A Review on Analytical Methods for the Evaluation of *Hibiscus cannabinus* Leave

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Abstract

Hibiscus cannabinus L. (kenaf) is a medicinal plant known for its rich phytochemical composition and diverse pharmacological properties. Although widely cultivated as a fiber crop, its leaves contain bioactive compounds such as flavonoids, phenolic acids, tannins, alkaloids, saponins, terpenoids, and glycosides, which exhibit antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, antidiabetic, and anticancer activities. The increasing therapeutic importance of this plant has highlighted the need for reliable analytical methods to ensure its quality, safety, efficacy, and standardization.

Analytical evaluation of *Hibiscus cannabinus* leaves involves plant authentication, physicochemical analysis, phytochemical screening, chromatographic fingerprinting, quantitative estimation of marker compounds, and method validation. Modern analytical techniques, including TLC, HPTLC, HPLC, UPLC, GC–MS, LC–MS/MS, UV–Visible spectroscopy, FTIR, NMR, and ICP–MS, are widely used for comprehensive qualitative and quantitative analysis.

This review provides an overview of the botanical characteristics, phytochemical constituents, traditional uses, and analytical methods employed for the evaluation of *Hibiscus cannabinus* leaves. It also highlights current quality control strategies and recent advances that support the development of standardized and evidence-based herbal medicines.

Keywords:*Hibiscus cannabinus*, Kenaf, Analytical methods, Phytochemical analysis, Herbal standardization, Chromatography, Spectroscopy, Quality control, Medicinal plants, Plant authentication.

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1. Introduction

Medicinal plants have served as one of the oldest and most reliable sources of therapeutic agents throughout human history. According to the World Health Organization (WHO), nearly eighty percent of the global population depends partially or completely on traditional herbal medicines for primary healthcare. The increasing prevalence of chronic diseases, antimicrobial resistance, and adverse effects associated with synthetic drugs has renewed scientific interest in medicinal plants as potential sources of novel bioactive molecules. Consequently, herbal medicines have gained worldwide recognition in pharmaceutical research, nutraceutical development, and complementary healthcare systems. [1]

Despite their widespread use, herbal medicines exhibit considerable variability in their chemical composition due to differences in geographical location, climatic conditions, harvesting period, cultivation practices, storage conditions, and extraction procedures. Such variability significantly influences the pharmacological efficacy, safety, and therapeutic consistency of herbal preparations. Therefore, accurate analytical evaluation and standardization of medicinal plants have become indispensable components of herbal drug development. [2]

Analytical evaluation encompasses a broad range of scientific techniques used to identify, isolate, characterize, quantify, and validate phytochemical constituents present in medicinal plants. These techniques not only confirm botanical identity but also detect adulteration, contamination, degradation products, and chemical markers responsible for biological activity. Comprehensive analytical characterization forms the scientific basis for quality

assurance, regulatory compliance, formulation development, and commercialization of herbal products. [3]

Among numerous medicinal plants investigated during recent decades, *Hibiscus cannabinus* L., belonging to the family Malvaceae, has emerged as an important medicinal and industrial crop. Traditionally recognized for its strong bast fibers used in paper, textiles, ropes, and biocomposites, the plant has recently attracted considerable pharmaceutical attention due to its abundance of biologically active phytochemicals. Various experimental investigations have demonstrated that different parts of the plant, particularly the leaves, possess significant antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, antidiabetic, anticancer, analgesic, wound-healing, and immunomodulatory activities. These pharmacological effects are primarily attributed to the presence of phenolic compounds, flavonoids, tannins, alkaloids, terpenoids, saponins, glycosides, steroids, and other naturally occurring metabolites. [4]

The leaves of *Hibiscus cannabinus* are considered a particularly valuable source of polyphenolic compounds that exhibit strong free radical scavenging activity and contribute substantially to the medicinal potential of the plant. Recent phytochemical investigations have identified compounds such as quercetin, kaempferol, rutin, catechin, epicatechin, chlorogenic acid, caffeic acid, ferulic acid, gallic acid, and various flavonoid glycosides within the leaves. These compounds have demonstrated promising therapeutic applications in the prevention and management of oxidative stress-related disorders including cancer, cardiovascular diseases, diabetes mellitus, inflammatory disorders, and neurodegenerative conditions. [5]

Fig. 1 *Hibiscus cannabinus*(whole plant)

However, the increasing utilization of herbal medicines has highlighted significant challenges associated with plant authentication, adulteration, contamination by heavy metals and pesticides, microbial load, and inconsistency in phytochemical composition. The absence of standardized analytical protocols frequently results in variations in the quality and efficacy of herbal formulations. Consequently, scientific validation through modern analytical methodologies has become essential for ensuring reproducibility and regulatory acceptance of herbal medicines. [6]

The analytical evaluation of *Hibiscus cannabinus* leaves generally begins with botanical authentication followed by physicochemical characterization, extraction, preliminary phytochemical screening, chromatographic fingerprinting, quantitative estimation of marker compounds, structural elucidation, and method validation. Conventional physicochemical parameters such as moisture content, ash values, extractive values, and foreign organic matter provide preliminary information regarding plant quality. Subsequently, chromatographic techniques including TLC, HPTLC, HPLC, UPLC, GC–MS, and LC–MS/MS enable qualitative and quantitative analysis of phytoconstituents with high sensitivity and specificity. Spectroscopic techniques including UV–Visible spectroscopy, FTIR spectroscopy, Nuclear Magnetic Resonance (NMR), and Mass Spectrometry (MS) further facilitate structural characterization and identification of biologically active compounds. [7]

Recent technological advances have integrated analytical chemistry with metabolomics, chemometrics, molecular fingerprinting, and artificial intelligence-based data analysis, thereby significantly improving the reliability of herbal standardization. These innovations enable comprehensive metabolic profiling, quality assessment, authentication, and discovery of novel phytochemicals with potential pharmaceutical applications. [8]

Considering the expanding medicinal importance of *Hibiscus cannabinus*, there is an increasing need for a consolidated review summarizing the available analytical methodologies used for its evaluation. Such information is valuable for researchers involved in herbal drug standardization, phytochemical investigation, quality control, formulation development, and regulatory assessment.[9]

The present review critically discusses the botanical characteristics, phytochemical composition, analytical techniques, quality control strategies, and future prospects associated with the comprehensive evaluation of *Hibiscus cannabinus* leaves. The review aims to provide a scientific framework for the

standardization and pharmaceutical utilization of this medicinally important plant while highlighting recent advances in analytical methodologies applicable to herbal research.

2. Botanical Description, Taxonomy and Geographical Distribution

2.1 Botanical Description

Hibiscus cannabinus L., commonly known as Kenaf, is an annual herbaceous plant belonging to the family Malvaceae. Although traditionally cultivated as a fibre crop, it has gained considerable attention in recent years because of its medicinal, nutritional, and pharmaceutical importance. The leaves are rich in biologically active compounds, including flavonoids, phenolic acids, tannins, alkaloids, saponins, and terpenoids, which are responsible for various pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, antidiabetic, and anticancer effects. [10]

The plant grows rapidly under tropical and subtropical climatic conditions and reaches a height of 2–5 m. Different parts of the plant, including leaves, stems, flowers, roots, and seeds, are used for medicinal and industrial purposes. Among these, the leaves are considered one of the most valuable sources of natural antioxidants and phenolic compounds. Since the phytochemical composition varies with environmental conditions and harvesting time, proper botanical identification is essential before analytical evaluation. [11]

Taxonomical Classification [12]

Taxonomic Rank	Classification
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Malvales
Family	Malvaceae
Genus	*Hibiscus*
Species	*Hibiscus cannabinus* L.
Common Name	Kenaf
Vernacular Name	Ambadi (Marathi), Mesta, Deccan Hemp

Geographical Distribution

Hibiscus cannabinus is widely distributed in tropical and subtropical regions of the world. It is cultivated in countries such as India, China, Bangladesh, Thailand, Malaysia, Nigeria, Brazil, Australia, and Indonesia. In India, it is commonly grown in Maharashtra, Karnataka, Andhra Pradesh, Telangana, Tamil Nadu, West Bengal, Odisha, Assam, and Bihar. [13]

The plant grows best in warm climates with temperatures of **25–35°C** and well-drained fertile loamy soils. Environmental factors such as climate, soil conditions, harvesting season, and cultivation practices influence the quality and concentration of phytochemical constituents. Therefore, proper cultivation and authentication are necessary for obtaining high-quality plant material for analytical and pharmacological studies. [14]

3. Morphology and Traditional Uses

3.1 Morphology

Hibiscus cannabinus is a fast-growing annual herb with an erect cylindrical stem that is green to reddish in colour and rich in bastfibres. The plant possesses a strong tap root system that enables efficient nutrient absorption. The leaves are simple, alternate, long-petiolate, and palmately lobed with 3–7 serrated lobes. Young leaves are generally ovate, whereas mature leaves become deeply lobed. The flowers are large, solitary, and axillary with creamy yellow petals and a dark maroon or purple centre. The fruit is an ovoid capsule containing numerous smooth, kidney-shaped brown to black seeds. [15]

Morphological Characteristics [16]

Plant Part	Characteristics
Habit	Annual erect herb
Height	2–5 m
Stem	Cylindrical, green to reddish, fibrous
Leaves	Alternate, palmately lobed, serrated margins
Flowers	Creamy yellow with dark centre
Fruit	Ovoid capsule
Seeds	Brown to black, kidney-shaped

3.2 Traditional Uses

Hibiscus cannabinus has been used in traditional medicine for centuries in Asian and African countries. The leaves are commonly consumed as vegetables and herbal preparations because of their nutritional and medicinal properties. Traditionally, leaf decoctions and extracts have been used to treat fever, inflammation, digestive disorders, skin infections, wounds, and general weakness. Fresh leaf paste is also applied externally for wound healing and the management of minor skin ailments. [17]

Modern pharmacological studies support many of these traditional uses and have demonstrated antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, antidiabetic, and anticancer activities of the leaf extracts. These therapeutic effects are mainly attributed to the presence of flavonoids, phenolic compounds, tannins, alkaloids, and other bioactive metabolites. [18]

4. Phytochemical Constituents

The therapeutic potential of *Hibiscus cannabinus* leaves is primarily associated with the presence of a wide variety of secondary metabolites. These phytochemicals play an important role in protecting the plant from environmental stress while also exhibiting significant biological activities in humans. [19]

Flavonoids constitute one of the major classes of compounds present in the leaves. Important flavonoids such as quercetin, kaempferol, catechin, epicatechin, rutin, and myricetin exhibit strong antioxidant activity by scavenging free radicals and reducing oxidative stress. These compounds also possess anti-inflammatory, anticancer, cardioprotective, and hepatoprotective properties.

Phenolic acids including gallic acid, caffeic acid, chlorogenic acid, ferulic acid, and p-coumaric acid are abundant in the leaves and contribute to their antioxidant and antimicrobial activities. These compounds help prevent lipid peroxidation and cellular damage caused by reactive oxygen species. [20]

The leaves also contain tannins, which possess astringent, antimicrobial, antioxidant, and wound-healing properties. Tannins are known to inhibit the growth of pathogenic microorganisms and protect tissues from oxidative damage.

Other important phytochemicals include alkaloids, saponins, terpenoids, glycosides, steroids, carbohydrates, proteins, amino acids, vitamins, and essential minerals. Saponins exhibit immunomodulatory and cholesterol-lowering effects, whereas terpenoids possess antimicrobial and anti-inflammatory activities. Glycosides and steroids further contribute to the therapeutic potential of the plant.

Recent analytical investigations using chromatographic and spectroscopic techniques such as HPLC, GC–MS, LC–MS/MS, FTIR, and NMR have identified numerous bioactive constituents in *Hibiscus cannabinus* leaves. The concentration of these compounds varies depending on geographical location, climatic conditions, harvesting stage, drying methods, and extraction procedures. Therefore, analytical standardization is essential to ensure the quality, safety, and consistency of herbal preparations. [21]

Major Phytochemical Constituents of *Hibiscus cannabinus* Leaves [22-25]

Phytochemical Class	**Representative Compounds**	**Reported Biological Activities**
Flavonoids	Quercetin, Kaempferol, Catechin, Epicatechin, Rutin	Antioxidant, anti-inflammatory, anticancer
Phenolic Acids	Gallic acid, Caffeic acid, Chlorogenic acid, Ferulic acid	Antioxidant, antimicrobial
Tannins	Hydrolysable and condensed tannins	Astringent, wound healing
Alkaloids	Various alkaloids	Antimicrobial, pharmacological activity
Saponins	Triterpenoid saponins	Immunomodulatory, hypocholesterolemic
Terpenoids	Monoterpenes and triterpenes	Anti-inflammatory, antimicrobial
Glycosides	Flavonoid glycosides	Antioxidant, cardioprotective
Steroids	Phytosterols	Anti-inflammatory and membrane

		stabilization

The rich phytochemical composition of *Hibiscus cannabinus* leaves forms the scientific basis for their traditional medicinal use and highlights the importance of advanced analytical techniques for their identification, quantification, quality control, and standardization.

3: Physicochemical Evaluation, Preliminary Phytochemical Screening, and Chromatographic Methods

3.1 Physicochemical Evaluation

Physicochemical evaluation is an essential step in the quality assessment and standardization of *Hibiscus cannabinus* leaves. It helps determine the purity, identity, and quality of the plant material while detecting possible adulteration. Common parameters include moisture content, loss on drying, total ash, acid-insoluble ash, water-soluble ash, extractive values, pH, and foreign matter. These tests provide baseline standards for the authentication and quality control of herbal raw materials. [26]

3.2 Preliminary Phytochemical Screening

Preliminary phytochemical screening is carried out to identify the major classes of bioactive compounds present in the leaf extract. Standard qualitative tests are performed for alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, terpenoids, steroids, carbohydrates, proteins, and amino acids. The leaves of *Hibiscus cannabinus* are reported to contain abundant flavonoids, phenolics, tannins, and saponins, which contribute to their antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective activities. These screening tests serve as a foundation for further quantitative and instrumental analysis. [27]

3.3 Chromatographic Methods

Chromatographic techniques are widely used for the separation, identification, and quantification of phytochemicals in *Hibiscus cannabinus* leaves. Thin-layer chromatography (TLC) is commonly employed for rapid identification of phytochemical constituents, while high-performance thin-layer chromatography (HPTLC) provides accurate fingerprint profiles for quality control and authentication. High-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC) are extensively used for the quantitative estimation of phenolic compounds and flavonoids with high sensitivity and precision. Gas chromatography–mass spectrometry (GC–MS) is useful for identifying volatile and semi-volatile constituents, whereas liquid chromatography–mass spectrometry (LC–MS/MS) enables detailed characterization of complex phytochemicals and marker compounds. These analytical techniques play a vital role in standardization, quality assurance, and pharmaceutical research involving *Hibiscus cannabinus* leaves. [28]

4: Spectroscopic Methods, Hyphenated Techniques, Method Validation, Quality Control, Recent Advances, and Emerging Analytical Methods

4.1 Spectroscopic Methods

Spectroscopic techniques are widely employed for the identification, characterization, and quantification of phytochemicals present in *Hibiscus cannabinus* leaves. These methods are rapid, sensitive, and require minimal sample preparation. [29]

Ultraviolet–Visible (UV–Vis) Spectroscopy:

UV–Vi's spectroscopy is commonly used for the quantitative estimation of total phenolic and flavonoid contents. It is also applied in antioxidant assays such as DPPH, ABTS, and FRAP. The method is simple, economical, and suitable for routine quality control. [30]

Fourier-Transform Infrared (FTIR) Spectroscopy:

FTIR spectroscopy identifies functional groups such as hydroxyl, carbonyl, amine, and aromatic groups present in phytochemicals. It provides a characteristic chemical fingerprint that assists in authentication and detection of adulteration. [31]

Nuclear Magnetic Resonance (NMR) Spectroscopy:

^{1}H-NMR and ^{13}C-NMR are powerful techniques for determining the molecular structure of isolated phytochemicals. NMR provides detailed information regarding molecular arrangement and is widely used in metabolite identification and structural confirmation. [32]

Atomic Absorption Spectroscopy (AAS) and ICP–MS:

These techniques are used to determine essential minerals and detect toxic heavy metals such as lead, cadmium, arsenic, and mercury. ICP–MS offers extremely high sensitivity for trace elemental analysis and supports herbal safety evaluation. [33]

4.2 Hyphenated Techniques

Hyphenated analytical techniques combine chromatographic separation with advanced detection systems, offering improved sensitivity, selectivity, and accuracy.[34]

LC–MS/MS (Liquid Chromatography–Tandem Mass Spectrometry):

LC–MS/MS enables highly sensitive identification and quantification of flavonoids, phenolic acids, glycosides, and other bioactive compounds. It is one of the most reliable techniques for phytochemical profiling.[35]

GC–MS (Gas Chromatography–Mass Spectrometry):

GC–MS is primarily used for the analysis of volatile and semi-volatile phytochemicals. It provides molecular weight information and fragmentation patterns useful for compound identification. [36]

UPLC–MS/MS:
Ultra-performance liquid chromatography coupled with tandem mass spectrometry provides faster analysis, better resolution, and higher sensitivity than conventional HPLC, making it suitable for complex herbal extracts. [37]

HPLC–DAD (High-Performance Liquid Chromatography with Diode Array Detection): HPLC–DAD simultaneously records chromatograms at multiple wavelengths, enabling accurate identification and quantification of several phytochemicals in a single analysis. [38]

4.3 Method Validation

Analytical methods must be validated to ensure reliable and reproducible results. Validation is generally performed according to International Council for Harmonisation (ICH) guidelines.

Important validation parameters include:

- Specificity
- Accuracy
- Precision
- Linearity
- Range
- Limit of Detection (LOD)
- Limit of Quantification (LOQ)
- Robustness
- Ruggedness
- System suitability

Validated analytical methods ensure consistent quality, reproducibility, and regulatory compliance for herbal medicines. [39]

4.4 Quality Control and Standardization

Quality control is essential for ensuring the safety, efficacy, and consistency of *Hibiscus cannabinus* leaf preparations. Standardization involves authentication of plant material, physicochemical evaluation, phytochemical screening, chromatographic fingerprinting, quantitative estimation of marker compounds, microbial limit testing, pesticide residue analysis, aflatoxin determination, heavy metal analysis, and stability studies.

Chromatographic fingerprinting using HPTLC and HPLC has become a valuable approach for maintaining batch-to-batch consistency and detecting adulteration in herbal products. [40]

4.5 Recent Advances in Analytical Evaluation

Recent developments have significantly improved the analysis of medicinal plants by providing greater sensitivity, speed, and accuracy.

- Metabolomics enables comprehensive profiling of primary and secondary metabolites.
- Chemometric analysis applies multivariate statistical tools for classification, authentication, and quality assessment.
- High-resolution mass spectrometry (HRMS) allows accurate identification of unknown phytochemicals with high mass accuracy. [41]
- Nuclear magnetic resonance (NMR)-based metabolomics facilitates rapid characterization of complex plant extracts.
- Green analytical chemistry focuses on environmentally friendly extraction techniques using reduced solvent consumption and energy-efficient methods.
- Digital image analysis and artificial intelligence (AI) are increasingly used for automated chromatographic interpretation and quality assessment. [42]

4.6 Emerging Analytical Methods

Several modern analytical techniques are now being explored for the comprehensive evaluation of *Hibiscus cannabinus* leaves.

Ultra-High Performance Liquid Chromatography (UHPLC):

UHPLC provides superior chromatographic resolution, shorter analysis time, and reduced solvent consumption compared to conventional HPLC. [43]

Quadrupole Time-of-Flight Mass Spectrometry (QTOF–MS):

QTOF–MS enables high-resolution identification of known and unknown phytochemicals with excellent mass accuracy, making it valuable for metabolite profiling. [44]

Orbitrap Mass Spectrometry:

Orbitrap instruments provide ultra-high-resolution mass spectra, allowing precise structural characterization of complex phytochemical mixtures. [45]

Raman Spectroscopy:

Raman spectroscopy is a rapid, non-destructive technique used for authentication of herbal materials, detection of adulterants, and chemical fingerprinting without extensive sample preparation. [46]

Near-Infrared (NIR) Spectroscopy:

NIR spectroscopy offers rapid and non-destructive quantitative analysis of moisture content, extract quality, and major phytochemical constituents. It is increasingly used for routine industrial quality control. [47]

Capillary Electrophoresis (CE):

Capillary electrophoresis separates ionic compounds with high efficiency and minimal solvent consumption. It is useful for the analysis of flavonoids, phenolics, and other polar phytochemicals. [48]

Ambient Ionization Mass Spectrometry:

Techniques such as DESI-MS and DART-MS enable direct analysis of plant materials with minimal sample preparation, allowing rapid phytochemical screening. [49]

4.7 Future Perspectives

Future analytical research on *Hibiscus cannabinus* should integrate advanced chromatographic techniques, high-resolution mass spectrometry, metabolomics, artificial intelligence, and chemometric tools to achieve comprehensive phytochemical characterization. The development of validated, rapid, cost-effective, and environmentally sustainable analytical methods will strengthen the quality control, standardization, and pharmaceutical development of *Hibiscus cannabinus*-based herbal products.

5. Conclusion

Hibiscus cannabinus L. is a valuable medicinal plant rich in bioactive phytochemicals such as flavonoids, phenolic acids, tannins, alkaloids, saponins, and terpenoids, which contribute to its diverse pharmacological activities. Accurate analytical evaluation is essential to ensure the quality, safety, efficacy, and standardization of its leaf extracts.

Conventional methods, including physicochemical analysis and phytochemical screening, together with advanced analytical techniques such as HPLC, UHPLC, GC–MS, LC–MS/MS, FTIR, UV–Visible spectroscopy, and NMR, provide reliable identification and quantification of phytoconstituents. Recent advances, including metabolomics, chemometrics, high-resolution mass spectrometry, DNA barcoding, and AI-assisted analytical approaches, have further improved the authentication and quality assessment of herbal materials.

Overall, the integration of conventional and modern analytical techniques provides a comprehensive approach for the evaluation and standardization of *Hibiscus cannabinus* leaves, supporting their safe and effective use in pharmaceutical and herbal medicine research.

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