International Journal of Basic & Clinical Pharmacology
Khan S et al. Int J Basic Clin Pharmacol. 2026 Jan;15(1):27-34
http://www.ijbcp.com
pISSN 2319-2003 | eISSN 2279-0780

DOI: https://dx.doi.org/10.18203/2319-2003.ijbcp20254149

Original Research Article

Unraveling *Iris ensata* Thunb: a pharmacognostical, physicochemical and HPLC-assisted phytochemical investigation

Sania Khan[1], Shamshad Alam[1], Mohd Azhar[2]*, Sameera Shamshad[3], Malik Nuzhat Fatima[4], Samreen Farha[5], Saleha Hasan[6]

[1]Department of Ilmul Advia, Aligarh Muslim University, Aligarh, Uttar Pradesh, India
[2]Department of Tahaffuzi Wa Samaji Tib, Eram Unani Medical College and Hospital, Lucknow, Uttar Pradesh, India
[3]Department of Ilmul Amraz, Aligarh Muslim University, Aligarh, Uttar Pradesh, India
[4]Department of Amraze Jild Wa Zohrawiya, Aligarh Muslim University, Aligarh, Uttar Pradesh, India
[5]Department of Ilaj Bit Tadbeer, Aligarh MuslimUniversity, Aligarh, Uttar Pradesh, India
[6]Department of Tahaffuzi Wa Samaji Tib, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Received: 26 August 2025
Revised: 15 October 2025
Accepted: 16 October 2025

***Correspondence:**
Dr. Mohd Azhar,
Email: azhar.ak503@gmail.com

ABSTRACT

Background: *Iris ensata* Thunb, commonly known as the Japanese iris, is a species of flowering plant in the *Iris* family (Iridaceae). Native to Japan, China, Korea, and Russia, it thrives in wetland environments and is often found near ponds and streams.
Methods: To standardize the test drug, various analytical techniques were employed, including organoleptic evaluation, extractive value determination, ash content analysis, phytochemical screening, elemental analysis, fluorescence study, and HPLC profiling.
Results: The findings revealed that the total ash content was 6.98%, while the water-soluble and alcohol-soluble extractive values were 13.3% and 9.81%, respectively. The loss on drying was recorded at 4.21%, foreign matter was 0.66% and the bulk density was found to be 0.63%. The HPLC analysis displayed 18 peaks, with peak 02 and peak 03 being the most significant, showing area concentrations and retention times of 54.676% at 2.828 minutes and 43.822% at 3.051 minutes, respectively.
Conclusions: This study provided essential reference data for ensuring the quality, purity, and identification of future batches of *I. ensata* Thunb contributing to its standardization in medicinal applications.

Keywords: *Iris ensata*, Herbal medicine, Standardization

INTRODUCTION

Plant-derived bioactive compounds continue to serve as a rich frontier in drug discovery and therapeutic innovation. According to WHO estimates, around 80% of the world's population especially in developing countries still depends primarily on traditional medicinal plants for health care.[1,2] This persistent reliance underscores the critical importance of phytochemicals not just historically, but as sources of new pharmacological leads. Within the pantheon of medicinal genera, *Iris* (family Iridaceae) stands out for both taxonomic richness and chemical diversity. The genus comprises roughly 300 perennial species, making it one of the largest and most complex lineages in Iridaceae.[3-5] *Iris* species are distributed predominantly across the temperate regions of the northern hemisphere (Eurasia, North Africa, North America), and exhibit remarkable habitat plasticity from wetlands and semi-arid zones to rocky and dry terrains.[6,7] Their presence in countries such as China, Turkey, Pakistan, India, Egypt, Morocco, and Iran attests both to their ecological extensiveness and ethno botanical significance.[8-11]

Beyond their ornamental and cultural appeal, *Iris* species are increasingly scrutinized for their medicinal potential. Extracts and isolated compounds from various *Iris* species have shown activity against pathogens (bacteria, fungi, protozoa), antioxidant and cytotoxic effects, and modulatory effects on inflammation and metabolism.[12,13] For example, a recent review of the genus *Iris* documents updated occurrences of antibacterial, antifungal, and antiparasitic properties across multiple species.

Among the *Iris* species, *Iris ensata* Thunb. (commonly called "Irsa" or "Sosan" in Indian subcontinental vernaculars) is of considerable traditional and phytochemical interest. It thrives natively in the Western Himalayas at altitudes of 1500-3000 m, achieving flowering and fruiting between June and September.[14] Ethnobotanically, its leaves are used for fodder, thatching, matting, and basket weaving, while its roots have traditional applications as alteratives, blood purifiers, and treatments for venereal and liver disorders, and dropsy.[15,16]

Recent studies have sharpened our understanding of *I. ensata*'s phytochemistry and biological potential, offering fresh evidence for its therapeutic value. A 2025 comparative analysis of botanical versus market *"Irsa"* samples established critical diagnostic, pharmacognostic, and phytochemical distinctions underscoring the risk of adulteration in herbal formulations.[17] A meta-review (2024) likewise affirms that *I. ensata* contains flavonoids, isoflavonoids, phenolics, terpenoids, steroids, and glycosides, and that several of the traditionally ascribed pharmacological properties (anti-inflammatory, mucolytic, antimicrobial, bronchial effects) have been corroborated in modern assays.[18]

In the wider *Iris* genus, the antioxidant activities of *I. ensata* extracts (petroleum ether, ethyl acetate, methanol) have been characterized in recent work, with correlations drawn between total phenolic/isoflavonoid content and radical-scavenging potential.[19] Moreover, isoflavones such as Iridin have been further implicated in cytotoxic, immunomodulatory, and anti-inflammatory pathways, advancing the biological plausibility of *Iris*-derived leads.[20]

Taken together, these updated findings point to *Iris ensata* as not merely an ornamental or traditional plant, but as a scientifically validated reservoir of bioactive compounds with diverse biological effects. A deeper investigation into its phytochemistry, standardization, and mechanism-based pharmacology offers a compelling path toward novel therapeutics, particularly in disease domains like inflammation, oxidative stress, infection, and cancer.

Scientific classification

Kingdom: Plantae, Division: Tracheophyta, Class: Magnoliopsida, Order: Asparagales Family: Iridaceae, Genus: Iris, Species: *Iris ensata*.

METHODS

Plant material

Fresh rhizomes of *Iris ensata* were obtained from a local supplier at the Khari Baoli market in Delhi and were authenticated by Prof. Wazahat Husain, a taxonomist from the department of botany, Aligarh Muslim University (AMU), Aligarh. For future reference, a voucher specimen was deposited at the Ibne Baitar Museum, department of ilmul advia, AMU, Aligarh. All impurities and foreign materials were manually removed, after which the rhizomes were dried in an oven at 40°C for one hour and subsequently powdered using an electric grinder. The study period for the present study was from March 2023 to October 2024.

Plant extract

The rhizomes of *Iris ensata* were dried at 40°C in a hot air oven. Once dried, they were ground into a fine powder and passed through a sieve of mesh size 80. The powdered sample was then subjected to extraction using a Soxhlet apparatus placed on a heating mantle for six hours, employing a range of solvents including water, acetone, petroleum ether, chloroform, and alcohol. The resulting extracts were filtered and concentrated by evaporation on a water bath. The dried extracts were stored at low temperature for further analysis.

Macroscopic and microscopic analysis

Organoleptic evaluation.

Organoleptic evaluation was performed by visually examining the plant material and recording key diagnostic parameters such as color, appearance, texture, taste, and odor.

Transverse section study

Several transverse sections of the rhizome were carefully cut using a sharp blade, stained with various dyes, and preserved in glycerine for microscopic examination. The stained sections were thoroughly cleaned and mounted in glycerine for observation under a microscope. Additionally, images were captured at different magnifications using a binocular microscope.

Physicochemical parameters

The physicochemical standardization of *Iris ensata* rhizome was carried out following established protocols outlined in various pharmacopoeias and the guidelines provided by the World Health Organization (WHO). Standardization involved evaluating key physicochemical parameters, including solubility in water and alcohol, loss on drying, total ash content, water-soluble ash, acid-insoluble ash, and extractive values in different solvents.

Phytochemical screening

Preliminary phytochemical screening of various extracts of *Iris ensata* Thunb was conducted to identify the presence of bioactive constituents such as alkaloids, glycosides, tannins, carbohydrates, flavonoids, proteins, saponins, terpenoids, and phytosterols.

Fluorescence analysis

Crude powdered drug was reacted with different chemicals and observed for characteristics fluorescent colours under visible light, short and long UV wavelength regions.

HPLC profile

A general HPLC profile of the methanolic extract of Iris ensata rhizome was developed using a Cyber Lab HPLC system equipped with a single pump and a C18 column (4.6 × 250 mm, 5 µm particle size, porous silica). Chromatographic separation was monitored using software-generated peaks (Figure 4). The analysis was conducted at a temperature of 25°C, with a system pressure of 7 Pa and a flow rate of 1.0 ml/minute. Detection was performed at 254 nm using a UV detector. The mobile phase consisted of a mixture of acetonitrile and 3% acetic acid in a 60:40 ratio. The methanolic extract was prepared by subjecting the coarsely powdered rhizome to Soxhlet extraction for six hours. After extraction, the solution was filtered and concentrated by evaporation on a water bath. For HPLC analysis, 20 mg of the dried alcoholic extract was dissolved in 1 ml of HPLC-grade methanol.

EDEX (energy dispersive x-ray spectroscopy)

This analytical technique is used to determine the elemental composition or chemical characteristics of a sample. It operates based on the interaction between the sample and an x-ray excitation source. The fundamental principle is that each element possesses a unique atomic structure, which produces a characteristic set of emission peaks in its electromagnetic spectrum. When atoms are in their ground state, exposure to an incident x-ray beam can excite electrons to higher energy levels. The resulting energy difference is emitted and can be detected, often recorded on x-ray film.[21]

Elemental analysis of the powdered test drug was carried out using the JSM-6510LV, manufactured by JEOL, Japan.

SEM (scanning electron microscopy)

The dried material was mounted directly on stubs using double-side adhesive tape, and sputtered with a thin layer of gold in a JEOL JSM-1200 Fine Coater. The electron micrographs were obtained in a JEOL JSM-6510LV scanning electron microscope with an integrated digital image acquisition system.

RESULTS

Organoleptic features of Iris ensata Thunb

The organoleptic properties of *Iris ensata* Thunb., including its color, appearance, texture, taste, and smell, are presented in Table 1.

Table 1: Organoleptic description of test drug.

Parameters	Observation
Color	Woody brown
Appearance	Wrinkled
Texture	Rough
Taste	Bitter
Smell	Aromatic
Size and shape	Small and varied

Macroscopic study of Iris ensata Thunb

The macroscopic examination of the dried rhizome of *Iris ensata* Thunb. revealed small, elongated fragments of irregular shape. The outer surface was dark brown with prominent transverse wrinkles, while the inner surface appears light brown. The rhizome exhibited a hard and fibrous fracture. It was characterized by a pungent odor and a slightly bitter, aromatic taste (Figure 1A and B).

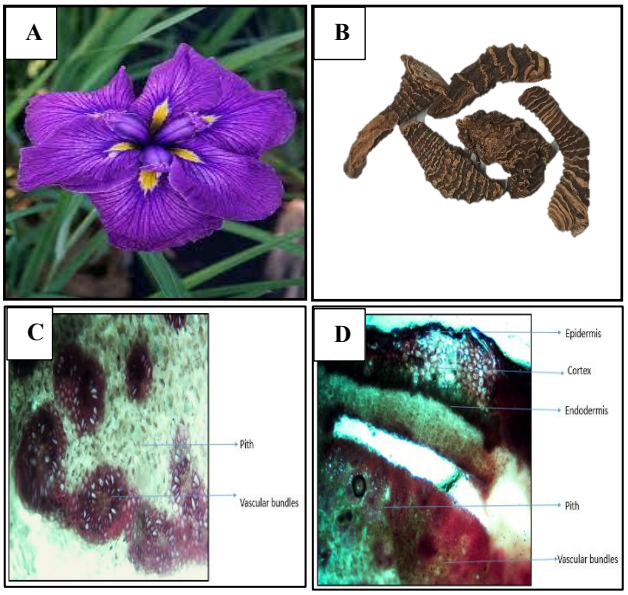

Figure 1: A) Plant of *I. ensata*; B) Dried Rhizome; C, D) Microscopic features of *I. ensata* rhizome.

Microscopic study of Iris ensata Thunb

The transverse section of *Iris ensata* rhizome displays a single-layered epidermis composed of parenchymatous cells with thickened outer walls. Beneath this lies a cortex made up of several layers of rectangular to oval parenchyma. The endodermis is a compact, single cell layer backed by 4-5 layers of thick walled polygonal to

oval cells. Within the inner region, numerous, scattered vascular bundles which are rounded to tapered and appear just interior to the endodermis; each bundle contains phloem and scattered xylem elements encased in a lignified fibrous sheath (1-3 cells thick). Finally, the central pith comprises thin-walled, tightly packed polygonal to oval parenchymatous cells (Figure 1C and D).

Physicochemical analysis of Iris ensata Thunb

The rhizome powder of *Iris ensata* demonstrated the following physicochemical properties: total ash- 6.98% w/w, acid-insoluble ash- 1.07% w/w, water-soluble ash- 5.91% w/w, loss on drying- 4.21% w/w, foreign matter- 0.66% w/w, bulk density- 0.63%, and pH- 5.68 (Table 2).

Table 2: Physicochemical parameters.

Parameters	Value (%w/w)
Foreign matter	0.66
Loss on drying	4.21
Total ash value	6.98
Acid insoluble Ash	1.07
Water soluble Ash	5.91
pH	5.68
Bulk density	0.63
Pet. ether soluble extractive value	2.6
Chloroform soluble extractive value	1.4
Acetone soluble extractive value	5.3
Water soluble extractive value	13.3
Alcohol soluble extractive value	9.81

Table 3: Fluoresce analysis of *Iris ensata* Thunb.

Powdered drug + chemical reagents	Day light	UV Short	UV Long
P. drug + conc. HNO_3	Brownish orange	Yellowish brown	Greenish black
P. drug + conc. HCl	Chocolate brown	Greenish brown	Greenish yellow
P. drug + conc. H_2SO_4	Yellowish black	Jet black	Blackish green
P. drug + Gl. acetic acid	Dark brown	Dark green	Greenish black
P. drug + NH_3	Yellowish brown	Grass green	Yellowish black
P. drug + Petroleum ethe	Woody brown	Olive green	Dark green
P. drug + Ethanol	Brown	Olive green	Greenish black
P. drug + Ethyl acetate	Light brown	Light green	Dark olive green
P. drug + Benedict's reagent	Greyish green	Dark green	Bluish black
P. drug + Fehling's Reagent	Dark green	Algae green	Bluish green

Table 4: Qualitative analysis of the phytochemicals present in *Iris ensata* Thunb.

Chemical constituents	Test/reagents	Result
Alkaloids	Dragendroff's test	+ve
	Hager's test	+ve
	Wagner's test	+ve
	Mayer's test	+ve
Carbohydrate	Fehling's solution test	+ve
	Molisch's test	+ve
	Benedict's test	+ve
Flavonoids	Shinoda test	+ve
	Alkaline reagent test	+ve
Glycosides	Borntrager's test	+ve
	Salkowski's test	+ve
	Keller-kiliani test	+ve
Tannins	Ferric chloride test	+ve
	Lead subacetate test	+ve
Proteins	Millon's reaction	-ve
Proteins	Biurette's test	-ve
Starch	Xanthoproteic test	-ve
	Iodine test	-ve
Phenols	Lead acetate test	+ve
Phenols	Ferric chloride test	+ve
Sterols/Terpenes	Liebermann test	+ve
	Salkowski's test	+ve

Continued.

Chemical constituents	Test/reagents	Result
Sterols/Terpenes	Liebermann-Burchard's test	+ve
Amino Acids	Ninhydrin test	-ve
Resins	Acetic anhydride test	+ve
Saponins	Honeycomb test	+ve
Fixed oil	Spot test	+ve

Table 5: Peak profile of HPLC of methanolic extract of *Iris ensata* Thunb.

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.159	149202	21759	0.278	0.519
2	2.828	21326275	2291376	39.709	54.676
3	3.051	31601478	1836528	58.842	43.822
4	4.112	35457	5888	0.066	0.140
5	4.258	62482	9640	0.116	0.230
6	4.808	22671	1911	0.042	0.046
7	5.318	54202	3000	0.101	0.072
8	6.093	23693	1861	0.044	0.044
9	6.881	7540	816	0.014	0.019
10	7.222	3873	381	0.007	0.009
11	7.883	10827	881	0.020	0.021
12	10.381	2689	216	0.005	0.005
13	10.903	5319	401	0.010	0.010
14	11.291	42717	2401	0.080	0.057
15	11.746	246104	9547	0.458	0.228
16	12.987	58482	1890	0.109	0.045
17	13.429	42220	1977	0.079	0.047
18	13.913	10499	368	0.020	0.009
Total		53705729	4190842	100.000	100.000

Fluorescence and phytochemical analysis of Iris ensata Thunb

Preliminary phytochemical screening of *Iris ensata* Thunb. confirmed the presence of various compounds, including steroids, terpenoids, saponins, fatty acids, flavonoids, phenolic compounds, glycosides, and carbohydrates. The detailed results of the Fluorescence and phytochemical analysis are provided in Tables 3 and 4.

HPLC profiling of methanolic extract of Iris ensata Thunb

The HPLC chromatogram of the methanolic extract of *Iris ensata* revealed a total of 18 distinct peaks. Among them, peak 2 was the most prominent, exhibiting a concentration of 54.676% and a retention time of 2.828 minutes. This was followed by peak 3 with a concentration of 43.822%, and peak 1 with 0.519%. Detailed chromatographic results are illustrated in Figure 2 and summarized in Table 5.

EDEX (Energy dispersive x-ray spectroscopy) of Iris ensata Thunb

The elemental analysis of *Iris ensata* revealed the presence of essential elements, including carbon, oxygen, magnesium, potassium, phosphorus, and silicon. The results are presented in Table 6 and illustrated in Figure 3.

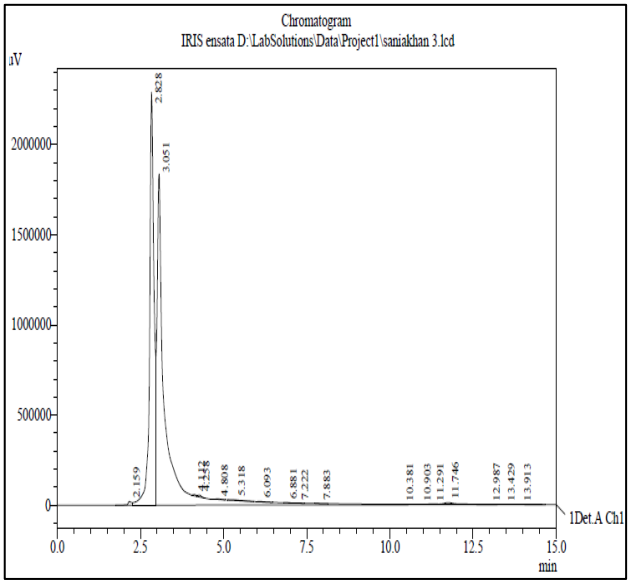

Figure 2: Peak profile of HPLC of methanolic extract of *Iris ensata* Thunb.

Table 6: Elemental composition of Iris ensata Thunb.

Element	Atomic %	Weight %
Carbon	42.69	50.89
Oxygen	52.65	47.11
Magnesium	0.23	0.13
Potassium	2.67	0.98
Phosphorus	0.24	0.11
Silicon	1.53	0.78

SEM (scanning electron microscopy) of I. ensata Thunb rhizome

SEM revealed the presence of parenchyma tissue containing secretory ducts and calcium oxalate crystals embedded in the parenchyma and abundant starch granules in cortical parenchymal tissue (Figure 4).

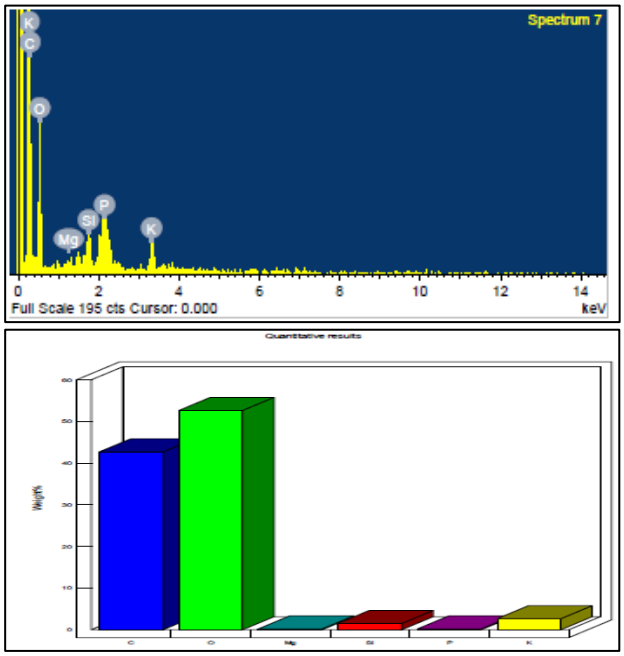

Figure 3: Analysis of elemental composition of *Iris ensata* Thunb by EDAX.

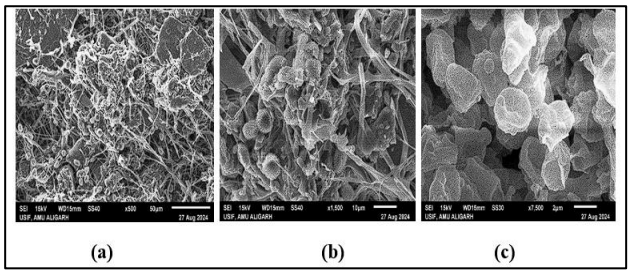

Figure 4: a) SEM micrograph with a detail of the parenchyma tissue containing secretory ducts. Scale bar =50 µm; b) calcium oxalate raphide crystals embedded within parenchyma tissue. Scale bar =10 µm; c) starch granules located in the cortical parenchyma. Scale bar =2 µm.

DISCUSSION

Modern medicine is grounded in rigorous experimental evidence, including toxicity studies and human clinical trials. However, a significant gap exists in the pharmacopoeial standards for raw materials and finished herbal products, resulting in inadequate quality control measures. This lack of standardization has led to adverse effects, varying from mild to severe. Consequently, the standardization of herbal ingredients is crucial to ensure their identity, purity, safety, and overall quality.[22] Herbal medicines are traditionally regarded as safe and are often consumed without medical supervision. However, certain herbal products may cause adverse health effects, lack therapeutic efficacy, or interact negatively with other medications. Therefore, standardization is essential for assessing the quality, purity, and authenticity of herbal drugs. This is achieved through the evaluation of physicochemical parameters, HPLC profiling, and identification of active constituents.[23] A well-standardized, high-quality herbal drug is essential for ensuring therapeutic efficacy and gaining global acceptance. *Iris ensata* Thunb., a well-known medicinal plant in the Unani system of medicine, is traditionally used for managing various inflammatory conditions. The present study aims to standardize *I. ensata* by evaluating its physicochemical properties, including organoleptic features, ash values, extractive values, moisture content, solubility, fluorescence characteristics, qualitative phytochemical screening, and HPLC profiling.

Organoleptic evaluation plays a vital role in the preliminary identification and consumer acceptance of herbal drugs. Key sensory parameters such as visual appearance, color, texture, taste, odor, and fracture are commonly assessed during macroscopic examination to distinguish crude plant materials. The organoleptic properties of *Iris ensata* Thunb including its color, appearance, texture, taste, and smell are given in Table 1. Standardization and quality control of herbal drugs is necessary because adulteration is found in market herbal medicines. Microscopic analysis is among the cheapest of methods to correctly identify the particular herbal drug in powder form.[24,25] Powder microscopy and anatomical features provide useful knowledge about quality control of medicinal powder drugs.[26] Therefore, we have established standards for controlling adulteration and correct identification of this plant for future use.

Physicochemical parameters such as ash values, extractive values in alcohol and water, moisture content, and weight loss upon drying at 105°C are critical indicators of the purity, quality, and authenticity of crude drugs. These values are fundamental for the standardization of herbal medicines. Total ash, acid-insoluble ash, and water-soluble ash help in detecting the presence of inorganic impurities or adulterants in plant materials. Likewise, water- and alcohol-soluble extractive values indicate the concentration of bioactive compounds extractable in respective solvents, with deviations potentially pointing to

adulteration, improper processing, or the use of exhausted plant material.[27,28] Moisture content also plays a vital role, as high moisture levels can encourage microbial growth and lead to deterioration of the drug's therapeutic efficacy.[28] In the present study all the physicochemical parameters were found within permissible limits indicating Test drug's purity and quality (Table 2).

Fluorescence analysis is a valuable tool in the pharmacognostic evaluation of crude drugs, as certain phytoconstituents exhibit characteristic fluorescence when exposed to daylight or ultraviolet (UV) light. Even if a substance does not naturally fluoresce, the application of specific reagents can induce fluorescence, aiding in its identification. This property is commonly employed for the qualitative analysis of herbal drugs and serves as an important parameter in assessing their authenticity and purity.[29]

The therapeutic efficacy of herbal medicines largely depends on the presence of secondary metabolites such as alkaloids, glycosides, and other bioactive compounds. The detection of these phytoconstituents serves as a key indicator of the quality and medicinal potential of the crude drug.[29] Hence, the evaluation of secondary metabolites is a critical step in the standardization process. Preliminary phytochemical screening of *Iris ensata* Thunb confirmed the presence of various compounds, including steroids, terpenoids, saponins, fatty acids, flavonoids, phenolic compounds, glycosides, and carbohydrates (Table 4).

Preparative and analytical high-performance liquid chromatography (HPLC) is widely employed in the analysis of herbal medicines due to its high resolution and superior separation efficiency. This technique enables the identification and quantification of nearly all constituents within herbal formulations, provided that an optimized method is established. Key factors in method development include the appropriate selection of mobile and stationary phases, as well as other chromatographic parameters.[30] HPLC is also instrumental in detecting adulteration and impurities; deviations in peak number, retention time, or peak area from standard profiles may suggest degradation or adulteration of the sample. The HPLC chromatogram of the methanolic extract of *Iris ensata* revealed a total of 18 distinct peaks. Among them, peak 2 was the most prominent, exhibiting a concentration of 54.676% and a retention time of 2.828 minutes. This was followed by peak 3 with a concentration of 43.822%, and peak 1 with 0.519% (Figure 2 and Table 5).

The elemental composition of the powdered herbal drug was determined using energy-dispersive x-ray spectroscopy (EDX) coupled with scanning electron microscopy (SEM). This technique provided detailed insights into both the chemical composition and the spatial distribution of elements within the sample. The elemental analysis of *Iris ensata* revealed the presence of essential elements, including carbon, oxygen, magnesium, potassium, phosphorus, and silicon (Table 6 and Figure 3).

SEM is a powerful method for the investigation of surface structures of herbal medicines. Scanning electron microscopy is operated in the vacuum and focuses the electron beam and magnifies images with the help of electromagnetic lenses.[29] Results for SEM analysis of test drug were depicted in Figure 4a, b and c.

CONCLUSION

The present study demonstrates that the standardization and identification of *Iris ensata* Thunb, through organoleptic evaluation and physicochemical analysis, provide essential parameters for verifying the authenticity and ensuring the quality of the test drug. Adhering to high-quality standards is critical for maintaining the therapeutic efficacy of herbal medicines. Furthermore, the results of phytochemical screening and HPLC fingerprinting offer valuable insights for the identification and authentication of *I. ensata*. These analytical tools not only contribute to quality assurance but also support the plant's traditional use in managing various health conditions. The findings of this study will serve as a reference for the identification, standardization, and quality control of *Iris ensata* and support the development of its monograph for inclusion in national and international pharmacopoeias.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Orhan I, Nasim S, Sener B, Ayanoglu F, Ozguven M, Chaudhary MI, et al. Two isoflavones and bioactivity spectrum of the crude extracts of *Iris germanica* rhizomes. Phytother Res. 2003;17(5):575-7.
2. Benoit-Vical F, Imbert C, Jean-Paul B, Sauvaire Y. Antiplasmodial and antifungal activities of iridal, a plant triterpenoid. Phytochemistry. 2003;62(5):747-51.
3. Wang H, Cui Y, Zhao C. Flavonoids of the genus *Iris* (Iridaceae). Mini Rev Med Chem. 2010;10(7):643-61.
4. Rahman A, Nasim S, Baig I, Sener B, Orhan I, Ayanoglu F, et al. Two new isoflavanoids from the rhizomes of Iris soforana. Nat Prod Res. 2004;18(5):465-71.
5. Choudhary MI, Hareem S, Siddiqui H, Anjum S, Ali S, Atta-Ur-Rahman, et al. A benzil and isoflavone from *Iris tenuifolia*. Phytochemistry. 2008;69:1880-5.
6. Ibrahim SR, Mohamed GA, Zayed MF, Ross SA. 8-Hydroxyirilone 5-methyl ether and 8-hydroxyirilone, new antioxidant and α-amylase inhibitors isoflavonoids from *Iris germanica* rhizomes. Bioorg Chem. 2017;70:192-8.
7. Basgedik B, Aysel U, Nurdan S. Antimicrobial, antioxidant, antimutagenic activities, and phenolic compounds of Iris germanica. Ind Crops Prod. 2014;61:526-30.

8. Nazir N, Koul S, Qurishi MA, Taneja SC, Ahmad SF, Khan B, et al. Immunomodulatory activity of isoflavones isolated from *Iris germanica* (Iridaceae) on T-lymphocytes and cytokines. Phytother Res. 2009;23(3):428-33.
9. Mohamed GA, Ibrahim SR, Ross SA. New ceramides and isoflavone from the Egyptian *Iris germanica L*. rhizomes. Phytochem Lett. 2013;6(3):340-4.
10. Roger B, Jeannot V, Fernandez X, Cerantola S, Chahboun, J. Characterization and quantification of flavonoids in *Iris germanica L.* and *Iris pallida Lam*. resinoids from Morocco. Phytochem Anal. 2012;23(5):450-5.
11. Mozaffarian V. Identification of Medicinal and Aromatic Plants of Iran. Tehran: Farhang-e Moaser. 2012.
12. Kukula-Koch W, Sieniawska E, Widelski J, Urjin O, Głowniak, P, Skalicka-Wo´zniak, K. Major secondary metabolites of Iris spp. Phytochem Rev. 2015;14(1):51-80.
13. Asghar SF, Habib-ur-Rehman, Atta-ur-Rahman, Choudhary MI. Phytochemical investigations on *Iris germanica*. Nat Prod Res. 2010;24(2):131-9.
14. Anonymous. The Unani Pharmacopoeia of India, Part-I, Vol- II. Government of India; 2009.
15. Ministry of Health and Family Welfare. Department of AYUSH, New Delhi; 2007:57-58.
16. Anonymous, The Wealth of India: a dictionary of raw materials and industrial products. Raw Materials. Vol-V. Council of Scientific and Industrial Research, Delhi; 1997:255.
17. Kirtikar KK, Basu BD. Indian Medicinal Plant. Vol-IV. International Book Distributors, Dehradun; 1987:2460.
18. Salim S, Khanday S, Kalam MA, Yousuf A, Rifat I, Butt T. Exploring morphological and phytochemical differences in *Iris ensata* (Sosan asmanjuni) and market varieties. Tradition Med. 2025;6(1):32.
19. Momin A, Zaibunnisa B, Ather MR. Therapeutic Insights into *Iris ensata* Thunb. (Irsa): bridging Unani wisdom and modern pharmacology. Int J Creat Res Thoughts. 2025;13(8):402-15.
20. Khatib S, Faraloni C, Bouissane L. Exploring the use of iris species: antioxidant properties, phytochemistry, medicinal and industrial applications. Antioxidants. 2022;11(3):526.
21. Sumaiya S, Siddiqui A, Chaudhary SS, Aslam M, Jamal A, Phytochemistry, traditional uses and biological activities of Irsa (*Iris ensata* Thunb): a multi potent Unani medicine. JBPAS. 2023;12(4):1770-80.
22. Neeraj C, Bhupinder SS. An overview of advances in the standardization of herbal drugs. J Pharm Educ Res. 2011;2(2):55-70.
23. Akbar S, Hanif U, Ali J, Ishtiaq S. Pharmacognostic studies of stem, roots and leaves of *Malva parviflora L*. Asian Pac J Trop Biomed. 2014;4(5):410-5.
24. Uza NU, Dastagir G. Microscopic and pharmacognostic standardization of *Astragalus scorpiurus* Bunge. Microsc Res Tech. 2022;85(1):324-38.
25. Nisar MF, Khadim M, Rafiq M, Chen J, Yang Y, Wan CC. Plant complexes and their isolated compounds for oxidative stress and inflammation: from basic research to clinical evidence. Oxidat Med Cell Long. 2021.
26. Latif A, Rehman S. Standardization of an herbal medicine- *Swertia chirayita linn*. Pharmacophore. 2014;5(1):98-108.
27. Zameer M, Rauf A, Qasmi IA. Standardization and safety evaluation of a polyherbal formulation "Kabideen (Syrup). Intern J Adv Res. 2015;3(9):1105-14.
28. Mohammad R, Shariq S, Roohi Z. Preliminary physico-chemical evaluation of sunun poste mughilan. Hippocrat J Unani Med. 2014;2:9-19.
29. Sumitra C. Importance of pharmacognostic study of medicinal plants: an overview. J Pharmacogn Phytochem. 2014;2(5):69-73.
30. Kirti MK, Leena SP, Khanvilkar VV, Vilasrao JK. Fingerprinting techniques in herbal standardization. Indo Am J Pharm Res. 2014;4(2):1049-62.
31. Flegler SL, Heckman Jr JW, Klomparens KL. Scanning and transmission electron microscopy. W. H. Freeman and Company, New York; 1993.

Cite this article as: Khan S, Alam S, Azhar M, Shamshad S, Fatima MN, Farha S, et al. Unraveling *Iris ensata* Thunb: a pharmacognostical, physicochemical and HPLC-assisted phytochemical investigation. Int J Basic Clin Pharmacol 2026;15:27-34.