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QUALITY ASSESSMENT OF LAKSHADI GUGGULU VATI: A POLYHERBAL FORMULATION

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ABSTRACT
Lakshadi Guggulu Vati (LGV) is one of the widely used poly herbal formulations in Ayurvedic clinical practice since decades for the treatment of various conditions like fractures (*Kanda Bhagna*), pain (*Ruja*), dislocation of joints (*Sandhi Bhagna*) etc. However, comparative analysis of different marketed samples of LGV needs to be carried out to ensure uniformity in the quality of raw drugs and pharmaceutical process. In this germane, present study has been carried out with an objective to generate and compare the analytical profile of different marketed samples of LGV and validate its quality standards as per official publications. Various marketed samples of LGV have been procured and comparative evaluation for their quality control parameters along with HPTLC finger printing as per guidelines of Ayurvedic Pharmacopoeia of India (API) have been carried out. The study revealed significant variation in values of quality control parameters of all four marketed samples of LGV. It can be inferred from the study that dissimilitude in quality control parameters of all marketed sample is evident. Therefore, analytical evaluation and standardization of raw drugs of all patent as well as classical formulations should be performed to optimize the final product quality according to the standards, which would otherwise affect the therapeutic activity of the finished products.

KEYWORDS: HPTLC, *Lakshadi Guggulu Vati*, Phytochemical screening, Quality control.

INTRODUCTION
Medicinal herbs and human beings have distinctive connection since ancient time. Man's profound appeal in plants, chiefly as a source of nutrition, dates back to the very origin of human civilization. Herbs are environment's "Chemical Niche" and richest available source of biological substances on earth. Various herbs have been used as folk medicine to treat numerous illnesses and ailments since centuries.[1] World Health Organization (WHO) has its specific focus on standardization and quality control of plant crude drugs. It emphasizes on physicochemical assessment of raw drugs covering the aspects of the selection and handling of crude material along with safety, efficacy, stability assessment of finished product. It also ensures certification of safety, adverse effect established on experience, provision of product information to consumer and product promotion.[2] The quality evaluation of Ayurvedic formulations is crucial in order to validate their effectiveness in recent culture of medicine but only few studies.[3,4] pertaining to standardization of herbal formulations have been done. The major lacuna of ayurvedic drug industry to establish quality, safety and efficacy is the non-availability of adequate data and documentation of quality control profiles for herbal raw drug and their preparations. It has been observed that a greater proportion of Ayurvedic formulations present in the market are not properly evaluated for their quality standards. Therefore, to ensure the safety and effectiveness of drug it is the need of time to generate standard quality profile for Ayurvedic drugs and document them scientifically. Considering above

facts, the present study has been planned to generate the analytical profile of *LGV*. It is a poly herbal preparation containing of 6 ingredients with *Guggulu* as the key one. It is indicated in the management of Fractures (*Bhagna*) etc. in various Ayurvedic texts.[5] It contains six ingredients, and descriptions are available in various classical texts like *Gadnigrah*[6], *Yogaratnakara*[7], *Bhaishajya Ratnavali*[8], *Madhava Nidana*[9], *Vangasen*[10] and *Ayurveda Sara Sangraha*.[11]

MATERIAL AND METHODS

Procurement of Sample

Various marketed samples (GMP certified pharmacies) of LGV been procured from local market in New Delhi and coded as Brand A, Brand B, Brand C and Brand D. The following tests have been conducted to establish the quality standards for LGV.

A. Organoleptic evaluations

All the organoleptic tests like colour, odour, taste etc. of the LGV were executed as per standard method.

B. Physico-Chemical Evaluation

Physicochemical parameters like moisture content (Loss on Drying)[12], pH, total ash[13], acid Insoluble ash[14], water-soluble extractive value[15] and alcohol soluble extractive values[16] of all samples have been evaluated as per standard procedure mentioned in Ayurvedic Pharmacopoeia of India (API) which is as follows.

C. Pharmaceutical evaluations

Pharmaceutical parameters like Hardness[17], Friability[18] Disintegration time[19], Uniformity of weight[20] were performed.

D. Phytochemical Evaluation[21]

The obtained alcoholic extracts of the drug were subjected to initial phytochemical screening. These tests revealed the presence of many bioactive secondary metabolites which might be accountable for their medicinal characteristics. Methods adopted for initial qualitative phytochemical examinations of the drug extracts are placed in Table 1. Obtained values were compared with those available in API as applicable.

Table 1: Methods adopted for phytochemical evaluations.

S. No.	Phytoconstituents	Name of Tests	Procedure	Observation
1	**Alkaloid**	Mayer's test	2 ml of extract+ few drop of HCl+ few drop of Mayer's reagent	Cream precipitation
		Hager's test	2 ml of extract+ few drop of HCl+ few drop of Hager's reagent	Yellow precipitation
		Wagner's test	2 ml of extract+ few drop of HCl+ few drop of Wagner's reagent	Reddish brown colour
		Dragondraff's test	2 ml of extract+ few drop of HCl+ few drop of Dragondraff's reagent	Reddish brown ppt
2	**Carbohydrates**	Molish Test	2ml extract+ 2 drops of Molish reagent+ few drops of conc. H_2SO_4	Violet or Reddish colour
3	**Terpenoids**	Copper acetate	2ml of extract + 3-4 drops of copper acetate solution	Emerald green colour
4	**Phenols**	Ferric chloride test	2-3ml of extract+ few drop of 5% of $FeCl_3$	Deep blue black colour
		Lead acetate test	2-3 ml of extract+ few drop of lead acetate solution	White precipitate
5	**Tannins**		2-3ml of extract+ 2ml of H_2O+ few drop of 5% of $FeCl_3$	Blue green or blackish colour
6	**Phytosteriods**	Salkowaski test	2ml extract+ 2ml chloroform+ 2ml conc. H_2SO_4	Chloroform layer appears red and acid layer shows greenish yellow fluorescence

E. High-performance Thin-layer Chromatography (HPTLC)[22]

Chromatographic conditions

6 µl methanol extract of LGV was applied through CAMAG Linomat V" Linomat 5_230245" S/N230245 (1.00.13), Syringe size- 100 micro litre, Band width- 8.00 mm, Filtering System- Whatman filter paper No. 1, Plate size-10×10 cm, Material- TLC plates silica gel 60 F 254, Manufacturer- E. MERCK KGaA, Development mode- CAMAG TLC Twin Trough Chamber 20×10 cm, Chamber saturation time- 30 minutes, Mobile phase: Toluene: Acetone (9:1), Drying device- CAMAG TLC Plate Heater, Temperature- 600C, Visualization- 254 and 366 nm, Detection instrument- CAMAG TLC Scanner_230698 (2.01.02), Slit Dimensions- 6.00 × 3.00 mm, micro, Scanning speed- 20nm/s, Lamp- D2 and Hg, Measurement type- Remission, Measurement mode- Absorption.

RESULTS

Organoleptic, pharmaceutical, physico chemical and phyto chemical evaluation of samples of different brands of *LGV* are depicted in Table 2, 3, 4, 5 respectively. R_f values observed in HPTLC fingerprinting of all samples have been presented in Table 6. Peaks and Band development have been shown in Graph 1 & 2 and Figure 1 & 2 respectively.

Table 2: Organoleptic findings of marketed samples of *LGV*.

S. No.	Properties	Brand A	Brand B	Brand C	Brand D	Standard API (Part II, Vol. II)
1	Description/ Appearance	Solid Tab.	Solid Tab.	Solid Tab.	Solid Tab.	Solid
2	Colour	Greyish Black	Brownish black	Brownish black	Black	Black
3	Odour	Agreeable	Agreeable	Agreeable	Agreeable	Agreeable
4	Taste	Bitter	Bitter	Bitter	Astringent	Bitter

Table 3: Pharmaceutical evaluation of marketed samples of *LGV*.

S. No.	Properties	Brand A	Brand B	Brand C	Brand D	Standard API
1	Weight variation	**Passed**	**Passed**	**Passed**	**Passed**	Absent
2	Hardness	5.28 kg/cm^2	4.6 kg/cm^2	4.0 kg/cm^2	3.9kg/cm^2	
3	Friability	0.53% w/w	0.49% w/w	0.32% w/w	0.49% w/w	
4.	Disintegration time	37 min. 08 secs.	3 hrs. 45 min. 55 secs.	1 hr. 15 min.	58 min. 17 sec.	NMT 60 min.

Table 4: Physico-chemical values of marketed samples of *LGV*.

S. No.	Properties	Brand A	Brand B	Brand C	Brand D	Standard API (Part II, Vol. II)
1	Loss on drying at 105°C	7.3%	6.5%	8%	5.1%	NMT 12%
2	Alcohol soluble extractive	5.6%	16.8%	2.6%	4%	NLT 22%
3	Water soluble extractive	12.6%	13.8%	6.8%	4%	NLT 17.5%
4	Total ash	8.8%	8.2%	3.7%	15%	NMT 11%
5	Acid insoluble ash	0.48%	0.43%	0.36%	0.41%	NMT 2.5%
6	Ph at 25°C	4.2	4.15	7.6	4.6	4.71-5.19

NMT- Not more than; NLT- Not less than

Table 5: Observations for the phytochemical evaluation of marketed samples of LGV.

S. No.	Properties	Tests	Brand A	Brand B	Brand C	Brand D
1	Alkaloid	Mayer's test	+	+	+	+
		Hager's test	+	+	+	+
		Wagner's test	+	+	+	+
		Dragondraff's test	-	+	+	-
2	Carbohydrates	Molish Test	-	-	-	-
3	Phytosteriods	Salkowaski test	+	-	+	+
4	Phenols	Ferric chloride test	-	-	-	-
		Lead acetate test	+	+	+	+
6	Tannins		+	+	+	+
7	Terpenoids	Copper acetate	+	+	+	+

+ Present, - Absent

Table 6: HPTLC profiles of marketed samples of *LGV*.

Track position	Name of Sample (Methanolic Extract)	254 nm		366 nm	
		No. of spots	Rf Values	No. of spots	Rf Values
1.	Brand A	**10**	0.01, 0.03, 0.20, **0.26, 0.32,**0.55, 0.62, 0.65, 0.70, 0.80,	**8**	0.11, 0.17, **0.25,** 0.46, 0.58, 0.64, 0.71, 0.92
2.	Brand B	**9**	0.01, 0.18, **0.26**, **0.35**, 0.60, 0.69. 0.71, 0.92, 0.99	**9**	0.01, 0.04, 0.18, **0.26,** 0.46, 0.61, 0.69, 0.71, 0.92
3	Brand C	**11**	0.01, 0.05, 0.24, **0.29, 0.34**, 0.50, 0.57, 0.64, 0.74, 0.87, 0.93,	**11**	0.01, 0.06, 0.24, **0.29**, 0.42, 0.49, 0.55, 0.58, 0.64, 0.71, 0.93
4	Brand D	**9**	0.01, 0.14, 0.18, **0.25,** 0.41, 0.57, 0.64, 0.71, 0.93	**9**	0.01, 0.05, 0.14, **0.29,** 0.41, 0.57, 0.64, 0.71, 0.93

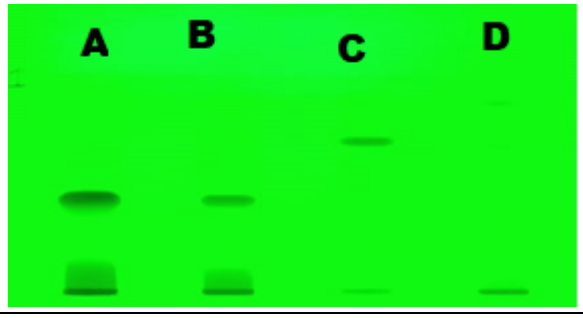


Figure 1: Band separation visualized under 254 nm in UV Cabinet.

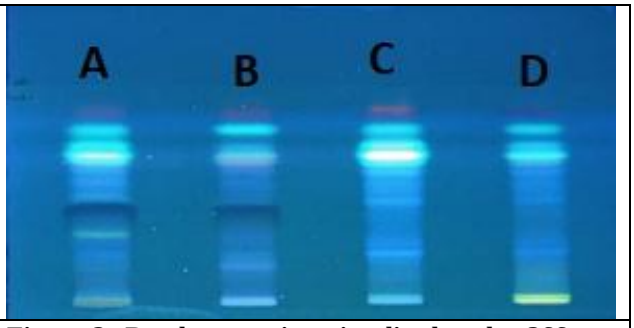
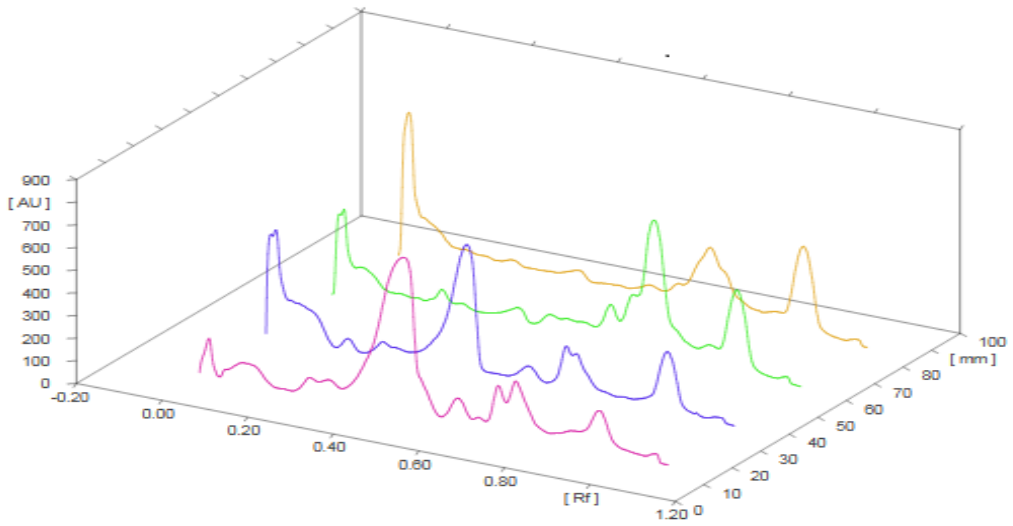
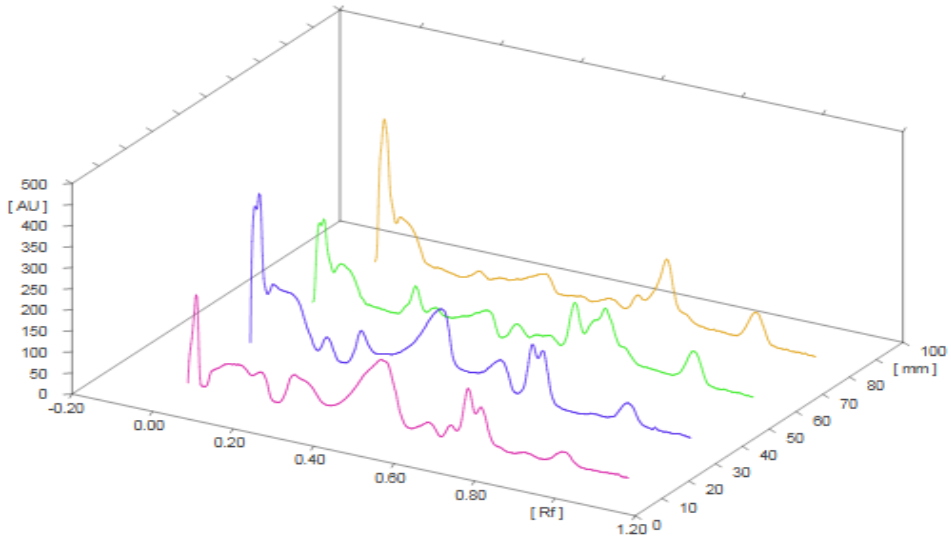


Figure 2: Band separation visualized under 366 nm in UV Cabinet.

Track positioning: From left-right
Track 1: Brand A, **Track 2:** Brand B, **Track 3:** Brand C, **Track 4:** Brand D



Graph 1: Observed peak for Rf values of all samples at 254 nm.



Graph 2: Observed peak for Rf values of all samples at 366 nm.

DISCUSSION

Single drug or compound Ayurvedic formulations comprise various compounds in sophisticated complex matrices in which numerous active constituents are responsible for the therapeutic activity. This leads to difficulty in establishing quality control standards and

standardization of finished product. In Ayurveda, quality control aspect of drugs has been specifically detailed through keen and careful observation. Seers have mentioned various physical parameters and guidelines for raw drugs and finished product as well. Contemporary quality control parameters are the extension of guidelines mentioned by the Acharyas of Ayurveda. LGV is a widely used formulation used in Ayurvedic system of medicine. For LGV labelling and packaging guidelines is complied on Drug and Cosmetic Act Rule 161 1945.

On Organoleptic evaluation i.e. touch, taste and odour, no variation has been found between different marketed samples of LGV and were as per standard characteristics but there was a huge difference in a color of these different marketed sample of Vati. LOD can be defined as the loss of weight due to reduction or elimination of water content and volatile substances under specific conditions. Moisture content plays a crucial role in the drug shelf life. Moisture content above permissible limit may lead to the microbial growth in the product and affects its quality, safety and efficacy. Therefore, it is recommended to have no or minimum moisture content in the solid formulations.[23] In present study, LOD of all samples were found to be in permissible limits of NMT 12 % as per API. It is found in the bracket of 5.1 % (Brand D) - 8 % (Brand C) **(Table-4).** Extractive values evaluate the amount of active constituents present in a drug material when extracted with different solvent. It indicates amount of principle constituents which is readily soluble in aqueous and alcohol media. This value plays an important role in evaluation of efficacy of drugs. An extractive value below the standard value indicates presence of adulterants, substandard material or incorrect processing.[24] Water and alcohol soluble extractives of all samples of LGV were found to be much less than the recommended value of NLT 17.5 % and 22 % respectively. It has been found 4 % (Brand D) - 12.6 % (Brand A) for water soluble extractive and 4 % (Brand D) - 16.8 % (Brand B) for alcohol soluble extractive **(Table-4).** The total ash is the residue remaining after incineration of drug powder. It not only represents the inorganic salts naturally occurring in the drug and adhering to it, but also indicates inorganic matter like metal, salts and silica etc. added for the purpose of adulteration. Total ash and acid insoluble ash contents are important parameters to determine quality and purity of drug. Total ash value was found 8.8% (Brand A), 8.3% (Brand B), 3.7% (Brand C) which are within permissible limit of NMT 11 % except 15 % in Brand D. It is probably due to improper processing or adulteration **(Table-4)**. The pH affects Solubility, Stability and Permeability of the drug.[25] For a drug to cross a membrane barrier it must normally be soluble in the lipid material of the membrane to get into membrane and it has to be soluble in the aqueous phase as well to get out of the membrane. pH of Brand A (4.2), Brand B (4.15), Brand D (4.6) of LGV has been found acidic and below the prescribed standard of 4.71 – 5.19 except Brand C (7.6). This is possible due to adulteration and deterioration of drug **(Table-4)**. Hardness of samples was found to be in the range of 5.28 kg/cm^2 (Brand A) to 4.6 kg/cm^2 (Brand B) **(Table-3).** Hardness indicates the structural integrity and strength of tablets to withstand the impact of force during storage, packaging and transportation. These observed values were range the recommended value of 4-6 N **(Table-3)** which shows the low bonding strength, weak integrity, deterioration and improper processing of tablets. Friability of samples were found ranging from 0.32 % (Brand C) - 0.53 % (Brand A) which is within the conventionally recommended range of 0-1 % **(Table-3).** Disintegration time indicate that whatever dosage forms such as tablets, capsules etc. disintegrate within a permit limit time when placed in a liquid medium under the prescribed experimental conditions. It shows the quality of drug and type of binding agent. If disintegration time is not standardized to all batches of preparations, it indicates the inappropriate SOP. More DT also will affect the absorption of drug on oral consumption. Official publication by Ministry of AYUSH state that *Vatis* prepared with *Guggulu* as an ingredient should have DT value of not more than 60 min.[26] In view of this, all samples except Brand B and C comply with this parameter **(Table-3).** A general trend is observed amongst physicians wherein *Vati* containing *Guggulu* are advised to be chewed before swallowing owing to its more DT. Weight variation is major quality control factor which gives knowledge about the uniformity of dosage form. Weight variation of LGV was found to be in the permissible limits. Phytochemical screening revealed the presence of alkaloids, phytosteroids, tannins and terpenoids in extracts of all the samples. It also revealed that carbohydrate and phenols were found absent in methanolic extract for all samples **(Table-5).** HPTLC profile of samples revealed almost similar Rf values and number of spots in all samples at both the wavelengths.

Comprehensively, physical profile has shown gross variation in various standard parameters in comparison with API whereas chemical profile (phytochemical analysis and HPTLC) profile has shown relative similarity. Different methods for processing, automatic/semiautomatic methods, excipients added, condition for storage, packing and transport may be the possible reasons for these variations. Relative less difference in chemical profile indicate that the variations may be due to geographical variations, stage, source, season and method of collection of raw material. Ayurvedic formulations are gaining huge popularity and demand in the society especially in this time of COVID-19 pandemic. Therefore, it should be mandatory to standardize the collection and storage practices of raw materials to the finished product i.e. formulations in order to maintain the quality, safety and efficacy of the drug. One of limitation of the study was that only four marketed samples were taken for study. Such type of market surveillance studies can be undertaken on a

greater number of marketed samples of same formulation.

CONCLUSION

The present study reveals the non-uniformity in quality control parameters among the various marketed samples of same drug. Although, HPTLC profile has shown relatively greater uniformity among samples as well as in comparison with API, this variation can be accepted unless it does not drastically affect the biological activity and safety profile of the formulation. The quality parameters for *Vati* / tablet that are not reported by API need to be taken up so that a standard would be available for researchers to follow.

REFERENCES

1. Egwaikhide PA, Okeniyi SO, Gimba CE. Screening for anti-microbial activity and phytochemical constituents of some Nigerian medicinal plants. Journal of Medicinal Plants Research, 2009; 3(12): 1088-91.
2. Sharma PC, Yelne MB, Dennis TJ, Joshi A, Billore KV. Database on medicinal plants used in Ayurveda. Vol.1. New Delhi; Central Council for Research in Ayurveda, 2000; 336-9.
3. Yadav NP, Dixit VK. Recent advances in herbal drug standardization. Int J Integrat Biol, 2008; 2: 195–203.
4. Dubey NK, Kumar A, Singh P, Shukla R. Microbial contamination of raw materials: A major reason for the decline of India's share in the global herbal market. Curr. Sci, 2008; 95: 717–8.
5. Sharma PV. Chakradatta, Chap.49/12-13, 1st ed., Chaukhambha Orientalia, Varanasi, 2007; 386.
6. Shodhala. Gadanigraha with Vidyotini Hindi Commentary, 2nd Part, 3rd ed., Chaukhambha Sanskrit Sansthan, Varanasi, 1999.
7. Laxmipatishashtri. Yogaratnakar, Uttarardha, Lakshaguggulu- 11/1, Reprint ed., Chaukhambha Prakashan, Varanasi, 191.
8. Ambikadatta Shashtri. Bhaishajya Ratnavali, Chap.49/14, reprint ed., Chaukhambha Sanskrita Sansthan, Varanasi, 2009; 775.
9. Tewari, PV, Asha Kumari. Vrindamadhava, 1st ed., Chaukhambha Visvabharati, 2007.
10. Tripathi HP. Vangsena Samhita, Vatarakta-Adhikara, Chaukhamba Sanskrit Series Office, Varanasi, 2009; 858.
11. Ayurveda Sara Sangraha, Sri Baidhyanath Ayurveda Bhavan Ltd., Allahabad, 2018; 601.
12. Anonymous. The Ayurvedic Pharmacopoeia of India. Part II, Vol- I, Appendix 2.2.8. Govt. of India, Ministry of Health and Family Welfare, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homeopathy (AYUSH). 1st ed., New Delhi; The Controller of Publication, 2006; 161.
13. Anonymous. The Ayurvedic Pharmacopoeia of India. Part II, Vol- I, Appendix 2.2.8. Govt. of India, Ministry of Health and Family Welfare, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homeopathy (AYUSH). 1st ed., New Delhi; The Controller of Publication, 2006; 159.
14. Anonymous. The Ayurvedic Pharmacopoeia of India. Part II, Vol- I, Appendix 2.2.8. Govt. of India, Ministry of Health and Family Welfare, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homeopathy (AYUSH). 1st ed., New Delhi; The Controller of Publication, 2006; 159.
15. Anonymous. The Ayurvedic Pharmacopoeia of India. Part II, Vol- I, Appendix 2.2.8.Govt. of India, Ministry of Health and Family Welfare, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homeopathy (AYUSH). 1st ed., New Delhi; The Controller of Publication, 2006; 141.
16. Anonymous. The Ayurvedic Pharmacopoeia of India. Part II, Vol- I, Appendix 2.2.8. Govt. of India, Ministry of Health and Family Welfare, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homeopathy (AYUSH). 1st ed., New Delhi; The Controller of Publication, 2006; 141.
17. Lachman L, Leiberman AH. The Theory and Practice of Industrial Pharmacy, Section III. Special Indian Edition, New Delhi; CBS Publisher and Distributers Pvt. Ltd, 2009; 297.
18. Lachman L, Leiberman AH. The Theory and Practice of Industrial Pharmacy, Section III. Special Indian Edition, New Delhi; CBS Publisher and Distributers Pvt. Ltd, 2009; 297-98.
19. Lachman L, Leiberman AH. The Theory and Practice of Industrial Pharmacy, Section III. Special Indian Edition. New Delhi; CBS Publisher and Distributers Pvt. Ltd.:2009, 301.
20. Lachman L, Leiberman AH. The Theory and Practice of Industrial Pharmacy, Section III. Special Indian Edition, New Delhi; CBS Publisher and Distributers Pvt Ltd, 2009; 300-301.
21. Khandelwal KR. Practical Pharmacognosy-Techniques and Experiments, 26th ed., Pune; Nirali Prakashan, 2016.
22. Rich E, Schibli A. HPTLC for the Analysis of Medicinal Plants. Thieme Medical Publisher, Inc: ISBN, 13: 978-1-58890-409-6(US).
23. Paritosh jha. Revalidating Standard Manufacturing Procedures of Guduchi Ghana and GuduchiSatva and their comparative β-cell regeneration and glucose uptake activities in experimental animals. PG Dissertation, Discussion, Deptt. of Rasa Shastra and Bhaishajya Kalpana, AIIA, Delhi University, 2019.
24. Chandel HS, Pathak AK, Tailang M. Standardization of some herbal antidiabetic drugs in polyherbal formulation. Pharmacognosy research, 2011; 3(1): 49.
25. http://phcalculator.blogspot.com/2013/04/importance-of-ph-in-pharmacyand.html.accessed on 10.04.21 at 10.30 am.
26. Anonymous. Indian Pharmacopoeia. Vol 1, Ghaziabad, New Delhi; Indian Pharmacopoeial Commission, 2007, 2016–26.