



Research Article

COMPARATIVE PHYTOCHEMICAL FINGERPRINTING OF CLASSICAL AND SUGAR-FREE
CHYAVANAPRASA USING HPTLC

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ABSTRACT

Chyavanaprasa is a classical Ayurvedic *Rasayana* formulation widely used for health promotion and rejuvenation. Conventional *Chyavanaprasa* contains a substantial quantity of sugar and honey, whereas sugar-free formulations are increasingly being developed to improve suitability for health-conscious individuals and those requiring sugar restriction. Analytical comparison of these formulations is essential to ensure that modification of sweetening agents does not compromise their phytochemical integrity. Objective: To compare the HPTLC fingerprint profiles of classical *Chyavanaprasa* and sugar-free *Chyavanaprasa* and evaluate their phytochemical similarity for quality control and standardisation. **Materials and Methods:** Both formulations were analysed using High-Performance Thin-Layer Chromatography (HPTLC). Samples were applied on silica gel 60 F254 plates using a CAMAG Linomat 5 applicator. Classical *Chyavanaprasa* used Toluene:Ethyl acetate:Methanol:Formic acid (3:3:0.8:1.0), while sugar-free *Chyavanaprasa* used Ethyl acetate:Methanol:Water (15:1:1). Plates were derivatized with anisaldehyde-sulphuric acid reagent and scanned at 254nm and 366nm. Three replicate tracks were analyzed for each formulation to evaluate reproducibility and chromatographic consistency. **Observation:** The sugar-free formulation produced multiple distinct peaks with consistent R_f values across all replicate tracks. Similar chromatographic characteristics were observed in the classical formulation. Major bands appeared at corresponding chromatographic positions in both preparations, indicating preservation of principal phytochemical constituents. **Result:** The sugar-free formulation showed 9–11 characteristic peaks with major components distributed across the chromatogram, while the classical formulation demonstrated a comparable fingerprint pattern, indicating preservation of major phytochemical constituents despite the absence of sugar and honey. **Conclusion:** The chromatographic profiles of both formulations demonstrated excellent reproducibility.

INTRODUCTION

Chyavanaprasa is one of the most important *Rasayana* formulations described in Ayurvedic classics. The reference of *Chyavanaprasa* for the study is taken from *Caraka Samhita*^[1]; the same reference is standardised in API Part II, Volume I^[2]. Other references are *Ashtanga sangraha*^[3], *Ashtanga Hridayam*^[4], *Sarngadhara Samhita*^[5],

Bhaishajya Ratnavali^[6], *Cakradatta*^[7], *Vangasena Samhita*^[8], *Sahasrayoga*^[9], and *Yogaratanakara*^[10]. The content of *Chyavanaprash* can be classified into the following categories *Pradhana Dravya* (Main ingredient), *Dravadravya (Kashaya dravya)*, *Sneha dravya* (lipid media) *Madhura dravya* (preserving agents) and *Prakshepa dravyas* (flavouring agents^[11]. Traditionally prepared using *Amalaki* and numerous medicinal herbs, it is renowned for its rejuvenating, immunomodulatory, antioxidant, and adaptogenic properties. The *Kashaya* group of drugs forms an essential component of the *Chyavanaprasa* formulation. These herbs are primarily used to prepare the decoction (*Kashaya*), which serves as the first stage of *Avaleha* preparation. 36 drugs are included in the

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Kashaya preparation they are *Bilwa*, *Agnimantha*, *Syonaka*, *Kasmari* (*Gambhari*), *Patala*, *Bala*, *Salaparni*, *Prsniparni*, *Mudgaparni*, *Masaparni*, *Pippali*, *Swadamshttra*, *Brihati*, *Kandakari*, *Karkatakasrngi*, *Tamalaki* (*Bhumyamalaki*), *Draksha*, *Jivanthi*, *Jivanthi*, *Pushkara*, *Agaru*, *Abhaya* (*Hareethaki*), *Amrutha* (*Guduchi*), *Rddhi*, *Jeevaka*, *Rrshabhaka*, *Sati*, *Mustha*, *Punarnava* (*Rakta Punarnava*), *Meda*, *Ela*, *Chandana* (*swetha Chandana*), *Utpala*, *Vidari* (*Kanda*), *Vrsamula* (*Vasa*), *Kakoli*, *Kakanasika*. These include *Dasamula* and *Jeevaneeya* groups of drugs. The decoction prepared from these herbs helps extract water-soluble phytoconstituents and creates a therapeutic foundation into which the remaining ingredients—including *Amla*, *ghee*, *Taila*, and *Yogavahi-dravyas* are later incorporated. The *Kashaya* group thus supports digestion, metabolism, and rejuvenation while enhancing the stability and efficacy of the final formulation of *Chyavanaprasa*. Classical *Chyavanaprasa* contains *Matsyandika sarkara* (sugar candy), and honey, which contribute to preservation, palatability, and consistency. Growing concerns regarding excessive sugar intake have encouraged the development of sugar-free *Chyavanaprasa*

formulations. However, removing sugar should not compromise the phytochemical composition responsible for its therapeutic activity. Therefore, comparative phytochemical fingerprinting is essential to establish equivalence between classical and modified formulations. High-Performance Thin Layer Chromatography (HPTLC) is a rapid, reliable, and reproducible technique widely used for authentication and standardization of herbal formulations. Comparative HPTLC fingerprinting provides qualitative evidence regarding the preservation of phytochemical constituents after formulation modification.

HPTLC Fingerprinting Analysis of *Chyavanaparasa*

HPTLC is used to identify chemical components and create a fingerprint profile of herbal formulations. The sample was applied to silica gel plates, developed in a solvent system, Toluene + Ethyl acetate + Methanol + Formic acid. The sample solvent is methanol, and HPTLC plates were visualised at 254nm and 366 nm wavelengths.

A. Mean Area and peaks of methanol extract of sample of *Chyavanaprasa* at 254nm on all three tracks

Table 1: Mean Area and peaks of methanol extract of sample of *Chyavanaprasa* at 254nm on all three tracks

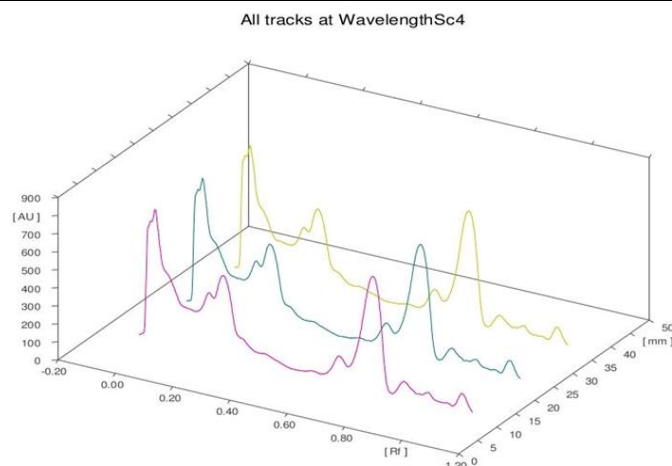
Peak	Mean Rf	Mean Peak Area (AU)	Mean Peak Area %
1	-0.03	35800.2	34.10
2	0.16	10096.6	9.62
3	0.21	19444.1	18.54
4	0.35	2046.6	1.95
5	0.52	642.9	.61
6	0.62	4472.5	4.26
7	0.74	28557.3	27.22
8	0.84	2454.5	2.34
9	0.93	1225.8	1.17
10	0.99	308.5	.29

Values are expressed as the mean of three replicate tracks.

Peak 10 was detected in Tracks 1 and 2 but was absent in Track 3.

Data Interpretation

The representative HPTLC chromatogram of the methanolic extract of classical *Chyavanaprasa* at 254nm demonstrated a reproducible fingerprint profile with 9–10 characteristic peaks. Major peaks were observed at Rf -0.03 (34.10%), Rf 0.21 (18.54%), and Rf 0.74 (27.22%), indicating the predominance of the phytochemical constituents. The close agreement in Rf values and peak areas among the three replicate tracks confirmed the reproducibility and consistency of the chromatographic method, supporting its suitability for quality control and standardization of *Chyavanaprasa*.



Graph 1: Graph of all tracks of *Chyavanaprasa* at wavelength of 254nm

Area and peaks of the methanol extract of the sample of *Chyavanaprasa* at 366nm

Mean area and peaks of the methanol extract of the sample of *Chyavanaprasa* at 366nm on all three tracks

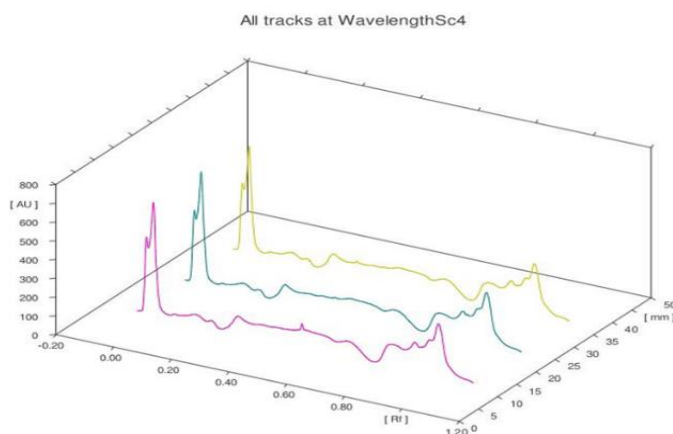
Table 2: Mean Area and peaks of the methanol extract of the sample of *Chyavanaprasa* at 366nm on all tracks

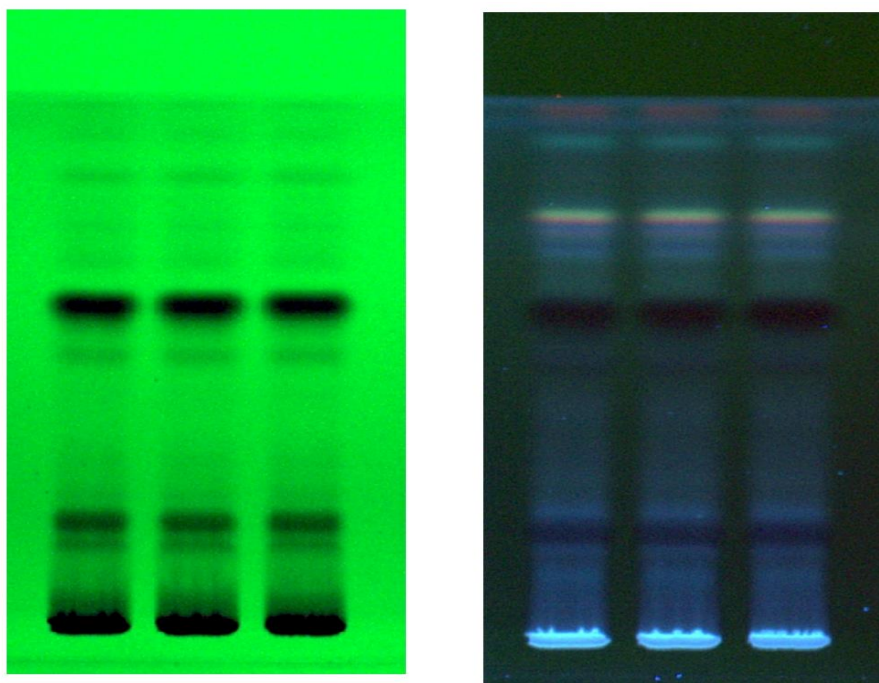
Peak	Mean Rf value	Mean Peak area (AU)	Mean Peak Area %
1	-0.03	11683.1	23.85
2	0.05	419.8	.86
3	0.12	1905.9	3.89
4	0.17	576.8	1.18
5	0.27	3296.9	6.73
6	0.49	5226.5	10.69
7	0.53-0.64	4667.5	9.39
8	0.65-0.80	4607.8	9.07
9	0.88	4192.7	8.55
10	0.96	10859.7	22.17

Minor variation in Rf values among replicate tracks due to chromatographic separation.

Data Interpretation

The HPTLC fingerprint of the methanolic extract of Classical *Chyavanaprasa* at 366nm demonstrated a reproducible chromatographic profile with 10–11 characteristic peaks across the three replicate tracks. The chromatograms showed major fluorescent peaks at approximately Rf –0.03, 0.49–0.53, 0.64–0.80, and 0.96, indicating the presence of major phytochemical constituents. Minor variations in Rf values and peak areas were observed among replicate tracks; however, the overall chromatographic pattern remained consistent, confirming the repeatability of the analytical method. The representative chromatogram can therefore serve as a characteristic fingerprint



Graph 2: Graph of all tracks of the wavelength of *Chyavanaprasa* at 366nm**Fig 1: HPTLC Plate of track 1, 2, 3 at 254nm Fig 2: HPTLC Plate of track 1, 2, 3 at 366nm****HPTLC Fingerprinting Analysis of *Chyavanaprasa* without Sugar**

HPTLC is used to identify chemical components and create a fingerprint profile of herbal formulations. The sample was applied to silica gel 60 F254 plates, The mobile phase was Ethyl acetate: Methanol: Water (15:1:1). The sample solvent was methanol, and HPTLC plates were visualised at 254 nm and 366 nm wavelengths

Mean area and peaks of methanol extract of sample of *Chyavanaprasa* without sugar at 254 nm on all three tracks

Table 3: Representative HPTLC Fingerprint Chromatogram of Sugar-free *Chyavanaprasa* at 254 nm on three tracks

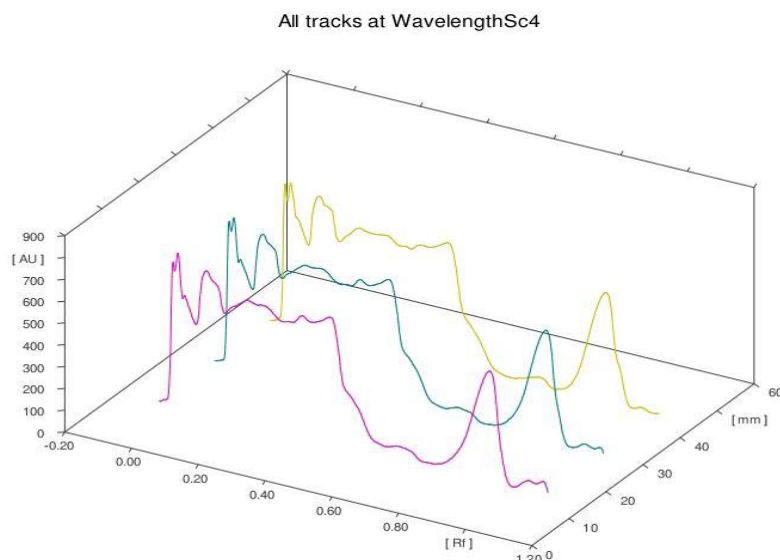
Peak	Representative Rf value	Representative Peak Area	Representative Peak Area %
1	-0.03	22796.0	11.41
2	0.06	29600.2	140.86
3	0.19	30052.4	15.08
4	0.23	24677.1	12.39
5	0.35	20135.8	10.04
6	0.44	39244.5	19.69
7	0.64	2410.2	1.20
8	0.91	27943.3	14.02
9	1.03	686.0	0.34

Track 3 showed splitting of the initial peak (Rf -0.04 and -0.02), whereas Tracks 1 and 2 showed a single peak at Rf -0.03. Minor variations in Rf values (± 0.01 – 0.02) were observed among replicate tracks.

Data Interpretation

The methanolic extract of sugar-free *Chyavanaprasa* exhibited a reproducible HPTLC fingerprint profile at 254nm. The chromatograms showed 9–10 well-resolved peaks across the three replicate tracks. Major peaks were consistently observed at Rf values around 0.06, 0.19, 0.44, and 0.91, indicating the presence of predominant phytochemical constituents. Minor variations in Rf values and peak areas among the replicate tracks were noted, particularly in the initial peak region, but the overall chromatographic pattern remained highly consistent. The representative chromatogram can therefore be used as a characteristic fingerprint for the authentication, quality control, and standardization of sugar-free *Chyavanaprasa*.

All tracks of *Chyavanaprasa* without sugar, 254nm wavelength



Graph 3: Graph of all tracks of *Chyavanaprasa* without sugar, 254nm wavelength

Area and peaks of the methanol extract of sample of *Chyavanaprasa* without sugar at 366nm on all three tracks

Table 4: Representative HPTLC Fingerprint Chromatogram of Sugar-free *Chyavanaprasa* at 366nm

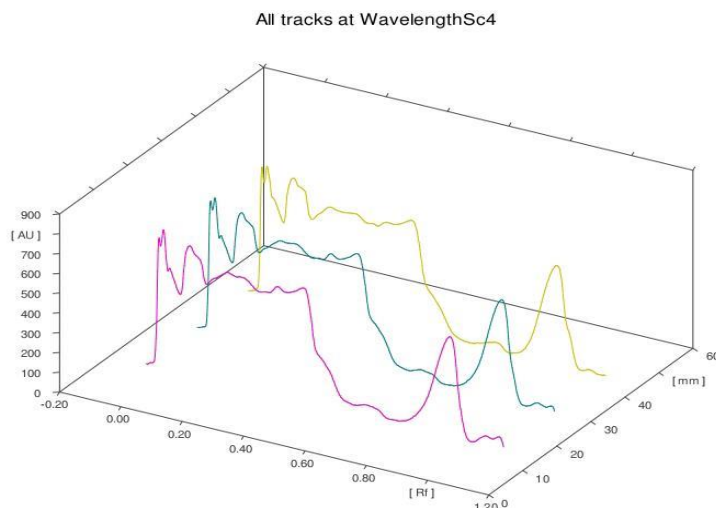
Peak	Representative Rf value	Mean peak area (AU)	Mean peak Area %
1	-0.03	15708.3	55.95
2	0.04	1834.7	6.53
3	0.08	1206.5	40.29
4	0.10	1155.1	4.12
5	0.23	478.5	1.70
6	0.36	765.6	2.72
7	0.54	1775.3	6.28
8	0.64	1110.8	3.99
9	0.71	1173.3	3.53
10	0.80	1037.1	3.69
11	1.00	2638.8	9.40

Note: Minor variations in Rf values (± 0.01 – 0.03) were observed among replicate tracks. Peaks at Rf 0.64–0.82 showed slight shifts due to chromatographic variation.

Data Interpretation

The methanolic extract of sugar-free *Chyavanaprasa* exhibited a reproducible HPTLC fingerprint profile at 366 nm. The chromatographic analysis demonstrated 9–11 characteristic fluorescent peaks across the three replicate tracks. Major peaks were consistently observed at Rf values around -0.03 , 0.04 , 0.54 , and 1.00 , while minor peaks were detected between Rf 0.08 and 0.82 . Slight variations in peak intensity and Rf values were observed among the replicate tracks, particularly in the region between Rf 0.64 and 0.82 ; however, the overall chromatographic pattern remained highly consistent. The representative chromatogram confirmed the reproducibility of the analytical method and can serve as a characteristic fingerprint for the authentication, quality control, and standardization of sugar-free *Chyavanaprasa*.

All tracks of *Chyavanaprasa* without sugar, 366nm wavelength as the representative chromatogram.



Graph 4: All tracks of *Chyavanaprasa* without sugar in 366nm

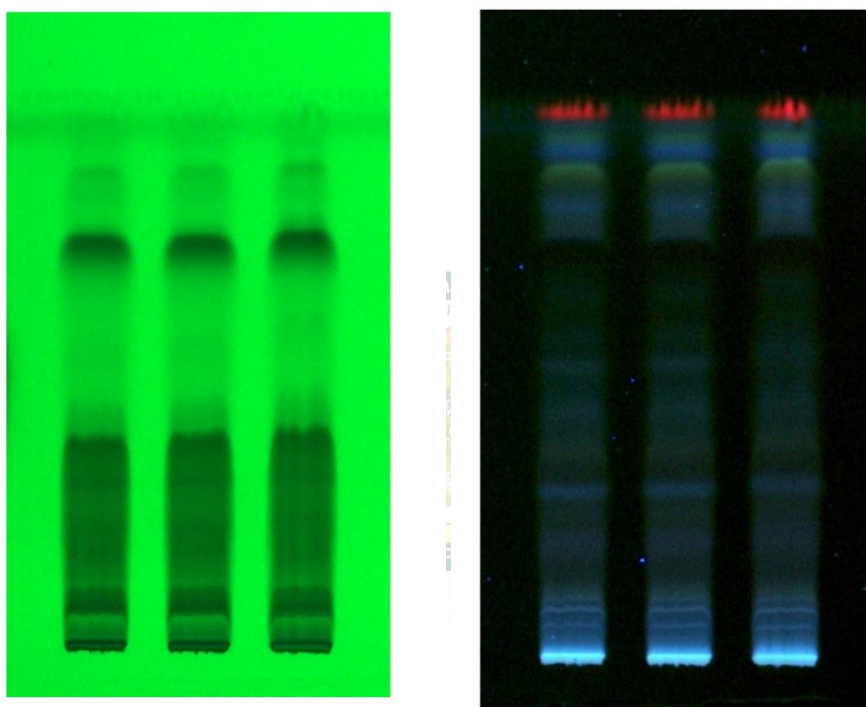


Figure 3: HPTLC Plate of track 1, 2, 3 at 254nm Figure no 5: HPTLC Plate of track 1, 2, 3 at 366nm

RESULT

Comparative Interpretation

Both preparations generated multiple resolved peaks at 254 and 366nm, demonstrating the chemical complexity expected of a polyherbal formulation. Replicate tracks were broadly consistent within each preparation, supporting repeatability of sample application and chromatographic development. The sugar-free formulation retained a rich multi-peak fingerprint despite omission of sugar and honey. Nevertheless, the classical and sugar-free samples were developed using different mobile phases; therefore, apparent differences or similarities in Rf position, peak number or intensity cannot by themselves establish chemical equivalence or identify common compounds. Confirmation of shared marker compounds would require analysis under a common validated chromatographic system with suitable

reference standards. The present fingerprints are best used as formulation-specific reference profiles for authentication, batch consistency and future standardization.

DISCUSSION

The classical *Chyavanaprasa* showed a reproducible multi-peak HPTLC profile at both wavelengths. At 254nm, prominent peaks occurred at Rf -0.03, 0.21, and 0.74; at 366 nm, major responses were observed around Rf -0.03, 0.49-0.53, 0.64-0.80, and 0.96. The sugar-free formulation also produced a consistent fingerprint, with major peaks around Rf 0.06, 0.19, 0.44, and 0.91 at 254nm and around Rf -0.03, 0.04, 0.54, and 1.00 at 366nm. Minor Rf shifts among replicate tracks were observed, but the overall

patterns remained consistent. Both formulations therefore retained multiple phytochemical constituents, although differences in peak number, position, and intensity were evident, which may be related to differences in formulation composition and chromatographic systems. HPTLC/HPLC analysis of Patanjali Special *Chyawanprash* has similarly reported gallic acid, ellagic acid, piperine, eugenol, cinnamic acid, glycyrrhizin, corilagin, and chebulagic acid, with gallic acid as a predominant marker^[12]. The present study did not identify or quantify individual marker compounds because reference standards were not used; therefore, the obtained profiles should be interpreted as comparative fingerprints for authentication, quality control, and standardization.

CONCLUSION

Classical and sugar-free *Chyavanaprasha* each produced reproducible HPTLC fingerprints with multiple characteristic peaks at 254 and 366 nm. The results document the phytochemical complexity of both formulations and provide formulation-specific reference profiles that may be useful for authentication and quality-control assessment. Since different mobile phases were used for the two preparations, direct compound-level equivalence cannot be concluded from R_f correspondence alone. Future studies using a common mobile phase and authenticated marker standards are recommended for definitive comparative profiling.

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