) respectively. Finally, the chloroform and ethanol extracts were evaporated to obtain 19.8 g and 17.4 g of crude material, respectively.

Because the acetone extract yielded a higher crude material, it was chosen for pilot-scale extraction.

Pilot-scale Extraction: For pilot-scale extraction, 2 kg of dried aerial parts of *C. halicacabum* were macerated with 1000 mL of petroleum ether and maintained at 60-80°C for 8 hours, and the operation was repeated four times. The defatted marc was then extracted with 3 litres of cold acetone, and the combined acetone extracts were evaporated to yield 100 g of crude material.

Preliminary Phytochemical Screening: Phytochemical screening of the extracts was conducted to identify a range of bioactive secondary metabolites. The plant has been screened for the presence of carbohydrates, alkaloids, steroids, sterols, glycosides, saponins, flavonoids, triterpenoids, proteins, and amino acids. A range of chemical tests was conducted to verify the presence of these compounds, with positive results for carbohydrates, alkaloids, and flavonoids, among others.

Isolation of Active Compounds: Two isolation methods were employed to isolate bioactive compounds from the ethanol extract of *Cardiospermum halicacabum*.

Isolation Procedure 1: Column Chromatography

Column chromatography was performed on the ethyl acetate extract using silica gel as the stationary phase and different solvent systems for elution. The silica gel column was conditioned by filling a glass column with 400 g of silica gel (60-120 mesh) and eluted using hexane, benzene, chloroform, acetone, ethyl acetate, and methanol gradient. The fractions were dried, collected, and evaluated using thin-layer chromatography (TLC). Fractions with similar TLC patterns were combined, leading to the isolation of a compound with an Rf value of 0.16 and a melting point of 144-146°C, which was identified as a sterol.

Isolation Procedure 2: Solvent Extraction and Recrystallization

The acetone extract was loaded onto column-grade silica gel, which was further extracted with toluene and filtered to obtain the residue. The residue was refluxed in petroleum ether and filtered to produce a precipitate, which was crystallized from diethyl ether to obtain the purified compound. The compound was purified by recrystallization to yield 280 mg of pure substance.

Thin Layer Chromatography (TLC): TLC was utilized to check the separation of compounds during the course of isolation. A range of solvent systems was tested, of which the ethyl acetate:methanol:water (8.5:2.5:0.5) system resulted in the best separation of the identified compounds. TLC plates were developed, scanned at 366 nm, and visualized using vanillin-sulfuric acid reagent.

High-Performance Thin Layer Chromatography (HPTLC): HPTLC was carried out with a CAMAG system comprising a Linomat 5 applicator and a multi-wavelength scanner. The test samples were prepared by extracting the leaves of C. halicacabum with methanol, which was then concentrated and spotted onto silica gel plates. The plates were developed using a solvent mixture of ethyl acetate, methanol, and water in the ratio 8.5:2.5:0.5, and scanned for fingerprints of compounds.

HPLC Analysis: The separated compounds were additionally analyzed by High-Performance Liquid Chromatog-

raphy (HPLC) using a Phenomenex C18 column. Water and methanol (55:45) were used as the mobile phases, and a flow rate of 1.0 mL/min was used for detection at 350 nm. The purities of the separated compounds were determined by comparing the sample peaks with those of the reference standard.

Spectral Characterization: Pure compounds were identified using different spectroscopic methods. Functional groups were identified by recording spectra between 4000-400 cm^{-1} by the KBr pellet method using Fourier-transform infrared (FT-IR) spectroscopy. Structural elucidation of the compounds was performed using nuclear magnetic resonance (NMR) spectroscopy by examining their chemical shifts, integration, and multiplicity. Mass spectrometry (MS) was used to determine the molecular weights and identify the chemical structures of the compounds by examining their mass-to-charge ratios. Through extraction, isolation, and characterization, a thorough investigation of the chemical constituents of C. halicacabum was conducted, providing insight into its pharmaceutical potential.

RESULTS

Chemical analysis of plant extracts revealed the presence of saponins, phytosterols, triterpenoids, proteins, free amino acids, and alkaloids. The chemical investigation of *Cardiospermum halicacabum*, a Sapindaceae plant, provided a detailed profile of its chemical constituents using preliminary and elaborate analyses. Authenticated leaves of C. halicacabum were obtained from Green Chem. Pvt. Ltd., and underwent physicochemical analysis. Phytochemical screening of the aqueous and acetonic extracts revealed the presence of various bioactive compounds, as presented in Table 1.

Table 1: Phytoconstituents identified in different solvent extracts of *C. halicacabum*

S. No	Phyto constituents	Aqueous Extract	Acetonic Extract
1	Alkaloids	+	+
2	Carbohydrates	+	+
3	Fixed oils and fats	-	-
4	Saponins	+	+
5	Tannins and phenolic compounds	-	-
6	Proteins and free amino acids	+	+
7	Flavonoids	-	-
8	Terpenoids	+	+
9	Phytosterol	+	+
10	Glycosides	-	-

(- : absent; +: present)

Both extracts contained alkaloids, carbohydrates, saponins, proteins, free amino acids, terpenoids, and phytosterols, whereas flavonoids, tannins, phenolic compounds, and glycosides were absent in the aqueous extract. The acetonic extract also contained alkaloids, carbohydrates, saponins, proteins, free amino acids, terpenoids, and phytosterols but lacked tannins and flavonoids. The petroleum ether and ethyl acetate extracts provided further insights into the chemical profile of the plant. The petroleum ether extract contained phytosterols, triterpenoids, and fats, while the ethyl acetate extract contained sterols, tannins, and flavonoids.

A significant compound was successfully isolated from the aerial parts of *Cardiospermum halicacabum*, exhibiting the following characteristics: molecular formula $C_{39}H_{56}O_2$, molecular weight 557, and melting point 145°C. The compound appeared as a pale yellow crystalline solid soluble in alcohol and chloroform, with an Rf value of 0.16. The yield of the isolated compound was 280 mg.

The isolated compounds were structurally elucidated by various spectroscopic techniques. High-Performance Thin-Layer Chromatography (HP-TLC) (Figure 1), infrared (IR) spectroscopy, Mass Spectrometry (MS), and Nuclear Magnetic Resonance (NMR) spectroscopy were used to confirm the structure. The IR spectrum displayed absorption bands corresponding to hydroxyl groups (3418.21 cm^{-1}) and substituted double bonds (1634.38 cm^{-1} and 1017.27 cm^{-1}). Mass spectrometry identified a molecular ion peak at m/z 414.38, which matched the molecular formula of $C_{39}H_{56}O_2$. The 1H-NMR spectrum showed characteristic resonances indicative of an ursane skeleton with multiple methyl groups and olefinic protons, further confirming the structure of the compound. The final structure of the isolated compound was determined to be [(3S,4aR,6aR,6bS,8aR,11R,12S,12aR,14aR,14bR)-4,4,6a,6b,8a,11,12,14b-octamethyl-2,3,4a,5,6,7,8,9,10,11,12,12a,14,14a-tetradecahydro-1H-picen-3-yl] (E) 3-phenylprop-2-enoate (Figure 2).

These findings illustrate the wide range of chemical constituents found in *Cardiospermum halicacabum*, providing a comprehensive overview of its pharmacological potential. The isolation and characterization of the compound adds to the increasing knowledge of the bioactive nature of the plant.

DISCUSSION

In the present work, chemical profiling of *Cardiospermum halicacabum* concurs with earlier research findings, indicating the plant's varied chemical profile and pharmacological relevance. Both the initial phytochemical screening and sophisticated analytical methods have provided an in-depth understanding of the plant's bioactive compounds.

A comparative account of the research conducted by Grace et al. is marked by a consistent pattern in the isolation and characterization of bioactive compounds from *Cardiospermum halicacabum*. In their work, mucilage was purified and identified, which also accounts for the

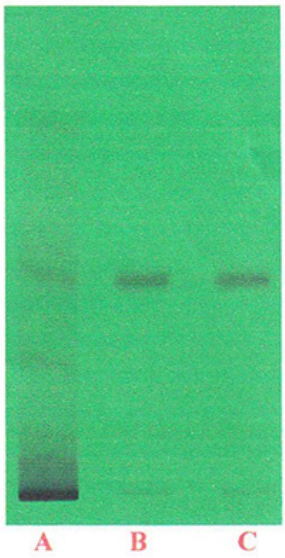

Fig. 1: HP-TLC profile A-C. halicacabum extract, B-Isolated compound from C. halicacabum extract, C-Reference standard at 254 nm

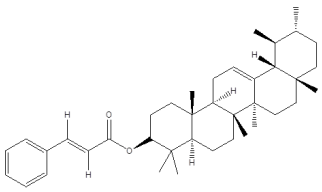

Fig. 2: Structure of the isolated compound [(3*S*,4*aR*,6*aR*,6*bS*,8*aR*,11*R*,12*S*,12*aR*,14*aR*,14*bR*)-4,4,6a,6b,8a,11,12,14b-octamethyl-2,3,4a,5,6,7,8,9,10,11,12,12a,14,14a-tetradecahydro-1H-picen-3-yl] (E) 3-phenylprop-2-enoate

pharmacological activity of the plant[16]. Our findings build on this by the identification of a wider variety of compounds, including alkaloids, saponins, phytosterols, and terpenoids, which have been reported to possess a variety of therapeutic activities, including anti-inflammatory, antimicrobial, and antioxidant activities. These results are consistent with the traditional application of C. halicacabum in the treatment of inflammatory diseases and other ailments.

The phytochemical profiles noted in the present study are consistent with those recorded by Patil et al., who have standardized C. halicacabum stem material. Their emphasis on HPTLC fingerprinting and pharmacognostic analysis provided a reference point for the identification of plant material, an imperative requirement for quality control in herbal medicines[17]. The occurrence of alkaloids, saponins, and terpenoids in aqueous and acetonic leaf extracts of the plant under investigation is further evidence of the plant's chemical uniformity, which is necessary for the therapeutic performance of herbal products.

Isolation of an important compound with a molecular formula of $C_{39}H_{56}O_2$ and molecular weight of 557 is a valuable contribution to the field of knowledge related to *Cardiospermum halicacabum*. This compound, designated as [(3S,4aR,6aR,6bS,8aR,11R,12S,12aR,14aR,14bR)-4,4,6a,6b,8a,11,12,14b-octamethyl-2,3,4a,5,6,7,8,9,10,11,12,12a,14,14a-tetradecahydro-1H-picen-3-yl] (E) 3-phenylprop-2-enoate, has been characterized with the help of IR, MS, and NMR studies. Spectroscopic information, especially the 1H-NMR and mass spectrometry findings, was in accordance with the ursane skeleton, which has been reported to have anti-inflammatory and antimicrobial activities. The results are in agreement with literature reports, e.g., Muthumani et al., in which chemical constituents of C. halicacabum were reported to have strong pharmacological activity, including anti-inflammatory activity[18].

Compared with the review by Murti et al., which reported the pharmacological attributes of *Cardiospermum halicacabum*, this study offers a more profound chemical basis for its therapeutic effects. The presence of saponins, alkaloids, and terpenoids in the plant vindicates the reports of Murti et al., who highlighted the anti-inflammatory and analgesic actions of the plant[19]. The isolated compound may account for the plant's legendary effectiveness in traditional medicine, especially for the treatment of inflammatory disorders and skin diseases.

The presence of phytosterols, triterpenoids, and fats in the petroleum ether extract and sterols, tannins, and flavonoids in the ethyl acetate extract further enhanced the chemical complexity of *Cardiospermum halicacabum*. These compounds are commonly associated with antioxidant, anti-inflammatory, and lipid-regulating activities, which further support the value of plants for use in the treatment of a range of diseases. The purification of a crystalline compound with a melting point of 145°C and alcohol and chloroform solubility also indicated that this compound may have potential utility in pharmaceutical formulations, particularly in the targeting of diseases associated with inflammation and oxidative stress.

CONCLUSION

In this study, we isolated and identified a bioactive phytoconstituent of C. halicacabum using advanced chromatography and spectroscopy techniques. The molecular structure and chemical properties of the compound were confirmed through a detailed analysis, providing valuable insights into the chemical composition of the plant. The isolation and characterization of the identified compound contribute significantly to ongoing research into the therapeutic applications of this plant, providing promising avenues for further pharmacological studies and the development of herbal medicines.

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