Exploring Morphological and Phytochemical Differences in *Iris ensata* (*Sosan asmanjuni*) and Market Varieties

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ABSTRACT

Iris has taken its name from the Greek word meaning rainbow because of its colorful flowers. The genus Iris is widely distributed throughout the North Temperate Zone. There is considerable variation in their habitat, ranging from cold mountainous regions to meadow lands and riverbanks across Europe and Asia. In Kashmir, they are commonly found in alpine or sub-alpine regions, grasslands, graveyards, and stream banks. Most species with medicinal properties grow wild. Various species of iris found in Kashmir are *Iris ensata* Thunb, *Iris hookeriana, Iris germinaca. Iris kashmiriana* and Iris *hookeriana* grow wild throughout Kashmir and Ladakh. In Kashmir, the botanical source of Iris is derived from Iris ensata Thunb, while a different species available in the market is known as Irsa, which is used medicinally. This comparative study is aimed to have the detailed study of Pharmacognostical parameters along with physiochemical and Phytochemical study of the botanical source and market sample of Irsa.

Keywords: *Iris ensata Thunb, Iris, Sosan asmanjuni, Irsa,* Physiochemical, Phytochemical

INTRODUCTION

In the current health sector, there is a notable shift from communicable to non-communicable diseases, with conditions like Type II diabetes mellitus, hypertension, obesity, and cardiovascular diseases becoming increasingly prevalent. These diseases can often be prevented or effectively treated using the Unani System of Medicine (USM), which primarily relies on herbal remedies. Surveys indicate that 80% of the global population depends on herbal medicine for primary healthcare [1]. USM utilizes

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approximately 25,000 plant-based formulations documented in indigenous medical texts [2], with its treatments derived mainly from plants, followed by animal and mineral origins.

In USM, diseases are treated using either single herbs or compound formulations composed of multiple herbs. However, the efficacy of these treatments depends on the accurate identification and knowledge of each herb's properties. Adulteration, whether intentional or accidental, poses significant risks, including diminished therapeutic efficacy and harmful side effects. This highlights the necessity for robust identification and standardization parameters for each herb to prevent adulteration and ensure safety and efficacy.

One common form of adulteration in USM is the substitution of herbs with similar species. For instance, Iris (commonly known as Irsa in USM) is often confused in the Indian market with other species such as Iris ensata Thunb, Iris germanica, Iris kashmiriana, and Iris versicolor. The genus Iris is large and taxonomically complex, with about 12 species found in India, particularly in the Kashmir and Ladakh regions, where many species like Iris ensata and Iris kashmiriana grow wild [3-7].

To address this issue, the present study aims to 1) establish diagnostic keys for the identification and differentiation of Iris ensata Thunb from other species and market samples labeled as Irsa.

2) Conduct a detailed comparative pharmacognostic evaluation of the rhizomes from identified botanical sources and market samples. Develop macroscopic and microscopic profiles to differentiate between authentic and adulterated samples, thereby ensuring quality and authenticity in USM formulations. This study provides a critical foundation for preventing adulteration and enhancing the therapeutic reliability of Unani medicines.

***Iris ensata* thunb Rhizome (Botanical source)**

***Iris ensata* thunb (Market sample)**

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Action and Uses: The main action of the rhizome is Mushil-i-safrā' wa Balgham; it is also Mundij Muhallil, Musakkin, Musaffī-i-Dam, Munaffith-i-Balgham and Mukhrij-i-Balgham. Therapeutically it is used in illnesses like Dhāt al-Janb and Dama.It can be used in hard enlargements, splenomegaly, tumours etc. [8-10].

Major Chemical Constituents: The Phytochemical constituents reported from the plant are resins, sterols, phenols, terpenoids, glycosides, flavonoids, proteins and carbohydrates [11].

MATERIALS AND METHODS

Plant Material Collection and Authentication

Fresh rhizome of *Iris ensata* Thunb was collected from herbal garden of RRIUM Srinagar in June 2018. Authentication of specimen of Iris *ensata* Thunb.was done in Centre for Biodiversity and Taxonomy Department of Botany, University of Kashmir, vide voucher no. CBT/KASH/Vouch. Dated 19/11/2018. The market sample of *Irsa* was collected from different markets of Jammu and Kashmir but the dried rhizome could not be identified for its botanical source as its full plant was not available for proper identification, However, it was confirmed from all the outlets that the rhizome is being used as *Irsa*.

Macroscopic Evaluation:

The detailed macroscopic evaluation was carried out to differentiate between the Iris ensata thunb obtained from botanical source and *Irsa* market sample.

Powder study: The plant sample of both Iris *ensata* thunb and *Irsa* market sample were powdered and both stained and unstained slides were prepared. These slides were then observed under microscope.

Microscopic Evaluation:

Physico-Chemical Evaluation:

Physiochemical parameters of the rhizome of *Iris ensata* Thunb and *Irsa* market sample were evaluated as per Indian pharmacopeia, 1996. Ash values (Total Ash, Acid insoluble ash, water soluble ash, and sulphated ash), Water and Alcohol soluble extractive values, loss on Drying, etc were carried out [12-14].

Preparation of extracts:

The dried, free from any foreign material and coarsely powdered rhizome of *Iris ensata* Thunb and *Irsa* market sample (200gm each) were subjected to successive extraction in Soxhlet apparatus with different solvents in increasing order of polarity, viz; pet ether 60/80, ethyl acetate, methanol, hydro-alcoholic and water. Extraction was performed using continuous soxhlation [15].

Preliminary Phytochemical screening

The various extracts of *Iris ensata* Thunb and *Irsa* market sample were subjected to Phytochemical screening to identify various constituents like alkaloids, glycosides, tannins, carbohydrates, flavonoids, proteins, saponins, terpenoids and phytosterols, present in them [12,15].

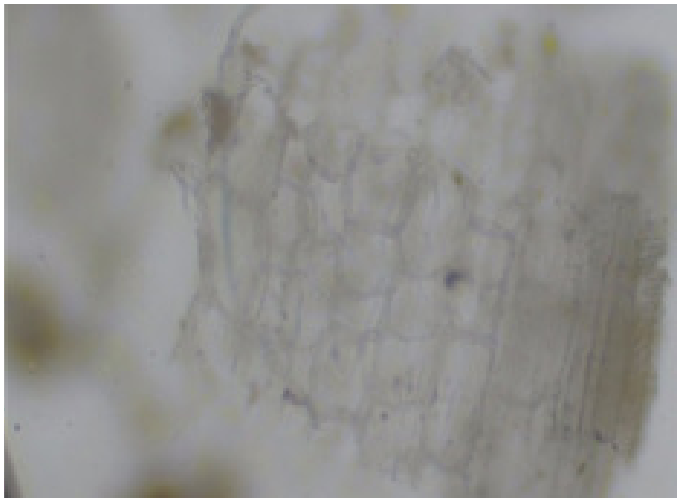
Thin layer chromatography: The Pet ether, Ethyl acetate and Methnolic extracts of *Iris ensata* Thunb (IER) and *Iris* market sample (IMS) were subjected to TLC profiling after resolving in their corresponding solvents and developing suitable solvent system for each of the extract. For Pet ether extract of Iris *ensata* Thunb (IER) and *Irsa* market sample (IMS), the solvent system that was developed was 10% ethyl acetate in 90% of Hexane. For Ethyl acetate extract of *Iris ensata* Thunb and *Irsa* market sample the solvent system that was developed was 1% ethyl acetate in 99% of chloroform. Similarly, for Methnolic extract of *Iris ensata* Thunb and *Irsa* market sample, the solvent system that was developed was 40% Ethyl acetate in 60% Methanol.

RESULTS

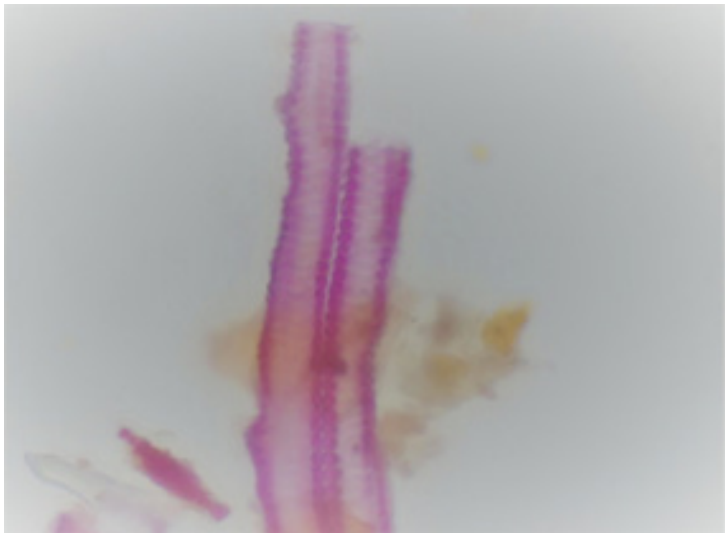
Macroscopic characters of rhizomes of Iris *ensata Thunb*. Rhizome (IER) and *Irsa* market (IMS) sample

S.No	Parameters	*Iris ensata* Thunb. (IER)	*Irsa*market sample(IMS)
1.	Colour	Dark brown externally yellow internally	Dark brown externally yellow internally
2.	Odour	Characteristic	Characteristic
3.	Taste	Bitter	Bitter
4.	Length	6.2-8.1cm(Root lets)	2.4-4.3cm
5.	Diameter	1.1-1.52 cm	1.3-2.4cm
6.	Shape	Irregular	Irregular

Microscopical findings of Iris *ensata Thunb*. Rhizome and *Irsa* market sample



Leaf *Iris ensata* Thunb. (Epidermal cells)



Spiral vessels of *Iris ensata* Rhizome

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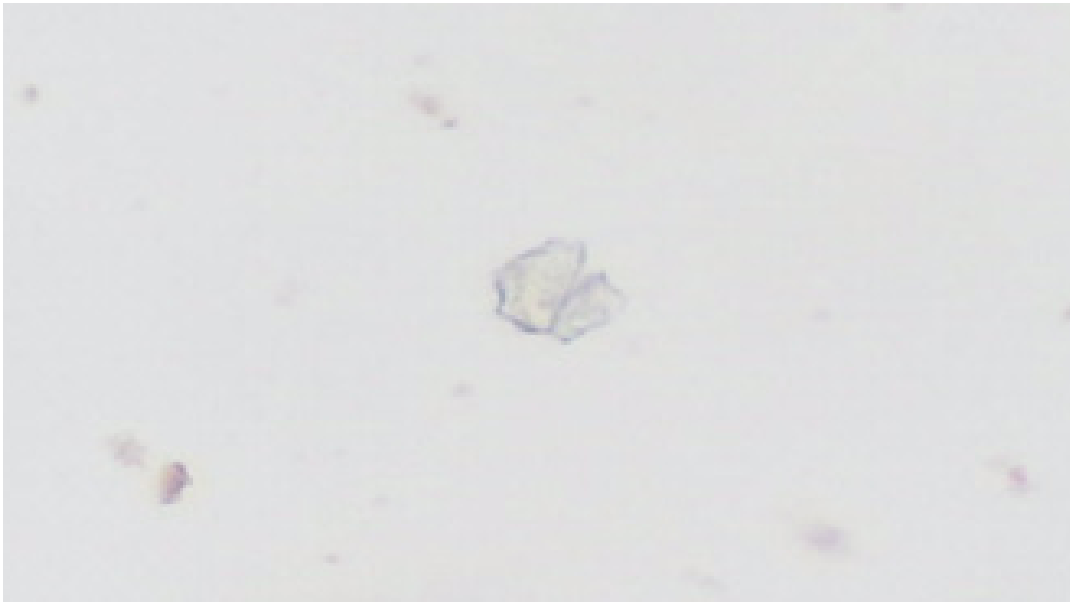
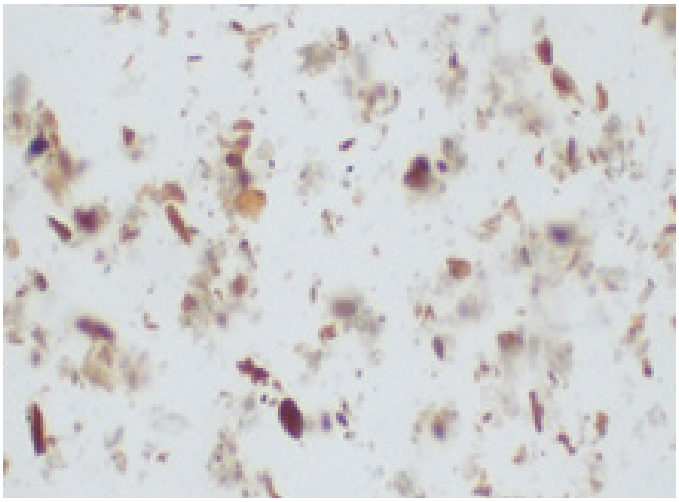
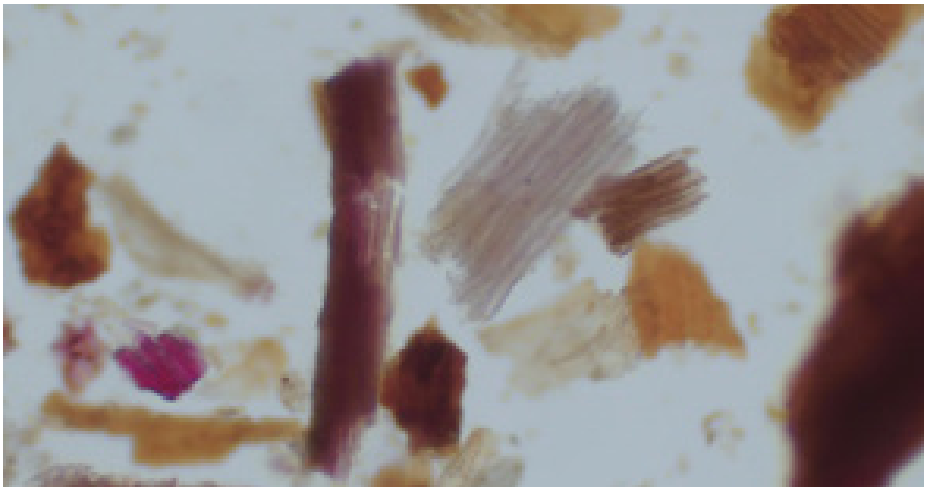


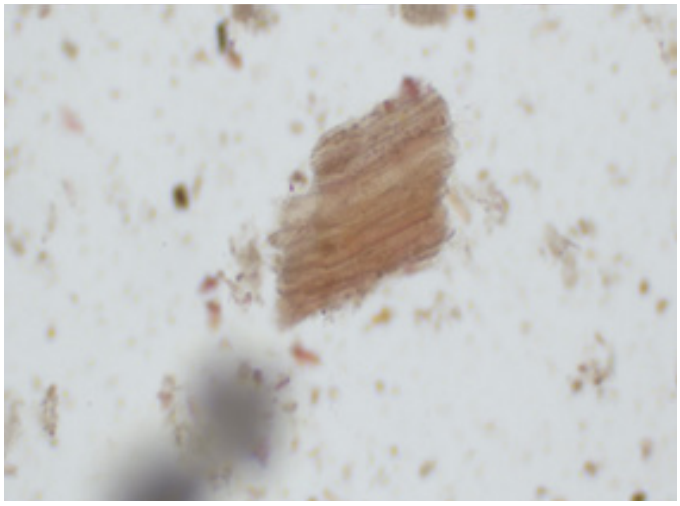
Presence of Prismatic Calcium Oxalate Crystals in Iris *ensata* Thunb. Rhizome



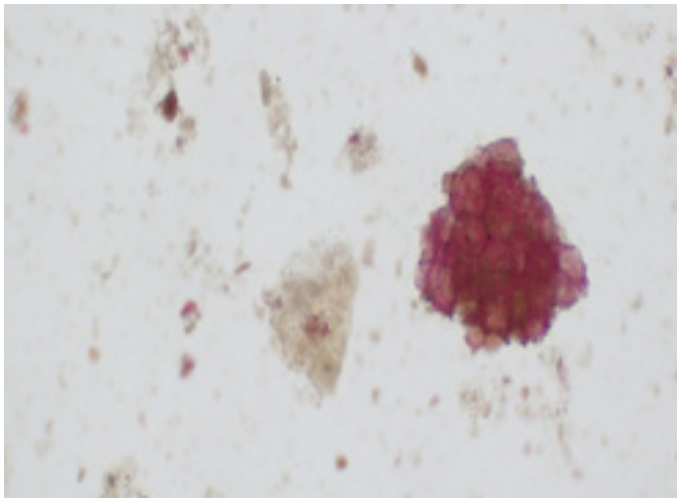
Presence of starch granules in *Iris ensata* thunb Rhizome



Presence of group of Fibro-Vascular Tissue in *Iris ensata* Thunb. Rhizome



Part of Fibro Vascular Tissue *Irsa* Market sample



Endospermal cells *Irsa Market* sample

Physico-Chemical Evaluation:

Parameters	Values *Iris ensata* Thunb	Values *Irsa* market sample
Total Ash value	11.33%	4.33%
Acid insoluble ash	3.60%	0.67%
Water soluble ash	1.40%	18%
Sulphated ash	15%	6.50%
Water soluble extractive value	1.40%	10%
Alcohol soluble extractive value	0.40%	4.80%
Loss on drying	14.60%	9.70%

Preliminary screening of major phyto-chemicals of *Iris ensata* rhizome:

Tests for	Name of the Test	Pet ether extract	Ethyl-acetate extract	Methanol extract	Hydro –Alcoholic Extract	Aqueous Extract
Alkaloids	Mayor's Test	-ve	-ve	-ve	-ve	-ve
	Tannic acid	-ve	-ve	-ve	-ve	-ve
	TestWagner's	-ve	-ve	-ve	-ve	-ve
	TestDragen-	-ve	-ve	-ve	-ve	-ve
	droff's Test	-ve	-ve	-ve	-ve	-ve
	Hager's Test	-ve	-ve	-ve	-ve	-ve
Glycosides	Borntrager's Test	-ve	-ve	-ve	-ve	-ve
	Keller Killani Test	+ve	+ve	+ve	+ve	-ve
	Legal's Test	-ve	-ve	+ve	+ve	-ve
Tannins	Ferric chloride Test	-ve	-ve	+ve	+ve	-ve
	Lead acetate Test	-ve	-ve	+ve	+ve	+ve
Carbohy-drates	Molisch's Test	+ve	+ve	+ve	+ve	+ve
	Benedict's Test	-ve	+ve	+ve	+ve	+ve
	Fehling's Test	+ve	+ve	+ve	+ve	+ve
	Barford's Test	-ve	-ve	+ve	+ve	+ve
Flavonoids	Alkaline reagent test	-ve	-ve	+ve	-ve	-ve
	Zinc test	-ve	-ve	+ve	-ve	-ve
Proteins	Nin Hydrin Test	-ve	-ve	+ve	+ve	+ve
	Million's Test	-ve	-ve	+ve	+ve	+ve
Saponin's	Lead acetate Test	-ve	-ve	+ve	+ve	+ve
	Froth Test	_	_	_	+ve	_
	Foam Test	_	_	_	+ve	_
Terpenoids	Salkowski Test	+ve	+ve	-ve	-ve	-ve
Sterols	Salkowski Test	+ve	-ve	-ve	-ve	-ve

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Preliminary screening of major phyto-chemicals of *Irsa* Market sample:

Tests for	Name of the Test	Pet ether extract	Ethyl-acetate extract	Methanol extract	Hydro – Alcoholic Extract	Aqueous Extract
Alkaloids	Mayor's Test	-ve	-ve	-ve	-ve	-ve
	Tannic acid Test	-ve	-ve	-ve	-ve	-ve
	Wagner's Test	-ve	-ve	-ve	-ve	-ve
	Dragendroff's Test	-ve	-ve	-ve	-ve	-ve
	Hager's Test	-ve	-ve	-ve	-ve	-ve
Glycosides	Borntrager's Test	-ve	-ve	-ve	-ve	-ve
	Keller Killani Test	+ve	+ve	+ve	+ve	-ve
	Legal's Test	-ve	-ve	+ve	+ve	-ve
Tannins	Ferric chloride Test	-ve	-ve	+ve	+ve	-ve
	Lead acetate Test	-ve	-ve	+ve	+ve	+ve
Carbohydrates	Molisch's Test	+ve	+ve	+ve	+ve	+ve
	Benedict's Test	-ve	+ve	+ve	+ve	+ve
	Fehling's Test	+ve	+ve	+ve	+ve	+ve
	Barford's Test	-ve	-ve	+ve	+ve	+ve
Flavonoids	Alkaline reagent test	-ve	-ve	+ve	-ve	-ve
	Zinc test	-ve	-ve	+ve	-ve	-ve
Proteins	Nin Hydrin Test	-ve	-ve	+ve	+ve	+ve
	Million's Test	-ve	-ve	+ve	+ve	+ve
	Lead acetate Test	-ve	-ve	+ve	+ve	+ve
Saponin's	Froth Test	–	–	–	+ve	–
	Foam Test	–	–	–	+ve	–
Terpenoids	Salkowski Test	+ve	+ve	-ve	-ve	-ve
Sterols	Salkowski Test	+ve	-ve	-ve	-ve	-ve

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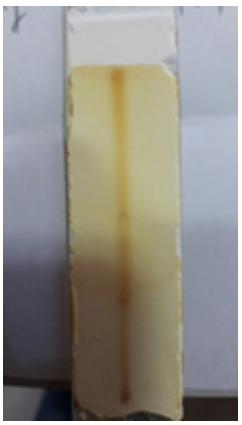
Thin Layer Chromatography:

TLC profile of Pet ether extract of *Iris ensat*a Thunb and *Irsa* market sample in Ethyl acetate (10%) in Hexane (90%) solvent system

No of spots in Pet ether extract of IER	R_f values	No of spots in Pet ether extract of IMS	R_f values
Spot 1	0.384 (visible at UV 365nm)	Spot 1	0.18
Spot 2	0.60	Spot 2	0.433
Spot 3	0.692	Spot 3	0.516
	0.769	Spot 4	0.966

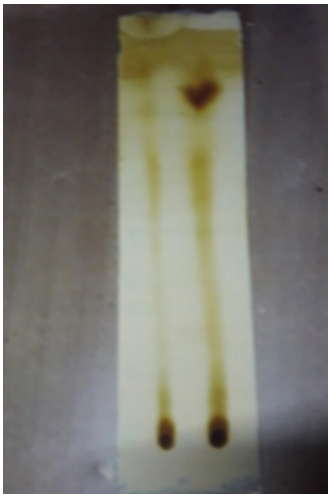
TLC plate of pet ether extract IER UV Visible Spot at 365



TLC Plate of pet ether extract IMS showing spots as mentioned in the Table above

TLC profile of Ethyl acetate extract of iris ensata Thunb and Irsa market sample in Ethyl acetate (1%) in Chloroform (99%) solvent system

No of spots in Ethyl acetate extract of IER	R_f values	No of spots in Ethyl acetate extract of IMS	R_f values
Spot1	0.492	Spot 1	0.127
Spot 2	0.569	Spot 2	0.509
Spot 3	0.646	Spot 3	0.618
-	-	Spot 4	0.745

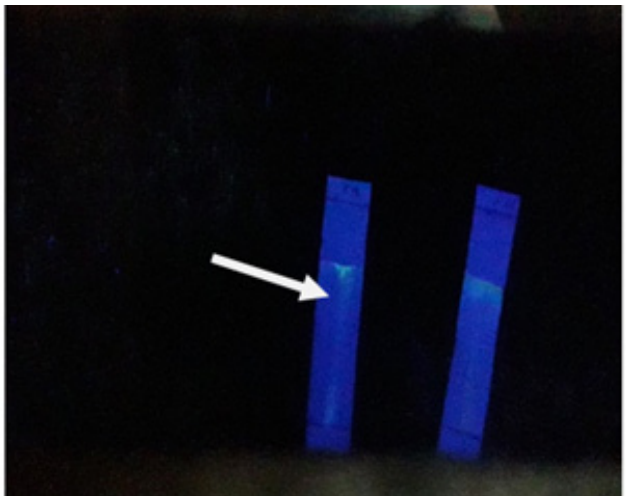


TLC plate of ethyl acetate extract of IER Showing visible spots as mentioned the above table.

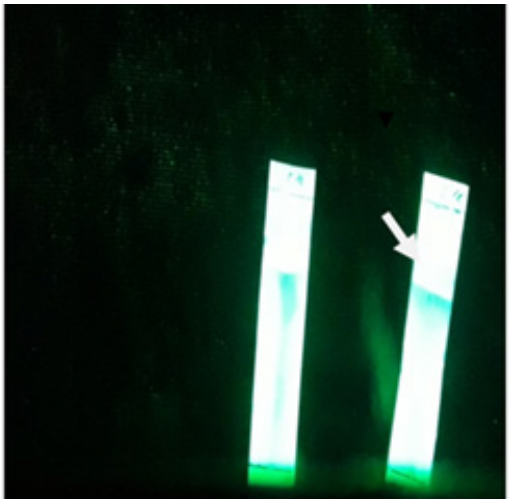
TLC plate of ethyl acetate extract of IMS showing visible spots as mentioned in the above table.

TLC profile of Methnolic extract of Iris ensata Thunb and Irsa market sample in Ethyl acetate (50%) in Methanol (50%) solvent system.

No spots in Methnolic extract of IER	R_f values	No spots in Methnolic extract of IMS	R_f values
Spot 1	0.741(visible at UV365)	Spot 1	0.779 (visible at UV254)
Spot 2	0.833(visible at UV365)	-	-



TLC plates of Methnolic extract of IER (Left side with arrow) UV visible spots At 365nm



TLC plates of Methnolic extract of IMS with UV visible spots at 254nm (on right with arrow)

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DISCUSSION AND CONCLUSION

The present study has been carried out at the department of *Ilmul Advia* (Pharmacology), Regional Research Institute of Unani Medicine (RRIUM) Srinagar and department of Pharmacy, University of Kashmir-Srinagar, to fulfill the aim of establishing a comprehensive scientific way of comparative study between two species of *Irsa*, a medicinally active drug, by adapting the comparative investigation procedures such as Physiochemical and Phytochemical procedures.

The macroscopic study of roots of Iris *ensata* Thunb revealed that the roots were irregular, dark brown externally and yellow internally with a characteristic odor and bitter taste. The length of the root measures about 4-6 cm.While as the macroscopic study of *Irsa* market sample revealed that roots were also dark brown externally and yellow internally with characteristic bitter taste.

The length of the root measures 2.4-4.3cm.

The powder microscopy of Iris *ensata* Thunb revealed presence of epidermal cells, starch granules, vascular fibers, spiral vessels prismatic calcium and oxalate crystals. Similarly, the powder microscopy of *Irsa* market sample revealed presence of fibro vascular tissue and Endospermal cells.

In physicochemical investigation all the parameters were taken to analyze Iris ensata Thunb Rhizome and Irsa market sample. Total ash, Acid insoluble Ash, water soluble Ash, sulphated-Ash, water soluble extractive, alcohol soluble extract value and loss on drying were determined for Iris ensata thunb and were found to be 11.33%, 3.6%, 1.4%, 15%,1.4%,0.4%, and 14.6% respectively. For Irsa market sample. Total ash, Acid insoluble Ash, water soluble Ash, sulphated-Ash, water soluble extractive, alcohol soluble extract value and loss on drying were determined were found to be 4..33%, 0.67%, 18%, 6.5%,10%,4.8%, and 9.7% respectively.

The preliminary phytochemical screening was carried out for the identification and nature of phyto-constituents present in various root extracts of *Iris ensata* Thunb and *Irsa* market sample. For Phytochemical investigation *Iris ensata* Thunb Rhizome and *Irsa* market sample were subjected to successive extraction in Soxhlet apparatus in Solvents of increasing order of polarity like Pet ether, Ethyl acetate, Methanol, Hydro alcoholic and Aqueous It was observed that both rhizomes contains glycosides, tannins, carbohydrates, flavonoids, proteins, saponins, terpenoids and sterols. However, alkaloids were not found in any extract of both rhizomes.

TLC profiling was done on three different extracts of *Iris ensata* Thunb namely pet ether, ethyl acetate, and methanolic extract. Maximum number of spots (4) was identified in pet ether extract whereas in ethyl acetate and methanolic extract they were found to be 3 and 2 respectively.

Similarly, TLC profiling was done on three extracts of *Irsa* market sample namely pet ether, ethyl acetate and methanolic extracts. Maximum number of spots (4 each) were identified in pet ether and ethyl acetate extracts while as only one spot was found in methanolic extract.

These parameters always remain constant for any plant material and thus considered as a reliable source to confirm any type of adulteration. The results of Iris ensata Thunb Rhizome and *Irsa* market sample were recorded and put into the table form for future reference.

There is tremendous scope for drugs of herbal origin globally and most of the world population relies on herbal medicine for their various types of ailments so the need of hour is to standardize the herbal drugs on scientific parameters so as to check adulteration as well as enhance the efficacy of herbal drugs.

The comparative study was, therefore, designed in such a way so that important Pharmacognostical techniques can be employed and the results yielded can be used for future reference of the *Iris ensata* Thunb (from botanical source) and *Irsa* (market sample). For this purpose, Macroscopical, Microscopical, Physical, Chemical, instrumental methods of determination of the characteristics of the drug *Sosan Asmanjuni* (*Iris ensata* Thunb) and Irsa market sample were planned. For Phytochemical investigation *Iris ensata* Thunb Rhizome (IER) as well as *Irsa* market sample (IMS) were subjected to successive extraction in Soxhlet apparatus in Solvents of increasing order of polarity like Pet ether, Ethyl acetate, Methanol, Hydro alcoholic and Aqueous. In case of Iris ensata Thunb Rhizome (IER) maximum yield was observed in Methanol (15.32%) followed by Aqueous (3.67%), while as in case of Irsa market sample (IMS) maximum yield was observed in Methanol (19.58%) followed by Ethyl acetate (!8.79%). High yield of Methnolic extract in both cases indicate that both samples have fairly good percentage of polar substances. Phytochemical investigations were carried on the Pet ether, Ethyl acetate, Methnolic, Hydro Alcoholic and Aqueous Extracts of *Iris ensata* Thunb Rhizome (IER) and *Irsa* market sample (IMS). All the extracts of both the samples tested negative for Alkaloids and positive for Cardiac Glycosides,

Tannins, Carbohydrates, Proteins, saponins, Terpenoids and Sterols. Flavonoids were observed in case of *Iris ensata* Thunb Rhizome (IER) only.

This comparative study thus indicated that the two drugs have potential of being a substitute to one another and both can be effectively used in the name of Irsa for the treatment of the various diseases already mentioned.

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CONFLICT OF INTEREST

The authors have no conflicts of interest.

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