Comparative Standardization of the Ayurvedic Formulation Trikatu Churna: A Polyherbal Mixture

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Abstract

People are shifting to using herbal medical goods in the current global healthcare system. The utilization of herbs and awareness in conventional medicine has increased exponentially. The majorities of the ancient systems of medicine (namely Ayurveda, Unani, Homeopathy, and Yoga) is effective but have certain gaps in standardizations, hence it is crucial to properly integrate current scientific procedures and old knowledge. An Ayurvedic polyherbal composition called Trikatu Churna is effective for treating a variety of illnesses and problems. The authenticity of the herbs included in a preparation determines its effectiveness. The first and most important stage in standardizing herbal formulation is the authentication of plants through anatomical research. Aim: The objective of the study is comparative standardization of commercial and in-house ayurvedic formulation Trikatu Churna by it pharmacognostic examinations, including macroscopic, microscopic, and chemical tests including preliminary phytochemical, physico-chemical constants, and TLC profile. Methods: The quality paper's standard methods were followed. Results: Macro-microscopic, preliminary phyto-chemical, and TLC analyses of the in-house and commercial formulation has been compared and documented. Conclusion: The study's findings are useful in standardizing the polyherbal Ayurvedic formula Trikatu Churna, which will increase its reputation as an Ayurvedic medicine around the world.

1. Introduction

Ayurveda, Siddha, and Unani are a few of the diverse ancient medical systems that make up India's rich past. The primary component of these traditional remedies is phyto-constituents. It is possible to conserve this traditional legacy and justify the use of natural products in healthcare by developing these orthodox medicine approaches with both the dimensions of safety, quality and efficacy [1]. The

practice of using botanicals as therapies is the traditional type of healthcare known to humanity, and it has been exercised throughout history in all cultures [2]. Due to its natural origin, efficacy, safety and minimum of side effects, traditional herbal medicine and its formulations have been widely utilised for thousands of years in both developed and developing countries [3-4].

However, the majority of Ayurvedic formulas lack a defined quality control criteria and an evaluation mechanism [5]. The World Health Organization has stressed the necessity of ensuring the safety of medicinal plant products by employing contemporary controlled technique and adopting appropriate criteria. Standardization is the process of assessing the quality and purity of crude pharmaceuticals using different criteria, such as morphological, microscopical, physical, chemical, and biological observations [6]. The harvesting of the herbal substance, its packaging, and its use as medicine are all standardized. The variety of phytoconstituents found in herbal medicines is influenced by changes in the environment and variations in the chemical makeup of the soil [7].

As a result, adulterants or imitations are added to the herbal medication. The safety and effectiveness of the medicine can alter if replacements and adulterants are added. Although churna, one such ayurvedic composition, is described in the Ayurvedic school of medicine as a powder form of drug or medicament, there is a need to standardized herbal treatments. If stored in an airtight container, the churna is freely flowing and keeps its efficacy for a year. They resemble powder compositions used in the allopathic medical system. Because of the size of its particle, these medication types are typically recommended. The better the bioavailability, the larger the rate of absorption from G.I.T. is when the particle size is smaller [8].

The goal of the current study was to standardise commercial versions of the "Trikatu Churna" ayurvedic polyherbal compound. The Ayurvedic definition of "Trikatu" is "containing a Katu-Tikta rasa" (bitter, pungent, or acrid flavour), "Usna" (hot), "Virya" (potency), "Madhura rasa" (sweet taste), and "Vata-kapha Nasaka" (destroyer of air and mucus under disease states) [9].

Trikatu, which is composed of three herbs i.e. fruits of *Piper nigrum* L. (Maricha) and *Piper longum* L. (Pippali) and rhizomes of *Zingiber officinale* Rosc. (Sunthi) which shows potent antibacterial, rejuvenator, and stimulant properties, is also used as a digestive tonic to help the body assimilate other foods [10]; it also aids in reducing gas forming in the

abdomen; and it has analgesic and antibacterial properties [11]. Additionally, anthelmintic action has been observed for aqueous extract of churna [12]. Trikatu speeds up nutritional absorption, which improves the metabolic process [13]. It has an alkaloid called "Piperine" as an active component that increases the bioavailability of medications and minerals [14]. These plant products are also utilised as spices all throughout the world. They are also employed as significant components in folkloric medicine and Ayurvedic, Siddha, and Unani (ASU) medications. Trikatu Churna intake has a number of positive health effects due to its numerous therapeutic benefits, including those for fever, asthma, colds, and cough [15]. Additionally, it is used as a digestive aid, a carminative, and for both acute gouty arthritis and rheumatic diseases [16]. The WHO establishes standards for herbal drugs and focuses on the present and future developments in analytical techniques based on a number of factors, including the accurate identification of the specimen, sensory properties assessment, pharmacognostic assessment, volatile matter, quantitative evaluation (ash values, extractive values), and phytochemical evaluation. Of these factors, the phytochemical profile is particularly significant because it directly affects the quality of the herbal drug. The term "phytochemical standardization" refers to the collection of all available data pertaining to the chemical components found in herbal medicines. While phytochemical profiling of the medicine is guided by the fingerprint profiles to ensure quality. The goal of the current study was to standardize Trikatu Churna and establish uniform guidelines for maintaining drug quality.

2. Methodology

Raw Material Sourcing

Fruits of *Piper longum* L. (Pippali), *Piper nigrum* L. (Maricha), and rhizomes of *Zingiber officinale* Rosc. (Sunthi) were collected as crude drug ingredients from Abbumiya Herbs, Nagpur, India. The drug where treated for size reduction and in-house formulation was prepared, based on the ayurvedic parameters such as (colour), *gandha* (odour), *ruchi* (taste), *aakruti varna* (shape) and *parimana* (size).



Figure 1. Raw crude drugs used in In-House formulation of Trikatu Churna

Purchasing of Commercial formulation

Trikatu Churna purchased from a Community Ayurveda Pharmacy, Nagpur, India (Table 1).

Table 1. Purchasing of Commercial formulation

S. No	Name of Product	Mfg by	Batch No
1	Trikatu Churna	Vyas Pharmaceuticals, Haridwar	Ref: AFI-I-89
2	Raw Crude Drug Ingredients	Abbumiya Herbal Drugs, Nagpur	

Preparation of in-house polyherbal formulation

All of the individual crude drug ingredients were cleansed by hand picking and dried in the shade. After being individually pulverized with a grinder, the medications were subsequently put via mesh no. 80. To create the formulation, an equal part of each powdered medication was taken (Table 2). The various medications were then weighed according to the appropriate dosage. Utilizing a double cone blender, the medicines were geometrically blended. Weighed, and packed into containers was the combined In-House formulation of Trikatu Churna [17].

Organoleptic Assessment

Organoleptic assessment and variables: The Macroscopic assessment of crude drugs as well as commercial and in-house formulated Churna was carried out to assess the distinct characteristics as well as its size, colour, odour, and taste.

Microscopic Assessment

It involves a thorough assessment of herbal medications and is employed to identify organized drugs based on their recognized histological characteristics. With the aid of a microscope, it is frequently employed for qualitative investigation of organized crude medicines in whole and powder form. A circular or oval form characterizes the inner pseudo-parenchyma cells. They have fixed oil and protein. By cutting thin TS (Transverse Section) and LS (Longitudinal Section) into a bark, wood, or leaf, crude pharmaceuticals can be microscopically detected.

Powder Microscopy

The analysis of powder is crucial in identifying crude drugs. These characteristics will aid in the detection of adulterants and the proper variety identification. One of the easiest and cheapest ways to use to identify the source materials correctly is powder microscopy. It aids in the standardization of plant material and further pharmacological and medicinal evaluation. Dried powder is typically used to examine cellular

contents (type/shape) under a microscope to determine whether they are present or absent.

Physical Assessment

Each monograph includes detailed botanical, macroscopic, and microscopic descriptions along with thorough physical evaluation pictures that offer visible proof of correctly identified material. A microscopic examination confirms the authenticity of the substance and serves as a preliminary check for contaminants [18-19].

Determination of pH [20]

1 g of churna, precisely weighed, was added to a 100-ml volumetric flask, and the remaining volume was filled with distilled water to the required level. For roughly 10 minutes, the solution was sonicated. Using a digital pH metre, pH was measured.

Foreign organic matter (FOM): 50gm of the specimen should be precisely weighed and then distributed in a single sheet. Use a 6 X lens or unaided dye to examine the sample, and then manually remove the foreign organic materials as completely as you can [21].

Moisture (Loss on drying [LOD]): 2g of the churna was weight in a porcelain dish that is both flat and thin. Dry in the oven for 30 minutes at 100-105°C. Weigh the contents after cooling. Keep the contents in a hot air oven and repeat the drying process for 30 minutes at 100-105°C. (Care should be taken that the contents should not get charred off). Cool the weight and its contents. Up until two concordant weights are recorded, repeat the drying and weighing process [21].

Total Ash Determination: For 30 minutes, heat a silica or platinum crucible to red heat. After it has cooled in a desiccator, weigh it. Distribute the item under examination uniformly throughout the crucible after precisely weighing 2g of it. Dry for an hour at 100 to 105°C, and then ignite to constant weight at 600° to 250° C in a muffle furnace. After each ignition, let the crucible cool in a desiccator. Throughout the process, the material shouldn't catch fire at any point. The charred mass should be extinguished with hot water if a carbon-free ash cannot be produced after a lengthy ignition process. The residue should then be collected on an ash-free filter paper and incinerated until the ash turns white or

almost so. Determine the percentage of ash using the air-dried medication as a reference [21].

Determination of Acid-insoluble Ash: After being heated for 5 minutes using 25ml of 2M hydrochloric acid, the total amount of ash is filtered through ashless filter paper. Within silica crucible, the filter paper is fired, cooled, and the acid-insoluble ash is then weighed [21].

Determination of Water-soluble Ash: Ash that dissolves in water is the weight difference between total ash and the residual that results from boiling total ash. The complete amount of ash should be heated in 25 cc of water for five minutes. The insoluble material can be gathered on ash-free filter paper or in a Gooch crucible. It should be burned for 15 minutes at a temperature of no more than 4500C after being cleansed with hot water. The weight of the ash should be deducted from the overall weight of the insoluble materials. The water-soluble ash is represented by this weight disparity. Calculating the proportion of water soluble ash should be done in relation to the medicine that has been air dried [21].

Water Soluble Extractive Value: Put 5g of precisely weighed coarsely powdered, air-dried material in a conical flask with a glass stopper. To get the overall weight with the flask included, weigh after adding 300ml of water. Give a good shake and let stand for an hour. Connect a reflux condenser to the flask and slowly boil for 6 hours. After cooling, weigh the mixture, and then quickly filter it through a dry filter. The extracted powder should be dried in the oven until the weight is constant. Determine the extractable matter content in mg per g of air-dried material [21].

Alcohol Soluble Extractive Value: Put 5g of precisely weighed coarsely powdered, air-dried material in a conical flask with a glass stopper. To get the overall weight with the flask included, weigh after adding 100ml of water. Give a good shake and let stand for an hour. Connect a reflux condenser to a flask and slowly boil for 6 hours. After cooling, weigh the mixture, and then quickly filter it through a dry filter. The extracted powder should be dried in the oven until the weight is constant [21].

Determination of Physical Characteristic [22]

Bulk Density

Bulk density, also referred to as fluff density, is the ratio of a given quantity of powder to its bulk volume. It is estimated by transferring a precisely weighed amount of powder sample to the graduated cylinder using a funnel. The initial volume was described as being poured or uncapped.

Tapped Density

A graduated cylinder is filled with a predetermined amount of powder (25 grammes), and the measurement is made by tapping the cylinder repeatedly. The beginning volume was observed. The grade cylinder was continuously tapped for 10 to 15 minutes.

Angle of Repose

Angle of repose has had in fact been used as a de facto indirect method of evaluating the capacity of powder to flow because of its relationship to particle cohesiveness. The angle of repose seems to be the natural angle formed by the surface of the powder pile and the horizontal surface. The powder is funnelled through a 4 cm-high funnel linked to a burette. A sheet of paper is placed on the table close to the funnel. Both the height and radius of the pile were measured.

Hausner Ratio

It is related to interparticle friction and, as a result, can be used to predict the properties of powder flow. Low interparticulate friction powders, such coarse spheres, have a Hausner ratio of about 1.2, whereas more cohesive, less flow able powder particles, like flakes, have a ratio of more than 1.6.

Carr's Index

Another technique for inferring the powder flow indirectly using bulk density is Carr's index.

Chemical Assessment

The majority of medications have specific chemical components that are active and are in charge of their biological and pharmacological action. Drug quality and purity are determined by a qualitative chemical test. Phytochemicals are chemicals derived from plants and the term is often used to describe the large number of secondary metabolic compounds found in plants. Phytochemical screening assay is a simple,

quick, and inexpensive procedure that gives the researcher a quick answer to the various types of phytochemicals in a mixture and an important tool in bioactive compound analyses. Chemical evaluation also includes preliminary phytochemical research [23].

A brief summary of the experimental procedures for the various phytochemical screening methods for the secondary metabolites is shown in Table 7. After obtaining the crude extract or active fraction from plant material, phytochemical screening can be performed with the appropriate tests as shown in the Table 7 to get an idea regarding the type of phytochemicals existing in the extract mixture or fraction.

Chromatographic Profile (TLC profile)

Marker compounds are chemical components of medication that can be used to verify its authenticity or potency. Since the marker molecules occasionally behave as chemicals or active components that confirm the true botanical identity of the starting material. It is extremely difficult to identify the appropriate marker molecules for all traditional drugs because some of them have active compounds that are unidentified and others contain several active components. A chromatography profile of a medicinal herb is a chromatographic profile of an extract containing multiple widely distributed chemical components having physiologically active and/or chemical qualities. Chromatographic fingerprints can be used to correctly determine the validity and identity of herbal medicines even when the amount and/or concentration of the chemically unique constituents are not consistent among medicinal samples [24].

Therefore, it's critical to produce reliable chromatographic fingerprints that show the chemically unique and physiologically active components of an herbal medicine.

Extract preparation for TLC

3g of each drug sample including formulated and marketed sample were immersed separately in hydroalcohol (50:50) for the entire night, refluxed over a water bath for 30 min., and then filtered. The filtrates were prepared up to 10 ml in a standard flask separately and concentrated on a water bath. Technique for creating TLC Chromatographic

separation was accomplished using a slide support coated with silica gel G of approx. 0.2 mm thickness. Capillary tubes were used to spot the samples. Toluene, ethyl acetate, and glacial acetic acid were used as the mobile phase in a glass beaker throughout the development of the plate (8:2:0.1). The plate was dried after development, and 10% sulfuric acid was utilised as a spraying reagent. In the UV Cabinet at UV-254 the dry plate was seen. The spots are found, and the RF value is determined.

3. Result and Discussion

Although Ayurvedic formulations claim to be made in accordance with established guidelines, maintaining uniformity in formulations is very difficult. This difficulty may be caused by natural heterogeneity, as the quality of herbal materials collected from wild collections exhibits increasing seasonal variations [25].

Preparation of in-house polyherbal formulation

All of the individual crude drug ingredients were cleansed by hand picking and dried in the shade. After being individually pulverized with a grinder, the medications were subsequently put via mesh no. 80. To create the formulation, an equal part of each powdered medication was taken (Table 2). The various medications were then weighed according to the appropriate dosage. Utilizing a double cone blender, the medicines were geometrically blended. Weighed, and packed into containers was the combined In-House formulation of Trikatu Churna.

Table 2. Trikatu Churna In-house formulation

S. No.	Herbs	Commo n Name	Botanical Name with family	Parts	
1	Peeper	Pimpli	Piper longum L. (Piperaceae)	All	
2	Black Pepper	Maricha	Piper nigrum L. (Piperaceae)	ingredient in	
3	Dried Ginger	Sunthi	Zingiber officinale Roscoe (Zingiberaceae)	equal parts	

Organoleptic Assessment

Based on the information in **Table 3 and 4**, except for flavour/taste, the formulations are equivalent according to an organoleptic study. It is used to identify organized pharmaceuticals based on their

identified histological properties to thoroughly examine herbal treatments. It is widely used for qualitative analysis of organized crude medications with whole and form of powder with the use of a microscope.

Table 3. Macroscopic assessment of individual crude drugs

Paramet er	Piper nigrum	Piper longum	Zingiber officinale
Color	Grayish black or a dark brown colour	Light to dark brown	Buff color
Odour	Pungent and aromatic	Aromatic spicy	Aromatic
Size	3.5-6mm in diameter	2 to 5 cm in length 0.4 to 0.5cm in diameter	Rhizomes of ginger are about 5-15×1.5-6.5cm
Taste	Pungent	Sweet and Hot taste	Pungent
Shape	Circular	Circular and sweet taste	Irregular

Table 4. Macroscopic assessment of commercial and In-house formulated Churna

S. No.	Trikatu Formulation	Appearance	Color	Odou r	Taste
1	Commercial Churna	Fine, evenly powder	Light brown	Punge nt	Astringent and acidic
2	In-House Churna	Fine, evenly powder	Slight greenish brown	Punge nt	Bitter in taste

Microscopic Assessment

The pericarp and perisperm of *Piper nigrum* are thick and contain lignified stone cells. Epicarp is one-layered, while mesocarp is wide. Numerous Sclerenchymatous cells with a yellow tinge and oil globules had been seen.

A thick cuticle that covers the outer epidermis is visible on *Piper longum*. The endocarp included grains of starch, while the mesocarp was composed of large cells which had collapsed.

According to *Zingiber officinale*, the cortex is devoid of starch and is made up of endoderm, reddish-brown oleo resin, and isodiametric thin-walled parenchyma. Vascular bundles are known to exist.

Microscopy of Piper nigrum: Piper nigrum under the microscope reveals a thick pericarp and an inner layer of perisperm with lignified stone cells. Mesocarp is broad, and epicarp is single-layered. Oil globules and several Sclerenchymatous cells with a yellow tint had been seen (Figure 2).

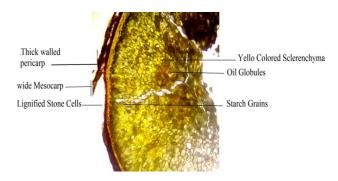


Figure 2. Transverse Section of Piper nigrum

Microscopy of Piper longum: The transverse section reveals a thick cuticle covering the outer epidermis. Large cells that had collapsed made up

the mesocarp, while the endocarp contained grains of starch (**Figure 3**).

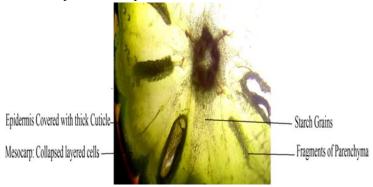


Figure 3. Transverse section of *Piper longum*

Microscopy of Zingiber officinale: The transverse section reveals that the cortex is free of starch and is composed of isodiametric thin-walled parenchyma,

reddish-brown oleo resin, and endoderm. Vascular bundles have been observed (**Figure 4**).

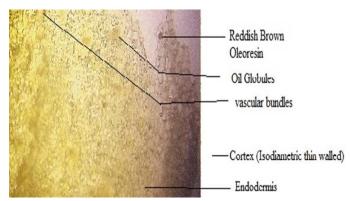


Figure 4. Transverse section of Zingiber officinale

Powder Microscopy

The identification of crude medicines requires the study of powder. These traits will help in the appropriate variety identification and the detection of adulterants. It helps to standardise plant-based materials. Under a microscope, dried powder is

frequently used to check the presence or absence of cellular components. It demonstrates the presence of starch grains, oil globules, parenchymateous cell fragments, and sclerenchymatous cell fragments. Geographical conditions of agriculture parameters cause the fluctuation (**Figure 5 and 6**).

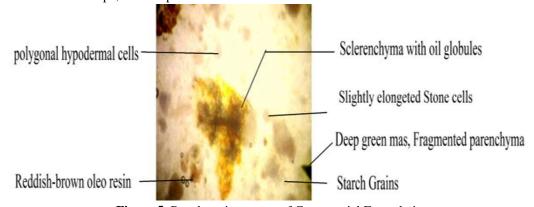


Figure 5. Powder microscopy of Commercial Formulation

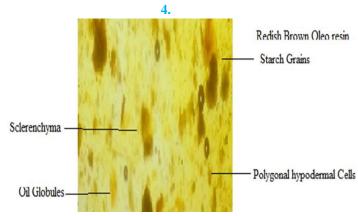


Figure 6. Powder microscopy of In-house Formulation

Physical Assessment

The obtained pH value was discovered to be within the norms. The obtained ash values, acid insoluble and water soluble were discovered to be within the accepted ranges. Both the alcohol- and water-soluble extractive values in the current analysis were discovered to be higher than the benchmark values. Physical characterization revealed similarities between the outcomes of the commercial formulations and the in-house formulation. The in-house formulation had acceptable physical and chemical properties, but it was unacceptable since it had higher LOD and extractive values (**Table 5**).

Table 5. Physico-Chemical Parameters

S. No	Physico-Chemical Parameters	Commercial Churna	In-House Churna
1	рН	5.8	6.1
2	Moisture (Loss on drying)	0.4231gm/cm ³	0.5181gm/cm ³
3	Total Ash Determination	3.6	3.1
4	Acid-insoluble Ash	1.8	1.5
5	Water-soluble Ash	2.6	2.9
6	Water Soluble Extractive Value	1.9gm	0.7gm
7	Alcohol Soluble Extractive Value	0.9gm	0.15gm

Determination of Physical Characteristic

Poor formulation flow ability was discovered in both commercial and in-house formulations, which was further supported by high Hausner ratio values. Although the physical characteristic values of each formulation varied, they were all above the allowed range and therefore could not be effectively regarded as meeting standards. To prevent gastrointestinal irritation, the physical characteristics such as pH and moisture content were assessed, as well as any weight gain brought on by moisture absorption (**Table 6**).

Table 6. Physical characteristics

S.	Physical Parameters	Commercial Churna	In-House Churna
No			
1	Bulk Density	0.421gm/cm ³	0.679gm/cm ³
2	Tapped Density	0.635gm/cm ³	0.991gm/cm ³
3	Angle of Repose	430	440
4	Hausner Ratio	0.673gm/cm ³	0.591gm/cm ³
5	Carr's Index	38.91	41.47

Chemical Assessment According to qualitative phytochemical examination, all samples contain alkaloids, carbohydrates, saponin, phenol, flavonoids,

and tannin, all of which can be further investigated and quantified (**Table 7**).

Table 7. Preliminary Phytochemical Screening of hydro-alcoholic extracts of Commercial and In House formulated Trikatu Churna

Phytochemical s	Commercial Churna	In-House Churna
Alkaloids	+	+
Carbohydrates	+	+
Saponins	+	+
Phenols	+	+
Flavonoids	+	+
Protein	-	-
Tannins	+	+
Terpenoids	-	-
Steroids	-	-
Glycosides	-	-

Chromatographic Profile (TLC profile)

The hydro-alcohol extract of the samples showed the highest resolution in the solvent system on the TLC chromatogram. Ethyl acetate, glacial acetic acid, and toluene (8:2:0.1). We can illustrate how many active principles are present by counting the number of spots.

Samples BP, PL, G, LF, and MF display a variety of spots, some of which have the same Rf value but a varied intensity (area under the curve), indicating that the amount of the active ingredient is not uniform (Figure 7) and (Table 8).



Figure 7. TLC of Drugs, Marketed and In House formulation of Trikatu Churna

(BP: Black Pepper; PL: Long Pepper; G: Ginger; LF: Lab formulation and MF: Marketed formulation)

Table 8. RF value of visible spots

Sr. No/ Spot No	Dist travel by Solute/ distance travel by Solvent	Rf Value
1	0.7/5.2	0.1
2	2.5/5.2	0.5
3	3.1/5.2	0.5
4	3.4/5.2	0.6
5	3.6/5.2	0.6
6	4/5.2	0.7
7	4.5/5.2	0.8
8	4.9/5.2	0.9

4. Conclusion

With the use of a comparative analysis of in-house and a commercial formulation, the objective of this evaluation study was to determine the quality, purity, and integrity of trikatu churna. It is concluded that there is uniformity in formulation except variation in LOD and extractive values, which may be caused by the variety of environments in which these plants grow. Physicochemical parameters like water-soluble, alcohol-soluble, and moisture content, bulk density, tapped density, Carr's index, Hausner's ratio, pH, water-soluble ash, acid-insoluble ash, and Organoleptic characteristics can be effectively used for standardizing polyherbal formulations.

The study's findings could be used as a guide for establishing upper and lower bounds for reference standards for these medications' quality assurance and control.

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