Journal of Herbal Drug
journal homepage: www.jhd.iaushk.ac.ir

Pharmacognostical and physico-phytochemical evaluation of Trisama-an unexplored ayurvedic formulation

Amit G. Patel[*1], Mukeshkumar B. Nariya[1], CR Harisha[2], V. J. Shukla [3]

[1]*Pharmacology Laboratory, Institute for Post Graduate Teaching & Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India;*
***Email:** *amitptl99@gmail.com*

[2]*Pharmacognocy Laboratory, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India;*

[3]*Pharmaceutical Chemistry Laboratory, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India;*

ARTICLE INFO

Type: *Original Research*
Topic: *Medicinal Plants*
Received *October 29th 2018*
Accepted *April 25th 2019*

Key words:
- ✓ *Trisama powder*
- ✓ *Decoction*
- ✓ *Pharmacognosy*
- ✓ *Physicochemical*
- ✓ *Phytochemical*

ABSTRACT

Background & Aim: The major problem of pharmaceutical industry for herbal product in current scenario is authentication of raw material, and availability of standards. The quality control and quality assurance of herbal drugs still remains a challenge because of the high variability of chemical components involved.

Experimental: In present research work details of *Trisama* formulation were noted including preliminary pharmacognostical study. The physicochemical and phytochemical evaluation of *Trisama* formulation was done using various laboratory chemicals and reagents.

Results: Microscopical characters like boarder pitted vessel, brown content, compound starch grain, cork in surface view, stone cell, lignified collenchyma cell, sclerides, scleriform vessel etc are seen. Physicochemical and phytochemicals parameters also performed in both dosage forms however difference is seen in parameters result. Still both the dosage forms fulfill the minimum qualitative standards at a preliminary level. The following study will improve the quality of drugs as well as help to get more involved in the standardizations of formulations.

Recommended applications/industries: The abundant chemical constituents and significant potential could be well and new source of unexplored Ayurvedic formulation in industry.

1. Introduction

In ancient time, *Acharyas* prepared medicine on individual basis, and prepare drug according to the requirement of the patient. But now scenario has been changed; herbal medicines are being manufactured on the large scale in Pharmaceutical units, where manufacturers come across many problems such as availability of good quality raw material, authentication of raw material, and availability of standards, proper standardization methodology of single drugs and formulation, quality control parameters. The quality control and quality assurance of herbal drugs still remains a challenge because of the high variability of chemical components involved (Muthugala *et al.*, 2013). Most of the herbal formulations, especially the classical formulations of traditional medicine, are

Polyherbal. Each formulation contains 5-10 or more than one ingredients; For such formulations it is very difficult to establish parameters for quality control (Patwardhan, 2000; Shinde *et al.*, 2008). Hence the first important task is to evolve such parameter by which the presence of the entire ingredient can be identified, Spectrophotometric methods and evaluation of physicochemical properties can be tried to evolve pattern for identifying the presence of different ingredient. Separation of individual components from the herbal mixture is the key step to enable identification and bioactivity evaluation.

Indian system of medicine has a longstanding history of using medicinal plants for the prevention and treatment of various health ailments. *Trisama* is an ancient Ayurvedic preparation can be prescribed for a wide range of disorder but not popular as many other Ayurvedic herbal formulations. *Trisama* is preparing by mixing the *Sunthi* (*Zingiber officinale* Linn.), *Haritaki* (*Terminalia chebula* Retz.), and *Guduchi* (*Tinospora cordifolia* Willd Meirs) in equal proportion. *Trisama* is a Polyherbal Ayurvedic formulation used in inflammation and metabolic disorder (Patel et al., 2017). In present research work an attempt was made to setting a preliminary profile for further references and to authenticate it and standardize *Trisama* formulation (powder and decoction) containing *Sunthi*, *Haritaki* and *Guduchi* with the help of Pharmacognostical evaluation (organoleptic, microscopical), Physicochemical evaluation as well as Phytochemical (Qualitative and quantitative) evaluation.

2. Materials and Methods

2.1. Collection and authentication of raw drugs

The all ingredients of *Trisama* (*Sunthi, Haritaki and Guduchi)* were collected near Mangrol. Dist. Surat as per standard procedure. The ingredients with botanical source and parts used are mentioned in Table 1. Pharmacognostical authentication of all the raw drugs was done based on the morphological features, organoleptic characters and powder microscopy of individual drugs. The API standards were used for authentication (Anonymous, 2001).

Table 1: Ingredients of Trisama formulation

Drug	Latin name	Part used
Sunthi	*Zingiber officinale*	Rhizome
Haritaki	*Terminalia chebula*	Fruit
Guduchi	*Tinospora cordifolia*	Stem

2.2. Preparation method for Trisama dosage forms

Ingredients enlisted in Table no.1 were made into fine powder and sieved in Mesh no.80. The powder was mixed well in mass mixing machine till the homogeneous mixture was obtained and *kwath* (decoction) of *Trisama* also prepared as per standard method with the use of four times water (Bramhanand, 2006).

2.3. Pharmacognostic standardization

Raw drugs were identified and authenticated by the pharmacognosy laboratory, I.P.G.T. & R.A, Jamnagar. The identification was carried out based on organoleptic characters of powder. Later pharmacognostical evaluation of the powder was carried out. One by one for all the three ingredients and then mixed formulation studied under the Carl-Zeiss Trinocular microscope attached with camera, with stain and without stain. The microphotographs were also taken under the microscope (Kokate, 2008; khandelwal, 2008). Organoleptic evaluation shows various characters such as colour, odour, taste and touch of drugs was observed and recorded (Kokate, 2008).

2.4 Microscopic study

Trisama powdered and dissolved with water and microscopy of the sample was done without stain and after staining with Phloroglucinol+HCl. Microphotographs of *Trisama* powder was also taken under Carl-zeiss trinocular microscope (Khandelwal, 2008).

2.5. Physico-phytochemical evaluations

Pharmaceutical Evaluation *Trisama* powder and decoction was analyzed using qualitative and quantitative parameters at the pharmaceutical laboratory, I.P.G.T & R.A., Jamnagar. The common parameters mentioned for powder and decoction in Ayurvedic Pharmacopeia of India and Central Council

for Research in Ayurvedic Sciences guidelines are total Ash value, pH value, Water and Methanol soluble extracts (Aggrawal et al., 2011). On these bases, the parameters were selected. The presence of more moisture contents in a sample can create preservation problem. Hence, loss on drying was also selected as one of the parameters (API, 2008; Anonymous, 1998).

3. Results and discussion

Standardization of Ayurvedic formulations is an important aspect of research in Indian system of medicine and essential for worldwide acceptance and globalization of Ayurveda. So, there is need of method development to ascertain standards for quality and purity of raw drugs as well as formulations to maintain their therapeutic efficacy.

Trisama is a classical Ayurvedic formulation. Although there is a very wide use of *Trisama* formulation but, till date, no any scientific study is reported on *Trisama* formulation. The initial purpose of the study was to confirm the authenticity of the drugs used in the preparation of *Trisama* powder. For this, coarse powder of all the ingredients was subjected to Organoleptic, Microscopic, and Physico-phytochemical evaluation separately to confirm the genuineness of all the raw drugs.

3.1. Pharmacognostical study

Organoleptic characters are experience based judgment of the colour, touch, odour, taste. On these bases it was observed that *Trisama* having mixed taste as Astringent pungent bitter which is responsible from their all the three ingredients. The astringent tastes in *Trisama* sample is due to haritaki where pungent taste owing to *Sunthi* and bitter taste is due to *Guduchi*. The colour change of the *Trisama* sample may be due to the overwhelming colour property of *Haritaki*. The characteristic and aromatic odour in the *Trisama* sample is due to the presence of *Sunthi*.

3.2. Microscopic characters identified

Microscopic evaluation was conducted by powder after dissolving it in the distilled water and studied under a microscope for the presence of characteristics of ingredient drugs. Powder microscopy of final product showed all the characters of individual three drugs of *Trisama* powder. The diagnostic characters of *Trisama* powder without stain boarder pitted vessel of *Guduchi*, brown content of *Guduchi*, compound starch grain of *Haritaki*, cork in surface view of *Guduchi*, stone cell of *Haritaki*, iodine stained simple starch grain of *Sunthi*, lignified collenchymas cell of *Guduchi*, sclerides of *Haritaki*, scleriform vessel of *Sunthi*, starch grain of *guduchi*. Furthermore after staining with iodine starch grain of *Haritaki* and *Guduchi*, compound starch grain of *Haritaki* and *Guduchi*, starch grain of *Sunthi* were observed. And after staining with Phloroglucinol+HCL following characters are observed sclerides of Haritaki, collenchymas cell of *Guduchi*, stone cell of *Guduchi*, boarder pitted vessel of *Guduchi* are seen. (Figure 1: A to L).

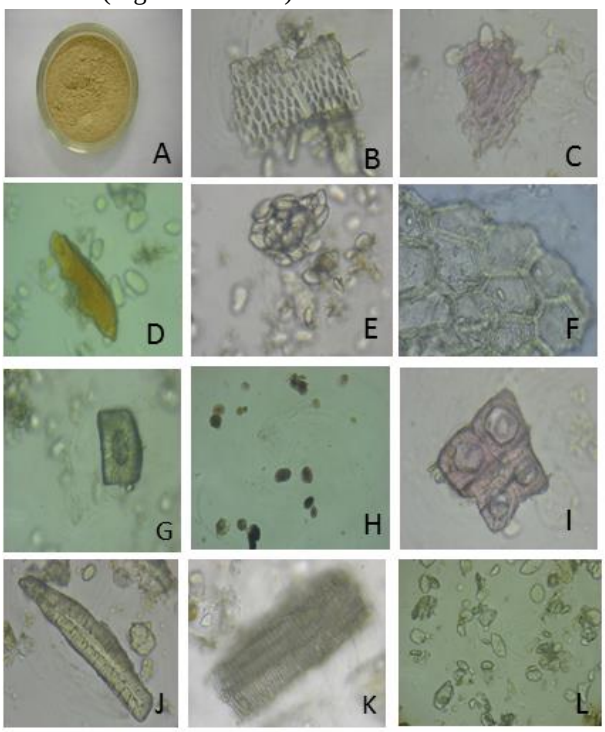

Fig 1. A: *Trisama* powder; **B**: Boarder pitted vessel of *Guduchi*; **C:** Lignified Boarder pitted vessel of *Guduchi;* **D**: Brown content of *Guduchi*; **E:** Compound starch grain of *Haritaki;* **F:** Cork in surface view of *Guduchi;* **G:** Stone cell of *Haritaki*; **H:** Iodine stained simple starch grain of *Sunthi;* **I:** Lignified collenchymas cell of *Guduchi;* **J:** Sclerides of *Haritaki*; **K:** Scleriform vessel of *Sunthi;* **L:** Starch grain of *Guduchi.*

Powder microscopy of *Trisama* powder showed the striking characters of all individual three drugs of final product. This confirms the ingredients present in the finished product and there is no major change in the microscopic structure of the raw drugs during the pharmaceutical processes of preparation of powder.

3.3. Organoleptic analysis

The characters of the sample are tabulated in Table 2.

Table 2: Organoleptic parameters of Trisama ingredients and formulation

Parameters	Sunthi	Haritaki	Guduchi	Trisama
Taste	Pungent	Astringent sweetish	Bitter mucilaginous	Astringent, pungent, bitter
Colour	Creamish	Golden yellow	Creamish ash	Creamish yellow
Odour	Characteristic aromatic	Slightly aromatic	Characteristic	Pungent
Touch	Fine powder	Fine powder	Fine powder	Fine powder

3.4. Physicochemical evaluation

At present official pharmacopoeial standard are stick to the pharmacognostic and physic-chemical parameter. Physicochemical parameters of the *Trisama* powder and decoction are total ash value, pH value, water and methanol soluble extracts, loss on drying, refractive index, specific gravity, viscosity. The results are placed at Table 3 & 4. Results indicate that dryness of the sample is important in size reduction, preservation and preventing hydrolytic degradation of active components of herbal formulations. Higher moisture content promotes growth of microbes and hence spoilage of formulations. The moisture content was found to be low (8.32% w/w) in the *Trisama* Powder sample.

Table 3: Physicochemical parameters of Trisama powder

Powder parameters	Results Mean±SD
pH	4±1.10
Loss on drying (%w/w)	08.32±1.90
Ash value (%w/w)	12.74±1.12
Acid insoluble ash (%w/w)	0.613±0.37
Alcohol soluble extractive(%w/w)	27.06±1.91
Water soluble extractive (%w/w)	25.84± 2.41

Ash value is such a parameter by which purity of drug can be measured. Amount of material remain after ignition is the total ash, where as acid insoluble ash is non physiological ash, basically inorganic substance such as silica. Water soluble ash is ash of physiological material plant itself. The sample of *Trisama* powder contained 12.74% w/w total ash and acid insoluble ash 0.613% w/w which is quiet normal. Extractive value indicate amount of chemical constituents of the drug. Water soluble extractive value indicates its soluble material such as sugar, carbohydrate, glycoside, tannin. *Trisama* powder contain 25.84% w/w water soluble extractives, where as methanol soluble extractive is 27.06%w/w. which indicates that *Trisama* powder is easily soluble in water as well as alcohol.

Table 4: Physicochemical parameters of Trisama decoction

Decoction Parameters	Results
pH	4±0.90
Total solid (%w/v)	6.00±0.0
Refractive index	1.35±0.0
Specific gravity	1.02±0.0
Viscosity (poise)	1.093±0.5

The term total solid is applied to the residue obtained when the prescribed amount of the preparation is dried to constant weight under the specified condition. This parameter was important for the pharmacokinetic and pharmacodynamics activity of drug because of the bioavailability condition. Result of total solid is found to be 6%w/w which is quiet normal. such a high value is due to the intermediatory process of *Trisama* decoction preparation involved where by water soluble constituents such as Carbohydrates, Glycosides, Amino acids, Proteins etc. get extracted, which are usually present in large amount in plants.

Refractive index has the large number of applications. It is mostly applied for identify a particular substance, confirm its purity, or measure its concentration. Generally it is used to measure the concentration of a solute in an aqueous solution. Refractive index *Trisama* decoction is 1.354 at 40^0 C.

Viscosity is an important property of fluids which describes a liquids resistance to flow and is related to the internal friction within the fluid. viscosity and drug dissolution share an inverse relationship Change in viscosity may affect the drug absorption by

mechanisms like; Modification in gastric emptying rate, Modification in intestinal transit rate, Change in the rate of drug molecules from lumen to the absorbing membrane. The viscosity of *Trisama* decoction sample was 1.039 poise which was quiet high but it is included in normal range. Here, the value of Specific gravity of Trisama decoction sample is found to be in normal range (1.02).

The pH value indicates the relative concentration of hydrogen ion in the solution compared with that of standard solution that represents the relative acidity or alkalinity of solution. The pH of 10% solution of *Trisama* powder and *Trisama* decoction was 4 which indicates that both the dosage form of *Trisama* having acidic in nature.

3.5. Phytochemical evaluation

Quantitative test of *Trisama* powder and decoction: Total alkaloid, total saponins, tannin estimation are performed from both the dosage form of *Trisama* and results are placed in Table 5. The Quantitative estimation of total alkaloid and total saponins and total tannin by are quite satisfactory. Besides, powder sample gives quiet higher range then decoction. Such a high value is due to the intermediately process of decoction preparation involved where by water soluble constituents such as Carbohydrates, Glycosides, Amino acids, Proteins etc. get extracted, which are usually present in large amount in plants.

Table 5: Quantitative analysis of Trisama formulation

Parameters	Results	
	Powder	Decoction
Total Alkaloid%	1.96±0.23	0.04±0.20
Total saponins%	5.30±0.90	0.92±0.12
Tannin estimation%	4.10±1.11	1.01±1.40

Qualitative test of Trisama powder

The methanol extract of the sample was analyzed qualitatively for different functional groups. Details are placed at Table 6. Qualitative phytochemical tests of *Trisama* powder confirms the presence of certain chemical constituents like, alkaloids, flavonoids, tannin, carbohydrate, steroid, glycosides, and saponins.

Study on the *Trisama* is a effort towards pharmacognostical and Physicochemical standardization of herbal drugs in powder form.

Table 6: Qualitative analysis of Trisama formulation

Name of the test	Presence/Abs
Carbohydrates	++
Starch	--
Protein	--
Amino acid	--
Steroid	++
Glycoside	++
Flavonoids	++
Tannin	++
Alkaloid	++

4. Conclusion

Pharmacognostical study reveals genuineness as that all the ingredient microscopic characters were observed which shows the authenticity of ingredients which is used to form *Trisama*. Its results confirm the ingredients at a preliminary level in pharmacognostical and pharmaceutical analysis of *Trisama* powder and decoction. Though the groundwork for the standardization of *Trisama* dosage forms was sheltered in this study, further important analysis and investigations are essential for advance identification of all the active chemical constituents. The result of this study may be supportive as the reference for the further research work.

5. References

Aggrawal, B.B., Prasad, S., Reuter, S. 2011. Identification of Novel Anti-inflammatory agents from Ayurvedic Medicine for Prevention of chronic diseases: Reverse pharmacology and -bedside to bench‖ approach. *Current Drug Targets*, 12: 1595-1653.

Anonymous. 1998. Quality control methods for medicinal plant materials. Geneva: World Health Organization.

Anonymous. 2001. The Ayurvedic Pharmacopoeia of India. Part 1, Vol. 1, 2, 4, 5 1st ed. New Delhi: Ministry of Health and Family welfare, Department of AYUSH Government of India.

Anonymous. 2008. The Ayurvedic Pharmacopoeia of India, Part 2. 1st ed., vol.1. New Delhi: Department of

AYUSH, Ministry of Health and Family Welfare, Government of India. pp 140.

Bramhanand, T. 2006. *Sharangdhar Samhita*, *Madhyam khanda*. 2nd chapter, Varanasi: Choukhamba Surbharati Prakashana; Reprint 2006. pp 133.

Khandelwal, K.R. 2008. *Practical Pharmacognosy techniques and experiments*. 19th ed. Pune: Nirali Prakashan. pp 149-66.

Kokate, C.K., Purohit, A.P., Gokhale, S.B. 2008. *Pharmacognosy*. 42nd ed. Pune: Nirali *Prakashan*. pp 102.

Muthugala, M., Harisha, C.R., Goyal, Mandip., Dave, A.R., Shukla, V.J. 2013. pharmacognostical and pharmaceutical analysis of parasikayavanyadi churna. *International Journal of Science Inventions* Today, 2(3): 184-193.

Patel, A.G., Nariya, M.B., Shukla, V.J., Patel, B.R. 2017. Review on *Trisama*- an unexplored ancient Ayurvedic formulation. *Journal of Ayurveda and Herbal Medicine*, 3(3):163-168.

Patwardhan, B. 2000. Ayurveda: the designer medicine: a review of ethnopharmacology and bioprospective research. *Indian Drugs*, 37(5): 213-222.

Separation from herbal mixture identification and bioactivity evaluation. Available from http://pac.iupac.org/publications/pac/pdf/2008/pdf/8010x2195.pdf.

Shinde, V., Dhalwal, K., Mahadik, K.R. 2008. Issues related to pharmacognosy. *Pharmacognosy Reviews*, 2(3): 1-5.