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A COMPARATIVE ANALYSIS OF *HARIDRA CHURNA* (*CURCUMA LONGA* LINN.) AND *AMALAKI* (*EMBLICA OFFICINALIS* GAERTN.) *SWARASA BHAVITA HARIDRA* (*CURCUMA LONGA* LINN) *CHURNA* THROUGH HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY

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ABSTRACT

Introduction: High-Performance Thin-Layer Chromatography (HPTLC) is an advanced version of Thin-Layer Chromatography (TLC) used for the separation, identification, and quantification of compounds in a sample. This analytical technique employs a stationary phase, which consists of a thin layer of adsorbent (typically silica gel or alumina) coated on a flat surface like a glass, plastic, or aluminium plate, along with a mobile phase that is a solvent or a mixture of solvents. HPTLC has a wide range of applications across various industries, including quality control, the quantification and detection of adulterants, authenticity and purity testing, additive analysis, and drug analysis. Its sensitivity, versatility, and capability to analyze complex mixtures make it a valuable tool in these fields.

Materials and Methods: *Haridra churna* (powder of Curcuma longa Linn.) and *Amalaki* (*Emblica officinalis* Gaertn.) *swarasa bhavita haridra* (*Curcuma longa* Linn) *churna* were prepared using the same methodology for both preparations. The HPTLC fingerprinting of both samples was conducted using a methanolic extract. The analysis of the bands was performed at wavelengths of 254 nm and 366 nm using the CAMAG Linomat V Automatic Sample Spotter. The peaks and areas were compared for evaluation.

Results and Discussion: HPTLC chromatogram of methanolic extracts of *churna* and *bhavita churna* showed 9 peaks at 254nm with a total area of 77202.6 AU and 18549 AU respectively. At 366 nm *churna* showed 5 peaks with a total area of 51440.9AU and *bhavita churna* showed 8 peaks with a total area of 49365.9 AU.

Conclusion: At 254 nm, both *churna* and *bhavita churna* showed the same number of peaks. However, *bhavita churna* exhibited a greater number of larg er value peaks at 366 nm, indicating that *bhavita churna* is more potent compared to *churna*.

Keywords: *Curcuma longa* Linn., *Emblica officinalis* Gaertn., High-Performance Thin-Layer Chromatography (HPTLC)

1. INTRODUCTION

High performance thin-layer chromatography (HPTLC) is the most advanced form of thin-layer chromatography (TLC) and provides sharper and more distinct separation of compounds, even in complex mixtures, due to improved resolution. It allows detection of trace elements and can be used for both qualitative and quantitative identification of each compound present in the sample. Also, they share advantages such as visual chromatogram results, simplicity, multiple sample handling, single use of the plate, rapid results, flexibility and the possibility of multiple detection. HPTLC can be applied to ensure the purity of drugs and their proper formulation, authenticity of medicines, detection of adulterants and for a wide range of substances, such as drugs, pesticides, food additives, essential oils and environmental pollutants. Instrumentation for HPTLC can be simple to sophisticated and allows obtaining traceable digital images and a deeper exploitation of the data contained in

these images.[1]The peaks in HPTLC are integral to identifying, analyzing, and quantifying the phytoconstituents of plant extracts.

Haridra churna (powder of *Haridra*), botanically identified as *Curcuma longa* Linn. contains various phytoconstituents such as curcumin, demethoxycurcumin, tetrahydroxycurcumin, 1,7-bis(4-hydroxyphenyl)-1-heptene-3,5-dione, (Z)-ferulic acid, (E)-ferulic acid, phenolic compounds like vanillin, Vanillic acid, turmerones like Ar-turmerone, a-turmerone and b-turmerone.[2] *Ashtangahrdayam Uttarastanam* in the context of *Agroushadi* mentioned *Amalaki* and *Haridra* as the best remedy for *Prameha.*[3]*Haridra churna* is used in a modified dosage form by doing *bhavana* (process of trituration) with the juice of *Amalak*i in diabetic dyslipidemia. The process of *bhavana* is done for 7 times as per the reference of *Bhaishajya Ratnavali*.[4] It increases the potency and reduces the dose of the drug by reducing the particle size of the plant material and increase the surface area of active compounds, thereby enhancing their absorption. Furthermore, trituration has evolved significantly both **pharmacognostically** and **phytochemically.** The use of **HPTLC** to distinguish ***bhavita*** and ***abhavita churna*** is highly effective due to its ability to **separate, identify, and quantify** chemical components in a mixture. The processing methods used to prepare these *churna* result in **distinct chemical signatures,** which can be visualized and compared using HPTLC. This study seeks to compare the peaks obtained from the HPTLC chromatogram of *Haridra churna* and *Amalaki swarasa bhavita Haridra churna* and to substantiate the enhanced potency of *bhavita churna* in relation to its counterpart.

2. MATERIALS AND METHODS

2.1 Preparation of *Haridra churna* (powder)

Freshly collected *haridra* rhizomes were washed thoroughly under running water to remove the physical impurities. The rhizomes were taken in a vessel and sufficient amount of water enough to cover the rhizomes was added. Boiling was continued until the froth and fumes were released with a typical aromatic smell of *haridra*. Boiling was considered complete after pressing with a pointed stick into the rhizomes with slight pressure. The rhizomes' softness and ease of breaking when squeezed between the forefinger and thumb is another

sign that the process is complete. An effective cooking time of 60 to 90 minutes are considered essential.[5] Later, the cooked rhizomes were dried in the sun for 10-12 days. The dried rhizomes were then made into fine powder and sieved through mesh size-85 and stored in air tight containers.

2.2. Preparation of *Amalaki swarasa* (juice) for *bhavana* (trituration)

Amalaki fruits were purchased from the vegetable market, near Kanjirapally in Kerala. Swarasa (juice) was prepared based on the reference mentioned in *Sarangadhara Samhita*.[6] The purchased *Amalaki* fruit were washed thoroughly under running water to remove the physical impurities like soil, dust particles, etc. Then the fleshy part got detached using a clean knife and cut into small pieces and then grinded. It was then placed in a clean cloth, pressed and squeezed out through the cloth.The expressed juice was collected in a clean stainless- steel container.

2.3. Preparation of *Bhavita churna* (triturated powder)

Amalaki swarasa bhavita haridra churna was prepared according to the reference of *Bhaishajya ratnavali*. The prepared *haridra churna* was spread evenly in the stainless-steel tray to form a thin layer (1cm thickness). Freshly prepared *swarasa* of *Amalaki* was then gradually poured into the powder of *Haridra* so that the *swarasa* gets easily absorbed into the powder. Using a sharp thin rod, it was enusured that each fine particle of *churna* gets completely soaked in the *swarasa.* The *swarasa* was poured continuously until a thin layer was seen on the surface of the *haridra churna*. It was then kept under sunlight after covering with a clean cloth to avoid contamination and during night time, it was kept under shade. On the next day morning, *churna* was stirred and spread uniformly without any lumps on the tray and freshly prepared *Amalaki swarasa* was poured until it is soaked. The same procedure was performed continuously for 7 consecutive days with sufficient quantity of *swarasa*. After the 7th *bhavana* the *churna* was kept under sunlight until it was dried completely (4 days). Ensure that there was no contamination. *Haridra churna* was made into fine powder using a mixer. This sieved *bhavita churna* was stored in air tight containers.

a. Procedure of HPTLC

2.4.1 HPTLC conditions

The HPTLC plate used measured 4.0 x 10.0 cm and was pre-coated with silica gel 60 F254 (E. Merck KGaA), with a thickness of 0.2 mm supported by an aluminum sheet. The experiment utilized a CAMAG Linomat V Automatic Sample Spotter (Camag, Muttenz, Switzerland) with a 100 µL syringe from Hamilton for sample application. The developing chamber consisted of a twin trough chamber with dimensions of 20 x 10 cm, also provided by CAMAG. Post-chromatographic derivatization was performed using a chromatographic sprayer with a vanillin-sulfuric acid reagent solution. Detection of the tracks was accomplished with a CAMAG TLC Scanner linked to WINCATS software.

2.4.2 Method

The same methodology is applied for both *churna* and *bhavita churna*. A twin trough chamber is pre-saturated with a solvent mixture of toluene, ethyl acetate, and acetic acid in a 6:3:1 ratio for 30 minutes. The plate is then developed with an 8 mm band length after washing the syringe with methanol and applying 5.0 µl of the methanolic extract. Once dried, the plate is scanned using a Deuterium, Tungsten, or Mercury lamp under ultraviolet (UV) light at 254 nm and 366 nm. The UV spectrum of each scan is analyzed, and tracks are set to observe their fingerprints. Finally, UV spectral spots are compared, and all scanning and spectrum parameters are recorded after opening a file.

4. RESULTS

HPTLC fingerprinting profile of *Haridra churna* (powder of *Curcuma longa* Linn.) and *Amalaki swarasa bhavita Haridra churna* (triturated powder of *Haridra* with *Amalaki swarasa*) were performed. The observations found are being tabulated.

3.1 HPTLC finger printing profile of methanolic extract of *Haridra churna* (powder of *Curcuma longa* Linn.)

3.1.1 Area and peaks of methanolic extract of *Haridra churna* (powder of *Curcuma longa* Linn.) at 254nm

HPTLC fingerprinting profile of *Haridra churna* (powder of *Curcuma longa* Linn.) showed 9 peaks at the wavelength of 254 nm in a total area of 77202.6 AU. These 9 peaks were defined at the maximum Rf value of -0.03 with area 19049.6 AU, maximum Rf value of 0.03 with area 10530.2 AU, maximum Rf value of 0.10 with area 20531.8 AU, maximum Rf value of 0.18 with area 418.0 AU, maximum Rf value of 0.30 with area 1799.2 AU, maximum Rf value of 0.46 with area 694.1 AU, maximum Rf value of 0.58 with area 1197.7 AU, maximum Rf value of 0.66 with area 4231.3 AU, maximum Rf value of 0.83 with area 18750.7 AU respectively.

Figure No: 1 HPTLC plate view of methanolic extract *Haridra churna* (powder of *Curcuma longa* Linn.) at 254nm wavelength.

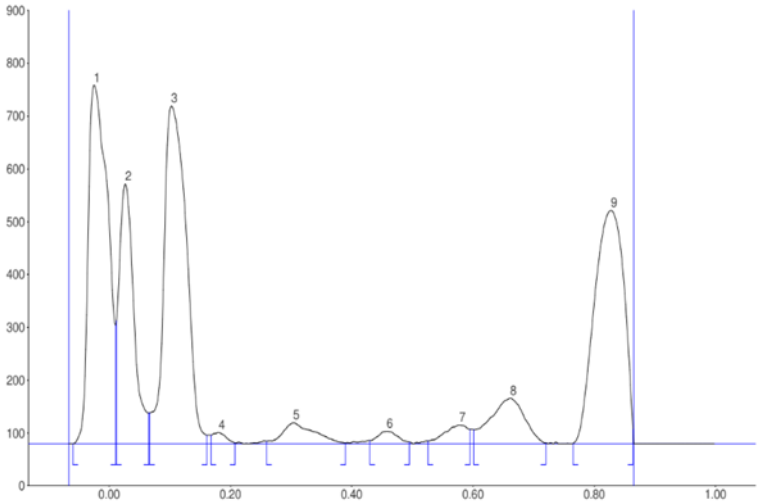

Chart No:1 Overview graph of methanolic extract of *Haridra churna* (powder of *Curcuma longa* Linn.) at 254nm wavelength.

Table No:1 Peak and area of methanolic extract of *Haridra churna* (powder of *Curcuma longa* Linn.) at 254nm

Peak no.	Start Rf	Max Rf	End Rf	Area (AU)	% Area (AU)
1.	-0.06	-0.03	0.01	19049.6	24.67
2.	0.01	0.03	0.07	10530.2	13.64
3.	0.07	0.10	0.16	20531.8	26.59
4.	0.17	0.18	0.21	418.0	0.54
5.	0.26	0.30	0.39	1799.2	2.33
6.	0.43	0.46	0.50	694.1	0.90
7.	0.53	0.58	0.60	1197.7	1.55
8.	0.60	0.66	0.72	4231.3	5.48
9.	0.77	0.83	0.87	18750.7	24.29

3.1.2 Area and peaks of methanolic extract of *Haridra churna* (powder of *Curcuma longa* Linn.) at 366nm wavelength

HPTLC fingerprinting profile of *Haridra churna* (powder of *Curcuma longa* Linn.) showed 5 peaks at the wavelength of 366 nm in a total area of 51440.9 AU. These 5 peaks were defined at the maximum Rf value of 0.00 with area 21909.1 AU, maximum Rf value of 0.04 with area 9771.8 AU, maximum Rf value of 0.09 with area 5630.5 AU, maximum Rf value of 0.14 with area 11824.6AU, maximum Rf value of 0.33 with area 2304.5 AU respectively.

Figure No: 2 HPTLC plate view of methanolic extract of *Haridra churna* (powder of *Curcuma longa* Linn.) at 366 nm wavelength.

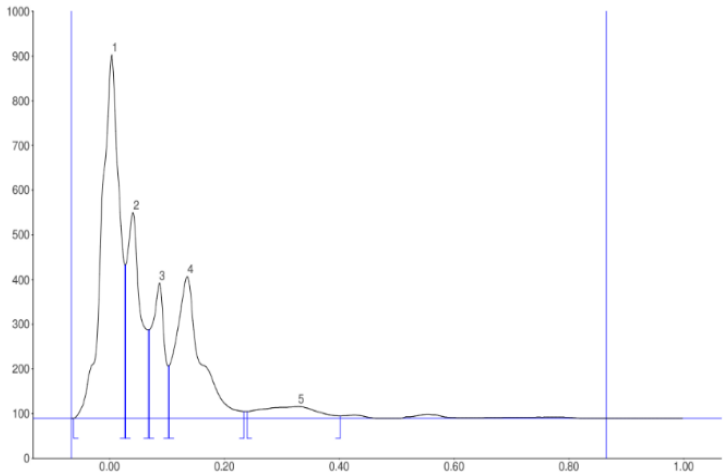

Chart No: 2 Overview graph of methanolic extract of *Haridra churna* (powder of *Curcuma longa* Linn.) at 366nm

Table No: 2 Peak and area of methanolic extract of *Haridra churna* (powder of *Curcuma longa* Linn.) at 366 nm wavelength

Peak no.	Start Rf	Max Rf	End Rf	Area (AU)	% Area (AU)
1.	-0.06	0.00	0.03	21909.1	42.59
2.	0.03	0.04	0.07	9771.8	19.00
3.	0.07	0.09	0.10	5630.5	10.95
4.	0.10	0.14	0.23	11824.6	22.99
5.	0.24	0.33	0.40	2304.9	4.48

3.2 HPTLC finger printing profile of *Amalaki swarasa bhavita Haridra churna* (triturated powder of *Haridra* with *Amalaki swarasa*)

3.2.1 Area and peaks of methanolic extract of *Amalaki swarasa bhavita Haridra churna* (triturated powder of *Haridra* with *Amalaki swarasa*) at 254nm

HPTLC fingerprinting profile of *Amalaki swarasa bhavita Haridra churna* (triturated powder of *Haridra* with *Amalaki swarasa*) showed 9 peaks at the wavelength of 254 nm in a total area of 18549 AU. These 9 peaks were defined at the maximum Rf value of -0.02 with area 30.69 AU, maximum Rf value of 0.03 with area 111475.0 AU, maximum Rf value of 0.11 with area 26.96 AU, maximum Rf value of 0.19 with area 1159.2 AU, maximum Rf value of 0.32 with area 1328.8 AU, maximum Rf value of 0.47 with area 615.9 AU, maximum Rf value of 0.58 with area 1288.5 AU, maximum Rf value of 0.68 with area 691.2 AU, maximum Rf value of 0.82 with area 14019.4 AU respectively.

Figure No: 3 HPTLC plate view of methanolic extract of *Amalaki swarasa bhavita Haridra churna* (triturated powder of *Haridra* with *Amalaki swarasa*) at 254 nm

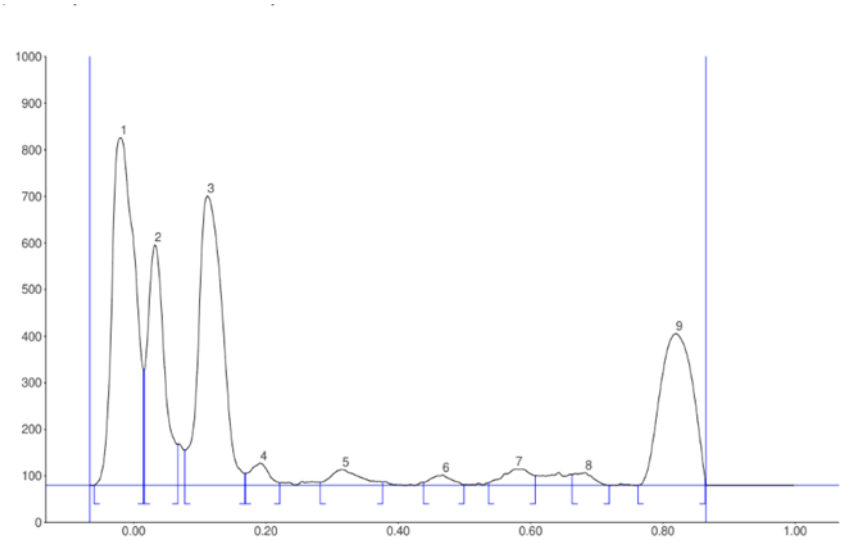

Chart No: 3 Overview graph of methanolic extract of *Amalaki swarasa bhavita Haridra churna* (triturated powder of *Haridra* with *Amalaki swarasa*) at 254nm

Table No: 3 Peak and area of methanolic extract of *Amalaki swarasa bhavita Haridra churna* (triturated powder of *Haridra* with *Amalaki swarasa*) at 254 nm

Peak no.	Start Rf	Max Rf	End Rf	Area (AU)	% Area (AU)
1.	-0.06	-0.02	0.01	22166.7	30.69
2.	0.02	0.03	0.07	11475.0	15.89
3.	0.08	0.11	0.17	19472.3	26.96
4.	0.17	0.19	0.22	1159.2	1.61
5.	0.28	0.32	0.38	1328.8	1.84
6.	0.44	0.47	0.50	615.9	0.85
7.	0.54	0.58	0.61	1288.5	1.78
8.	0.66	0.68	0.72	691.2	0.96
9.	0.76	0.82	0.87	14019.4	19.41

3.2.2 Area and peaks of methanol extract of *Amalaki swarasa bhavita Haridra churna* (triturated powder of *Haridra* with *Amalaki swarasa*) at 366nm

HPTLC fingerprinting profile of *Amalaki swarasa bhavita Haridra churna* (triturated powder of *Haridra* with *Amalaki swarasa*) showed 8 peaks at the wavelength of 254 nm in a total area of 49365.9 AU. These 8 peaks were defined at the maximum Rf value of -0.03 with area 1230.5 AU, maximum Rf value of 0.01 with area 19933.9 AU, maximum Rf value of 0.05 with area 9208.4 AU, maximum Rf value of 0.10 with area 6548.5 AU, maximum Rf value of 0.14 with area 402.4 AU, maximum Rf value of 0.56 with area 412.2 AU respectively.

Figure No: 4 HPTLC plate view of methanol extract of *Amalaki swarasa bhavita Haridra churna* (triturated powder of *Haridra* with *Amalaki swarasa*) at 366nm

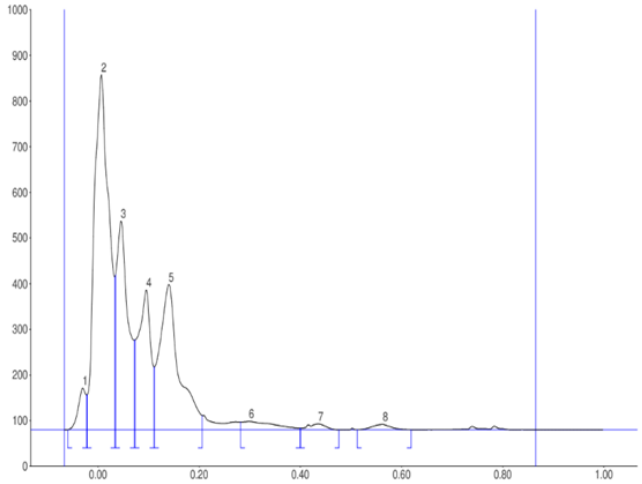

Chart No: 4 Overview graph of methanol extract of *Amalaki swarasa bhavita Haridra churna* (triturated powder of *Haridra* with *Amalaki swarasa*) at 366 nm

Table No: 4 Peak and area of methanol extract of *Amalaki swarasa bhavita Haridra churna* (triturated powder of *Haridra* with *Amalaki swarasa*) at 366 nm.

Peak no.	Start Rf	Max Rf	End Rf	Area (AU)	% Area (AU)
1.	-0.06	-0.03	-0.02	1230.5	2.49
2.	-0.02	0.01	0.03	19933.9	40.38
3.	0.03	0.05	0.07	9208.4	18.65
4.	0.07	0.10	0.11	6548.5	13.27
5.	0.11	0.14	0.21	10566.9	21.41
6.	0.28	0.30	0.40	1063.1	2.15
7.	0.40	0.44	0.48	402.4	0.82
8.	0.51	0.56	0.62	412.2	0.84

5. DISCUSSION

HPTLC fingerprinting profile of methanolic extract of *churna* and *bhavita churna* were demonstrated in this study and the bands are analysed at 254 nm and 366nm. The peaks and area obtained are compared with the previous research works. It was found that both the *churna* and *bhavita churna* obtained 9 peaks at 254 nm with a total area of 77202.6 AU and 18549 AU respectively. At 366 nm *churna* showed 5 peaks with a total area of 51440.9AU and *bhavita churna* showed 8 peaks with a total area of 49365.9 AU. In a previous research work using toluene - ethyl acetate in the ratio7:1 as the mobile phase, showed 12 peaks and 11 peaks for 254nm and 366nm respectively. Peak intensities were different in both samples

and *bhavita churna* shows a higher concentration of a particular compound at 366 nm compared to regular *churna*, possibly indicating a greater number of phytoconstituents due to the *bhavana* process.

6. CONCLUSION

HPTLC fingerprinting profile of *churna* and *bhavita churna* of were demonstrated in methanolic extract. At 254nm both samples obtained same number of peaks. Since the *bhavita churna* possess the greater number of large value peaks in 366nm, it is clear that *bhavita churna* is more potent in comparison with *churna*.

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8. CONFLICTS OF INTEREST: Nil.

REFERENCE

1. Prof. Salvador Cañigueral, Unitat de Farmacologia, Farmacognòsia i Terapèutica, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona, Av. Joan XXIII, 27-31, 08028 Barcelona, Spain. E-mail: s.canigueral@ub.edu.
2. Shiyou Li, et.al, Chemical Composition and Product Quality Control of Turmeric (Curcuma longa L.), Pharmaceutical Crops, 2011, 2, 28-54.
3. Srikantha Murthy KR. Vagbhatas Astangahrdyam, Chaukhamba krishnadas academy,

Varanasi (2016), Vol 3. Chapter 40, p.154.

4. Govinda Das. Translated by Bhishagrathna shri Brahmasankar Misra. Bhaishajya rathnavali. Varanasi:Chaukambha prakasan; Misraprakarana. Chapter 4 Paribhasha prakaranam: Sloga 89; p. 313.
5. Krishi Vigyan Kendra Knowledge Network [Internet]. Icar.gov.in. 2019 [cited 2024 Jun 15]. Available from: https://kvk.icar.gov.in.
6. Sarangadhara. Translated by Himasagara Chandra Murthy. Sarangadhara Samhita.Varanasi: Chaukhambha Sanskrit Series Office; 2013.Madhyamkanda. Chapter 1 Swarasa Kalpana. Sloga 2.p.102.