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Original Article

Comparative HPTLC Fingerprinting Analysis of *Yashtimadhu Medhya Rasayana* in Different Media

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ABSTRACT

Background: *Yashtimadhu* (*Glycyrrhiza glabra* Linn.), classified as a *Medhya Rasayana* in Ayurveda, is traditionally administered with milk (*Ksheera anupana*) to enhance cognitive function. Despite these claims, the phytochemical basis and role of milk as an adjuvant in influencing the availability of active constituents like glycyrrhizin remain insufficiently explored. **Objectives:** To investigate the effect of different extraction media: water, milk and *ksheerapaka* on the glycyrrhizin content in *Yashtimadhu* using High-Performance Thin Layer Chromatography (HPTLC). **Materials and Methods:** TLC was initially conducted to screen phytochemical profiles of Yashtimadhu extracts (aqueous, milk, and ksheerapaka (milk-based decoction)). Based on these findings, HPTLC analysis was performed using methanolic extracts to identify and quantify glycyrrhizin, with comparison to a standard marker at 254 nm and 366 nm. **Results:** Glycyrrhizin was identified in all preparations, with R_f values ranging from 0.26 to 0.30. The milk extract (Y+M) showed the highest glycyrrhizin content (35,523.1 AU), followed by aqueous extract (31,329.1 AU) and *ksheerapaka* (28,220.9 AU). **Conclusion:** This study provides preliminary evidence supporting the Ayurvedic rationale of *anupana*. Milk appears to enhance glycyrrhizin availability, likely due to its amphipathic nature, highlighting its role in modulating herbal efficacy. Further *in vivo* and pharmacokinetic studies are warranted to validate these findings and optimize formulation strategies.

Keywords: *Yashtimadhu*, *Glycyrrhiza glabra*, *Medhya Rasayana*, Glycyrrhizin, *Anupana*, HPTLC.

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INTRODUCTION

Medhya Rasayana, as outlined in the *Charaka Samhita* of *Ayurveda*, refers to a class of herbal formulations renowned for their cognitive-enhancing properties. The term '*Medhya*' denotes agents that improve intellect, while '*Rasayana*' signifies rejuvenation therapies in Ayurveda that, when used consistently, are believed to support memory, intelligence, vitality, and overall well-being.[1] This concept highlights the use of various nootropic herbs in distinct forms, such as the fine powder (*churna)* of *Yashtimadhu* (*Glycyrhhiza glabra* Linn.) with milk, undiluted juice (*Swarasa*) of *Mandukaparni* (*Centella asiatica* Linn.) and *Guduchi* (*Tinospora cordifolia* Miers.), as well as the paste form (*Kalka*) of *Shankhapushpi* (*Convolvulus pluricaulis* Choisy.) It also underscores that the pharmacologically active components responsible for nootropic effects are optimally expressed only when the herbs are prepared and administered in their specific traditional forms.

Among all the *Medhya rasayana, Yashtimadhu* is a commonly used herb proven to improve cognition and memory.[2,3] The combination of *Yashtimadhu* powder with milk (*ksheera anupana*) holds special therapeutic significance. Milk, in this context, is not merely a carrier but functions as an *anupana*, an Ayurvedic adjuvant believed to modulate the pharmacological activity and absorption of the herb. An *anupana* in Ayurveda has numerous roles, out of which, enhancing bio-availability, target delivery and distribution, mitigation of side effects and drug toxicity, synergism, dosha-specific effectiveness are the main functions.[4]

Despite this traditional knowledge, the precise role of milk in influencing the therapeutic efficacy of *Yashtimadhu* particularly its interaction with key phytoconstituents such as glycyrrhizin remains scientifically underexplored. Glycyrrhizin, the principal active component of *Yashtimadhu*, exhibits limited oral bioavailability due to its amphipathic molecular structure, possessing both hydrophilic and hydrophobic domains.[5,6] This raises the possibility that milk may facilitate either the enhanced

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release or improved absorption of glycyrrhizin, thus potentiating the herb's *Medhya* effect.

To explore this hypothesis, a comparative HPTLC fingerprinting study of different *Yashtimadhu* preparations namely aqueous extract, milk extract and *ksheera paka* (ayurvedic milk-based extraction) against the standard glycyrrhizin is proposed. Such an approach can provide valuable insights into the influence of extraction media and *anupana* on the availability of glycyrrhizin, potentially bridging the gap between Ayurvedic theory and modern phytochemical understanding.

This study is thus warranted to scientifically validate the traditional claims and to better understand the pharmacological synergy between *Yashtimadhu* and milk, with implications for optimizing dosage forms and therapeutic outcomes in Ayurvedic practice.

MATERIALS AND METHODS

Materials

The raw drug *Yashtimadhu* and *Yashtimadhu churna* were procured from GMP certified pharmacy and authenticated by experts at AYUSH approved Drug Testing Laboratory KAHER Shri BMK Ayurveda Mahavidhyalaya, Belagavi. Milk (*Ksheera*) was procured from the local market (Nandini Homogenized pasteurized cow milk). The marker compound glycyrrhizin, was procured from Yucca Enterprisers, Mumbai.

The TLC study was carried out in AYUSH approved Drug Testing Laboratory KAHER Shri BMK Ayurveda Mahavidhyalaya, Belagavi. HPTLC study was done at Center of Medicinal Plants Research (CMPR) - Arya Vaidya Sala (AVS) Kottakkal, Kerala.

Methods

Preparation of the study samples

Sample 1: Yashtimadhu phanta (Hot aqueous extract of Yashtimadhu: Y+W)

6 g of fine powder of *Yashtimadhu* was weighed and mixed with 48 mL of boiled drinking water. The mixture was stirred well and was kept undisturbed till the mixture attained room temperature. Later the contents were macerated well and then filtered.

Sample 2: Yashtimadhu Medhya Rasayana (Y+M)

6 g of fine powder of *Yashtimadhu* was weighed and taken. 48 mL of boiled cow's milk was added. The contents were stirred well.

Sample 3: Yashtimadhu ksheera paka (YKP)

6 g of coarse powder of *Yashtimadhu* was mixed with eight parts of cow's milk (48 mL) and 32 parts of boiled drinking water (192 mL).[7] All the contents were made as a mixture together and boiled in mild fire with vessel open until the milk portion in the mixture remained. Later the contents were strained and subjected for further analyses.

Chromatographic Study: Thin Layer Chromatography

Raw drug Test solution preparation for TLC and HPTLC

5 g of *Yashtimadhu* coarse powder was taken in flask and 100 mL methanol was added in it. Flask was kept in orbital shaker incubator for 6 hr followed by rest for 24 hr. Later the mixture was filtered through filter paper and kept for drying on hot water bath. Sample extract was collected after drying.

Final samples test solution preparation TLC and HPTLC

All the three samples i.e. Sample 1 (*Yashtimadhu* + water), Sample 2 (*Yashtimadhu*+milk) and Sample 3 (*Yashtimadhu Ksheerpaka*) were taken in the quantity of 20 mL each.[8] Each sample was taken in flask and 50 mL methanol was added. Flasks were kept in orbital shaker incubator for 6 hr then were kept aside with intermittent shaking. After 24 hr, mixture was filtered through Whatman filter paper and kept for drying on hot water bath. Sample extracts were collected after drying (10% Extracts were taken).

Procedure of TLC

Test Solutions

The above extracts of raw drug and final samples were mixed with a sufficient quantity of methanol to obtain clear solutions, which were used as the test solutions for TLC analysis.

Mobile Phase

n-Butanol: Glacial Acetic Acid: Water (7:2:1, v/v/v).

Chromatographic Conditions

TLC was performed on precoated silica gel 60 F_{254} plates with a uniform thickness of 0.2 mm. A volume of 10 µL of each test solution was applied as a discrete spot or band onto the plate. The plates were developed in a twin trough chamber pre-saturated with the mobile phase, and the development was carried out up to a distance of 8 cm from the point of application.

Visualization

Following development, plates were air-dried and visualized under ultraviolet light using a TLC UV cabinet at both short wavelength (254 nm) and long wavelength (366 nm). The R_f values and the color of resolved bands were recorded for each extract.

Based on the TLC bands in 254 nm and 366 nm and qualitative confirmation of R_f values corresponding to active constituent glycyrrhizin further proceeded for HPTLC fingerprinting analysis.

HPTLC Study

All solvents and reagents used in the study were of HPTLC grade to ensure analytical precision. Glycyrrhizin, used as the reference standard, was procured from Yucca Enterprisers, Mumbai (99% purity). HPTLC analysis was conducted using a CAMAG Linomat 5 system (Model: Linomat5_160447, S/N 160447, Version 1.00.13) equipped with an automatic TLC sample applicator. The system also included a CAMAG TLC Visualizer and a CAMAG Automatic Developing Chamber, operated via winCATS software (version 1.4.1). A 100 µL Hamilton syringe was employed for precise sample loading, and 5 µL aliquots of each sample extract were applied to aluminum-backed precoated silica gel 60 F_{254} plates (Merck) using a CAMAG Pro automatic TLC applicator to ensure uniform spotting.

Chromatographic Conditions

The chromatographic separation was carried out using a twin trough glass chamber (20 × 10 cm, CAMAG). The mobile phase consisted of *n-butanol: water: glacial acetic acid* in the ratio of 7:2:1 (v/v/v). Plates were developed to a migration distance of 80 mm, facilitating optimal resolution of the analytes on the silica gel stationary phase.

Following development, plates were air-dried and visualized under UV light at 254 nm and 366 nm using a CAMAG UV cabinet. Densitometric scanning was performed using a CAMAG TLC Scanner 3 in absorbance mode at the same wavelengths, managed via winCATS software (version 1.4.1). A total of five tracks were scanned, each spaced 15.5 mm apart. Scanning was carried out at a speed of 20 mm/s with a slit dimension of 6.00 mm × 0.30 mm. The analysis yielded distinct chromatographic profiles for each sample, with R_f values, number of peaks, and peak areas recorded for comparative evaluation.

RESULTS

Macroscopic Evaluation

The organoleptic evaluation of raw Yashtimadhu confirmed the use of root as the medicinal part. It exhibited a yellowish-brown color, sweet taste and characteristic odour, consistent with standard descriptions.

TLC Results

Thin-layer chromatographic analysis under UV light at 254 nm and 366 nm revealed multiple resolved bands across all samples and the raw *Yashtimadhu* extract. The standard data of glycyrrhizin exhibited a R_f value of 0.25, at 254 nm, which was consistently observed in all test samples (Y+W, Y+M and YKP) as well as in the raw drug under both wavelengths, confirming the presence of glycyrrhizin in each preparation.

In addition to the glycyrrhizin band, each sample displayed distinct patterns of other phytoconstituent bands, indicating variations in compound profiles depending on the extraction medium. Sample 2 (Y+M) and Sample 3 (YKP) showed more resolved bands at higher R_f values (up to 0.96 at 366 nm), suggesting enhanced extraction of non-polar compounds in the presence of milk or during *ksheerpaka* preparation as evident in Figures 1, 2 and Table 1.

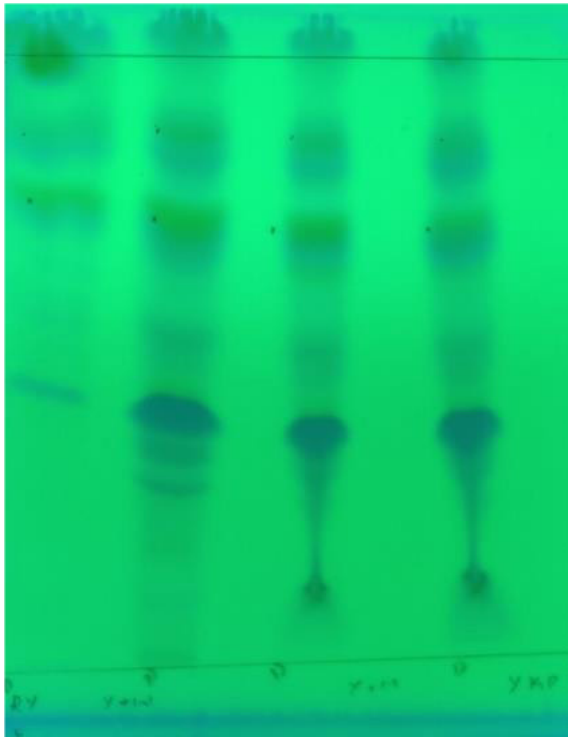

Figure 1: TLC of raw drug and all samples at 254 nm.

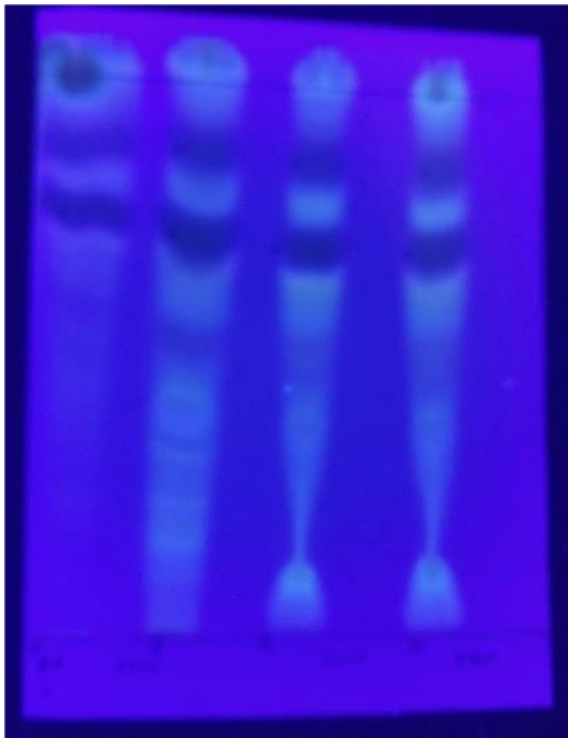

Figure 2: TLC of raw drug and all samples at 366 nm.

Table 1: **TLC Results of raw drug and all final samples.**

Raw Yashtimadhu		Sample 1 (Y+W)		Sample 2 (Y+M)		Sample 3 (YKP)	
254 nm	366 nm	254 nm	366 nm	254 nm	366 nm	254 nm	366 nm
0.12	0.50	0.12	0.17	0.18	0.13	0.13	0.13
0.25	0.67	0.18	0.25	0.25	0.38	0.25	0.40
0.36	0.87	0.25	0.67	0.42	0.67	0.42	0.67
0.51		0.47	0.77	0.60	0.76	0.56	0.82
0.77		0.67	0.85	0.81	0.83	0.67	0.96
		0.85			0.93	0.75	
		0.96				0.93	

HPTLC Results

This section presents the HPTLC fingerprinting analysis of five samples, examined under ultraviolet illumination at 254 nm and 366 nm.

Standard marker compound Glycyrrhizin

The HPTLC chromatographic profile of the standard marker compound glycyrrhizin was evaluated at two wavelengths 254 nm and 366 nm to assess its retention behavior and spectral response. At 254 nm, glycyrrhizin exhibited a well-defined chromatogram comprising multiple peaks. The principal peak was observed at R_f=0.30, with a peak height of 393.4 AU and area of 8133.1 AU, accounting for 55.33% of the total peak area, confirming it as the characteristic band for glycyrrhizin. (Figure 3) Additional peaks at R_f=0.26 (10.83% area) and R_f=0.48 (17.92% area) likely correspond to structurally related saponins or minor constituents commonly associated with glycyrrhizin-rich extracts as depicted in Figure 4 and Table 2.

In contrast, scanning at 366 nm revealed a single peak at R_f=0.95, which contributed 100% of the detected area (1508.8 AU) (Figure 5). This peak does not coincide with the primary R_f of glycyrrhizin identified at 254 nm, indicating that the compound detected at 366 nm is not native glycyrrhizin as evident from Figure 6 and Table 2. Due to the lack of R_f correspondence and diagnostic specificity, the 366 nm findings were not considered reliable for glycyrrhizin identification and were thus excluded from final interpretation.

Raw *Yashtimadhu*

HPTLC analysis of raw *Yashtimadhu* was also done, which revealed eleven distinct peaks at 254 nm, indicating a diverse phytochemical composition. A notable peak at R_f 0.28, with a substantial area, corresponded to glycyrrhizin.

Sample 1 (Y + W)

It revealed a significant peak at R_f 0.29 with an area of 31329.1 AU, which closely matches the major glycyrrhizin peak in the standard. This strong signal indicates the presence of glycyrrhizin in considerable quantity as evident from Figure 7 and Table 3.

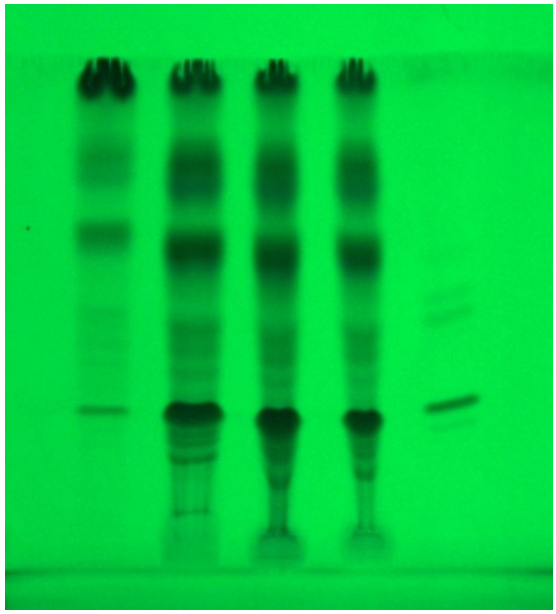

Figure 3: HPTLC Bands at 254 nm.

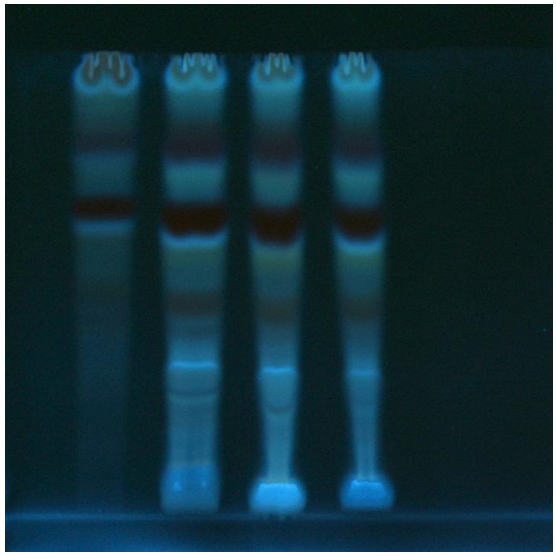

Figure 4: HPTLC Bands at 366 nm.

Sample 2 (Y + M)

It displayed a prominent peak at R_f 0.28 with an area of 35523.1 AU. This peak correlate precisely with the glycyrrhizin marker peak, suggesting that Sample 2 contains the highest concentration of glycyrrhizin among the tested samples as in Figure 8 and Table 3.

Sample 3 (YKP)

It also exhibited a notable peak at Rf 0.27 with an area of 28220.9 AU, also within the range of glycyrrhizin's Rf. While glycyrrhizin is present, its quantity is relatively lower compared to Samples 1 and 2 as shown in Figure 9 and Table 3.

Table 2: HPTLC Areas and peaks of Marker Compound Glycyrrhizin.

HPTLC: Areas and peaks of Marker Compound Glycyrrhizin at 254 nm									
Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	0.16	2.4	0.20	15.2	2.38	0.22	10.9	430.6	2.93
2	0.23	13.5	0.26	71.7	11.21	0.28	43.2	1592.1	10.83
3	0.28	43.3	0.30	393.4	61.50	0.35	9.3	8133.1	55.33
4	0.42	6.6	0.48	83.1	12.98	0.50	35.6	2634.5	17.92
5	0.51	36.0	0.52	54.6	8.53	0.55	5.4	1335.8	9.09
6	0.58	7.8	0.60	21.7	3.40	0.63	4.7	574.1	3.91
HPTLC: Areas and peaks of Marker Compound Glycyrrhizin at 366 nm									
1	0.91	12.2	0.95	38.5	100.00	0.98	25.4	1508.8	100.00

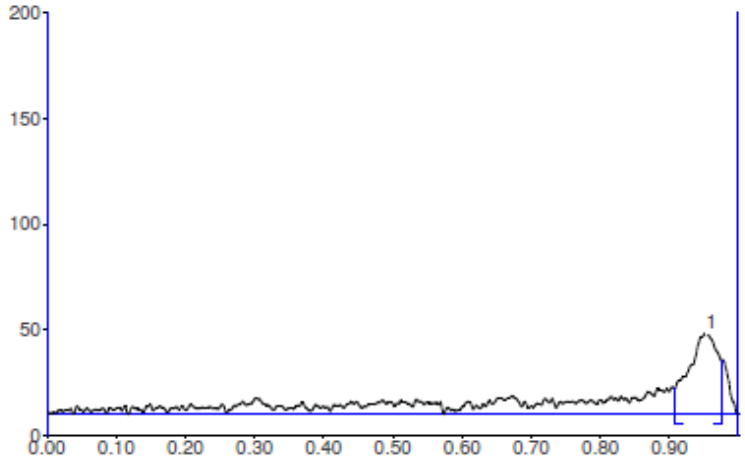

Figure 5: Standard glycyrrhizin densitogram at 366 nm.

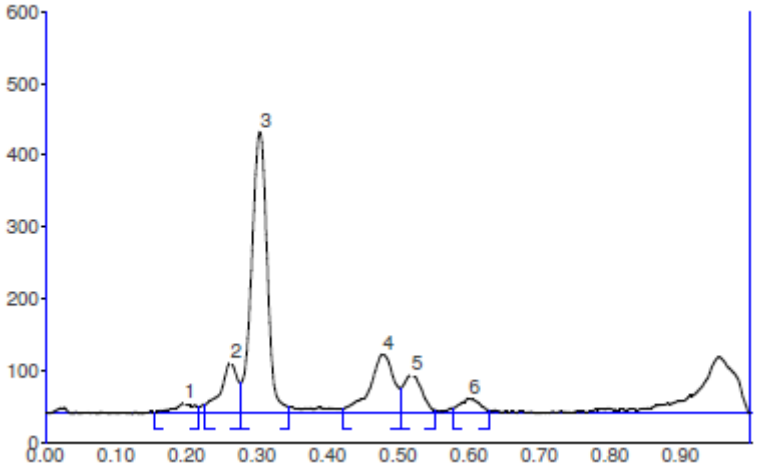

Figure 6: Standard glycyrrhizin densitogram at 254 nm.

Table 3: HPTLC Areas and peaks of methanolic extract of Sample 1, 2 and 3 at 254 nm.

Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
Sample 1									
1	0.01	6.0	0.04	37.1	0.77	0.06	6.3	894.7	0.33
2	0.07	6.4	0.09	169.7	3.53	0.11	63.3	2707.5	0.99
3	0.13	65.5	0.19	340.1	7.08	0.20	233.8	10441.7	3.81
4	0.21	234.3	0.24	398.7	8.30	0.25	382.6	10992.4	4.01
5	0.25	384.0	0.29	655.1	13.64	0.33	185.6	31329.1	11.43
6	0.33	185.7	0.36	264.5	5.51	0.38	230.1	8697.4	3.17
7	0.38	230.5	0.40	355.5	7.40	0.44	200.0	25822.0	9.42
Sample 2									
1	0.01	0.8	0.05	182.5	5.10	0.08	46.7	5187.2	2.07
2	0.11	52.2	0.15	208.0	5.81	0.17	193.0	6798.4	2.71
3	0.21	348.1	0.28	581.5	16.24	0.32	155.5	35523.1	14.18
4	0.32	156.0	0.34	207.7	5.80	0.36	190.9	6053.5	2.42
5	0.36	191.1	0.40	285.4	7.97	0.40	282.9	8522.8	3.40
6	0.40	283.1	0.42	301.4	8.41	0.47	198.8	15113.7	6.03
Sample 3									
1	0.01	0.9	0.04	161.9	5.46	0.05	120.7	3572.7	2.12
2	0.05	121.3	0.06	150.6	5.08	0.10	50.5	3072.8	1.82
3	0.12	62.8	0.16	227.1	7.66	0.18	179.9	7300.3	4.32
4	0.23	284.7	0.27	578.4	19.50	0.32	130.2	28220.9	16.72
5	0.32	130.3	0.34	168.5	5.68	0.36	151.1	5082.1	3.01
6	0.36	151.3	0.43	258.9	8.73	0.48	151.3	19391.5	11.49

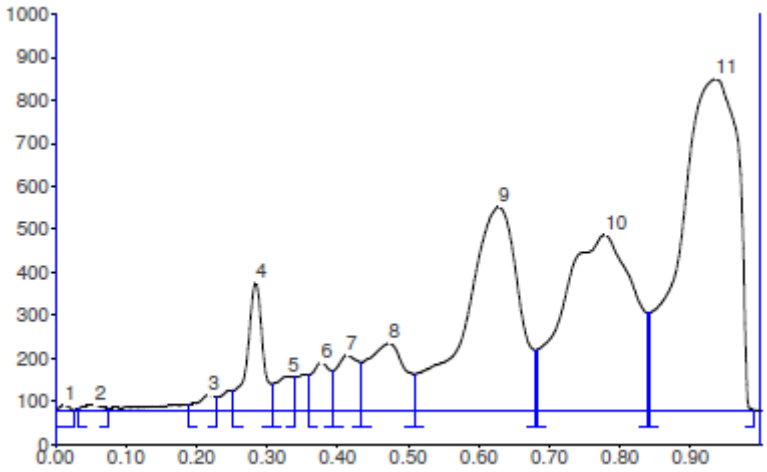

Figure 7: Sample 1 (Y+W) densitogram at 254 nm.

HPTLC analysis was performed to assess the presence and relative concentration of glycyrrhizin in the methanolic extracts of three samples, using standard glycyrrhizin as a qualitative reference. All samples exhibited peaks within the standard R_f range (0.26-0.30), confirming the presence of glycyrrhizin. Sample 2 showed the highest content (R_f 0.28; area 35,523.1 AU), followed by Sample 1 (R_f 0.29; 31,329.1 AU, ~88% of Sample 2), indicating high presence, and Sample 3 (R_f 0.27; 28,220.9 AU, ~79% of Sample 2), indicating moderate presence as summarized in Table 4.

DISCUSSION

The present study investigated the glycyrrhizin content in various traditional preparations of *Yashtimadhu*, a well-established *Medhya Rasayana* in Ayurveda, through comparative HPTLC

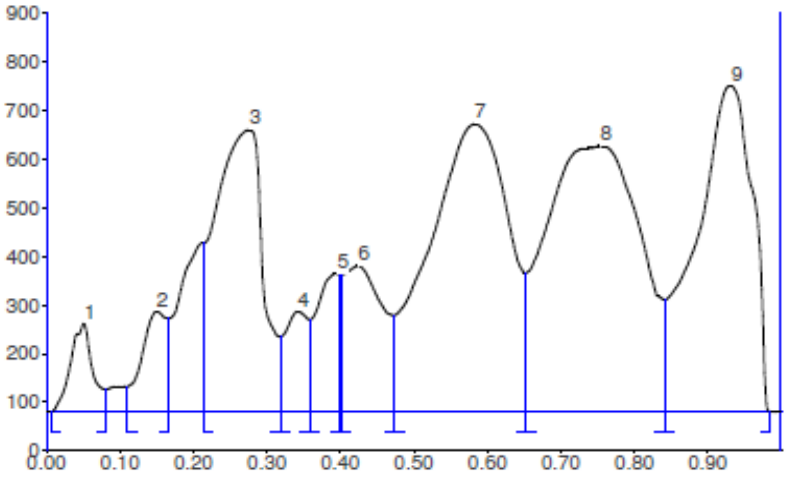

Figure 8: Sample 2 (Y+M) densitogram at 254 nm.

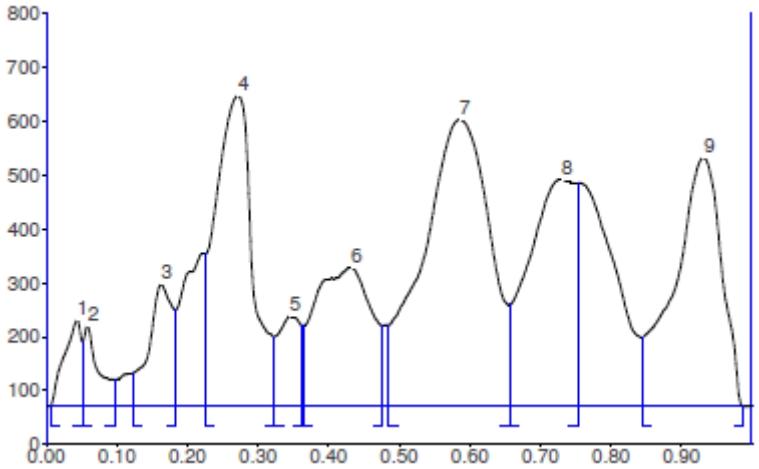

Figure 9: Sample 3 (Y KP) densitogram at 254 nm.

Table 4: Comparative Summary of HPTLC Profiles with Reference to Std. Glycyrrhizin.

Sample	Glycyrrhizin R_f Range	Peak R_f	Area (AU)	Interpretation
Std. Glycyrrhizin	0.26-0.30	0.30	8133.1	Reference standard
Sample 1	0.26-0.30	0.29	31329.1	High presence
Sample 2	0.26-0.30	0.28	35523.1	Highest content
Sample 3	0.26-0.30	0.27	28220.9	Moderate presence

profiling. The study aimed to explore the impact of different extraction media specifically water, milk and on the presence and availability of glycyrrhizin, the principal bioactive component associated with the herb's nootropic activity.

Our findings confirmed the consistent presence of glycyrrhizin across all preparations, evidenced by peaks within the R_f range of 0.26-0.30, when compared against the standard marker compound (R_f 0.30; area 8133.1 AU). Among the samples analyzed, the milk extract (Sample 2) demonstrated the highest glycyrrhizin content (R_f 0.28; area 35,523.1 AU), followed by the aqueous extract (Sample 1; R_f 0.29; area 31,329.1 AU) and *ksheerapaka* (Sample 3; R_f 0.27; area 28,220.9 AU). These findings suggest that milk, either alone or as a decoction medium, enhances glycyrrhizin availability in *Yashtimadhu* preparations.

Milk's role as an *anupana* is highlighted in Ayurvedic pharmaceutics for its capacity to enhance bioavailability, facilitate targeted delivery and reduce toxicity of herbs.[9] Modern pharmacokinetic studies have supported this by demonstrating the absorption enhancing effects of milk for various lipophilic and amphipathic compounds.[10] Glycyrrhizin, being amphipathic

in nature, has limited oral bioavailability due to poor membrane permeability and first-pass metabolism.[11,12] Our data support the hypothesis that milk-based extraction may facilitate solubilization and/or stabilization of glycyrrhizin, thereby enhancing its extractability.

Furthermore, the enhanced band resolution at higher R_f values (up to 0.96 at 366 nm) in the milk-based preparations suggests co-extraction of additional non-polar phytoconstituents. This aligns with previous findings where milk decoctions resulted in broader phytochemical diversity compared to aqueous extracts, which may contribute to synergistic therapeutic effects.[13] Though the 366 nm scan in standard glycyrrhizin revealed a prominent band at R_f 0.95, which did not correspond with glycyrrhizin's known retention behavior at 254 nm, and thus it was excluded from glycyrrhizin quantification. This is consistent with prior studies indicating poor UV fluorescence of glycyrrhizin at higher wavelengths.[14]

The observation that glycyrrhizin content was highest in the milk extract rather than the *ksheerapaka* suggests that prolonged heat exposure during traditional decoction may lead to partial degradation or transformation of glycyrrhizin or its associated saponins. This hypothesis aligns with earlier reports indicating thermal sensitivity of glycyrrhizin and its derivatives.[15]

Collectively, these results lend scientific credibility to the traditional Ayurvedic assertion that milk enhances the therapeutic efficacy of *Yashtimadhu*. More specifically, the use of milk as an *anupana* or extraction medium appears to optimize the phytochemical yield of glycyrrhizin, potentially improving the herb's cognitive-enhancing properties as described in the *Medhya Rasayana* context.

Further studies using pharmacokinetic modeling, LC-MS quantification and *in vivo* bioavailability assessments are warranted to confirm these findings and elucidate the precise mechanisms by which milk modulates glycyrrhizin's pharmacological profile.

CONCLUSION

This study analytically validates the traditional Ayurvedic concept that the method of formulation and choice of *anupana* critically influence the phytochemical yield of *Yashtimadhu*. HPTLC profiling demonstrated consistent presence of glycyrrhizin across all preparations, with the milk-based extract (Y+M) yielding the highest concentration, indicating superior extraction efficiency compared to the aqueous and *ksheerapaka* preparations.

These findings suggest that milk, owing to its amphipathic composition, may enhance the solubility and potential bioavailability of glycyrrhizin. This underscores the relevance of traditional Ayurvedic processing techniques in optimizing therapeutic outcomes. Further pharmacokinetic and biological studies are warranted to elucidate the mechanistic role of milk as an adjuvant and to support its integration into evidence-based formulations targeting cognitive enhancement

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

The present work was carried out under the funds sanctioned by CCRAS for SPARK project.

ABBREVIATIONS

TLC: Thin Layer Chromatography; **HPTLC:** High performance thin layer chromatography.

SUMMARY

This study investigates the *Ayurvedic Anupana* concept, that milk improves the therapeutic effects of *Yashtimadhu* (*Glycyrrhiza glabra* Linn.), a well-known *Medhya Rasayana* used to improve cognition. The study quantitatively compared the glycyrrhizin, a crucial bioactive compound for improved cognition, in *Yashtimadhu* extracts made with water, milk and *ksheerapaka* (milk-based decoction) using High-Performance Thin Layer Chromatography (HPTLC). According to the results, milk extract had the highest levels of glycyrrhizin followed by aqueous extract and *ksheerapaka*. The amphipathic nature of milk, which probably increases solubility and extraction efficiency, is responsible for the increased glycyrrhizin yield in the milk-based preparation. These results highlight the role of milk in increasing the bioavailability of active phytoconstituents and offer scientific evidence for its use as an *anupana* (adjuvant) in *Ayurveda*. The study underscores the importance of traditional preparation methods and suggests the need for further pharmacokinetic and *in vivo* studies to fully understand and optimize the therapeutic potential of *Yashtimadhu* formulations.

REFERENCES

1. Kulkarni R, Girish KJ, Kumar A. Nootropic herbs (Medhya Rasayana) in Ayurveda: an update. Pharmacogn Rev. 2012; 6(12): 147-53. doi: 10.4103/0973-7847.99949, PMID 23055641.

2. Chitrakar MY, Pawar V, Wange M, Bhapkar VK. A systematic review investigating the impact of *Glycyrrhiza glabra* on cognitive function and memory: A comprehensive analysis. J Nat Rem. 2024: 2667-77. doi: 10.18311/jnr/2024/44205.
3. Chakravarthi KK, Avadhani R. Beneficial effect of aqueous root extract of *Glycyrrhiza glabra* on learning and memory using different behavioral models: an experimental study. J Nat Sci Biol Med. 2013; 4(2): 420-5. doi: 10.4103/0976-9668.117025, PMID 24082744, PMCID PMC3783792.
4. The science behind Anupana: Ayurveda's approach to safe and effective medicine; 2024. CayeIT. Available from: cayeit.com.
5. Jin S, Fu S, Han J, Jin S, Lv Q, Lu Y, *et al.* Improvement of oral bioavailability of glycyrrhizin by sodium deoxycholate/phospholipid-mixed nanomicelles. J Drug Target. 2012; 20(7): 615-22. doi: 10.3109/1061186X.2012.702770, PMID 22726209.
6. Selyutina OY, Polyakov NE. Glycyrrhizic acid as a multifunctional drug carrier–from physicochemical properties to biomedical applications: A modern insight on the ancient drug. Int J Pharm. 2019; 559: 271-9. doi: 10.1016/j.ijpharm.2019.01.047, PMID 30690130.
7. Kushwaha IS, Behera B, Bhushan V. Mohapatra S. HPTLC study of Yashtimadhu (*Glycyrrhiza glabra*) – an approach for raw drug standardization. World J Ayurvedic Sci. 2017; 2(2): 168-73.
8. Kushwaha IS, Behera B, Bhushan V. Mohapatra S. HPTLC study of Yashtimadhu (*Glycyrrhiza glabra*) – an approach for raw drug standardization. World J Ayurvedic Sci. 2017; 2(2): 168-73.
9. Sharma PV. Charaka Samhita. Vol. 2. Varanasi: Chaukhambha Orientalia; 2001. p. 273-5.
10. Sabu MC, Kuttan R. Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. J Ethnopharmacol. 2002; 81(2): 155-60. doi: 10.1016/s0378-8741(02)00034-x, PMID 12065146.
11. Liu J, Wang S, Liu H, Yang L, Nan G. Absorption, distribution, metabolism and excretion of glycyrrhizin and its metabolites in rats. Acta Pharmacol Sin B. 2013; 3(5): 360-5.
12. Iino N, Takai S, Yokota N, *et al.* A pharmacokinetic study of glycyrrhizin in rats: high exposure of 18β-glycyrrhetinic acid in liver and kidney after oral administration. Biol Pharm Bull. 2002; 25(7): 902-5.
13. Jadhav AN, Bhutani KK. Ayurveda and guggul: biological effects of *Commiphora mukul* (guggul) resin. Curr Sci. 2005; 88(4): 517-24.
14. Pawar HA. Lalitha KG. HPTLC profiling of glycyrrhizin and isoliquiritigenin in *Glycyrrhiza glabra* root extract. Pharmacogn Res. 2015; 7(2): 134-8.
15. Li C, Lin G, Zuo Z. Pharmacological effects and pharmacokinetics properties of *Radix Glycyrrhizae* (licorice): a review. Phytother Res. 2012; 26(7): 863-72.

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