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STUDY OF VARIOUS VALIDATION PARAMETERS OF TRIPHLA CHURNA

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ABSTRACT

The study was designed to formulate and pharmaceutically evaluate a herbal powder drug. Triphala Churna into Triphala Tablet by incorporation of Amla, Bahera, Harad fruits powder. The raw materials were procured, authenticated and standardized to determine their ash value, extractive value, moisture content and its chromatographic studies. The in house tablet was formulated and evaluation parameters were studied for its weight variation, hardness testing, disintegration time test, friability test. Triphala churna is used as a daily tonic to improve the digestion with a mild laxative properties. We have incorporated Amla fruits powder to the powdered drugs and formulated in to a tablet dosage form to improve its therapeutic activity with respect to its laxatives effect and the tablet form of triphala is considered as one of the most preferred ways of consuming it.

Keywords: Triphala Churna, Ash value, Extractive values, Chromatography, Validation Parameters, Phytochemical Investigation.

INTRODUCTION

Herbal medicines are consistently getting used in the various countries as their requirement is increasing in the developed as well as developing countries. The purpose is the primary healthcare due to their broad spectrum biological activities, higher safety margins and cheaper costs. Herbal medicines has been popularizing among the customers throughout the world. The herbal drug market in our country is about $ 01 billion and plant based crude drugs are exported around $ 80 million (Singhai *et al.*, 2012; Aithal and Day, 1999). The lack of standard quality control profiles are generating problems for the acceptance of the Ayurvedic or Herbal formulations (Akerele, 1993). The QC of herbal medicine is the profile of the constituents in the final product has implications in efficacy and safety. The complex nature and inherent variability of the chemical constituents of the plant based drugs is the major problem to establish quality control parameters and through modern analytical techniques (Tiwari, 2008).

Herbal medicines are also referred as botanical medicine or phytomedicine (refers to using a plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes). They are not only used by about eighty percent of the world population basically in the developing countries for primary health care system but also widely preferred for their safety, efficacy, cultural acceptability and lesser side effects whenever they got compared to the allopathic system of medicines. The phyto-constituents present in herbals are part of the physiological functions

of living flora and hence they are believed to have better compatibility with the human body.

Importance of Herbal Medicine

There are a number advantages associated with using herbal medicines as opposed to pharmaceutical products. Examples include the following:

- Reduced risk of side effects and low cost (Satoskar *et al.*, 2002).
- Effectives with chronic disorder for long term treatment with widespread availability.
- For certain ailments, herbal medicines are considered to be more effective than allopathic medicines.
- These types of medicines are best for people who are allergic to various types of drugs (Thyagarajan *et al.*, 2002).
- Herbal medicine are also effective in boosting the mental health.
- Herbal medicine can be used to reduce weight by regulating appetite.
- The natural detoxification process of the body is effectively enhanced by herbal medicines.

Triphala

Triphala is a combination of the three fruits of *Emblica officinalis* Gaertn., *Terminalia chebula* Retz and *Terminalia belerica* Roxb in equal proportion.The formulation is regarded as an important rasayana. It is also termed as **"nectar of life"**. Previously it was called by some other names as referred in Charaka Samhita and Susruta Samhita (Ponnusankar *et al.*, 2011).

Triphala powder used in various health conditions as it contains very effective herbs in appropriate proportion. Triphala has many benefits and used in various ways for different health condition (Naik, 2006).

Plant Profile:

***Emblica officinalis* Gaertn (Amla):** Amla is also known as Indian Gooseberry. Amla Botanical name is *Emblica officinalis*. It belongs to Phyllanthaceae family.

***Terminalia balerica* ROXB (Bahera):** *Terminalia balerica*, known as baheda, bahera, behada, beleric or bastard myrobalan is a large deciduous tree in the Combretaceae family.

***Terminalia chebula* Retz. (Harad):** *Terminalia chebula* Retz. (Fam. Combretaceae), is called the 'King of Medicine' in Ayurveda as well as Tibetan medicine because of its extraordinary power of healing.

Preparation of Triphala Churna: The Triphala churna can be prepared by taking dried Indian Gooseberry, Haritaki and Bibhitaki in equal quantity (Gupta, 2010). The powdered forms of these 3 ingredients are blended. This powder is commonly called as Triphala churna. Some use it for reducing weight and some to cure any problem related to the digestive system. This churna comprised of the combination of the above herbs. It stimulates the metabolism and hence takes care of our stomach and digestive system.

Rasayana Preparations: Rasayana preparation is providing multidimensional benefits and important role and significance in ayurveda. It is a rejuvenation therapy. That restores the basic homeostatic balance of the human body.

Vati preparations: Medicaments in the form of tablets or pills are known as vatika or gutika. These are made by single or combinations of vegetable, mineral or animal drugs.

Standardization and validation: **"**Standardization"expression encompasses the entire field of study from birth of a plant to its clinical application. It also means adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity respectively by adding excipients or by mixing herbal drugs or herbal drug preparations. A standardized herbal drug has to be defined under the following healings: Botanical, Chemical, Chemo Profiling. Standardization problem arises from the complex composition of drugs, which are used in the from of whole plant part, the plant abstracts. The large number of Ayurvedic Formulations available in the market. A Pharmacopeia standard for the drug used in traditional system of medicine including herbal is considered being a very important and challenging task. The bioactivity still remains within tolerable limits.

Basic features and requirements for control and validation procedures of herbal medicinal products:

These extracts are multicomponents systems containing:

- Pharmacologically active substances
- Compounds which by themselves are not pharmacologically active but influence the
- Biological effectiveness of active substances, i.e. supportive substances, Neutral, bulk material.

MATERIALS AND METHODS

Materials

The chemicals and reagents used in the study were of analytical and HPLC grade. Petroleum ether, water (HPLC grade), methanol, phosphate buffer saline, and methanol (HPLC grade) were procured from Merck, Mumbai. Gallic acid was obtained from Jay Appliances, Sagar.

Methods

Collection, Authentication and drying of plant material: The fruits of (Amla) *Emblica officinalis*, (Harad) *Terminalia chebula*, (Bahera) *Terminalia belerica* was collected from Sagar (local vendor). The plant was identified, confirmed and authenticated from Department of Botany, Dr. H.S. Gour Central University, Sagar (M.P.). A Herbarium has been deposited in the Botany Department. The fruits of Triphala were shade dried at room temperature and powdered through grinder to make coarse powder.

Preparation of extract: Triphala fruits powder (200gm) were packed in Soxhlet apparatus and defatted with petroleum ether (60-80ºC), to ensure complete defatting, the marc was dried at room temperature and extracted with distilled water by hot maceration technique then filtered and evaporate the filterate and dried. The yield of aqueous extract was calculated. The schematic representation of extraction processes is given as follows:

Formulations:

Formulation of Triphala Churna: The Triphala churna can be prepared by taking dried Indian Gooseberry, Haritaki and Bibhitaki in equal quantity. The powdered forms of these 3 ingredients are blended. This powder is commonly called as triphala churna (Juss, 1997).

Formulation of Triphala Vati: The Triphala vati can be prepared by taking dried Indian Gooseberry, Haritaki and Bibhitaki in equal quantity (Thakur *et al.,* 1988). The powdered forms of these 3 ingredients are blended with suitable quantity of honey. This mixture after suitable consistency was rounded to form vati of uniform size and weight.

Physiochemical Parameters: These parameter gives the idea of the physical characteristics and the chemical constituents present in the herbal drugs. These parameters involve the determination of ash values, foreign matter, extractable matter, volatile oil content of the preparations or individual drugs.

Physical Evaluation:

Determination of foreign matter: 100gm of the sample was weighed and spread on a white tile uniformly without overlapping. Then the sample was inspected by means of 5x lens and the foreign organic matter was separated. After complete separation the matter was weighed and percentage w/w was determined (Prasad, 2005) (Table-4).

Determination of solvent extractive values:

Determination of water-soluble extractive value: 5gm of powdered drug was macerated with 100ml of water closed flask for 24hrs and was occasionally Shaked with 6hrs time period and was allowed to stand for 18hrs. After filtration the 25ml of the filterate evaporated to dryness in a tared bottomed shallow dish. Dry at 105ºC and weighed. Percentage of water-soluble extractive value was calculated with reference to the air-dried drug (Table-3).

Determination of alcohol soluble extractive value: 5gm of powdered drug was macerated with 100ml of water closed flask for 24hrs and was occasionally Shaked with 6hrs time period and was allowed to stand for 18hrs. After filtration the 25ml of the filterate evaporated to dryness in a tared bottomed shallow dish (Singh, 2005). Dry at 105C and weighed. Percentage of ethanol soluble extractive value was calculated with reference to the air-dried drug (Table-1).

Determination of Ash values:

Determination of total ash: Total ash was determined by weighing 2-3gm of the air-dried crude drug in the tared platinum or silica dish and incinerated at a temperature not exceeding 450C until free from carbon and then was cooled and weighed. (Table-2)

Determination of acid insoluble ash: Ash insoluble in HCl is the residue obtained after extracting the sulphated or total ash with HCl and calculated with reference to 100gm of drug. The ash obtained from the previous process was boiled with 25ml of 2M HCl for 5min. and the insoluble matter was collected on ash -less filter paper and was washed with hot water, ignited, cooled in a desiccators and weighed. Percentage of acid insoluble ash was calculated with reference to the air-dried drug. (Table-2)

Determination of water-soluble ash: The ash was boiled with 25ml of water for 5min. and the insoluble matter was collected on ashless filter paper and was washed with hot water, ignited for 15min. at a temperature not

exceeding 450ºC. The weight of the insoluble matter was subtracted from the weight of the ash and this represents the water-soluble ash. Percentage of water-soluble ash was calculated with reference to the air-dried drug (Table-2).

Physical Characteristics

Determination of volatile oil content: The volatile oil content was determined by the 'Clevenger Apparatus'. It is hydro-distillation method in which the drug is distilled with the drug is distilled with water or glycerine and the distillate is collected in a graduated tube from which the aqueousprotein of distillate is automatically returned to the distillation flask. The procedure followed was as the following:

50gm of accurately weighed powdered drug with 250ml of water was taken in 1L distillation flask and few pieces of porcelain were added in order to avoid bumping distillation. The assembly was arranged and the graduated receiver was filled with water avoiding any air bubbles. The upper end of the receiver was loosely packed with cotton. After starting the heating by mantle it was kept for continuous distillation for 4-5hrs. The distillate was collected in the graduated receiver, from where the volatile oil was collected and measured. The quantity as %v/w on a dry weight basis was calculated (Table-3)

Phytochemical Investigation:

Test for Carbohydrates:

MolischTest: 2Ml of aqueous extract was treated with 2 drops of alcoholic naphthol solution in a test tube and then 1ml of concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicate the presence of carbohydrates.

Barfoed's Test: 1ml of extract and Barfoed's reagent were mixed in a test tube and heated on water bath for 2min. red colour due to formation of cupric oxide indicates the presence of monosaccharide (Harborne, 1984).

Test for Protein and Amino acids:

Million's Test: 3ml of extract was mixed with 5ml of million's reagent. White precipitate formed which on heating turned to brick red, indicating the presence of proteins.

Ninhydrin Test: 3ml of the test solution was heated with 3 drops of 5% Ninhydrin solution in a water bath for 10min. Formation of blue colour indicates the presence of amino acids.

Test for Glycosides:

Legal's Test: 1ml of test solution was dissolved in pyridine.1ml of sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink to blood red colour indicates the presence of cardiac glycosides (Modi, 2002).

Test for saponin:

Froth Test: The extract was diluted with distilled water and shaken in graduated cylinder for 15 mins.The formation of layer of foam indicates the presence of saponins (Mukherjee, 2003).

Test for Alkaloids:

Mayer's Test: to 2-3ml of filtrate, few drops of Mayer's reagent were added along sides of tube. Formation of while or creamy precipitate indicates the presence of alkaloids.

Test for flavonoids:

Lead Acetate Test: The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate may indicate the presence of flavonoids.

Test for Triterpenoids and steroids:

Liebermann-Burchard's Test: The extract was treated with chloroform. To this solution few drops of acetic anhydride were added, boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. Formation of brown ring at the junction of two layers, if upper layer turned green, indicate presence of steroids and formation of deep red colour indicate presence of triterpenoids.

Test for Tannin and Phenolic compounds:

Dilute Iodine solution Test: To 2-3ml of extract, few drops of dilute iodine solution were added. Formation of transient red colour indicates presence of phenolic compounds (Kokate *et al.,* 1996).

Test for Fats and Oils:

Solubility Test: To2-3mlof the alcoholic solution of extract, add few ml of chloroform and solubility was observed. To 2-3ml of the alcoholic solution of extract, add few ml of 90% ethanol and solubility was observed.

In-process evaluation parameters:

For churna preparation

Bulk density:

Bulk density = W/V_{ut} g/ml

Where, W = Mass of the powder, V_{ut} = Untapped volume

Tapped density:

Tapped volume = W/V_{tv} g/ml

Where, W = mass of the powder, V_{tv} = tapped volume

Compressibility index:

% Compressibility = [(tapped density – bulk density)] / tapped density] x 100

Hausners ratio:

Hausner ratio = Tapped density/bulk density

Angle of Repose:

Angle of Repose = tan-1(h/r)

Where, h = Height of the pile, r = Radius of the pile

RESULTS AND DISCUSSION

The individual drugs were standardized pharmacognostically, chemically and analytically. Macroscopic and microscopic features of crude plant drugs were studied to confirm identity of the drugs. Various qualitative chemical tests were found positive for formulations like volatile oil, glycosides, carbohydrates, tannins, saponins, and terpenoids in formulations. Some constituents had shown the presence in the churna preparations marketed as well as in the inhouse formulated preparations but are absent in the individual drug showing the mixture property of formulation. Process evaluation parameters like bulk density, tapped density, compressibility index and angle of repose for the marketed and formulated churna preparation can be said under quality control standard limits. As preparations has shown the compressibility index limit in the poor, fair to passable limits, which is also reflected by the angle of repose studies for the flow property studies. It was also observed that as the formulation is prepared from the individual drugs the flow property of the formulation changes and altering the compressibility index. All the examined results in marketed and formulated preparations were compared and the results are almost alike. Heavy metal concentrations and microbial contamination were found to be below than standard limits. All the pesticides were found absent in all samples i.e. concentration of pesticides was found under limit (<5ppm). Various parameters established could be employed as quality control standards for evaluating identity,

quantity and quality of preparation of routine analysis.

Table 1: Solvent extractive values of crude drugs & preparations

S. No.	Name of the drug	Water soluble extractive value % w/w		Alcohol soluble extractive value % w/w	
		Mentioned	Obtained	Mentioned	Obtained
1.	*E. Officinalis*	>50	70.28	>40	58.86
2.	*T. Chebula*	>60	70.77	>40	58.41
3.	*T. Belerica*	>35	49.57	>80	09.57
4.	UTC	>48	62.13	>30	51.81
5.	STC	>48	68.25	>30	53.36
6.	FC	>48	52.24	>30	48.43
7.	MTCR	>57	57.98	>55	61.22
8.	FCR	>57	59.33	>55	63.43
9.	STV	>50	60.45	>42	63.80
10.	FTV	>50	61.47	>42	64.14

UTC- Uma ayurved marketed preparation, STC- Sharmayu ayurved marketed preparation, FC- In house Triphala churna, MTCR- VPK maharshi ayurved marketed rasayan preparation, FCR- Inhouse formulated triphala rasayan, STV- Sharmayu triphala vati, FTV- Formulated inhouse triphala vati.

Table 2: Physical Characteristics

S. No.	Name of the drug	Foreign organic matter	Total ash value	Acid insoluble ash value	Water soluble ash value
1.	*E. officinalis*	<1.0%	<7%	<2%	<1.5%
		0.86%	3.26%	0.61%	1.02%
2.	*T. chebula*	<1.0%	<5%	<5.88%	<1.45%
		Nil	4.44%	0.98%	1.74%
3.	*T. belerica*	<2.0%	<7.0%	<0.97%	<1.05%
		0.75%	2.04%	0.22%	0.86%
4.	UTC	<1%	<19.5%	<2.2%	<4.31%
		Nil	18.34%	2.11%	4.02%
5.	STC	1%	<19.5	<2.2%	<4.31%
		Nil	18.27%	2.04%	4.30%
6.	FC	<2%	<2%	<2.2%	<4.31%
		Nil	19.48%	2.17%	4.29%
7.	MTCR	<0.2%	<42.0%	<1.98%	<4.75%
		0.75%	32.14%	1.42%	2.89%
8.	FCR	<2%	<42.5%	<1.98%	<4.75%
		0.15%	39.32%	1.11%	4.02%
9.	STV	<1%	21.5%	<1.5%	<4.54%
		Nil	19.79%	1.04%	4.42%
10.	FTV	<1%	<21.5%	<1.5%	,4.54%
		Nil	20.81%	1.17%	4.44%

Table 3: Volatile content of drugs and preparations

S. No.	Name of drugs	Distillation time	%V/W Yield
1.	*E. officinalis*	5hrs	0.67
2.	*T. chebula*	6hrs	0.98
3.	*T. belerica*	5hrs	0.11
4.	UTC	6hrs	1.82
5.	STC	6hrs	1.77
6.	FC	6hrs	2.11
7.	MTCR	6hrs	2.13
8.	FCR	6hrs	2.94
9.	STV	6hrs	1.22
10.	FTV	6hrs	1.79

Table 4: Qualitative phytochemical tests

S. No.	Compound	Exracts of drugs and preparations									
		EO	TC	TB	UFC	SFC	FC	MTCR	FCR	STV	FTV
1.	Alkaloids	-	-	-	-	-	-	-	-	-	-
2.	Carbohydrates	+	+	+	+	+	+	+	+	+	+
3.	Glycosides	-	+	+	+	+	+	+	+	+	+
4.	Volatiles	+	+	+	++	++	++	++	++	+	+
5.	Proteins	+	-	-	-	-	-	-	-	-	-
6.	Terponoids	+	+	+	+	+	+	+	+	+	+
7.	Gums and resins	-	-	-	-	-	-	-	-	-	-
8.	Tannins	+	+	+	++	++	++	++	++	++	++
9.	Fixed oils and fats	-	-	-	-	-	-	-	-	-	-
10.	Saponins	-	-	+	+	+	+	+	+	+	+

Where, EO- *Emblica officinalis*, TC- *Terminalia chebula*, TB- *Terminalia balerica*, UFC- Uma ayurved formulated churna, SFC- Sharmayu ayurved formulated churna, FC- In house formulated churna, MTCR- Maharshi Triphala Rasayana, FCR- In house formulated rasayana, STV- Sharmayu Triphala vati, FTV- In house formulated triphala vati;+ for compound present; - for compound absent.

Table 5: Bulk density

S. No.	Name of drugs	Bulk density
1.	*E. officinalis*	0.34
2.	*T. chebula*	0.38
3.	*T. belerica*	0.39
4.	UTC	0.47
5.	STC	0.48
6.	FC	0.44

Table 6: Tapped density

S. No.	Name of drugs	Tapped density
1.	*E. officinalis*	0.40
2.	*T. chebula*	0.42
3.	*T. belerica*	0.43
4.	UTC	0.65
5.	STC	0.66
6.	FC	0.65

Table 7: Compressibility index

S. NO.	Name of drugs	Compressibility index
1.	*E. officinalis*	25.3
2.	*T. chebula*	21.4
3.	*T. belerica*	22.2
4.	UTC	31.0
5.	DTC	31.0
6.	FC	30.1

Table 8: Hausners Ratio

S .No.	Name of drugs	Hausners ratio
1.	*E. officinalis*	1.33
2.	*T. chebula*	1.34
3.	*T. belerica*	1.31
4.	UTC	1.43
5.	STC	1.46
6.	FC	1.44

Table 9: Angle of Repose

S. No.	Name of drugs	Angle of repose
1.	E.officinalis	32.7
2.	T.chebula	32.4
3.	T.belerica	31.3
4.	UTC	36.2
5.	STC	35.7
6.	FC	35.4

For Rasayana preparation

Table 10: Determination of pH

S. No.	Name of drugs	pH
1.	MTRC	6.8
2.	FCR	6.5

Table 11: Determination of Spreadability

S. No.	Name of drugs	Consistency
1.	MTRC	Ideal
2.	FCR	Ideal

CONCLUSION

The present study involved the pharmacognostical, chemical, and analytical standardization of Triphala Churna and the development of its tablet dosage form prepared from the powdered fruits of Amla *(Emblica officinalis)*, Bahera *(Terminalia bellirica)*, and Harad *(Terminalia chebula)*. The macroscopic and microscopic evaluation confirmed the identity and purity of the crude drugs, while qualitative chemical tests revealed the presence of phytoconstituents such as volatile oils, glycosides, carbohydrates, tannins, saponins, and terpenoids, validating the formulation's authenticity. Physicochemical parameters like bulk density, tapped density, compressibility index, and angle of repose were within acceptable limits, ensuring good flow properties. The absence of heavy metals, microbial contamination, and pesticide residues confirmed the safety of the formulations. The formulated Triphala Tablet exhibited acceptable weight variation, hardness, friability, and disintegration time, indicating good tablet quality. Thus, the study successfully established quality control standards for Triphala Churna and demonstrated that its tablet form enhances convenience, stability, and therapeutic efficacy, making it a standardized and effective herbal dosage form for routine therapeutic use.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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