Research Article

www.ijrap.net (ISSN:2229–3566)

PHYTOCHEMICAL AND ELEMENTAL PROFILING AND STANDARDIZATION OF SOME AYURVEDA MEDICINES USED IN COVID-19 PANDEMIC

Priyanka Patil [1], Madhuree Gawhankar [1], Shivcharan Bidve [1], R V Gudi [2] and Atul Lavand [2]*

[1] Shree Dhootapapeshwar Ayurvedic Research Foundation, Veer Savarkar Chowk, Panvel, Navi Mumbai, Maharashtra, India

[2] Shree Dhootapapeshwar Limited, Veer Savarkar Chowk, Panvel, Maharashtra, India

Received on: 08/07/21 Accepted on: 02/08/21

***Corresponding author**
E-mail: atul.lavand@teamsdl.in

DOI: 10.7897/2277-4343.1204110

ABSTRACT

Tribhuvankirti Rasa is a herbo-mineral Ayurvedic medicine regularly used to treat different types of fever. It has antipyretic and analgesic activities. It is an effective medicine for the common cold, flu and other Vata kapha problems. Laghumalini Vasanta is also Ayurvedic medicine, used to treat chronic fever and effective in pitta disorders. Ministry of AYUSH, Government of India also recommended these medicines to prevent the severe conditions of Cov-2 infection. Review of literature suggested that phytochemical and elemental characterization parameters of Tribhuvankirti Rasa and Laghumalini Vasant are not reported. The objective of this study is to report phytochemical and elemental profiling and to standardize Tribhuvankirti Rasa (TKR) and Laghumalini Vasant (LMV) to confirm quality and purity. Tribhuvankirti Rasa and Laghumalini Vasant evaluated for phytochemical and elemental parameters by HPTLC and ICP-OES respectively. HPTLC analysis confirms LMV contains Piperine and TKR contains Piperine and 6-Gingerol. The solvent systems toluene: ethyl acetate (7: 3) v/v for Piperine & Hexane: Ethyl acetate: Formic acid (4 : 6 : 0.1) v/v for 6-Gingerol were optimized. ICP-OES analysis confirms presence of Zn in LMV and Hg in TKR. HPTLC and ICP-OES methods were validated successfully for Tribhuvankirti Rasa and Laghumalini Vasant. The characterization and method validation parameters presented in this paper may serve as standard reference for quality control analysis of Tribhuvankirti Rasa and Laghumalini Vasant.

Keyword: Covid-19, Ayurveda, Anti-viral, Anti-inflammatory, Piperine, 6-Gingerol

INTRODUCTION

Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) also known as COVID-19, is appeared as the global pandemic threat since its outbreak in Wuhan (China) in late 2019[1]. World Health Organization (WHO) is constantly monitoring situation worldwide. As on 13 June 2021 WHO has reported over 175.3 million cases and 3.7 million deaths globally since the start of the pandemic (https://covid19.who.int/). The COVID-19 virus spreads primarily through droplets of saliva or discharge from the nose when an infected person cough or sneezes[2]. It is being observed that COVID-19 have fastest frequency of replication in its positive strand which results in the quick development of new progeny viral cells inside the host cells. SARS-CoV-2 is a single-stranded RNA pathogen with high mutation rate[3,4].

Majority infected people will develop mild to moderate respiratory illness and recover without hospitalization. Peoples with medical problems related to diabetes, cardiovascular disease, cancer and chronic respiratory disease are more prone to develop serious illness[5]. There is a big challenge for the researcher community and health experts to discover solution for Covid-19 virus. Pathogenesis of Covid-19 disease causation is not completely understood and testing of drugs or vaccines against Covid-19 virus are still in process. Majority of doctors across the globe focusing on enhancing immunity to treat Covid-19 infected case[6]. In China, high doses of vitamin C are used to boost immunity in COVID-19 patients and showed promising results[7].

High fever, severe body pain and severe cough are main symptoms of Covid-19, along with many of the other associated symptoms[8]. The disease could be control within a short period by treating with exclusively on Ayurvedic medicines[9]. This demonstrates that there is a wide scope to explore the variety of relevant medicines present in Ayurvedic Pharmacopoeia which can be used more rationally to suit every stage of the disease. Ayurveda, one of the world-renowned forms of Indian traditional medicine, references several immunity boosting therapeutics. Several herbal products are found to normalize or regulate immune system and have antiviral property, so they can be used in prevention and control of COVID-19[10].

Medicinal plants have been used to treat several infectious diseases since ancient times. The benefit of using ayurvedic medicines in viral respiratory infections is to build immune-stimulating and inflammation-modulating effects to prevent severe life-threatening conditions[11,12].

Ministry of AYUSH, Government of India also recommended Ayurvedic medicines to boost immunity and to prevent the severe conditions of Cov-2 infection (https:// www.ayush.gov.in/ayush-guidelines.html). About 80% of COVID-19 patients have mild symptoms requiring only primary medical care. Advisory on Ayurved, Unani and Homeopathy for Covid-19, published the guideline for treating Covid-19 patient in mild symptom, moderate symptoms and Immuno Compromised Conditions stage (https://www.ayush.gov.in/). It includes Tribhuvankirti Ras (https://www.ayush.gov.in/docs/ayurved-guidlines.pdf), Laxmivilas Rasa, Laghumalini Vasant (https://health.ncog.gov.in/ayush-covid-dashbaord/assets/Classified/AYUSH_Advisory_Maharashtra.pdf), Arogyavardhini, Chyavanprash, etc. The present study was focused on the identification of active ingredients and elements of certain

Ayurveda medicines such as Tribhuvankirti Ras and Laghumalini Vasant.

Laghumalini Vasant contains Piperine and Tribhuvankirti Rasa contains Piperine and 6-gingerol as main active phyto-constituents. Piperine is widely used in fever, pain management, rheumatism arthritis, chills and influenza. Piperine is proved to have many biological activities such as anti-inflammatory, antimicrobial, anti-infective, insecticidal, antiamoebic, antiulcer and antidepressant[13,14]. Piperine is also used for the blood circulation enrichment, salivation and stimulation of appetite[14]. 6-Gingerol has been reported to have various biological properties including anti-inflammation, anti-fungal, anticancer, antioxidant and anti-platelet aggregation[15-17]. 6-Gingerol have been reported to be effective treatments for metabolic syndrome and infectious diseases[18]. The antibacterial activities of 6-gingerol have been also studied[19]. More recently, the antibiofilm activities of 6-gingerol against *P. aeruginosa* have been reported[20].
As per our knowledge there are no reports for phytochemical and elemental profiling for Tribhuvankirti Rasa and Laghumalini Vasant. The objective of the present investigation is the development of a simple, precise and reproducible HPTLC and ICP-OES method for the estimation of phytoconstituents and elements from commercially marketed formulation. As method validation is an essential constraint in analytical method development, the presented method has been validated following the guidelines of the ICH.

MATERIALS AND METHODS

Ayurvedic formulation, Laghumalini Vasant (LMV), formulated as per text reference Bharat Bhaishajya Ratnakar 4/6972 & Tribhuvankirti Rasa (TKR) formulated as per text reference Bharat Bhaishajya Ratnakar 2/2755 were procured from Shree Dhootapapeshwar limited stockiest. A complete list of ingredients of the formulation is listed in Table 1.

The proposed analytical methods were validated as per the ICH guidelines in terms of linearity, precision, robustness, accuracy, specificity, LOD (Limit of Detection) and LOQ (Limit of Quantification) as detailed below:

Chemicals and reagents

All chemicals and reagents (Toluene, Ethyl acetate, Methanol, Formic acid, Hexane, hydrochloric acid etc.) used for analysis, were of analytical reagent grade and purchased from Merck. The certified reference standards (CRS), such as Piperine of purity 97% (CAS 94-62-2) & 6-Gingerol of purity 97.90 (CAS 23513-14-6) were procured from Sigma Aldrich & from Natural Remedies Private Limited respectively for HPTLC Analysis.

The certified reference standards of Zinc (Zn) of 100 ppm (Batch no- HC90909387) and Mercury (Hg) of 1000 ppm (Batch no-HC72675526) traceable to NIST SRM were procured from Merck for elemental Analysis.

Chromatographic conditions

Camag HPTLC instrument with sample applicator Linomat 5, Densitometer TLC scanner 4 and Wincat Software was used for quantification of phytochemicals by chromatographic analysis (HPLTC). The pre-coated silica gel G60- F254 aluminium plates (E. Merck, Germany) 20 x 10 mm, thickness layer 0.2 mm were used as stationary phase. The solvent systems toluene: ethyl acetate (7: 3) v/v for Piperine & Hexane : Ethyl acetate : Formic acid (4 : 6 : 0.1) v/v for 6-Gingerol were selected which gave good resolution. Twin trough chamber was used for development of HPTLC plates. Prior to study chamber was saturated with mobile phase for 30 minutes at room temperature at a relative humidity 38 ± 2. Chromatographic run was roughly 90 mm and distance between two tracks was set to 11.5 mm. Photo documentation cabinet fitted with high resolution camera was used for capturing images at different wavelengths. Densitometer TLC scanner 4 equipped with deuterium (D_2) and tungsten (W) lamps were used to obtain spectra. The wavelengths of 336 nm & 568 nm were used for quantification of Piperine & 6-Gingerol in the formulations.

ICP-OES instrumental conditions

For elemental analysis ICP-OES instrument (PerkinElmer Avio-200 model) was used in radial mode using conditions mentioned in Table 2. For sample preparation Mars-6 microwave digestion system (CEM Corporation, USA) was used and instrumental parameters are presented in Table 3.

Preparation of standard and sample solutions

Standard stock solution for HPTLC

Piperine standard
Standard stock solution of 97 ng/ul piperine was prepared by dissolving 1mg of standard in 10 ml of volumetric flask containing methanol. Prepared solution was further diluted with methanol to obtain final concentration of 9.7 ng/ul.

6-Gingerol standard
Standard stock solution of 97 ng/ul 6-Gingerol was prepared by dissolving 1mg of standard in 10 ml of volumetric flask containing methanol. Prepared solution was further diluted with methanol to obtain final concentration of 50 ng/ul.

Sample stock solution for HPTLC

LMV and TKR sample solution
Approximately 1 gram of each sample were refluxed with 25 ml of Methanol for 2 hours. It was then filtered through a sonar filter paper no.1 and volume to 25 ml with methanol.

Standard solution for ICP-OES

Zinc (Zn) standard
100 ppm standard stock solution of Zn is prepared in double D/W. From stock standard solution 0.5-10.0 ppm solutions were prepared in double D/W.

Mercury (Hg) standard
1000 ppm standard stock solution of Hg is prepared in double D/W. From stock standard solution 0.5-10.0 ppm solutions were prepared in double D/W.

These solutions were aspirated in the plasma for linearity, which were then used to find out the concentration of the Zn and Hg in samples.

Sample stock Solution for ICP-OES

Sample preparation was performed using microwave assisted acid digestion. Accurately 100 mg of sample LMV and 100 mg of TKR were pre-digested with 10 mL conc. HCl and 10 mL Aquaria respectively. After digestion samples were diluted with double D/W to make final volume to 250 mL for LMV and 100 mL for TKR. These samples were aspirated on ICP-OES to get the ppm concentration and the mg/tablet concentration as follows:

$$\text{mg/tab (w/w) in sample} = \frac{\text{Concentration of sample (ppm) x Dilution factor x average wt.}}{\text{1000 x Weight of sample in mg}}$$

Method validation

The analytical methods were validated for Linearity, precision, accuracy, LOD and LOQ as per International Conference on Harmonization (ICH, 2005) guidelines Q_2 (R1) and USP <1225>.

For HPTLC

To determine linearity range of standard Piperine and 6-Gingerol, a series of spots of different volumes (1,2,3,4,5 uL) were applied to get appropriate quantity of standard per band. To determine linearity range different concentration of each of analytes Vs Peak area were observed in the range of 9.7-67.9 ng/spot for Piperine and 48.95-220.27 ng/spot for 6-Gingerol. The standard deviation (SD), Coefficient of variation (r), slope and intercept of calibration curves were used to determine linearity. Quantification of marker in samples were carried out by calibration curve.

Limit of detection (LOD) and Limit of quantification (LOQ) were calculated from standard deviation (SD) of intercept and Slope (S) of the calibration curve. For HPTLC, LOD were calculated from formula (LOD = 3.3*(SD/S)) and LOQ were calculated from (LOQ = 10* (SD/S)). The precision study was validated for repeatability, intra-day, inter-day and for different analyst. For repeatability approximately 1.0 gram of sample extracted and applied 10 times. Intra-day studies were carried out by taking approximately 1.0 gram of sample. Which then extracted and each prepared sample was applied three times and three different times in a day. For Inter-day precision same procedure is followed for three different days. Precision studies for different analysts were also performed with same procedure by three different analysts. For precision study %RSD was reported. Accuracy by recovery studies were carried out by spiking known concentration of standard to pre-analyzed samples. Each prepared sample was applied three times. The accuracy was calculated from following equation:

$$\% \text{ Recovery} = \frac{\text{Spiked concentration} - \text{Mean concentration}}{\text{Spiked concentration}} \text{ x } 100$$

For ICP-OES

Prepared standard stock solutions was diluted to different concentrations to obtain linearity. To determine linearity range different concentration of each of analytes Vs intensity were observed in the range of 0.25-1.5 ppm for Zn and 5.0-100.0 ppm for Hg. The standard deviation (SD), coefficient of variation (r), slope and intercept of calibration curves were used to determine linearity. Quantification of marker in samples were carried out by calibration curve.

Limit of detection (LOD) was calculated as three times the standard deviation on the measured concentrations for ten replicate blank samples. Limit of quantification (LOQ) was calculated as ten times the standard deviation on the measured concentrations for ten replicate blank samples. The precision study was validated for repeatability, intraday, inter-day and for different analyst. For repeatability approximately prepared sample applied for 10 times. Intra-day studies were carried out by taking prepared sample three times and three different times in a day. For Inter-day precision same procedure is followed for three different days. Precision studied for different analysts were also performed with same procedure by three different analysts. For precision study %RSD was reported. Accuracy by recovery studies were carried out by spiking known concentration of standard to pre-analyzed samples. Each prepared sample was applied three times and accuracy was calculated.

Table 1: Formulations with their ingredients

Laghumalini Vasant (LMV)		**Tribhuvankirti Rasa (TKR)**	
Ref: Bharat Bhaishajya Ratnakar 4/6972		Ref: Bharat Bhaishajya Ratnakar 2/2755	
Jasad Bhasma	10 parts	Shodhita Hingula	1 Part
Maricha	5 Parts	Shodhita Vatsanabha	2 Part
Navneet	1Part	Shodhita Tankan	3 Part
Processed in		Shunthi	4 Part
Nimbu Swarasa	q. s.	Maricha	5 Part
		Pippali	6 Part
		Pippalimula	7 Part
		Processed in	
		Adraka swarasa	q. s.
		Tulasi Patra Swarasa	q. s.
		Dhatturapatra Swarasa	q. s.

Table 2: Analytical parameters of the ICP-OES instrument (avio-200, Perkin Elmer)

Analytical parameters for ICP-OES	
RF Power (kW)	1300
Nebulizer gas flow rate (L/min)	0.6
Auxiliary gas flow rate (L/min)	0.2
Plasma gas flow rate (L/min)	10
Sample Flow (mL/min)	1
Pump Tubing Diameter (in)	0.045
Plasma Viewing Height (mm)	15
Plasma Viewing	Radial
Measurement Processing Mode	Peak Area
Background Correction	Manual Selection of Points- 2 points
Calibration equation	Linear Thru Calculated Intercept
Read Delay (sec)	30
Rinse Delay (sec)	30
Replicates	3
Wavelength (nm)	Zn = 213.857, Hg = 253.652

Table 3: Operating conditions for the microwave oven digestion (MARS6, CEM)

Sample	**Step**	**Power (W)**	**Pressure (psi)**	**Temperature (˚C)**	**Ramp time (min)**	**Hold time (min)**	**Cooling to 0W time (min)**
LMV	1	600	650	190	20	15	15
TKR	2	600	650	170	20	10	15

Table 4: Method validation parameters for the quantification of Piperine in LMV and TKR and 6-Gingerol in TKR.

Parameters	Results		
	Laghumalini Vasant	Tribhuvankirti Rasa	
	Piperine	Piperine	6-Gingerol
Wavelength (nm)	336	336	568
Retention factor (Rf)	0.25 ± 0.02	0.25 ± 0.02	0.47 ± 0.02
Linearity range (ppm)	9.70-67.90	9.70-67.90	48.95-220.27
Correlation coefficient (r)	0.9997	0.9997	0.9996
Slope	72.33	72.33	19.429
Intercept	246.61	246.61	363.2
SD of intercept	83.82	83.82	102.89
LOD (ng/spot)	3.82	3.82	17.48
LOQ (ng/spot)	11.59	11.59	52.96

Table 5: Intra-day and inter-day precision by HPTLC for LMV and TKR

Marker	Analyte	Amount of Sample (g)	Amount of drug detected (ng)	RSD (%)
Piperine	Laghumalini Vasant (LMV)	Intra-day precision (n = 3)		
		1.0215	37.02	0.019
		1.0001	33.59	0.017
		1.0023	32.28	0.005
		Inter-day precision (n = 3)		
		1.0001	33.14	0.012
		1.0005	32.97	0.017
		1.0025	32.5	0.016
Piperine	Tribhuvankirti Rasa (TKR)	Intra-day precision (n = 3)		
		1.1396	39.29	0.02
		1.0894	37.55	0.015
		1.0832	38.3	0.009
		Inter-day precision		
		1.0563	37.27	0.003
		1.0906	37.99	0.014
		1.1398	37.84	0.016
6-Gingerol	Tribhuvankirti Rasa (TKR)	Intra-day precision (n = 3)		
		1.0927	59.58	0.016
		1.0085	60.82	0.016
		1.0396	59.93	0.022
		Inter-day precision (n = 3)		
		1.0384	61.99	0.023
		1.0396	58.93	0.01
		1.0012	58.7	0.009

Table 6: Repeatability precision for LMV and TKR by HPTLC

Analyte	Amount of Sample (g)	Amount of drug detected (ng)	RSD (%)
Piperine in LMV	1.034	38.32	0.02
Piperine in TKR	1.0384	36.62	0.021
6-Gingerol in TKR	1.0384	59.35	0.018

Table 7: Different analyst precision for LMV and TKR by HPTLC

Analyte	Analyst	Amount of Sample (g)	Amount of drug detected (ng)	RSD (%)
Piperine in LMV	Analyst 1	1.0314	36.13	0.012
	Analyst 2	1.0003	35.27	0.013
	Analyst 3	1.0021	34.75	0.014
Piperine in TKR	Analyst 1	1.0020	28.23	0.004
	Analyst 2	1.0000	27.2	0.010
	Analyst 3	1.0002	28.83	0.014
6-Gingerol in TKR	Analyst 1	1.0025	58.26	0.016
	Analyst 2	1.0000	60.43	0.016
	Analyst 3	1.0020	60.77	0.017

Table 8: Accuracy studies of Piperine and 6-gingerol by proposed HPTLC method

Product	Amount of drug analyzed (ng)	Amount of drug added (ng)	Theoretical concentration (ng)	Amount of drug found (ng)	Recovery (%)	Mean Recover (%)
Piperine in LMV	37.66	30.13	67.79	73.58	108.54	100.02
	37.66	37.66	75.32	72.65	96.46	
	37.66	45.19	82.85	78.75	95.05	
Piperine in TKR	18.13	14.5	32.63	33.89	103.86	101.85
	18.13	18.13	36.26	35.51	97.93	
	18.13	21.76	39.89	41.39	103.76	
6-Gingerol in TKR	32.58	32.58	65.16	64.33	98.73	99.88
	32.58	39.06	71.64	72.09	100.63	
	32.58	45.6	78.18	78.39	100.27	

Table 9: Statistical analysis from Linearity curve for Zn and Hg

Parameters	Results	
	Zn	Hg
Wavelength (nm)	213	253
Linearity range (ppm)	0.25-1.5	5.0-100
Correlation coefficient (r)	0.9999	0.9992
Slope	95668	1176.3
Intercept	1356.3	5529.7

Table 10: Intra-day and inter-day precision by ICP-OES for LMV and TKR

Element	Analyte	Amount of Sample (g)	Amount of element detected (w/w)	RSD (%)
Zn	Laghumalini Vasant (LMV)	Intra-day precision (n = 3)		
		0.1386	31.27	0.10
		0.1476	31.31	0.10
		0.1533	31.28	0.10
		Inter-day precision (n = 3)		
		0.1355	31.29	0.09
		0.1243	31.19	0.40
		0.1138	31.30	0.14
Hg	Tribhuvankirti Rasa (TKR)	Intra-day precision (n = 3)		
		0.1009	8.58	0.24
		0.1011	8.58	0.35
		0.1029	8.57	0.31
		Inter-day precision (n = 3)		
		0.1004	8.7	0.4
		0.1001	8.5	0.73
			8.54	0.59

Table 11: Repeatability precision for LMV and TKR by ICP-OES

	Amount of Sample (g)	Amount of drug detected (w/w%)	RSD (%)
Zn in LMV	0.0865	31.31	0.43
Hg in TKR	0.0978	8.48	1.51

Table 12: Different analyst precision for LMV and TKR by ICP-OES

Element	Analyst	Amount of Sample (g)	Amount of drug detected (w/w%)	RSD (%)
		(n = 3)		
Zn in LMV	Analyst 1	0.1386	31.27	0.10
	Analyst 2	0.1476	31.31	0.10
	Analyst 3	0.1533	31.28	0.10
Hg in TKR	Analyst 1	0.1009	8.58	0.24
	Analyst 2	0.1011	8.58	0.35
	Analyst 3	0.1029	8.57	0.31

Table 13: Accuracy studies of Zn and Hg by proposed ICP-OES method

Element	Amount of drug analyzed (w/w%)	Amount of drug added (w/w%)	Theoretical concentration (w/w%)	Amount of drug found (w/w%)	Recovery (%)	Mean Recover (%)
Zn in LMV	31.25	5.26	36.51	37.58	102.93	102.15
	31.25	7.5	38.75	39.16	101.06	
	31.25	9.15	40.40	41.4	102.48	
Hg in TKR	8.51	1.00	9.51	9.53	100.21	98.76
	8.51	2.00	10.51	10.39	98.86	
	8.51	3.00	11.51	11.19	97.22	

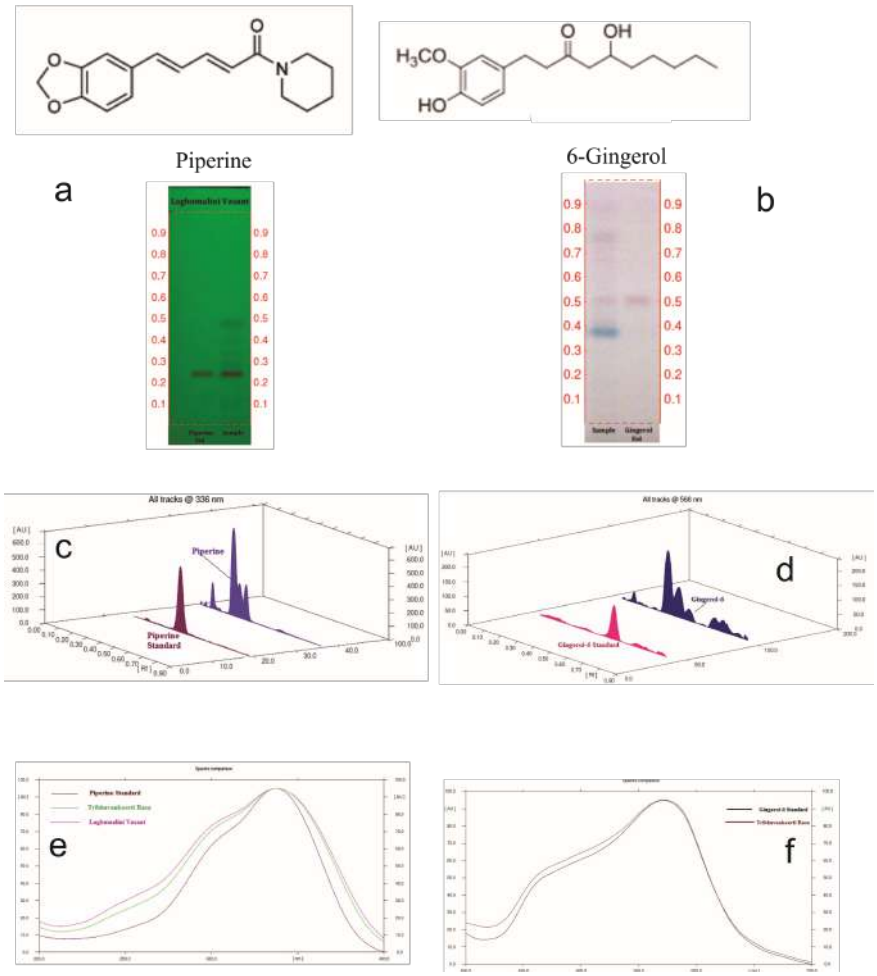

Figure 1 a. HPTLC plate for Piperine std and sample, b. HPTLC plate for 6-Gingerol standard and sample, c. densitogram of Piperine std and sample, d. densitogram of 6-Gingerol standard and sample, e. the spectral index for piperine standard and samples, f. spectral index for Gingerol-6 standard and samples

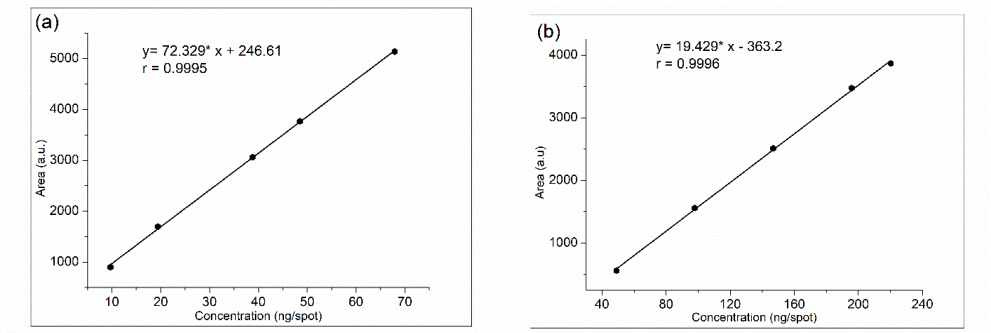

Figure 2: Calibration curve for (a) Piperine standard and (b) 6-Gingerol standard

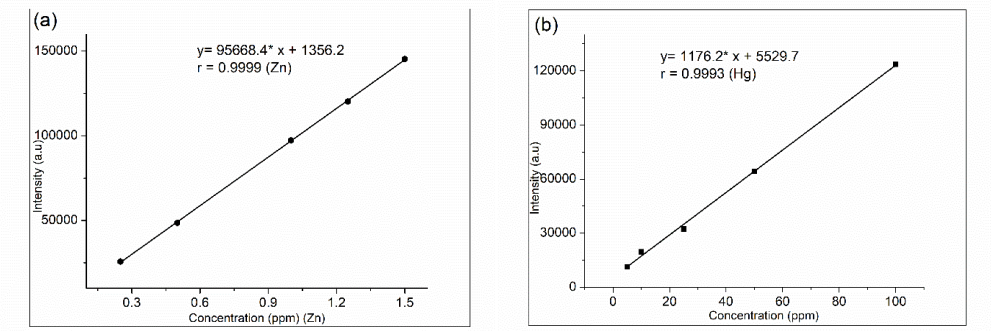

Figure 3: Calibration curve for (a) Zn standard and (b) Hg standard

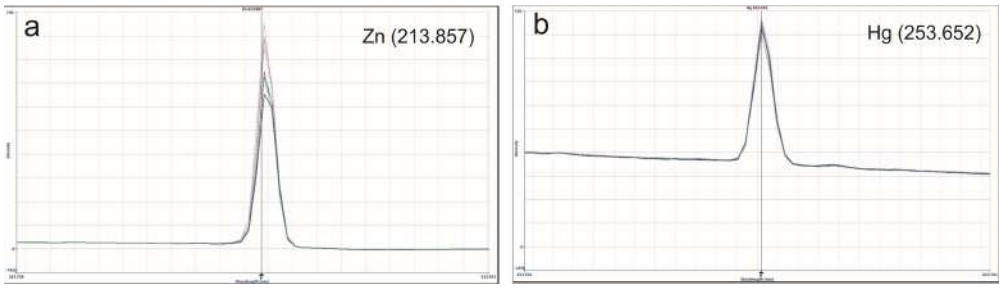

Figure 4: Intensity of ten replicates of a. Laghumalini Vasant for Zn and b. Tribhuvankirti Rasa for Hg

RESULT AND DISCUSSION

Method validation by HPTLC

Standard solutions of Piperine and 6-Gingerol are analysed by HPTLC method. HPTLC analysis of methanol extract of Laghumalini Vasant was carried out along Piperine reference standard and Tribhuvankirti Rasa was carried out along Piperine and 6-Gingerol reference standard. The solvent systems toluene: ethyl acetate (7: 3) v/v for Piperine & Hexane: Ethyl acetate: Formic acid (4 : 6 : 0.1) v/v for 6-Gingerol were optimized. The selected mobile phase composition showed good resolution under UV-254 and after spray with anisaldehyde sulfuric acid reagent. The developed densitogram showed characteristic peak corresponding Piperine at the R_f 0.25 ± 0.02, and 6-Gingerol at the R_f 0.47 ± 0.02 (Figure 1.a and 1.b). Chromatogram and 3D spectral display are presented in Figure 1.c. 1. d and Figure 1.e and 1.f. The spectral index confirmed that the peak obtained was that of Piperine at wavelength 336 nm and of 6-Gingerol at wavelength 568 nm (Figures 1.e and 1.f). In Tribhuvankirti Rasa formulation dried Shunthi powder was used, which is source of 6-Gingerol.

The chamber saturation time was optimized to 30 min at room temperature with relative humidity 38 ± 2%. Chromatographic run was roughly 90 mm and distance between two tracks was set to 11 mm. The optimized chromatographic conditions are given in Table 4.

Linearity

Different concentrations of standards were analyzed to get linearity for present method. Under optimized chromatographic conditions peak areas for corresponding standard were found to be proportional to concentrations of Piperine and 6-Gingerol (Figure 2). The statistical analysis of the linearity graph such as linearity range, correlation coefficient, slope, intercept, SD of intercept, LOD and LOQ were presented in Table 4. The linearity graphs are presented in Figure 2. The limit of detection (LOD) estimated for Piperine and 6-Gingerol was 3.82 ng/spot and 17.48 ng/spot respectively and limit of quantification (LOQ) for Piperine and 6-Gingerol was reported as 11.59 ng/spot and 52.96 ng/spot respectively.

Precision

Precision studies were carried out to show reproducibility of the method and presented in supporting data supporting Table 5-7. Both intra-day and inter-day were also calculated for each phytoconstituent. Precision studied for different analysts were also presented in Table 3. % RSD for all parameters were below 2% for Piperine and 6-Gingerol, which shows the proposed method has high level of precision (supporting data Table 5-7).

Accuracy (Recovery studied)

Accuracy studies were performed in acceptable range of concentrations in triplicate and presented in supporting data Table 8. The proposed method has recovery in-between ±10% which demonstrate ruggedness of proposed method too.

Specificity

The peak purity of Piperine and 6-Gingerol were assessed by comparing the spectra and Rf values and the overlay spectra were presented in Figure 1.e and Figure 1.f.

Method validation by ICP-OES

The developed analytical method was validated according to the same parameters as described in USP chapter <233> Elemental Impurities – Procedures. ICP-OES analysis of Laghumalini Vasant was carried out along Zn reference standard and Tribhuvankirti Rasa was carried out along Hg reference standard. (Table 9)

Linearity

Different concentrations of standard were analyzed to get linearity for present method. Under optimized conditions parameters the statistical analysis of the linearity graph such as linearity range, correlation coefficient, slope, intercept was presented in Table 9. The linearity graphs are presented in Figure 3.a and 3.b. We found r is 0.9999 for Zn and 0.9996 for Hg.

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) estimated for Zn and Hg was 0.0015 ppm and 0.34 ppm respectively and limit of quantification (LOQ) for Zn and Hg was 0.005 ppm and 1.14 ppm respectively.

Precision

Precision studies were carried out to show reproducibility of the method and presented in supporting Table 10-12. Both intra-day and inter-day were also calculated for Zn in LMV and Hg in TKR. Precision studied for different analysts were also presented in Table 3. % RSD for all parameters were below 2% for Zn and Hg, which shows the proposed method is very of précised (supporting Table 10-12). Repeatability of the proposed method is validated and intensity peaks of each sample overlays with each other very well (Figure 4).

Accuracy (Recovery studied)

Accuracy studies were performed in acceptable range of concentrations in triplicate and presented in supporting Table 13.

The proposed method has recovery in-between ±10% which demonstrate ruggedness of proposed method too.

CONCLUSION

COVID-19 is global pandemic, causing large number of deaths even in scientifically advanced countries like USA. There is no proper treatment for COVID-19 as of now due to unavailability of specific drug. Hence, there is a scope for Ayurvedic medicines for treatment of Covid-19 disease. Therefore, this paper focused on phytochemical and elemental profiling of some herbal medicine used during Covid-19, such as Laghumalini Vasant (LMV) and Tribhuvankirti Rasa (TKR). Major phytoconstituent of LMV is piperine and TKR is Piperine and 6-Gingerol. These phytoconstituent due to its antiviral, immune-modulatory, antioxidant, anti-inflammatory, anti-fungal, properties; seems to be effective in boosting immunity for the anticipation and reduction of viral disease complications. HPTLC study confirms the presence of Piperine and 6-Gingerol in TKR and Piperine in LMV. ICP-OES demonstrates presence of Hg in TKR and Zn in LMV. Both, HPTLC and ICP-OES methods were validated successfully.

ACKNOWLEDGMENT

We are thankful to Shree Dhootapapeshwar Ltd., for support throughout the work.

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Cite this article as:

Priyanka Patil *et al*. Phytochemical and Elemental Profiling and Standardization of Some Ayurveda Medicines used in COVID-19 Pandemic. Int. J. Res. Ayurveda Pharm. 2021;12(4):76-83
http://dx.doi.org/10.7897/2277-4343.1204110

Source of support: Nil, Conflict of interest: None Declared