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Pharmacognostic evaluation and development of quality control parameters for *Berg-e-Bed-Sada* (leaf of *Salix alba* L.)

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Abstract
In the Ayurvedic, Siddha and Unani (ASU) systems of medicine, *Salix alba* L. is used as a remedy for a number of illnesses like rheumatic inflammation, painful muscles, fever, diarrhea, jaundice, gout, leucoderma (vitiligo), jaundice, etc. The current study revealed macro-, micromorphology, powder microscopy, physicochemical, high-performance thin layer chromatography and quality control factors studies to authenticate and standardize the leaf of *S. alba*. The microscopic analysis of powder drug showed various identification characters such as trichomes, druses of calcium oxalate crystals, crystal fibers, etc. Whereas, different physicochemical parameters like moisture content, total ash, acid-insoluble ash, water-soluble ash, alcohol extractive, and water extractive were also standardized within permitted limits. Likewise, all quality control factors, including heavy metals, microbiological load, aflatoxins, and pesticide residue were determined to be within acceptable ranges, evidencing that the use of *Salix alba* leaf is safe and lacks of dangerous toxins. The purposeful and unintentional use of adulterants and substitutes are regular malpractices in the trade of herbal raw materials. As a result, this study helps to fix the standards for *S. alba* leaf, which can help in preventing drug adulteration, substitution, incorrect identification, and authentication.

Keywords: Authentication, bed sada, pharmacognosy, HPTLC, quality control, drug standardization

Introduction
Traditional medicine is still preferred as a primary health care system all over the world, especially in developing countries. The world health organization (WHO) estimated that 70-80% of the world's population relies on the traditional healthcare care system. Medicinal plants are playing a vital part in traditional medicine for the treatment of many diseases due to their potent healing abilities, low toxicity, better tolerated by patients, and negligible side effects, but they lack standardization (Saleem, 2008; Rivera *et al*., 2013; Shehla Akbar, 2014) [1, 2, 3]. Moreover, natural drugs obtained from medicinal plants are also used as an alternative to synthetic products which serve as starting points for the development of new drugs (Rates, 2001; Braithwaite, 2014) [4, 5]. Therefore, continuous efforts are being made to evaluate the scientific standards for herbal medicine, which would help to provide the genuineness and purity of the crude drug as well as help in finding adulterated materials.

Salix alba L., commonly known as white willow, is often cultivated around habitation, and occasionally found along water courses and wet situations. The species is naturally distributed throughout Europe, northern Africa, and the temperate regions of Asia. In India, it found in the Himalayas region of Himachal Pradesh, Uttarakhand, and Jammu and Kashmir between altitudes 1200-2000 m (Aswal and Mehrotra, 1994; Chandra Sekar and Srivastava, 2009) [6, 7]. The plant has many medicinal properties such as antibacterial, antibacterial and antifungal, analgesic, anti-inflammatory, febrifuge, therapeutically useful in the treatment of rheumatic inflammation, painful muscles, fever, diarrhea, gout, leucoderma/vitiligo, jaundice, dysentery, bleeding disorder (Jain, 1977; Ambasta, 1986; Agarwal, 2003; Khare, 2007; Zengion and Yarnell, 2011; Van Wyk and Wink, 2018; Maistra *et al*., 2019; Javeda *et al*., 2021; Tawfeek *et al*., 2021) [8, 9, 10, 11, 12, 13, 14, 15, 16]. The main constituents of plant are anthocyanins, apigenin, amentoflavone, rutin, isoquercitrin, salicin, fragilin, salicortin, (+)-catechin, (+)-gallocatechin, narcissin, isorhamnetin-3-O-β-D-glucoside (Wagner *et al* 1996; Esatbeyoglu *et al* 2010; Gligorić *et al*, 2018; Piątczak *et al* 2020; Bajraktari *et al*., 2022) [17, 18, 19, 20, 21]. Leaf of *Salix alba* contain many medicinally important phytoconstituents such as flavonoids, phenolic acids, their derivatives, and phenolic glycosides (Tawfeek *et al*, 2021; Gligorić *et al*, 2018) [16, 19].

Unani System of Medicine is an important segment of AYUSH (Ayurveda, Yoga and Naturopathy, Unani, Siddha, and Homoeopathy), which is based on the uses of plants, minerals, and animal products as a main source of drugs uses to cure various ailments. In this system, the dried leaves of *S. alba*, commonly known as *Berg-e-Bed Sada*, are used as the chief ingredient of the very common Unani compound formulation of *Araq-e-Bed Sada*, however, it is also used in many other Unani formulations, *viz. Araq-e-Aswad Barid*, *Araq-e-Muasaffi-e-Khoon Qawi* (Anonymous, 2006) [22].

In reference to medicine uses in Unani classical literature *Barg-e-Bed Sada* has some actions, like *Muqawwi-e-Dimag* (Brain Tonic), *Muqawwi-e-Qalb* (Cardiac Tonic), *Zof-e-Meda* (Cardiac Asthenia), *Mudir-e-Baul* (Diuretic), *Qabiz* (Astringent). So, it is used to cure many diseases such as *Tap-e-Haar* (Acute Fever), *Ramad* (Acute Conjectivitis), *Sara* (Epilepsy), *Yarqaan* (Jaundic), *Warm-e-Tihal* (Hepatomegelly), *Khafqaan* (Palpitation), *Jadri* (Chickenpox), *Ikhtinaq-i-Reham* (Hysteria), *Waja-ul-Mafasil* (Arthritis), *Niqras* (Gout) (Ghani,YNM; Kabir-ud-din, YNM; Kabeer Uddin,1930; Hakeem, 2002) [23, 24, 25, 26].

Keeping in view, the wide therapeutical uses of the *Salix alba* leaf, the aim of the present study is authentication and standardization of this drug through macro-morphology, micromorphology, powder microscopy, physicochemical studies, and High-Performance Thin Layer Chromatography (HPTLC) studies. Consequently, it will surely help to fix the standards for *Salix alba* leaf which can prevent adulteration, substitution, the wrong identification, and authentication of the drug.

Material and Methods

Collection and authentication of plant material

The leaves of the plant were collected from the campus of the Regional Research Institute of Unani Medicines (RRIUM), Srinagar, J&K, identified and authenticated by the Botany Section of Drug Standardization Research Institute (DSRI), Ghaziabad. The voucher specimens were later deposited in the herbarium of DSRI, Ghaziabad for future reference. The samples were cleansed from foreign matter and examined for macroscopic and microscopic characteristics. For powder microscopy, the drug was air-dried and ground to a coarse powder using a grinding paster. The powder was stored in airtight containers for further use. In order to authenticate and develop its pharmacopeia standards, WHO (2011) [27] guidelines were followed.

Organoleptic evaluation

The organoleptic characters of the samples were evaluated based on textual methods.

Microscopic studies

About 5 g of powder drug was taken and washed thoroughly with distilled water. Pour out the water by rejecting the supernatant and keeping the sediment; repeat the process without loss of material; mount a few mg in 50% Glycerine and dry, treat a few mg of washed material with Phloroglucinol and HCl and mount in 50% Glycerine; treat a few mg with Chloral hydrate, wash it in water and mount in 50% glycerine and observed under a digital microscope for its microscopic characters (Johansen, 1940; Trease and Evans, 1989; Wallis 2005) [28, 29, 30].

Physicochemical analysis

The physicochemical parameters such as moisture content, water, and ethanol extractive values, ash values, pH values were analyzed by standard methods (Anonymous, 1987, 1998) [31,32].

HPTLC profiling

The drug extract was prepared by sonicating 2g drug sample with 25ml of *Ethanol* for 30 minutes. The extract was filtered and concentrated up to 10 ml and used as test solution. 10µl of ethanol extract was applied on aluminum TLC plate pre-coated with silica gel 60 F^{254} (Merck) by employing CAMAG Linomat IV automatic sample applicator. The plate was developed up to a distance of 9 cm in twin trough glass chamber (10x10) using 10 ml of the solvent system Toluene: ethyl acetate: formic Acid (9: 1: 0.5) as mobile phase. The plate was air-dried at room temperature and observed under UV at wavelengths 254 nm and 366 nm. Further the plate was dipped in 1% Vanillin-sulphuric acid reagent and heated at 105ºC till coloured bands appeared. The plate was finally examined under visible light (Wagner and Bladt, 1996; Sethi, 1996; Ansari *et al*., 2020) [33, 34, 35].

Quality control analysis

a) Estimation of Heavy Metals and Aflatoxins

The analysis of heavy metals like lead, cadmium, mercury, and arsenic was carried out as per standard methods (WHO, 1998; Anonymous, 2005, 2007; Ansari *et al*., 2020) [36,37,38,35]. Atomic Absorption Spectrophotometer (AAS) model LABINDIA AA7000 was used for heavy metals analysis. The operating parameters were as follows:

Lead and Cadmium: Instrument technique-Flame atomization; wavelength (Lead) - 217 nm; wavelength (Cadmium) - 228.8 nm; slit width - 0. 5 mm; lamp current (Pb) - 4.0 mA; lamp current (Cd) - 3.0 mA; carrier gas and flow rate - air and acetylene, 1.1 L/min; sample flow rate - 2 ml/min.

Mercury: Instrument technique - Cold vapour technique; wavelength - 253.7 nm; slit width - 0. 5 mm; lamp current - 3.0 mA; carrier gas and flow rate - argon, 1.1 L/min; sample flow rate - 5ml/min.

Arsenic: Instrument technique - Cold vapour technique; wavelength - 193.7 nm; slit width - 0. 5 mm; lamp current - 6.0 mA; carrier gas and flow rate - acetylene, argon, 1.1 L/min; sample flow rate - 5ml/min. The hallow cathode lamps for Pb, Cd, Hg and As analysis were used as light source to provide specific wavelength for the elements to be detected.

b) Determination of pesticide residue

The estimation of pesticide residues was carried out as per the method described in AOAC, 2005 (Anonymous, 2005) [37]. The analysis was done by employing Gas Chromatography-Mass Spectrometry (Thermo Fisher TSQ 9000 Triple Quadrupole GC-MS/MS system).

c) Estimation of microbial load

Estimation of the microbial load was conducted as per the standard method (Anonymous, 2005) [37].

Result and Discussion

Herbal drug standardization is a crucial technique for establishing their identity, purity, safety, and quality control. Nowadays uses of adulterants and substitutes are regular malpractice in the trade of herbal raw materials. As a result, this study helps to fix the standards for *Salix alba* L. leaf, which can help in preventing any type of drug adulteration, substitution, incorrect identification, and authentication.

A) Taxonomical classification and identification

Taxonomic examination of the herbal drug is one of the most crucial procedures for maintaining quality control. The

efficiency of the herbal drugs is ensured only when there is proper identification and authentication of starting material. Sometimes, the unintentional use of adulterants and substitutes can occasionally result due to confusion in vernacular names used in regional dialects and indigenous medical systems. Therefore, correct identification and nomenclature of herbal drugs are the cornerstones of the safe use of herbal medicines.

Classification of *S. alba*

As per the latest system of classification (APG IV, 2016) [39] *Salix alba* L. belongs to the family Salicaceae (Table 1).

Table 1: Botanical classification of *Salix alba L.*

Kingdom:	Plantae
Phylum:	Tracheophyta
Class:	Equisetopsida C. Agardh
Order:	Malpighiales Juss. ex Bercht. & J. Presl
Family:	Salicaceae
Genus:	*Salix*
Species:	*Salix alba* L. (synonyms: *Salix regalis* Hort. Ex Wesm., *S. caerulea* Sm., *S. pameachiana* Barrat

Vernacular names

Unani – Bed Sada; English – Indian Willow, Huntingdon Willow, White Willow; French – Osier blane, Plon blane, Saule, Saule blanc; Greek – Itea; German – Dotterweide, Faelber, Felber, Fieberweide, Knak, Kneien, Weide; Hindi – Bod, Bains; Italian – Salcio, Salcio bianco; Kashmir – Vivir, Vir; Punjab – Bis. Bushan, Chamma, Changma, Chung, Kalchan, Malchang, Madana, Yur Madnu; Ladakh – Changma; Roumanian – Salcie; Russian – Iva, Verba; Spanish: Salce, Salce blanco, Sause, Sauce blanco; Swedish – Hvit pihl; Tamil – Atrupala (Kirtikar and basu, 1984; Ambasta, 1986) [40, 9].

Morphological characteristics of *S. alba*

It is a medium-sized to large deciduous tree growing up to 10-30 m tall, with a silky hairy young stem. Flowers unisexual, solitary, appear after the leaves, catkins terminating short, lateral, leafy shoots. Male catkins yellow, 1.5-2.5 cm long, drooping, dense flowering. Female catkins 4-7 cm long. Bracts yellow, oblong, ciliate. Stamens 2, protruding. Carpels 2, 1-chambered, with many ovules. Fruits (Capsules) conical, glabrous, subsessile. Seeds with a tuft of white silky hairs aid their dispersal (Fig. 1 A-D).

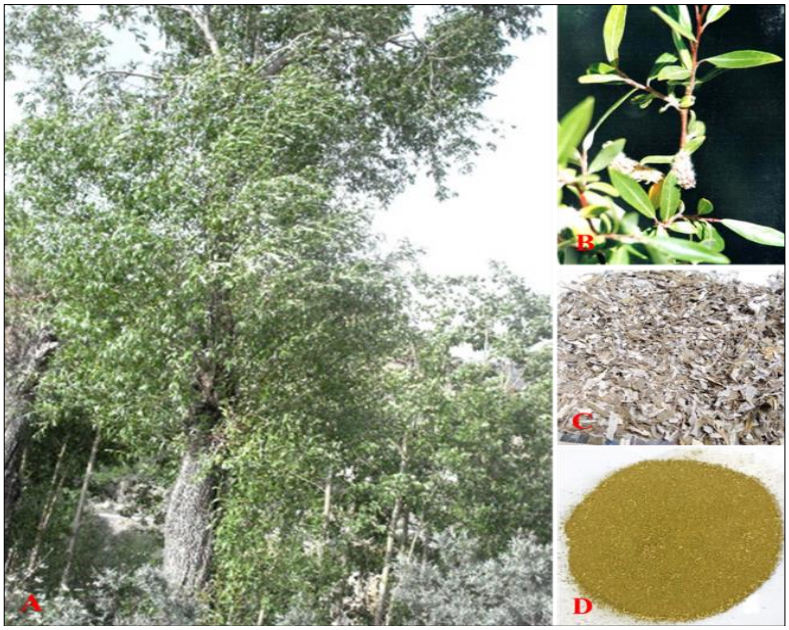

Fig 1: A-D): Morphological characters of *Salix alba* L. (A) Mature tree; (B) Fresh leaves; (C) Dried leaves; (D) Powder of leaves

B) Pharmacognostical studies

The anatomical features of herbal drugs provide salient diagnostic characteristics for the identification of both entire and powdered crude drugs and also help to detect adulterants in plant materials (Ghani, 1990; Atinga *et al*, 2021) [41,42]. Therefore, it is crucial to analyze the morphology and microscopic characteristics of the crude drug in order to standardize herbal medicines.

Macro-morphological characters of the leaf of *S. alba*

Leaves are dorsiventral, narrowly oblong, lanceolate, 5-10 x 1-2 cm long, finely serrated, shiny green above, nearly white and silky below, glabrous on maturity, base cuneate or convex, margins flat, serrate or serrulate. Petiole 7.5-10 mm, adaxially grooved shallowly, long silky abaxially, globous or scattered pubescent at the base (Fig. 1 B-C).

Micro-morphological characters of leaf sample of *S. alba*

T.S of leaf lamina: T.S of leaf lamina shows the layers from up to down respectively are trichomes, upper cuticle layer, upper epidermis, palisade parenchyma, spongy parenchyma, lower epidermis, lower cuticle layer, and trichomes. The upper epidermis is single layered consist of polygonal cells with uniformly thickened membranes. The lower epidermis is bilayer, cells are much smaller than the cells of the upper epidermis, covering simple, uni-cellular, thin-walled trichomes. Palisade cells are two layered presents adjacent to both the upper and lower epidermis. Rosette and prismatic crystals of calcium oxalate are present in a few mesophyll cells. The microscopic examination of the leaf peel showed anomocytic stomata with unicellular trichomes in the epidermal cells (Fig. 2C).

T.S of mid-vein: Epidermis cells of mid-vein are single layered at upper side and two-layered on at lower side covered with cuticles on both sides. Sub epidermal layer is represented by 5 or 6 rows of angular collenchyma immediately adjacent to the epidermis. Hypodermis layer consisting of parenchymatous cells. In the cells of the parenchyma, there are the presence of druses of calcium oxalate crystals. A few unicellular long trichomes are also present on the surface and have crescent-shaped collateral vascular bundle. The xylem of the mid-vein consists of spiral vessels. Core consists phloem of small cells. Vascular-fibre bundles have a crystalline lining. The anatomical structure of the petiole is similar to the structure of the midvein (Fig. 2A-B, D).

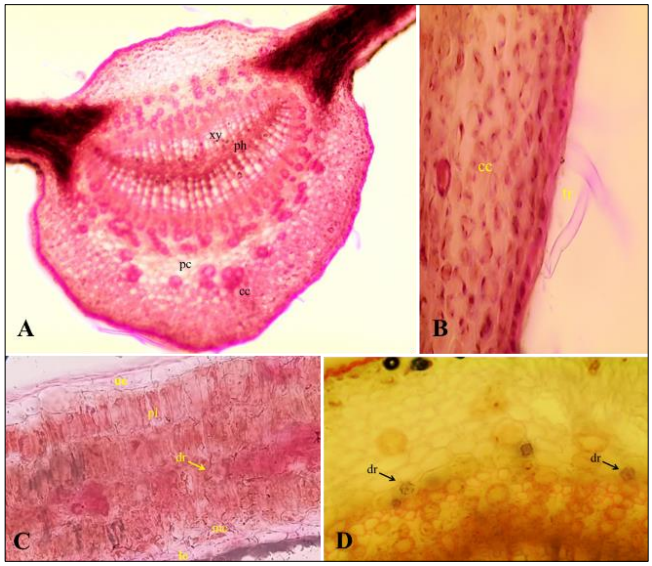

Fig 2: (A-D). Transvers Section (T.S.) of *Barg-e*-Bed Sada (Leaf of *S. alba*). A. T.S. of mid vein of leaf constituting different type of cells like xy – xylem, ph – phloem, pc – parenchyma, cc – collenchyma; B. Trichomes on leaf surface; C. T.S. of leaf lamina containing various type of cells such as ue – upper epidermis cells, pl – bi-layered palisade parenchyma cells, dr – druses of calcium oxalate crystals, mc – spongy mesophyll cells, le – lower epidermis cells; D. Druse of calcium oxalate crystals (dr).

Organoleptic features: Organoleptic evaluation of any crude drug is an essential component for establishing the authenticity, identity, and purity of medicinal plants. The drug powder of leaf of *Salix alba* is tea green, which is bitter in taste having characteristic odour and smell (Fig. 1D).

Powder microscopic analysis: Plant samples were subjected to microscopic examinations and qualitative criteria in order to establish relevant information that can be applied in the identification of crude drugs, particularly in powder form. After the examination under microscope powder of the drug shows the fragments of the epidermis with anomocytic stomata, fragments of spiral vessels, fragments of vein with spiral vessels and crystal fibers, fragments of leaf with druse of crystals in mesophyll cells, Crystal fibre containing prism of calcium oxalate crystals, collenchyma cells, parenchyma cells, unicellular trichomes, druse of calcium oxalate crystals, prismatic crystals of calcium oxalate (Fig. 3 A-K)

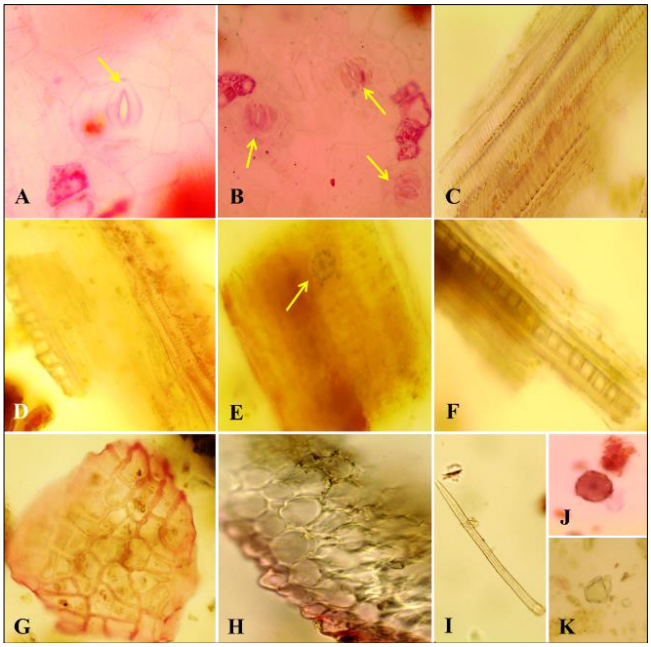

Fig 3: Powder microscopic characters of *Barg-e*-Bed Sada (Leaf of *Salix alba*). A. Lower epidermis with stomata; B. Upper epidermis with stomata; C. Spiral vessels; D. Fragment of vein with crystal fibre & spiral vessels; E. Fragments of leaf with druse of crystals in mesophyll cells; F. Crystal fibre containing prism of calcium oxalate crystals; G. Collenchyma cells; H. Parenchyma cells; I. Unicellular trichome; J. Druse of calcium oxalate crystals; K. Prismatic crystals of calcium oxalate.

C) Physicochemical evaluation

The results of physicochemical parameters of leaf sample of *S. alba* are depicted in table 2. Quantitatively evaluated data revealed that the moisture content of the drug ranged between 10.40 – 11.35% which is apropos in case of dry leaves. Total ash content of the drug was not more than 8.58% and acid insoluble ash remained as low as around 1% which indicated that siliceous matter was present in negligible amount. The water extractive values were significantly high ranged between 35.45 – 36.60% which indicated the extraction of inorganic substance & highly polar organic substance in the drug sample. The ethanol soluble extractive values came out to be on lower side; ranged between 9.75 – 10.90% which indicated the presence of distinctly polar constituents.

Table 2: Physicochemical parameters

S. No.	Parameters	Values
	Foreign Matter (%)	2.10
	Loss in weight on drying at 105^0 C (%)	10.40 – 11.35
	Total Ash (%)	7.80 – 8.58
	Acid insoluble ash (%)	0.92 – 1.22
	Ethanol Soluble Extractive (%)	9.75 – 10.90
	Water Soluble Extractive (%)	35.45 – 36.60
	Hexane Soluble Extractive (%)	1.95 – 2.50
	pH 1% Soln.	6.05 – 6.18
	pH 10% Soln.	5.70 – 5.88

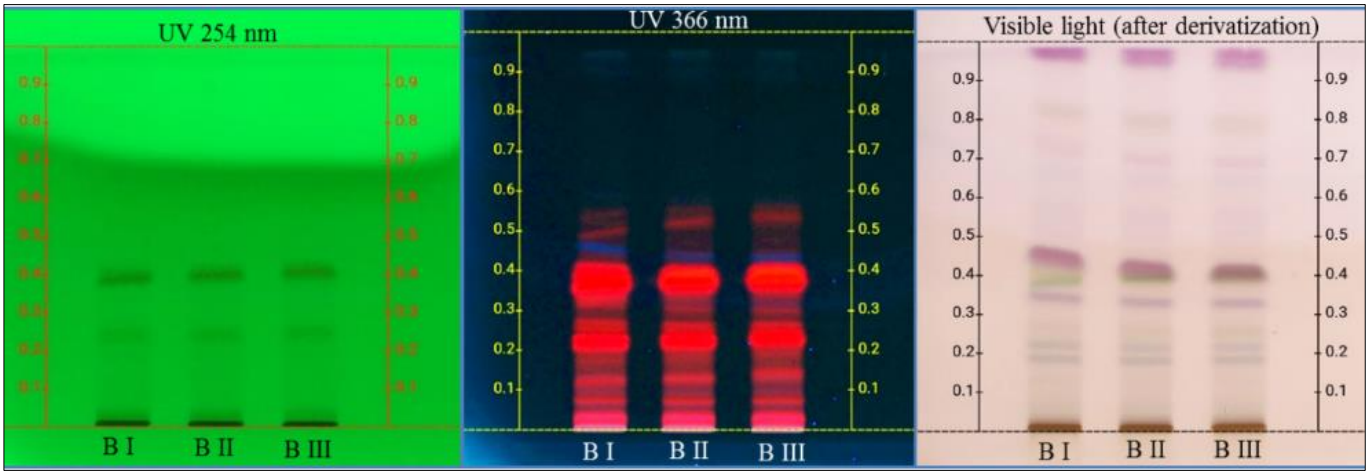

Fig 4: HPTLC fingerprint developed from ethanol extracts of leaf sample of *S. alba*.

D) HPTLC fingerprinting

The HPTLC (High Performance Thin Layer Chromatography) has become the most potent tool for quality control of herbal medicines because of its simplicity and reliability for phytochemical and biomedical analysis. The HPTLC fingerprinting of ethanol extracts of *Berg-e-Bed Sada (*three samples) was observed under UV 254nm, UV 366nm and under white light after derivatization. All the samples show similar colorful bands with similar R_f values (Fig. 4).

E) Quality control parameters

Quality assurance is the main concern for the development of pharmacopoeial standards of herbal medicines. So, the various quality control parameters, such as heavy metals, microbial load, aflatoxins, and pesticide residues were analysed to verify the required quality.

Heavy metal analysis

Heavy metals may enter medicines through contaminated agricultural resources and/or poor production practices (Street, 2012) [43]. There is a global threat of heavy metal contamination associated with the use of traditional medicines, and this could lead to serious human health problems. Therefore, the accurately quantified levels of heavy metals in herbal drugs seem necessary to assess and justify the dosage of herbal formulas. In present study the concentration of four heavy metal lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg) was investigated using Atomic Absorption Spectrophotometer (AAS) model LAB. Leaves of *Salix alba* were found to contain heavy metals content below the detection limit, indicating that the drug was not contaminated with any heavy metals. Moreover, the heavy metals *viz*. lead, cadmium, mercury and arsenic were found to be below detection Limit (LOD) (Table 3).

Table 3: Assessment of heavy metals in leaf sample of *S. alba.*

Sl. No	Element	Values	WHO Limits for internal use
1.	Lead	< LOD	10 ppm
2.	Cadmium		0.3 ppm
3.	Arsenic		3.0 ppm
4.	Mercury		1.0 ppm

Microbial load and aflatoxin analysis

The estimation of microbial growth is a very important parameter in traditional medicines which helps to verify the presence of bacterial and fungal pathogens to avoid the health risk during drug development. It indicates whether the drug contains disease-causing and spoilage micro-organisms in permissible limits. The assessment is done for evaluating the total bacterial count and total fungal count. Bacteria belonging to the Enterobacteriaceae members *viz*. *Escherichia coli*, *Salmonella* sp., *Shigella* sp., *Klebsiella* sp. & Specific objectionable pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans* were not detected. Aflatoxin-producing fungi *Aspergillus flavus* and *Aspergillus parasiticus* were also absent in the drug samples. The results of microbial load are shown in Table 4 which indicate that the drug is safe for internal use. Microbial loads *viz*. Total aerobic bacterial Count (TABC), Total yeast and molds count (TYMC) were found to be within permissible limits. Aflatoxins are toxic metabolites produced by a variety of molds such as *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. The results do not show the presence of any of the aflatoxins contents (B1, B2, G1, and G2) in drug. The results of microbial load are shown in Table 4 which is indicated that the drug is safe for internal use.

Table 4: Microbial load exhibited in leaf sample of *S. alba*

Total aerobic bacterial Count (TABC)	**2.8×10^3 CFU/gm**	**105 CFU / gm**
Total yeast and molds count (TYMC)	**1.1×10^2 CFU/gm**	**103 CFU / gm**
Enterobacteriaceae members		
Escherichia coli	ND	
Salmonella sp.	ND	
Shigella sp.	ND	
Klebsiella sp.	ND	
Specific objectionable pathogens		
Pseudomonas aeruginosa	ND	
Staphylococcus aureus	ND	
Candida albicans	ND	
Aflatoxin producing fungi		
Aspergillus flavus	ND	
Aspergillus parasiticus	ND	

*ND – Not detected

Pesticide analysis

Pesticides are highly toxic to humans and can cause both acute and chronic health effects, depending on the quantity and ways in which a person is exposed. Hence, it is essential to check the herbal material for pesticide residues before human consumption. The analysis shows that the pesticide residues such as organo chlorine group, organo phosphorus group, alachlor, aldrin, chlordane, DDT, endosulfan, heptachlor, lindane were also below the Limit of Quantification (LOQ) (Table 5).

Table 5: Pesticide residue found in leaf sample of *S. alba*

S. No.	Pesticide	Result (mg/Kg)	Permissible limit (mg/Kg)
1.	Alachlor	BLQ	0.02
2.	Aldrin (Aldrin and dieldrin combined expressed as dieldrin)	BLQ	0.05
3.	Azinophos-methyl	BLQ	1.0
4.	Bromopropylate	BLQ	3.0
5.	Chlordane (cis, tans and oxychlordane)	BLQ	0.05
6.	Chlorfenvinphos	BLQ	0.5
7.	Chlorpyrifos	BLQ	0.2
8.	Chlorpyrifos-methyl	BLQ	0.1
9.	Cypermethrin (and isomers)	BLQ	1.0
10.	DDT (all isomers, sum of p,p'-TDE (DDD) expressed as DDT)	BLQ	1.0
11.	Deltamethrin	BLQ	0.5
12.	Diazion	BLQ	0.5
13.	Dichlorvos	BLQ	1.0
14.	Dithiocarbamates (as CS2)	BLQ	2.0
15.	Endosulphan (sum of isomers & Endosulphan sulphate	BLQ	3.0
16.	Endrin	BLQ	0.05
17.	Ethion	BLQ	2.0
18.	Fenitrothion	BLQ	0.5
19.	Fenvalerate	BLQ	1.5
20.	Fonofos	BLQ	0.05
21.	Heptachlor (sum of Heptachlor & Heptachlor epoxi	BLQ	0.05
22.	Hexachlorobenzene	BLQ	0.1
23.	Hexachlorocyclohexane isomer (other than γ)	BLQ	0.3
24.	Lindane (γ – Hexachlorocyclohexane)	BLQ	0.6
25.	Malathion	BLQ	1.0
26.	Methidathion	BLQ	0.2
27.	Parathion	BLQ	0.5
28.	Parathion methyl	BLQ	02
29.	Permethrin	BLQ	1.0
30.	Phosalone	BLQ	0.1
31.	Piperonyl butoxide	BLQ	3.0
32.	Pirimiphos methyl	BLQ	4.0
33.	Pyrethrins (sum of isomers)	BLQ	3.0
34.	Quintozen (sum of Quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)	BLQ	1.0

*****BLQ – Below limit of quantification

Conclusion

Due to unique therapeutic characteristics and potential effects, there has been a sharp growth in the standardization of various medicinal plants in recent years. In the present study, the *Salix alba* leaf is authenticated by macroscopy, microscopy, HPTLC and various other quality control parameters. The macroscopy and microscopic characteristics of the plant material can provide the majority of the information about its identity, purity, and quality. Microscopically, the drug has crescent-shaped collateral vascular bundle, an anomocytic type of stomata with epidermis covering with cuticle and unicellular trichomes. The identifying characters of drug powder are crystal fibres, spiral vessels, druse of calcium oxalate crystals etc. Various physico-chemical parameters, HPTLC profile etc. provide criteria for easy identification of the drug, and quality control analysis ensures the authenticity, quality and efficacy of the medicine. The study indicates that the drug samples were found to be safe and free from any harmful toxins such as heavy metals, aflatoxins, and pesticide residues.

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