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DEVELOPMENT OF QUALITY PARAMETERS OF TALISADI CHURNA AND ITS MARKET FORMULATIONS

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ABSTRACT: Talisadi Churna is a classical Ayurvedic polyherbal formulation traditionally used in cough, bronchitis, fever, dyspepsia, anaemia and cardiac disorders. The present study was carried out for pharmacognostical, physicochemical and phytochemical evaluation of standard laboratory-prepared and marketed formulations in accordance with WHO guidelines. Diagnostic microscopical features of each crude drug such as lignified fibres, stone cells, starch grains, oleo-resinous parenchyma, silicate crystals and epidermal characters were confirmed in all the formulations, ensuring its identity and authenticity. Phytochemical screening detected alkaloids, flavonoids, phenolics, tannins, carbohydrates, steroids and triterpenoids. Comparative TLC fingerprinting confirmed the presence of all crude drugs in prepared and marketed formulations. HPTLC quantification using piperine as a marker revealed highest content in *P. nigrum* (4.86%w/w), followed by *P. longum* (4.809% w/w). Among formulations, laboratory prepared contained 4.88% w/w, with comparatively lower amounts in marketed formulations. The findings provide a comprehensive standardization profile for Talisadi Churna, ensuring quality of both laboratory-prepared and marketed formulations.

INTRODUCTION: Ayurveda has been an integral part of Indian medicine since ancient times, with a wide range of formulations such as Asava, Arka, Avaleha, Ghrita, Taila, Churna, and others. Ayurvedic formulations play a key role in enhancing immunity and disease resistance. One of the formulation, churnas are fine powdered preparations that may be simple (single ingredient) or compound (multiple ingredients). Their therapeutic effectiveness increases with finer particle size, and they are easy to administer, especially for children who have difficulty swallowing tablets or capsules [1].

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To meet modern challenges and achieve global acceptance, Ayurveda requires appropriate scientific adaptations while preserving its fundamental principles [1]. Talisadi Churna is well known Ayurvedic formulation used in cough, cold, respiratory diseases, bronchitis, vomiting, heart diseases, chronic fever, anorexia, stimulant, dyspepsia and anaemia. Talisadi Churna chiefly contains *P. longum* and *P. nigrum*, with piperine alkaloid as the major constituent. To ensure the quality, safety, and efficacy of such herbal formulations, standardization in accordance with WHO guidelines is essential.

Therefore, the present study evaluates Talisadi Churna using modern analytical techniques, including marker-based analysis, to establish quality-control and standardization parameters for ensuring its authenticity, consistency, and therapeutic reliability [2].

MATERIALS AND METHODS:

Collection and Authentication of Plant Material: All the crude drugs were collected from the local market of Ahmedabad. Their identification and authentication were done by taxonomist of Department of Botany, Gujarat University, Ahmedabad.

Preparation of Standard Churnas: Plant materials (as per **Table 1**) were taken together in mentioned quantity, powdered (60#), mixed properly and stored in airtight containers.

TABLE 1: CONTENT OF STANDARD FORMULATION

Name	Latin Name	Part used	Qty (Parts)
Talishpatra	*Taxus wallichiana*	Leaf	1
Black pepper	*Piper nigrum*	Fruit	2
Ginger	*Zingiber officinale*	Rhizome	3
Long pepper	*Piper longum*	Fruit	4
Vansalochana	*Bambusa arundinacea*	Unorganized part	5
Cardamom	*Elettaria cardamomum*	Seed	½
Cinnamon	*Cinnamomum zeylanicum*	Stem bark	½
Sarkara	-	-	32
	Total		48

Collection of Market Samples of the Formulation: Two market samples of Talisadi Churna were collected from the various Ayurvedic pharmacies of Ahmedabad.

1. Shri Narnarayan Ayurvedic Pharmacy (NAR)
2. Shri Baidyanath (BDN)

Pharmacognostical Studies: All the plant material were studied individually for morphological and microscopical characters. Powder study and quantitative microscopy were done for standard (STD) and market formulation (NAR and BDN) of Talisadi Churna. Physico- chemical parameters like ash values, extractive values and volatile oil content were performed for standard and market formulation [3,4].

Standard and market formulations were subjected to the following tests separately for the presence of various phytoconstituents like alkaloids, flavonoids, saponins, carbohydrates, sterols and terpenoids, anthraquinone glycosides, coumarins, tannins and phenolic compounds [5-10].

The content of alkaloids [11, 12], flavonoids [11, 13], carbohydrates [11, 14] and tannins [6] were also determined. Sodium and potassium ions were estimated by flame photometric method [6]. Fluorescence analysis was carried out using various solvents like water, methanol, ethanol, 1N HCI and 1N methanolic NaOH separately at short UV, long UV and visible wave length [6].

Thin Layer Chromatography (TLC) Analysis: TLC profiling of key ingredients in Talisadi Churna was performed using co-TLC with standard drugs and market formulations (NAR, BDN).

Sample Preparation: Powdered crude drugs (1 g) and formulations (2 g) were refluxed with suitable solvents (20 mL) for 30 min. Extracts were filtered, concentrated, and used for TLC.

Chromatographic Conditions:

1. *Cinnamomum zeylanicum*: Petroleum ether extract; mobile phase Toluene: Ethyl acetate (9:1); detection under UV.
2. *Zingiber officinale*: Chloroform extract; mobile phase n-Hexane: Ether (4:6); derivatized with vanillin–H_2SO_4; detection in visible light.
3. *Elettaria cardamomum*: Acetone extract; mobile phase Toluene: Ethyl acetate (9:1); detection under UV.
4. *Taxus wallichiana*: Chloroform extract; mobile phase Toluene: Ethyl acetate (9:1); derivatized with vanillin–H_2SO_4; detection under UV and visible light.
5. *Piper longum* & *Piper nigrum*: Methanolic extract; mobile phase Toluene: Acetone: Formic acid (5:2:1); detection under UV.
6. Stationary Phase: Precoated silica gel G 60 F254 plates (E. Merck).

HPTLC Analysis: HPTLC analysis of crude drugs and standard and market formulations of Talisadi Churna was carried out using silica gel 60 F_{254} plates (Merck) with Toluene:Acetone:Formic acid (5:2:1) as mobile phase. Methanolic extracts of samples were prepared by maceration. Samples were applied as bands (2–10 µL) and developed up to 8 cm after chamber saturation (45 min) at 25 ± 2°C. Densitometric scanning was performed at 337 nm under UV light. Piperine (1 mg/mL) was used as standard to construct calibration curve (1000–6000 ng/spot), and quantification in samples was carried out by comparing peak areas with the standard curve.

Validation of HPTLC Method: The method was validated as per ICH guidelines for linearity (1000–6000 ng/spot), precision (%RSD for intra- and interday), accuracy (recovery by standard addition), and specificity. Repeatability was confirmed (RSD <1% for peak area; <3% for application, n=7). LOD and LOQ were calculated as 3.3σ/S and 10σ/S, respectively.

RESULTS AND DISCUSSION:

Pharmacognostical Studies: Standard Talisadi Churna and its market formulations (NAR and BDN) were evaluated for morphological characters. Their findings are shown in **Table 2**. Microscopic features are presented in **Fig. 1**. Results of quantitative microscopy and physico-chemical parameters, including ash values, extractive values, and volatile oil content, were given in **Table 3** and **4.**

Phytochemical screening indicated the presence of alkaloids, flavonoids, sterols and triterpenoids, phenolics, tannins, and carbohydrates. Higher levels of alkaloids, flavonoids, phenolics, and sugars were observed in the standard formulation, while tannins were higher in NAR and starch content in BDN (**Table 5**).

Table 6 shows that sodium and potassium ion content was higher in NAR formulation. Fluorescence analysis results are presented in **Table 7.**

TABLE 2: MACROSCOPICAL CHARACTERS

Parameters	Talisadi Churna		
	STD	NAR	BDN
State	Fine	Fine	Fine
Colour	Off white	Buff	Buff
Odour	Aromatic	Aromatic	Aromatic
Taste	Sweet	Sweet	Sweet

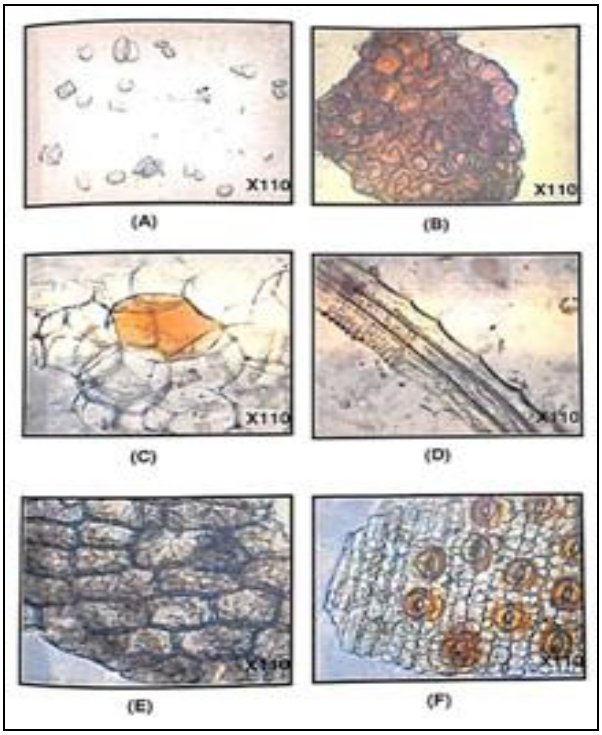

FIG. 1: POWDER STUDY OF STANDARD Talisadi Churna

TABLE 3: QUANTITATIVE MICROSCOPY OF Talisadi Churna

Parameters	Talisadi Churna		
	STD	NAR	BDN
Stone cell			
Length	22.2 - 207.2 µ	18.5 - 207.2 µ	18.5 - 207.2 µ
Breadth	18.5 - 70.3 µ	18.5 - 70.3 µ	18.5 - 70.3 µ
Phloem Fibre			
Length	379.9-727.8µ	429.1-700.7 µ	371.8-715.1 µ
Breadth	18.5 - 25.9 µ	18.5 - 25.9 µ	18.5 - 25.9 µ
No. of sclerenchyma cells per sq mm	3720 cells	3750 cells	3800 cells

(±SD); Standard deviation, Number of readings = 3)

TABLE 4: PHYSICAL PARAMETERS OF Talisadi Churna

Quality Parameters	Talisadi Churna (% w/w ± SD) (n=3)		
	STD	NAR	BDN
Ash value			
Total ash	11.36 ± 0.42	11.92 ± 0.30	11.33 ± 0.47
Acid insoluble ash	9.69 ± 0.48	8.44 ± 0.18	9.27 ± 0.144
Water soluble ash	1.42 ± 0.09	1.35 ± 0.10	0.93 ± 0.09
Extractive value			
Water soluble extractive	77.55 ± 0.65	65.80 ± 0.29	71.5 ± 1.17
Alcohol soluble extractive	2.62 ± 0.16	6.59 ± 0.11	4.76 ± 0.14
Volatile oil content(% v/w)	0.2 ± 0.02	0.2 ± 0.05	0.2 ± 0.02

Standard deviation (±SD); Number of readings (n) =3

TABLE 5: ESTIMATION OF PHYTOCONSTITUENTS OF Talisadi Churna

Phytoconstituents	Talisadi Churna (% w/w ± SD) (n=3)		
	STD	NAR	BDN
Alkaloids	0.602 ±0.002	0.574 ±0.016	0.547 ±0.001
Flavanoids	15.65 ± 0.083	7.081 ±0.105	13.05 ± 0.075
Phenolics	16.2 ± 0.007	7.4 ± 0.006	13.2 ± 0.01
Carbohydrates Sugar content	12.06 ±0.602	11.26 ±0.380	9.9±0.4
Starch	0.2 ±0.01	0.19 +0.004	0.23 ± 0.006
Tannins	0.202 ± 0.007	0.274 ± 0.008	0.132 ±0.008

Standard deviation (±SD); Number of readings (n) =3

TABLE 6: ESTIMATION OF SODIUM AND POTASSIUM IONS IN Talisadi Churna

Elements	*Talisadichurna* (% w/w)		
	STD	NAR	BDN
Sodium (Na^+)	0.425	0.514	0.335
Potassium (K^+)	1.278	1.532	1.032

TABLE 7: FLUORESCENCE ANALYSIS OF Talisadi Churna

Reagent	Wave length	Talisadi Churna		
		STD	NAR	BDN
Water	Visible	Yellow	Light Yellow	Brownish Yellow
	Short UV	Green	Green	Green
	Long UV	Whitish green	Whitish green	Whitish green
Methanol	Visible	Dark green	Yellowish green	Light green
	Short UV	Green	Green	Green
	Long UV	Brownish Green	Brownish Green	Brownish Green
Alcohol	Visible	Green	Green	Green
	Short UV	Green	Green	Green
	Long UV	Green	Green	Green
1N HCl	Visible	Light Yellow	Yellow	Yellow
	Short UV	Light Green	Green	Green
	Long UV	Yellowish green	Yellowish green	Yellowish green
Methanolic NaOH	Visible	Yellowish green	Brownish Green	Brownish Green
	Short UV	Green	Green	Green
	Long UV	Brownish Green	Brownish Green	Yellowish green

Visible wave length - 400-800nm, Short UV wave length - 254nm, Long UV wave length - 366nm

Comparative TLC Study of Talisadi Churna:

TLC of *C. zeylanicum* with Standard and Market Formulations: TLC of *C. zeylanicum* showed prominent spots at R_f 0.42 and 0.25 under short UV, which were also observed in standard and both marketed formulations, indicating the presence of similar phytoconstituents. Additional spots at lower R_f values (0.19 and 0.10) in formulations suggest the presence of other co-existing components **Fig. 2.**

FIG. 2: TLC OF *C. ZEYLANICUM* AND TALISADI CHURNA

TLC of *Z. officinale* with Standard and Market Formulations: In *Z. officinale*, after derivatization with vanillin–H_2SO_4, characteristic spots at R_f 0.92, 0.68, 0.50, and 0.38 were observed in both standard and formulations, confirming the presence of gingerol-related compounds **Fig. 3.**

FIG. 3: TLC OF Z. OFFICINALE AND TALISADI CHURNA

TLC of *E. cardomomum* with Standard and Market Formulations: TLC profile of *E. cardamomum* exhibited multiple spots under both short and long UV. Common R_f values (0.58, 0.49, 0.31, and 0.27) in standard and formulations indicated good correlation, while minor variations suggest formulation complexity **Fig. 4**.

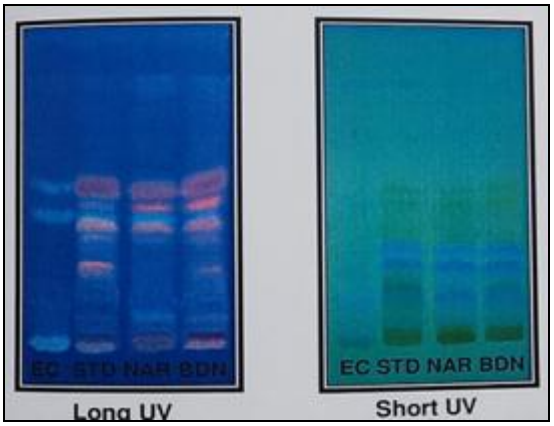

FIG. 4: TLC OF *E. CARDOMOMUM* MATON AND TALISADI CHURNA

TLC of *T. wallichiana* with Standard and Market Formulations: *T. wallichiana* showed spots at Rf0.58, 0.52, and 0.37 across crude drug, standard, and formulations under UV and after derivatization, confirming its presence **Fig. 5.**

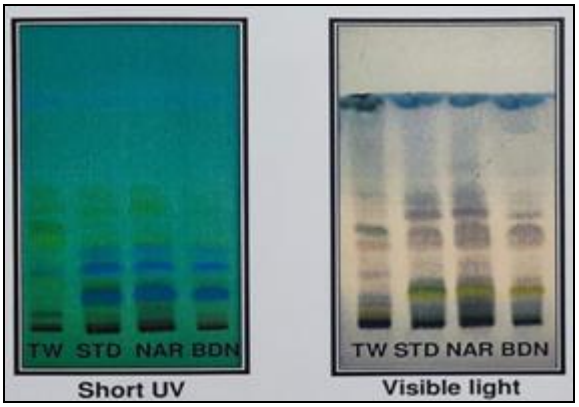

FIG. 5: TLC OF *T. WALLICHIANAAND* TALISADI CHURNA

TLC of *P. longum* and *P. nigrum* with Standard and Market Formulations: For *P. longum* and *P. nigrum*, major spots at R_f 0.77, 0.68, 0.59, and 0.55 were observed in all samples under short UV, indicating piperine-like constituents **Fig. 6.**

FIG. 6: TLC OF *P. LONGUM, P. NIGRUM* AND TALISADI CHURNA

HPTLC Study of Crude drugs, Standard and Market Formulations: Densitometric analysis confirmed the presence of piperine in all samples, with comparable peak areas, indicating uniform distribution of the bioactive marker as shown in **Fig. 7.**

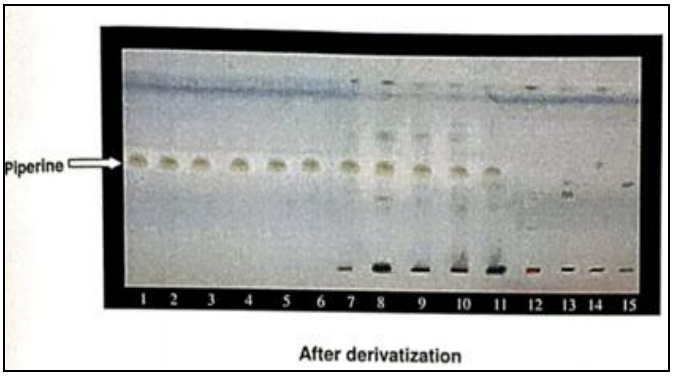

FIG. 7: CHROMATOGRAM OF PIPERINE AND TLC FINGERPRINTING OF TALISADI CHURNA

1. Std piperine (1000 ng)
2. Std piperine (2000 ng)
3. Std piperine (3000 ng)
4. Std piperine (4000 ng)
5. Std piperine (5000 ng)
6. Std piperine (6000 ng)
7. *P. longum*
8. *P. nigrum*
9. STD formulation
10. NAR formulation
11. BDN formulation
12. *T. wallichiana*
13. *C. zeylanicum*
14. *Z. officinale*
15. *E. cardamomum*

Calibration curve of Piperine: HPTLC analysis was performed using piperine as a marker compound. The calibration curve of piperine showed good linearity in the range of 1000–6000 ng/spot with a correlation coefficient (R^2 = 0.96), indicating reliable quantification **Fig. 8.**

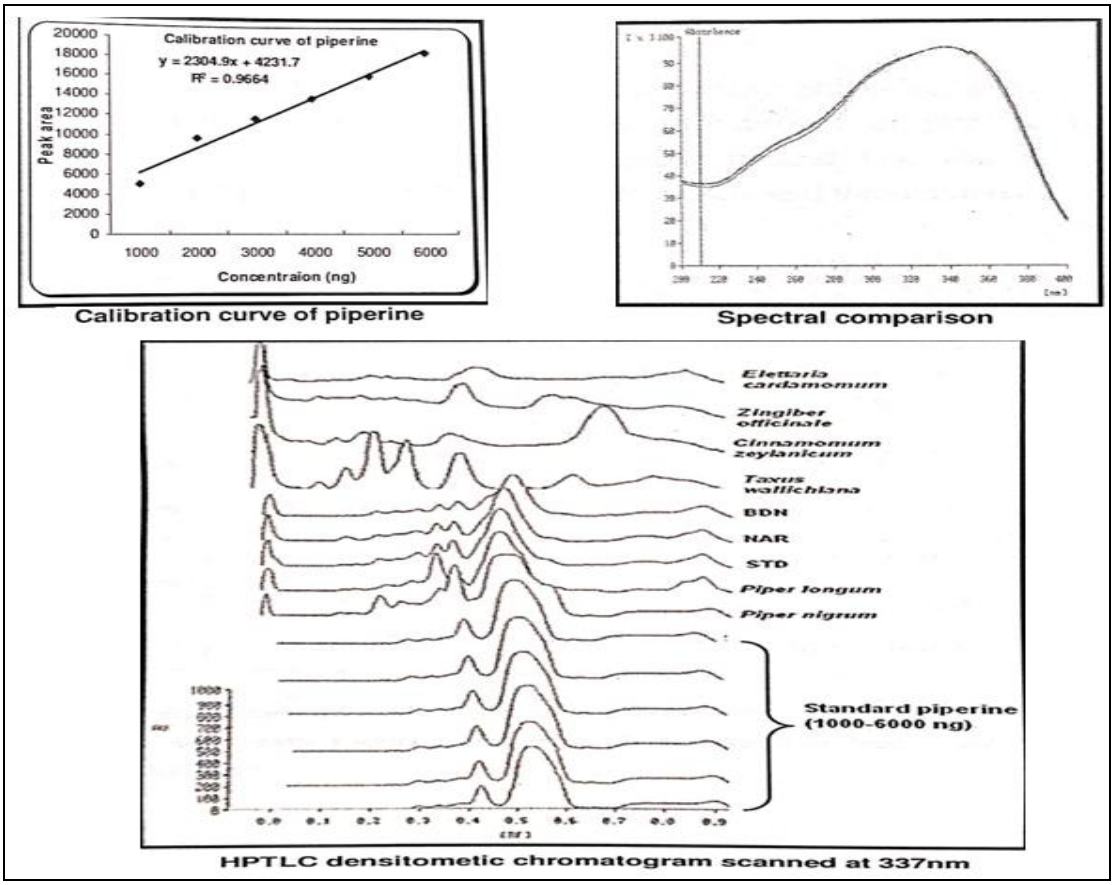

FIG. 8: HPTLC STUDY OF PIPERINE

Estimation of Piperine in *P. longum*, *P. nigrum* and all the three Formulations: The chromatographic profiles confirmed the presence of piperine in all samples without interference. **Table 8** shows a decrease in piperine content was observed in marketed formulations compared to the standard formulation.

TABLE 8: ESTIMATION OF PIPERINE IN *P. LONGUM, P. NIGRUM* AND TALISADI

Sample	Mean peak area (n=3)	Average amount of piperine (ng/spot)	Average %w/w of piperine ± SD	%C.V.
P. nigrum	41789	16290	4.86 ± 0.05	0.01
P. longum	42116	16430	4.809 ± 0.02	0.02
STD	27191	9960	4.88 ± 0.01	0.02
NAR	25965	9429	4.456 ± 0.0023	0.04
BDN	22064	7730	3.92 ± 0.0015	0.03

(± SD); Standard deviation, Number of readings S=3

Validation of HPTLC Method: The developed HPTLC method was validated as per standard guidelines and showed acceptable linearity, precision, accuracy, and specificity. The method was found to be reliable and suitable for routine estimation of piperine **Table 9.**

TABLE 9: SUMMARY OF VALIDATION PARA-METERS

Parameters	Results
Linearity	0.96
Precision (% C.V.)	
Repeatability of Measurement	0.395
Repeatability of Application	1.01
Interday	1.27-2.59%
Intraday	0.84-1.38%
Range	1000-6000 ng/spot
Limit of Detection	55.42 ng/spot
Limit of Quantification	516.19 ng/spot
Accuracy	99.26-99.60%
Specificity	Specific

CONCLUSION: The study established standardization of Talisadi Churna using pharmacognostical, physicochemical, phytochemical, TLC, and HPTLC analysis. Macroscopic and microscopic characters confirmed the identity of all ingredients in standard and marketed formulations. Physicochemical parameters and extractive values were comparable, indicating acceptable quality. Phytochemical screening revealed the presence of major bioactive constituents. TLC fingerprinting showed similar R_f values across crude drugs and formulations, confirming ingredient consistency. HPTLC analysis using piperine as a marker demonstrated good linearity and confirmed its presence in all samples with minor variation. These findings provide reliable parameters for quality control and authentication of Talisadi Churna.

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CONFLICT OF INTEREST: Nil

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