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COMPARATIVE STANDARDIZATION STUDY OF THREE TRIPHALA CHURNA FORMULATION

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ABSTRACT: In a few decades, there has been exponential growing in the field of herbal medicines. Most of the traditional system of medicine is effective, but they lack standardization. So, there is a need to develop a standardization technique. Standardization of herbal formulation is essential to assess the quality, purity, safety, and efficacy of the drug based on the concentration of their principles. This articles reports on standardization of Triphala churna. Polyherbal ayurvedic medicines used to treat constipation, gastric disorder. The present research study deal with the comparative Standardization of two reputed marketed Triphala churna formulation, from Patanjali, Shree Ayurveda and laboratory made churna. The standardization of this formulation, organoleptic characteristics, physical properties such as moisture content (LOD), ash value, extractive values, crude fiber content was carried out. The heavy metal content, tannin test, and alkaloid test study also carried out to ascertain the quality, purity, and safety of these herbal formulations.

INTRODUCTION: Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Today about 80% of people in developing countries still rely on traditional medicine based largely on the different species of plants for their primary health care. About 500% of plants with medicinal uses are mentioned in ancient literature, and 800 plants have been used in the indigenous system of medicine. The various indigenous system such as Ayurveda, Siddha, Unani use several plant species to treat different ailments [1, 2, 3]. Herbal medicines make up an essential component of the trend toward alternative medicine.

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A Harvard study recently found that one in three respondents acknowledged the use of at least one alternative therapy within the past year. Extrapolated, these findings suggest that up to $13.7 billion were spent in 1990 alone for these treatments [4]. Tyler defines herbal medicines as "crude drugs of vegetable origin utilized for the treatment of disease states, often of a chronic nature, or to attain or maintain a condition of improved health." [5] current demands for herbal medicines have resulted in an annual market of $1.5 billion and increasingly widespread availability [6].

Potential Benefits of Herbal Drugs: Historically, herbal medicines have played a significant role in the management of both minor and major medical illnesses. One example is foxglove, which contains cardiac glycosides, and serves as a classic treatment for congestive heart failure. Even now, physicians still use many drugs that possess botanical origins. Huxtable notes that one-quarter of the prescriptions

currently written in the United States are for plant products, while one quarter is for agents based on botanical compounds. The therapeutic potential of herbal medicines cannot be ignored and is highlighted in the three examples provided next [7].

Advantages of Herbal Medicine:

- They have a large amount of use.
- They have better patient tolerance as well as acceptance.
- The medicinal plants have a renewable source of cheaper medicines.
- Improvements in the quality, efficacy, and safety of herbal medicines with the development of science and technology.
- Prolong and uneventful use of herbal medicines may testify to their safety and efficacy.
- They are cheap.
- They are not harmful.
- They are more effective than any synthetic drug throughout the world herbal medicines have provided many of the most potent medicines to the vast arsenal of drugs available to modern medical science, both in crude form as well as a pure chemical upon which modern medicines are constructed [8, 9].

The Need for Standardization – Producers and Consumers Perspective: [10] In the global perspective, there is a shift towards the use of the medicine of herbal origin, as the dangers and the shortcoming of modern medicine are getting more apparent. It is the cardinal responsibility of the regulatory authorities to ensure that consumers get the medication, which guarantees purity, safety, potency and efficacy. The regulatory authorities rigidly follow various standards of quality prescribed for raw materials and finished products in pharmacopeias, formularies and manufacturing operation through statutory imposed good manufacturing practices. These procedures logically would apply to all types of medication whether included in the modern system of medicine or one of the traditional systems.

Though, herbal products have become increasingly popular throughout the world, one of the impediments in its acceptance is the lack of standard quality control profile. The quality of herbal medicine that is, the profile of the constituents in the final product has implications in efficacy and safety.

However, due to the complex nature and inherent variability of the constituents of plant-based drugs, it is difficult to establish a quality control parameter through the modern analytical technique is expected to help in circumventing this problem. Furthermore, the constituents responsible for the claimed therapeutic effects are frequently unknown or only partly explained. This is further complicated by the use of a combination of herbal ingredients as being used in traditional practice. It is common to have as many as five different herbal ingredients in one product. Thus, batch to batch variation starts from the collection of the raw material itself in the absence of any reference standard for identification.

These variations multiply during storage and further processing. Hence, for herbal drugs and products, standardization should encompass the entire field of study from the cultivation of medicinal plant to its clinical application. Plant materials and herbal remedies derived from them represent a substantial portion of the global market, and in this respect internationally recognized guidelines for their quality assessment and quality control are necessary.

Plan of Work: Comparative standardization of Triphala Churna formulated by Patanjali, Shree Shree Ayurveda and laboratory made Triphala churna was planned to carry out the development of quality standards for the finished marketed formulation. The method used for the comparative standardization was planned to be carried out as follows:

Development of standardization parameters for Triphala Churna:

Study of Organoleptic Characters:

- Colour
- Odor
- Taste

Determination of Physicochemical Parameters:

- Total ash
- Acid-insoluble ash
- Water soluble ash
- Moisture content/ Loss on drying
- Water-soluble extractive
- Alcohol soluble extractive
- Crude fiber contents

Evaluation of Churna:

- Powder fineness
- Bulk density
- Tap density
- Angle of repose
- Compressibility
- Hausner's ratio

Determination of pH:

Establishing the Safety about Heavy Metals & Microbial Load:

Fluorescence Analyses:

MATERIALS AND METHODS:

Samples Preparation: Triphala churn contains mainly three ingredients as Harad or Haritaki (*Chebulic Myrobalans* or *Terminalia chebula*), Baheda or Bibhitaki (*Terminalia Bellirica*) and Amla or Amalaki (Indian gooseberry or *Emblica officinalis*).

Sample No. 1: Patanjali Triphala Churna

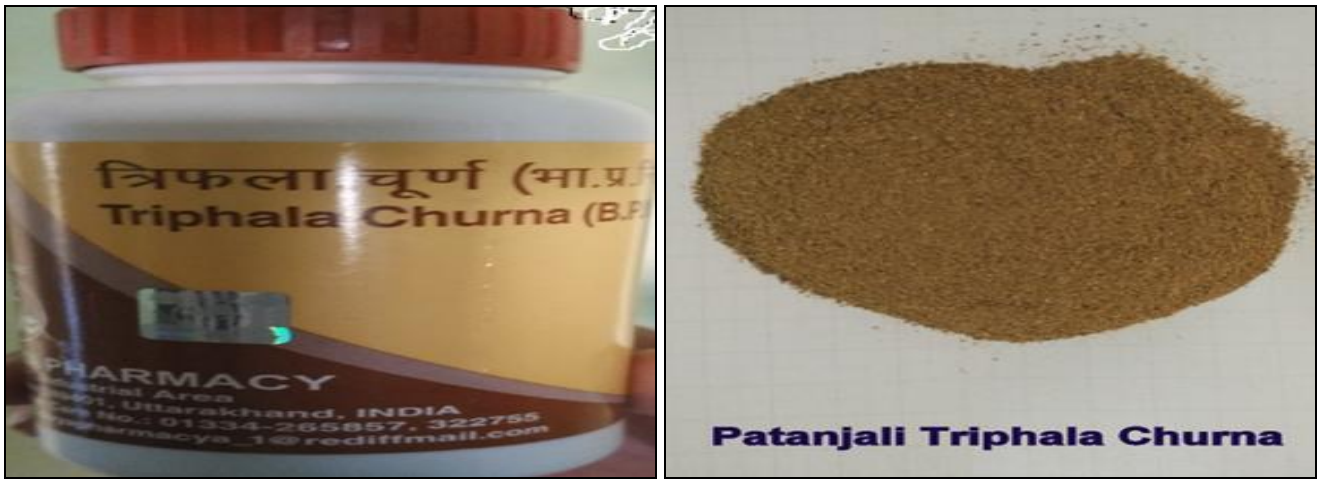

FIG. 1: SAMPLE NO. 1 PATANJALI TRIPHALA CHURNA

Sample No. 2: Shree Shree Ayurveda Triphala Churna

FIG. 2: SAMPLE NO. 2 SHREE SHREE AYURVEDA TRIPHALA CHURNA

Sample No. 3: Lab made Triphala churna

FIG. 3: SAMPLE NO. 3 LAB MADE TRIPHALA CHURNA

Method to Prepare the Triphala Churna: Harad or Haritaki (Chebulic Myrobalans or *Terminalia chebula*), Baheda or Bibhitaki (*Terminalia Bellirica*) and Amla or Amalaki (Indian gooseberry or *Emblica officinalis*) collected from the local market. The fine powder was made both by grinding and filtering them.

All the powders were mixed properly in a ratio 1:2:4. The Triphala Churna is prepared and ready to use. For future use, it can keep into a plastic box.

Developments of Standardization Parameters for Triphala Churna:

Study of Organoleptic Characters: The polyhedral formulation is studied for organoleptic characters like color, odor and taste using the sensory organs of our body.

Physicochemical Analysis: [11]

A) Ash Value:

Determination of Total Ash: About 2 to 3 g of sample was accurately weighed in a tarred silica dish at a temperature not exceeding 45 °C until it was free from carbon. Then it was cooled and weighed. The percentage of total ash was calculated concerning the air-dried drug.

Determination of Acid Insoluble Ash: The total ash obtained was boiled for 5 minutes with 25 ml of dilute hydrochloric acid; the insoluble matter obtained was collected on an ashless filter paper, washed with hot water and ignited to constant weight. The percentage of acid insoluble ash was calculated concerning the air-dried drug.

Determination of Water-soluble Ash: The ash obtained in the determination of total ash was boiled for 5 min with 25 ml of water. The insoluble matter was collected on an ashless filter paper and washed with hot water. The insoluble ash was transferred into a tarred silica crucible and ignited for 15 min at a temperature not exceeding 45 °C. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference in weight was considered as the water-soluble ash was calculated concerning the air-dried drug.

B) Determination of Loss and Drying: 10 g of the sample (without preliminary drying) was weighed and placed in a tared evaporating dish. It was dried at 105 °C for 5 h, and at 1-h interval until difference two successive weighing corresponded to not more than 0.25%.

C) Determination of Extractive Values:

Determination of Water-Soluble Extractive: 5 g of the test sample was weighed and macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, frequently shaking during six hours and allowing standing for eighteen hours. It was filtered rapidly, taking precautions against the loss of solvent. 25 ml of the filtrate was taken and evaporated to dryness in a tarred flat bottomed shallow dish at 105 °C, to constant weight and weighed the percentage of water-soluble extractive was calculated concerning the air-dried sample.

Determination of Alcohol-Soluble Extractive: Procedure for water-soluble extractive was followed for the determination of alcohol-soluble

extractive, but 90% ethanol was used instead of chloroform water.

D) Determination of Crude Fiber Content: Mix about 2g of the powdered drug in no.60 with 50 ml of 10% nitric acid. Bring to boil and maintain at the boiling point for 30 sec. Dilute with water and strain through a fine filter cloth held over the mouth of filter funnel. Transfer the washed residue to the beaker and boil further 30 seconds with 50 ml of a 2.5% solution of sodium hydroxide. Collect and wash residue as before, mount and examine.

Qualitative Phytochemical Screening: [12, 13, 14]

A) Detection of Tannins: 2-3 ml of aqueous or alcoholic extract of powders were tested carefully with various tannins test reagents as:

5% $FeCl_3$ Solution: A deep blue-black color indicates the test is positive.

Lead Acetate Solution: A white precipitate indicates the test is positive.

Bromine Water: Deceleration of bromine water indicates the test is positive.

Dilute Iodine Solution: Transient red color indicates the test is positive.

B) Detection of Alkaloids: 50 mg of solvent-free extract was hydrolyzed with dil. HCl and filtered. The filtrates were tested carefully with various alkaloid test reagents as follows

Dragendroff's Test: To a few ml of filtrates, 1 to 2 ml of Dragendroff's reagent was added. A prominent yellow precipitate indicates the test is positive.

Wagner's Test: To a few ml of filtrates, few drops of Wagner's reagent were added by the side of the test tube. A reddish-brown precipitate confirms the test as positive.

Mayer's Test: To a few ml of filtrates, few drops of Mayer's reagent were added by the side of the test tube. A white or creamy precipitate if obtained indicates the presence of alkaloids.

Determination of Physical Characteristics: [15]

A) Bulk Density: It is the ratio of the given mass of powder and its bulk volume. It is determined by transferring an accurately weighed amount of powder sample to the graduated cylinder with the aid of a funnel. The initial volume was noted. The ratio of the weight of the volume it occupied was calculated.

$$\text{Bulk density} = w / v_0 \text{ g/ml}$$

Where, w = mass of the powder, v_0 = untapped volume.

B) Tapped Density: It is measured by transferring a known quantity (25g) of powder into a graduated cylinder and tapping it for a specific number of times. The initial volume was noted. The graduated cylinder was tapped continuously for 10-15 min. The density can be determined as the ratio of the mass of the powder to the tapped volume.

$$\text{Tapped volume} = w/v_f \text{ g/ml}$$

Where, w = mass of the powder v_f = tapped volume.

C) Compressibility Index/ Carr's Index: It is the propensity of the powder to be compressed. Based on the apparent bulk density and tapped density the percentage compressibility of the powder can be determined using the following formula.

$$\text{Compressibility index/ Carr's index} = [(V_0-V_f)/V_0] \times 100$$

Or

% Compressibility/ Carr's Index = [(Tapped density – Bulk density)]/ Tapped density] × 100

D) Hausner's Ratio: It indicates the flow properties of the powder. The ratio of tapped density to the bulk density of the powder is called Hausner's ratio.

$$\text{Hausner's ratio} = \text{Tapped density/bulk density}$$

E) Angle of Repose: The internal angle between the surface of the pile of powder and the horizontal surface is known as the angle of repose. The powder is passed through funnel fixed to a burette at s height of 4 cm. A graph paper is placed below the funnel on the table. The height and radius of the pile were measured. The angle of repose of the powder was calculated using the formula

$$\text{Angle of repose} = \tan\text{-}1(h/r)$$

Where, h=height of the pile r = radius of the pile.

Determination of pH Range: The powder sample of Triphala churna was weighed to about 5g and immersed in 100 ml of water in a beaker. The beaker was closed with aluminium foil and left behind for 24-h s in room temperature. Later the supernatant solution was decanted into another beaker, and the pH of the formulation was determined using a calibrated pH meter.

Heavy Metals Test: [16]

TABLE 1: FOR CADMIUM

Test	Observation	Inference
NH_4OH added in the sample solution	White ppt. of cadmium hydroxide soluble in excess NH_4OH	Presence of cadmium
Potassium ferrocyanide added	White ppt. of cadmium ferrocyanide	Presence of cadmium

TABLE 2: FOR BISMUTH

Test	Observation	Inference
H_2S gas added in the sample solution	Dark brown ppt. soluble in hot dil. HNO_3 but insoluble in NH_4S	Presence of bismuth
NH_4OH	White ppt. insoluble in excess NH_4OH dissolved in dil. HCl	Presence of bismuth

TABLE 3: FOR LEAD

Test	Observation	Inference
Dil. HCl added in the sample solution	White ppt. of $CaCl_2$ soluble in boiled water & conc. HCl	Presence of lead
KI is added in the sample solution	Yellow ppt. soluble in boiling water	Presence of lead

Fluorescence Analysis: [14, 17] A little amount of churna was macerated with a small quantity of solvents like 1N sulphuric acid, 1N nitric acid, 1N hydrochloric acid, iodine, potassium hydroxide, ammonia, 1N sodium hydroxide for an hour and then filtered. The filtrate was then analyzed under daylight and UV light for color and fluorescence.

RESULTS AND DISCUSSION:

TABLE 4: DETERMINATION OF ORGANOLEPTIC CHARACTERS

Characteristics	Sample 1	Sample 2	Sample 3
Colour	Light yellow	Yellowish	Yellowish
Odor	Characteristics	Characteristics	Characteristics
Taste	Very bitter	Astringent	Astringent

TABLE 5: ASH VALUES

Type of ash	Sample 1	Sample 2	Sample 3
Total ash	6.65	7.45	6.35
Acid insoluble ash	2.55	3.4	3.4
Water soluble ash	2.20	4.55	3.5

TABLE 6: MOISTURE CONTENT/ LOSS ON DRYING

Characteristics	Sample 1	Sample 2	Sample 3
Moisture Content/ Loss On Drying	0.779	1.25	1.8

TABLE 7: EXTRACTIVE VALUES

Characteristics	Sample 1	Sample 2	Sample 3
Water	3.5	3.3	3.24
Alcohol	1.24	2.06	1.52

TABLE 8: QUANTITATIVE ESTIMATION

Test	Sample 1	Sample 2	Sample 3
Test of Tannin			
5% $FeCl_3$ solution	Positive	Positive	Positive
Lead acetate solution	Positive	Positive	Positive
Bromine water	Positive	Positive	Positive
Dilute iodine solution	Positive	Positive	Positive
Test for Alkaloids			
Dragendroff's test	Positive	Positive	Positive
Wagner's test	Positive	Positive	Positive
Mayer' test	Positive	Positive	Positive

TABLE 9: BULK DENSITY & TAP DENSITY

Characteristics	Sample 1	Sample 2	Sample 3
Bulk Density	0.666	0.476	0.555
Tap Density	0.909	0.625	0.80

TABLE 10: CARR'S INDEX & HAUSNER'S RATIO

Characteristics	Sample 1	Sample 2	Sample 3
Carr's index	26.73	23.84	30.625
Hausner's ratio	1.36	1.31	1.44

TABLE 11: ANGLE OF REPOSE

Characteristics	Sample 1	Sample 2	Sample 3
Angle of repose	36.50	39.69	35.75

TABLE 12: DETERMINATION OF PH SAMPLE

Characteristics	Sample 1	Sample 2	Sample 3
pH	5 (acidic)	6 (acidic)	6 (acidic)

TABLE 13: ESTIMATION OF CRUDE FIBER

Characteristics	Sample 1	Sample 2	Sample 3
Crude Fiber	4.7	4.15	4.2

Heavy Metal Test: Triphala Churna of Patanjali, Shree Shree Ayurveda, and Laboratory made.

TABLE 14: TEST FOR CADMIUM

Test	Observation	Result
NH_4OH added in the sample solution	White ppt. is absent	Absence of cadmium
Potassium ferrocyanide added	White ppt. is absent	Absence of cadmium

TABLE 15: TEST FOR BISMUTH

Test	Observation	Result
H_2S gas added in the sample solution	Dark brown ppt. is absent	Absence of bismuth
NH4OH	White ppt. is absent	Absence of bismuth

TABLE 16: TEST FOR LEAD

Test	Observation	Result
Dil HCl added in the sample solution	White ppt. of $CaCl_2$ is absent	Absence of lead.
KI is added in the sample solution	Yellow ppt. is absent	Absence of lead.

TABLE 17: FLUORESCENCE ANALYSIS FOR SAMPLE 1

Solvent added	Colour observed under		
	Daylight	Short UV wavelength (256 nm)	Long UV wavelength (365 nm)
1N Sulphuric acid	Light brown	Light green	Dark green
1N Nitric acid	Light brown	Light green	Dark green
1N Hydrochloric acid	Light brown	Light green	Dark green
Iodine	Greenish brown	Dark green	Dark blue
Potassium hydroxide	Brown	Green	Dark blue
Ammonia	Brown	Green	Dark blue
1N Sodium hydroxide	Dark brown	Dark green	Dark blue

TABLE 18: FLUORESCENCE ANALYSIS FOR SAMPLE 2

Solvent added	Colour observed under		
	Daylight	Short UV wavelength (256 nm)	Long UV wavelength (365 nm)
1N Sulphuric acid	Light brown	Light green	Green
1N Nitric acid	Light brown	Light green	Green
1N Hydrochloric acid	Light brown	Light green	Green
Iodine	Greenish brown	Dark green	Dark blue
Potassium hydroxide	Brown	Green	Dark blue
Ammonia	Brown	Green	Dark blue
1N Sodium hydroxide	Dark brown	Dark green	Dark blue

TABLE 19: FLUORESCENCE ANALYSIS FOR SAMPLE 3

Solvent added	Colour observed under		
	Daylight	Short UV wavelength (256 nm)	Long UV wavelength (365 nm)
1N Sulphuric acid	Light brown	Light green	Dark Green
1N Nitric acid	Light brown	Light green	Dark Green
1N Hydrochloric acid	Light brown	Light green	Dark Green
Iodine	Greenish brown	Dark green	Dark bluish
Potassium hydroxide	Light Brown	Green	Light bluish
Ammonia	Light Brown	Green	Light bluish
1N Sodium hydroxide	Brown	Dark green	Dark bluish

DISCUSSION: From the heavy metal test it is concluded that Triphala Churna of Patanjali, Shree shree Ayurveda and Lab made formulation are free from heavy metals.

CONCLUSION: From the present investigation various standardization parameters such as physicochemical standards like total ash, acid insoluble ash, water & alcohol-soluble extractive values, loss on drying, phytochemical analysis, flow properties, and safety evaluation were carried out, it can be concluded that the formulation of *Triphala* churna contains all good characters of an ideal churna and it was found to be harmless, more effective, and economic.

The comparison between the two marketed samples and lab-made churna have been done on the basis of the above mentioned parameters which shows satisfactory results, but the efficacy of the products can only be judged by doing the pharmacology of which is suggested as the future scope of R & D.

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CONFLICT OF INTEREST: Nil

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