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Development of Chromatographic Profile of Processed and non-processed *Piper nigrum* Linn.

Research Article

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Abstract

Advanced Chromatographic technique is one of the parameters for qualitative and quantitative assessment. In this study advanced chromatographic studies were conducted for the assessment of processed and non-processed *Piper nigrum* Linn. Ayurvedic pharmaceutical is enriched in a wide range of preparations. The specific methods are applied for different formulation preparations. *Laghumalini Vasant* is a herbomineral formulation in which one of the ingredients is *P. nigrum*. In this formulation, the special process is advocated for *P. nigrum*. *P. nigrum* should be soaked in buttermilk till the outer black covering is removed. There are possibilities that some changes may occur due to this process. Hence this study focused on the comparative assessment of processed *P. nigrum* and non-processed *P. nigrum*. For assessment chromatography techniques were used. HPLC and HPTLC methods were used to get more precise result**s.** High-Performance Thin Layer and High performance Liquid Chromatography were studied by using piperine as a reference standard. The piperine bands were seen in both processed and non-processed *P. nigrum* when analysed through both techniques. But, HPLC method has shown that piperine recovery is more in processed *P. nigrum*. In non-processed *P. nigrum* twenty seven peaks were observed but after processing with Buttermilk twenty one peaks were seen. The small six peaks were missing in processed *P. nigrum.*

Keywords: *HPTLC, HPLC, Unprocessed P. nigrum, Processed P. nigrum, Buttermilk*.

Introduction

The chromatographic study is useful in qualitative and quantitative analysis of herbs (1). This article is related to the chemical analysis of Unprocessed *P. nigrum* and the chemical changes that occurred in processed *P. nigrum*. Under the system of Ayurveda, there are many herbal and herbomineral formulations that are mentioned as antioxidants and immunomodulatory (2). One of the formulations mentioned is *Laghumalini Vasant* (LMV). *Laghu Malini Vasant* (LMV) is a zinc formulation mentioned in *Yogartnakar*, consisting of zinc carbonate and *P. nigrum* Linn as the basic ingredients.In the formulation, it is advocated to use processed *P. nigrum* (3). For the processing, *P. nigrum* was soaked in buttermilk (*Takra*) till the outer covering is removed. The chemical changes that occurred were assessed by HPLC and HPTLC techniques in both samples. This research article is an effort to explore the chemical changes that occurred in *P. nigrum* after processing with buttermilk by utilizing HPLC and HPTLC techniques.

Rasashastra and Bhaishajya Kalpana is an important branch of Ayurveda that deals with medicine manufacturing. In medicine manufacturing quality assurance and quality control plays an important part (4). However, in-process quality control (IPQC) plays an important role in the outcome of the product. Advanced analytical techniques help in the quality assurance of the raw products and finished formulations also (5). For assuring IPQC different parameters can be performed. In herbomineral formulations, many herbs are added. In *Laghumalini Vasant* (LMV) the processed P. *nigrum* should be chemically analyzed to assess chemical variations. LMV is useful to treat fever and associated diarrhea. It is said that the formulation is safe and efficacious in pregnant women and the pediatric age group also (6,7). The processed *P. nigrum* is having an important role in this formulation. Hence, the finger printing of *P. nigrum* is an important aspect to be studied.

The assurance of the safety, quality, and efficacy of medicinal plants and herbal products is turning into a vital issue as the demand for and commercial value of herbal medicines is rising dramatically. Chromatographic techniques in herbal elements play an important part. (8).

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The standardization of herbal raw materials and herbal formulations calls for the development of a systematic approach and well-designed techniques. All factors affecting the quality of herbal drugs should be taken into account when standardization techniques are used. Herbal medicines are quite variable because they are made up of numerous components. Therefore, obtaining trustworthy chromatographic fingerprints that represent the pharmacologically active and chemically distinctive components of herbal medicine is crucial (9, 10). Here, in LMV multiple processes are involved to obtain the final product. The stepwise involvement of proper processes has significance. However, the omission of one of the processes may result in the alteration of the properties. In LMV the processing of *P. nigrum* is involved. Here, the *P. nigrum* is required to be assessed to get the difference between raw and processed *P. nigrum* for chromatographic fingerprinting is an important tool.

The chromatography tool is used as an advanced analytical technique. HPLC, HPTLC and GC (Gas chromatography) are widely used in herbal pharmaceutical industries. These techniques are suitable for quantitative determinations of various bioactive components. For the qualitative estimation of formulation or single herb containing volatile principle, GC is recommended (11).

Materials and methods

In this study**,** *P. nigrum* was purchased from Shri Shail Pharma, Nagpur, Maharashtra. The market sample of the processed *P. nigrum* in the buttermilk was considered as *Shweta* Maricha. The specimens were preserved in the quality assurance lab of Dattatraya Ayurved Rasashala, Wardha, Maharashtra. The buttermilk required for the processing was used of Amul Company.

The raw *P. nigrum* was dried properly and after proper weighing, it was preserved. The sample was considered as non processed sample (*Maricha)*. The 100g of the raw was taken for processing and was soaked in buttermilk. The soaked *P. nigrum* was rubbed thoroughly and washed with water. Again the *P. nigrum* was soaked in Buttermilk. The process was repeated till the outer covering is removed. Every day the fresh buttermilk was used for soaking. It took seven days to get the *Shweta Maricha* (white *P. nigrum*) from raw *P. nigrum.*

In this study, the HPTLC and HPLC techniques were performed. Marketed samples as Raw is denoted as *Maricha* Processed sample purchased from the market is considered as *Shweta Marich* and the processing done in the laboratory with buttermilk is coded as *Takra Maricha*. All the samples were also compared by using Piperine as a reference standard.

For HPTLC the chemicals Toulene and Ethyl acetate were used and purchased from Merck (Mumbai, India). HPTLC analysis was performed on CAMAG TLC Scanner "Scanner_181112" S/N 181112 (2.01.02) equipped with winCATS Planar Chromatography Manager. CAMAG Linomat 5 “Linomat 5_180945" S/ N 180945 (1.00.13) Twin Trough Chamber 10x10cm with HPTLC plates silica gel 60 F 25. Including these, analytical balance (ME-205, Mettler-Toledo), pH meter (FiveEasy-A211, Mettler-Toledo), and ultra-sonicator (Labman®) were used throughout the HPTLC analysis. Linomat 5 application parameters Spray gas was used Inert gas Sample solvent type with methanol Dosage speed is 150 nl/s Pre dosage volume of 0.2 ul.

Accurately weighed each 300 mg of samples were taken in the 50 ml Toulene: Ethyl acetate in the concentration of 7:3.volumetric flasks and kept in the mobile phase for 24 hours overnight at 28˚C temperature. The solution was shaken intermediately. Furthermore, the sample was ultra-sonicated for 10 minutes and then filtered through 0.20µ nylon filters. The filtrate was collected in the fresh vial and used for HPTLC. The silica gel plate 60 F 254 manufactured by E. MERCK KGaA was used as the Stationary phase. The extracted solvent was spotted on the stationary phase by using linomat. The mobile phase used was Toulene: Ethyl acetate in the concentration of 7:3.The stationary phase spotted with extract was placed in mobile phase. The result was recorded at 254 and 366 nm.

Similarly for HPLC profile of raw and processed *P. nigrum*. HPLC grade chemicals and solvents including deionized water, methanol and acetonitrile and absolute ethanol were used and purchased from Merck (Mumbai, India). HPLC columns UltraSil-RP (150 x 4.6 mm. id, Particle size 5µ) were used throughout the analysis and purchased from UltraChrom Innovatives Pvt. Ltd. (Wardha, India). HPLC analysis was performed on Shimadzu Class A-10 VP instrument, equipped with UV-Vis detector (SPD-10A VP), binary pumps (LC-10AT VP), the system controller (SCL-10A VP) with manual rheodyne injector (20µl) which is moderated by LC-solution software. Including these, analytical balance (ME-205, Mettler-Toledo), pH meter (FiveEasy-A211, Mettler-Toledo), and ultra-sonicator (Labman®) were used throughout the HPLC analysis.

Accurately weighed each 300 mg of samples were transferred to the 50 ml volumetric flasks. All samples individually were mixed with water-methanol-acetonitrile (1:2:2, v/v) and kept for 24 hours overnight at 28˚C temperature. After 24 hours the sample was heated in the water bath to accelerate the release rate of all vital components (decoction method). Furthermore, sample was cooled to prevent from evaporation of solvent and then it was ultra-sonicated for 10 minutes and then filtered through 0.20µ nylon filters. The filtrate was collected in fresh vial and analysed by HPLC-UV technique. 20µL of freshly derived sample extract was injected into the UltraSil-RP column and eluted at the flow rate of 1ml/minutes using the eluents; 10mM ammonium acetate (A) and acetonitrile (B) by gradient elution technique of programming 0-5 mins, 90% A; 5-30 mins, 40% A; 30-70 mins, 20%A and finally it was re-equilibrated with initial gradient. HPLC-UV separation was carried out at 28˚C and recorded at 210 and 254 nm wavelength.

Observation and Results

This research work revealed chemical evaluation with HPTLC and HPLC techniques .This may be beneficial in quality control and therapeutic use of processed *P. nigrum*. This research work is beneficial to detect chemical changes after processing of *P. nigrum* with HPTLC and HPLC techniques both.

Based on chromatographic study, the quantitative estimation of Piperine was done along with alteration in the recovery assessed. Also, the peaks related to the study were compared with processed and non-processed *P. nigrum.* The HPTLC analysis of all three samples along with standards are mentioned in Figures No. 1 to 4 and in Table no. 1

HPLC analysis study is shown in Table no. 2 to 5 and Figure No. 5.

Table 1: HPTLC chromatography, Rf with regression

Track	Sample ID	Application volume	Starting Rf	Maximum Rf	Regression
1	Piperine (standard)	2 µl	0.36	0.43	Polynomial
2	*Maricha*	2 µl	0.36	0.43	Polynomial
3	*Takra Maricha*	2 µl	0.36	0.43	Polynomial
4	*Shweta Maricha*	2 µl	0.36	0.43	Polynomial

Table 2: HPLC pattern of Piperine – Standard

Peak#	Retention. Time (Ret. time)	Area	Height	Area%	T.Plate#	Resolution	k'
1	1.884	13970	1766	0.1031	3663.887	--	0
2	2.103	24300	5748	0.1793	5498.773	1.835	0.116
3	41.267	177782	6244	1.3118	54550.67	95.504	20.901
Piperine	43.873	13336012	413192	98.4058	53744.06	3.561	22.284

Table 3: HPLC pattern of Raw Black Maricha

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'
1	2.175	215619	25387	0.1555	1091.951	--	0
2	2.317	348657	23964	0.2514	829.855	0.484	0.065
3	17.618	151315	4240	0.1091	6335.434	25.353	7.1
4	26.378	336922	9771	0.2429	14548.78	9.953	11.127
5	28.708	1111775	36342	0.8016	30511.18	3.042	12.198
6	31.575	488520	12926	0.3522	22719.49	3.834	13.516
7	33.179	534070	18599	0.3851	36260.24	2.09	14.253
8	36.833	871291	37434	0.6282	8073.923	3.128	15.933
9	37.271	2097996	63295	1.5126	28543.71	0.347	16.135
10	39.016	119342	4684	0.086	60173.42	2.299	16.937
11	40.982	13342946	427794	9.62	50571.09	2.879	17.841
12	43.494	78093282	1123088	56.3039	11714.25	2.151	18.996
13	46.631	1878538	56863	1.3544	51560.75	2.583	20.438
14	48.003	9367259	377733	6.7536	60580.79	1.714	21.069
15	48.643	27071535	811866	19.5181	59785.94	0.812	21.363
16	50.215	139522	6511	0.1006	84957.78	2.118	22.086
17	50.585	104813	4848	0.0756	33396.01	0.412	22.256
18	52.483	150236	5796	0.1083	90383.29	2.102	23.128
19	53.801	273251	11891	0.197	140620.7	2.072	23.734
20	55.302	253362	10410	0.1827	121993.3	2.487	24.424
21	57.069	734337	32754	0.5294	164128.3	2.953	25.237
22	58.472	296292	12542	0.2136	184666	2.532	25.881
23	59.759	213677	10013	0.1541	153966.8	2.232	26.473
24	60.737	89226	5152	0.0643	250149.1	1.787	26.923
25	64.497	160473	7443	0.1157	194073.5	7.019	28.651
26	66.974	81281	5489	0.0586	14120.59	1.745	29.79
27	67.288	174060	6954	0.1255	78933.39	0.195	29.935

Table 4: HPLC pattern of *Shweta Maricha* (market sample)

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'
1	1.894	58985	8032	0.044	1146.277	--	0
2	2.183	108303	16688	0.0808	1931.926	1.365	0.152
3	2.327	303243	21151	0.2262	788.003	0.543	0.228
4	17.517	333923	8179	0.2491	5184.779	23.287	8.247
5	26.26	736118	20390	0.5491	13859.92	9.375	12.863
6	28.097	228266	9397	0.1703	3548.012	1.322	13.833
7	28.583	663588	24639	0.495	27223.98	0.377	14.089
8	33.066	464423	16558	0.3464	34992.09	6.404	16.455
9	36.736	661918	24307	0.4937	9488.741	3.314	18.393
10	37.205	1170946	31943	0.8734	23070.26	0.377	18.641
11	40.909	12991992	401553	9.6908	46525.39	4.261	20.596
Piperine	43.461	74745064	1120950	55.7525	16812.29	2.432	21.943
13	46.593	2537794	54485	1.8929	18336.23	2.306	23.597
14	47.978	9564361	371938	7.1341	33443.45	1.142	24.328
15	48.585	27679472	781910	20.6462	49915.01	0.632	24.648
16	53.751	193408	7959	0.1443	133105.1	7.081	27.375
17	55.246	245315	9972	0.183	120938.7	2.442	28.165
18	57.013	646314	28994	0.4821	161277.8	2.936	29.097
19	58.352	262036	10445	0.1955	163326.3	2.339	29.804
20	59.61	170045	8439	0.1268	187115	2.228	30.468
21	66.982	300277	7643	0.224	60873.76	9.006	34.36

Table 5: HPLC pattern of *Takra Maricha* processed in butter milk in the lab

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	k'
1	1.863	103332	8315	0.0729	589.092	0
2	2.195	109125	17101	0.077	2075.022	0.178
3	2.345	262060	20691	0.1848	886.428	0.259
4	17.543	42651	1238	0.0301	11250.01	8.417
5	25.858	194	84	0.0001	2238322	12.88
6	28.455	1422159	37347	1.0031	21591.97	14.275
7	31.416	157289	4938	0.1109	25636.49	15.864
8	32.901	372959	13328	0.2631	32030.42	16.661
9	34.962	32795	1673	0.0231	67751.98	17.767
10	36.928	3170004	76399	2.2359	28340.2	18.823
11	38.675	161110	6374	0.1136	57994.96	19.76
12	40.621	10738232	328069	7.5741	45101.49	20.805
Piperine	43.052	81535447	1125653	57.5099	11017.74	22.11
14	46.1	2699634	59845	1.9041	21781.42	23.746
15	47.452	7614137	317752	5.3705	20205.32	24.472
16	48.032	32218052	881576	22.7246	47632.21	24.783
17	51.823	186044	6482	0.1312	79406.41	26.818
18	53.082	149418	6476	0.1054	125874.2	27.494
19	54.577	250357	10585	0.1766	108096	28.297
20	56.305	345839	14925	0.2439	147461.8	29.224
21	57.746	66101	3484	0.0466	209349.1	29.998
22	58.998	139438	6747	0.0984	190034	30.669

In HPLC and HPTLC study piperine was considered as a standard and it was observed in all three samples of *P. nigrum* which were Raw *P. nigrum* , Lab Processed *P. nigrum* and a market sample of processed *P. nigrum*. The maximum Rf value found in all three samples is the same as that of the standard which is 43 and 44. The polynomial regression was observed in all the samples when going through HPTLC graphical representation considering time and area.

In the HPLC study the retention time observed in all three samples (Raw *P. nigrum* , lab Processed *P. nigrum* and market sample of processed *P. nigrum*) was found to be approximately the same. The retention time of standard, Piperine was 43.873. However, the retention time of Raw *P. nigrum* , lab Processed *P. nigrum* and a market sample of processed *P. nigrum was* 43.494,43.052,43.461 respectively. The peaks observed in Raw *P. nigrum* are 27 in number, but the peaks available in, lab Processed *P. nigrum* and a market sample of processed *P. nigrum* are 22 and 21 respectively. Through HPLC the recovery of Piperine observed is more in the lab Processed *P. nigrum* as shown in figure No.6.

Discussion

Rasashastra and Bhaishajya kalpana deal with pharmaceutical preparation which should be carried out with vigilance (12, 13). The stepwise mechanism mentioned in the text is to be carried out genuinely to obtain the best therapeutic outcome in terms of patient compliance. The processed *P. nigrum* is to be used while the preparation of LMV. LMV is indicated in *Garbhini Jwar and Atisar* (fever in ANC condition and diarrhea associated). It is also said that LMV can be safely used in neonates and children to treat diarrhea and fever (14, 15).*P. nigrum* the herb used is said to be used with processing only. However, in most of the Ayurvedic formulations, the *P. nigrum* is used as one of the ingredients (16.17, 18) without any processing**.** The chemical variations need to be observed within the processed and non-processed *P. nigrum* . In HPLTC chromatographical study the standard and all three samples were analyzed. The Rf value was observed in the range of 43-44 in all the samples of *P. nigrum.* That was matching with standard, piperine.

HPLC chromatograph was used to get more précised and focused results. The retention time in all the samples of *P. nigrum* was compared with the standard that was within the range of 43.052- 43.873. This study shows that piperine is present in all three samples of *P. nigrum.* The peaks observed in Raw *P. nigrum* were 27, however after processing the peaks reduced to 21. This shows that the buttermilk processing of *P. nigrum* results in the removal of some bioactive components. These bioactive substances were not identified. But, it may be possible that these constituents may be not desired for pregnant lady as well as in children and neonates. The therapeutic implications of this need to be validated. Apart from this, it was observed that the recovery of Piperine was more in Laboratory processed *P. nigrum* than in the raw sample but it was less in the market sample of processed *P. nigrum.* As per Ayurveda Raw, *P. nigrum* is very hot in potency hence, due to this process hot potency may be reduced as the process results in alteration of the property (19). The loss of peaks in processed *P. nigrum* may be indicating the same. This study contributes to the development of a standard of butter milk processed *P. nigrum*. The fingerprinting of the butter milk processed *P.Nigrum* is developed with this study. The LMV is one of the important formulations indicated in many ailments related to antenatal care. P. nigrum is considered a *Pramathi* (which clears the channel) (20). Hence, in febrile conditions the *P.Nigrum* is needed but, its hot potency should be reduced while recommendation in pregnant and pediatric age group. This may be the reason for processing the *P.Nigrum* with buttermilk.

Conclusion

In HPTLC, all three samples were compared with piperine and the Rf value observed in all the samples were similar, indicating qualitative estimation of Piperine. The HPTLC study reveals the polynomial regression. The Rf value was within the range of 0.43-0.44 in all three samples. The standard deviation was 3.73.

In HPLC the recovery of Piperine was more in the butter milk processed *P. nigrum*. The peaks were also reduced in processed samples as compared with the raw sample. This may indicate that the unwanted or undesired bioactive components may be removed with this process. The in vivo evidence of this needs to be done.

The recovery of piperine is found more in *P.Nigrum* processed with butter milk, which will give direction to pharmaceutical company as one of the approach for phytoconstituent recovery. However, by processing the Piperine enriched herbs with the butter milk the recovery of piperine can be observed.

Figure no. 1: Standard (Piperine) – HPTLC chromatograph showing maximum Rf 0.44 and maximum height 374.2 at 254 nm

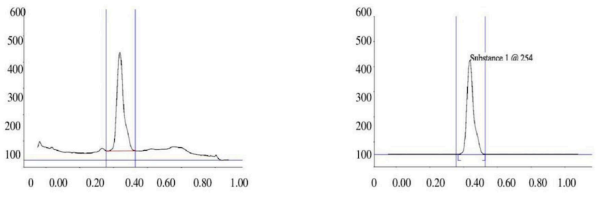


Figure no. 2: Shweta Maricha – HPTLC chromatograph showing maximum Rf 0.43 and maximum height 519.6 at 254 nm

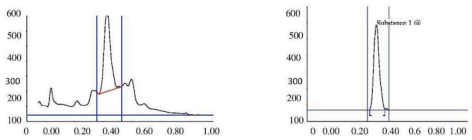


Figure no. 3: Takra Maricha – HPTLC chromatograph showing maximum Rf 0.43 and maximum height 421.1 at 254 nm

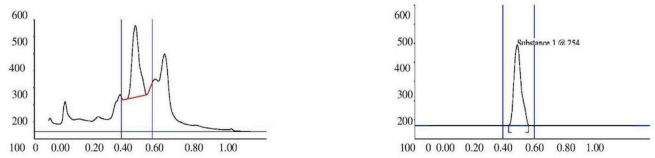


Figure no. 4: Raw Maricha – HPTLC chromatograph showing maximum Rf 0.43 and maximum height 443.0 at 254 nm

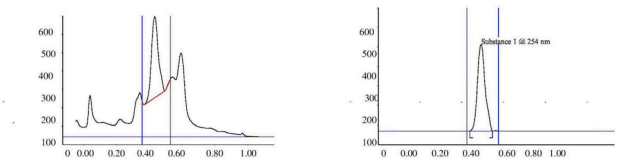


Figure no. 5:– HPLC chromatograph of Piperine, processed P.nigrum, Shweta maricha (market sample) and raw black maricha (p.nigrum) at 254 nm indicating Rf value within 43.052-43.873

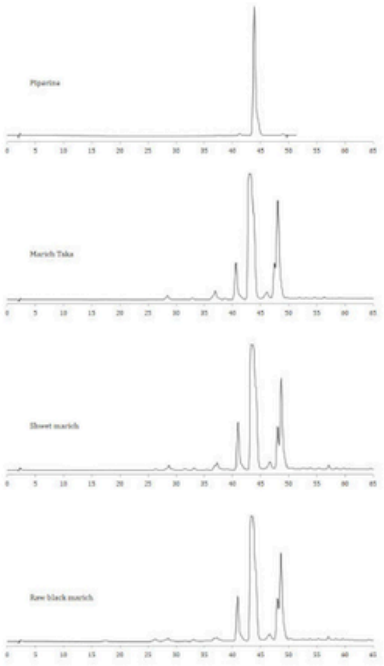
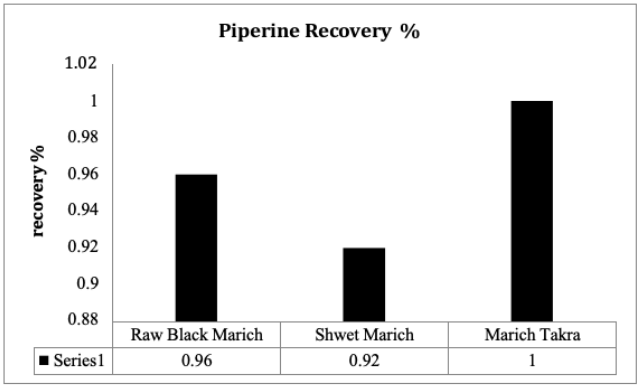


Figure no. 6 -showing recovery of Piperine, more in processed Maricha (Takra Maricha) as compared to Raw black maricha and Shwet Maricha



References

1. Loescher CM, Morton DW, Razic S, Agatonovic-Kustrin S. High performance thin layer chromatography (HPTLC) and high performance liquid chromatography (HPLC) for the qualitative and quantitative analysis of Calendula officinalis—Advantages and limitations. Journal of Pharmaceutical and Biomedical Analysis. 2014 Sep 1;98:52-9.
2. Singh R, Goel S, Bourgeade P, Aleya L, Tewari D. Ayurveda Rasayana as antivirals and immunomodulators: potential applications in COVID-19. Environmental Science and Pollution Research. 2021 Oct 1:1-27.
3. Sanjay LG, Mahesh JM, Rajender BR. Different Therapeutic Angles of Commonly used Swarna Kalpa. International Journal of Ayurveda and Pharma Research. 2022 Jul 10:90-5.
4. Katore AS, Wanjari A, Rathi B, Khan M, Sonare M. Pharmaceutical and Analytical Study of Gandharva Haritaki Prepared by Murchhit and Amurchhit Erand Tail and Comparative Assessment for In Vitro Bio-Accessibility. Systematic Reviews in Pharmacy. 2023 Mar 1;14(3).
5. Manghani K. Quality assurance: Importance of systems and standard operating procedures. Perspectives in clinical research. 2011 Jan;2(1):34.
6. Bhatted SK, Kabra DN. Ayurveda treatment for granulomatosis with polyangiitis: A case report. Journal of Indian System of Medicine. 2022 Jul 1;10(3):196.
7. Nakanekar A, Rathod P. The clinical evaluation of Basti along with Rasayana on symptoms of post-COVID-19 syndrome: an open-labeled proof of concept pragmatic study—a study protocol. Pilot and Feasibility Studies. 2023 Dec;9(1):1-0.
8. Mohammed Abubakar B, Mohd Salleh F, Shamsir Omar MS, Wagiran A. DNA barcoding and chromatography fingerprints for the authentication of botanicals in herbal medicinal products. Evidence-Based Complementary and Alternative Medicine. 2017 Apr 27;2017.
9. Bandaranayake WM. Quality control, screening, toxicity, and regulation of herbal drugs. Modern phytomedicine: turning medicinal plants into drugs. 2006 Sep 20:25-57.
10. Joshi DD. Herbal drugs and fingerprints: evidence based herbal drugs. Springer Science & Business Media; 2012 Nov 2.
11. Spangenberg B, Poole CF, Weins C. Quantitative thin-layer chromatography: a practical survey. Springer Science & Business Media; 2011 Jan 3.
12. Bhojashettar S, Jadar PG, Rao VN. Pharmaceutical study of Yashadabhasma. Ancient Science of Life. 2012 Jan;31(3):90.
13. Mukherjee PK, Harwansh RK, Bahadur S, Banerjee S, Kar A, Chanda J, Biswas S, Ahmmed SM, Katiyar CK. Development of Ayurveda–tradition to trend. Journal of ethnopharmacology. 2017 Feb 2;197:10-24.
14. Bhatted SK, Kumari K, Malhotra N, Nesari T. Safe and effective management of COVID-19 through ayurveda intervention: A case series. Journal of Ayurveda. 2021 Jul 1;15(3):237-44.
15. Patil P, Gawhankar M, Bidve S, Gudi RV, Lavand A. Phytochemical and elemental profiling and standardization of some Ayurveda medicines used

in COVID-19 pandemic. International Journal of Research in Ayurveda and Pharmacy. 2021:76-83.

16. Johri RK, Zutshi U. An Ayurvedic formulation 'Trikatu'and its constituents. Journal of ethnopharmacology. 1992 Sep 1;37(2):85-91.
17. Singh R, Rasheed H, Ahmed S, Singh H, Sharma A. Most Modern Approach to the Phytochemical Evaluation and Use of Pepper Species in Ayurvedic Formulations. InChemistry, Biological Activities and Therapeutic Applications of Medicinal Plants in Ayurveda 2022 Nov 9 (pp. 148-175). Royal Society of Chemistry.
18. Hazra AK, Chakraborty B, Mitra A, Sur TK. A rapid HPTLC method to estimate piperine in Ayurvedic formulations. Journal of Ayurveda and integrative medicine. 2019 Oct 1;10(4):248-54.
19. Sharma R, Prajapati PK. Liquid media's in Bhavana Samskara: A pharmaceutico-therapeutic prospect. J Phytopharm. 2015;4:49-57.
20. Shende HA, Bhatted SK. Effect of Vamana Karma (therapeutic emesis) in Mukhadushika (acne vulgaris)—A case study. Journal of Indian System of Medicine. 2022 Apr 1;10(2):146.

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