# A Study on Qualitative and Quantitative Phytochemical Analysis of Triphala Churna from Different Sources

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## ABSTRACT

Traditional medical systems like Ayurveda have been utilized for centuries in India and other South Asian nations for disease prevention and treatment. In contrast to Modern medicine, which focuses mainly on particular organs or ailments, Ayurveda prioritizes comprehensive healing and the upkeep of overall bodily harmony. Triphala Churna holds an important position among the various Ayurvedic preparations because of its numerous therapeutic uses. Triphala is a composite herbal mixture made from the desiccated fruits of three herbal species: Emblica officinalis, Terminalia chebula, and Terminalia bellirica. It is commonly recognized for its cleansing, revitalizing, digestive, and immune-enhancing qualities. In this context, the current research sought to assess and contrast laboratory-made and commercially sold Triphala Churna samples concerning their phytochemical makeup, physicochemical and properties. The research additionally concentrated on the qualitative and quantitative assessment of phytoconstituents and the biological functions related to Triphala Churna. The study took place in Joginder Nagar, District Mandi, Himachal Pradesh, India, situated in the northwestern Himalayan area between 31.9869° N latitude and 76.7899° E longitude, with an elevation varying from 1,220 to 2,500 meters above sea level.

Sensory and microscopic analysis verified the genuineness of the raw materials employed in the formulation. Physicochemical characteristics, such as ash content, extractive content, and moisture loss, were observed to be within permissible limits, reflecting high-quality processing of the herbal materials. Thin Layer Chromatography (TLC) analysis additionally verified the existence of significant phytoconstituents and showcased the chemical uniformity of the formulation. The results showed that the developed formulation met the criteria established by the Ayurvedic Pharmacopoeia of India. The research emphasizes the significance of quality assurance and standardization in Ayurvedic preparations to guarantee safety, effectiveness, and consumer trust in traditional herbal remedies.

**Key words:** Ayurvedic medicine, ethnomedicine, histochemical analysis, phytoconsitutents, Triphala Churna

## INTRODUCTION

Traditional medical practices from long ago, such as Ayurveda in India and other parts of South Asia, are being used today for both illness prevention and treatment. Unlike symptom centered modern therapeutics, Traditional medical systems emphasizes balance and long term physiological wellbeing [1]. Nearly 80% of the world's population, especially in poor nations, still uses traditional medical systems to meet their healthcare requirements, according to the World Health Organisation [2]. Herbal medicines are often believed to be safer, more accessible, and more economically feasible, which contributes to their ongoing trustworthiness. Traditional Indian medical practices such as Ayurveda, Siddha, and Unani all have an emphasis on the medicinal properties of native plants, and ethnobotany is an integral part of these traditions. Before modern pharmacology came along, ancient writings such as the Charaka Samhita and the Sushruta Samhita recorded the uses of hundreds of therapeutic plants [3]. Numerous contemporary medications have their origins in plant-

based chemicals that were first used in traditional contexts, demonstrating the significant impact of ethnobotanical knowledge on the field of drug development [4].

Ayurvedic medicine places Triphala Churna in a prominent position among the many herbal compositions that have endured. The dried fruits of three plants Emblica officinalis (Amla), Terminalia chebula (Haritaki), and Terminalia bellirica (Bibhitaki) form this traditional polyherbal composition. It has a long history of usage in the treatment of many diseases. It helps in detoxifying and revitalising along with digestion, immunity, and overall [5,6,7,8,9,10,11].

Although Triphala is widely used in traditional medicine, the claims made about its ethnomedical properties are typically unsubstantiated and based on anecdotes rather than solid scientific evidence. To verify and standardise its use across therapeutic situations, comprehensive phytochemical analysis and pharmacological research are necessary. There is a strong correlation between the chemical components of an herbal mixture and its medicinal efficacy. The phytochemical profile of medicinal plants may be greatly impacted by several aspects, including their geographic location, soil quality, harvesting processes, drying methods, and storage conditions[12]. Hence, Triphala Churna's composition and effectiveness might vary greatly among brands and batches. Therefore, it is essential to standardise phytochemicals in order to guarantee the reliability, excellence, and security of herbal remedies. This requires quantitative study to determine concentrations and qualitative investigation to identify important components such as tannins, flavonoids, alkaloids, and saponins. Since phytochemical content is often associated with biological activities like antioxidant capacity, evaluating these is particularly crucial [13]. These kinds of studies help close the gap between anecdotal evidence and hard scientific evidence, which in turn makes herbal remedies safer for more widespread medical use. Many different firms now make Triphala Churna, and each one uses slightly different ingredients, processing techniques, and packaging processes. The idea behind the formulation is straightforward, but since there isn't any control or standardisation, the quality of the final product might vary greatly [14].

Our aims in conducting this study was to compare and contrast commercially available and laboratory-grown Triphala Churna products in terms of their phytochemical characterizationof the phyto-constituents of Triphala Churna. To compare the phytochemical and biological activities between laboratory-prepared and commercially available Triphala Churna samples and to study the ethanomedicinal properties of Triphala Churna.

## MATERIALS AND METHODS

### Study area

The present study has been carried out at Joginder Nagar in the District Mandi, Himachal Pradesh, India which is located in the northwest Himalayas. It is located between the latitude 31.9869° N and 76.7899° E. between 1,220 and 2,500 meters above sea level. Temperatures in Joginder Nagar vary from 5 to $30^0$ C (Fig.1).

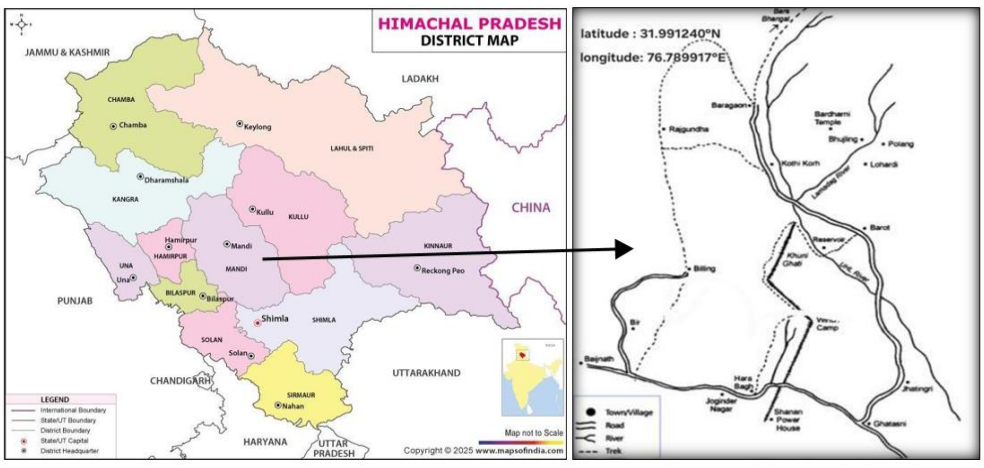



**Figure 1- Map of studied area: Jogindernagar, District Mandi, Himachal Pradesh, India**

**Material**

For the preparation of Triphala Churna, each of the three fruits Amla (Emblica officinalis**)**, Haritaki (Terminalia chebula), and Bibhitaki (Terminalia bellirica) thrive in this location because of the varied flora, which ranges from temperate to subtropical. The varied ecosystems of Joginder Nagar are supported by the rugged landscape, steep slopes, and deep valleys. The creation of Triphala Churna relies on plant species from the Combretaceae (Terminalia spp.) and Phyllanthaceae (Emblica officinalis) families, which are either naturally occurring or grown in the surrounding regions. Fruits of these three plants were processed with care to preserve their phytochemical and therapeutic qualities.

# METHODOLOGY

**Preparation of Triphala churana**

Triphala was made from three medicinal fruits that were picked when fully ripe and checked extensively to make sure they are not contaminated or infested. In keeping with conventional Ayurvedic wisdom, these fruits were each used in an equal amount. The preparation of a classical Ayurvedic Herbal Powder, Triphala Churana, involves a systematic approach that ensures quality, potency, and efficacy.

**Methods of analysis**

The Analysis of Triphala Churana involves various standard methods to determine its identity, purity, quality, and phytochemical profile.

**Preparation of extracts**

After the collection of plant material the fruits of all three plants were dried in shade. The dried plant sample were crushed and grinded. 50 gram dried powder was successively extracted with 100ml ethyl alcohol solvent. The extract was kept in rotatory shaker for 72 hour then it was filtered through Whatman No. 1 paper and evaporated on the water bath till it was finally reduced to get dry extracts. The extract was then transferred to previously weigh airtight container and stored in the refrigerator until it was screened for their phyto-constituents.

**Determination of moisture (loss on drying)**

The moisture content was determined by drying off the amount of volatile matter (i.e., water) from the drug. The evaporating dish (ED) should be cleaned and dried in an oven before being cooled for half an hour in a desiccator. The next step was to measure the empty ED's weight. After that, measure 5 g of the sample and bake it at 105°C for 5 hours until the weight remains consistent. Moisture content was calculated by:

$(W2 - W1) \times 100 \div W3$

W1: Weight of Empty Evaporating Dish

W2: Weight of Evaporating Dish after drying

W3: Weight of Sample

**Determination of total ash value**

The total ash value is the quantity of inorganic content in a sample; it is the residue left behind after burning the substance. For this, the sample precisely 2 g was weighed into a crucible that has been previously weighed. To achieve carbon-free combustion, the crucible was placed in a muffle furnace and heat it to 450°C. The crucible was reweigh after cooling it in a desiccator to prevent it from absorbing moisture.

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**Total Ash Value = (W2 - W1) × 100 ÷ W3**

W1: Weight of Empty Cruisible weight
W2: Weight of Cruisible after ignited
W3: Weight of Sample

**Determination of Acid-Insoluble Ash Value**

25 ml of diluted hydrochloric acid was boiled and added to the crucible that already contains the whole ash. The insoluble substances was separated using ashless filter paper. Then, the mixture was washed with hot water until it became neutral. The insoluble material- containing filter paper was placed in the initial crucible, light it to a consistent weight, it was cooled in a desiccator to prevent moisture absorption, and weighed it immediately. Afterwards, the amount of ash that was insoluble in acid was calculated:

Amount of Acid-Insoluble Ash Value = (W2 - W1) × 100 ÷ W3

Where, W1: Weight of Empty Cruisible

W2: Weight of Cruisible after ignited

W3: Weight of Sample

**Determination of alcohol soluble extract**

In a closed flask, 5 gm of coarsely powdered was infused with 90% alcohol at the stated strength for twenty-four hours, it was stirred often for the first six hours and then it was stand for remaining eighteen hours. It was then filtered quickly to prevent solvent loss.

In a tarred evaporating dish, 25 ml of the filtrate was evaporated until it reaches dryness at 105° until it reaches a constant weight, and then weigh it to determine what proportion of the extract was soluble in alcohol as follows:

Alcohol Soluble Extractive = (W2 - W1 × 400) ÷ W3

W1: Weight of Empty Evaporating Dish

W2: Weight of Evaporating Dish after drying

W3: Weight of Sample

**Determination of Water-Soluble Extract**

In a closed flask, 5 gm of coarsely powdered was infused with 100 ml of chloroform-water at the stated strength for twenty-four hours, it was stirred often for the first six hours and then it was stand for remaining eighteen hours followed by filtration. In an evaporating dish, 25 ml of the filtrate was evaporated until it reached dryness at 105° with a constant weight, and then weigh it. In relation to the air-dried extraction, we determine what proportion of the extract was water- soluble as follows:

Water-Soluble Extractive = (W2 - W1 × 400) ÷ W3

W1: Weight of Empty Evaporating Dish

W2: Weight of Evaporating Dish after drying

W3: Weight of Sample

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**Thin Layer Chromatography**

A typical technique may be followed in the laboratory to create silica gel plates for thin- layer chromatography. Clean, flat glass plates around 20 cm long were used to uniformly disperse this solution using a specialised spreading tool. After coating, the plates were baked at 100-105°C for at least an hour, then kept for air dry.

The initial step is conditioning the developing chamber to provide a saturated environment. This was accomplished by placing filter paper within the chamber's interior walls and then adding the mobile phase solvent until it forms a layer 5 to 10 mm deep at the bottom. At this point, the filter paper ought to be completely soaked. The next step was to seal the chamber and let it sit at room temperature for an hour. Next, apply the test and reference solutions to the plate in the shape of tiny circular spots or thin bands. These should be 20 mm from the bottom edge and at least 20 mm from the sides. The spots should be 2 to 6 mm in diameter or 10 to 20 mm in length and 2 to 6 mm wide. For optimal coverage, leave 15 mm of empty space between each area. It may be applied in repeated sections, with drying in between, if the amount of the sample was big or needs building up. After that, 15 cm above the sample line, draw a pencil line across the plate, and that was be the distance we want the solvent front to move.

When the sample solvent has evaporated, position the TLC plate vertically in the pre- equilibrated chamber such that the spots were above the mobile phase level. Once the solvent reached the indicated line, the compartment was shut and left undisturbed. After that, we take the plate out, let it dry, and it according to the method. To apply the reagents evenly without disrupting the spots, a fine mist sprayer was used when chemical detection is required.

**Visualization of Spots**

The components that were separated by TLC may be identified by examining the plate under ultraviolet (UV) light. Visualisation typically made use of two conventional wavelengths: 254 nm for short-wave UV and 365 nm for long-wave UV.

**Calculation of Rf Values**

Each compound's retention factor (Rf) was computed after visualisation to aid in identification.

Rf = Distance travelled by the spot

Distance travelled by the solvent front

The resulting Rf value was a unit-free number typically between 0 and 1, which can be compared with standard references for identification.

**Microscopy and Histochemical evaluation**

The fresh plant parts preserved in alcoholic acetic solutions which prepared by the mixture of 50% of alcohol, 40% of formalin, 2% of acetic acid and subjected for microscopic studies. The section cutting was performed of different parts of selected germplasm i.e. stem, leaf, petiole. Fresh plant material taken for microscopic studies. Section cutting were done with the help of blade. All section poured into a watch glass filled with water then put the thinnest section on the clean side. After this clean section firstly stain with Saffranin. Keep it for 4-5 minute and washed it and excess of stain was removed by acid alcohol and again wash the material with water. Now stain the material with few drop of fast green for few seconds. One thin section was taken out and mounted on clean glass side. A drop of glycerine was added and covered with the cover slip. The slide was observed under microscope with different magnification.

For histochemical analysis, free-hand sections of the plant rhizome were prepared and treated with specific reagents to identify cellular components such as, lignin, tannins, starch grains, calcium oxalate crystals and vascular bundles. Then comparison of intensity of staining and abundance of specific compounds in different

samples was performed.

**Phytochemical analysis**

Triphala churna was tested for the existence of bioactive substances using phytochemical screening. This study was useful for determining the formulation's medicinal potential since it identifies important components such glycosides, phenols, alkaloids, tannins, and saponins (table1).

Table 1- Qualitative Phytochemical test for confirmation of presence of compounds in samples

| Phyto-constituents | Test | Observations (Indicating Positive Test) | References |
|---|---|---|---|
| Protein | Biuret test | A pink colour solution (In ethanolic layer) | [15, 16] |
| Carbohydrates | Molish test | A violet ring | [16, 17] |
| Flavonoid | Lead acetate test | A yellow precipitate | [15,18] |
| Glycoside | Bromine water test | A yellow precipitate | [19, 20] |
| Amino acids | Ninhydrin test | A purple colure solution | [15, 16] |
| Phenol | Ferric chloride test | Dark / green bluish black solution | [16,18] |
| Saponins | Foam test | Formation of 2cm thick layer of foam | [16] |

## RESULTS AND DISCUSSION

Authenticity, effectiveness, and conformity with Ayurvedic Pharmacopoeial standards were guaranteed by a thorough quality assessment of the Triphala Churna formulation that was created for this research. Emblica officinalis (Amla), Terminalia chebula (Haritaki), and Terminalia bellirica (Bibhitaki) are the three medicinal herbs that make up Triphala, a traditional Ayurvedic polyherbal compound. Among its traditional uses were those of a digestive aid, immunological booster, gentle laxative, and revitalising tonic. Scientific validation and quality assessment of the created formulation were achieved using a number of tests including organoleptic, microscopic, physicochemical, chromatographic, and phytochemical evaluations.

**Comparative organoleptic evaluation of triphala in-house and triphala marketed:**

There were discernible variations in the sensory attributes of in-house and commercialised Triphala, including appearance, odour, taste, and texture, according to the organoleptic examination (table2). A uniform light brown colour, a strong herbal scent, and a distinctive mix of astringent, sour, and bitter flavours indicated freshness and little processing were characteristics of the in-house formulation, which was made using authorised raw ingredients in identical quantities. The formulation also had minimal processing. On the other hand, the samples that were sold had a wide range of colours and textures. Some of them had clearly been stored poorly, with diminished aromatic intensity and changed flavour profiles all symptoms of industrial processing and a too long shelf life. The significance of traditional preparation methods and quality control in guaranteeing the efficacy of herbal remedies, such as Triphala, was underscored by these results.

Table 2- Comparative Organoleptic Evaluation of Triphala In-house and Triphala Marketed

| Parameter | Observation Triphala In-house | Observation Triphala Marketed |
|---|---|---|
| Colour | Brownish to light brown | Dark brown |
| Odour | Characteristic herbal smell | Slightly pungent herbal odour |
| Taste | Astringent and slightly sour | Bitter and slightly acidic |
| Texture | Fine powder | Rough powder |

**Microscopic and histochemical evaluation**

The detection and assessment of unprocessed plant medicines rely heavily on microscopy. Cell kinds, tissue arrangement, trichomes, stomata, veins, crystals, and other diagnostic information that aren't always apparent to the human eye may be examined using this method. Confining a drug's botanical identification and

identifying adulterants or replacements were both made easier using microscopy's quantitative and qualitative analytical capabilities. It was a crucial tool in pharmacogenetic investigations because techniques such as powder microscopy, staining, and transverse sectioning make important traits more visible. Microscopy revealed characteristic features of each ingredient in Triphala (Figure3):

**Amla (Emblica officinalis)**

Microscopic evaluation of the powdered formulation of Emblica officinalis in Triphala Churna revealed the presence of several characteristics features (figure3a). Thin walled parenchymatous cells along with simple starch grains of oval to spherical shapes were observed. Numerous rosette or clustered forms calcium oxalate crystals were observed.

Heavily lignified stone cells with thick walls and narrow lumen were present in the powder sample. In addition, spiral and reticulate vascular elements, fibres, and epidermal cells of the pericarp were identified. These characteristic microscopic features confirmed the authenticity and purity of the Amla component used in the Triphala Churna formulation.

**Harad (Terminalia chebula)**

Microscopic analysis of the Harad (Terminalia chebula) powder in Triphala Churna revealed the presence of single or clusters of thick-walled stone cells with a small opening and noticeable fissures (figure3b). In parenchymatous cells, tannins were common having brown pigmentation. Simple tiny, often round or oval-shaped starch grains were observed.

Additionally, xylem channels that were spiral or reticulate and fibres with tapering ends were visible. Rosette shaped calcium oxalate crystals were found. Epidermal cells exhibited irregular or, wavy anticlinal walls. These microscopic characters were useful to differentiate Haritaki from other plant powders in triphala churna.

**Bahera (Terminalia bellirica)**

Under a microscope examination, Terminalia bellirica powder revealed a variety of plant tissues that may be used for identification purposes (figure3c). Large stone cells, either singly or in groups, with strong walls and tiny lumens were observed. Parenchymatous cells containing starch grains, tannins and oil globules were observed. Long fibres with thick wall were visible. Scalariform or reticulate thickening was found in vessels. Epidermal cells exhibited a pitted surface along with tiny prismatic crystals of calcium oxalate.

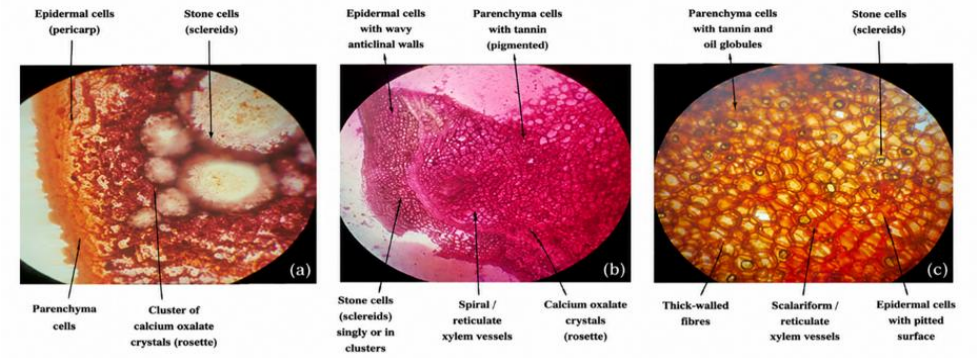



**Figure 1(a):** Transverse section of Emblica officinalis (Amla) showing a single-layered epidermis with polygonal-shaped cells in surface view, (b) Transverse section of Terminalia chebula (Harad) highlighting the presence of stone cells and mesocarp tissue, (c) Transverse section of Terminalia bellirica (Bahera) displaying palisade cells, mesocarp, and centrally located xylem.

**Microscopic characters of Triphala Powder**

**In-house**

Microscopical examination of the Triphala powder that was made in-house showed that it included typical

plant components such fibres, starch grains, and stone cells. The presence of many starch grains and well defined stone cells indicates that the fruits were little processed, which allowed their original tissue architecture to remain intact. These diagnostic traits provide evidence that the in-house preparation is genuine and of high quality.

**Marketed**

Microscopic analysis of the commercially available Triphala powder revealed the presence of characteristic plant fibres, calcium oxalate crystals, and starch grains. Plant fibres, calcium oxalate crystals, and starch grains were among the distinctive characteristics uncovered during microscopic analysis of the commercially available Triphala powder. Powdered Terminalia chebula, Terminalia bellirica, and Emblica officinalis fruits comprise the Triphala formulation, which is often linked with these structures. Although the amount and clarity of these characteristics may change according to processing and storage circumstances, their existence verifies the sample's botanical origin and lends credence to its validity as a true herbal product.

**Histochemical examination**

Powdered herbal medicines like Triphala depend on microscopy to verify their authenticity, quality, and purity. According to Kokata et al. (2010) [21], it allows for the identification of important diagnostic cellular features that help in the authentication of botanical origin and evaluation of raw material integrity. Emblica officinalis (Amla), Terminalia chebula (Harad), and Terminalia bellirica (Bahera) are the three fruits that make up Triphala, and their structural characteristics were identified by microscopic study of both commercially available and in-house powders (Figure4). According to Khare (2007) [22], the in-house preparation maintained the original architecture of the fruit with well-preserved and well- defined components including fibres, starch grains, and stone cells. In particular, the Amla powder exhibited characteristics consistent with traditional pharmacognostic accounts, such as parenchymatous cells with thin walls, starch grains that ranged from oval to rounded, rosette-type calcium oxalate crystals, lignified stone cells with narrow lumens, and pieces of spiral and reticulate vessels [23]. Kumar et al. (2015)[24] also noted that harad powder had parenchyma rich in tannins, tiny spherical starch grains, tapering fibres, spiral capillaries, and calcium oxalate crystals in rosette form. The powder also exhibited thick-walled stone cells with conspicuous pits. The hallmark microscopic features of Terminalia bellirica, such as big stone cells (either singly or in clusters), parenchymatous cells loaded with tannin, oil globules, prismatic calcium oxalate crystals, fibres with thick walls, and scalariform capillaries, were seen in bahera powder [25]. Alternatively, the commercially available Triphala product also had fibres, starch grains, and calcium oxalate crystals, all of which are signs of authentic plant matter. On the other hand, the cellular structures seemed less clear, which might be because of commercial processing, long storage, or lower-quality raw materials. These factors can impact how visible the structures are under the microscope and their therapeutic potential [26]. According to the World Health Organisation (2007) [27], powder microscopy is a valuable tool for standardising herbal products, checking for adulteration or replacement, and guaranteeing the validity of polyherbal compositions such as Triphala.

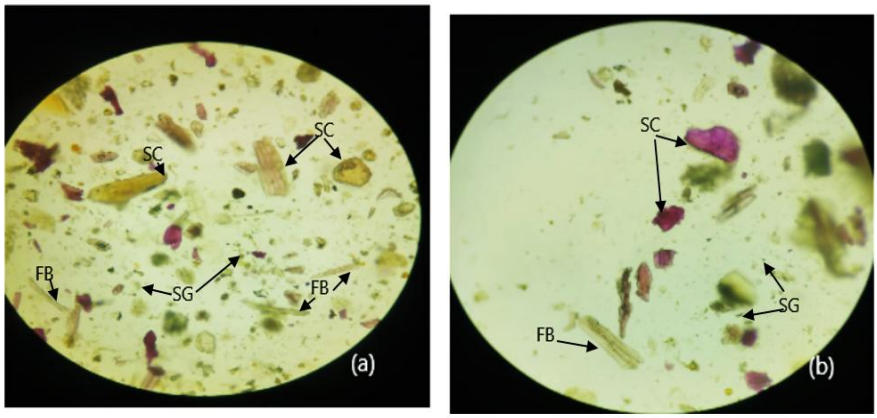



Figure 4- Microscopic study of Triphala (In-house) showing the presence of fibres (FB), starch grains (SG), and stone cells (SC) at 10X.

**Physiochemical evaluation of triphala churana**

Ayurvedic Pharmacopoeia of India quality criteria were met by both the in-house and commercialised Triphala churna samples, according to a comparative physicochemical study [28]. Improved formulation integrity and therapeutic potential were suggested by the slightly better features of the in-house formulation across many criteria. According to Kokata et al. (2010)[21], the in-house sample had a moisture content of 10.04 ± 0.11% and the marketed sample had a moisture content of 9.07 ± 0.11%. These values were within the permitted range for pharmacopoeial standards, suggesting that there is little chance of microbiological contamination and that the product will remain stable over time. The total ash values for the in-house sample were 2.50 ± 0.08% and for the marketed sample they were 2.09 ± 0.08%. The acid-insoluble ash values, meanwhile, were 1.02 ± 0.03% and 1.12 ± 0.03%, respectively (table3). According to Mukherjee (2002)[25], these results indicate that there were little amounts of inorganic and siliceous contaminants, such dirt or sand, which confirms that both samples were generally pure. According to Harborne (1998)[29], there was a clear difference in the extractive values between the in-house formulation and the marketed sample. The in-house formulation had higher concentrations of bioactive components such as tannins, phenolics, and flavonoids, as evidenced by higher water-soluble (49.00 ± 0.24%) and alcohol-soluble (39.34 ± 0.15%) extractives. The moderate acidity of both samples was consistent with the acidity of the fruits used, since both the in-house and marketed samples had slightly acidic pH values (3.40 ± 0.05 and 3.54 ± 0.05, respectively). Minor variances like this might arise from things like different raw material quality, different sourcing methods, different processing procedures, or different storage conditions [30]. Taken together, the findings confirm that both formulations are up to par, although the in-house Triphala churna showed somewhat better physicochemical properties, lending credence to its status as a trustworthy, premium herbal preparation.

**Table 3: Physiochemical test results of marketed triphala churna**

| **Parameter** | **In-house Triphala preparation** | | **Marketed Triphala sample** | |
|---|---|---|---|---|
| | Result (Mean ± SD) | Standard Limit | Result (Mean ± SD) | Standard Limit |
| Loss on Drying (%) | 10.04 ± 0.11 | Not More Than 12% | 9.07 ± 0.11 | Not More Than 12% |
| Total Ash Value (%) | 02.50 ± 0.08 | Not More Than 7.0% | 02.09 ± 0.08 | Not More Than 7.0% |
| Acid-Insoluble Ash (%) | 01.02 ± 0.03 | Not More Than 2% | 01.12 ± 0.03 | Not More Than 2% |
| Water Soluble Extractive (%) | 49 ± 0.24 | Not Less Than 30% | 41 ± 0.24 | Not Less Than 30% |
| Alcohol Soluble Extractive (%) | 39.34 ± 0.15 | Not Less Than 25% | 35.4 ± 0.15 | Not Less Than 25% |
| pH (1.0% Aq. Solution) | 3.40 ± 0.05 | - | 3.54 ± 0.05 | - |

**Thin Layer Chromatography (TLC) of Triphala in-house and triphala marketed**

In order to determine the important phytoconstituents in both the in-house and commercially available Triphala churna formulations, Thin Layer Chromatography (TLC) proved to be a dependable and repeatable method. Under ultraviolet light at 254 nm and 365 nm, the examination showed six separate spots in every sample, suggesting the existence of several bioactive chemicals [25]. Rf values of0.25, 0.36, 0.46, 0.67, 0.82, and 0.92 were produced by the 11.3 cm solvent front in the in-house sample. In a similar manner, the commercially available sample included a 12.1 cm solvent front with Rf values of0.25, 0.40, 0.51, 0.68, 0.90, and 0.94. According to research by Sharma et al. (2013)[30] and Harborne (1998)[29], ellagic acid normally has an Rf value of around 0.25 while gallic acid, which is recognised for its anti-inflammatory and powerful antioxidant characteristics, typically has an Rf value of about 0.36-0.40. Triphala has a large amount of

tannins, a kind of polyphenol, which are typically located in the lower to mid-Rf range (0.25-0.50), indicating that they are represented in the first three locations [21]. The secondary metabolites with greater Rf spots are probably less polar or are yet unknown (table4). Compared to the commercial sample, the in-house formulation's bands were much more intense and distinct, suggesting either increased phytochemical concentration, improved extraction efficiency, or higher-quality raw materials. In sum, the Triphala churna's phytochemical richness, uniqueness, and authenticity were all confirmed by the TLC profiles [25, 29].

**Table 4- Results of Thin Layer Chromatography and Rf Value of Triphala in- house**

| | In- house sample | | | Triphala marketed | | |
|---|---|---|---|---|---|---|
| Spot No. | Distance Travelled by Spot (cm) | Distance Travelled by Solvent Front (cm) | Rf Value | Distance Travelled by Spot (cm) | Distance Travelled by Solvent Front (cm) | Rf Value |
| 1 | 2.9cm | 11.3cm | 0.25 | 3.1 | 12.1 | 0.25 |
| 2 | 4.1cm | 11.3cm | 0.36 | 4.9 | 12.1 | 0.40 |
| 3 | 5.3cm | 11.3cm | 0.46 | 6.2 | 12.1 | 0.51 |
| 4 | 7.6cm | 11.3cm | 0.67 | 8.3 | 12.1 | 0.68 |
| 5 | 9.3cm | 11.3cm | 0.82 | 10.9 | 12.1 | 0.90 |
| 6 | 10.5cm | 11.3cm | 0.92 | 11.4 | 12.1 | 0.94 |

The results from the thin-layer chromatography (TLC) confirm that the commercial formulation contains important phytochemicals; nevertheless, differences in spot intensity and sharpness between the commercial formulation and the in-house sample may indicate differences in concentration or raw material quality.

**Phytochemical analysis**

Plant extracts may be qualitatively analysed for bioactive chemicals such alkaloids, glycosides, carbohydrates, flavonoids, tannins, and saponins using phytochemical analysis. The evaluation of the therapeutic potential of herbal formulations relies on these chemicals, which are responsible for the medicinal characteristics of herbs. The identification and standardisation of plant materials are aided by this analysis, which also aids in their preliminary screening (table5).

Table 5- Phytochemical analysis of the extracts of Triphala in- house and Triphala marketed:

| **Test for phytochemical** | **Triphala in- house** | | | **Triphala marketed** | | |
|---|---|---|---|---|---|---|
| | Water extracts | Methanol extracts | Ethanol extracts | Water extracts | Methanol Extracts | Ethanol extracts |
| Alkaloids | + | ++ | ++ | + | ++ | ++ |
| Glycosides | - | + | ++ | - | + | ++ |
| Carbohydrate | + | ++ | ++ | + | ++ | ++ |
| Tannins | ++ | +++ | +++ | ++ | +++ | +++ |
| Flavonoids | ++ | ++ | ++ | ++ | ++ | ++ |
| Saponin | + | + | + | + | + | + |
| Phenol | + | + | + | + | + | + |

Note: - absent, + indication, ++ significant, +++ requisite

Alkaloids, glycosides, tannins, phenolic chemicals, saponins, polysaccharides, and flavonoids were among the many secondary metabolites identified by qualitative phytochemical screening of the Triphala formulation (figure5). According to Mukherjee (2002)[25], these bioactive components play a major role in the medicinal effects of herbs. According to Trease and Evans (2002)[31], alkaloids are known for their pharmacological actions, which include antibacterial and analgesic potential. Mayer's test confirms this existence by forming a pale yellow precipitate. Triphala may have nutritional and prebiotic properties due to the natural carbohydrate content generated from its fruit components, as shown by the identification of reducing sugars by Fehling's test [29]. Triphala has a long history of usage as a general health tonic, and the foam test has shown that it contains saponins, which are recognised for their surface-active and immunomodulatory characteristics [32]. According to Kaur and Arora (2009)[33], Triphala is known for its digestive and wound-healing properties, and the positive ferric chloride test result validates the presence of tannins and phenolic compounds. These compounds have strong antioxidant, antibacterial, and astringent activities. According to Cowan (1999)[34], flavonoids, which are linked to powerful antioxidant and anti-inflammatory properties, were present when the Shinoda test revealed magenta colouration. There were also glycosides detected by the Keller-Killiani test; these included cardiac glycosides in particular, which improve cardiovascular function and may have an effect on the formulation's adaptogenic qualities [35]. The existence of these phytochemicals lends credence to Triphala's traditional medicinal uses, supports its wide range of therapeutic uses, and calls attention to its promising future in pharmacological and clinical research [25].The following bar graph visually represents these comparative findings, highlighting the observed differences between the two samples.

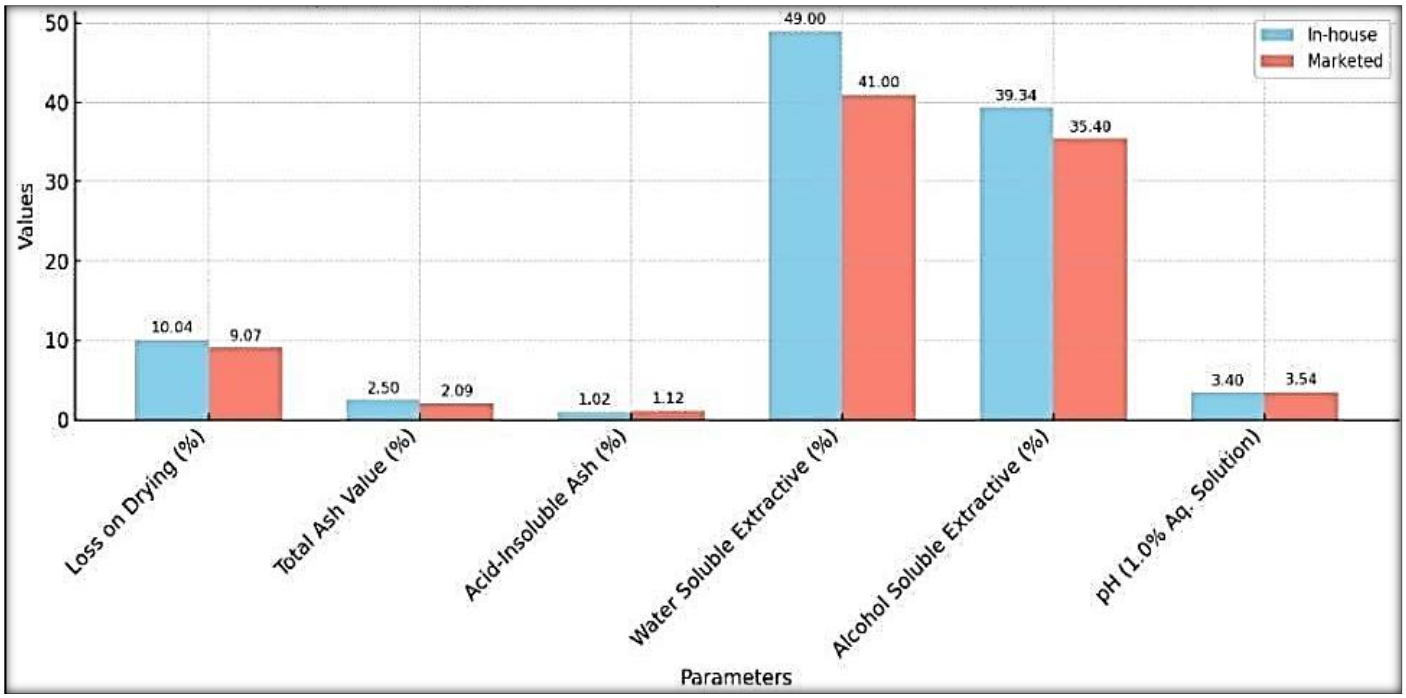



Figure 5- Comparative analysis of physiochemical parameters of Triphala In-house and Triphala Marketed

## CONCLUSION

Triphala Churna is a traditional Ayurvedic formula, and its main components -Terminalia bellirica (Bahera), Emblica officinalis (Amla), and Terminalia chebula (Harad) were determined to be genuine by organoleptic and microscopic testing. All of the physiochemical criteria, such as ash levels, extractive values, loss on drying, and overall quality, were determined to be within acceptable ranges, suggesting that the raw materials were handled correctly. Chemical consistency and therapeutic significance were further confirmed by the TLC profiling, which further corroborated the presence of essential phytoconstituents. These findings will be used in-depth organoleptic, microscopic, physicochemical, and chromatographic evaluations to determine its quality criteria. This created composition satisfies all requirements set forth by the Ayurvedic Pharmacopoeia of India, according to the findings. Following quality control standards is crucial for making Ayurvedic medicines, according to the research. Modernising the healthcare system by standardising polyherbal products

increases the effectiveness and safety of traditional treatments while also boosting customer confidence.

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### Conflict of interest

All authors declare that they have no conflict of interest.

### Author contribution

NS has conducted the conceptualization, experimentation, data curation and investigation. SNO and SG were responsible for supervision. MS has conducted statistical analysis, review editing, and plagiarism. All authors has finalized the manuscript.

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