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Original Article

Decoding *Vijaya* (*Cannabis sativa* L.) Leaves: A Comprehensive Analysis through Pharmacognosy, Physicochemical Profiling and Advanced Analytical Techniques

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ABSTRACT

Background: *Vijaya (Cannabis sativa* L.) is an incredible therapeutic potential drug; however, it is enlisted in *Upavisha*[1] (mild potency poison)[2] in Ayurvedic literature. And it is mentioned in Schedule E (1) of the Drug and Cosmetic Act and the Narcotic Drugs and Psychotropic Substances Act (leaves and seeds are exempted). It contains numerous constituents that interact with the body's endocannabinoid system, offering substantial medicinal benefits through its multi targeted approach. Early research has paid limited attention to the leaves of *Vijaya*, regarding their pharmacognostic, phytochemical, and qualitative and quantitative analysis of its primary cannabinoids, Δ^9-THC and CBD, for medicinal use. **Objectives:** To conduct comprehensive pharmacognostical, phytochemical, and analytical studies aimed at establishing the standardization and quality control of *Vijaya* (*Cannabis sativa* L.) leaves to revalidate its medicinal use. **Materials and Methods:** Organoleptic, Macroscopic, Microscopic, Powder Microscopy, Qualitative, Quantitative (cannabinoids - Δ^9-THC and CBD) phytochemical studies, Fourier-transform infrared spectroscopy, high-performance thin-layer chromatography, and high-performance liquid chromatography were performed as per the guidelines of the Ayurvedic Pharmacopeia of India, Quality control of medicinal plants. **Results:** In the plant sample FTIR showed the presence of alkanes, alkenes, aromatic amines, and nitro compounds, HPTLC showed the presence of CBN and CBD, and HPLC showed the CBDA 1.30%, CBD at 0.77%, Δ^9THCA 0.001%, and Δ^9THC at 0.74% (7417.38 mg/kg). **Conclusion:** This comprehensive study, which includes pharmacognostical and diverse analytical methods, provides comprehensive data essential for the standardization and quality assurance of *C. sativa* leaves for further medicinal use.

Keywords: *Vijaya*, Bhang, *Cannabis sativa*, Delta 9-tetrahydrocannabinol, Cannabidiol.

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INTRODUCTION

Traditional, Complementary, and integrative medicine (T and CM) practices encompass diverse health practices that have been an integral part of healthcare systems for centuries in various countries, including India and China. The World Health Organization recognizes the benefits of traditional, complementary, and alternative medicines.[3] Ayurveda, an ancient medical science and one of the traditional systems of India, has a holistic approach to the maintenance of health and the management of diseases through natural substances, including plants, minerals, and animal products. Nowadays, there is a vogue for integrating T and CM with the national health system and the mainstream health care system. Integrative medicine aims to connect traditional and modern medical practices by conducting thorough scientific research and clinical assessments, thus providing treatment options that are both safe and effective. Therefore, the standardization and quality control of Ayurvedic formulations and medicinal plants are necessary for evidence-based validation of traditional medicine to ensure its effectiveness and credibility in the current healthcare landscape.

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Vijaya, commonly known as Bhanga in Hindi and Cannabis in English, is an annual dioecious flowering plant whose first appearance was believed to have been found in Central Asia.[4] Cannabis is the preferred designation of the plants *Cannabis sativa* L. (*C. sativa*), *Cannabis indica*, and, of minor significance, *Cannabis ruderalis* from the Cannabaceae family.[5] The Genus name Cannabis means "cane-like," while the species name "sativa" means "planted or sown," indicating that the plant is propagated from seed.[6] For thousands of years, the plant has been utilized for fiber and oil production and for recreational and traditional purposes.[7] In ancient texts, bhang is described as one of the five sacred plants, symbolizing a source of happiness, joy, and salvation.[4] Several synonyms have been described in N*ighantus* (Materia Medica), including "*Matulani*" which denotes its intoxication effect and feminine gender of matul that is dhatura, "*Jaya*," which is noted for its efficacy in treating diarrhoea, "*Samvidamanjari*," referring to flowers that bloom in clusters or inflorescences, "*Bahuvadini*," (delirium), and "*Tandrakrita*", (drowsiness).[8] The *Rasapanchak*, pharmacotherapeutic principles of *Vijaya* are characterized by its *Rasa* (taste) as *Tikta* (bitter), *Guna* (attribute) as *Laghu* (light) and *Tiksna*, its *Vipaka* (post-digestive effect) as *Katu* (pungent), and its *Virya* (potency) as *Usna* (hot).[9] In the textbook *Anankand*, the distinctive characteristics of leaves are articulated as *Ekparna, Dwiparna, Triparna, Panchaparna*, Dashparna, and Triyodash Dala.[10] Approximately 191 formulations having cannabis as major ingredient or minor ingredient in 13 different dosage forms have been used in approximately 29 different diseased conditions, which signifies its beneficial effects.[11,12] The detailed description of *Vijaya*, including its origin, types, synonyms, morphology, cultivation techniques, useful parts, formulations, dose, Symptoms of overdose and treatment, is only referenced in *Anandakanda*, a classical text on *Rasashastra* within Ayurvedic literature.[13] *Vijaya* is included in *Upvisha* Varga, and it is advised to be used after the *shodhana* (method of purification).[11,13,14] The leaves of male and female *C. sativa* plants are called *Bhang; Ganja* is the dried inflorescence of the female plant, and *Charas* is the resin from the cannabis plant.[15] Over 500 components have been identified in cannabis, of which 125 have been classified as cannabinoids. The remaining constituents include non-cannabinoid phenols, flavonoids, terpenes, and alkaloids. Cannabinoids are terpenophenolic compounds that accumulate mainly in trichomes' cavities.[16] The primary compounds are delta 9-tetrahydro cannabinol (Δ^9THC, a psychoactive compound), Cannabidiol (CBD, a non-psychoactive compound), and Cannabinol (CBN). Due to its narcotic and addictive effects, this plant has been restricted for medicinal use. The United Nations' latest "World Drug Report" for 2023 sheds light on global substance use trends, emphasizing the sustained prevalence of drug consumption worldwide. A UN report states that cannabis is the most used substance on earth, it yet remains illegal almost everywhere. Recent research has identified cannabis as possessing medicinal properties that can alleviate nausea and vomiting associated with chemotherapy, as well as chronic pain, muscle spasms, and symptoms related to epilepsy, Alzheimer's disease, inflammatory bowel syndrome, and COVID-19. Additionally, it has been recognized for its sedative and intoxicant effects.[5,17,18] The efficacy of *Vijaya* leaves after *shodhan* (purification) and Ayurvedic formulations containing *Vijaya* leaves as ingredients may be revalidated in different disease conditions. So, there is a need to establish evidence-based scientific studies on *Vijaya* to optimize its use in the medical system. As phytochemical constituents, particularly cannabinoids, in *C. sativa* depend on many factors, such as species type, cultivation techniques, environmental conditions, and methods of collection and storage, standardization and quality control are needed for its revalidation in different disease conditions. Quality standard methods for herbal medicines have been established by the World Health Organization (WHO).[19] Various parameters, such as macroscopic, microscopic, physicochemical, and phytochemical evaluations, biological activity, and heavy metal analysis were used. Therefore, in this study, an evaluation of the pharmacognostic specifications of *C. sativa* leaves for their authenticity, purity, and quality control of plant material for Ayurvedic medicines was performed.

MATERIALS AND METHODS

Plant Sample Collection and Authentication

Plant material, *Vijaya* (*C. sativa*) leaves were collected from M/s Ravinder Chaudhary, a licensed vendor in Haridwar, Uttarakhand, India, after obtaining approval from the State Excise Department. The plant sample was authenticated from FRLHT Bangalore as *Cannabis sativa* L. from the Cannabaceae family, having herbarium specimen FRLH acc. no. 6302 dated 16.01.23. One voucher specimen was submitted to the Department of Dravyaguna, All India Institute of Ayurveda, New Delhi.

Chemical and Reagent

Standard or analytical grade chemicals/reagents used for the pharmacognostic study were procured from Sigma-Aldrich, India.

Pharmacognostical Studies

The collected plant material was washed with tap water to remove dust and foreign material and then dried in the shade. Macroscopic, Organoleptic, Microscopic, and DNA Barcoding analyses were performed in the present study.

Macroscopic Study

Fresh leaves were observed with the naked eye, and observations were recorded.

Organoleptic

The leaves were studied for their organoleptic characteristics, i.e., colour, texture, odour, and taste.

Microscopic study

Free-hand sections of various leaf parts were obtained. Then, photographs of safranin-stained samples were taken using a microscope attached to a primo star HD camera.

DNA BARCODING

DNA barcoding using the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit *(rbc*L) gene is a widely accepted molecular approach for the accurate identification of plant species. This study was conducted at the Centyle Biotech Analysis Laboratory.

Experimental Method

DNA isolation

Genomic DNA was isolated from the leaf samples of the collected plant specimens. The quality of the RNA was evaluated using a 1.0% Agarose Gel. This was started by grinding ~50-100 mg of leaf tissue using liquid nitrogen, followed by lysis with a buffer containing Sodium Dodecyl Sulfate (SDS) and guanidine hydrochloride, which helped break cell walls and denature proteins. The lysate was incubated at 65ºC, and optional RNase treatment was used to remove RNA. After precipitating proteins and contaminants with potassium acetate or ammonium acetate, the clear supernatant was transferred to a silica-based spin column, where the DNA was bound to the membrane upon centrifugation. The column was washed with ethanol-based buffers (70% ethanol) to remove residual impurities, followed by a dry spin to eliminate ethanol traces. DNA was then eluted using nuclease-free water or Tris-EDTA (TE) buffer and collected for quality assessment using a Nanodrop spectrophotometer (A260/A280 ratio of ~1.8-2.0) or agarose gel electrophoresis. This method ensures rapid, high-purity DNA extraction suitable for PCR, qPCR, sequencing, and other molecular biology applications.

PCR Amplification

PCR amplification of the gene for sequencing using *rbc*L primers was performed in a total reaction volume of 25 µL, containing 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of forward and reverse *rbc*L primers, 1 U of Taq DNA polymerase, and 50-100 ng of template DNA. The thermal cycling conditions included initial denaturation at 94 ºC for 3 min, followed by 35 cycles of denaturation at 94 ºC for 30 sec, annealing at 55 ºC for 30 sec, and extension at 72 ºC for 1 min. A final extension step was carried out at 72 ºC for 10 min to ensure complete amplification. The amplified products were confirmed through agarose gel electrophoresis using a 1.5% gel stained with ethidium bromide and visualized under UV light before sequencing. Forward and reverse DNA sequencing reactions of the PCR amplicon were carried out with forward primer (*rbc*L-B (ATGTCACCACAAACAGAAA) and reverse primer ((*rbc*L-724R (TCGCATGTACCTGCAGTAGC) primer using the BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. The consensus sequence of *the rbc*L gene was used to carry out a Basic Local Alignment Search (BLAST) with the National Center for Biotechnology Information (NCBI) gene bank database.

Physicochemical Parameters

Shade-dried *C. sativa* leaves were ground, and the fine powder was passed through a 120 mesh. This powder was stored in an airtight container for further physicochemical tests, such as loss on drying, total ash, acid-insoluble ash, alcohol-soluble extract, and water-soluble extract, as per the recommended protocols of the Ayurvedic Pharmacopeia of India (API) Vol. 1. All parameters were conducted in triplets.

Preliminary Phytochemical Analysis

A 5 g *C. sativa* leaf powder was added to 30 mL of chloroform water and 5 g to 30 mL of methanol (95%) in an Erlenmeyer flask for aqueous and alcohol extracts, respectively. Both flasks were placed on a rotatory shaker and agitated at a constant speed for 6 hr. The mixture remained undisturbed under static conditions for 18 hr to ensure complete analyte-solvent interaction. After a total incubation period of 24 hr, the extract was filtered using Whatman filter paper No. 1 to separate the dissolved constituents from the residual plant matrix. These extracts were used for further studies to detect the presence of constituents such as alkaloids, tannins, proteins, flavonoids, and saponins.

High-Performance Thin-Layer Chromatography (HPTLC)

Phytochemical Fingerprinting

An aliquot of 1 g of *C. sativa* leaf powder was added to 10 mL methanol (95%) in an Erlenmeyer flask, which was placed on a rotatory shaker and agitated at a constant speed for 6 hr. The mixture remained undisturbed under static conditions for 18 hr to ensure complete analyte-solvent interaction. After a total incubation period of 24 hr, the extract was filtered using Whatman filter paper No. 1 to separate the dissolved constituents from the residual plant matrix. The resulting extract was used for High-Performance Thin-Layer Chromatography (HPTLC) analysis.

Chromatographic Conditions

The methanolic extract of *C. sativa* leaves was applied as discrete bands onto a 10 cm × 10 cm Thin-Layer Chromatography (TLC) plate, precoated with silica gel 60 F254 (0.2 mm thickness) at volumes 8 µL using a CAMAG TLC applicator LINOMAT 5, equipped with a 100 µL Hamilton syringe. The plate was developed in a pre-saturated chromatographic chamber with a mobile phase of n-hexane: diethyl ether 8:2(v/v). The solvent was then allowed

to run up to the specified distance on the silica plate. The plate was then air-dried and visualized under visible light, UV light at 254 nm, and long-wave light UV at 366 nm. Seven visible bands were detected under UV light, and the plate was derivatized using an anisaldehyde-sulfuric acid reagent. Post-derivatization, it was dried at 105°C using a CAMAG plate heater. The developed plates were subsequently examined under white light and scanned at 540 nm using a CAMAG Scanner (Muttenz, Switzerland).

Powder Microscopy

The leaf powder was soaked in KOH overnight at room temperature for extraction. The sample was rinsed with distilled water. Subsequently, slides were prepared after staining with safranin, which was then examined under a microscope (40X), and images were captured using a Carl Zeis camera.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy was employed to identify and analyze the functional groups in *C. sativa* leaf powder. Finely ground *C. sativa* leaf powder (<1 g) was placed in the sample holder of a PerkinElmer UATR (Waltham, MA, USA). The sample was then scanned within the mid-infrared (mid-IR) range of 4000-400 cm^{-1} using a deuterated triglycine sulphate detector to ensure optimal signal acquisition.

High Performance Liquid Chromatography

High-Performance Liquid Chromatography (HPLC) was Conducted to quantify Cannabinoid Acid (CBDA), Cannabidiol (CBD), Delta-9-Tetrahydrocannabinol (Δ^9THC) and its precursor Delta-9-Tetrahydrocannabinoid Acid (Δ^9THCA). The sample was prepared by dissolving 0.5 g of the sample in 25 mL acetonitrile in a 50 mL volumetric flask. Then it was kept for sonication for 10 min and made up to 50 mL. The solution was filtered, and 20 µL was injected. Flow rate was 1.0 mL and run time was 60 min Chromatographic analysis was performed using the internal method by HPLC, utilizing an Agilent HPLC-1260 infinity UV instrument. Separation was accomplished using a 4.6X250 mm 5-micron C18 chromatographic column from Agilent Technologies. The mobile phase comprised of A- 0.1% Tri fluoracetic acid in water B- Acetonitrile Isocratic (A: B 30:70) and the column compartment temperature was consistently maintained at 30°C throughout the analysis. A UV detector was used at a wavelength of 228 nm to detect cannabinoid forms.

RESULTS

Macroscopy

The leaves were compound, palmate-shaped, lower opposite 5 to 7 foliate with long rachis, upper alternate 3 to 5 foliate, leaflet 5 to 8 cm long and 0.8 to 1.5 cm in width with linear lanceolate shape, acuminate apex, and serrate margins (Figure 1).

Organoleptic Characteristics

Colour

The leaf's adaxial surface was dark green, while its abaxial surface was light green.

Odour

The leaf's odour is slightly aromatic.

Taste

The leaf's taste is pungent and slightly acrid.

Figure 1: *Cannabis sativa* L., A: Whole plant; B: Herbarium Twig; C: Fan leaf showing abaxial and adaxial surfaces. Reproduce at column width.

Source: Figure 1, Digital photograph of (*Cannabis sativa*) captured by the author and Plant taxonomist (RRDR, AIIA) using a Flatbed Scanner (Expression 12000 XL) at Lab AIIA.

Microscopy of Leaf

The transverse section of the leaflet through the midrib showed a horse shoe shaped outline; cuticle single-layered; epidermis single-layered, cells barrel-shaped, trichomes unicellular, uniseriate; vascular bundle crescent-shaped, bicollateral; followed by parenchymatous ground tissue, cells isodiametric, without intercellular spaces, a few idioblasts containing rosette crystal (Figure 2).

The transverse section through the lamina revealed a single-layered cuticle and a single-layered epidermis with barrel-shaped cells. The trichomes are of two types: (a) glandular and (b) simple (non-glandular). Glandular trichomes were either sessile capitate or capitate with stalks, whereas non-glandular trichomes were either cystolithic and conical or non-cystolithic. The mesophyll parenchyma is categorized into two types: (i) palisade, which is single-layered, and (ii) spongy, also single-layered. The stomata were of the anisocytic type, with a stomatal index ranging from 12.8 to 19. The palisade ratio was between 3 and 7, the number of vein islets was 8 to 10, and the number of vein terminations was 7 to 11 (Figure 2).

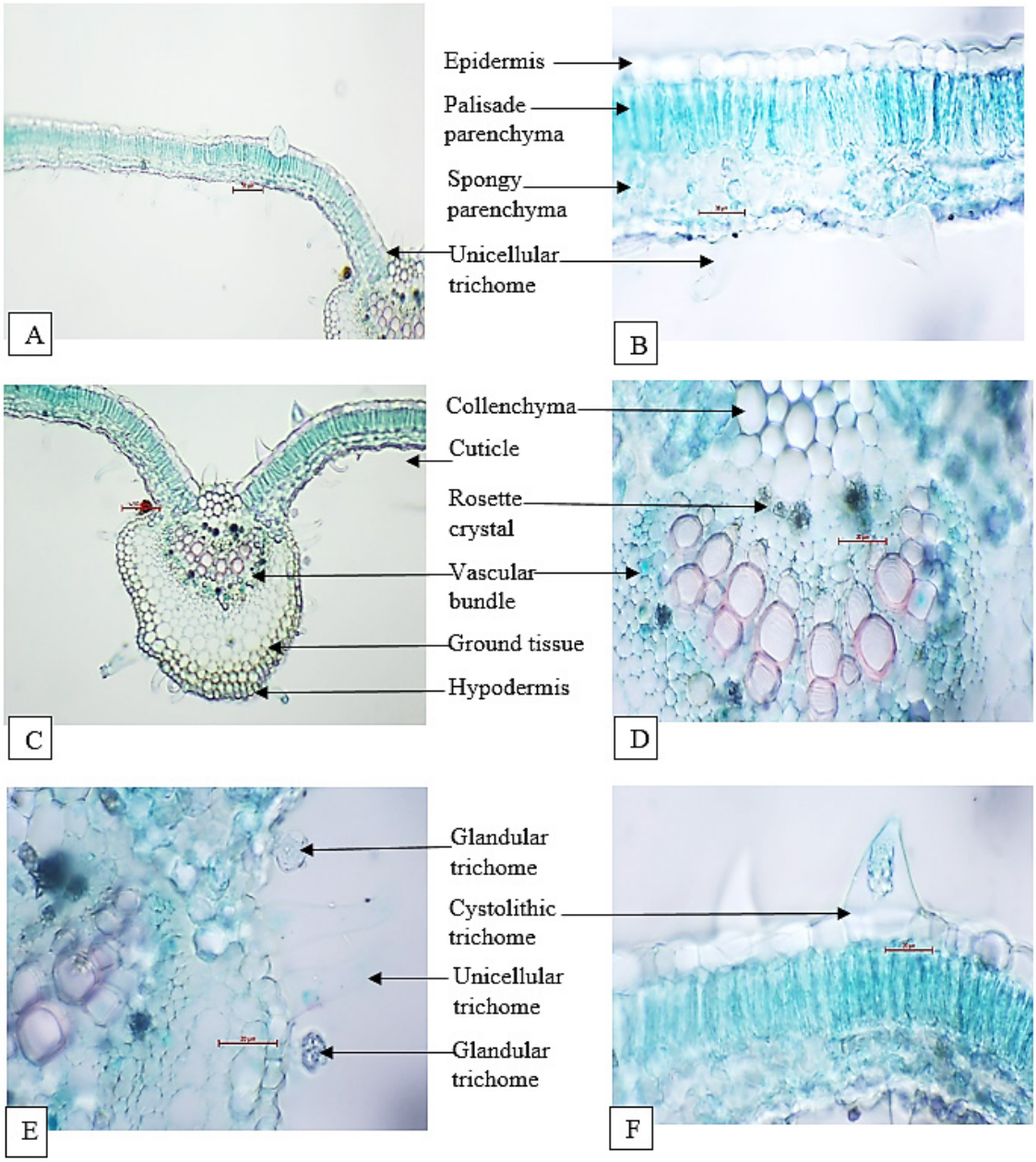

Figure 2: *Cannabis sativa* L., Leaf; A: Leaf, T.S. at 4X through lamina and midrib showing unicellular trichomes; B: T.S. at 40X through lamina showing epidermis, palisade parenchyma, spongy parenchyma, unicellular trichomes; C: T.S. at 10X through midrib and lamina showing cuticle, vascular bundle, ground tissue, hypodermis; D: T.S. at 40X through midrib showing collenchyma, rosette crystals, vascular bundle; E: T.S. at 40X through midrib showing glandular trichomes, unicellular trichomes; F: T.S. at 40X through lamina showing triangular cystolithic trichomes. Reproduce at full page width.

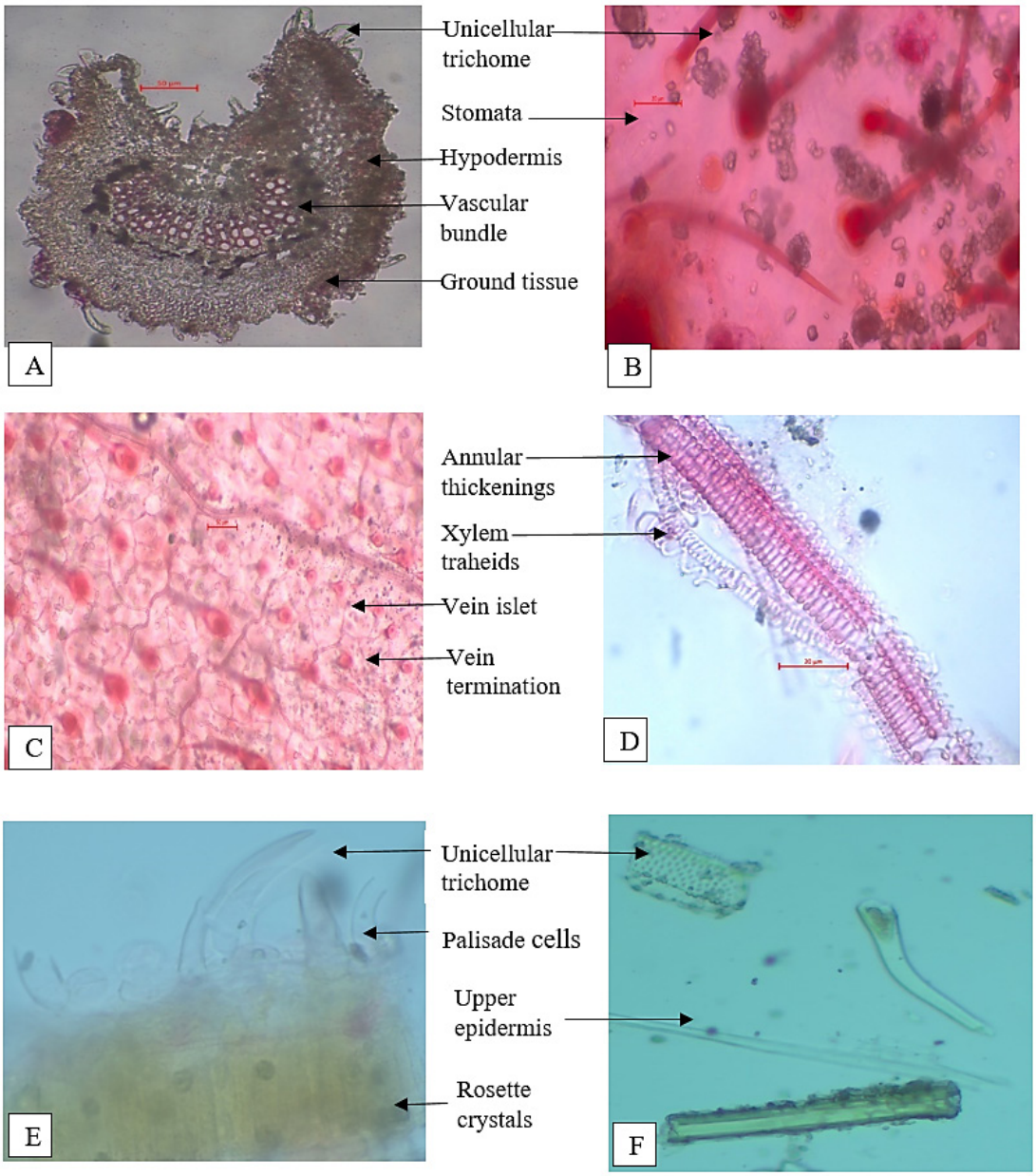

Figure 3: *Cannabis sativa* L., Leaf; A: Petiole, T.S. at 10X showing horse shoe shaped outline, hypodermis, vascular bundle, ground tissue, unicellular trichome; B: Leaf epidermis at 40X stained in safranin showing unicellular trichomes, anisocytic type stomata; C: Leaf epidermis at 10X stained in safranin showing vein islet and vein termination; D: Powder at 40X stained in safranin showing xylem tracheid with annular thickenings; E and F: Powder at 40X stained in safranin showing xylem pits, unicellular trichomes, upper epidermis, palisade cells,rosette crystals. Reproduce at full page width.

The transverse section of the petiole exhibited a horseshoe-shaped outline. The outermost layer, the epidermis, was single-layered with barrel-shaped cells, and the trichomes were unicellular and uniseriate. The vascular bundle was crescent-shaped and bicollateral, followed by parenchymatous ground tissue composed of isodiametric cells without intercellular spaces (Figure 3).

Powder Microscopy

Powder microscopy showed the presence of vascular strands, clusters of calcium oxalate crystals, xylem pits, fragments of upper epidermal cells, palisade cells, tracheids, conical trichomes, glandular trichomes, and spongy parenchyma (Figure 3).

DNA Barcoding

The consensus sequence of the *rbc*L gene established from both forward and reverse sequencing data using aligner software was then utilized to perform a Basic Local Alignment Search Tool (BLAST) analysis against the National Center for Biotechnology Information (NCBI) Gene Bank database. Based on the highest identity scores, the top ten sequences were selected and aligned using the Clustal W multiple alignment software. Subsequently,

a distance matrix was generated and a phylogenetic tree was constructed using MEGA version 7. The aligned sequences of taxonomically identified *C. sativa* leaves were queried for highly similar sequences in GenBank using the NCBI nucleotide BLAST tool. The analysis of nucleotide homology and phylogenetic relationships indicated a high similarity with *Cannabis sativa* L.

Physicochemical Study

The recorded physicochemical properties of *C. sativa* leaf powder were as follows: Loss on Drying, 10.04% w/w; Total Ash, 14.23% w/w; Acid-Insoluble Ash, 3.60% w/w; Alcohol-Soluble Extractive, 28.29%; and Water-Soluble Extractive, 20.48%. These values are in accordance with the limits defined by the API.

Qualitative Phytochemical Analysis

Preliminary phytochemical analysis revealed the presence of flavonoids, alkaloids, proteins, and tannins in the aqueous and alcoholic extracts of *C. sativa* Leaves powder.

High-Performance Thin Layer Chromatography (HPTLC)

High-performance thin-layer chromatography was employed to analyze the methanolic extract of *C. sativa* leaves, identifying six peaks with R_f values 0.05, 0.13, 0.18, 0.39, 0.48, and 0.57, at 254 nm and four peaks with R_f values 0.05, 0.16, 0.24 and 0.48 at 366 nm. After derivatization, dark brown, green, light brown, and pink bands were observed (Figure 4).

Fourier-Transform Infrared (FTIR)

The FTIR spectrum of *C. sativa* leaf powder shows eight distinct peaks revealed a diverse array of functional groups). Prominent absorption at 2918 and 2850 cm^{-1} correspond to aliphatic C-H stretching, suggesting alkane structures, at 1615 and 1566 cm^{-1} corresponds to aromatic C-C and C=C stretching suggesting the presence of unsaturated aromatic structures. The peak at 1373 cm^{-1} reflecting combined NO_2 stretching and C-H deformation, implies contributions from nitro-containing compounds or alkane segments. Additionally, the spectral region between 1255 and 1032 cm^{-1} exhibits pronounced C-N stretching vibrations, which are consistent with aromatic amines (1255 cm^{-1}), secondary amines (1188 cm^{-1}), and primary amines (1032 cm^{-1}).

High-Performance Liquid Chromatography (HPLC)

HPLC analysis revealed the following concentrations: Cannabidiolic Acid (CBDA) 1.30% (13013.17 mg/kg), Cannabidiol (CBD) at 0.77% (7779.08 mg/kg), Δ^9 Tetrahydrocannabinolic Acid (Δ^9THCA) 0.001% (11.24 mg/kg), and Δ^9 Tetrahydrocannabinol (Δ^9THC) at 0.74% (7417.38 mg/kg). HPLC analysis indicated that the sample contained a lower concentration of Δ^9THCA and total THC in comparison to the total CBD content.

DISCUSSION

The growing demand for herbal medicines necessitates rigorous standardization and quality control to ensure their safety, efficacy, and reproducibility. Accurate identification of medicinally important plant species is helpful for their effective use in medicine. Therefore, maintaining consistency in herbal medicines requires a thorough standardization approach that covers both raw materials and the finished products. The present study is a comprehensive proximate analysis of *C. sativa* leaves collected from Uttarakhand to aid in the identification, standardization, and quality control. The botanical description of the *Vijaya* plant, as presented in the *Anandkand* text, refers to it as "*Tri parna*," *"Pancha parna*," "*Das parna*," and "*Triyodash dal.*" These terms indicate the plant's

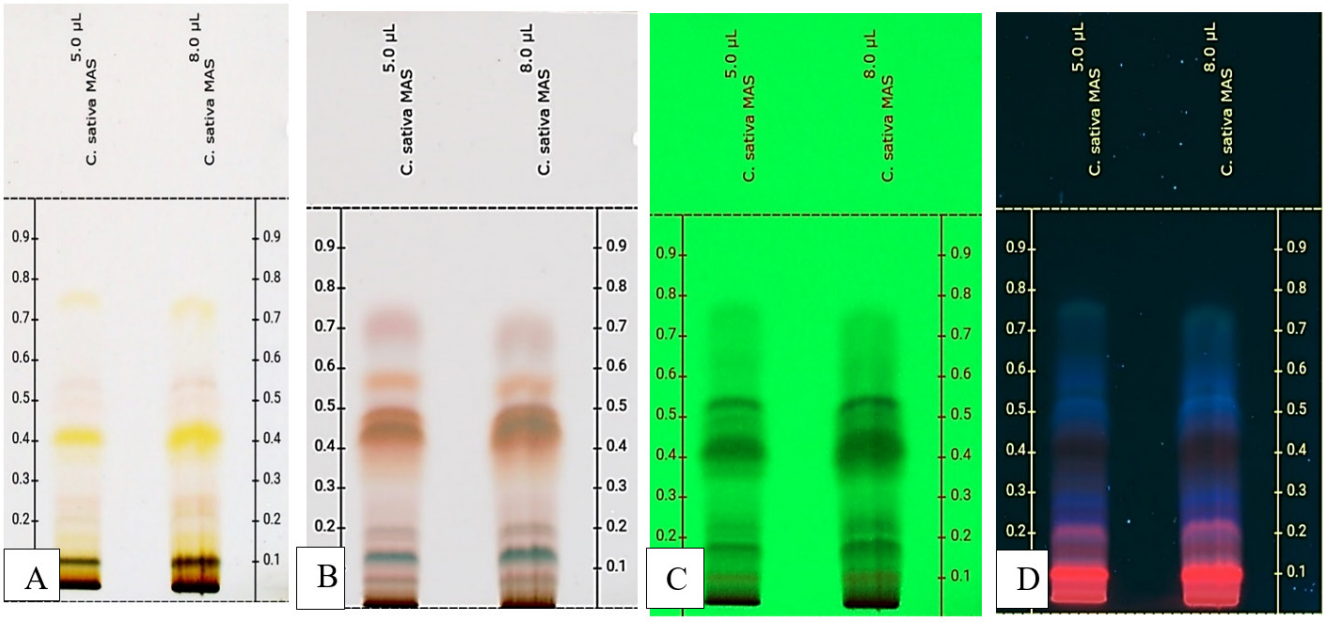

Figure 4: HPTLC profile of *C. sativa* leaf A. under white visible light B. After derivatization; C: Under 254 nm; D: Under 366 nm: Reproduce at column width.

palmate leaves, which exhibit variability in the number of leaflets, specifically 3, 5, 7, 10, and 13. The macroscopic characteristics of the leaves exhibit a palmate structure with serrated margins, dark green on adaxial surface, light green on abaxial surface, The lower leaves are opposite, consisting of 5 to 7 leaflets with a long rachis, whereas the upper leaves are alternate, comprising 3 to 5 leaflets aligned with the characteristics of *C. sativa* leaves as outlined in the *Anandkand*, Quality Standard of Medicinal Plants and The cannabis plants-botanical aspects.[6,13,20,21] Our observation aligns with that of Tavhare (2024), as leaves with a palmate structure, featuring leaflets measuring 5-8 cm in length and 0.8-1.5 cm in width, characterized by acute and serrate margins, can facilitate primary plant authentication. Microscopic examination of *C. sativa* leaves revealed several distinctive features that aid in their identification, including horseshoe-shaped outlines, bicollateral vascular bundles, and anisocytic stomata. Additionally, the leaves exhibit both glandular trichomes, which can be sessile, bulbous, or have long multicellular stalks, and non-glandular trichomes, which may be claw-shaped, cystolithic, or non-cystolithic/conical. Notably, there are abundant conical trichomes on the abaxial surface, and the simultaneous presence of cystolithic on the upper surface and non-cystolithic on the lower surface of the leaf is a characteristic of *C. sativa.*[6,22] The presence of trichomes may be associated with the production and quantity of cannabinoids that accumulate within the trichome cavity.[21] In DNA fingerprinting, the presence of high sequence similarity and strong phylogenetic clustering identified the sample as C*annabis sativa* L. This analysis provides a robust method for species identification using DNA barcoding of *rbc*L gene.[23,24]

Powder microscopy revealed the presence of conical and glandular trichomes, calcium oxalate crystals, xylem vessels, and tracheids, which may be key characteristics of the leaf powder. Physicochemical parameters, such as moisture content, total ash values, and extractive values, were within the pharmacopeial limits as described in API vol.1, indicating purity and minimal contamination by inorganic matter. In contrast to the findings of Tiwari and Rawat (2014), who reported a total ash content of 10.23% and an acid-insoluble ash content of 0.92%, the present study recorded higher values. This discrepancy may be attributed to variations in the plant material, such as the specific leaves that were analyzed in this study, or it could be influenced by geographical area, environmental, or storage conditions. The water-soluble extractive value (20.48%) was found to be lower than the alcohol-soluble extractive value (28.29%), indicating the presence of more alcohol-soluble compounds in the plant material. Preliminary Phytochemical analysis of the powdered material revealed the presence of flavonoids, alkaloids, proteins, saponins, and tannins in both water-soluble and alcohol-soluble extracts. Flavonoids are known for their antioxidant and anti-inflammatory properties.[21-26] In examining the HPTLC fingerprinting profile, seven distinct peaks were detected at a wavelength of 540 nm post-derivatization, corresponding to R_f values of 0.05, 0.12, 0.18, 0.25, 0.45, 0.53, and 0.66. The peak observed at an R_f value of 0.45 is indicative of Cannabinol (CBN), aligning with the R_f value specified for CBN in the Quality standards for medicinal plant.[20] R_f values at 0.40 and 0.48 align with literature-reported Δ^9-THC and CBD values, respectively.[27,28] After derivatisation, the enhanced band intensity for R_f values of 0.65 suggests the possible presence of glycosides. The derivatized plate revealed band colors at the following R_f values: 0.12 (bluish green), 0.18 (slight brown), 0.25 (pink), 0.45 (red-orange), 0.53 (pink), and 0.66 (pink). The bands were not distinctly discernible, as derivatization with the anisaldehyde-sulfuric acid reagent. In contrast, derivatization with the Fast Blue salt B reagent resulted in clearly distinguishable band colours when analyzing the constituents present in *C. sativa*. The HPLC results also confirmed the presence of Δ^9-THC and CBD in the plant material.

The FTIR analysis of the *C. sativa* leaf showed a C-H stretching region (~2850-2918 cm^{-1}), suggesting the presence of alkanes and lipids. Aromatic C- C stretch (1615 cm^{-1}, 1566 cm^{-1}) suggests the presence of aromatic compounds, possibly phenolic compounds or flavonoids. C-N stretch (1254 cm^{-1}, 1187 cm^{-1},1032 cm^{-1}) suggests amines, possibly protein. Therefore, FTIR suggests the presence of organic molecules, likely terpenes, flavonoids and proteins, which contribute their anti-oxidative, anti-inflammatory, anti-mutagenic, antimicrobial, antihyperglycemic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function.[25,29] High-performance liquid chromatography analysis revealed the following concentrations: CBDA 1.30%, CBD at 0.77%, Δ^9THCA 0.001%, and Δ^9THC at 0.74%**.** This indicates that the sample exhibited a lower concentration of Δ^9THC and total THC compared to the CBD and total CBD content. According to the guidelines delineated in the "Recommended Methods for the Identification and Analysis of Cannabis and Cannabis Products" by the United Nations Office on Drugs and Crime (UNODC), the concentration of Δ^9THC in cannabis leaves is typically around 1-2%. The slightly reduced concentration of total Δ^9THC in the plant sample may be attributed to storage or environmental conditions. The ratio of total CBD content to total Δ^9-THC content in *C. sativa leaves* are approximately 5:2, while the ratio of CBD to Δ^9-THC is approximately 1:1. These findings suggest the potential application of this compound as a medical cannabis, which may be beneficial for conditions such as inflammation, seizures, anxiety, chronic pain, nausea, and appetite loss by utilizing the effects of lower concentrations of Δ^9-THC and higher concentrations of CBD. Within conventional medical systems, the isolation and optimization of the Δ^9-THC to CBD ratio have been standardized at 2:1, 1:1, and 1:2 for application in various medical conditions. The leaves of *C. sativa*, when utilized in their entirety, contain cannabinoids (in a 1:1 ratio of Δ^9-THC and CBD), flavonoids, terpenes, sugars, steroids, fatty acids, non-cannabinoid phenols, and nitrogenous compounds that may reinforce a multi-target therapeutic strategy, and their interaction

with the endocannabinoid system could yield beneficial outcomes, potentially endorsing their medicinal use across a range of disease conditions. *Shodhan* (purification) is a procedure referenced in Ayurvedic literature and is recommended prior to the medicinal application of *C. sativa* leaves. This process may aid in reducing the concentration of Δ^9-THC, thereby allowing the leaves to be used medicinally without inducing psychoactive effects. In different studies, it was found that after the purification process, the Δ^9-THC percentage was lower. However, this Evidence-based health benefits of *C. sativa* may lead to consideration of its legal and ethical frameworks.

CONCLUSION

This comprehensive study on *Vijaya* (*Cannabis sativa* L.) leaves was conducted to offer valuable knowledge on their pharmacognostic characteristics, phytochemical composition, and qualitative and quantitative analysis of the main cannabinoids, Δ^9-THC and CBD, for medicinal use. This multifaceted approach, which includes macroscopic, microscopic, DNA barcoding, and various analytical techniques, provides robust data for the standardization and quality control *of C. sativa* leaves. These results lay the foundation for developing evidence-based herbal medicines and may contribute to the integration *of Cannabis sativa* into healthcare system. Further research is required to validate the evidence regarding Ayurvedic formulations containing *Vijaya* (*C. sativa*) and its standalone use.

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ABBREVIATIONS

C. sativa: *Cannabis sativa* L.; **FTIR:** Fourier-Transform Infrared Spectroscopy; **HPTLC:** High-Performance Thin-Layer Chromatography; **HPLC:** High-Performance Liquid Chromatography; **rbcL:** Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase Large Subunit; **BLAST:** Basic Local Alignment Search Tool; **NCBI:** National Centre for Biotechnology Information; **CBDA:** Cannabidiolic Acid; **CBD:** Cannabidiol; **Δ^9THCA:** Δ^9 Tetrahydrocannabinolic Acid; **Δ^9THC:** Δ^9 Tetrahydrocannabinol.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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LIMITATION OF THE STUDY

This study focused on the quantification of only major cannabinoids that is Δ9-THC, CBD, and their precursors. This narrow focus may overlook the potential significance of other compounds. The chemical composition of cannabis plants is influenced by various factors, including geographical location, cultivation practices, the timing of harvest, and storage methods. These variables were not considered in this analysis.

SUMMARY

This study provides detailed characterization of leaves of *Cannabis sativa* L. Using multiple analytical techniques for developing evidence-based herbal medicines containing it. Macroscopic and microscopic analysis confirmed characteristic features of *C. sativa* leaves, including palmate structure, serrated margins, and presence of glandular and non-glandular trichomes. DNA barcoding using the *rbc*L gene confirmed the identity as *Cannabis sativa* L.

Physicochemical parameters were within pharmacopoeial limits, indicating purity. Phytochemical screening revealed presence of flavonoids, alkaloids, proteins, and tannins. FTIR analysis suggested presence of organic molecules like terpenes, flavonoids and proteins.

HPTLC analysis revealed seven distinct peaks, with R_f values indicative of cannabinoids like CBN and CBD. HPLC quantification found CBDA: 1.30%, CBD: 0.77%, Δ^9-THCA: 0.001%, Δ9-THC: 0.74%. The CBD to Δ^9-THC ratio was approximately 1:1. The comprehensive pharmacognostic and analytical data provides a basis for standardization and quality control of *Cannabis sativa* L. leaves for potential Ayurvedic medicinal use.

Further research is needed to validate Ayurvedic formulations containing *Vijaya (C. sativa)* leaves and its standalone use.

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