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Development and standardisation of *Laghu Sudarshan Churna* – An Ayurvedic polyherbal formulation

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Laghu Sudarshan Churna, LSC is an Ayurvedic polyherbal formulation employed for different types of *jvaras* (fevers). The present study was undertaken to prepare its standardised formulation and to standardise the finished product using quality control procedures mentioned in Ayurvedic Pharmacopoeia of India (API). For this, four batches of the finished products were prepared on a laboratory scale and performed the pharmacognostical parameters (macroscopic, microscopic and powder drug analysis); thin layer chromatography; quantitative physicochemical evaluation including loss on drying, total ash, acid-insoluble ash, alcohol & water soluble extractive values, and pH; & measuring the level of aflatoxins, microbial load, heavy metals and pesticide residues of the finished product. This study is the foremost effort to develop the standardised formulation along with the evaluation parameters for *LSC.* Thus, obtained results would be beneficial and will act as the reference for the standardisation of *LSC*.

Keywords: *Laghu Sudarshan Churna*, Pharmacognosy, Standardisation, Thin layer chromatography

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The standardisation protocol of natural products leads to the assurance of its quality, safety, efficacy and reproducibility. The quality assessment of a final product can be measured by some basic standard parameters and its method of preparation. Thus, standardisation is an essential tool for the reproducibility and quality control process.

In the case of synthetic products, there are numerous methods for its testing. But the standardisation of herbal preparations is a huge task for any scientist assessing the quality of a formulation because of the ecological variations. In order to make the Ayurvedic formulations globally acceptable, standardisation of the formulation as well as their ingredients (raw drugs) is the need of the hour[1,2].

The commonly prescribed Ayurvedic formulation for all types of fevers i.e., '*Laghu Sudarshan Churna' (LSC)* mentioned in *Ayurved Sar Sangrah* (*Yogratnakar*) was selected for this study. The *LSC* basically comprises *Guduchi, Pippalimula, Pippali, Haritaki, Shunthi, Nimba, Kutaki, Lavanga, Shveta chandana* and *Kiratatikta* as therapeutically active raw drugs. The formulation is useful in *tridosha jvara* (*Sannipata-jvara*) and all types of fevers. Apart from these, it is also beneficial in conditions such as drowsiness (*Tandra*), giddiness (*Bhrama*), polidypsia (*Trishna*), anemia (*Pandu*), jaundice (*Kamala*) and pain in the hip, back and flank region[3,4].

Notwithstanding such usefulness, no standardisation data is available till date even in reference texts. To meet this lacuna and to assess the quality parameters; the method of preparation for the *churna* and its standardisation parameters have been developed in the present study.

Materials and Methods

Collection & authentication of ingredients (raw drugs) of LSC

The ingredients of *LSC* were obtained from four different shops of Punjab for four different batches of the formulation. All the ingredients were identified/authenticated at CARI, Patiala (peripheral unit of CCRAS) as per the API standards

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and deposited in the Institute Pharmacy with voucher specimen numbers as NIAPR/M-1 (for *Shunthi*), NIAPR/M-2 (for *Guduchi*), NIAPR/M-5 (for *Pippalimula*), NIAPR/M-6 (for *Pippali*), NIAPR/M-7 (for *Haritaki*), NIAPR/M-8 (for *Nimba*), NIAPR/M-9 (for *Kutaki*), NIAPR/M-10 (for *Lavanga*), NIAPR/M-11 (for *Shveta chandana*) and NIAPR/M-12 (for *Kiratatikta*)[5-10].

Method of preparation of LSC

The formulations in four batches were manufactured from the authenticated ingredients and labeled as *LSC1, LSC2, LSC3, and LSC4*. All the ingredients of the formulation (given in Table 1) were taken, cleaned, dried and chopped into small pieces. These were pulverized individually and then, sieved through IS mesh no. 80. All the plant materials were weighed individually as per specified quantity in Table 1 and mixed homogeneously. The whole weight of this formulated *Laghu Sudarshan Churna* was measured. Then, it was packed, labelled and preserved in the containers to avoid light and moisture contact.

Pharmacognostical analysis of LSC

Macroscopic characters

Organoleptic and morphological features like colour, odour, taste and appearance of *LSC* were observed and noted[11,12].

Powder microscopy

The sample of *LSC* (1 mg) was stained with I_2 solution (composed of 2 g iodine and 3 g potassium iodide in 100 mL of water) and then mounted in 50% glycerine on one slide. On another slide, the sample of 2 mg was first clarified with chloral hydrate; then washed in water and after that mounted in 50% glycerine. The microscopical characteristics of both the prepared slides were observed using Magnus MLM microscope. Micro photographs were taken through the versatile digital microscope model no. Deno Capture 2.0 version 142D. The diagnostic features were studied and line drawings were made with the help of Camera Lucida prismatic type[11,12].

Physicochemical analysis

Physicochemical analyses such as loss on drying (LOD), alcohol & water soluble extractive values, total ash value, acid-insoluble ash, and pH were carried out[11,12].

Thin-layer Chromatographic (TLC) analysis

The sample extract (chloroform) of *LSC* was prepared and the sample was loaded on a Merck pre-coated silica gel 60 F_{254} TLC plate. Different solvent systems were tried along with their different ratios. The plates were placed in a CAMAG twin trough chamber and saturated with the solvent system for resolving the components. The plates were observed under UV light at $\lambda_{max.}$ 254 and 366 nm, and then it was sprayed with anisaldehyde sulphuric acid reagent for derivatising the components. The R_f values were measured and the colors of the bands (representing the constituents of the formulation) were noted. The photographs under short & long UV wavelength and after derivatization were taken using digital Camera (SLR Canon)[11,12].

Microbial count analysis

The total viable aerobic (microbial) count was determined[11,12].

Aflatoxins analysis

The levels of the B1, B2, G1 and G2 aflatoxins were measured according to AOAC[11-13].

Heavy metals analysis

The heavy metals (arsenic, cadmium, lead, and mercury) were estimated using Inductively coupled plasma mass spectrometry (ICPMS)[11,12].

Table 1 — Ingredients of the polyherbal formulation, *LSC*[3-4]

S. No.	Sanskrit name	Botanical name	Part used	Quantity taken
i.	*Shunthi* API	*Zingiber officinale* Roscoe	Rhizome	100 g
ii.	*Guduchi* API	*Tinospora sinensis* (Lour.) Merr.	Stem	100 g
iii.	*Pippalimula* API	*Piper longum* L.	Stem	100 g
iv.	*Pippali* API	*Piper longum* L.	Fruit	100 g
v.	*Haritaki* API	*Terminalia chebula* Retz.	Pericarp	100 g
vi.	*Nimba* API	*Azadirachta indica* A. Juss.	Stem bark	100 g
vii.	*Kutaki* API	*Picrorhiza kurroa* Royle ex Benth.	Rhizome	100 g
viii.	*Lavanga* API	*Syzygium aromaticum* (L.) Merr. & L.M. Perry	Flower bud	100 g
ix.	*Shveta chandana* API	*Santalum album* L.	Heart Wood	100 g
x.	*Kiratatikta* API	*Swertia chirata* Buch.-Ham. ex Wall.	Whole plant	450 g

Pesticide residue analysis

The quantification of pesticide residues was measured through QuEChERS extraction method coupled with gas chromatography-tandem mass spectrometry (GC-MS/MS)[11,12].

Results

Pharmacognostical analysis of LSC

Macroscopic characters

The finished product was found to have brown colour, characteristic odour, mild bitter taste and fine texture. The powder could completely pass through IS sieve no. 80. (Fig. 1)

Powder microscopy

Under trinocular compound microscope, the sample of *LSC* revealed the occurrence of thick-walled polygonal cork cells which are of different shapes viz., hexagonal (*Shunthi* rhizome), rectangular (*Nimba* stem bark), pentagonal (*Guduchi* stem) and some cork cells are enclosed with starch grains (*Haritaki* pericarp); phloem fibre with prism-shaped crystals of calcium oxalate (*Guduchi* stem); annular and bordered pitted vessels (*Kutaki* rhizome); spiral vessels (*Kiratatikta* whole plant); spindle shaped lignified sclereids (*Pippalimula* stem); spindle-shaped lignified fibre (*Shveta chandana* heartwood); stone cells of oblong shape (*Haritaki* pericarp) and oval shaped enclosed with brown matter (*Guduchi* stem); and polygonal endosperm filled with granular matter (*Pippali* fruit). The other diagnostic features like calcium oxalate prismatic crystals (which may be from *Haritaki* pericarp, *Guduchi* stem, *Shveta chandana* heartwood and *Nimba* stem bark); simple and compound starch grains (*Shunthi* rhizome, *Pippalimula* stem, *Haritaki* pericarp, *Guduchi* stem, *Nimba* stem bark and *Kutaki* rhizome); and volatile oil globules (*Lavanga* flower bud and *Shunthi* rhizome) were also observed. (Fig. 2 and 3)

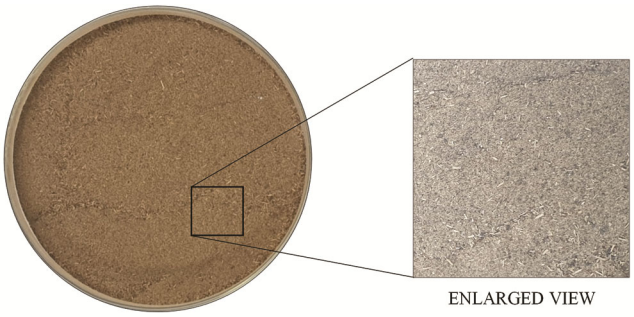

Fig. 1 — *Laghu Sudarshan Churna (LSC)*

Physicochemical analysis

The quantitative physicochemical analyses are depicted in Table 2.

TLC analysis

No. of chromatograms were developed with different solvent systems for TLC of the chloroform extract of the *LSC*, but the ratios of Toluene: Ethyl acetate: Formic acid (6: 4: 1) was selected as best solvent system in which the components were properly resolved. The chromatographic profile showed eight bands under short UV and seven bands under long UV scanning. Six bands were visualized on spraying with anisaldehyde H_2SO_4 reagent. The details of the Rf values and its corresponding band colours are depicted in Table 3 and Fig. 4.

Microbial count analysis

The total viable aerobic count was determined and found to be 1.8 x 10^3 cfu*/g which is under the API permissible limits (Table 4).

Aflatoxins analysis

The level of B1, B2, G1 and G2 aflatoxins were not present in the formulation (Table 5).

Heavy metals analysis

The cadmium (Cd) & mercury (Hg) metals were not found in the formulation; whereas, arsenic (As) & lead (Pb), were present in 1.44 and 3.33 ppm concentration, which is permissible (Table 6).

Pesticide residue analysis

The toxic pesticide residues such as organochlorine, organophosphorus, and pyrethroids were absent in the formulation (Table 7).

Discussion

The *LSC* possesses brown colour, characteristic odour along with mild bitter taste. The microscopic analysis showed the existence of various distinguished features, due to the multiple ingredients, such as thick-walled polygonal cork cells (hexagonal, rectangular, pentagonal) and some cork cells are enclosed with starch grains; phloem fibre with prism-shaped crystals of calcium oxalate; annular and bordered pitted vessels; spiral vessels; spindle shaped lignified sclereids; spindle-shaped lignified fibre; stone cells (oblong, oval); polygonal endosperm filled with granular matter; calcium oxalate prismatic crystals; simple and compound starch grains; and oil globules. These diagnostic microscopical characters

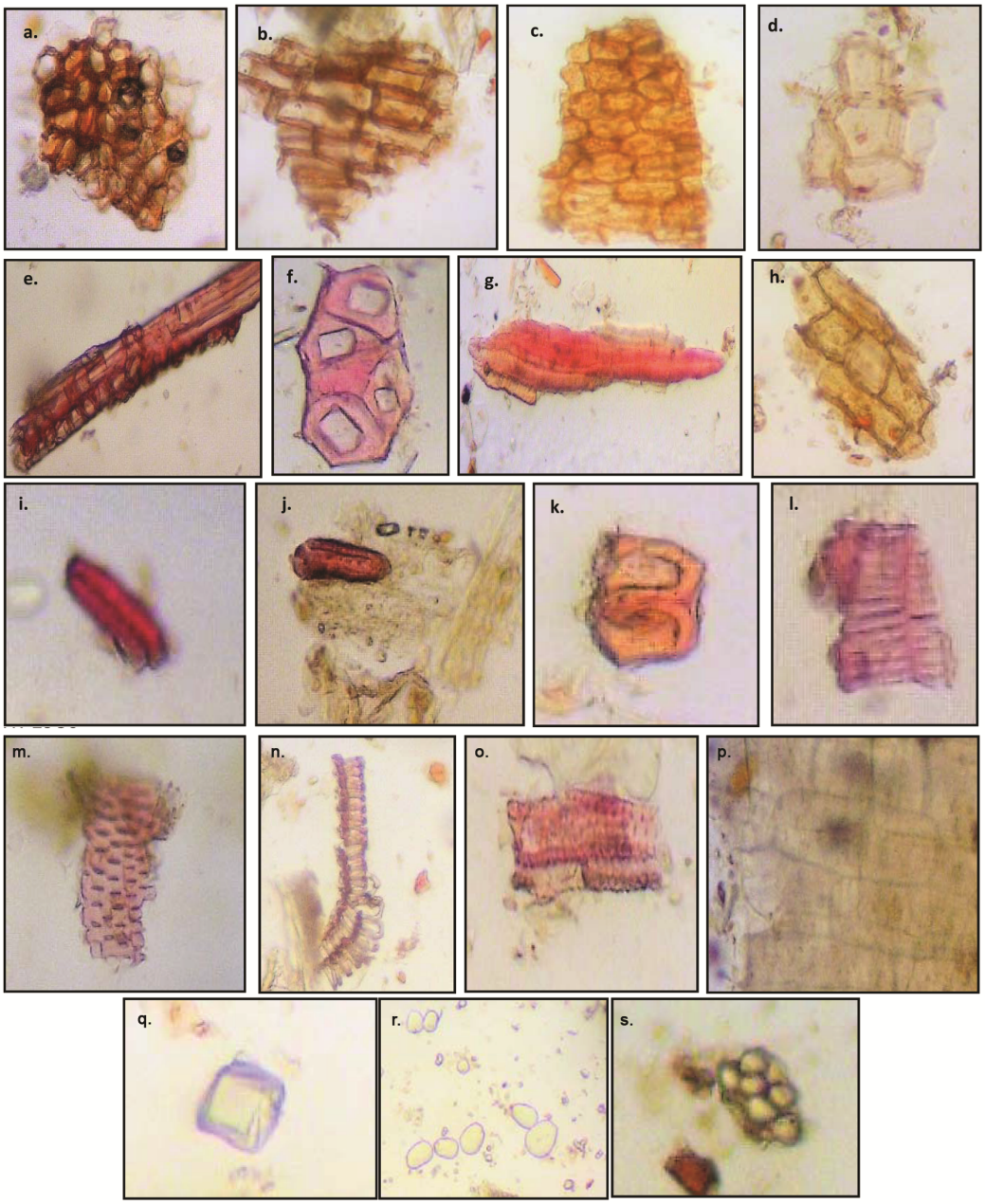

Fig. 2 — Powder microscopic analysis of *LSC* (a. Thick walled polygonal cork in sectional view, b. Sectional view of thick walled polygonal cork cells, c. Sectional view of thick walled polygonal cork cells with starch grains, d. Sectional view of thick walled polygonal cork cells, e. Crystalloid phloem fibre in longitudinal section, f. Crystalloid fibre of phloem in sectional view, g. Spindle shape lignified fibre, h. Polygonal cork in surface view, i-k. Stone cells, l. Annular vessel, m. Bordered pitted vessel, n. Spiral vessel, o. Sclereids, p. Endosperm, q, calcium oxalate crystals (prismatic), r. volatile oil, s. compound starch grains.)

are can be utilized for the identity of the standardised formulation and to know the presence of the respective ingredients[5,6,8-10] in the formulation.

The chromatographic profile showed UV-active components under $\lambda_{max.}$ 254 nm at 0.11, 0.39, 0.50, 0.61, 0.70, 0.77 (lt. black), 0.18, 0.58 (dk. black); and under $\lambda_{max.}$ 366 nm at 0.18 (dk. black), 0.27 (lt. blue), 0.39 (lt. purple), 0.50 (lt. purple), 0.58 (blue), 0.77 (lt. blue), 0.83 (lt. blue); and after spraying with anisaldehyde H_2SO_4 reagent showed some coloured prominent bands at 0.18 (lt. purple), 0.35 (lt. purple), 0.39 (pink), 0.58 (lt. green), 0.70 (lt. purple), 0.77 (lt. orange). The chromatographic analysis depicting R_f values (along with its band colors) which

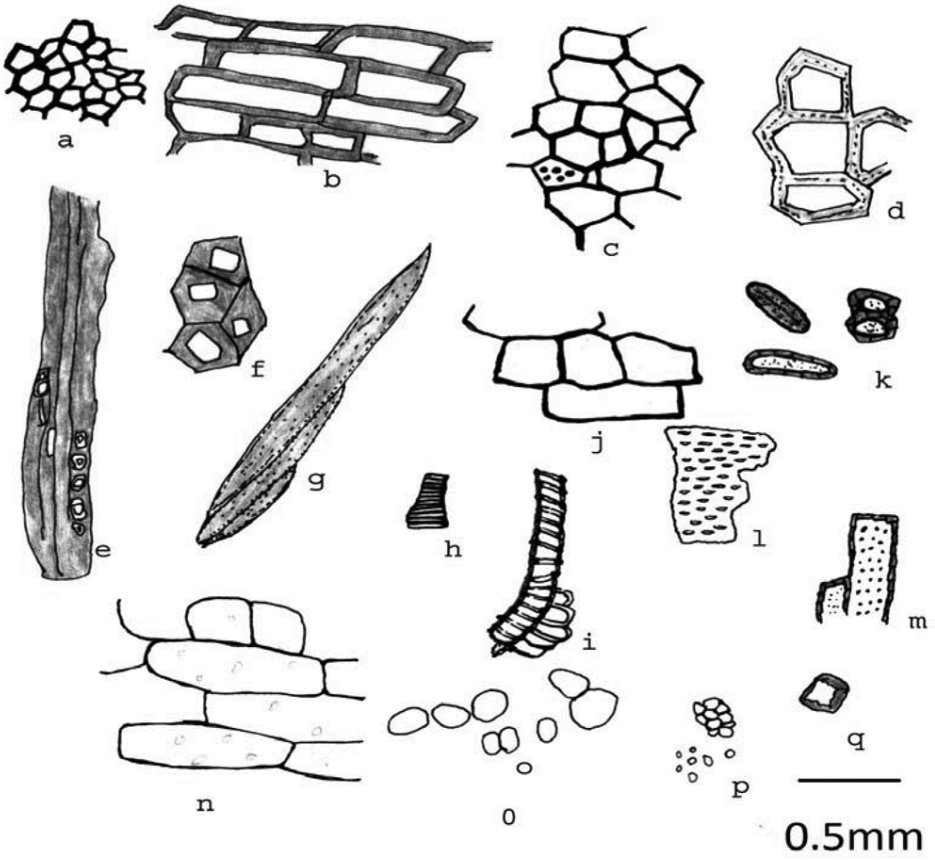

Fig. 3 — Line drawings of *LSC* (a. Thick walled polygonal cork in sectional view (*Shunthi*), b. Sectional view of thick walled polygonal cork cells (*Nimba)*, c. Sectional view of thick walled polygonal cork cells with starch grains (*Haritaki*), d. Thick walled polygonal cork in sectional view (*Guduchi*), e. Crystalloid fibre of phloem in longitudinal section (*Guduchi*), f. Crystalloid fibre of phloem in sectional view (*Guduchi*), g. Spindle shape lignified fibre (*Shveta chandana*), h. Annular vessel (*Kutaki*), i. spiral vessel (*Kiratatikta)*, j. Polygonal cork in surface view (*Nimba)*, k. Stone cells *(Haritaki* and *Guduchi)* l. Bordered pitted vessel (*Kutaki*), m. vessel fragment (*Kiratatikta)*, n. Endosperm (*Pippali)*, o. volatile oils (*Lavanga* and *Shunthi*), p. Compound and simple starch grains (*Shunthi*, *Pippalimula*, *Haritaki*, *Guduchi*, *Nimba* and *Kutaki*) and q. Prismatic crystal of calcium oxalate (*Haritaki*, *Guduchi*, *Shveta chandana* and *Nimba*)

Table 2 — Physicochemical analysis of *LSC*

S.No.	Quantitative Parameters	*LSC1*	*LSC2*	*LSC3*	*LSC4*	Mean ± S.D.	Developed standard limits
a.	Loss on drying, LOD	8.2	8.47	8.51	8.06	8.31 ± 0.19	NMT 9%
b.	Total ash value	5.02	5.03	5.34	5.62	5.25 ± 0.29	NMT 6%
c.	Acid insoluble ash value	1.23	1.24	1.3	1.44	1.30 ± 0.10	NMT 1.5%
d.	Alcohol soluble extractive value	18.85	18.84	17.96	19.46	18.78 ± 0.62	NLT 18%
e.	Water soluble extractive value	23.75	23.33	23.12	24.21	23.60 ± 0.48	NLT 23%
f.	pH	5.75	5.6	5.85	5.9	5.78 ± 0.13	5.5 – 6.0

(where, NMT = Not more than, NLT = Not less than)

corresponds to the respective chemical entity of the ingredients of the formulation finds its importance in its identification and authentication.

The physicochemical analysis showed NLT 18% alcohol soluble and NLT 23% of water-soluble extractive values which showed to be more polar components in the formulation. The total ash i.e., NMT 6% and acid insoluble ash i.e., NMT 1.5% may indicate the earthy constituents of the grounded raw materials. The moisture content, LOD was recorded to be NMT 9% and pH was between 5.5 to 6.0.

The microbial load, heavy metals, aflatoxins and pesticide residues were either absent or found as per the API permissible limits indicating the formulation to be safe and free from toxicity.

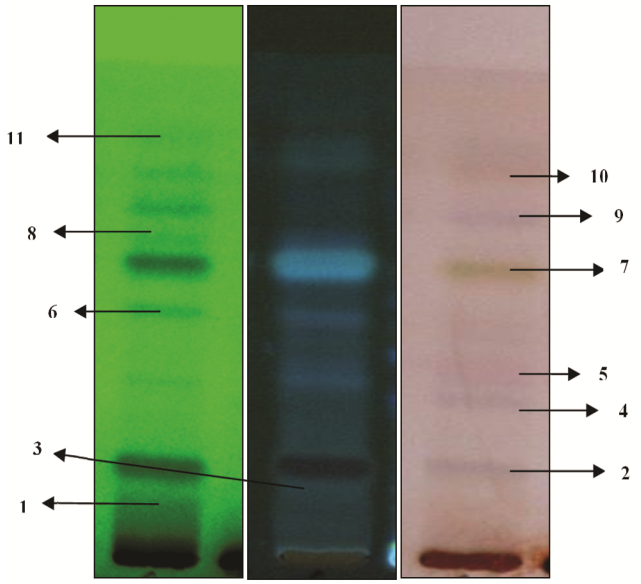

Fig. 4 — TLC of *LSC* at $\lambda_{max.}$ 254 nm UV, $\lambda_{max.}$ 366 nm UV and after spraying

Table 3 — R_f values and its corresponding band colours under 254 nm UV, 366 nm UV and after spraying

S.No.	R_f values	At $\lambda_{max.}$ 254 nm	At $\lambda_{max.}$ 366 nm	After spraying
1.	0.11	lt. black	-	-
2.	0.18	dk. black	dk. black	lt. purple
3.	0.27	-	lt. blue	-
4.	0.35	-	-	lt. purple
5.	0.39	lt. black	lt. purple	Pink
6.	0.50	lt. black	lt. purple	-
7.	0.58	dk. black	Blue	lt. green
8.	0.61	lt. black	-	-
9.	0.70	lt. black	-	lt. purple
10.	0.77	lt. black	lt. blue	lt. orange
11.	0.83	-	lt. blue	-

(where, lt. = light, dk. = dark)

Table 4 — Microbial count analysis of *LSC*

S. No.	Analysis Parameter	Observation	API limits (ppm)
a)	Total viable aerobic count	1.8 x 10^3 cfu*/g	10^5 cfu*/g

(*colony forming units)

Table 5 — Aflatoxins analysis of *LSC*

	Analysis Parameter	Observation	API limits (ppm)
a)	Aflatoxin B1	Absent	0.5
b)	Aflatoxin B2	Absent	0.1
c)	Aflatoxin G1	Absent	0.5
d)	Aflatoxin G2	Absent	0.1

Table 6 — Heavy metals analysis of *LSC*

S. No.	Analysis Parameter	Observation	API limits (ppm)
a)	Arsenic (As)	1.44	3
b)	Cadmium (Cd)	Absent	0.3
c)	Lead (Pb)	3.33	10
d)	Mercury (Hg)	Absent	1

(where, MDL = 0.2 ppb)

Table 7 — Pesticide residue analysis of *LSC*

S. No.	Analysis Parameter	Observation	API limits (ppm)
1. Pesticide Residuals of Organochlorine			
a.	DDT (p,p'-DDD & o,p'-DDD, p,p'-DDE, p,p'-DDT, o,p'-DDT)	Absent	1.0 (sum of)
b.	Endosulfan (α, β isomers and endosulfan sulphate)	Absent	3.0 (sum of)
c.	Aldrin & Dieldrin	Absent	0.05 (sum of)
d.	Methoxychlor	Absent	0.05
e.	BHC (α, β, δ isomers)	Absent	0.3 (sum of)
f.	BHC (γ-isomer)	Absent	0.6
g.	Heptachlor & heptachlor epoxide	Absent	0.05 (sum of)
2. Pesticide Residuals of Organophosphorus			
a.	Chlorfenvinphos	Absent	0.5
b.	Chlorpyrifos	Absent	0.2
c.	Diazinon	Absent	0.5
d.	Dichlorovos	Absent	1.0
e.	Ethion	Absent	2.0
f.	Fenthion its oxygen analogues, their sulphoxides & sulphones	Absent	0.05 (sum of)
g.	Malathion	Absent	1.0
h.	Malaoxon	Absent	Not included
i.	Methyl paraoxon	Absent	Not included
j.	Phorate sulphones	Absent	Not included
k.	Phorate sulphoxides	Absent	Not included
l.	Phorate	Absent	Not included
m.	Parathion-methyl	Absent	0.2
3. Pesticide Residuals of Pyrethroids			
a.	Permethrin	Absent	1.0
b.	Cypermethrin	Absent	1.0

(where, MDL = 0.01 ppm)

Conclusion

LSC being a polyherbal Ayurvedic formulation consisting of ten ingredients of pharmacopoeial quality, was prepared and analysed w.r.t. its pharmacognostical characters, TLC R_f values and quantitative physicochemical analysis. This analytical data may be used to establish the standards and further as the reference tool for the quality evaluation of *LSC*.

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Conflict of Interest

There are no conflicts of interest declared by the authors.

Authors' Contributions

AKM conceived the presented idea; RT, HJ, SBP, HS, BSS performed the experimentation and analysis, AKM & HJ wrote the original manuscript, reviewed and edited under the support and supervision of NS & KSD. All authors discussed the findings and contributed to the final manuscript.

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