Article

Drug Standardization, HPTLC Finger Printing, Toxicity Research Studies of ASU Herbaceous Plant Fruit Seeds Part Samples of *Ipomoea nil* (Linn.) Roth

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Abstract: This study aims to evaluate the drug standardization research, physico-chemical, toxicity research studies of the fruit seeds part of plant of I N. drug standardization research, physico-chemical, toxicity research studies of ASU (*Ayurveda, Siddha, Unani*) herbal products remains a big challenging task. There needs to be more than the advance investigation research studies and screening parameters to validation, authenticate and differentiate adulterants in *Ipomoea nil* (Linn.) Roth medicinal plant is one of the herb used to treat various health wellness and therapeutic illness of public mankind. Plant samples of I N. powder were carried out using standard methods. The Quality, safety and toxicity effects of the tested drug samples were also investigated. estimated and investigated research studies data's of I N. have shown that all the parameters were within the AYUSH/WHO permissible limits. The tested drug samples showed significant Quality, safety and toxicity studies against certain pathogens organisms and promising anti-pathogenic activity. In the investigated studies of DSR, HPTLC finger printing investigation, QC, Toxicity research findings revealed that the revalidated test drug samples was free from adulterations. This investigated herb research data confirmed to drug standardization and therapeutically may treat that the drug is safe for internal use and cures in Antioxidant, Anti-Inflammatory, Antimicrobial, Antispasmodic, Anticancer, Anti-tumor, Antiproliferative Activities, Multidrug-Resistance Efflux-Inhibiting Activity, bronchodilatory along with Antiasthmatic potential, Diuretic, anthelminthic and deobstruant and are prescribed for dropsy and constipation, Antihypertensive and cardio-protective effects, Investigated drug had classically, traditional and alternative ASU medicine I N. used as a Cold, Dropsy, Gout, Joint pain, Vitiligo, Itching, Asthma and Anthelmintic.

Keywords: *Ipomoea nil* (Linn.) Roth. DSR; HPTLC finger printing; QC and QA; Toxicity; Pharmacological Quality; safety and toxicity studies

1. Introduction

The World Health Organization (WHO) reports that herbal medicines are able to fulfill the health requirements of approximately 80% of the global population, particularly those residing in rural regions of developing nations. The quality assurance and quality control of herbal crude drugs and formulated products are important in justifying their acceptability in modern system of medicine. Hence it is required to conduct the research on drugs standardization and product validation to provide effective, curable and safe drugs to the needy mass suffering from various ailments [1–10]. I N. (Convolvulaceae) is known as ivy morning glory; seed usage is common, and flowers are produced from June to October. It is indigenous to Punjab and distribution is in Pakistan in addition to India. Folkloric reputation as anti-inflammatory, blood purifier, anthelmintic, astringent, laxatives, anti-emetic, carminatives and is considered useful to treat a series of ailments like abdominal diseases, asthma, hypertension, disease of liver and joints, drying the phlegm [3,11,12]. Investigated I N herbaceous plant, Ivy leaf morning-glory, an annual climber is locally known as "Kaladana". Commonly found in gardens and wild fields, areas along railway tract and roadsides. Cambodia, China, Columbia, England, Japan, Korea, Mexico, Nigeria and Philippines [3,13]. The top role of these mediators is central in asthmatic problems, as labelled in miscellaneous

studies beforehand [3,11]. Epithelial cells, pseudo stratified cells, dendritic cells, mast cells and mucus secreting cells are present in the airway's lumen. Vagus sub-epithelial cells, in addition to receptors, discharge acetylcholine on bronchial muscles. Parasympathetic action cause airways to narrow, so, when this is going to antagonize, generate dilatation of airways so anti-muscarinics are used in asthma, COPD. and other respiratory infections [3,11]. Different medicines, e.g., short and/long acting beta 2 agonists, and corticosteroids have been engaged as therapeutic agents as desirable treatment plus prevention for these sorts of disorders to treat broncho-constriction, asthma and lung dam age [3,11]. However, tenacious treatments, besides their immediate termination outcomes as side effects, may demonstrate as being lethal for the social life cycle and express necessity to sightsee traditional medicinal flora to discover new reliable along with life-saving medicinal elements [3,11]. erectile dysfunction and for lubrication obligations. Application of seeds paste is used for cosmetic purposes i.e., dry skin, freckles, etc. In addition, it is also hepato protective, anti-diabetes, anti-interleukin-8 (IL-8), and anti-inflammatory [3,11]. Phyto analysis exposed the occurrence of alkaloids—(chanoclavine-18, penniclavine, elymoclavine)-methyl-oxybutyric acid, pharbitinic acid, phytoecdysone, tiglic acid, plytosteratin, lysergol [14], ecdysteriods, hederasterones, stigmasterol 3-O-D-glucoside, 20-hydroxyecdysone, sitosterol-3-O-D-glucoside, ethylcaffeate, anthocyanin,i.e., cyanidin, cyanidin 3-sophoroside and-d glucopyranoside [3,11]. The utmost contemporary investigated phytochemicals are hederaceterpenol, hedera terpenoside, triterpenoid plus stigmast-5-en-3-O--D-glucopyranoside [3]. The aim of the current research effort on the aqueous-methanolic crude extract of I N. seeds was commenced to observe its antioxidant, bronchodilator, anti-asthmatic and enzyme inhibition action with mechanistic approaches [11]. The seeds are diuretics (antihypertensive), anthelmintic, aphrodisiacs, blood purifiers, carminative, laxative and useful for headache, abdominal inflammation, bronchitis, hearts diseases and high blood pressure. Seeds are also prescribed to induce menstruation and cause abortion in high doses. Seeds are also useful in gout, scabies and leucoderma. Whereas, few phytochemical compounds including chanoclavine I, elymoclavine, lysergol, penniclavine and isopenniclavine have also been reported previously, antifungal and antibacterial, analgesic and CNS stimulant, Flavonoids, saponins, tannins, mucilage and proteins. Phytochemical studies have revealed the presence of different phytochemical classes including alkaloids, reducing sugars, terpenoids. Hepato-protective. and treatment of skin diseases [3,12,14–17]. Its quality, safety, and efficacy are affected due to adulterants and contamination of herbal products; therefore, its purity, safety, potency, and efficacy are major problems associated with the quality of ingredients. The regulatory bodies will have to ensure that medications given to consumers are of good quality with assurance. The regulatory authority should implement good manufacturing practices at the manufacturing operation unit and develop a quality control unit for raw materials and finished products as per the Pharmacopoeia [3,7,18]. The World Health Organization (WHO) Assembly and Ayurvedic Pharmacopeia Commission have expressed the need to use modern technology and appropriate standards like HPLC, HPTLC, and Spectroscopy to ensure the quality of Ayurvedic medicines and their products [19–21]. The study plant I N. fruit seeds parts active phytochemical constituents shown as Secondary metabolites, such as Alkaloids, Triterpenes, Flavonoids, Norisoprenoids, Glycosides, Tannins, Coumarins, Carbohydrates, Phenols, Saponins, Phlobatannins, and Steroids, Steroids/triterpenes, alkaloids, glycosides, flavonoids, resins, reducing sugars, tannins, proteins, fibers, lipids, carbohydrates, minerals (potassium, calcium, phosphorus and magnesium), amino acids, fatty acids (palmitic acid, linoleic acid, linolenic acid, and oleic acid) and seed oils [3,14,22]. Plant extract was found to contain alkaloids, saponins, anthraquinones, sterols, tannins, coumarins, flavonoids and terpenes [3,11]. Alkaloids, coumarins, anthraquinones, saponins, tannins, flavonoids, polyphenolics compounds and terpenes as potential foremost elements of the plant [3,11]. Steroids/triterpenes, alkaloids, glycosides, flavonoids, resins, reducing sugars, tannins, proteins, fibers, lipids, carbohydrates, minerals (potassium, calcium, phosphorus and magnesium), amino acids, fatty acids (palmitic acid, linoleic acid, linolenic acid, and oleic acid) and seed oils [3,14,15,17] and their therapeutics medicinal potential and pharmacological activities shown as Antioxidant, Anti-Inflammatory, Antinociceptive, Antimicrobial, Collagenase Inhibitory, Antispasmodic, Anticancer, Antitumor, Antiproliferative Activities, Multidrug-Resistance Efflux-Inhibiting Activity [3,14,22]. Antioxidant, bronchodilatory along with Anti-asthmatic potential [3,11,14,23]. Diuretic, anthelminthic and deobstruant and are prescribed for dropsy and constipation, and to promote menstruation and cause abortion [3,14,15,17]. Anti- hypertensive and cardio-protective effects [3,13,15]. I N. Investigated herbasious medicinal plant studies Graphical Illustration, Confirmation and identification shown in Figure 1, respectively.

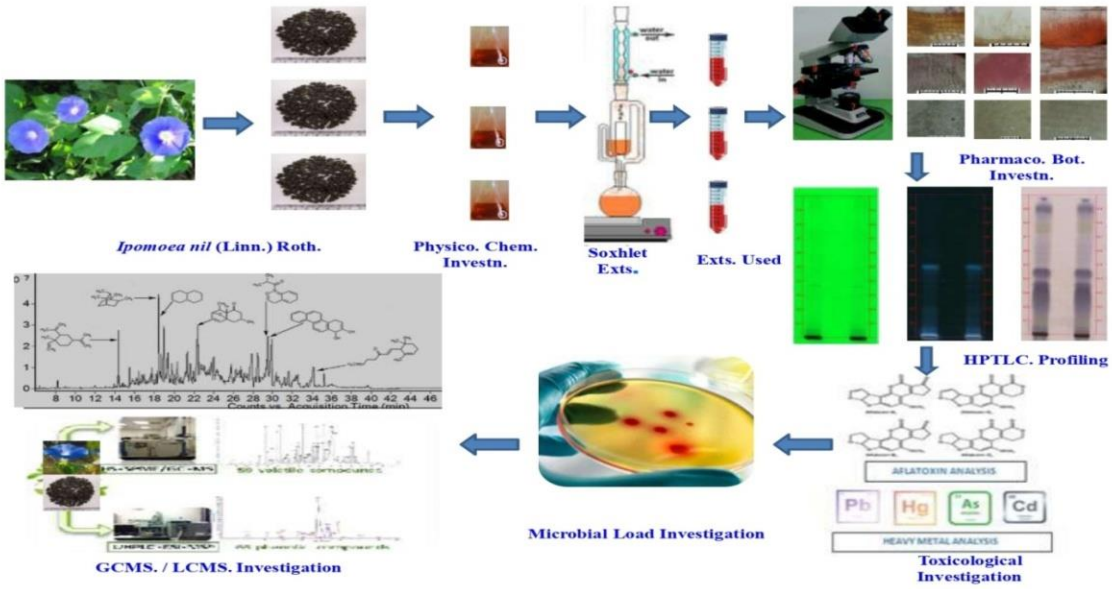


Figure 1. Graphical Illustration.

Geographical, biodiversity and Natural Occurrence: The investigated herbaceous medicinal plant Fruit seed part of I N. was shown and worldwide occurred and found in Alabama, Angola, Bangladesh, Bermuda, Burkina, California, Cambodia, Cameroon, Cape Provinces, Central African Republic, Chad, China North-Central, China South-Central, China Southeast, Christmas I., Comoros, Congo, Equatorial Guinea, Eritrea, Ethiopia, Florida, Ghana, Greece, Guinea, Gulf of Guinea Is., Hainan, India, Inner Mongolia, Ivory Coast, Jawa, Korea, KwaZulu-Natal, Laos, Lesser Sunda Is., Louisiana, Madagascar, Malaya, Maluku, Maryland, Mauritius, Myanmar, Namibia, Nansei-shoto, Nepal, New Caledonia, New Guinea, Nigeria, North Carolina, Northern Territory, Oman, Pakistan, Philippines, Queensland, Rodrigues, Réunion, Saudi Arabia, Senegal, Seychelles, Sierra Leone, Socotra, South China Sea, Sri Lanka, Sudan, Sulawesi, Sumatera, Tanzania, Texas, Thailand, Tibet, Uganda, Vietnam, Western Australia, Yemen, Zambia, Zaïre, Zimbabwe, East Himalaya and West Himalaya of Indian, Southern region of India and Pakistan, Asian region climatically and biodiversity presence appeared of herbaceous medicinal plant I N. having investigated bioactive phytochemacal constituents with immense pharmacological action properties, medicinal and therapeutic potential [3,11,12,14,15,17].

2. Material and Method

Taxonomical and Pharmacognostical Studies: The fruits seeds samples of the plant I N. were procured from the local market of Central NCR Region, New Delhi, India, Southern Region, Tamil Nadu State, India, Northern Region of Uttarakhand State, Indiaand authenticated by Botany and Pharmacognosy Laboratory Section, Researcher Scientific Staff of Regional Research Institute of Unani Medicine, Royapuram, TN. State, Drug Standardization Research Institute, Ghaziabad, UP. and PCIM&H, Ghaziabad UP.

2.1. Collection and Authentication of Ipomoea nil Samples

Fresh *Ipomoea nil* (L.) Roth samples were collected from [Delhi NCR, Chennai and Haridwar commercial source] under controlled conditions. The plant material was authenticated by a qualified taxonomist at [RRIUM Chennai], following standard botanical identification keys and comparison with herbarium specimens. A voucher specimen (No: RRIUM(C)13718) was deposited in the herbarium for future reference. The authentication was based on macroscopic and organoleptic characteristics, including leaf morphology, venation patterns, and seed characteristics.

Standard Methods applied for Detection and investigation of Physicochemical, HPTLC Fingerprinting, Toxicology parameters used and applied Advance sophisticated Instruments and operating parameters, detections and investigations of Heavy Metals-Pb, As, Cd, Hg by Atomic Absorption Spectrometer (AAS-GF), Aflatoxins-B1, B2 and G1, G2 were estimated by Kobra cell techniques. Pesticide residues and Microbial Load contamination-TBC/TFC detect in cfu/gm. *Escherichia coli*, *Salmonella typhai* spp. *Staphylococcus aurous*author pathogenic, detection and estimation in Heavy Metals-Pb, Hg, Cd and As, Aflatoxins B1, B2 and G1, G2 andPesticide Residues-Organo chlorine, pesticides, Organo phosphorus pesticides, Pyrethroids etc, in ppm levels concentrations

as per WHO/AOAC/AYUSH-API/UPI Pharmacopeial permissible limits and standard methods basis [1–10,12,14,19,24–38].

2.2. Physicochemical Parameter Analysis Studies

2.2.1. Moisture Content

The Loss on Drying (LOD), (*w*/*w*)%, method was used to determine moisture content by heating the sample at 105 °C until a constant weight was achieved, ensuring stability and preventing microbial contamination.

2.2.2. Ash Value Analysis

Ash content was evaluated to detect inorganic impurities and ensure sample purity:

- Total Ash, (*w*/*w*)%—Indicates overall mineral content by incinerating the sample at 550 °C.
- Acid-Insoluble Ash, (*w*/*w*)%—Assesses contamination from siliceous matter such as sand or soil by treating the total ash with hydrochloric acid.

2.2.3. Extractive Values, (*w*/*v*)%

Extractive values were determined to quantify the amount of active phytoconstituents soluble in specific solvents:

- Alcohol-Soluble Extractive—Indicates the presence of polar and moderately nonpolar compounds.
- Water-Soluble Extractive—Represents the proportion of water-soluble constituents.

2.2.4. pH Analysis

pH Determination

The pH of aqueous extracts was measured to assess the acidity or alkalinity of the plant material, which can influence stability and bioavailability.

- Method: pH was determined using a digital pH meter calibrated with standard buffer solutions (pH 4.0, 7.0, and 9.2).
- Procedure: A 1% *w*/*v* aqueous extract of *Ipomoea nil* was prepared and measured at room temperature (25 °C ± 2 °C).
- Regulatory Range: As per pharmacopeial standards, ensuring consistency and quality of herbal formulations.

3. HPTLC Fingerprinting Analysis

3.1. Sample Preparation

Dried *Ipomoea nil* seeds were powdered and subjected to Soxhlet extraction using 100% ethanol as the solvent. The extract was concentrated under reduced pressure, filtered, and stored at 4 °C for analysis.

3.2. Chromatographic Conditions

HPTLC analysis was performed using a CAMAG TLC system with a Linomat 5 sample applicator, TLC scanner, and Win CATS software.

- Stationary Phase: Pre-coated silica gel 60 F254 plates.
- Mobile Phase: Optimized solvent system (Toluene: Ethyl Acetate: Formic acid) in 9:1:0.5 ratio.
- Sample Application: 10 µL of extract was applied in 6 mm bands at a distance of 10 mm from the plate edges.
- Development Chamber: Twin-trough glass chamber, pre-saturated with mobile phase vapor for 20 min.
- Development Distance: 80 mm.
- Detection:
  - UV Light: 254 nm (shortwave) and 366 nm (longwave).
  - Post-Derivatization: Anisaldehyde–Sulfuric Acid reagent was used for visualizing chemical components.
- Retention Factor (R^f) Values: Used for fingerprinting and comparison with reference standards.
- Densitometric Scanning: Performed at 366 nm to quantify specific bioactive markers.

4. Toxicity and Quality Control Measurement Studies

To ensure the safety and purity of *Ipomoea nil*, comprehensive quality control parameters were analyzed, including heavy metal analysis, pesticide residue screening, aflatoxin contamination assessment, and microbial load evaluation.

4.1. Heavy Metal Analysis

The presence of toxic heavy metals (Lead [Pb], Arsenic [As], Cadmium [Cd], and Mercury [Hg]) was assessed using Atomic Absorption Spectroscopy (AAS).

- Permissible Limits: As per WHO standards.

4.2. Pesticide Residue Analysis

Pesticide contamination was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS/MS).

- Screening for all the 34 Pesticides as mentioned in UPI and API like Organophosphates, Organochlorines, Pyrethroids, and Carbamates.
- Detection Limits: As per WHO guidelines.

4.3. Aflatoxin Contamination

Aflatoxins (B1, B2, G1, and G2) were analyzed by employing Kobra cell techniques.

- Regulatory Limits: Aflatoxin B1 < 5 ppb; Total Aflatoxins < 10 ppb (as per WHO standards).

4.4. Microbial Load Assessment

Microbial contamination was assessed as per Pharmacopeial Microbial Limits using standard culture methods.

- Total Bacterial Count (TBC) and Total Fungal Count (TFC) were determined in colony-forming units per gram (CFU/g).
- Pathogenic microorganisms were screened to ensure microbiological safety, including:
  - *Escherichia coli*, *Salmonella typhi* spp., *Staphylococcus aureus*
- Acceptable Limits: As per WHO, Unani Pharmacopoeia India and Ayurvedic Pharmacopoeia India guidelines.

5. Toxicological Investigation Study

Standard Methods applied for Detection and investigation of HPTLC Fingerprinting, Toxicology parameters used and applied of Advance sophisticated Instruments and operating parameters detections and investigations of Heavy Metals—Pb, As, Cd, Hg in ppm. levels by Thermo Fisher M Series, 650902 V1.27 model Atomic Absorption Spectrometer with Graphite Furnace (AAS-GF), Aflatoxins—B1, B2 and G1, G2 in ppm. levels were estimated by Kobra cell techniques using Agilent HPLC or CAMAG or Anchrom HPTLC instrument, Revolution Front (R^f) values detect in cm. range, Detector—UV-Visible detector, Detector temperature: 25 °C, Sample injector volume range 5.0 µL to 20 µL range, Pesticide residues in mg/kg and bioactive components were analyzed using Gas Chromatography Mass Spectra (GC-MS) (Instrument-Thermo Scientific, Model TSQ9000, Waltham, MA, USA), detector-mass selective detector or Triple Quadrupole mass analyzer detector, column specification-TG-5MS/Run time - usually for the investigated compounds vary according to the method of GC and temperature programming whereas roughly all the relevant bio active phyto-chemical constituents usually appear in an around 0–50 min in the GC column i.e., the Retention time, Run time usually varies with the method and GC temperature program. Mass value range 0.0 to 650 amu, Sample injector volume range 1.0 µL to 5.0 µL. Microbial Load contamination-TBC/TFC detect in cfu/gm. *Escherichia coli*, *Salmonella typhai* Spp. *Staphylococcus aurous* author pathogenic, detection and estimation in Heavy Metals-Pb, Hg, Cd and As, Aflatoxins B1, B2 and G1, G2 and Pesticide Residues- Organo chlorine, pesticides, Organo phosphorus pesticides, Pyrethroids etc., in ppm levels concentrations as per WHO/AOAC/AYUSH-API/UPI Standard methods basis [1–10,12,14,19,24–38].

6. Result and Discussion

6.1. Identification of Key Marker Compounds

Comparative analysis with established reference standards facilitated the tentative identification of key marker compounds in *Ipomoea nil*. The chemical nature of these constituents was inferred based on R^f values, spectral characteristics, and prior literature reports. Shown in Table 1. The presence of flavonoids, alkaloids, phenolic acids, and triterpenoids suggests the potential therapeutic efficacy of *Ipomoea nil* in traditional medicine, supporting its pharmacological applications in *Ayurveda, Siddha, and Unani* (ASU) medicine.

Table 1. key marker compounds in *Ipomoea nil*.

R^f Value's	Putative Compound		Pharmacological Relevance
0.32	Flavonoid glycoside	-	Antioxidant, anti-inflammatory
0.58	Phenolic acid	-	Antimicrobial, hepatoprotective
0.75	Alkaloid derivative	-	Neuroprotective, hypotensive
0.89	Glycoside	-	Cardioprotective, adaptogenic
0.65	Triterpenoid	-	Immunomodulatory, wound healing
0.78	Steroidal compound	-	Hormonal modulation, analgesic

6.2. Comparative Analysis with Existing Literature

The fingerprinting results were compared with previously reported HPTLC profiles of *Ipomoea nil* and related Convolvulaceae species to establish consistency in phytochemical markers and therapeutic applications.

- Flavonoids (R^f - 0.32, 0.75): Consistent with prior studies reporting quercetin and kaempferol derivatives in *Ipomoea* spp., known for antioxidant and anti-inflammatory activities [11,39].
- Steroidal compounds (R^f - 0.78): Align with ethno botanical studies indicating hormonal and anti-inflammatory effects in related *Ipomoea* species [11,15].
- Phenolic acids (R^f - 0.58, 0.89): Previously linked to hepatoprotective properties in the Convolvulaceae family [3,11,31]

The presence of characteristic marker compounds in this study reinforces prior findings on the phytochemical richness and medicinal potential of *Ipomoea nil*, supporting its therapeutic use in traditional medicine.

6.3. Implications for Standardization & Quality Control

The study contributes significantly to the quality assessment, authentication, and standardization of *Ipomoea nil* in herbal medicine.

6.4. Establishment of a Reference HPTLC Fingerprint

- The obtained HPTLC profile serves as a unique chemical fingerprint, ensuring authenticity and minimizing adulteration risks.
- The data can be utilized in pharmacopeial monographs for regulatory acceptance and standardization of *Ipomoea nil* extracts.

7. Quality Control Implementation

To ensure consistency, safety and toxicity in ASU., herbal formulations, quality control parameters were systematically evaluated.

Heavy Metal Analysis

- The levels of toxic metals (Lead [Pb], Arsenic [As], Cadmium [Cd], and Mercury [Hg]) were assessed using AAS, confirming compliance with WHO and USP limits.
- Ensures that *Ipomoea nil* extracts meet regulatory safety thresholds for heavy metal contamination.

Pesticide Residue Screening

- GC-MS analysis was employed to screen for pesticide residues, including organochlorines, organophosphates, pyrethroids, and carbamates.
- No detectable levels of harmful pesticide residues were found, supporting the safety of the plant material for therapeutic use.

Aflatoxin Contamination Assessment

- Kobra Test Method was used to detect aflatoxins (B1, B2, G1, G2).
- The levels were found to be within acceptable limits (<10 ppb), as per WHO guidelines.

Microbial Load and Pathogenic Contamination

Microbial safety was assessed using standard pharmacopeial methods, with results confirming the acceptability of *Ipomoea nil* for medicinal use:

7.1. Contribution to Herbal Standardization and Therapeutic Application

This study reinforces the scientific standardization of *Ipomoea nil* by providing a reliable HPTLC fingerprint and comprehensive quality control data, bridging the gap between traditional knowledge and modern pharmacognosy.

Key Contributions:

(1) Fingerprint Consistency: Ensures batch-to-batch reproducibility in herbal products. Marker Compound Standardization: Facilitates accurate quality assessment of commercial extracts.
Regulatory Compliance: Supports the inclusion of *Ipomoea nil* in pharmacopeial monographs (WHO, API, AYUSH).
(2) Therapeutic Validation: Aligns with phytochemical and pharmacological evidence, supporting traditional Ayurvedic and Unani medicine applications.
(3) Physicochemical Analysis
(4) The physicochemical parameters were analyzed to ensure consistency and purity. The results are summarized below:
(5) HPTLC Fingerprinting [12,37,38].
(6) The ethanolic extract of I N was analyzed using HPTLC to identify characteristic phytoconstituents. The results are presented in terms of R^f values under different detection methods [12,35–38].

7.2. Quality Control Measures

Microbial Load Analysis: Microbial contamination was evaluated using standard methods prescribed by WHO/AOAC/AYUSH/API/UPI. The results confirm that I N meets regulatory standards:

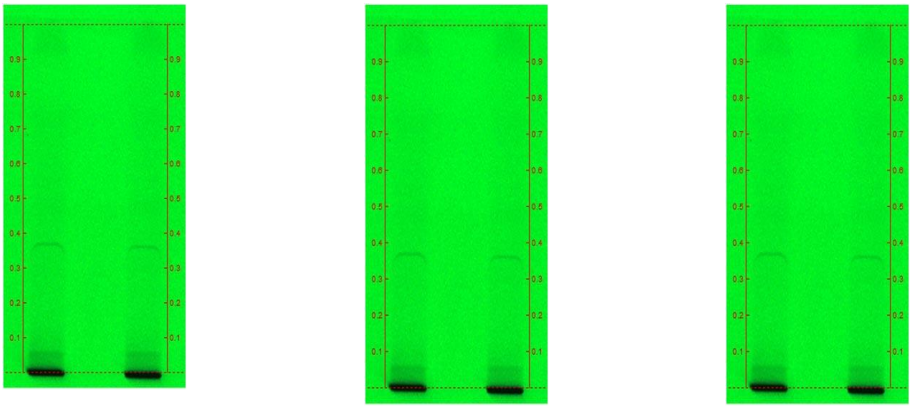
Heavy Metal Analysis: The concentration of heavy metals was evaluated using Atomic Absorption Spectroscopy (AAS-GF). The results indicate that all values fall within permissible limits:

Aflatoxin Contamination: The presence of aflatoxins was assessed using HPTLC, with no detectable levels observed.

Pesticide Residue Analysis: The analysis of pesticide residues by GC-MS confirmed no detectable residues in any sample.

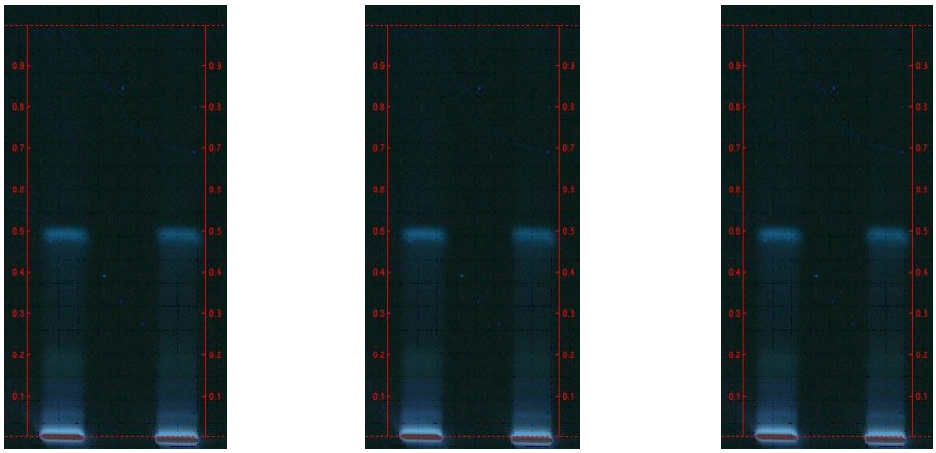
8. HPTLC Investigated Profiling

Extract 2 g of sample with 20 mL of alcohol separately and reflux on a water bath for 30 min. Filter and concentrate to 5 mL and carry out the thin layer chromatography. Applied Ethanol extract on precoated aluminium TLC plate of silica gel 60 F_{254} used HPTLC automatic sample applicator. Developed the plate in Toluene-Ethyl acetate-Formic Acid (9:1:0.5) solvent system. Allowed the plate to dried in air and examine under UV (254 nm). It shown 4 major spots at R^f - 0.93 (Green), 0.73 (Green), 0.46 (Green) and 0.36 (Dark green). Under UV (366 nm), it shown 1 major spot at R^f - 0.49 (Blue), and dipped the plate in 1% Vanillin—Sulphuric acid reagent followed by heated at 105 °C for 5 min and examined under visible light. Observed shown 6 major Spots at R_f 0.90 (Dark grey), 0.76 (Yellow), 0.43 (Dark grey), 0.36 (Dark grey), 0.28 (Dark grey) and 0.10 (Yellow). Shown in investigated Figures 2–4, their densitogram shown in Figures 5 and 6 respectively, R^f - values shown in Table 2 respectively. Resulted the all investigated Physicochemical, HPTLC Finger printing profiling data's, Toxicology research parameters found in the study medicinal plant shown complies and not found of any hazardous or highly toxic contamination in the investigated drug. It had fit for internal used. Investigated results shown in Tables 3–7 respectively [1–12,14,19,24–39].



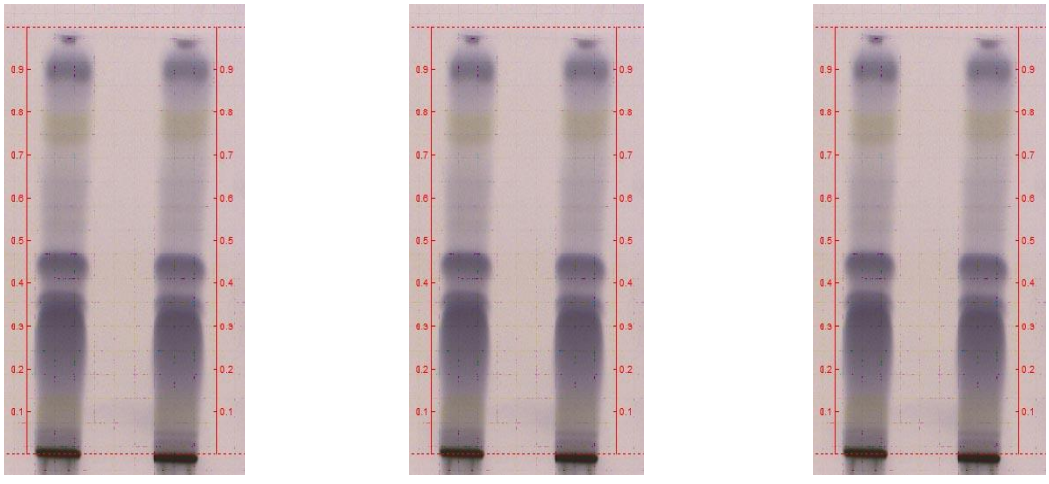
(**a**) (**b**) (**c**)

Figure 2. Thin layer chromatography (alcohol extract). (**a**) I N-1, UV-254 nm. (**b**) I N-2, UV-254 nm. (**c**) I N-3, UV-254 nm.



(**a**) (**b**) (**c**)

Figure 3. Thin layer chromatography (alcohol extract). (**a**) I N-1, UV-366 nm. (**b**) I N-2, UV-366 nm. (**c**) I N-3, UV-366 nm.



(**a**) (**b**) (**c**)

Figure 4. Thin layer chromatography (alcohol extract). (**a**) I N-1, V-S Reagent. (**b**) I N-2, V-S Reagent. (**c**) I N-3, V-S Reagent. Solvent System: Toluene:Ethyl acetate (9:1).

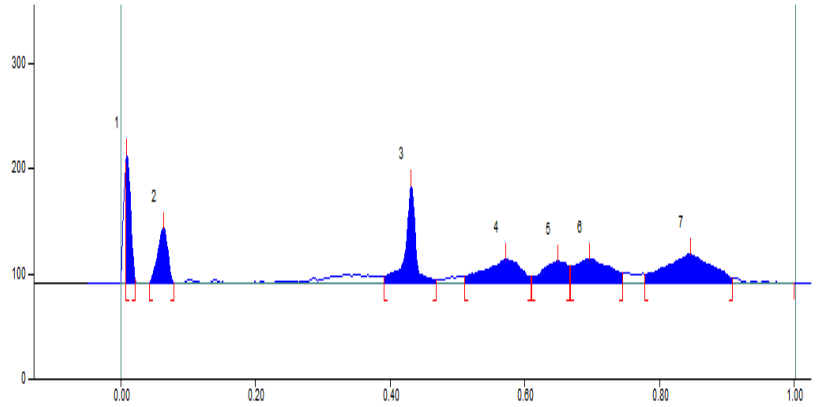


Figure 5. HPTLC finger print of Habb-ul-Neel—Alcohol extract at 254 nm.

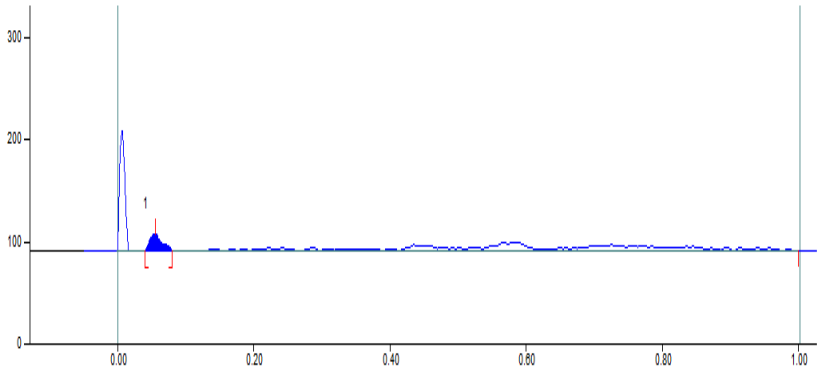


Figure 6. HPTLC finger print of Habb-ul-Neel—Alcohol extract at 366 nm.

Table 2. R^f values of Ethanolic extract: (By HPTLC).

Solvent System	R^f Values								
	UV Light at 254 nm			UV Light at 366 nm			1% *Vanillin—Sulphuric acid* Reagent		
Toluene: Ethyl acetate: Formic acid (9:1:0.5)	0.93 (Green)	0.92 (Green)	0.93 (Green)	0.49 (Blue)	0.48 (Blue)	0.49 (Blue)	0.90 (Dark grey)	0.89 (Dark grey)	0.90 (Dark grey)
	0.73 (Green)	0.72 (Green)	0.73 (Green)				0.76 (Yellow)	0.75 (Yellow)	0.76 (Yellow)
	0.46 (Green)	0.45 (Green)	0.46 (Green)				0.43 (Dark grey)	0.42 (Dark grey)	0.43 (Dark grey)
	0.36 (Dark green)	0.35 (Dark green)	0.36 (Dark green)				0.36 (Dark grey)	0.35 (Dark grey)	0.36 (Dark grey)
							0.28 (Dark grey)	0.27 (Dark grey)	0.28 (Dark grey)
							0.10 (Yellow)	0.09 (Yellow)	0.10 (Yellow)

Table 3. Physico-Chemical investigation tests.

Sr. No.	Analyzed Parameters	Results			Mean/Average Value
		I N-1	I N-2	I N-3	
1.	Colour	Brownish Black	Brownish Black	Brownish Black	Brownish Black
2.	Odour	No Characteristic	No Characteristic	No Characteristic	No Characteristic
3.	Taste	Sweetish Bitter	Sweetish Bitter	Sweetish Bitter	Sweetish Bitter
4.	Foreign matter, *w*/*w*%	ND	ND	ND	0.56%
5.	Loss in wt on drying at 105 °C	4.06%	4.14%	4.10%	3.81%
6.	Total Ash, *w*/*w*%	5.70%	5.76%	5.82%	5.51%
7.	Acid insoluble ash, *w*/*w*%	10.98%	10.96%	10.98%	4.20%
8.	Alcohol Soluble Extract, *w*/*v*%	10.98%	10.96%	10.98%	1.31%
9.	Water Soluble Extract, *w*/*v*%	26.95%	27.53%	27.34%	2.76%
10.	pH (1% Solution)	6.79	6.79	6.77	5.28
11.	pH (10% Solution)	5.94	5.94	5.92	5.37

N/D = Not Detect.

Table 4. Analysis of Microbial load (By WHO/AOAC/AYUSH/API/UPI Std. Methods).

S.No.	Parameter Analyzed	Results			WHO Limit
		I N-1	I N-2	I N-3	
1.	Total Bacterial Count	580 cfu/gm	583 cfu/gm	584 cfu/gm	10^5 cfu/gm
2.	Total Fungal Count	630 cfu/gm	636 cfu/gm	634 cfu/gm	10^3 cfu/gm
3.	*Escherichia coli*	Absent	Absent	Absent	Absent
4.	*Salmonella typhai Spp.*	Absent	Absent	Absent	Absent
5.	*Staphylococcus aurous*	Absent	Absent	Absent	Absent

Table 5. Estimation of Heavy Metals (By AAS-GF).

S.No.	Parameter Analyzed	Results			WHO Limit
		I N-1	I N-2	I N-3	
1.	Lead	3.02 ppm	3.04 ppm	3.03 ppm	10 ppm
2.	Cadmium	0.04 ppb	0.05 ppb	0.04 ppb	0.3 ppm
3.	Mercury	N/D	N/D	N/D	1.0 ppm
4.	Arsenic	0.05 ppm	0.06 ppm	0.04 ppm	3.0 ppm

N/D = Not Detect.

Table 6. Estimation of Aflatoxins (By HPTLC).

S.No.	Parameter Analyzed	Results			WHO Limit
		I N-1	I N-2	I N-3	
1.	Aflatoxin, B1	N/D	N/D	N/D	0.5 ppm
2.	Aflatoxin, B2	N/D	N/D	N/D	0.1 ppm
3.	Aflatoxin, G1	N/D	N/D	N/D	0.5 ppm
4.	Aflatoxin, G2	N/D	N/D	N/D	0.1 ppm

N/D = Not Detect.

Table 7. Estimation of Pesticide Residues (By GC-MS).

S.No.	Parameter Analyzed	Results			WHO Limit (mg/kg)
		I N-1	I N-2	I N-3	
1.	DDT (all isomers, sum of ρ, ρ'-DDT, α, ρ' DDT, ρ, ρ'-DDE and ρ, ρ'-TDE (DDD expressed as DDT)	N/D	N/D	N/D	1.0
2.	HCH (sum of all isomers)	N/D	N/D	N/D	0.3
3.	Endosulphan (all isomers)	N/D	N/D	N/D	3.0
4.	Azinphos-methyl	N/D	N/D	N/D	1.0
5.	Alachlor	N/D	N/D	N/D	0.02
6.	Aldrin (Aldrin and dieldrin combined expressed as dieldrin)	N/D	N/D	N/D	0.05
7.	Chlordane (cis & tans)	N/D	N/D	N/D	0.05
8.	Chlorfenvinphos	N/D	N/D	N/D	0.5
9.	Heptachlor (sum of heptachlor and heptachlor epoxide expressed as heptachlor)	N/D	N/D	N/D	0.05
10.	Endrin	N/D	N/D	N/D	0.05
11.	Ethion	N/D	N/D	N/D	2.0
12.	Chlorpyrifos	N/D	N/D	N/D	0.2
13.	Chlorpyrifos-methyl	N/D	N/D	N/D	0.1
14.	Parathion methyl	N/D	N/D	N/D	0.2
15.	Malathion	N/D	N/D	N/D	1.0
16.	Parathion	N/D	N/D	N/D	0.5
17.	Diazinon	N/D	N/D	N/D	0.5
18.	Dichlorvos	N/D	N/D	N/D	1.0
19.	Methidathion	N/D	N/D	N/D	0.2
20.	Phosalone	N/D	N/D	N/D	0.1
21.	Fenvalerate	N/D	N/D	N/D	1.5
22.	Cypermethrin (including other mixtures of constituent isomers sum of isomers)	N/D	N/D	N/D	1.0
23.	Fenitrothion	N/D	N/D	N/D	0.5
24.	Deltamethrin	N/D	N/D	N/D	0.5
25.	Permethrin (sum of isomers)	N/D	N/D	N/D	1.0
26.	Pirimiphos methyl	N/D	N/D	N/D	4.0

N/D = Not Detect.

Toxicological Investigated Study

I N. having investigated bioactive phytochemacal constituents with immense pharmacological action properties shown in Table 1 respectively, and toxicologically investigated of collected samples of various 3 regions from India, seeds part 3 samples of I N. medicinal plant shown, quality, safety, toxicity QC, QA research data's. Safety and Toxicity investigated research parameters revealed results were shown with in prescribed WHO/AYUSH Pharmacopeial permissible standard Limits in as Microbial Load contaminations-TBC/TFC detect in cfu/gm. *Escherichia coli*, *Salmonella typhai* spp. *Staphylococcus aurous* author pathogenic, detection and in Heavy Metals-Pb, Hg, Cd and As, Aflatoxins B1, B2 and G1, G2 and Pesticide Residues-Organo chlorine, pesticides, Organo phosphorus pesticides, Pyrethroids etc., potentially toxic elements detections in ppb. levels. Resulted the all investigated Safety, Toxicity research parameters found in the study medicinal plant investigated 3 samples shown complies and not found of any hazardous or highly toxic contamination in the investigated drug samples. It had fit for internal used. Investigated results shown in Tables 3–7 respectively. Result of Analytical and Advance Sophisticated instrumentations estimation and analysis HPTLC finger print of alcohol extract:TLC plate was developed using Toluene: Ethyl acetate: Formic acid (9:1:0.5) as mobile phase. After development allow the plate to dry in air, record the fingers print at 254 nm and 366 nm in identification of various bioactive phytochemical secondary metabolites, Quality, Safety and Toxicity research Studies [1–10,12,14,19,24–38].

9. Conclusions

The results investigated and revalidated, tested drug samples I N-1, I N-2 and I N-3 were found and confirmed that I N meets regulatory standards for quality, safety and toxicity in ASU herbal drugs. HPTLC fingerprinting revealed key phytochemical markers useful for authentication. The absence of microbial contamination, heavy metals, aflatoxins, and pesticide residues ensures its suitability for medicinal use. This study contributes to the standardization protocols of I N. enhancing its reliability in herbal medicine formulations. I N. may be treat and therapeutically used in various ASU traditional and alternative medicines confirmed and Investigated In-vitro, In-vivo research reports data's basis and shown therapeutics medicinal potent values as a Antioxidant, Anti-Inflammatory, Antinociceptive, Antimicrobial, Collagenase Inhibitory, Antispasmodic, Anticancer, Anti-tumor, Antiproliferative Activities, Multidrug-Resistance Efflux-Inhibiting Activity, bronchodilatory along with Antiasthmatic potential, Diuretic, anthelminthic and deobstruant and are prescribed for dropsy and constipation, Antihypertensive and cardio-protective effects, Investigated plant has been used classically, traditional and alternative ASU medicine as Cold, Dropsy, Gout, Joint pain, Vitiligo, Itching, Asthma and Anthelmintic disorder from since ancient time. I N. drug can be incorporated of pharmacopeial standard monograph development, DSR, QC, QA, PV aspects. However, further advance research studies on the isolation and characterisation, Structural detection upon GC-MS, LC-MS, XRD, SEM-EDX Advance sophisticated instrumental techniques of these investigated drug scan still be carried out for purposes of advance research investigation of isolated novel bioactive phytochemical, constituents, compounds, novel drug discovery, drug mechanism of action upon isolated active phytochemical constituents apply advance In-vitro or In-vivo studies trial's, thus studies on the these plant parts of I N. also be done to discover potential bioactive compounds that can be explored for discovery of novel drug development and health advantages.

10. Limitations and Future Remarks of the Study

The present study's drug standardization, physicochemical, HPTLC finger printing profiling toxicology profiles show the reconfirmation and presence of DSR, QC, QA of fruit seeds part of PG. plant. In the future, investigated data may be used to drug standardization research, pharmacopeial monographs profiling and confirm these investigated resulted data's.

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References

1. Sagar, P.K.; Sajwan, S.; Murugeswaran, R. *Bauhinia Spp.*,- Kachnar: Medicinal plant biodiversity, geographical distribution, bioactive phytochemical constituents and their ethno pharmacology, therapeutics medicinal values. *Asian J. Pharma. Res. Dev*. **2025**, *13*, 55–65.
2. Sagar, P.K.; Sajwan, S.; Sri, P.M.D.; et al. Scientific DSR validation, pharmacognostical, biodiversity, toxicological research studies of *Nyctanthes arbortristis* L.- aerial leaves part and their pharmacological, therapeutic medicinal values. *Int. J. Pharma. Sci*. **2025**, *3*, 1641–669.
3. Sagar, P.K.; Sajwan, S.; Mageswari, S.; et al. Scientific assessment, research studies of botanical, pharmacognostical,bio diversity and toxicological of ASU herbal drug Kaladana / Habb-ul-Neel (*Ipomoea nil* (Linn.) Roth.) seeds. *J. Pharmacogn. Phytochem.* **2024**, *13*, 112–123. https://doi.org/10.22271/phyto.2024.v13.i6b.15171.
4. Sagar, P.K.; Ahmed, M.W.; Kashyap, S.; et al. Drug standardization, HPTLC finger printing, toxicological research studies of ASU herbal drug fruit seeds part of *Peucedanum grande* C.B. Clarke. *Int. J. Herb. Med.* **2024**, *12*, 15–23. https://doi.org/10.22271/flora.2024.v12.i6a.954.
5. Sagar, P.K.; Mageswari, S.; Ahmed, M.W. An conscious review validation biodiversity, pharmacognostical, pharmacological, toxicological research studies and ther apeuticpotential of ASU herbal drugs-Seed part of Baphali /Duku/ Duqu (*Peucedanum grande* C.B. Clarke). *J. Pharmacogn. Phytochem.* **2024**, *13*, 396–404. https://doi.org/10.22271/phyto.2024.v13.i4e.15030.
6. Sagar, P.K.; Kashyap, S.; Kurele, R.; et al. Quality assurance, shelf life, stability studies of ASU herbal, medicinal plants - *Syzygium aromaticum* (Linn.) Merr LM Perry and *W. fruticosa* (Linn.) Kurz. *J. Pharmacogn. Phytochem.* **2024**, *13*, 323–333. https://doi.org/10.22271/phyto.2024.v13.i4d.15025.
7. Sagar, P.K.; Kashyap, S.; Kurele, R.; et al. Scientific standardization, quality control, antimicrobial potential, toxicology studies having effective therapeutic uses of (*Woodfordia Fruticosa* (Linn.) kurz) flowers part. *World J. Pharm. Pharm. Sci.* **2024**, *13*, 1301–1323.
8. Sagar, P.K.; Ahmed, N.Z.; Khan, A.S.; et al. Pharmacopeial standard development, pharmacognosy, HPTLC. fingerprinting and physicochemical research studies of *Beninca Hispida* (Thunb.). *Int. J. Recent Sci. Res.* **2024**, *15*, 4644–4651.
9. Sagar, P.K.; Khan, A.S.; Sajwan, S.; et al. Anconcise over view on standardization research of ASU-TAM herbal formulated and single drugs, products. *Int. J. Pharm. Pharm. Sci.* **2024**, *6*, 86–95. https://doi.org/10.33545/26647222.2024.v6.i1b.91.
10. World Health Organization. Quality Control Methods for Medicinal Plant Materials (1998). Available online: https://www.who.int/docs/default-source/medicines/norms-and-standards/guidelines/qualitycontrol/quality-control-methods-for-medicinal-plant-materials.pdf?sfvrsn=b451e7c6_0 (accessed on 1 July 2024).
11. Alqahtani, T.; Parveen, S.; Alghazwani, Y.; et al. Pharmacological validation for the folklore use of *Ipomoea nil* against asthma: In-vivo and in-vitro evaluation. *Molecules* **2022**, *27*, 4653. https://doi.org/10.3390/molecules27144653.
12. Ministry of Health and Family Welfare, Government of India. The Ayurvedic Pharmacopoeia of India. Available online: https://www.ayurveda.hu/api/API-Vol-1.pdf (accessed on 1 July 2024)
13. Chaudhry, M.A.; Mushtaq, M.N.; Bukhari, I.A.; et al. Ipomoea hederacea Jacq.: A plant with promising anti-hypertensive and cardio-protective effects. *J. Ethanopharmacol.* **2021**, *268*, 113584.
14. *Ipomoea nil* (L.) Rath. Available online: https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:1071575-2 (accessed on 1 January 2020).
15. Khare, C.P. Ipomoea nil (Linn.), Roth; In Indian Medicinal Plant; Springer: New York, NY, USA, 2014, pp. 1. https://doi.org/10.1007/978-0-387-70638-2_800.
16. Kirtikar, K.; Basu, B. Indian Medicinal Plants; International Book Distributors: New Delhi, India, 1987; vol. 1.
17. Satyavati, G.V.; Raina, M.K.; Sharma, M. Medicinal Plants of India, In Indian Council of Medical Research; New Delhi Indian Council of Medical Research: New Delhi, India, 1987; vol. 2, pp. 398–399.
18. Kadam, D.K.; Ahire, P.D.; Bhoye, J.V.; et al. Comparative standardization study of three *Triphala churna* formulation. *Int. J. Pharmacog.* **2018**, *4*, 71–78.

19. Parihar, A.K.S.; Sahu, U.; Karbhal, K.S.; et al. Quality Control of *Triphala Churna*. *J. Med. Nat. Prod.* **2024**, *1*,100003. https://doi.org/10.53941/jmnp.2024.100003.
20. Singh, D.P.; Govindarajan, R.; Rawat, A.K.S. High-performance liquid chromatography as a tool for the chemical standardization of *Triphala*- An Ayurvedic formulation. *Phytochem. Anal.* **2008**, *19*, 164–168. https://doi.org/10.1002/pca.1032.
21. Jain, V.; Saraf, S.; Saraf, S. Standardization of *Triphala churna*: Spectrophotometric approach. *Asian J. Chem.* **2007**, *19*, 1406.
22. Akinniyi, G.; Jeonghee Lee, J.; Kim, H.; et al. A medicinal halophyte *Ipomoea pescaprae* (Linn.) R.Br.: A review of its botany, traditional uses, phytochemistry, and bioactivity. *Mar. Drugs* **2022**, *20*, 329. https://doi.org/10.3390/md20050329.
23. Govaerts, R.; Nic Lughadha, E.; Black, N.; et al. The World Checklist of Vascular Plants, a continuously updated resource for exploring global plant diversity. *Sci. Data* **2021**, *8*, 215. https://doi.org/10.1038/s41597-021-00997-6.
24. Sagar, P.K.; Ahmed, N.Z. DSR, physicochemical HPTLC, GC-MS and anti-bacterial potential, toxicology studies of achloroform and ethanolic extracts of *Tagetes Erecta* L. flowers part. *World J. Pharm. Pharm. Sci.* **2024**, *13*, 1278–1300. https://doi.org/10.20959/wjpps20246-27457.
25. Sagar, P.K.; Khan, A.S.; Sajwan, S.; et al. Scientific standardization, quality control screening study on effective therapeutic uses of Dhataki/ DhaiPhool/ Gul-e-Dhawa (*Woodfordia fruticose* (Linn.) Kurz). *Int. J. Appl. Res.* **2024**, *10*, 39–53. https://doi.org/10.22271/allresearch.2024.v10.i5a.11729.
26. Sagar, P.K.; Khan, A.S. Pharmacognosy, physicochemistry, toxicity and quality development study Gul-e-Saad Barg (Marigold)-*Tagetes erecta* L. flower of plant. *Int. J. Appl. Res.* **2024**, *10*, 54–66. https://doi.org/10.22271/allresearch.2024.v10.i5a.11730.
27. Sajwan, S.; Negi, R.; Sagar, P.K. Pharmacognostic, physicochemical and therapeutic potential of *Woodfordia Fruticosa* (L.) Kurz. *Adv. Int. J. Multidiscip. Res.* **2024**, *2*, 1–5.
28. Sagar, P.K.; Ahmed, N.Z.; Khan, A.S.; et al. Pharmacopeial standard development, HPTLC finger printing and physicochemical, research studies of polyherbal classical drug *Raughan-E-Mukhtara*. *J. Sci. Res. Chem.* **2023**, *8*, 38–52.
29. Sagar, P.K.; Khan, A.S.; Meena, R.P.; et al. Scientific standard validation and HPTLC fingerprinting studies of polyherbal unani formulation *Majoon-e-Maddat-ul-Hayat Jadwari*, *J. Sci. Res. Chem.* **2023**, *8*, 14–29.
30. Sagar, P.K.; Khan, A.S.; Sonali, S. Pharmacognosy, physicochemical, toxicity quality evolution studies of Marigold-*Tagetes erecta* L. flower of plant. *Int. J. Pharm. Pharm. Sci.* **2022**, *4*, 57–66. http://doi.org/10.33545/26647222.2022.v4.i2a.90.
31. Kumar, J.; Sagar, P.K.; Sajwan, S.; et al. Pharmacpgnostical and HPTLC fingerprinting physcico-chemical studies of single drug Ban Ajwan or Hasha (*Thymus Serpyllum* L.) (WholePlant). *Int. J. Multidiscip. Educ. Res.* **2021**, *10*, 1.
32. Ministry of AYUSH, Govt. of India. Habb-ul- Neel (Seed); In The Unani Pharmacopeia of India; PCIM&H: Ghaziabad, India, 2007; Part I, Volume II, pp. 47–48.
33. WHO global atlas of traditional, complementary and alternative medicine. Available online: https://www.who.int/publications/i/item/9241562862 (accessed on 1 January 2025)
34. EMEA. Quality of herbal medicinal products/traditional herbal medicinal products - Scientific guideline. Available online: https://www.ema.europa.eu/en/quality-herbal-medicinal-products-traditional-herbal-medicinal-products-scientific-guideline (accessed on 1 January 2025)
35. Official Methods of Analysis of AOAC International, 18thed.; Horwitz, W., Latimer, G.W.; Eds.; AOAC International: Rockville, MD, USA, 2005; Chapter 3, pp. 10–11; Chapter 10, pp. 18–23; Chapter 26, p.17.
36. WHO. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine (2000). Available online: https://www.who.int/publications/i/item/9789241506090 (accessed on 1 January 2025)
37. Sethi, P.D. High Performance Thin Layer Chromatography, 1st ed.; CBS Publishers and Distributers: New Delhi, India, 1996; Volume X; pp. 1–56.
38. Wagner, H.; Biadi, S. Plant Drug Analysis—A Thin Layer Chromatography Atlas, 2nd ed.; Springer: Berlin/ Heidelberg, Germany, 1996.
39. Sharma, S.K.; Alam, A. Identification of secondary metabolites, antioxidantactivity, and GC-MS analysis of *Launaea procumbens* (Roxb.) Amin (Angiosperm: A steraceae): Aneglected plant with therapeutic value. *J. Nat. Resour. Hum. Health* **2023**, *3*, 462–468. http://doi.org/10.53365/nrfhh/174619.