Natural Resources for Human Health

visagaa publishing house

Research Article

View Article online

Check for updates

Received 22 October 2025
Revised 25 January 2026
Accepted 25 January 2026
Available Online 02 September 2026

Edited by Ricardo Diego de Albuquerque

KEYWORDS:
Hyoscyamus niger
Cleome viscosa L. Authentication
Profiling
Macro-microscopic studies
HPTLC and HPLC

Natr Resour Human Health 2026; 6 (4): 506–515
https://doi.org/10.53365/nrfhh/217340
eISSN: 2583-1194

High-Throughput Chromatographic and Spectroscopic Profiling for Reliable Differentiation of *Hyoscyamus niger* and *Cleome viscosa* L.

Santosh Kumar[1], Sapna Salar[2], Manju Singh[3], Dinesh Kumar Yadav[4], Anjali Singh[5] and Mhaveer Singh[6*]

[1]Department of Botany, Maharaja Bijli Pasi Government Post Graduate College, Ashiyana, Lucknow-226012, India
[2]Centre of Excellence in Pharmaceutical Sciences, Guru Gobind Singh Indraprastha University, Dwarka Sector 16(C), Delhi-110078, India
[3]Department of Pharmaceutics, Dr KN Modi Institute of Pharmaceutical Education and Research, Kapda Mill, opposite SBI Main Branch, Modinagar, Uttar Pradesh-201204, India
[4]Department of Pharmacognosy, SGT College of Pharmacy, SGT University, Gurugram Haryana, India-122505
[5]Department of Pharmacology, Institute of Applied Medicines and Research, 9th KM Stone Delhi Meerut Road, NH 9, Duhai, Ghaziabad, Uttar Pradesh-201206, India
[6]Department of Pharmacognosy, School of Pharmaceutical Sciences, Faculty of Pharmacy, IFTM University, Moradabad, Uttar Pradesh-244102, India

ABSTRACT: Quality evaluation of herbal medicines is essential to ensure their safety, efficacy, and consistency. The present study focused on the quality control and authentication of *Hyoscyamus niger* (henbane) and its adulterant *Cleome viscosa* L. using modern analytical techniques. Both plants possess significant medicinal value but are often adulterated, affecting therapeutic reliability. The study aimed to differentiate and authenticate *H. niger* and *C. viscosa* through macro- and microscopic characterization, followed by chemical profiling using High-Performance Thin-Layer Chromatography (HPTLC) and High-Performance Liquid Chromatography coupled with Mass Spectroscopy (HPLC–MS). These advanced tools enabled rapid, simultaneous analysis of multiple phytoconstituents, facilitating efficient quality assessment. The results revealed distinct differences in macro- and microscopic features between the two species. Chemical profiling identified characteristic marker compounds specific to each plant, allowing clear discrimination between authentic and adulterated samples. Quantitative estimation of total alkaloid content showed a notable variation, with *H. niger* containing 0.1737 ± 0.0023% and *C. viscosa* 0.0441 ± 0.0018% (w/w). Overall, the findings highlight the effectiveness of combining high-throughput screening with advanced analytical approaches for the authentication of medicinal plants. This integrated strategy strengthens quality control measures, ensuring the authenticity, potency, and safety of herbal formulations, and supports the development of robust quality assurance protocols in the herbal industry.

* Corresponding author.
E-mail address: mhaveer.singh@iftmuniversity.ac.in (Mhaveer Singh)

1. INTRODUCTION

Ensuring the safety, effectiveness, and consistency of herbal medicines requires quality control and medicinal plant authentication. Adulteration, whether intentional or accidental, poses significant challenges to the integrity of herbal medicines, leading to variations in therapeutic efficacy and potential risks to consumer health. To address these concerns, rigorous quality control measures must be implemented throughout the production process, from cultivation and harvesting to processing and distribution. Authentication techniques such as macroscopic and microscopic examination, chemical profiling, and DNA barcoding as well as High-performance analytical methods, including chromatography, spectroscopy, and mass spectrometry used to discriminate bioactive compounds have been abundantly employed to quality based evaluation and verification of therapeutic plants and the products (Gautam et al., 2023; Salar et al., 2023).

Among these, High-Performance Thin-Layer Chromatography (HPTLC), High-Performance Liquid Chromatography (HPLC), are the most utilized analytical techniques for chemical profiling and quantification of key bioactive constituents in plant samples (Gaurav et al., 2023b, 2022). This quick, cost-effective, and accurate approach has benefits like high sample throughput, little preparation of samples, and simultaneous analysis of several chemicals (Attri et al., 2023; Gaurav et al., 2020). HPTLC was employed for preliminary screening of plant extracts to identify characteristic chemical fingerprints while HPLC employed for quantitative analysis of specific marker compounds and known for its high sensitivity, accuracy, and reproducibility for validating medicinal plants and their derived formulations, scientifically (Karthika and Paulsamy, 2015).

H. niger, commonly known as henbane, is a plant species belonging to the Solanaceae family. It is native to Europe, Asia, and North Africa but has been naturalized in various regions worldwide. Henbane typically thrives in disturbed habitats, including waste areas, roadsides, and cultivated fields (AZHAR and MUSTEHASAN, 2020). Chemically, *H. niger* contains several pharmacologically active compounds, with tropane alkaloids being the most significant (Attri et al., 2023). The major tropane alkaloids found in henbane include hyoscyamine, scopolamine, and atropine, which exert anticholinergic effects on the nervous system. Traditionally, it has been used for its antispasmodic, sedative, and analgesic actions, though improper use can cause severe toxicity (AZHAR and MUSTEHASAN, 2020; Mohammad Salehudin, 2020; Wicaksono, 2020). Moreover, *H. niger* exhibits a range of pharmacological activities due to its alkaloid content. The plant has been traditionally used for its antispasmodic, sedative, and analgesic properties. Its alkaloids act as muscarinic receptor antagonists, blocking the action of acetylcholine and thereby relaxing smooth muscles. Additionally, henbane has been employed as a remedy for various ailments, including asthma, gastrointestinal disorders, and motion sickness (Carod Artal, 2015). Despite its therapeutic potential, *H. niger* contains toxic alkaloids and must be used with caution. Improper dosing or ingestion of large quantities can lead to severe toxicity, including hallucinations, delirium, and respiratory depression. Therefore, it is essential to consult healthcare professionals before using henbane or products derived from it for medicinal purposes (Azhar and Mustehasan, 2020; Mahfouze and Ottai, 2011; Stenzel et al., 2006).

H. niger and *C. viscosa* L. are two plants with significant medicinal value, commonly used in traditional and folk medicine systems. However, due to increasing demand and limited availability of authentic botanical materials, adulteration has become a prevalent concern in the herbal industry. Adulteration not only compromises the therapeutic efficacy of herbal preparations but also poses potential risks to consumer health. Therefore, robust analytical methods are imperative for accurate identification, quality assessment, and authentication of botanical materials (Seraglio et al., 2019). In this study, we present a comprehensive study on the quality control and authentication profiling of *H. niger* and its adulterant *C. viscosa* L. using high-throughput screening with modern analytical techniques. *H. niger*, belonging to the Solanaceae family, is renowned for its pharmacological properties, including antispasmodic, analgesic, and sedative effects. Conversely, *C. viscosa* L., a member of the Cleomaceae family, has been traditionally used for its anti-inflammatory, analgesic, and antimicrobial properties (Muhammad et al., 2018; Olaoluwa and Azeez, 2022).

Despite their medicinal significance, both plants are susceptible to adulteration, which can occur due to factors such as misidentification, substitution, or intentional mixing with lower-cost substitutes. Adulteration compromises the authenticity and quality of herbal preparations, leading to variations in therapeutic efficacy and safety (Kumar et al., 2023). Therefore, the development of reliable analytical methods for the detection and differentiation of authentic botanical materials from adulterants is imperative for ensuring the integrity of herbal products (Kumar, 2025; Kumar et al., 2025). Overall, this study highlights the efficacy of high-throughput screening combined with modern analytical techniques in quality control and authentication profiling of *H. niger* and *C. viscosa* L. The developed methods offer rapid, reliable, and cost-effective solutions for the detection and differentiation of authentic botanical materials from adulterants, thereby enhancing the integrity and safety of herbal products in the market (Singh et al., 2024; Vandana et al., 2024).

2. MATERIAL AND METHODS

2.1. Chemical, reagents and instrumentations

High-Performance Thin-Layer Chromatography (HPTLC) equipped with Linomat 5, Scanner 4 Camag Derivatizer, High-Performance Liquid Chromatography (HPLC), *H. niger* and *C. viscosa* L., BOROSIL Laboratory filter media (Grade: B59501), High-Performance Liquid Chromatography (Water 1525, Binary HPLC Pump equipped with UV/Visible detector: 2489 and Acquity QDa detector). Furthermore, analytical grade chemicals and solvents used to conduct the study.

2.2. Collection and preparation of extract

H. niger and *C. viscosa* L. seeds were procured form the local market, khari baoli, Delhi, India and authenticated as per Ayurvedic Pharmacopoeia of India. 1 g of *H. niger* and *C. viscosa* L. seeds powder of each plants were weighed accurately and extracted using sonication method with 20 ml of methanol at room temperature for 2 hrs. After extraction, the content was filtered through BOROSIL Laboratory filter media (Grade: B59501) paper to separate the plant material residue. The obtained filtrate was concentrated on a water bath at 70 ± 5°C to achieve its volume up to 5 ml that was directly used for TLC analysis (Gaurav et al., 2023a, 2020).

2.3. Macroscopical and microscopical examination

For the macro and microscopical examination of *H. niger* and *C. viscosa* seeds, dissecting microscopes and inverted microscopes (OPTIKA Microscope, Italy) were utilized. Initially, the seeds were observed under a dissecting microscope to assess their macroscopic characteristics such as size, shape, color, and surface texture. Horizontal and vertical views of the seeds were examined to capture variations in morphology. Subsequently, the seeds were prepared for microscopic examination using an inverted microscope. Thin sections of the seeds were obtained and mounted on glass slides with a suitable mounting medium. The slides were then observed under the inverted microscope to analyze the internal structures of the seeds at higher magnifications (Kumar et al., 2023). Microscopic features including the shape and arrangement of cells, presence of characteristic structures such as oil bodies, starch grains, or trichomes, and any other distinguishing features were carefully examined. Horizontal and vertical sections of the seeds were examined to capture variations in internal morphology in macroscopic examination. The combination of dissecting microscopes and inverted microscopes allowed for a comprehensive analysis of both the macroscopic and microscopic characteristics of *H. niger* and *C. viscosa* seeds, facilitating accurate identification and differentiation between the two species (Shrestha et al., 2014).

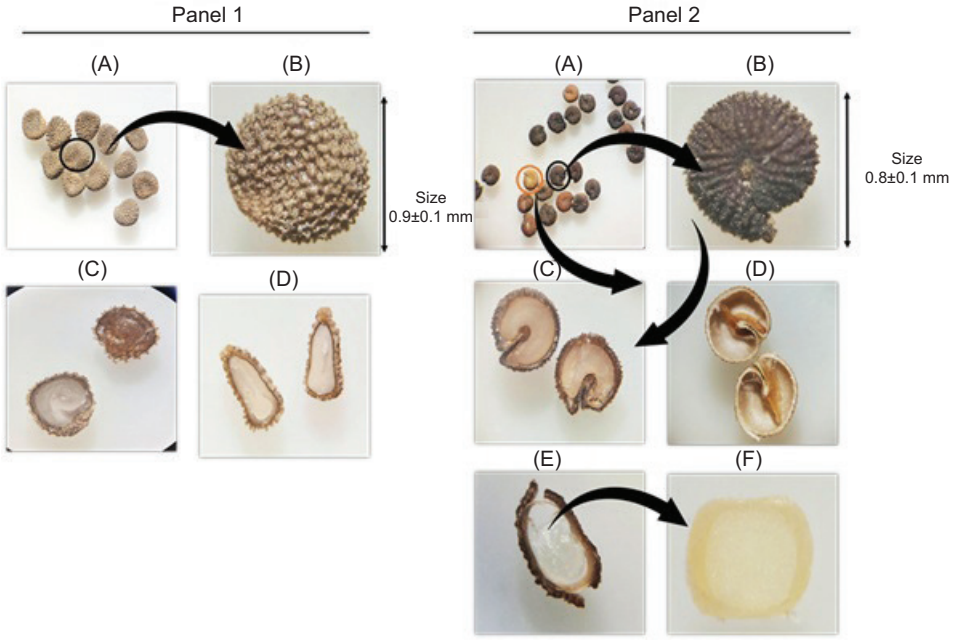

Figure 1. Macroscopical examination of *H. niger* and *C. viscosa.* Panel 1 and 2 (Figure A & B) represents the morphological state of *H. niger and C. viscosa*. Figure C represents horizontal (transverse) section showing compact cellular arrangement and prominent oil bodies. Figure D in panel 2 represent the horizontal section of immature seed of *C. viscosa* represented in circle (Yellow color seed) while Figure D in panel 1 and figure E and F (Panel 2) represents the sagittal (longitudinal) section of *H. niger* and *C. viscosa.*

2.4. Quantitative estimation of total alkaloids

For the quantitative estimation of total alkaloids in the methanolic extracts of *H. niger* and *C. viscosa*, a gravimetric method based on acid-base extraction was employed. Initially, 5 g of each powder was extracted in methanol (100 ml) using sonication method at room temperature for 2 hrs. the content was filtered and concentrated till its 10 ml volume on a water bath. Thereafter, the residues of each extract was suspended separately in 50 ml of acidified water (pH 2-3) in conical flasks to make alkaloidal salt of present alkaloids and partitioned with the chloroform (50 ml x 3). Thereafter, aqueous layer was collected and subsequently the solutions were basified (pH 9-10) by adding 10% ammonia solution dropwise to the sample, the alkaloids were then extracted into chloroform (50 ml x 3) by vigorous shaking for 5 minutes. The solvent was evaporated to dryness on a water bath after the chloroform layer containing the alkaloids was separated. The residue was then dried to a constant weight at an appropriate temperature (e.g., 60°C) to eliminate any residual solvent. The mass of the desiccated residue indicated the overall alkaloid concentration in the original extract and was quantified as a percentage of the initial botanical material (Ajanal et al., 2012).

2.5. TLC fingerprint densitometry analysis of methanolic extract and alkaloids enriched fraction of *H. niger* and *C. viscosa*

The TLC profiling of methanolic extracts of *H. niger* and *C. viscosa* prepared as described earlier was performed in two step. In first step, the mother extract directly applied on TLC onto silica gel TLC plates (pre-coated with a 0.25 mm layer). Application volume ranged from 2-5 µL using a microsyringe (100µl). The plates were prepared using a solvent mixture of toluene, ethyl acetate, and formic acid (6:3:1 v/v) in a glass chamber until the solvent front approached the upper edge of the plate (75 mm). Subsequent to development, the plate was desiccated and examined at 254 nm and 366 nm, followed by derivatization using Anisaldehyde sulfuric acid reagent. The images at each phase were taken for documentation purposes.

In second step, another plate with pre-application of sample was developed in n-Butanol: wate: Liq. Ammonia (6: 3.5: 0.5 v/v), dried and visualized under UV light (254 nm and 366 nm) for visualization of separated bands and derivatized using dragondroff reagent to visualized alkaloids bands in the methanolic extracts of *H. niger* and *C. viscosa*, facilitating authentication and quality control assessments (Gaurav, 2022; Gaurav et al., 2022).

2.6. HPLC profiling of alkaloids enriched fraction of *H. niger* and *C. viscosa*

For the HPLC profiling of alkaloids enriched fractions from *H. niger* and *C. viscosa*, a C18 column was utilized. The mobile phase included acetonitrile (ACN) and water in a 65:35 v/v ratio. The flow rate was established at 1 ml/min. Before injection, the samples were solubilized in methanol and subjected to filtration using a 0.45 µm membrane filter. Ten microliters of each sample were injected onto the C18 column, which was kept at an appropriate temperature of 25°C. Detection of alkaloids was performed using a UV detector at an appropriate wavelength (254 nm). Chromatographic separation was achieved by eluting the compounds through the column with the mobile phase at the specified flow rate. Retention times of peaks corresponding to alkaloids were recorded and compared between the samples to determine qualitative differences in alkaloid composition between *H. niger* and *C. viscosa* (Nejadhabibvash et al., 2012).

2.7. Statistical analysis

The data are represented in Mean ± SD via following each values measurements in triplicate.

3. RESULTS AND DISCUSSION

3.1. Macroscopical and microscopical examination

The macroscopic examination of *H. niger* seeds revealed a comparatively higher size (0.9 ± 0.1) then *C. viscosa* (0.8 ± 0.1), oval-shaped structure with a smooth surface and a brown coloration. In contrast, *C. viscosa* seeds exhibited a larger, spherical shape with a rough surface texture and a greenish-brown hue. Both horizontal and vertical views of the seeds highlighted distinct morphological differences between the two species, aiding in their differentiation. Upon microscopic examination using an inverted microscope, thin sections of the seeds revealed intricate internal structures. *H. niger* seeds displayed characteristic features such as compactly arranged cells, prominent oil bodies, and starch grains, while *C. viscosa* seeds exhibited a looser arrangement of cells with fewer oil bodies and starch grains. Trichomes were observed in both species but were more abundant in *C. viscosa* seeds. Overall, the combination of dissecting microscopes and inverted microscopes facilitated a comprehensive analysis of the macroscopic and microscopic characteristics of *H. niger* and *C. viscosa* seeds. These observations provided valuable insights

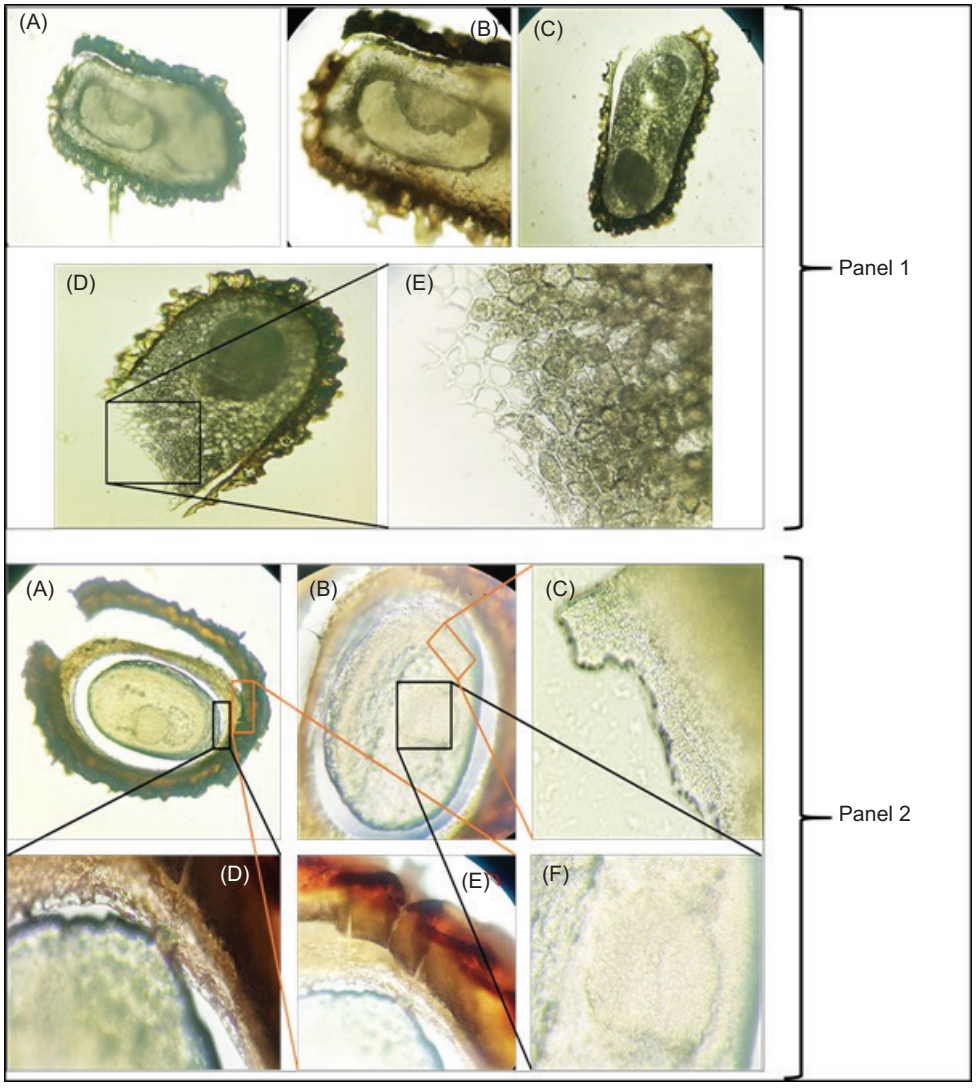

Figure 2. Microscopic characteristic of *H. niger* (Panel 1) and its adulterant *C. viscosa* (Panel 2). Panel 1 (Figure A, B and C) represents sagittal and parasagittal view of *H. niger* while Figure (D and E) represents complex oil bodies in *H. niger.* In panel 2 (Figure A, B, and C) represent the sagittal section, resolved view of internal seed and oil bodies, respectively. Figure (D, E and F) represent resolved view of mucilage and testa (Seed coat), endosperm palisade layer, dentils, and compacted phloem globules with oil bodies.

for accurate identification and differentiation between the two species, crucial for botanical authentication and quality control purposes.

3.2. Quantitative estimation of total alkaloids

Estimation of total alkaloids in methanolic extract of *H. niger* and *C. viscosa* was estimated through gravimetric method based on acid-base extraction. The outcome of the study showed that the content of total alkaloids in *H. niger* and *C. viscosa* was found with a significant difference in their content as follows 0.1963 ± 0.0023% and 0.0693 ± 0.0018 % (w/w), respectively.

3.3. TLC fingerprint densitometry analysis of methanolic extract and alkaloids enriched fraction of *H. niger* and *C. viscosa*

The HPTLC fingerprinting of *Hyoscyamus niger* (S) and *Cleome viscosa* (A) revealed distinct metabolite profiles, as observed under UV light at 254 nm, 366 nm, and after derivatization with anisaldehyde reagent (ASR). At 254 nm, very few spots were detected, with both plants showing bands at Rf

0.28, indicating the presence of common metabolites. Under 366 nm, *H. niger* displayed multiple fluorescent bands at Rf 0.13, 0.21, 0.26, 0.32, 0.40, 0.44, 0.51, and 0.68, while *C. viscosa* showed fewer bands, primarily at Rf 0.21, 0.28, 0.30, 0.36, and 0.40. This highlights the relatively higher chemical diversity in *H. niger* at this wavelength.

Post-derivatization with ASR, both species exhibited enhanced colorimetric resolution. Shared bands were noted at Rf 0.21, 0.40, and 0.72, suggesting the presence of similar classes of secondary metabolites, possibly terpenoids or phenolics. However, unique bands for *H. niger* (Rf 0.10, 0.13, 0.44) and *C. viscosa* (Rf 0.09, 0.30) indicate species-specific phytoconstituents. Overall, the fingerprinting analysis demonstrates both overlapping and distinct metabolite patterns, validating the utility of HPTLC in differentiating these two species. Such profiling provides a valuable tool for authentication, quality control, and comparative phytochemical assessment.

3.4. HPLC profiling of alkaloids enriched fraction of *H. niger* and *C. viscosa*

In HPLC chromatographic separation profiling of alkaloids enriched fractions of *H. niger* and *C. viscosa*, several significant discriminants emerge even under identical conditions. These discriminants play a crucial role in distinguishing between the chemical compositions of the two plant species. One significant discriminant is the retention time of peaks corresponding to different phytochemicals. Additionally, the peak shape and width can vary between *H. niger* and *C.*

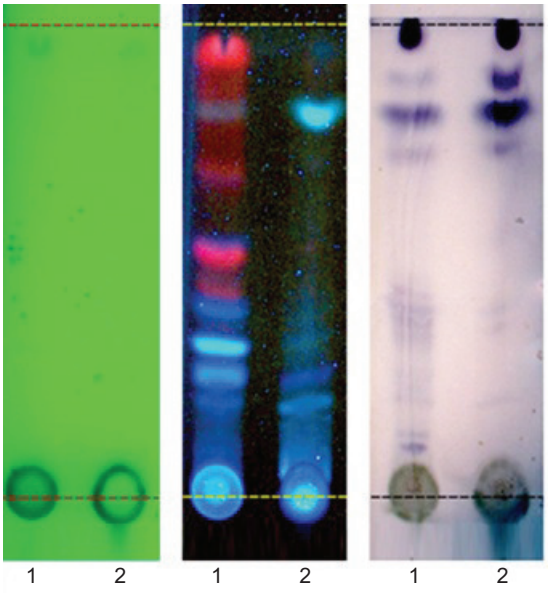

Figure 3. TLC analysis of *H. niger* (1) and *C. viscosa* (2) for comparative assessment in the metabolites fingerprint. The developed plate was viewed at 254 nm, 366 nm and derivatised with anisaldehyde reagent.

Table 1
TLC ingerprinting profile of *H. niger* and *C. viscosa.*

Sr. no.	R_f values	At 254 nm		At 366 nm		Derivatised with ASR	
		H. niger	*C. viscosa*	*H. niger*	*C. viscosa*	*H. niger*	*C. viscosa*
1	0.09	-	-	-	+	-	-
2	0.1	-	-	-	-	+	-
3	0.13	-	-	+	-	+	-
4	0.21	-	-	+	+	+	+
5	0.26	-	-	+	-	-	-
6	0.28	+	+	-	+	-	-
7	0.30	-	-	-	+	-	-
8	0.32	+	-	+	-	-	-
9	0.36	-	-	-	+	-	-
10	0.40	-	-	+	+	+	+
11	0.44	-	-	+	-	+	-
12	0.51	-	-	+	-	-	-
13	0.68	-	-	+	-	-	-
14	0.72	-	-	+	-	+	+
15	0.81	-	-	+	+	+	+
16	0.89	-	-	-	-	+	+
Total metabolites		**02**	**01**	**10**	**07**	**08**	**05**

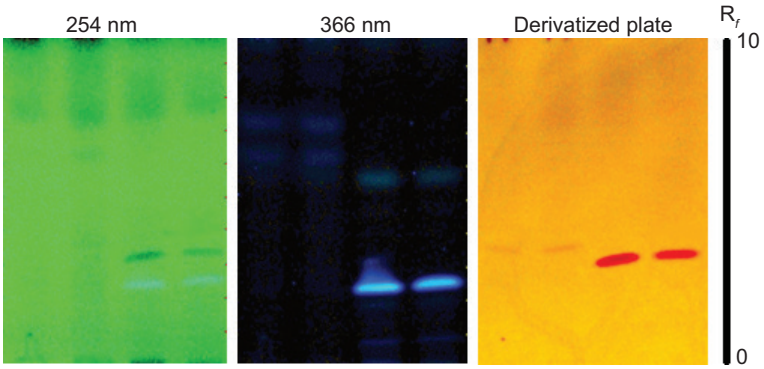

Figure 4. TLC fingerprinting profiling of *H. niger* (*H. niger* STD; Track 3-4) and *C. viscosa* (*H. niger* Adulterant; Track 1-2) alkaloids enriched fraction. The outcomes of the fingerprinting have been depicted in different panels. Panel 1 represents the TLC plate developed in n-Butanol: wate: Liq. Ammonia (6: 3.5: 0.5 v/v) and visualised at day light, 254 nm, 254 nm modified view, 366 nm and derivatised with Dragendorff's reagent.

viscosa due to differences in compound concentrations and interactions with the chromatographic system. However, one of the common major components found at retention time 45.73 min. Moreover, variations in peak intensity or area under the curve can also serve as discriminants, reflecting differences in alkaloid content between the two plant species. Overall, these discriminants in chromatographic separation are essential for accurate authentication and quality control of *H. niger* and *C. viscosa*, providing valuable insights into their chemical compositions and medicinal properties.

4. DISCUSSION

Quality evaluation of herbal medicines is paramount to ensure their safety, efficacy, and consistency in therapeutic outcomes. In this study, the focus was on the quality control and authentication profiling of *H. niger* and its adulterant *C. viscosa* using high-throughput screening with modern analytical techniques. Both plants possess significant medicinal value but are susceptible to adulteration, which can compromise the efficacy and safety of herbal preparations. The study aimed to unravel the macroscopic and microscopic characteristics of *H. niger* and *C. viscosa*, followed by High-Performance Thin-Layer Chromatography (HPTLC) and High-Performance Liquid Chromatography (HPLC) equipped with mass spectroscopy for comprehensive chemical profiling (Durrani et al., 2008; Khan et al., 2024; Zahiruddin et al., 2017).

The utilization of these advanced analytical techniques allowed for rapid and simultaneous analysis of multiple compounds, facilitating efficient quality assessment and authentication. The results revealed significant differences in the macroscopic and microscopic characteristics of *H. niger* and *C. viscosa*, aiding in their discrimination. Moreover, key chemical markers specific to each plant were identified, enabling differentiation between authentic samples and adulterants (Gaurav et al., 2023a; Gautam et al., 2023). Quantitative estimation of total alkaloid content revealed substantial variation between the two species, underscoring the value of such assessments in defining quality parameters and maintaining consistency in herbal formulations (Nejadhabibvash et al., 2012; Stenzel et al., 2006).

The findings underscore the efficacy of high-throughput screening combined with modern analytical techniques in distinguishing between H. niger and *C. viscosa*, thereby enhancing quality control measures and safeguarding the integrity of herbal products. This approach holds promise for ensuring the authenticity, potency, and safety of botanical medicines, contributing to the advancement of quality assurance protocols in the herbal industry. The successful application of HPTLC and HPLC in this study underscores their crucial role in herbal drug evaluation, offering rapid, accurate, and reproducible analysis for the identification and quantification of bioactive constituents. These techniques offer rapid and accurate analysis of herbal extracts, allowing for the identification and quantification of active constituents. By leveraging mass spectroscopy, the study further enhanced the chemical profiling of the herbal samples, providing valuable insights into their composition and authenticity.

Jaremicz et al. (2014) described a methodology for the creation of a high-performance thin-layer chromatography (HPTLC) technique for the isolation and quantification of the principal tropane alkaloids, hyoscyamine and scopolamine, together with their direct precursors, littorine and anisodamine, as well as cuscohygrine, in the leaves and roots of *H. niger* and *Atropa baetica*. HPTLC Si60 F254 plates,

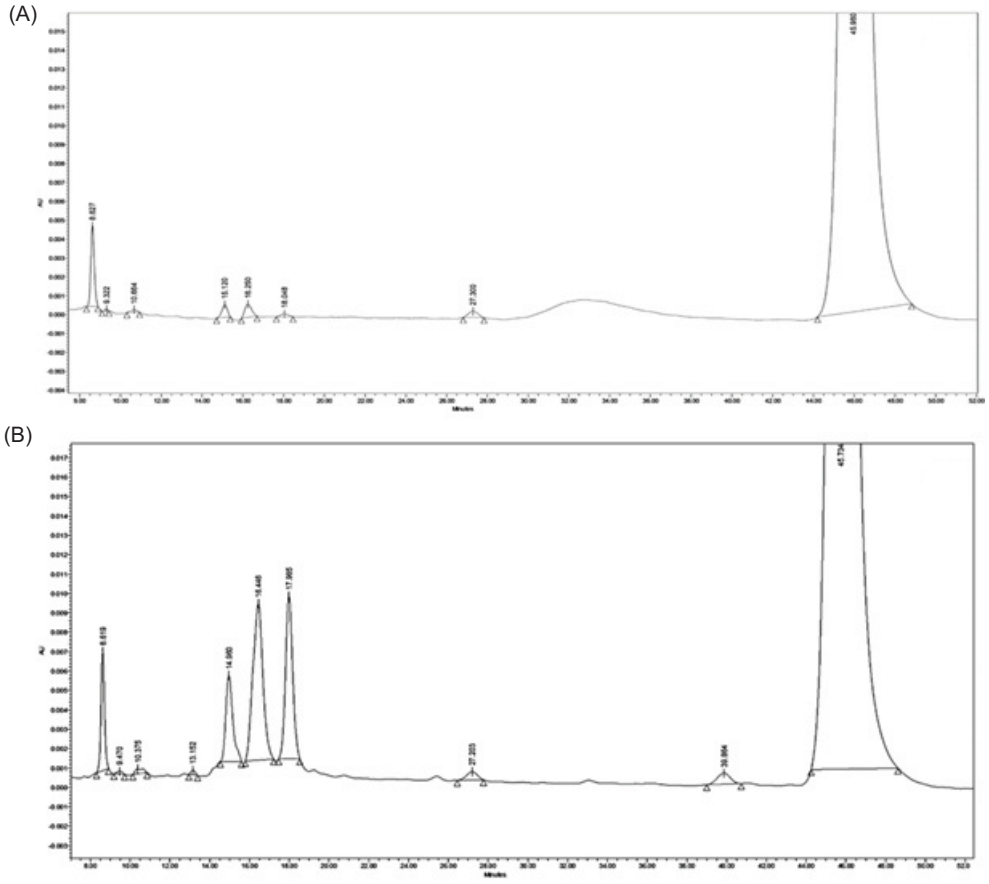

Figure 5. HPLC chromatogram of *H. niger* (A) and *C. viscosa* (B) represents the significant different in the chromatographic pattern. However, one of the common major components found at retention time 45.73 min.

Table 2
Fingerprinting profile of *H. niger* and *C. viscosa.*

Sr. No	Retention time	Metabolites found in *H. niger*	Metabolites found in *C. viscosa*
1	8.627	+	+
2	9.322	+	+
3	10.664	+	+
4	13.152	-	+
5	15.120	+	+
6	16.250	+	+
7	18.048	+	+
8	27.300	+	+
9	39.864	-	+
10	45.960	+	+
Total metabolites		**12**	**16**

preconditioned with mobile phase vapors (chloroform: methanol: acetone: 25% ammonia ratios of 75:15:10:1.8, v/v/v/v), were utilized for TLC development and densitometric detection at 190 and 520 nm for the quantification of hyoscyamine, scopolamine, anisodamine, littorine, and cuscohygrine in various plant materials, including *H. niger*. The results of the study showed that anisodamine (0.011 ± 0.003 mg/g), cuscohygrine (0.079 ± 0.013 mg/g), and scopolamine (0.157 ± 0.013 mg/g) found in leaves of *H. niger*, while hyoscyamine (0.208 ± 0.03), anisodamine (0.051 ± 0.006), cuscohygrine (0.861 ± 0.12), and scopolamine (0.285 ± 0.05) found in root of *H. niger.* The study provides a robust approach for quality based identification of *H. niger* (Jaremicz et al., 2014).

Singh et al. (2018) presented a quantitative method for estimating salicylic acid via HPTLC densitometry in *C. viscosa* seeds. The study's findings indicated that the TLC chromatogram exhibited a distinct spot for salicylic acid at Rf 0.51, with a concentration of 0.9 ± 0.04 µg/g of seed. The limit of detection (LOD) for salicylic acid was 4 ng, whereas the limit of quantification (LOQ) was 8 ng. This created approach yielded improved resolution of additional compounds contained in the extract, demonstrating simplicity, precision, and accuracy. Final Assessment the research indicated that Salicylic acid is the active ingredient found in the seeds of *C. viscosa* (Singh et al., 2018).

Additionally, the macroscopic and microscopic examination of the plants proved instrumental in identifying morphological differences that can aid in their authentication. These traditional methods, when combined with modern analytical techniques, create a robust framework for comprehensive quality assessment of herbal medicines (Jakabová et al., 2012). However, it is essential to acknowledge some limitations of the study. Nevertheless, certain limitations should be acknowledged. While high-throughput screening offers rapid and reproducible analysis, it may not fully capture the complexity of plant matrices or minor constituents contributing to therapeutic activity. Future work could incorporate complementary techniques such as NMR spectroscopy, metabolomics, or DNA barcoding for enhanced precision (Dodds and Reynolds, 1980). Moreover, the integration of high-throughput screening with modern analytical techniques represents a promising approach for quality evaluation and authentication of herbal medicines. By combining traditional knowledge with cutting-edge technology, researchers can effectively ensure the authenticity, potency, and safety of botanical medicines, thereby advancing quality assurance protocols in the herbal industry.

5. CONCLUSION

In conclusion, this study underscores the critical importance of quality control and authentication profiling in ensuring the safety, efficacy, and consistency of herbal medicines. Using an integrated approach combining macroscopic and microscopic analysis with advanced chromatographic techniques (HPTLC and HPLC–MS), clear distinctions were established between *Hyoscyamus niger* and *Cleome viscosa*. Characteristic of chemical markers specific to each plant enabled discrimination between authentic samples and potential adulterants, while quantitative analysis provided valuable insights into variations in alkaloid content. By contributing to the advancement of quality assurance protocols in the herbal industry, this approach holds promise for ensuring the authenticity, potency, and safety of botanical medicines, ultimately benefiting both consumers and practitioners of herbal medicine.

ORCID

Santosh Kumar 0009-0000-4372-5691
Sapna Salar 0009-0005-3164-3505
Manju Singh 0000-0003-4295-8519
Dinesh Kumar Yadav 0000-0003-1559-9354
Anjali Singh 0009-0003-9970-2861
Mhaveer Singh 0000-0001-9951-5685

REFERENCES

Ajanal, M., Gundkalle, M., Nayak, S., 2012. Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer. Ancient Science of Life. https://doi.org/10.4103/0257-7941.107361

Attri, D.S., Rathour, A., Ray, R.K., Kumar, V., 2023. Formulation and evaluation of hydrogel for topical drug delivery of Zingiber officinale Rosc . and Withania somnifera (L .) Dunal to increase the bioavailability of oils for the treatment of arthritis 12, 1–10.

AZHAR, M., MUSTEHASAN, 2020. PHYTOPHARMACOLOGY OF AN IMPORTANT UNANI DRUG BAZR-UL-BANJ (HYOSCYAMUS NIGER LINN.) – REVIEW. Asian Journal of Pharmaceutical and Clinical Research. https://doi.org/10.22159/ajpcr.2020.v13i9.38166

Carod Artal, F.J., 2015. Adverse Neurological Effects Caused by the Ingestion of Plants, Seeds, and Fruits, in: Bioactive Nutraceuticals and Dietary Supplements in Neurological and Brain Disease: Prevention and Therapy. https://doi.org/10.1016/B978-0-12-411462-3.00023-0

Dodds, J.H., Reynolds, T.L., 1980. A Scanning Electron Microscope Study of Pollen Embryogenesis in Hyoscyamus niger. Zeitschrift für Pflanzenphysiologie. https://doi.org/10.1016/s0044-328x(80)80080-8

Durrani, F.R., Chand, N., Jan, M., Sultan, A., Durrani, Z., Akhtar, S., 2008. Immunomodulatory and growth Promoting Effects of Neem Leaves Infusion in Broiler Chicks. Sarhad Journal of Agriculture 24, 655–659.

Gaurav, 2022. GC–MS metabolomics and network pharmacology-based investigation of molecular mechanism of identified metabolites from Tinospora cordifolia (Willd.) miers for the treatment of kidney diseases. Pharmacognosy Magazine 18, 548–558. https://doi.org/10.4103/pm.pm_582_21

Gaurav, Anwar, N., Zahiruddin, S., Ahmad, S., 2023a. TLC-bioautography-MS-based Identification of Antioxidant, α-Amylase and α-Glucosidase Inhibitory Compounds in a Polyherbal Formulation "Sugreen-120." Pharmacognosy Magazine. https://doi.org/10.1177/09731296221145064

Gaurav, Khan, M.U., Basist, P., Zahiruddin, S., Ibrahim, M., Parveen, R., Krishnan, A., Ahmad, S., 2022. Nephroprotective potential of Boerhaavia diffusa and Tinospora cordifolia herbal combination against diclofenac induced nephrotoxicity. South African Journal of Botany 000. https://doi.org/10.1016/j.sajb.2022.01.038

Gaurav, Sharma, I., Khan, M.U., Zahiruddin, S., Basist, P., Ahmad, S., 2023b. Multi-Mechanistic and Therapeutic Exploration of Nephroprotective Effect of Traditional Ayurvedic Polyherbal Formulation Using In Silico, In Vitro and In Vivo Approaches. Biomedicines 11. https://doi.org/10.3390/biomedicines11010168

Gaurav, Zahiruddin, S., Parveen, B., Ibrahim, M., Sharma, I., Sharma, S., Sharma, A.K., Parveen, R., Ahmad, S., 2020. TLC-MS Bioautography-Based Identification of Free-Radical Scavenging, α-Amylase, and α-Glucosidase Inhibitor Compounds of Antidiabetic Tablet BGR-34. ACS Omega. https://doi.org/10.1021/acsomega.0c02995

Gautam, G., Parveen, R., Ahmad, S., 2023. LC-MS-based Metabolomics of Medicinal Plants, in: Omics Studies of Medicinal Plants. https://doi.org/10.1201/9781003179139-9

Jakabová, S., Vincze, L., Farkas, Á., Kilár, F., Boros, B., Felinger, A., 2012. Determination of tropane alkaloids atropine and scopolamine by liquid chromatography-mass spectrometry in plant organs of Datura species. Journal of Chromatography A. https://doi.org/10.1016/j.chroma.2012.02.036

Jaremicz, Z., Luczkiewicz, M., Kisiel, M., Zárate, R., Jaber-Vazdekis, N. El, Migas, P., 2014. Multi-development-HPTLC method for quantitation of hyoscyamine, scopolamine and their biosynthetic precursors in selected solanaceae plants grown in natural conditions and as in vitro cultures. Phytochemical Analysis. https://doi.org/10.1002/pca.2455

Karthika, K., Paulsamy, S., 2015. TLC and HPTLC fingerprints of various secondary metabolites in the stem of the traditional medicinal climber, solena amplexicaulis. Indian Journal of Pharmaceutical Sciences. https://doi.org/10.4103/0250-474X.151591

Khan, M.U., Basist, P., Gaurav, Zahiruddin, S., Penumallu, N.R., Ahmad, S., 2024. Ameliorative effect of traditional polyherbal formulation on TNF-α, IL-1β and Caspase-3 expression in kidneys of wistar rats against sodium fluoride induced oxidative stress. Journal of Ethnopharmacology. https://doi.org/10.1016/j.jep.2023.116900

Kumar, N., Kumar, B., Sisodia, V.K., Rathour, A., Roy, R.K., Kumar, V., 2023. Determination of antibacterial and anti-inflammatory effect of developed ointment formulation containing rosmarinic acid 12, 1–11.

Kumar, V., 2025. A Comprehensive Review of Risk Factors, Diagnosis, and Treatment Options for Oral Squamous Cell Carcinoma. Current Cancer Therapy Reviews 21, 1–8. https://doi.org/10.2174/0115733947379301250703053245

Kumar, V., Jain, P., Parveen, S., Khatkar, D., Siddiqui, A., Ali, A., Fairy, Mirza, M.A., Iqbal, Z., 2025. Advances in Nanoformulation Strategies for Targeted Therapy and Management of Oral Squamous Cell Carcinoma. Current Nanoscience 21, 1–19. https://doi.org/10.2174/0115734137385293250711220844

Mahfouze, S.A., Ottai, M.E.S., 2011. Assessment of genetic variability for some Hyoscymus species using biochemical and molecular markers. Journal of Applied Sciences Research.

Mohammad Salehudin, H.J.S., 2020. Penggunaan Multimedia Berbasis Teknologi Bagi Pendidikan Profesi Guru (PPG): Analisis User Experience (UX) Mohammad. Molecules.

Muhammad, N., Wadood, S.F., Khan, W., Ali, N., Nisar, M., 2018. Intra-species profiling of Cleome viscosa growing in Swat district (Pakistan). Biosystems Diversity. https://doi.org/10.15421/011808

Nejadhabibvash, F., Rahmani, F., Heidari, R., Jamei, R., Azimi, F., 2012. Study of inheritance and environment on tropane alkaloids within Hyoscyamus species. Australian Journal of Crop Science.

Olaoluwa, E.A., Azeez, S.O., 2022. Chromosomal and reproductive barriers among three species of Cleome L. from Ile-Ife, Nigeria. Nordic Journal of Botany. https://doi.org/10.1111/njb.03534

Salar, S., Gaurav, Sharma, P., 2023. Quality Control and Multi-targeted Therapeutic Approach of Nyctanthes arbor-tristris for Management of Hepatic Disease and Associated Complications. Pharmacognosy Magazine. https://doi.org/10.1177/09731296231189619

Seraglio, S.K.T., Silva, B., Bergamo, G., Brugnerotto, P., Gonzaga, L.V., Fett, R., Costa, A.C.O., 2019. An overview of physicochemical characteristics and health-promoting properties of honeydew honey. Food Research International. https://doi.org/10.1016/j.foodres.2019.01.028

Shrestha, S., Kaushik, V.S., Eshwarappa, R.S.B., Subaramaihha, S.R., Ramanna, L.M., Lakkappa, D.B., 2014. Pharmacognostic studies of insect gall of Quercus infectoria Olivier (Fagaceae). Asian Pacific Journal of Tropical Biomedicine. https://doi.org/10.1016/S2221-1691(14)60205-7

Singh, D., Kharb, R., Sharma, S.K., Kumar, V., 2024. Phytochemical and Pharmacological Intervention of Indole and their Derivatives: A Comprehensive Review. Natural Resources for Human Health 4, 308–317. https://doi.org/10.53365/nrfhh/188891

Singh, H., Mishra, A., Mishra, A.K., 2018. Isolation, characterization and quantification of a bioactive compound from Cleome viscosa L. seeds by HPTLC-Densitometric method. South African Journal of Botany. https://doi.org/10.1016/j.sajb.2018.05.007

Stenzel, O., Teuber, M., Dräger, B., 2006. Putrescine N-methyltransferase in Solanum tuberosum L., a calystegine-forming plant. Planta. https://doi.org/10.1007/s00425-005-0077-z

Vandana, K., Kumar, V., Rathour, A., 2024. Advancements In Herbal-Nano Formulations : A Systematic Review 30, 11099–11109. https://doi.org/10.53555/kuey.v30i5.4901

Wicaksono, S.S., 2020. Analisis Dampak Pandemi Covid-19 Terhadap Bisnis Hotel Syariah Di Yogyakarta (Studi Kasus Hotel Unisi Yogyakarta). Molecules.

Zahiruddin, S., Khan, W., Nehra, R., Alam, M.J., Mallick, M.N., Parveen, R., Ahmad, S., 2017. Pharmacokinetics and comparative metabolic profiling of iridoid enriched fraction of Picrorhiza kurroa – An Ayurvedic Herb. Journal of Ethnopharmacology. https://doi.org/10.1016/j.jep.2016.07.072