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Comparative Phytochemical and Structural Characterization of Powder and Ethanolic Extract of *Hedyotis diffusa*

KANIGA PANDI*, NIMITHASREE KEERTHIVASAN, MRIDULA DHANAPAL SARAVANAN and DHANAVARSHA GOVINDARAJ

Department of Pharmaceutical Chemistry, Saveetha College of Pharmacy, SIMATS, Chennai-602105, India

*Corresponding author: E-mail: kanigapharma@gmail.com

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The present investigation focuses on the comparative phytochemical and structural evaluation of *Hedyotis diffusa* in powder form and its ethanolic extract. A combination of phytochemical screening and analytical techniques, namely Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and scanning electron microscopy (SEM), was utilized to examine their chemical composition and surface characteristics. FTIR analysis revealed the presence of important functional groups, including hydroxyl, carbonyl, and phenolic constituents, as indicated by prominent absorption bands at 3400 cm^{-1} (O–H stretching), 1635 cm^{-1} (C=O stretching), and 1050 cm^{-1} (C–O stretching). These groups are associated with potential involvement in metal ion interaction and stabilization mechanisms. The XRD pattern of the powdered sample exhibited well-defined diffraction peaks, confirming its semi-crystalline structure, while the ethanolic extract showed a predominantly amorphous profile due to the abundance of organic compounds. SEM observations indicated an irregular and finely textured surface morphology, which may contribute to increased surface activity. The comparative findings underscore notable differences between the two forms, offering valuable insight into their physico-chemical and functional properties.

Keywords: *Hedyotis diffusa*, Phytochemical analysis, Characterization.

INTRODUCTION

The herb *Hedyotis diffusa* Wild. (Rubiaceae) is widely distributed in the southern regions of China and has long been recognised as an important folk remedy [1]. Traditionally, it has been used for its heat-clearing, detoxifying, pain-relieving, mass-dispelling, diuretic and dehumidifying properties [2]. In traditional Chinese and Ayurvedic medicine, *H. diffusa* has been prescribed for the treatment of various ailments, including urinary tract infection, lung, asthma and cough, appendicitis, gastroenteritis, sore throat, intestinal carbuncles and diarrhea and even malignant tumors [2]. Phytochemical investigations have identified more than 180 bioactive compounds from *H. diffusa*, containing volatile oils, polysaccharides, phenolics, anthraquinones, iridoids and flavonoids [3-8]. These phytoconstituents contribute to its diverse pharmacological activities, such as anti-inflammatory, antioxidant, hepatoprotective, anti-angiogenic, apoptotic and anticancer effects [3,8-16].

Understanding the properties of plant materials is a key step in green nanoparticle synthesis, as their chemical composition strongly affects the nanoparticles form, remain stable and function. Plants contain a range of bioactive compounds, such as alkaloids, flavonoids, terpenoids, phenolics and proteins, that play an important role in reducing metal ions and stabilising the formed particles. To explore these roles in detail, modern techniques like FTIR, XRD and SEM are commonly employed. FTIR helps in the identification of the functional groups responsible for the reduction processes, XRD provides information about crystalline structure and SEM offers insight into surface features and texture. Together, these analyses enable a better understanding of plant-mediated synthesis and help achieve more consistent nanoparticle characteristics [17].

As described earlier, *H. diffusa* is known for its diverse range of bioactive compounds and established biological properties, making it an attractive candidate for the development of new therapeutic materials. The presence of polyphenols, flavonoids, and similar constituents suggests that the plant can effectively participate in reduction and stabilisation processes, which are important for eco-friendly nanoparticle formation. To utilise such potential effectively, a clear under-

standing of its chemical and structural characteristics is necessary. In this study, both the powdered plant material and its ethanolic extract were systematically analysed to explore their composition and physical properties. Techniques such as Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and scanning electron microscopy (SEM) were used to identify functional groups, evaluate crystallinity and observe surface morphology. By comparing these two forms, the study aims to link the presence of bioactive compounds with their possible role in the green synthesis of nanoparticles and its applications.

EXPERIMENTAL

All chemicals and solvents employed in this work were of analytical grade and used without any additional purification. The functional groups present in *Hedyotis diffusa* powder and its ethanolic extract were examined using Fourier Transform Infrared (FTIR) spectroscopy. For analysis, finely powdered samples were blended with spectroscopic-grade KBr in a 1:100 ratio and compressed into pellets, followed by scanning in the 4000-400 cm^{-1} range using a Shimadzu IR Affinity-1 instrument. The crystalline characteristics and phase composition were investigated through X-ray diffraction (XRD) using a PANalytical X'Pert PRO diffractometer equipped with Cu*K*α radiation (λ = 1.5406 Å), operating at 40 kV and 30 mA, within a 2θ range of 10-80º. Surface morphology and microstructural details were further analysed using scanning electron microscopy (SEM) on a JEOL JSM-6390LV microscope at an accelerating voltage of 10-15 kV, where samples were mounted on aluminium stubs and coated with a thin layer of gold to enhance conductivity.

Plant collection: The whole *Hedyotis diffusa* plant was gathered from Megamalai Hills of the Western Ghats in Tamil Nadu state of India and a certified botanist verified its authenticity. The collected plant material was properly cleaned with distilled water to remove dirt and other impurities. After 15 days of shade drying at room temperature to preserve its phytochemical integrity, it was ground into a powder for use in further extraction processes.

Preparation of ethanolic extract: The dried plant material was then finely powdered using a mechanical grinder. The ethanolic extract was prepared using the cold maceration method by soaking 15 g of powdered plant material in 250 mL of ethanol. The mixture was kept at room temperature for 48 h with occasional shaking to facilitate efficient extraction. After the extraction period, the mixture was filtered and the obtained extract was collected and stored under refrigeration.

Phytochemical screening: Phytochemical screening was conducted to identify the biologically active constituents associated with medicinal properties. Qualitative analysis of the plant extracts was performed to detect major classes of secondary metabolites, using standard biochemical tests for the identification of specific compounds [18].

RESULTS AND DISCUSSION

The phytochemical analysis of *Hedyotis diffusa* in both powdered form and ethanolic extract revealed the presence of several important secondary metabolites associated with its biological activity. Compounds such as flavonoids, phenolics, alkaloids, tannins, terpenoids, saponins and glycosides were identified in both samples, while steroids and carbohydrates were present in moderate amounts. A significant difference was observed in their relative abundance, with the ethanolic extract showing a higher concentration of these constituents compared to the crude powder (Table-1). This suggests that ethanol is more effective in extracting bioactive compounds, likely due to its ability to dissolve a broader range of phytochemicals.

The enriched presence of these metabolites, particularly phenolic and terpenoid compounds, indicates an enhanced potential of the extract to participate in reduction and stabilisation processes. In addition, triterpenoids such as ursolic acid, previously reported in *H. diffusa*, further support its role as a natural reducing and stabilising agent [19]. Thus, these findings highlight the ethanolic extract as a richer source of functional phytochemicals, making it more suitable for applications where such bioactive compounds are required.

FTIR spectral studies: The FTIR spectra of *H. diffusa* powder and its ethanolic extract (Fig. 1a-b) reveal significant similarities in functional group distribution, alongside distinct differences in peak intensity and resolution. Both samples exhibit a broad absorption band in the 3397-3375 cm^{-1} region, corresponding to O–H stretching vibrations, confirming the presence of hydroxyl-rich compounds such as phenolics and alcohols. The bands observed 2974-2925 cm^{-1} region in both spectra are attributed to aliphatic C–H stretching, indicating

TABLE-1
QUALITATIVE PHYTOCHEMICAL SCREENING DATA OF *Hedyotis diffusa* POWDER AND ITS ETHANOLIC EXTRACT

Phytochemical constituents	Powder	Ethanolic extract	Inference
Alkaloids	++	+++	Higher concentration in extract
Flavonoids	++	+++	Efficiently extracted by ethanol
Phenolic compounds	++	+++	Major antioxidant components
Tannins	+	++	Moderately present
Terpenoids (ursolic acid)	++	+++	Supports reducing and stabilizing potential, contribute to metal ion reduction
Saponins	+	++	Slightly higher in extract
Glycosides	++	++	Present in both forms
Steroids	+	++	Extract enhances solubility
Carbohydrates	+	+	Trace amounts in both
Proteins & amino acids	+	++	Indicate bioactivity support

(+) = present; (++) = moderately present; (+++) = highly present

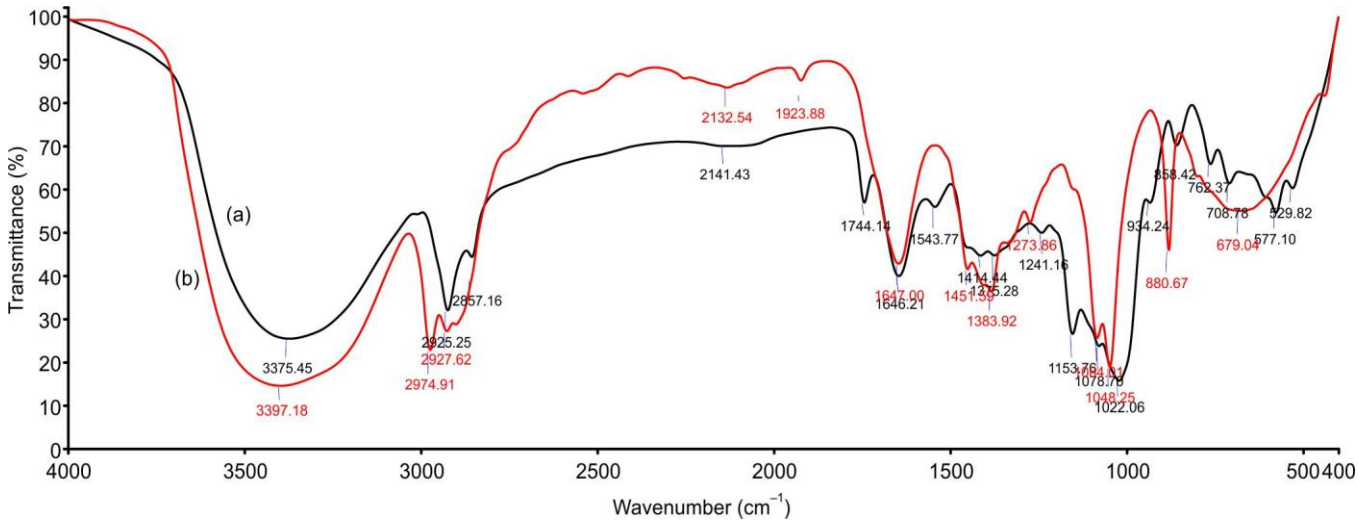

Fig. 1. FTIR spectra of (a) *H. diffusa* powder and (b) ethanolic extract of *H. diffusa*

the presence of hydrocarbon chains and terpenoid constituents. A prominent peak around 1648-1647 cm^{-1} is evident in both cases, which can be assigned to C=O stretching vibrations of carbonyl-containing compounds, including flavonoids and proteinaceous materials. Moreover, the absorption bands within the range of 1450-1375 cm^{-1} correspond to C–N stretching and O–H bending vibrations, while peaks between 1240-1040 cm^{-1} are associated with the C–O and C–O–C stretching of alcohols, ethers and glycosidic linkages. The fingerprint region below 1000 cm^{-1} further supports the presence of complex aromatic and aliphatic frameworks in both samples.

Despite these similarities, a clear distinction is observed in the spectral profiles. The ethanolic extract exhibits sharper, more intense and well-defined peaks compared to the powder, indicating a higher concentration and better availability of phytochemical constituents. In contrast, the powdered sample shows relatively broader and less resolved bands, likely due to the presence of a complex and heterogeneous matrix. Thus, the comparative enhancement in peak clarity and intensity in the extract suggests that ethanol effectively solubilises and concentrates bioactive compounds, particularly phenolics, flavonoids, and terpenoids. These functional groups are known to play a significant role in reduction and stabilisation processes. Therefore, the ethanolic extract demonstrates greater functional potential than the crude powder, highlighting its suitability as an active phytochemical source for green synthesis and related biomedical applications.

XRD studies: The X-ray diffraction (XRD) patterns of *H. diffusa* powder and its ethanolic extract (Fig. 2a-b) reveal distinct differences in their structural organization and degree of crystallinity. The powdered sample exhibits broad diffraction peaks at 2θ values of approximately 14.80º, 22.02º, 55.85º and 62.97º, indicating a semi-crystalline nature. The presence of a relatively intense peak around 22.02º suggests localized ordered regions within the plant matrix, which may be attributed to structured arrangements of phytoconstituents such as flavonoids, alkaloids and glycosides. However, the broadness and low intensity of the peaks reflect limited long-range order, confirming the coexistence of crystalline and amorphous phases typical of complex plant materials.

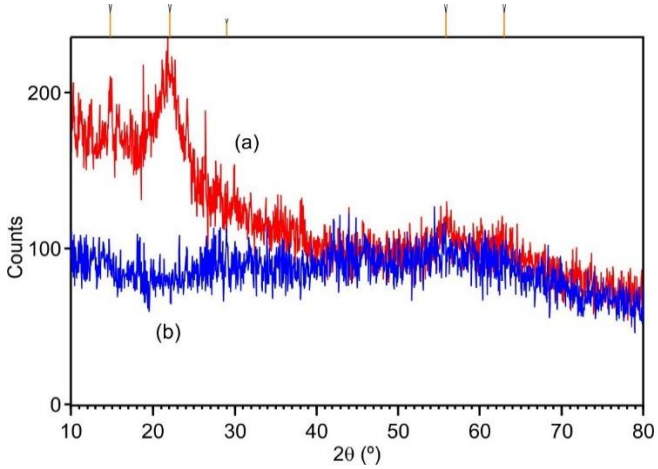

Fig. 2. X-ray diffraction (XRD) patterns of (a) *H. diffusa* powder and (b) ethanolic extract of *H. diffusa*

In contrast, the XRD pattern of the ethanolic extract displays a single broad and diffused peak centered around 2θ ≈ 29.03º, corresponding to a *d*-spacing of approximately 3.07 Å. The absence of sharp and well-defined diffraction peaks indicates a predominantly amorphous structure. This transition from semi-crystalline to amorphous nature suggests that the extraction process disrupts the ordered arrangement present in the raw plant matrix, likely due to the dissolution and redistribution of phytochemical constituents in ethanol. Furthermore, the ethanolic extract demonstrates a higher degree of amorphization than the powdered form, which can be correlated with the enhanced availability of bioactive compounds in a more disordered state. Such an amorphous structure is advantageous, as it facilitates greater surface reactivity and accessibility of functional groups. This structural transformation is particularly significant for applications involving green synthesis, where increased molecular dispersion and reduced crystallinity can promote efficient interaction with metal ions, thereby enhancing reduction and nucleation processes.

SEM studies: The SEM images of *H. diffusa* powder and its ethanolic extract (Fig. 3a-b) reveal significant differ-

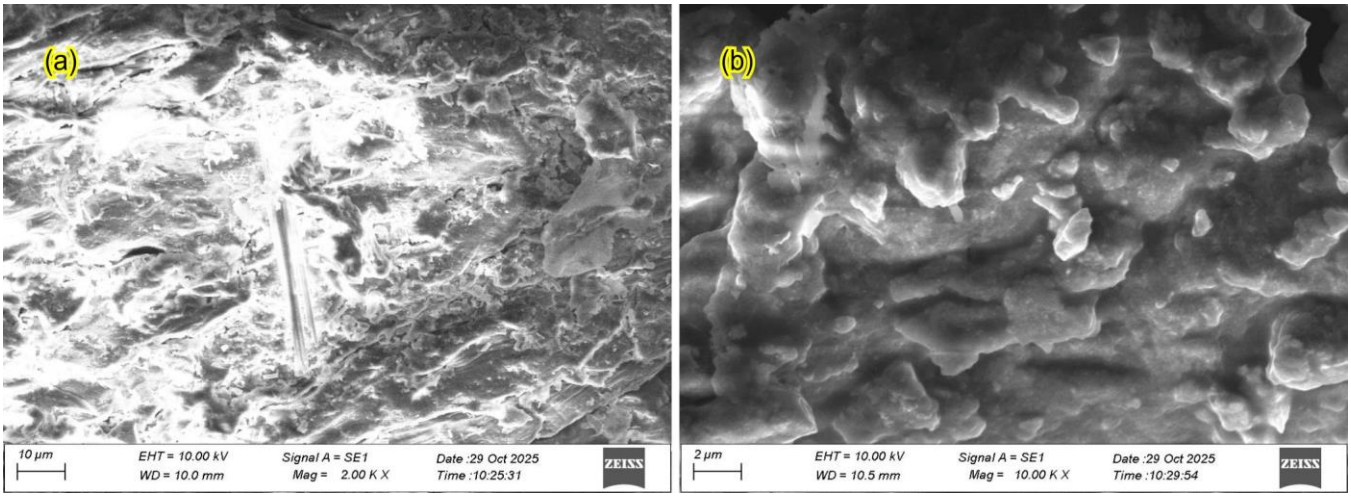

Fig. 3. SEM micrographs of (a) *H. diffusa* powder and (b) ethanolic extract of *H. diffusa*

ences in surface morphology and microstructural organisation. The powdered sample exhibits a heterogeneous surface characterized by irregular, coarse, and compact particles with rough textures. The presence of sharp edges and pronounced aggregation indicates intact fibrous structures and rigid plant cell wall components, such as cellulose and lignin. This compact and uneven morphology reflects the unprocessed nature of the plant material, where phytoconstituents remain embedded within a complex structural matrix.

The ethanolic extract demonstrates a markedly altered morphology, displaying smoother surfaces with finer and more uniformly distributed particles. The reduction in particle size and decreased aggregation suggest partial disruption of the plant matrix during solvent extraction. This structural transformation can be attributed to the dissolution and removal of non-soluble components, leading to the exposure and enrichment of bioactive constituents. The smoother and less compact morphology indicates improved dispersion and increased surface accessibility. Comparatively, the ethanolic extract shows a more refined and homogeneous microstructure than the crude powder. This enhancement in surface characteristics is particularly important, as finer and less aggregated particles provide a larger effective surface area and more accessible active sites. Such features are advantageous for interactions at the molecular level, especially in processes involving adsorption, reduction and stabilisation. These morphological evolution supports the improved functional performance of the ethanolic extract in applications requiring enhanced surface activity.

Conclusion

The present study demonstrated the comparative phytochemical and spectral characterisation of *Hedyotis diffusa* powder and its ethanolic extract using FTIR, XRD and SEM analyses. The results revealed that *H. diffusa* contains abundant bioactive compounds, including flavonoids, phenolics, terpenoids and alkaloids, which are responsible for various biological activities. The capacity of plant to function as a reducing and stabilising agent in green synthesis was demonstrated by the FTIR spectra, which verified the presence of crucial functional groups such hydroxyl, carboxyl and carbonyl groups. The XRD pattern showed the semi-crystalline nature of the sample, while SEM images revealed a heterogeneous and fine surface morphology suitable for nanoparticle formation. These findings establish *H. diffusa* as a promising natural precursor for the eco-friendly synthesis of calcium nanoparticles. Its rich phytochemical profile and favourable structural characteristics make it suitable for biomedical and dental applications, especially in the development of biocompatible, non-toxic and sustainable nanomaterials for future therapeutic formulations.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language. The authors reviewed and edited the content and take full responsibility for the published work.

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