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Article

Phytochemical Profiling, DSR, Quality Control and Safety Evaluation Studies of *Lavandula stoechas* L. Aerial Parts Using HPTLC and GC-MS

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Abstract: Ensuring the quality and safety of herbal medicines through drug standardization (DSR), quality control (QC), and quality assurance (QA) remains a major challenge, particularly for authenticating and differentiating adulterants in aromatic herbaceous plants. *Lavandula stoechas* L. (LSL), known as Jarub-e-Dimagh or Ustukhuddus in the Unani system of medicine, has long been used as a brain stimulant and nervine tonic for neurological disorders, insomnia, amnesia, melancholia, stress, anxiety, depression, and vertigo. This study evaluated the DSR, QC, safety profile, high-performance thin-layer chromatography (HPTLC), and gas chromatography–mass spectrometry (GC-MS) fingerprints of the aerial parts and extracts of LSL. Safety assessments included heavy metals, aflatoxins, pesticide residues, and microbial contamination, all of which were within permissible limits. Physicochemical and phytochemical analyses further supported the quality of the samples. HPTLC fingerprinting revealed multiple characteristic bands under UV 254 nm, UV 366 nm, and visible light after vanillin–sulfuric acid derivatization, using toluene: ethyl acetate: formic acid (7.6:2.4:0.01 *v/v*) as the solvent system. GC-MS profiling confirmed the presence of major bioactive phytochemical compounds (MBPCC). The integrated DSR, QC, safety, HPTLC, and GC-MS findings demonstrate that the tested samples of LSL were authentic and free from adulteration. These profiles provide valuable reference data for future standardization and support its traditional therapeutic applications, particularly in neurological and psychological disorders.

Keywords: *Lavandula stoechas* L. (LSL); DSR; QC; HPTLC; GC-MS; fingerprinting; MBPCC

1. Introduction

With the rapid growth of the global herbal medicine market, contamination and adulteration of herbal drugs have become serious concerns. Many countries have therefore established regulations and guidelines to standardize herbal medicines [1]. Drug standardization (DSR), quality control (QC), and quality assurance (QA) are essential to ensure authenticity, safety, and efficacy, while also supporting credibility, reproducibility, and global acceptability of herbal products, as well as preclinical and clinical studies [2,3]. Such standardization is equally critical for understanding bioactivities, identifying potential adverse effects of active components, and improving product quality. The QA and QC of herbal crude drugs and formulations are thus indispensable to justify their acceptability in modern medicine. Hence, research on drug standardization and product validation is required to provide safe and effective therapies to patients suffering from various ailments [4–18].

Lavandula stoechas L. (LSL), commonly known as Ustukhuddus, is a key member of the Lamiaceae family and has long been used in folk medicine worldwide [2,19,20]. The genus Lavandula includes about 39 species, numerous hybrids, and ~400 registered cultivars, with LSL being one of the most economically significant [2,21]. In India, it is found in Bihar, Bengal, and Jammu & Kashmir, while its flowers are also imported from the Persian Gulf [19,20]. The flowers are greyish-blue, bitter in taste, and camphor-scented. LSL is extensively distributed across the Mediterranean and cultivated in France, Spain, and Italy. In Spain, it is called “Romero Santo” (sacred

rosemary) [22,23]. In Unani medicine, LSL is recognized as a highly valued drug for its therapeutic virtues, particularly in neurological disorders. It is described as "Jarub-e-Dimagh" (broom of the brain) for its cognition-enhancing effects [19,21,22,24,25]. Historically, its medicinal importance was acknowledged by Romans, Greeks, and Arabs, and it continues to be used in Spain as a wild edible medicinal plant [26,27].

Phytochemical studies have identified diverse bioactive constituents in LSL, including terpenes, triterpenoids (e.g., camphor, fenchone), phenolics (rosmarinic, caffeic, ferulic, vanillic, gallic acids), flavonoids (apigenin, rutin, epicatechin), and essential oils (1,8-cineole, borneol, linalool, menthol, menthone, pulegone, α-thujone) [21,28–43]. These compounds underpin its reported pharmacological activities such as antimicrobial, antioxidant, anti-inflammatory, analgesic, sedative, antidiabetic, anticancer, nootropic, and anticonvulsant effects [2,19,21,22,24,25,28,29,31–33,35–54]. Such evidence corroborates its traditional therapeutic applications in conditions like insomnia, amnesia, melancholia, stress, anxiety, depression, epilepsy, vertigo, and related neurological disorders. Bio active phyto-chemical constituents present of *L. stoechas*. Shown in Table 1, Given its pharmacological potential and industrial use in Unani formulations, the standardization and validation of LSL are crucial for ensuring authenticity, safety, and therapeutic efficacy. Classical important Unani formulations and it's industrial uses Shown in Table 2 respectively.

Medicinal herbs plant parts of *Lavandula stoechas* L. (**A**) wild wide natural occurrences, (**B**) fresh arial parts, (**C**) fresh flower's & flowers buds., Dried samples-LSL-1, LSL-2, LSL-3 of medicinal herbs plant parts of *Lavandula stoechas* L., and Therapeutics medicinal potent values and pharmacological activities of *Lavandula stoechas* L., shown in Figures 1–4 respectively.

(**A**) (**B**) (**C**)

Figure 1. Medicinal herbs plant parts of *Lavandula stoechas* L. (**A**) wild wide natural occurrences, (**B**) fresh arial parts, (**C**) fresh flower's & flowers buds.

LSL-1 LSL-2 LSL-3

Figure 2. Dried samples—LSL-1, LSL-2, LSL-3 of medicinal herbs plant parts of *Lavandula stoechas* L.

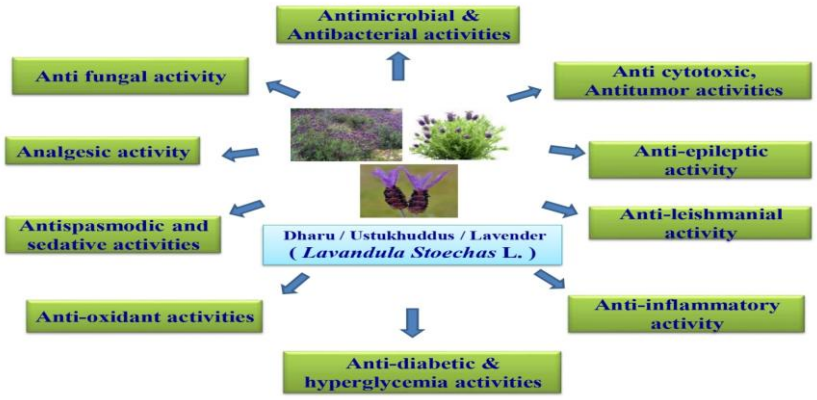


Figure 3. Therapeutics medicinal potent values and pharmacological activities of *Lavandula stoechas* L.

Table 1. Bio active phyto-chemical constituents present of *L. stoechas*.

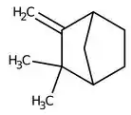
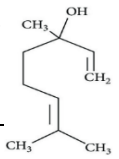
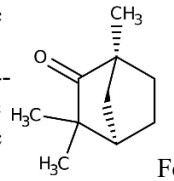
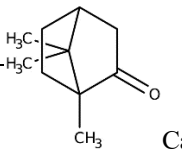
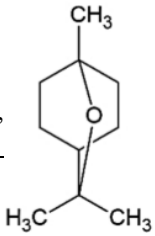
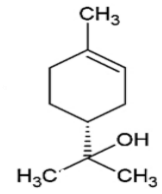
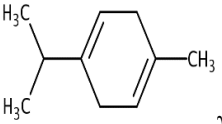
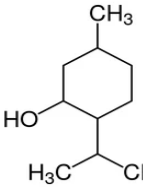
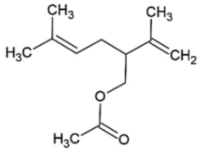
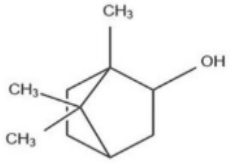
Lavandula Specie	Bio Active Phyto-Chemical Constituents Present	Statures of Major Compounds of LSL with Their Molecular Formulas	References
L. stoechas—flower's part	Terpene-coumarin, fatty acids-rosmarinic acid, ferulic acid, vanillic acid, protocatechic acid, gallic acid, lavender flower's include linalyl acetate, camphor, γ-terpinene, linalool, 1,8-cineole, fenchone and myrtenyl acetate.	 Camphene (C10H16),  Linalool (C10H180),	[2,21,28–31,37,41,55]
L. stoechas—aerial's part	Terpene, triterenoids-fenchane and camphor, phenethylamine and α-tocopherol, fatty acids-caffeic acid, rosmarinic acid, 4-hydroxybenzoic acid, gentisic acid, ferullic acid, P-coumaric acid, vanillic acid, pretocatechic acid, salicylic acid, camphene	 Fenchone (C10H16O),  Camphor (C10H160),	[21,28–35,37,38]
L. stoechas—leaves part	Terpene, triterenoids-fenchane and camphor, epicatechin, epicatechin gattate, apigenin, rutin, pinoresinol, fatty acids - caffeic acid, sinapic acid, cinnamic acid, Leaves include fenchone contained 1,8-cineole, α-cardinol and camphor.	 1,8-Cineole (C10H18O),  α-Terpineol (C10H18O),	[2,21,28–31,33,37,38,56]
L. stoechas—aerial's part—essential oil content's	Triterenoids and polyphenols-1,8 cineole, 10s,11s-himachala-3(12),4diene, borneol, camphor, cubenol, fenchone, linalool, menthol, menthone, pulegone, terpineol, α-thuyone, γ-guijunene, The essential oil of *L. stoechas* contains several bioactive constituents with camphor, borneol, fenchone, 1,8-cineole, linalool, camphene, caryophyllene and lavandulyl acetate being the major components	 γ-Terpinene (C10H16),  Menthol (C10H20O),  Lavandulyl acetate (C12H20O2),  Borneol (C10H18O)	[2,21,28,34–36,38–43,56–58]

Table 2. Classical important Unani formulations and it's industrial uses.

Lavandula Specie	Classical Important Unani Formulations and It's Industrial Uses	References
L. stoechas	Majun Najah, Majun Khadar, Itrifal Ustukhudoos, Itrifal Aftimoon, Itrifal Sanai, Itrifal Ghududi, Itrifal Muqaww-i-Dimagh Lavender—LSL widely used in aromatherapy, manufacturing of Lavender fragrance and perfume, use of this drug as a significant result in the maintenance of black hair, use as cosmetics hair colour dyes, bath, message, oils and Lavender based luxury shops, oils, foods, manufacturing of traditional meal and herbal tea and for making liqueur preparations.	[2,19,20,25,53,55,59–68]

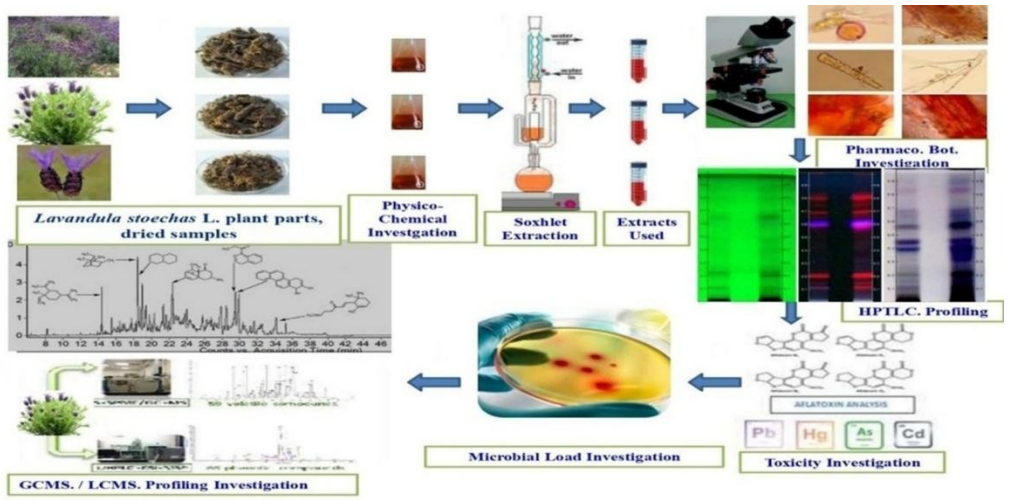


Figure 4. Graphical Illustration of LSL research investigations.

2. Materials and Methods

2.1. Source of Data Collection

All plant material of *Lavandula stoechas* L. (LSL) used in this study was obtained from authorized herbal drug suppliers in India. Sample LSL-1 was procured from M/s DKC Agrotech (P) Ltd., Khari Baoli, Delhi, India; LSL-2 from M/s Chennai Herbal Store, Palavakkam, Tamil Nadu, India; and LSL-3 from M/s Arif & Sons Jadibooti, Ayurvedic Herbal Store, New Salempur, Delhi-53.

The drugs were authenticated and reconfirmed by the Regional Research Institute of Unani Medicine, Chennai (Central Council for Research in Unani Medicine, Ministry of AYUSH, Government of India, Tamil Nadu, India) with reference ID No. DSM-84. Re-authentication was further carried out at PARC, Chennai (Ref. ID No. PARC/2021/4489), and at the Drug Museum of NIUM, Bengaluru (voucher specimen No. 115/IS/res/2022). Additional verification was performed by pharmacognosy and botany experts at the Drug Standardization Research Institute (DSRI), PCIM&H Campus, Ghaziabad, UP, India.

2.2. Procurement of Chemicals, Reagents, and Pathogens

All chemicals and reagents used were of analytical grade. Chloroform, ethanol, petroleum ether, formic acid, hexane, hydrochloric acid, lead acetate trihydrate, sodium hydroxide, Fehling's A and B solutions, toluene, and double-distilled or Millipore water were procured from Merck Life Sciences Pvt. Ltd., India. Ethyl acetate, sulfuric acid, ninhydrin, and ferric chloride were obtained from Fisher Scientific, India. Acetonitrile, methanol, and HPLC-grade solvents were sourced from the Drug Testing Laboratory, Regional Research Institute of Unani Medicine, Chennai, and from the Sophisticated Instrumentation Laboratory, Department of Chemistry, DSRI, PCIM&H Campus, Ghaziabad, UP, India.

Microbial strains and pathogens were obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, Punjab, India; the National Culture Collection (NCC), Pune, Maharashtra, India; and the American Type Culture Collection (ATCC), USA.

2.3. Preparation of LSL Extracts

Water, chloroform, ethanol, hydroalcoholic (50:50), and hexane extracts of the dried aerial parts of LSL-1, LSL-2, and LSL-3 were prepared according to Kancherla et al. (2023) [69]. The plant material was shade-dried, ground into coarse powder, and 50 g of each sample was extracted with 300 mL of the respective solvents using a Soxhlet apparatus for 6–8 h. After continuous extraction, the solutions were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator and water bath. The extracts were dried in a hot air oven at 105 °C for 2 h, scraped out with a spatula, and stored in airtight, temperature-resistant containers. The dried ethanolic extracts were preserved at 4 °C for further analysis.

2.4. Physicochemical Standardization

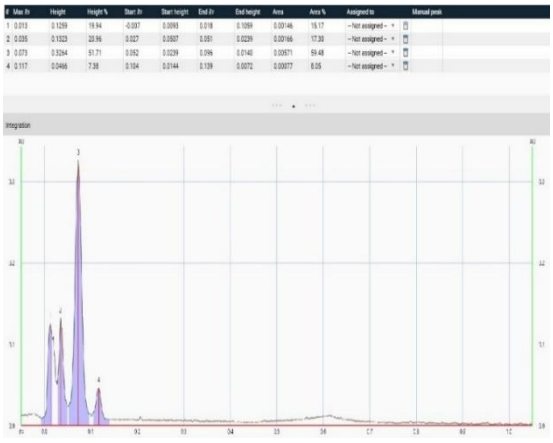
Organoleptic properties (color, odor, and taste) of the aerial parts of LSL were evaluated following the methods of Kancherla et al. (2023) and Siddiqui (1995) [69,70]. Standard physicochemical parameters, including foreign matter, loss on drying at 105 °C, total ash, acid-insoluble ash, extractive values, successive extractive values, and pH, were determined according to standard procedures [10,52,69,71–73].

2.5. High-Performance Thin Layer Chromatography (HPTLC)

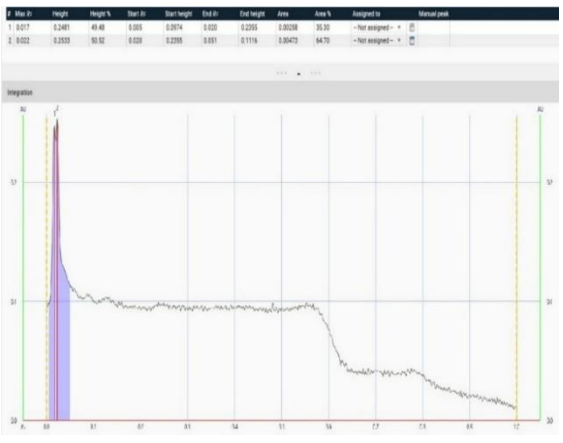
HPTLC fingerprinting of hydroethanolic, chloroform, and hexane extracts of LSL was performed using a CAMAG HPTLC system (Switzerland). Pre-coated silica gel 60 F254 plates (5 cm × 10 cm; Merck, Germany) were used. Five microliters of each extract were applied to the aluminum plates using a CAMAG ATS4 sample applicator. The mobile phase consisted of toluene: ethyl acetate: formic acid (9:1:0.5, *v*/*v*/*v*). Chromatography was carried out in a CAMAG twin-trough chamber (10 cm × 10 cm) pre-saturated for 30 min. The plates were developed up to 8 cm at room temperature, dried, and visualized under UV light at 254 and 366 nm, as well as after derivatizationith vanillin–sulfuric acid (V-S reagent) in the visible region. Chromatograms were recorded using a CAMAG TLC Scanner, and Rf values were calculated with WINCATS software, version-4.1 (Figure 5) [10,69,74,75].

Chromatogram-1, LSL-1, HCL ext. at 254 nm

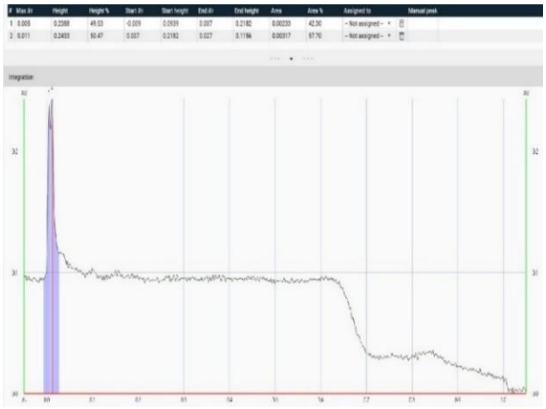
Chromatogram-2, LSL-2, HCL ext. at 366 nm



Chromatogram-3, LSL-3, HCL ext. apply VSDR

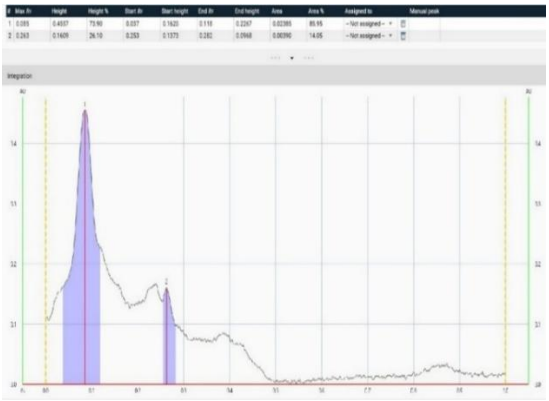


Chromatogram-4, LSL-1, HAL ext. at 254 nm



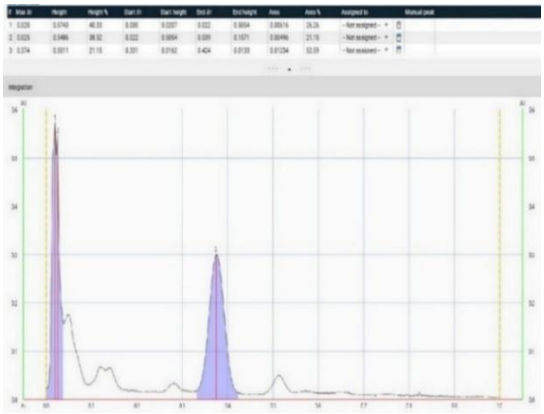
Chromatogram-5, LSL-2, CHLO. ext. at 254 nm

Chromatogram-6, LSL-3, HXAN. ext. at 254 nm



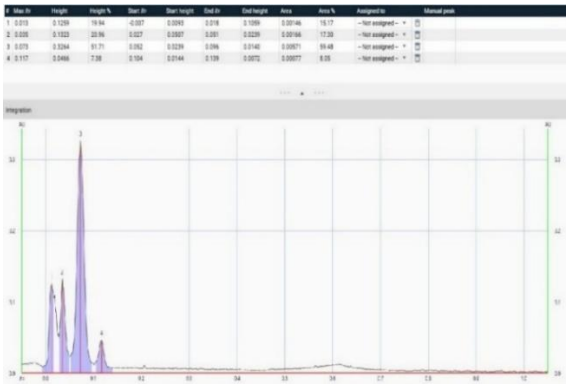
Chromatogram-7, LSL-1, HAL. ext. at 366 nm

Chromatogram-8, LSL-2, CHLO. ext. at 366 nm



Chromatogram-9, LSL-3, HXAN. ext. at 366nm

Chromatogram-10, LSL-1, HAL. ext. after applied VSDR at visible region



Chromatogram-11, LSL-2, CHLO. ext. after applied VSDR at visible region

Chromatogram-12, LSL-3, HEXN. ext. after applied VSDR at visible region

Figure 5. HPTLC Chromatogram 1 to 12 of LSL-1,2 &3 of HCL, HAL, CHLO & HEXN extracts detect in various region at 254 nm, 366 nm & VSDR.

2.6. Gas Chromatography-Mass Spectrometry

The analysis of pesticide residues and confirmation of bioactive phytochemical constituents was carried out in accordance with AOAC guidelines [72]. The ethanolic extract of T. E. was analyzed using Gas Chromatography–Mass Spectrometry (GC-MS) (Thermo Scientific, TSQ 9000). The system was equipped with a triple quadrupole mass analyzer and a TG-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness), with helium as the carrier gas at a flow rate of 1.0 mL/min. The transfer line and ion source temperatures were maintained at 220 °C. Data acquisition was performed with a scan time of 0.2 s and an interval of 0.1 s, over a mass range of 40–600 Da. Identification of compounds was achieved by comparing the obtained spectra with those in the NIST Library database [69,74,76].

2.7. Atomic Absorption Spectroscopy with Graphite Furnace

The estimation of heavy metals such as lead, cadmium, mercury, and arsenic was performed in accordance with WHO guidelines [10,72,77]. Heavy metals were analyzed using Atomic Absorption Spectroscopy (AAS) with a Thermo Fisher M Series 650902 V1.27 model and Thermo Scientific ICE 3000 spectrophotometer (Germany), operated with SOLAAR AA Software (Version 11.10). Operating parameters:

Lead (Pb) and Cadmium (Cd): Flame technique; wavelengths 217 nm (Pb) and 228.8 nm (Cd); slit width 0.5 mm; lamp currents 4.0 mA (Pb) and 3.0 mA (Cd); carrier gas air–acetylene at 1.1 L/min; sample flow rate 2 mL/min.

Mercury (Hg): Cold vapor technique; wavelength 253.7 nm; slit width 0.5 mm; lamp current 3.0 mA; carrier gas argon at 1.1 L/min; sample flow rate 5 mL/min.

Arsenic (As): Flame technique; wavelength 193.7 nm; slit width 0.5 mm; lamp current 6.0 mA; carrier gas acetylene–argon at 1.1 L/min; sample flow rate 5 mL/min.

Hollow cathode lamps specific to Pb, Cd, Hg, and As were used as light sources to provide element-specific wavelengths for quantification (in ppm concentrations).

3. Results and Discussion

3.1. Phytochemical Screening

Preliminary phytochemical screening of LSL-1, LSL-2, and LSL-3 samples was conducted in hydroalcoholic (50:50), chloroform (100%), and hexane (100%) extracts of LSL. Qualitative evaluation revealed the presence of alkaloids, triterpenoids, glycosides, flavonoids, polyphenols, proteins, and amino acids. The results are presented in section 3.4 [10,72,74,78–80].

3.2. GC-MS Profiling

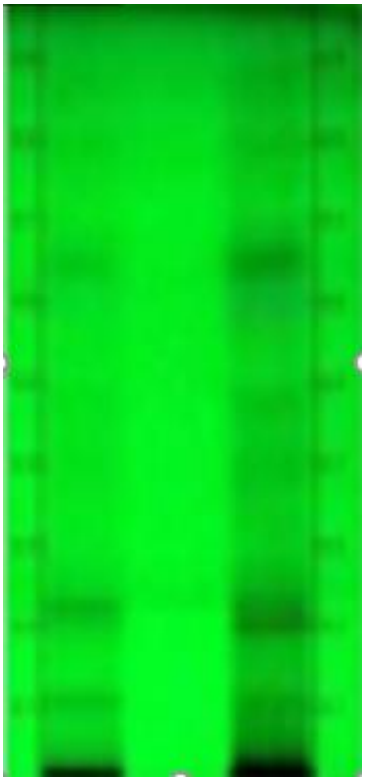
GC-MS profiling of the aerial parts (leaves and flowers) of LSL revealed both qualitative and quantitative data for major bioactive phytochemical constituents (MBPCC). Compounds identified included Camphene (2.42–3.5%), Linalool (1.44–7.5%), Fenchone (30.5–44.8%), Camphor (14.71–48.10%), 1,8-Cineole (3.4–17.8%), α-Terpineol (0.33%), γ-Terpinene (11.2%), Menthol (2.69%), Lavandulyl acetate (5.6%), and Borneol (0.88%, trace). The retention times (Rt) of identified compounds were 13.254, 17.322, 18.033, 20.129, 21.304, 21.564, 23.542, 25.632, 29.242, and 33.624 min, respectively [10,21,69,72,74–77,80–82].

3.3. HPTLC Fingerprinting

Hydroalcoholic (50:50), chloroform (100%), and hexane (100%) extracts (10 µL each) were applied on precoated silica gel 60F254 HPTLC plates (5 cm × 10 cm, Merck, Germany) as stationary phase. Plates were developed in a mobile phase of toluene:ethyl acetate:formic acid (9:1:0.5 mL). After separation, 2–14 distinct spots were visualized at 366 nm (UV), 240–450 nm (UV), and 540 nm (visible), corresponding to alkaloids, flavonoids, polyphenols, glycosides, triterpenoids, and proteins/amino acids. Results are shown in Figure 6a–c,g–l and chromatograms 1–12 for LSL-1, LSL-2, and LSL-3, respectively [10,74,77–80].

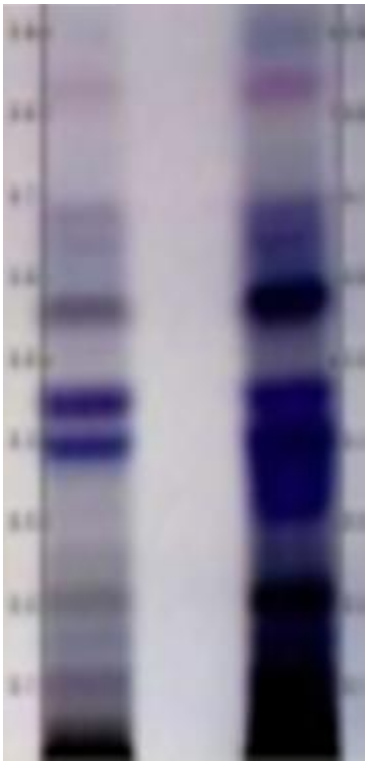
Note: HPTLC fingerprinting provides qualitative identification and quality confirmation of active phytochemical constituents by comparing Rf and HRf values with reference standards.

GC-MS/LC-MS profiling offers further qualitative identification and confirmation of MBPCC using retention time (RT) and spectral data, supporting advanced research in ASU herbal drugs.

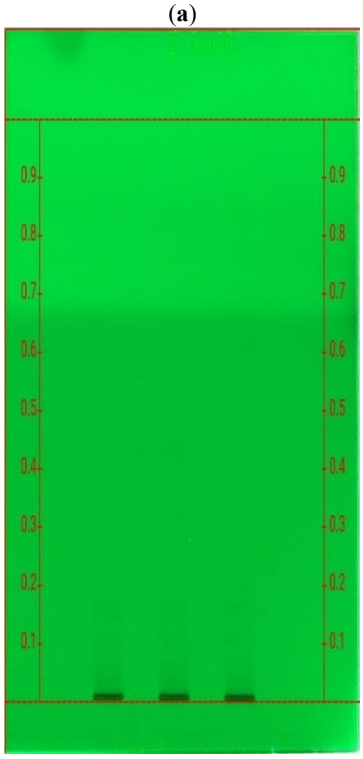


(**a**)

(**b**)



(**c**)


0.9
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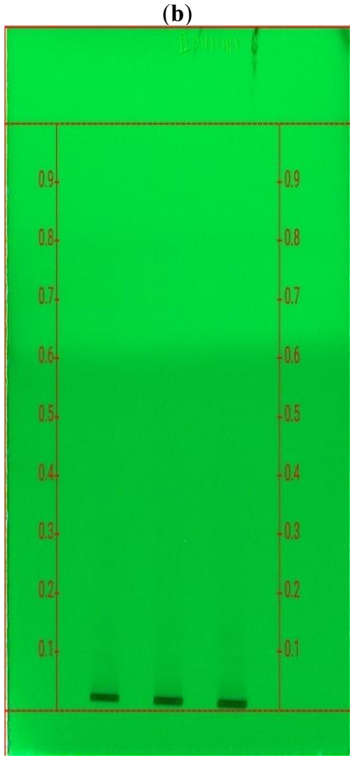
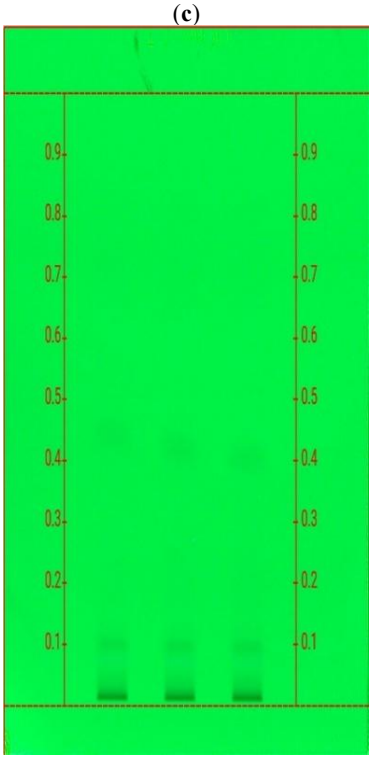
(**d**)


0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1

(**e**)


0.9
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(**f**)

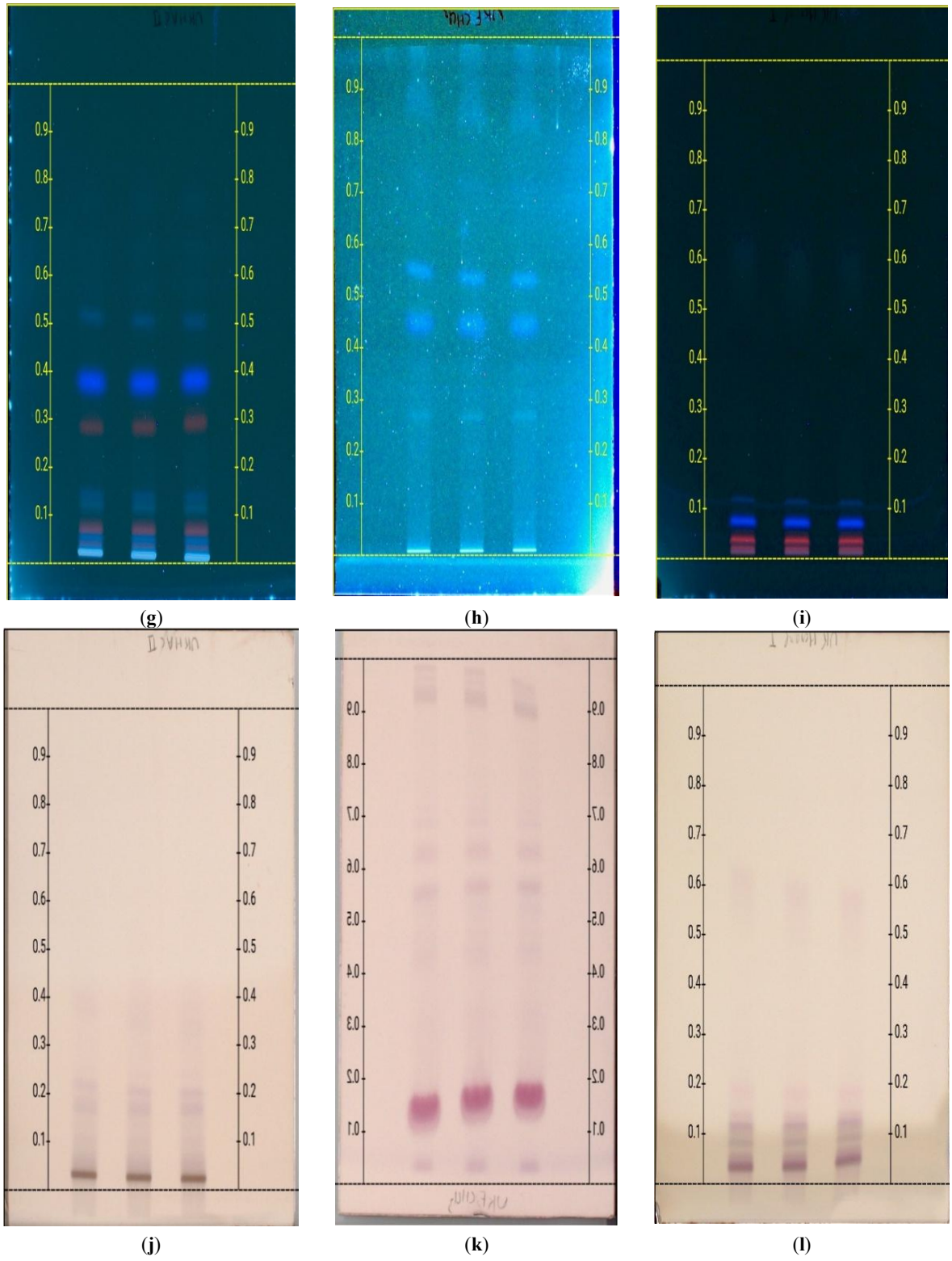

Figure 6. (**a**) LSL-1,-alcoholic and chloro. ext. at 254 nm. (**b**) LSL-2,-alcoholic and chloro. ext. at 366 nm (**c**) LSL-3,-alcoholic and chloro. ext. apply VDSR. (**d**) LSL-1, hydro-alcoholic ext. at 254 nm. (**e**) LSL-2, chloroform ext. at 254 nm. (**f**) LSL-3, hexane ext. at 254 nm. (**g**) LSL-1, hydro-alcoholic ext. at 366 nm. (**h**) LSL-2, chloroform ext. at 366 nm. (**i**) LSL-3, hexane ext. at 366 nm. (**j**) LSL-1, hydro-alco. ext. applied VSAR at visible regions. (**k**) LSL-2, chloroform ext. applied VSAR at visible regions. (**l**) LSL-hexane ext. applied VSAR at visible regions.

3.4. Phytochemical Screening (Repeated Study)

A second phytochemical screening of LSL-1, LSL-2, and LSL-3 samples confirmed the presence of alkaloids, triterpenoids, glycosides, flavonoids, polyphenols, proteins, and amino acids in hydroalcoholic (50:50), chloroform (100%), and hexane (100%) extracts. The results are shown in Table 3 [10,52,73,79,80,82].

Table 3. Phyto-chemical studies of *L. stoechas*.

Phytochemical Tests	Positives Performing Result	Observed Result		
		Water Extract (100%)	Hydro Alcoholic Extract (50:50%)	Ethanol Extract (100%)
Polyphenols:				
Ferric chloride test	dark green and bluish-black colour	++ve	++ve	+ve
Flavonoids:				
Lead acetate test	yellow precipitate	++ve	++ve	+ve
Shinoda's test	red colour	++ve	++ve	+ve
Glycosides				
Keller-killani test	brown ring between layers	++ve	++ve	++ve
Kedde's test	disappearing violet colour	++ve	++ve	++ve
Liebermann burchard test	colors ranging from blue to green, violet and red colour	++ve	++ve	++ve
Tannins:				
Gelatin test	white colour precipitate	−ve	−ve	−ve
Alkaloids:				
Mayer's test	creamy white/yellow precipitate	++ve	++ve	+ve
Wagner's test	brown/reddish precipitate	++ve	++ve	+ve
Dragendorff's test	red/reddish-brown precipitate	++ve	++ve	+ve
Hager's test	creamy white/yellow precipitate	++ve	++ve	+ve
Saponins:				
Foam test	foam persists for 10 min	−ve	−ve	−ve
Steroids and Triterpenoids:				
Salkowski test	appearance of performing colour	−ve,−ve	+ve,+ve	++ve, ++ve
Liebermann burchard test	colours ranging from blue to green, violet and red	++ve, ++ve	++ve,++ve	++ve,++ve
Test for Quinones:	appearance of red colour indicates the presence of quinone	−ve	−ve	−ve
Test for Carbohydrates:				
Fehling's test	appearance of performing colour,	−ve	−ve	−ve
Tollen's test	reddish violet or purple colour ring at the	−ve	−ve	−ve
Benedict's test	intersection of two liquids shows	−ve	−ve	−ve
Proteins/amino acids:				
Millon's test	appearance of performing colour	++ve	++ve	−ve
Biuret test	appearance of purple colour indicates	++ve	++ve	−ve
Starch	Potassium iodide solution	−ve	−ve	−ve

+ indicates present, ++ indicates adequately present, − absent.

3.5. HPTLC Fingerprinting (Detailed Study)

Hydroalcoholic, chloroform, and hexane extracts (10 µL each) were spotted on silica gel 60F254 plates and developed in toluene: ethyl acetate: formic acid (9:1:0.5 mL). Low-polar phytochemicals eluted earlier, while high-polar constituents eluted later. Separated spots were visualized at 366 nm, 240–450 nm, and 540 nm, as shown in Figure 6a–c,g–l, and chromatograms LSL-1 to LSL-9.

3.6. Quality Control and Quality Assurance Parameters

Organoleptic evaluation: LSL aerial parts were light bluish to reddish brown, with a camphoraceous odor, bitter-pungent taste, and indistinct aroma. No foreign matter was detected (Table 4, entries 1–4). Findings matched botanical literature.

Physicochemical parameters: (Detected by qualitative & quantitative slandered methods).

Moisture content (LOD at 105 °C): 4.08%, 4.06%, 4.11%.

Ash values: Total ash 5.70–5.80%; acid-insoluble ash 10.94–10.98%.

Extractive values: ASEM 9.85–10.98%, WSEM 18.29–18.31%, WSSEM 11.16–11.17%, ASSEM 6.54–6.58%, CSSEM 3.65–3.73%, ESSEM 5.46–5.84%.

pH: 1% solution 5.36–5.37; 10% solution 5.05–5.06.

Total phenolics: 3.50–3.53% (Detect by U Visible spectroscopy standard qualitative methods).

Total resins: 5.51–5.52%, (Table 4, entries 5–13, shown respectively).

All parameters were within permissible limits per the Ayurvedic Pharmacopoeia of India (API) and Unani Pharmacopoeia of India (UPI) [74,77].

Microbial load: Total bacterial count (TBC), total fungal count (TFC), Enterobacteriaceae, *E. coli*, *Salmonella* spp., and Staphylococcus aureus were assessed per standard methods; results are in Table 5 [1,10,15–17,73].

Heavy metals: Pb, Cd, As, and Hg were within WHO limits (Table 6) [10,73,75,79,80,82].

Aflatoxins: B1, B2, G1, and G2 were estimated by Kobra cell technique using CAMAG/Anchrom HPTLC instruments as per ASTA (1997). Results are shown in Table 7 [10,73,75,79,80,82].

Pesticide residues: Organochlorine, organophosphorus, and pyrethroid pesticides were analyzed using GC-MS (Thermo Scientific TSQ 9000) as per AOAC guidelines; results are in Table 8 [10,72,73,75,79,82].

Table 4. Physicochemical investigation tests.

Sr. No.	Analyzed Parameters	Results			Std. Deviation, (SD), Values	Related Std. Deviation, (RSD) Values, (Should Be NMT-5.0%)
		LSL-1	LSL-2	LSL-3		
1.	Colour	Light Bluish, Reddish Brown	Light Bluish, Reddish Brown	Light Bluish Reddish Brown	N/R	N/R
2.	Odour	Aromatic characteristics with camphor aroma smell	Aromatic characteristics with camphor aroma smell	Aromatic characteristics with camphor aroma smell	N/R	N/R
3.	Taste	Bitter and pungent	Bitter and pungent	Bitter and pungent	N/R	N/R
4.	Foreign matter, *w*/*w*, % -	N/D	N/D	N/D	N/R	N/R
5.	Loss on drying at 105 °C, % -	4.08%,	4.06%	4.11%	0.025	0.624
6.	Total ash, *w*/*w*, % -	5.70%	5.76%	5.80%	0.057	1.008
7.	Acid insoluble ash, *w*/*w*, % -	10.94%	10.96%	10.98%	0.020	0.182
8.	ASEM, *w*/*v*, % -	10.21%	10.96%	10.98%	0.268	2.530
9.	WSEM, *w*/*v*, % -	18.29%,	18.31%	18.30%	0.010	0.054
10.	WSSEM, *w*/*v*, % -	11.16%	11.17%	11.17%	0.005	0.044
11.	ASSEM, *w*/*v*, % -	6.54%	6.58%	6.56%	0.020	0.304
12.	CSSEM, *w*/*v*, % -	3.65%	3.73%	3.71%	0.042	1.138
13.	ESSEM, *w*/*v*, % -	5.46%	5.75%	5.70%	0.391	5.001
10.	pH, (1% solution)	5.36	5.37	5.37	0.007	0.131
11.	pH, (10% solution)	5.05	5.06	5.06	0.010	0.198
12.	Total phenolics, %	3.52%	3.50%	3.53%	0.017	0.492
13.	Total resins, %	5.51%	5.52%	5.52%	0.010	0.181

N/D = Not Detect, N/R = Not Required.

Table 5. Analysis of microbial load (By WHO/AOAC/AYUSH/API/UPI Std. Methods).

S. No.	Parameter Analyzed	Results			WHO Limit
		LSL-1	LSL-2	LSL-3	
1	Total bacterial count	570 cfu/gm	572 cfu/gm	576 cfu/gm	10^5 cfu/gm
2	Total fungal count	620 cfu/gm	624 cfu/gm	628 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 6. Estimation of heavy metals (By AAS-GF).

S. No.	Parameter Analyzed	Results			WHO Limit
		LSL-1	LSL-2	LSL-3	
1	Lead	3.01 ppm	3.02 ppm	3.02 ppm	10 ppm
2	Cadmium	0.05 ppb	0.04 ppb	0.04 ppb	0.3 ppm
3	Mercury	N/D	N/D	N/D	1.0 ppm
4	Arsenic	0.02 ppm	0.02 ppm	0.04 ppm	3.0 ppm

Table 7. Estimation of aflatoxins (By HPTLC).

S. No.	Parameter Analyzed	Results			WHO Limit
		LSL-1	LSL-2	LSL-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

Table 8. Estimation of pesticide residues (By GC-MS).

S. No.	Parameter Analyzed	Results			WHO Limit (mg/kg)
		LSL-1	LSL-2	LSL-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.

4. Conclusions

The tested drug samples of LSL (LSL-1, LSL-2, and LSL-3) were found to be of high quality and free from impurities, hazardous toxins, and adulterants, as evidenced by phytochemical, physicochemical, and quality control safety studies. All phytochemical ranges and physicochemical constants used for quality evaluation of the aerial parts of LSL were within acceptable bioactive levels and permissible limits. The presence of MBPCC and several secondary metabolites- such as alkaloids, triterpenoids, flavonoids, polyphenols, glycosides, and proteins/amino acids was confirmed through phytochemical screening, HPTLC, and GC-MS fingerprinting. These findings support the therapeutic potential of LSL as a nervine tonic, brain stimulant, and remedy for neurological and oxidative stress related disorders, including insomnia, amnesia, anxiety, depression, headaches, and epilepsy. However, this study has certain limitations. The analysis was limited to in vitro phytochemical, physicochemical, and chromatographic evaluations; comprehensive in vivo safety assessment and pharmacological validation were not performed. Moreover, the exact bioactive compounds responsible for therapeutic efficacy remain to be fully characterized. Future research should focus on in vivo safety evaluation, bioassay-guided fractionation, and structural elucidation of the bioactive constituents using advanced techniques such as GC-MS/MS, LC-MS/MS, XRD, and SEM-EDX. Clinical validation and pharmacological trials are also required to substantiate the traditional claims and to facilitate the development of standardized formulations. Establishing these advanced

datasets will strengthen the potential inclusion of LSL in pharmacopoeial monographs and support its application in novel drug discovery.

Author Contributions: P.K.S. performed Manuscript work designed and carried out, data interpretation's, review research data's profiling revalidation, Manuscript written, reconfirmation of laboratory data's interpretation, summarizing and supervised. S.K. carried out all required instrumentation, laboratory data collection works. H.C. and R.M. performed Manuscript checked and revised manuscript review. A.S., R.M. and S.S. have been performed and carried out LSL aerial plant parts raw material collections, initial plant authentication and identification, literature review of botanical, pharmacognosy and taxonomy confirmation of works designed and Research Material's Collection. All authors have read and agreed to the published version of the manuscript.

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