Article

Quality Control of Triphala Churna

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Abstract: Objectives: Triphala Churna (TC) is a tridoshashamak Ayurvedic rasayana used for treating various diseases such as skin diseases, diabetes, and as a mild laxative. Methods: In this study, TC was prepared and quality standardized standardized using various parameters such as macroscopical evaluation, physical properties, physicochemical properties, and phytochemical analysis (HPTLC fingerprinting and quantification) methods to assess its quality and purity. Results: The qualitative and quantitative phytochemical assays via HPTLC revealed concentrations of Gallic acid as 3.62 ± 0.05%, 3.67 ± 0.04%, 1.52 ± 0.03%, and 2.96 ± 0.02% for Amlaki (*Emblica officinalis*), Haritiki (*Terminalia chebula*), Vibhitki (*Terminalia bellerica*), and TC, respectively. Conclusion: This study provides valuable insights into establishing robust quality control measures and developing reliable assays for both Triphala Churna (TC) and its individual components.

Keywords: Gallic acid; HPTLC fingerprinting; mild laxative; quality standardization; Triphala Churna

1. Introduction

Ayurvedic medicines are effectively used to treat various chronic diseases as well as modern lifestyle disorders. They are mainly composed of herbal plants, minerals, aquatic drugs, and their combinations used for the treatment of diseases. They do not induce side effects and toxicity. Therefore, the demand for herbal sources is increasing daily, and it is difficult to fulfill the demand. Excessive demand increases the chances of adulteration and substitution. Herbal raw materials and their products need identification and standard parameters, which help to develop quality medicines and minimize adulteration and substitution.

The Ayurvedic medicine TC possesses tridoshashamak properties and is mainly used to treat various diseases and for detoxification. Acharya Charak said that "daily intake of TC with honey/ghee can make a person live for one hundred years devoid of old age diseases," whereas Acharya Sushrut revealed that it is useful for treating ulcers and wounds. Modern science indicates in-vivo studies on melanoma cancer [1,2]. TC is non-habit forming, is a mild laxative, and maintains healthy digestive power; hence, it is recommended for overall health. The effectiveness of this churna is due to its chemical constituents, which are mainly present in Amalki pulp (*Embelicaofficinelis*), epicarp of Haritki (*Terminalia chebula*), and epicarp of Vibhitki (*Terminalia bellerica*). The chief chemical constituents are tannin (35%), a phenolic compound (25–38%), Gallic acid (3–7%), chebulagic acid (5%), chebulinic acid (5%), ellagic acid (2%), and a small amount of flavonoids and saponins (0.053–0.33%). Gallic acid is a common chemical constituent, so it is used to assay TC and its ingredients. Gallic acid is also used as an anti-inflammatory and for treating hypoglycemia. It has antioxidant properties and gamma-ray protection [3] and is used to treat various oral diseases [4].

Its quality, safety, and efficacy are affected due to adulterants and contamination of herbal products; therefore, its purity, safety, potency, and efficacy are major problems associated with the quality of ingredients. The regulatory bodies will have to ensure that medications given to consumers are of good quality with assurance. The regulatory authority should implement good manufacturing practices at the manufacturing operation unit and develop a quality control unit for raw materials and finished products as per the Pharmacopoeia [5]. The World Health Organization (WHO) Assembly and Ayurvedic Pharmacopeia Commission have expressed the need to use modern technology and appropriate standards like HPLC, HPTLC, and Spectroscopy to ensure the quality of Ayurvedic medicines and their products [6,7]. Hence, in this connection, an attempt has been made to develop the

quality control parameter of TC and its raw materials, which would be useful in the Pharmacognosy, Phytochemistry, Botany, and Herbal industry for further research activities.

2. Materials

2.1. Collection of Plant Material

The Ayurvedic formulation Triphala Churna (TC) contains Amalki pulp (AP), epicarp of Haritki (HE), and epicarp of Vibhitki (VE). It was obtained from an Ayurvedic pharmacy in Raipur, Chhattisgarh, India. The authentication was performed in the Drug Testing Laboratory AvamA nusandhan Kendra.

2.2. Method of Preparation of Triphala Churna

Triphala Churna was prepared in the laboratory using a pulverization method with slight modifications. Equal amounts of Amalki pulp, epicarp of Haritki, and Vibhitki were mixed and converted into a fine powder. The fine powder was passed through sieve no. 44 for uniform size distribution [8]. It was further used for quality control assessment.

2.3. Extracts Preparation of Plant

The powdered sample of TC (10 g) was defatted using 200 mL of petroleum ether. The defatted sample was extracted with 300 mL of distilled water using a Soxhlet apparatus until the cycle turned transparent. The aqueous extract thus obtained was dried in a water bath at 90 °C. The dried sample was weighed and transferred into different sample bottles for storage in a refrigerator at 4 °C until required for further analysis. The same procedure was applied for AP, HE, and VE samples.

3. Methods of Standardization

3.1. Macroscopic Standardization

Triphala Churna, AP, HE, and VE samples were evaluated according to sensory observations such as color, odor, and taste.

3.2. Physical Characteristics Standardization

The physical characterization of TC was determined using parameters such as particle size distribution, angle of repose, Hausner's ratio, bulk density, and Carr's index.

3.2.1. Bulk Density

The Bulk density was determined using USP guidelines. In which 10 g of TC sample was taken into a 25 mL graduated measuring cylinder, and measured the bulk volume. Bulk density was calculated by using given formula.

$$\text{Bulk density} = \frac{\text{Weight of sample taken}}{\text{Bulk volume}} \quad (1)$$

3.2.2. Tapped Density

Triphala Churna (10 g) was taken in a graduated measuring cylinder and tapped on a wooden surface from the height of 2.5 cm at the second interval and after tapping measured the tapped volume. Tapped density was calculated by using given formula.

$$\text{Tapped density} = \frac{\text{Weight of sample taken}}{\text{Tapped volume}} \quad (2)$$

3.2.3. Angle of Repose

The angle of repose was estimated using the funnel method. The height of the funnel was fixed at 6 cm from the bottom surface. Ten grams of the powdered sample of TC was poured to flow through a funnel fixed on a stand.

A heap was formed, and its height and radius were measured. The angle of repose was calculated using the given formula.

$$\text{Angle of repose}(\theta) = \tan - 1\{\frac{\text{Height of heap (h)}}{\text{Radius of heap (r)}}\} \quad (3)$$

3.2.4. Compressibility / Carr's Index

Compressibility/Carr's Index is estimated using a previously applied procedure of tapped density and bulk density. The formula applied is as follows:

$$\text{Compressibility / Carr's Index} = \frac{\text{Tappeddensity} - \text{Bulk density}}{\text{Tappeddensity}} \quad (4)$$

3.2.5. Hausner's Ratio

The ratio of tapped density to bulk density is called Hausner's Ratio. The Hausner's Ratio is calculated using the following formula:

$$\text{Hausner's Ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \times 100 \quad (5)$$

3.3. Physicochemical Standardization

The physicochemical parameters mentioned, namely foreign matter, loss on drying, total ash, acid insoluble ash, and extractive values, are used to identify and assay the formulation according to the Ayurvedic Pharmacopoeia of India.

3.3.1. Loss on Drying

The powdered sample of TC (2 g) was accurately weighed on a watch glass plate. The weighed sample was spread evenly on the plate, dried in an oven at 105 °C, cooled, and then re-weighed. The procedure was repeated until a constant weight was obtained. The weight loss of the sample was recorded and calculated as a percentage. The same procedure was applied to all samples (AP, HE, and VE) in triplicate.

3.3.2. Total Ash Value

The powder sample of TC (3 g) was accurately weighed into a silica crucible. The crucible samples were placed in a muffle furnace at 450 °C for 3–4 h until complete combustion occurred. After cooling, the crucible was re-weighed, and the percentage of total ash was calculated. The same procedure was applied to all samples (AP, HE, and VE) in triplicate.

3.3.3. Acid Insoluble Ash

The total ash from TC was obtained as described in the above method. The ash was treated with 25 mL of 6 N HCl on a water bath with continuous heating until a solution was obtained. The solution was filtered through ashless Whatman filter paper no. 41. The residue from the filtrate was transferred into a silica crucible, ignited for 2 h, then cooled, re-weighed, and the percentage of acid-insoluble ash was calculated. The same procedure was applied to all samples (AP, HE, and VE) in triplicate.

3.3.4. Extractive Values

Determination of Alcohol Soluble Extractive

The powdered sample of TC (5 g) was macerated in 95% alcohol with continuous shaking for 6 hand left to stand overnight in a flask. Twenty-five milliliters of the filtrate was evaporated to dryness in a China dish at 105 °C on a water bath. The dried sample was weighed, and the percentage of alcohol-soluble extract was calculated. The same procedure was applied to all samples (AP, HE, and VE) in triplicate.

Determination of Water-Soluble Extractive

Five gramspowder sample of TC was taken and macerated in chloroform water with continuous shaking for 6 h and stand flask overnight. 25 mL of filtrate evaporated to dryness in a China dish at 105 °C on water bath. The dried sample was weighted and the percentages of alcohol soluble extract were calculated. The same procedure was applied to all samples (AP, HE and VE) in triplicate.

3.3.5. Determination of pH

The solution of TC (10% *w*/*v*) was prepared in distilled water. The pH of the solution was then measured using a pH meter. The same procedure was applied to all samples (AP, HE, and VE) in triplicate.

3.4. High-Performance Thin-Layer Chromatography (HPTLC) Analysis

The aqueous extract of TC (100 mg) was dissolved in 1 mL of methanol and centrifuged at 1000 rpm for 5 min. The same procedure was applied to all samples (AP, HE, and VE). The upper layer of the solution was used as the test solution for HPTLC fingerprinting and quantitative analysis (densitometry). A standard sample of Gallic acid (1 mg/mL) was prepared in methanol. The chromatographic analysis was performed on a Merck HPTLC plate silica gel 60F 254 measuring 200 × 100 mm. The standard sample solution of Gallic acid (1.0–7.0 µL) and test solutions of Amalki, Vibhitki, Haritki, and Triphala (2 and 5 µL) were applied to the plates as 8 mm bands with an 11.4 mm distance between bands using Linomat 5 instruments (CAMAG, Muttenz, Switzerland). The loaded sample plate was placed in a twin developing chamber with a pre-filled solvent of Chloroform:Acetone:Formic acid (7:2:1 *v*/*v*/*v*) as the mobile phase. The plate was placed in the solvent chamber for development in the relevant mobile phase until the solvent front reached 70 mm. After development, the plate was dried with hot air to evaporate solvents. Images of the plate were captured using a CAMAG TLC Visualizer chamber (S/N: 150503) under white light, at 254 nm and 366 nm, and scanned using a TLC Scanner 4 (Version 2.5.18262.1 - Anchrom, Mumbai, India). The peak table, display, and densitogram were recorded at 281 nm [9,10]. The calibration curve of Gallic acid is shown in Figure 1.

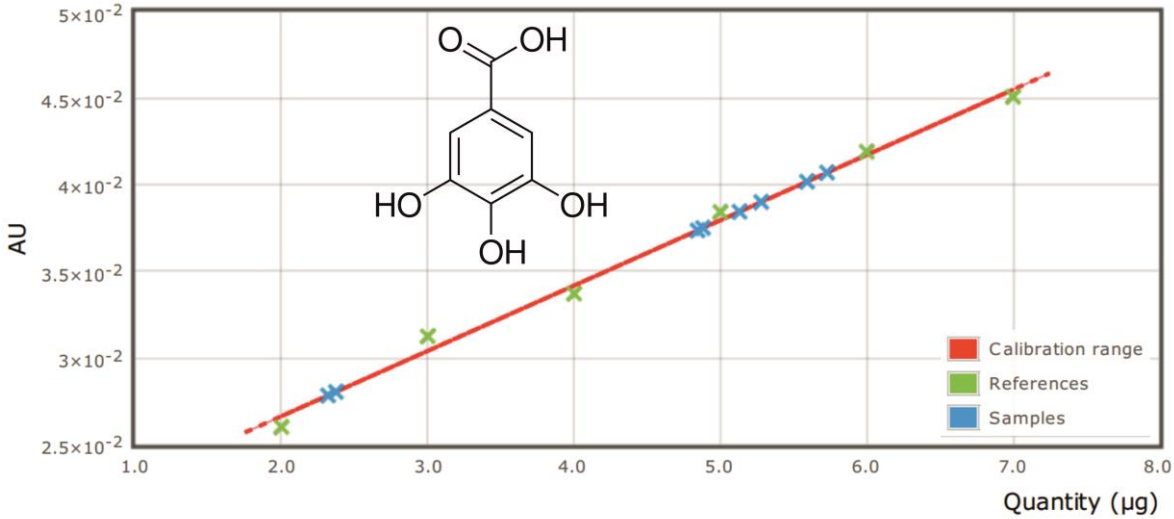


Figure 1. The calibration curve of Gallic acid.

4. Results

The present study of TC was prepared in accordance with the Ayurvedic Formulary of India to ensure the quality and efficacy of the product. The quality standardization of Ayurvedic TC was conducted using macroscopic and microscopic evaluations, physicochemical properties, physical properties, and the HPTLC method. Standard procedures as per the Ayurvedic Pharmacopoeia and other specific authorities were applied to establish suitable quality control parameters. Results were expressed as the mean (± SD) of three experiments for TC, AP, HE, and VE, as shown in Table 1.

Table 1. Standardization method for TC, AP, HE and VE.

No.	Parameters	Triphala Churna (TC)	Amalki Pulp (AP)	Epicarp of Haritki (HE)	Epicarp of Vibhitki (VE)
1.	Colour	Light Brown	Graish- Black	Yellowish Brown	Graish- Brown
2.	Odour	Characteristic	Aromatic-Sweet	Characteristic	Characteristic
3.	Taste	Characteristic	Sour - astringent	Astringent	Astringent
4.	Particle fitness	Fine to very fine powder	-	-	-
5.	Bulk density	0.4 ± 0.02 g/mL	-	-	-
6.	Tapped density	0.5 ± 0.04 g/mL	-	-	-
7.	Angle of Repose	0.25 ± 0.01	-	-	-
8.	Hausner's Ratio	1.25 ± 0.05	-	-	-
9.	Compressibility / Carr's Index	0.2 ± 0.001	-	-	-
10.	pH (10% aqueous sol.)	3.40 ± 0.07	-	-	-
11.	Loss of Drying	3.6 ± 0.06	4.55 ± 0.05	8.4 ± 0.07	4.05 ± 0.05
12.	Total Ash	6.33 ± 0.05	4.86 ± 0.03	4.43 ± 0.04	5.76 ± 0.06
13.	Acid Insoluble Ash	1.93 ± 0.04	1.63 ± 0.01	0.96 ± 0.02	1.66 ± 0.01
14.	Water soluble extractive	50.34 ± 0.08	49.4 ± 0.03	22.4 ± 0.07	43.74 ± 0.05
15.	Alcohol soluble extractive	43.68 ± 0.05	21.32 ± 0.02	12.08 ± 0.04	30.6 ± 0.03
16.	Conc. Of Gallic acid by HPTLC	2.92 ± 0.02 %	3.62 ± 0.05%	3.67 ± 0.04%	1.52 ± 0.03%

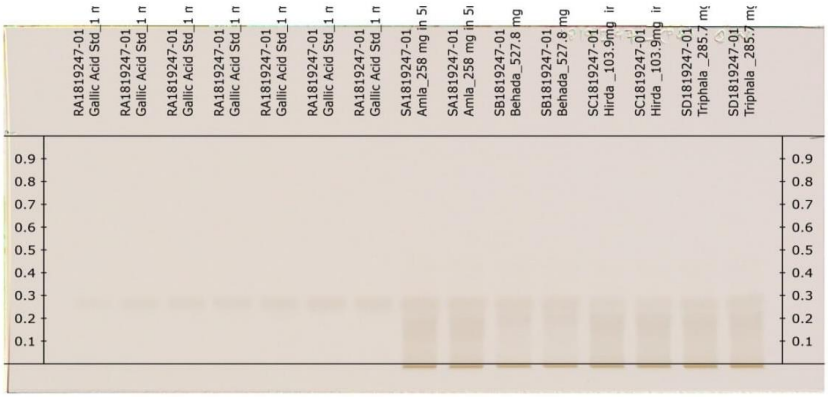
The macroscopic observation of TC reveals a reddish-brown color with a pungent odor and spicy, pungent taste. The Amalki pulp appears grayish-black, the epicarp of Vibhitki is grayish-brown, and the epicarp of Haritki exhibits a yellowish-brown color. The taste of Amalki is generally sour, while that of the other ingredients is astringent.

The physical properties of TC, such as bulk density, tapped density, and angle of repose, were measured as 0.4 ± 0.02 g/mL, 0.5 ± 0.04 g/mL, and 25 ± 1 degrees, respectively, indicating good flow properties. These flow properties were further confirmed by the Hausner ratio and Carr's index values, which were found to be 1.25 ± 0.05 and 20 ± 1%, respectively, for TC.

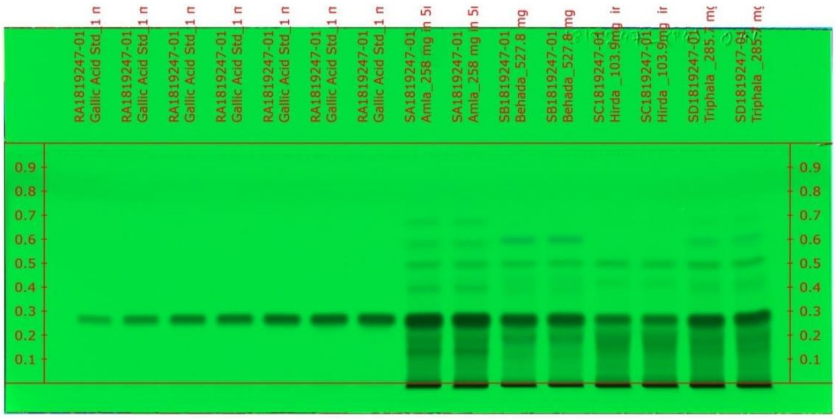
The physicochemical properties of TC, AP, HE, and VE are summarized in Table 1. The moisture content of TC, AP, HE, and VE falls within the acceptable range (3–9%), ensuring their stability during storage and protection against microbial degradation. The total ash values for TC, AP, HE, and VE were measured as 6.33 ± 0.05%, 4.86 ± 0.03%, 4.43 ± 0.04%, and 5.76 ± 0.06%, respectively, indicating the presence of inorganic components. The acid-insoluble ash value of TC (1.93%) indicates the presence of a small amount of acid-insoluble substances such as silica or sand. Moreover, the extractive values of TC, AP, HE, and VE were higher in water compared to alcohol.

The phytochemical assay was conducted using an HPTLC method for quality and quantitative analysis, with Gallic acid serving as the standard sample. HPTLC fingerprinting and quantitative analysis are effective methods for quality control in Ayurvedic medicine. Gallic acid, being a major phytoconstituent in TC, AP, HE, and VE, was used as a reference for chromatographic quantification. An optimized mobile phase (Chloroform:Acetone:Formic acid, 7:2:1 *v*/*v*/*v*) was employed to separate the phytoconstituents present in TC, AP, HE, and VE. The concentrations of Gallic acid in TC, AP, HE, and VE were determined using an HPTLC densitometer at 281 nm, yielding concentrations of 2.96 ± 0.02%, 3.62 ± 0.05%, 3.67 ± 0.04%, and 1.52 ± 0.03%, respectively. The fingerprinting at 252 nm, 366 nm, and 514 nm is shown in Figure 2, while the chromatogram of TC, AP, HE, and VE at the same wavelengths is shown in Figure 3.

RWhite



R254



R366

Figure 2. Fingerprinting of Triphala in white light,254nm, and 366nm

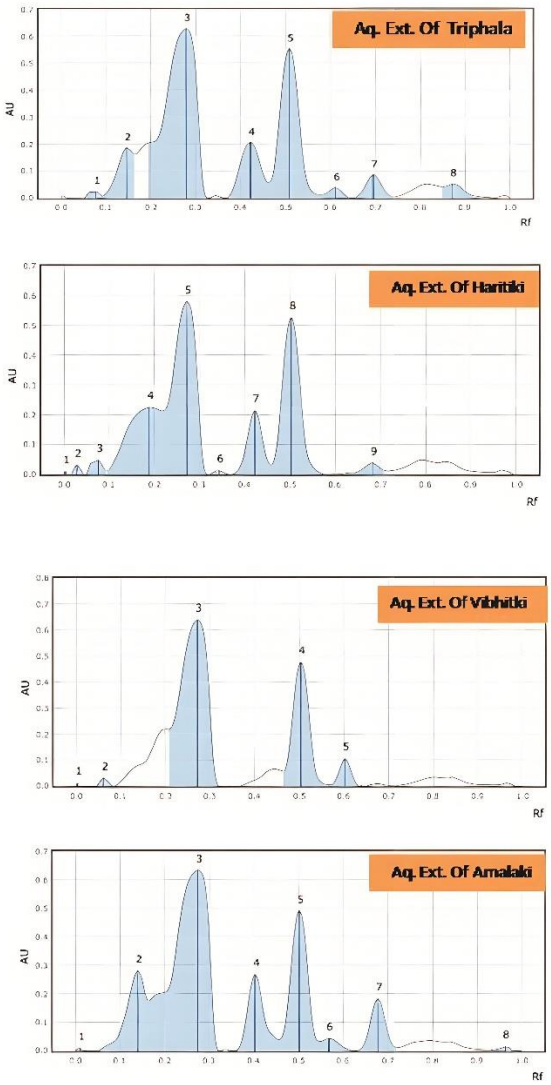


Figure 3. Chromatogram of TC, AP, HE and VE at 252, 366 and 514 nm.

5. Discussion

Ayurvedic medicine often combines herbal, mineral, and occasionally animal-based substances. Triphala Churna (TC), specifically, comprises three herbal drugs. Multiple manufacturers produce TC to treat various illnesses, but the lack of standardized parameters results in varying quality across the industry. Therefore, ensuring quality assurance for these products and their ingredients is crucial. Quality assurance is essential to guarantee consistent and reproducible medicine. TC, an Ayurvedic rasayana formulation, serves as a mild laxative for constipation and irritable bowel syndrome. Its antioxidant properties also enhance immunity and act as a detoxifying agent, making it a component in nearly 219 other Ayurvedic formulations. Thus, ensuring the qualitative standardization of these methods is necessary for safety and quality.

The World Health Organization (WHO) and India's Pharmacopeia Commission are working to establish quality standards for Ayurvedic formulations and their ingredients, addressing the challenge of standardization in this article. The organoleptic characteristics are important for identifying and ensuring the quality of TC, as they help detect adulteration, contamination, and spoilage. TC typically exhibits a reddish-brown color, but at times, it may darken to a dark brown shade due to variations in the quality or species of the herbal ingredients, namely AP, HE, and VE [11,12].

The physical characteristics of Churna include flowability, compressibility, density, and mechanical strength. Bulk density reflects the packing of particles, while the angle of repose indicates the powder's flowability and interparticle cohesion. Hausner ratio describes powder flow properties based on interparticle friction. According to Ajazuddin et al., a Hausner ratio less than 1.25 indicates good flow properties, whereas a ratio greater than 1.25 suggests poor flow [13]. The results of TC indicated good flow properties, which are crucial for the absorption and efficacy of medicines

The physico-chemical tests confirm the quality, safety, and stability of TC. Parameters such as loss on drying were observed and found to comply with ICH guidelines. No degradation was observed during the study, demonstrating the product's safety and efficacy [14,15] value and acid insoluble ash value serve as criteria to identify the purity and quality of the Ayurvedic formulation. The low acid-insoluble ash indicates minimal adulteration, such as silica or rice husk, ensuring effective absorption in the gastrointestinal tract (GIT). The water-soluble extractive was higher than the alcohol-soluble extractive, suggesting that water is a more efficient solvent for Triphala than ethanol. These values are detailed in Table 1. The correlation between pH and microbial contamination was studied suggested that a neutral or alkaline pH favors higher microbial contamination in herbal preparations [16–18].

HPTLC is crucial for qualitative and quantitative estimation of phytochemical constituents in herbal drugs and their formulations, making it vital for quality standardization [19]. TC and its ingredients contain Gallic acid, an important phytochemical easily isolated from plants, used for identification, quality control, and assay purposes. The fingerprinting of TC, AP, HE, and VE is shown in Figure 2, while their chromatograms is shown in Figure 3. Gallic acid was identified using a standard reference, and Scanner 4 quantified its percentage in TC, AP, HE, and VE. This HPTLC method serves as a valuable tool for qualitative and quantitative assays of these ingredients, playing a crucial role in their quality assessment [20].

6. Statistical Analysis

The statistical analysis applied in this work was conducted using SPSS 17 (SPSS Inc., Chicago, IL, USA). The results are presented as mean ± SD (n = 3).

7. Conclusion

Ayurvedic medicines require careful attention to manufacturing processes and quality control parameters to ensure their quality and safety. The present study focuses on developing quality control and assay methods for TC and its ingredients. This includes organoleptic and microscopic characterization, physicochemical properties, physical properties, and phytochemical qualitative and quantitative assays using HPTLC. Additionally, HPTLC densitometry proves useful for assaying Ayurvedic medicines with applied standard references.

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