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Original Article

HPTLC-Based Phytochemical Profiling and Quality Control Evaluation of *Sarivadi Lauha* with Relevance to Diabetic Neuropathy

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ABSTRACT

Background: *Sarivadi Lauha* is a classical Ayurvedic herbo-mineral formulation widely used in the management of metabolic disorders. Standardisation of such formulations is essential to ensure quality, safety, and reproducibility. **Objectives:** To evaluate the physicochemical parameters and develop an HPTLC fingerprint profile of *Sarivadi Lauha* tablets for analytical standardisation. **Materials and Methods:** *Sarivadi Lauha* tablets were evaluated for organoleptic characteristics and physicochemical parameters including pH, loss on drying, ash values, extractive values, hardness, friability, and disintegration time according to Ayurvedic Pharmacopoeia of India guidelines. Methanolic extract of the formulation was analysed by High-Performance Thin Layer Chromatography (HPTLC) using silica gel 60 F254 plates and a mobile phase consisting of toluene: ethyl acetate: formic acid (5:2:0.5 v/v/v). Densitometric scanning was performed at 254 nm and 366 nm. **Results:** Physicochemical parameters were within acceptable limits for herbo-mineral formulations. HPTLC analysis revealed multiple well-resolved peaks at characteristic R_f values indicating the presence of phenolic, flavonoid, tannin, coumarin, and terpenoid constituents. **Conclusion:** The developed physicochemical standards and HPTLC fingerprint provide reliable analytical parameters for quality control and authentication of Sarivadi Lauha.

Keywords: Ayurvedic formulation, Diabetic Neuropathy, HPTLC , Phytochemical fingerprinting, *Sarivadi Lauha*.

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INTRODUCTION

Standardisation and quality assurance of herbal and herbo-mineral formulations are essential to ensure their safety, efficacy, and reproducibility. Traditional Ayurvedic medicines contain multiple plant and mineral constituents whose therapeutic activity depends on proper authentication, processing, and batch-to-batch consistency. The World Health Organisation has emphasised the importance of quality control methods for medicinal plant materials to ensure purity and identity (World Health Organisation, 2011). High-Performance Thin Layer Chromatography (HPTLC) is widely used for phytochemical fingerprinting, detection of adulteration, and evaluation of formulation uniformity due to its simplicity, precision, and cost-effectiveness (Wagner & Bladt, 2009; Sethi, 1996).

Diabetic neuropathy is one of the most common chronic complications of diabetes mellitus and significantly contributes to morbidity and reduced quality of life (Pop-Busui *et al*., 2017). Persistent hyperglycaemia triggers multiple metabolic disturbances including activation of the polyol pathway, formation of Advanced Glycation End products (AGEs), oxidative stress, and microvascular dysfunction, ultimately leading to progressive nerve damage (Feldman *et al*., 2019; Vincent *et al*., 2004). Despite conventional glycaemic control strategies, adjunct therapies targeting oxidative and inflammatory pathways are being explored to provide comprehensive management (Tesfaye *et al*., 2010). Similar chromatographic approaches have also been applied for the quality control and standardisation of herbal formulations.

Sarivadi Lauha is a classical Ayurvedic herbo-mineral formulation described in *Bhaishajya Ratnavali* for the management of Prameha and its complications (Angadi, 2018). The formulation contains medicinal plants such as *Hemidesmus indicus, Tinospora cordifolia*, and *Terminalia chebula*, which are reported to possess antioxidant, anti-inflammatory, and immunomodulatory activities (Aneja *et al*., 2008).

These pharmacological properties are relevant in conditions associated with oxidative stress, inflammation, and metabolic dysfunction involved in diabetic neuropathy.

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Considering the complexity of its composition, establishing validated physicochemical parameters and chromatographic fingerprint profiles is necessary to ensure formulation integrity and reproducibility. Therefore, the present study aims to evaluate the physicochemical characteristics and develop an HPTLC fingerprint profile of *Sarivadi Lauha* to provide scientific evidence for its analytical standardisation.

Chromatographic fingerprinting has become an essential requirement for herbal drug standardisation and is recommended by regulatory authorities for ensuring batch-to-batch consistency of polyherbal formulations.

MATERIALS AND METHODS

Procurement and Authentication of Raw Materials

All raw materials of *Sarivadi Lauha* were procured from an authorised Ayurvedic pharmacy. The herbal ingredients were authenticated based on macroscopic and organoleptic characteristics in accordance with standards described in the Ayurvedic Pharmacopoeia of India (API). The herbo-mineral ingredient, *Lauha Bhasma* (*Kanta Loha Bhasma*), was evaluated as per the Pharmacopeial Laboratory for Indian Medicines (PLIM) guidelines and API specifications. The composition of the formulation is presented in Table 1.

Authentication of *Bhasma*

Kanta Loha Bhasma was evaluated as per the Pharmacopoeia Standards for Ayurvedic Formulations (PSAF) and the Ayurvedic Pharmacopoeia of India (API) standards (Table 2).

Method of Preparation

All ingredients were received in powdered form and individually weighed as per classical proportions described in *Bhaishajya Ratnavali* (Angadi, 2018). The powders were passed through a 60# sieve to ensure uniform particle size and removal of foreign matter. The sieved powders were blended uniformly in a mass mixer for 30 min.

A binding solution of Gum Acacia was prepared in distilled water and added gradually to obtain a uniform wet mass. The wet mass was dried in a tray dryer at a temperature not exceeding 60°C until adequate moisture reduction was achieved. The dried material was granulated, lubricated, and compressed into tablets using a tablet compression machine. In-process quality parameters such as tablet weight variation, hardness, and physical appearance were monitored during manufacturing. The finished tablets were stored in airtight containers for further analysis.

Analytical Parameters

High-Performance Thin-Layer Chromatography (HPTLC)

To evaluate its phytochemical profiling, the *Sarivadi Lauha* methanolic extract was analysed using HPTLC, a widely used technique for chromatographic fingerprinting of herbal medicines and polyherbal formulations (Zlatkis & Kaiser, 1977).

Preparation of Test Solution

The sample (2.5 g) was weighed and added to 20 mL of Methanol. Sonicate for 30 min and filter with simple filter paper. The filtrate is used as a Test solution and thus obtained for HPTLC fingerprinting. The details of HPTLC conditions are outlined in Table 3.

RESULTS

Macroscopic Analysis

The tablets are blackish. It has a characteristic taste and bitter odor.

Table 1 : Ingredients of *Sarivadi Lauha*.

Sl. No.	Ingredients	Part used	Quantity
1.	*Sariva*	Root	1 Part
2.	*Neelini*	Root/ Whole plant	1 Part
3.	*Rasana*	Leaf, Rhizome	1 Part
4.	*Guduchi*	Stem	1 Part
5.	*Ela*	Seed	1 Part
6.	*Chitraka*	Root bark	1 Part
7.	*Manakandasuran*	Rhizome	1 Part
8.	*Sankhini*	Leaves	1 Part
9.	*Trivrut*	Root bark, Leaf	1 Part
10.	*Suddha Bhallataka*	Fruit	1 Part
11.	*Haritaki*	Fruit	1 Part
12.	*Lauha Bhasma*		Part

Organoleptic Evaluation

The organoleptic analysis is as follows (Table 4).

Physicochemical Analysis

The physicochemical characterization of the test drug, *Sarivadi Lauha* tablet samples, was carried out in the analytical laboratory. The sample of test drugs was evaluated with various physicochemical parameters like tablet hardness, friability test, pH value, ash value, disintegration time, loss on drying, acid-insoluble ash, water-soluble extract, and alcohol-soluble extractive values. Physicochemical parameters were as in API (Ayurvedic Pharmacopoeia of India) and IP (Indian Pharmacopoeia) standards for the analysis of the tablet (*Sarivadi Lauha*).

Data Analysis

The observations of various physicochemical analyses are mentioned below (Table 5).

HPTLC Analysis

The HPTLC Chromatograph of *Sarivadi Lauha* developed at a wavelength of 254 nm with the volume of 10.0 µL and 20.0 µL is depicted in Figures 1 and 2 respectively. Chromatograph developed at a wavelength of 366 nm with a volume of 10.0 µL and 20.0 µL is depicted in Figures 3 and 4 showing the R_f Values,

Table 2: Authentication of *Lauha Bhasma*.

Sl. No.	Parameters	*Kant Loha Bhasma*
1.	Characteristic	Soft and Lustreless
2.	Colour	Dark red
3.	Consistency	Fine

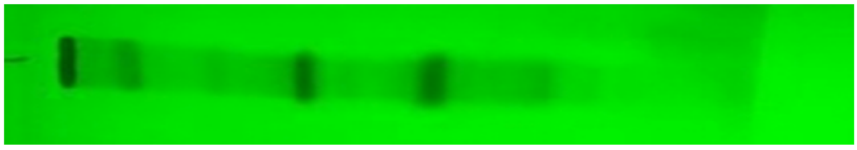

Figure 1a

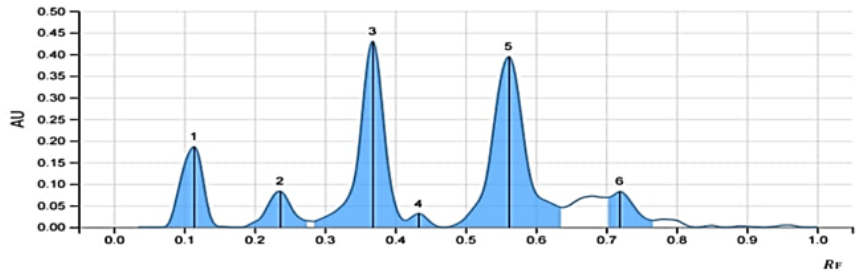

Figure 1b

Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.069	0.0000	0.114	0.1857	15.40	0.151	0.0016	0.00681	12.32
2	0.181	0.0000	0.236	0.0831	6.89	0.282	0.0135	0.00361	6.53
3	0.283	0.0134	0.368	0.4293	35.59	0.414	0.0158	0.01808	32.73
4	0.414	0.0158	0.433	0.0315	2.61	0.468	0.0000	0.00093	1.69
5	0.468	0.0000	0.561	0.3941	32.67	0.637	0.0455	0.02225	40.28
6	0.700	0.0686	0.719	0.0824	6.83	0.767	0.0156	0.00356	6.45

Figure 1c

Figure 1: HPTLC Chromatograph of *Sarivadi Lauha* @254 nm and 10.0 µL volume (A=Fingerprint, B= Peak height, C= Rf Value and Area percentage).

Chromatograph fingerprint, peak height, and percentage area. The obtained R_f values were compared with reported phytochemical data from literature and PubChem databases to identify probable compounds, which are presented in Table 6 along with their reported bioactivities.

DISCUSSION

HPTLC and physicochemical evaluation are important analytical tools for ensuring the quality, authenticity, and consistency of Ayurvedic formulations. Analytical techniques such as physicochemical testing and chromatographic fingerprinting are essential for evaluating the identity, purity, and consistency of herbal formulations. These methods detect contaminants, heavy metals, and pesticides, safeguarding consumer health. By standardising formulations, they support clinical research, enhance therapeutic efficacy, and advance evidence-based Ayurveda for better patient care (World Health Organization, 2011).

The physicochemical parameters of *Sarivadi Lauha* tablets were evaluated in accordance with the general quality control guidelines prescribed in the Ayurvedic Pharmacopoeia of India (API) (Ministry of AYUSH, 2016; Ministry of AYUSH, 2007). The pH value of 5.2 indicates a mildly acidic nature, which is acceptable for oral Ayurvedic formulations and supports formulation stability and gastrointestinal compatibility, as described under API, Part II, General Tests, pH determination (Section 2.3.3) The loss on drying at 110°C was found to be 4.42% w/w, which complies with API limits for finished dosage forms and indicates low moisture content and reduced susceptibility to microbial deterioration (API, Part II, General Tests, Loss on Drying; Section 2.4.1) (Ministry of AYUSH, 2007).

The total ash value (49.70% w/w) and acid-insoluble ash value (47.95% w/w) were comparatively high when assessed against purely herbal formulations; however, as per API guidelines, elevated ash values are expected and acceptable in herbo-mineral preparations containing *Lauha Bhasma* (API, Part I, General Notices for Herbo-mineral Formulations; Section 1.1.4) (Ministry of AYUSH, 2007). In such formulations, total ash reflects the inherent inorganic content rather than extraneous contamination, while acid-insoluble ash confirms the presence of stable mineral fractions, particularly iron-based components (API, Part II, General Tests, Ash Values; Sections 2.4.7 and 2.4.8) (Ministry of AYUSH, 2007; Lohar, 2007). The water-soluble extractive value (40% w/w) exceeded the alcohol-soluble extractive value (23% w/w), consistent with API observations that formulations rich in polar phytoconstituents such as tannins and glycosides demonstrate higher aqueous extractability (API, Part II, Extractive Values; Sections 2.4.10 and 2.4.11) (Ministry of AYUSH, 2007).

Tablet evaluation parameters complied with API general requirements for solid oral dosage forms, including acceptable average weight, hardness (4 kg/cm²), zero friability, and a disintegration time of 7 min, ensuring adequate mechanical strength and timely drug release (API, Part II, General Tests for Tablets; Sections 2.5.1-2.5.4) (Ministry of AYUSH, 2007). Overall, the results confirm that the observed physicochemical characteristics align with API standards and substantiate the quality and authenticity of *Sarivadi Lauha* as a classical herbo-mineral formulation.

Diabetic neuropathy is a common complication of chronic hyperglycaemia and is characterised by progressive damage to peripheral nerves. Hyperglycaemia activates several metabolic pathways including the polyol pathway, formation of Advanced Glycation End products (AGEs), protein kinase C activation, and oxidative stress, which collectively lead to neuronal injury and microvascular dysfunction (Pop-Busui *et al*., 2017; Feldman *et al*., 2019; Vincent *et al*., 2004). These pathological mechanisms contribute to axonal degeneration, demyelination, and impaired nerve conduction.

The HPTLC fingerprint of *Sarivadi Lauha* demonstrated a phytochemical profile rich in phenolic and flavonoid constituents, which are mechanistically relevant to the pathogenesis of diabetic neuropathy. At 254 nm, the presence of four major peaks ($R_f \approx 0.37$, 0.48, 0.56, and 0.63) indicates a predominance of aromatic and conjugated polyphenols, characteristic of *Hemidesmus indicus, Tinospora cordifolia*, and *Terminalia chebula*. These compounds are known to exert strong antioxidant and antiglycation activities,

Table 3 : Chromatographic conditions for HPTLC of *Sarivadi Lauha*.

Chromatographic Conditions	
Application Mode	CAMAG Linomat 5 (S/N: 280008) Applicator
Filtering System	Simple filter
Stationary Phase	MERCK - HPTLC Silica gel 60 F_{254} on TLC plates
Application (Y axis) Start Position	8.0 mm
Development End Position	80 mm from the plate base
Sample Application Volume	10 µL and 20 µL
Distance Between Tracks	13.4 mm
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	20 minutes
Mobile Phase (MP)	Toluene: Ethyl acetate: Formic acid (5:2:0.5v/v/v)
Visualization	@ 254 nm and @366 nm
Drying Mode, Temp. & Time	At room temperature for 5 min

which are critical in attenuating oxidative stress and AGE formation—two central pathways involved in diabetic nerve damage (Feldman *et al*., 2019; Vincent *et al*., 2004).

At 366 nm, the prominent fluorescent band at $R_f \approx 0.48$ (area ≈ 29%) corresponds to coumarin and phenolic glycosides derived from *Sariva*, suggesting a major contribution of coumarin-rich fractions. Coumarins and related phenolics have been reported to suppress reactive oxygen species, inhibit NF-κB-mediated inflammation, and protect mitochondrial function, thereby limiting axonal degeneration and demyelination (Pop-Busui *et al*., 2017; Feldman *et al*., 2019).

The additional mid-range bands at $R_f \approx 0.56$ and 0.63 indicate flavonoid and tannin fractions from *Guduchi, Haritaki*, which are known to inhibit protein kinase C activation, improve microcirculation, and enhance neurotrophic signaling (Pop-Busui *et al*., 2017; Vincent *et al*., 2004). High-R_f bands (0.76-0.90) representing terpenoid and resinous fractions from Chitraka and Bhallataka further contribute to anti-inflammatory and immunomodulatory effects.

There is an increase in glucose in the polyol pathway; that results in the production of sorbitol due to the presence of aldose reductase. However, sorbitol accumulation along with NADPH loss causes osmotic stress and oxidation, which then leads to impaired function of the Schwann cells along with early axonal

Table 4: Organoleptic characteristics of *Sarivadi Lauha*.

Sl. No.	Parameters	Result
1.	Odor	Bitter
2.	Color	Dark brown
3.	Taste	Bitter
4.	Consistency	Solid

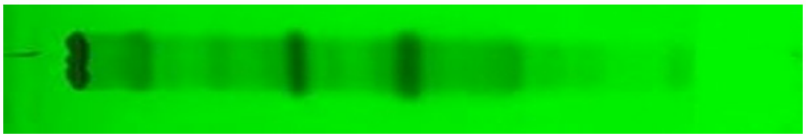

Figure 2a

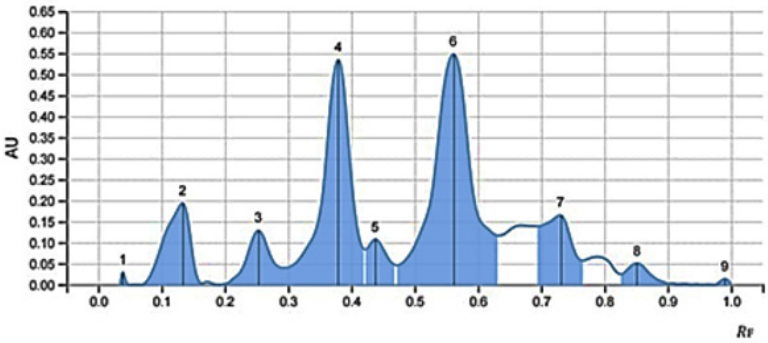

Figure 2b

Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.035	0.0000	0.039	0.0282	1.60	0.047	0.0000	0.00019	0.19
2	0.069	0.0000	0.133	0.1932	10.93	0.164	0.0032	0.00885	9.22
3	0.194	0.0000	0.253	0.1282	7.26	0.292	0.0399	0.00564	5.88
4	0.292	0.0399	0.379	0.5336	30.20	0.421	0.0839	0.02715	28.30
5	0.422	0.0838	0.438	0.1075	6.08	0.471	0.0463	0.00398	4.15
6	0.472	0.0462	0.561	0.5474	30.98	0.632	0.1167	0.03870	40.34
7	0.694	0.1387	0.731	0.1647	9.32	0.765	0.0570	0.00909	9.47
8	0.826	0.0247	0.851	0.0506	2.86	0.901	0.0024	0.00214	2.23
9	0.975	0.0000	0.990	0.0133	0.75	1.000	0.0028	0.00020	0.21

Figure 2c

Figure 2: HPTLC Chromatograph of *Sarivadi Lauha* @254 nm and 20.0 µL volume (A=Fingerprint, B= Peak height, C= Rf Value and Area percentage.

Table 5: **Physicochemical Parameters of *Sarivadi Lauha*.**

Sl. No.	Parameters	Result	Reference Standard (Ministry of AYUSH, 2016; Ministry of AYUSH, 2007; Lohar, 2007)
1.	pH	5.2	5.5-7.5
2.	Loss on drying (%w/w at 105°C)	4.42	≤ 15%
3.	Total Ash (%w/w)	49.70	High values expected in herbo-mineral formulations containing Lauha Bhasma
4.	Acid insoluble ash (%w/w)	47.95	(Variable)
5.	Water soluble extractive (%w/w)	40	15-30%
6.	Alcohol soluble extractive (%w/w)	23	20-40%
7.	Average tablet weight (g)	0.522	-
8.	Tablet hardness (Kg/cm^2)	4	4-12
9.	Friability test (%)	0	≤ 2%
10.	Disintegration time (min)	07	(Variable)

Figure 3a

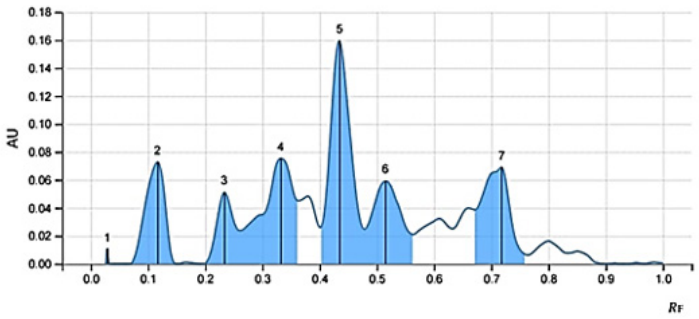

Figure 3b

Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.026	0.0000	0.029	0.0105	2.12	0.033	0.0000	0.00004	0.16
2	0.068	0.0000	0.117	0.0727	14.63	0.149	0.0000	0.00286	12.10
3	0.197	0.0000	0.233	0.0510	10.27	0.261	0.0237	0.00179	7.55
4	0.261	0.0237	0.332	0.0754	15.17	0.362	0.0450	0.00481	20.36
5	0.401	0.0260	0.435	0.1593	32.05	0.478	0.0247	0.00668	28.24
6	0.478	0.0247	0.515	0.0591	11.90	0.563	0.0210	0.00350	14.79
7	0.671	0.0386	0.718	0.0689	13.87	0.762	0.0062	0.00397	16.80

Figure 3c

Figure 3: HPTLC Chromatograph of *Sarivadi Lauha* @366 nm and 10.0 µL volume (A=Fingerprint, B= Peak height, C= Rf Value and Area percentage).

Table 6 : Rf (avg) value and probable compound, source drug and pharmacological action.

R_f (avg)	Area % (20 µL)	Visualisation	Probable Compound/ Phytochemical Class	Probable Source Drug (from formulation)	Reported Pharmacological Actions & References
0.05-0.10	~3-4 %	254 nm	Very polar small molecules - organic acids / salts	*Lauha Bhasma matrix, Guduchi* (*Acidic glycosides*)	Chelating ions, metal complexing, mild antioxidant activity due to phenolic acids and glycosides (Som *et al*., 2017).
0.16-0.18	~4 %	Both nm	Low-polarity phenolic compounds	*Guduchi, Haritaki, Neelini*	Immunomodulatory and antioxidant activities attributed to tinosporaside and gallic acid (Chaudhuri *et al*., 2018; Sharma *et al*., 2019).
0.27-0.32	~6 %	Both nm	Flavonoid glycosides (kaempferol, apigenin derivatives)	*Rasna, Ela, Guduchi*	Anti-inflammatory and digestive support from flavonoid glycosides (Singh *et al*., 2018; Gupta *et al*., 2017).
0.37-0.40	~15 %	254 nm strong absorbance	Coumarins / phenolic esters	*Sariva, Sankhini, Trivrut*	Hepatoprotective and antioxidant properties attributed to hemidesmin I/II and syringin (Sinha *et al*., 2018; Kumar *et al*., 2016).
0.48-0.50	29 %	Strong fluorescence at 366 nm	Major phenolic / coumarin compound (hemidesmin I/II, syringin)	*Sariva* (*Hemidesmus indicus*)	Hepatoprotective, antioxidant, anti-inflammatory, adaptogenic activity (Sinha *et al*., 2018; Kumar *et al*., 2016; Patel *et al*., 2020).
0.56	17 %	Both nm	Flavonoid aglycones (quercetin, rutin derivatives)	*Haritaki, Guduchi, Neelini*	Antioxidant and neuroprotective activity of quercetin and rutin (Srinivasan *et al*., 2018; Singh *et al*., 2019).
0.63-0.67	10-15 %	254 nm	Tannins/polyphenols (gallic and ellagic acids)	*Haritaki, Guduchi*	Astringent and antimicrobial effects from tannins and ellagic acid (Mandal *et al*., 2018; Shukla *et al*., 2020).
0.76-0.78	9-10 %	366 nm weak fluorescence	Terpenoids/steroids (β-sitosterol)	*Chitraka, Rasna, Suran*	Anti-inflammatory and digestive effects of β-sitosterol and plumbagin (Tiwari *et al*., 2018; Dubey *et al*., 2017).
0.90-0.92	8-9 %	254 nm	Non-polar pigments/resins/ triterpenes	*Suddha Bhallataka*	Immunostimulant and antimicrobial activity due to cardanol and anacardic acids (Kirti & Nandini, 2019; Singh *et al*., 2012).

damage. *Sarivadi Lauha* contains phenolic acids, glycosides, quercetin, gallic acid, and tinosporaside, which have been established to inhibit aldose reductase enzyme action along with antioxidant properties. The inhibition of aldose reductase along with a reduction in sorbitol content along with reinstatement of antioxidant action has been attributed to the potential of such plant compounds to reduce oxidative stress along with providing protection to peripheral nerves is outlined in Figure 5.

Hyperglycaemia leads to increased formation of AGEs products, which activate inflammatory signaling and cause progressive nerve damage. The HPTLC profile of *Sarivadi Lauha* showed the presence of coumarins, phenolic acids, syringin, and quercetin, which are known to reduce AGE formation and suppress NF-κB-mediated inflammation. By limiting AGE accumulation and inflammatory nerve injury, these phytochemicals help preserve neuronal structure and prevent further nerve damage. This suggests that *Sarivadi Lauha* may exert a protective effect in diabetic neuropathy by modulating the AGE pathway is outlined in Figure 6.

Chronic hyperglycaemia can elevate intracellular diacylglycerol concentrations, hence activating Protein Kinase C (PKC). PKC overactivation contributes to endothelial damage, decreased

Figure 4a

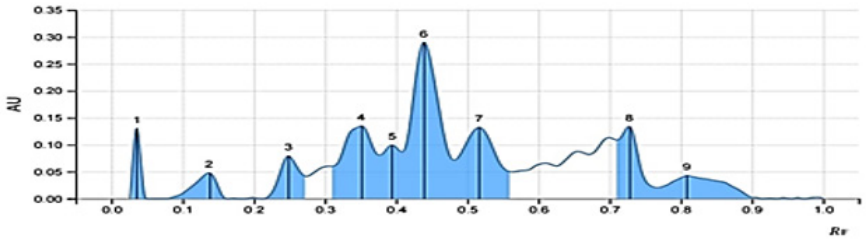

Figure 4b

Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.026	0.0000	0.036	0.1295	11.91	0.049	0.0000	0.00155	3.46
2	0.078	0.0000	0.138	0.0475	4.37	0.163	0.0000	0.00177	3.95
3	0.211	0.0000	0.249	0.0789	7.26	0.274	0.0413	0.00263	5.88
4	0.310	0.0601	0.351	0.1346	12.38	0.376	0.0806	0.00693	15.50
5	0.376	0.0806	0.394	0.0992	9.13	0.408	0.0866	0.00292	6.52
6	0.408	0.0866	0.439	0.2893	26.61	0.481	0.0718	0.01300	29.07
7	0.482	0.0716	0.517	0.1321	12.15	0.563	0.0494	0.00756	16.91
8	0.710	0.1088	0.728	0.1335	12.28	0.767	0.0206	0.00450	10.06
9	0.767	0.0206	0.808	0.0427	3.93	0.903	0.0021	0.00387	8.65

Figure 4c

Figure 4: HPTLC Chromatograph of *Sarivadi Lauha* @366 nm and 20.0 µL volume (A=Fingerprint, B= Peak height, C= R_f Value and Area percentage).

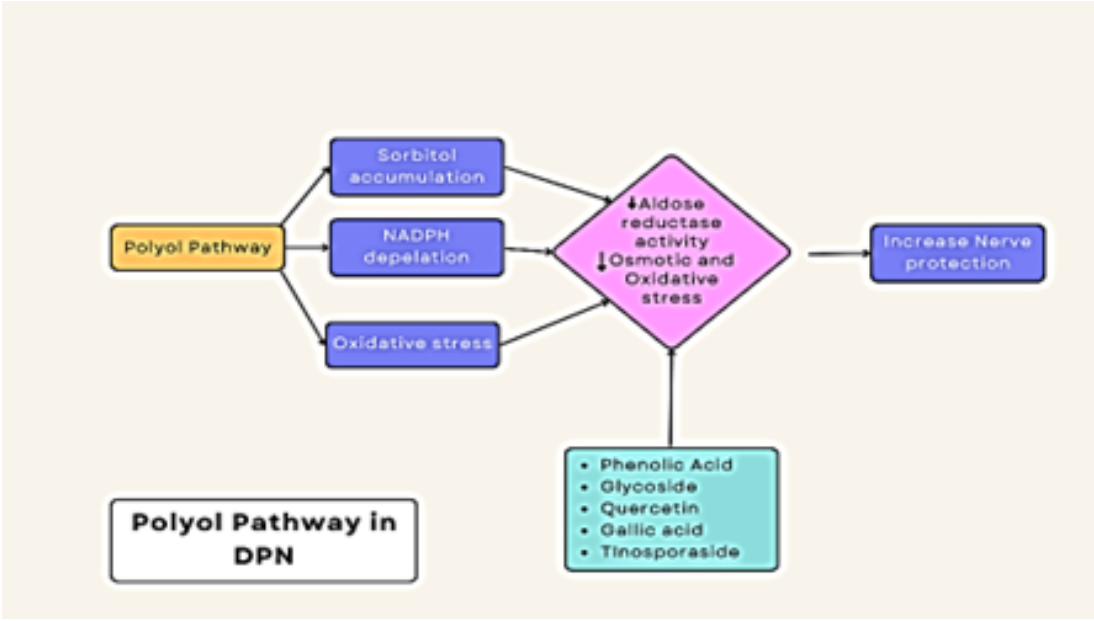

Figure 5: Probable action of *Sarivadi Lauha* on Polyol Pathway.

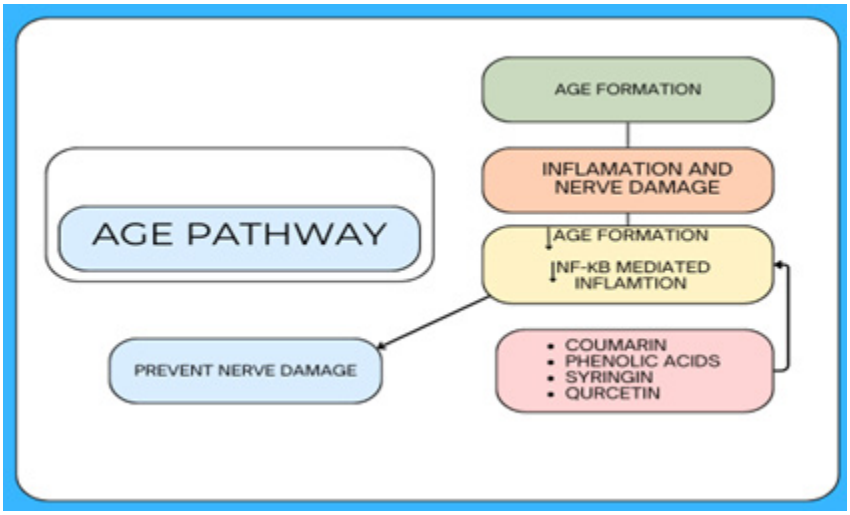

Figure 6: Probable action of *Sarivadi Lauha* on AGE Pathway.

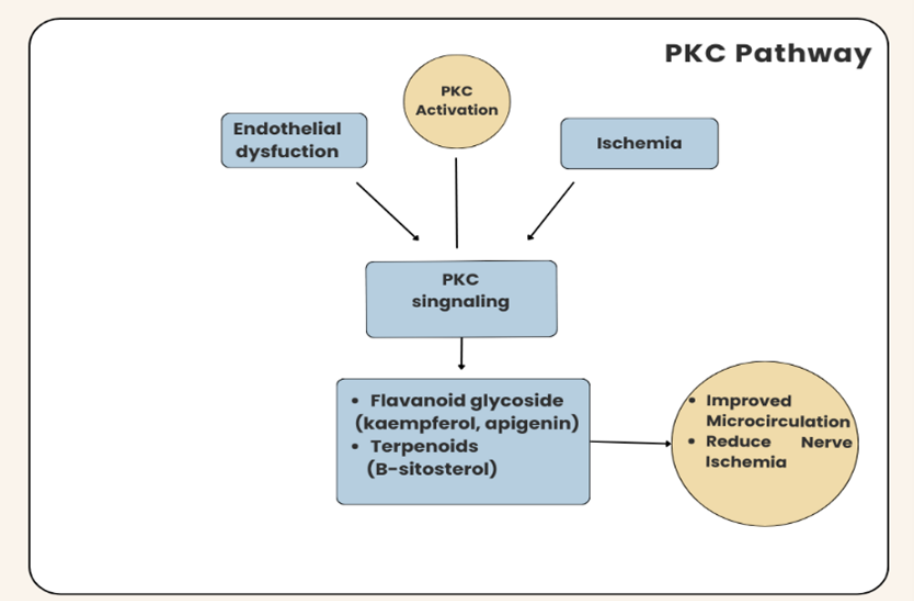

Figure 7: Probable action of *Sarivadi Lauha* on PKC Pathway.

nitric oxide production, impaired nerve blood flow, and microvascular ischemia, thereby contributing to progressive nerve damage (Koya & King, 1998; Geraldes & King, 2010). The HPTLC pattern of chemical analysis of *Sarivadi Lauha* indicated the existence of components like flavonoid glycosides and terpenes including β-sitosterol, which have been demonstrated to have potential effects of blocking protein kinase C signaling and hence endothelial dysfunction. These phytochemicals can hence protect against nerve injuries caused by ischemia due to their effects of reducing PKC overactivation and hence improving microcirculation is outlined in Figure 7.

Oxidative stress, rooted in chronic hyperglycaemia-induced mitochondrial overproduction of reactive oxygen species, has been identified as the central factor in the pathogenesis of diabetic neuropathy, leading to membrane lipid peroxidation, mitochondrial dysfunction, and axonal degeneration (Feldman *et al*., 2019; Vincent *et al*., 2004). *Sarivadi Lauha* has shown the presence of quercetin, coumarins, gallic acid, ellagic acid, and tinosporaside, which have been reported to have strong antioxidant activity. These phytochemicals scavenge ROS, thereby maintaining mitochondrial integrity, reducing oxidative damage to neuronal membranes, and retarding the progression of diabetic neuropathy by inhibiting oxidative stress. Chronic low-grade inflammation accelerates nerve injury in diabetic neuropathy by activating immune cells that account for the excessive release of TNF-α, IL-1β, and IL-6 (Pop-Busui *et al*., 2017; Vincent *et al*., 2011). The presence of tannins, polyphenols, and non-polar resinous fractions from *Bhallataka*, which exert immunomodulatory and anti-inflammatory actions.

CONCLUSION

Hence, the HPTLC profile demonstrates that *Sarivadi Lauha* contains multiple phytochemical classes capable of modulating oxidative stress, AGE formation, PKC activation, microvascular

ischemia, and neuroinflammation. This multi-targeted phytochemical composition provides a strong mechanistic basis for its potential neuroprotective and supportive role in diabetic neuropathy. Further analyses, such as gas chromatography, liquid chromatography, and experimental and clinical studies, are necessary to substantiate the therapeutic potential of *Sarivadi Lauha* in diabetic neuropathy.

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ABBREVIATIONS

R_f: Retention factor; **AGEs:** Advanced glycation end products; **NF-κB:** Nuclear factor kappa-light-chain-enhancer of activated B cells; **NADPH:** Nicotinamide adenine dinucleotide phosphate; **ROS:** Reactive oxygen species; **TNF-α:** Tumor necrosis factor alpha; IL-1β: Interleukin 1 beta; **IL-6:** Interleukin 6; **API:** Ayurvedic Pharmacopoeia of India; **PLIM:** Pharmacopeial Laboratory for Indian Medicines; **PSAF:** Pharmacopoeia Standards for Ayurvedic Formulations; **IP:** Indian Pharmacopoeia; **CAMAG:** Computer Assisted Measurement and Analysis of Gas; **HPTLC:** High Performance Thin Layer Chromatography; **PKC:** Protein Kinase C.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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PLAGIARISM STATEMENT

The manuscript has been checked using a standard plagiarism detection tool, and similarity was found within acceptable limits.

ETHICAL APPROVAL

Not applicable, as the study involved analytical evaluation of a marketed formulation and did not include human or animal subjects.

AUTHOR CONTRIBUTIONS

Denil Patel contributed to study design, experimental work, data analysis, and manuscript preparation. Haresh Soni supervised the research work, contributed to manuscript editing, and approved the final manuscript.

SUMMARY

This study examined the quality and phytochemical profile of *Sarivadi Lauha*, a classical Ayurvedic herbo-mineral formulation. Standard physicochemical tests confirmed that the formulation met acceptable quality parameters according to API guidelines. HPTLC analysis identified several phytochemical groups, including phenolics, flavonoids, tannins, coumarins, and terpenoids. These compounds are known for their antioxidant and neuroprotective properties, which may be helpful in conditions such as diabetic neuropathy. The findings provide useful analytical standards for the identification, authentication, and quality control of *Sarivadi Lauha*.

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