ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

A PHARMACEUTICO-ANALYTICAL & ANTIMICROBIAL STUDY OF *TRIVARGTRITYADI YOGA* (TTY)

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Article info

Article History:
Received: 24-10-2022
Revised: 13-11-2022
Accepted: 28-11-2022

KEYWORDS:
TT yoga, Ingredients, Toothpaste, *Churna,* Antimicrobial, Pharmaceutical & analytical.

ABSTRACT

In current scenario herbal formulations containing natural ingredients is more acceptable in public belief than chemical based synthetic formulations due to their safety and efficacy in reducing dental caries, pyorrhoea and preventing other dental and oral issues to which this generation is prone to. Clinically more effective and tolerable treatments should improve patient compliance with oral hygiene practices, further supporting medical professionals' efforts to avoid disease. Controlling the creation of dental plaque is the most efficient method of preventing the onset of dental disease. Microbes have an impact on its formation. Ayurveda recommends some daily use therapeutic procedures for the prevention and maintenance of oral health. These include: *Pratisarana* (gentle massage over tissue), *Dant Dhavani* (brushing), *Jivha Lekhana* (tongue scrapping) and *Gandoosha* (gargling) or oil pulling. The present study focused on preparation and validation of the classical formulation *Trivargtrityadi yoga (TT yoga)* which mentioned in *Ashtang Sangraha* by *Acharya Vagbhata* described in *Dantadhawanacharya*. It contains *Triphala*, *Trikatu*, *Trijatak* and *Kuth.* These extracts have a variety of beneficial characteristics, including those that are anti-cancer, anti-fungal, and anti-ulcer, anti-caries, anti-bacterial and wound healing. The preparation of the formulation in accordance with traditional references and evaluation of its analytic and antibacterial properties are the objectives of this study.

INTRODUCTION

In the modern era, fast moving hectic lifestyle is creating many health problems. Due to improper eating habits like junk food, fast food, ice-creams, sweets, chocolates and addictions like tobacco, gutakha, smoking and alcohol consumption, oral unhygienic problems are arising progressively. So we need to educate people on dental hygiene awareness, to prevent them from acquiring different types of diseases of oral cavity which can occur at various stages of life. Ayurveda recommends some daily use therapeutic procedures for the prevention and maintenance of oral health.

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Quick Response Code

https://doi.org/10.47070/ijapr.v10i11.2605

These include: *Pratisarana* (gentle massage over tissue), *Danta Dhavana* (brushing), *Jivha Lekhana* (tongue scrapping) and *Gandoosha* (gargling) or oil pulling. Acharya Sushruta and Vagbhata described dental diseases in detail. *Upkusha* is also described by Acharya Sushruta and Vagbhata. *Upkusha* can be compared with Pyorrhea.

Here I discuss *Trivargtritvadi yoga* (TT yoga) which mentioned in Ashtanga Sangraha. Acharya Vagbhata described this yoga in *Dantadhawan* for dental care in *Churna* form[1]. In the present era people are lacking interest in *Churna kalapana* because of its discomfort while using. Therefore here I am going to prepare other types of formulation currently running in dental care now days. The formulations of *Trivargtritvadi yoga* are-

***Churna Kalapana* (Powder)-** It is a *Upkalpana* of *Kalka kalpana*. It is fine powder of a drug. In Ayurved therapeutics, the *Churna* is used for both internal and external application.

Paste (Toothpaste)- It can also be considered as *Upkalpana* of *Kalka* and used as a *'Pratisarana chikitsa*. Toothpaste is a dentifrice used to clean, maintain and improve the health of teeth. Toothpaste is mainly used to promote oral cleanliness and also acts as an abrasive that helps to prevent the dental plaque and food particles from the teeth, aids in the removing and or veiling of halitosis*. TT yoga* contains *Triphala*, *Trikatu*, *Trijatak* and *Kuth*. *TT yoga* mixed with honey should be rubbed on teeth for *Dantadhavan* purpose twice in a day.

Pharmaceutical Study[2,3]

The formulations of '*Trivargtrityadi' yoga* (*TT*) will be prepared as per the reference of *Ashtang sangraha* in the Departmental Laboratory & Pharmacy of *Rasashastra*.

This study will be carried out in two groups:

1. Preparation of *TT Churna* as per classical texts.
2. Preparation of *TT* toothpaste as per modern pharmaceutical process.

Pharmaceutical Preparation of *TT Churna*

Following steps were performed for pharmaceutical processes

1. Collection of raw drugs of *TT yoga*
2. *Churna* of each drug separately
3. Sieving of each drug separately
4. Mixing of each drugs in equal quantity
5. Mass mixing procedure
6. Packaging and labeling

Ingredients

Table 1: Showing Ingredients of TT *Churna*

S.N.	Ingredients	Used Part	Raw drug wt. (in gm)	Powdered drug wt. (in gm)	Loss in gm	% loss	Powdered drug colour
1.	*Haritaki*	Fruit	500	487	13	2.6	Orange-brown
2.	*Amalaki*	Fruit	500	467	33	6.6	Dark brown
3.	*Bibhitaka*	Fruit	500	487	13	2.6	Pale brown
4.	*Pippali*	Fruit	500	455	45	9	Greenish brown
5.	*Maricha*	Fruit	500	417	83	16.6	Blackish
6.	*Sunthi*	Rhizome	500	462	38	7.6	Whitish brown
7.	*Twak*	Stem bark	500	440	60	12	Red
8.	*Sukshmaila*	Fruit	500	410	90	17	Greenish black
9.	*Tejpatra*	Leaves	500	427	73	14.6	Green
10.	*Kutha*	Root	500	408	92	18.4	Greyish brown
	Total	**-**	**5000**	**4460**	**540**	**10.8**	**Light brown**

Observation

- Each drug shown their different colour mentioned in table.
- The final mixture of *TT churna* colour was light brown.
- *Pipalli* and *Kutha* drugs gain moisture easily.
- When *TT Churna* was rubbed between index finger and thumb and its particles was present in grooves of finger.
- Percentage loss was to be higher in *Kutha*, *Suksmaila* and *Maricha*.
- Small amount of *Churna* had been lost during grinding and sifting the materials.

Result:

- Total duration required for preparation of *TT Churna* – 4 days
- Total powder taken – 400gm×10 = 4000gm
- Final weight obtained after mass mixing and packing of *TT Churna* - 3960gm
- Total loss of weight - 40gm
- Percentage of loss of weight – 1%
- The colour of *TT churna* was light brown.

Pharmaceutical Preparation of *TT* paste

Ingredients of TT Toothpaste

Table 2: Showing Ingredients of *TT Paste*

S.N.	Ingredients	Quantity
1.	Calcium carbonate	5.372kg
2.	Multani mitti	345gm
3.	Sorbitol	3.150kg

4.	Glycerine	450gm
5.	Honey	500gm
6.	*Triphala kwath*	600gm
7.	Remaining 7 drug extracts	175gm
8.	Carboxy methyl cellulose (CMC)	12.6gm
9.	Xanthum/ Acacia gum	18.7gm
10.	Sodium lauryl sulphate (SLS)	35gm
11.	Preservatives (MPS/PPS)	0.2%
12.	Aromatic agent	q.s.

Following steps were performed for pharmaceutical processes

1. Extraction of 7 drugs- Water soluble extraction of *Pippali*, *Marich*, *Sunthi*, *Twak*, *Suksmaila*, *Tejpatra*, *Kutha* as per API.
2. Preparation of *Triphala kwath*
3. Making Calcium carbonate and fuller's earth (*multani* soil) very fine powder
4. Seven drug extracts are mixed in *Multani* soil
5. CMC and Xantham gum are mixed in calcium carbonate
6. mixing (*Multani* + 7 extract) + (CMC + Xantham & calcium carbonate)
7. These mixture were shifted in stainless steel vessel
8. Adding of sorbitol, glycerine and honey
9. Adding of *Triphala kwath*
10. Blending of whole mixture
11. Adding of foaming agent
12. Adding of essential oil and peppermint
13. Adding of preservatives
14. Toothpaste tube filling
15. Packaging of toothpaste tubes
16. Labeling

Observation

- The water soluble extractive yield of *Kutha* was less than (9.4%) as mentioned in API (20%).
- The final product of *TT* paste colour was dark brown.
- *Pipalli* and *Kutha* drug extract gain moisture easily.
- When *Kwath* started to boil a characteristic smell was observed.
- The colour of obtained *Triphala Kwath* was dark brown.
- Calcium carbonate, *Multani* soil, CMC and Xanthum gum was rubbed between index finger and thumb and its particles was present in grooves of finger.
- In the process of blending when SLS (sodium lauryl sulphate) was added the texture of the mixture got improved and it became more even.

Result

- Total duration required for preparation of *TT* paste – 2 days
- Final weight obtained of *TT* paste - 10.658kg
- Total loss of weight after filling of tube– 9.400kg
- Total loss of weight after packing of tube (5 tubes are destroy) – 8.900kg
- Percentage of loss of weight – 16.49%

Analytical Study[4,5]

Analytical study deals with the analysis of the values of some physical constants and chemical values of prepared formulations. In present research work both formulations were tested on various preliminary standardization parameters as well as some of the sophisticated analytical tests. Analytical evaluation can be done on following parameters.

Organoleptic Evaluation

Organoleptic evaluation of drugs refers to the evaluation of a drug by color, odor, appearance, taste, texture etc. In the present study two samples of *TT Churna* and *TT* paste were analyzed by above mentioned parameters.

RESULTS

Table 3: Organoleptic characters of the sample of *TT Churna* and TT Paste

S. N.	Parameters	*TT Churna*	*TT* Paste
1.	Appearance	Fine powder	Thick viscous paste
2.	Color	Brown	Brown
3.	Odor	Aromatic	Aromatic

In the context of organoleptic characters, the TT *Churna* was observed brown in colour with aromatic odor and its appearance was fine powder. TT Paste texture was thick viscous with brown in colour and aromatic odor. Its aromatic odor is due to *Trikatu* and *Trijata*, and the colour is due to maximum raw drugs color is brown.

Table 4: TT *Churna* Parameters

A.	**Physiochemical Analysis**			
S.No.	**Parameters**	**Unit**	**TT *Churna***	
1.	Foreign matter	%w/w	0.10	
2.	pH (2% Aq. Solution)	-	4.41	
3.	Loss on drying	%w/w	10.80	
4.	Total ash	%w/w	4.63	
5.	Acid insoluble ash	%w/w	1.02	
6.	Water soluble extractive	%w/w	28.29	
7.	Alcohol soluble extractive	%w/w	27.90	
8.	Particle size (#120 No., 125 µm)	%w/w	73.08	
9.	Bulk density	g/ml	0.4290	
10.	Tap density	g/ml	0.6129	
11.	TLC (Toluene: Ethyl Acetate: Formic acid 6.0:4.0:0.5)	-	RF Value 366nm- 0.25, 0.44, 0.56, 0.97 254nm- 0.20, 0.25, 0.44, 0.56, 0.67, 0.76, 0.87, 0.97 White light- 0.16, 0.25, 0.44, 0.56, 0.97	
12.	Powder microscopy	-	Data attached	
B.	**Metal Analysis**			
13.	Lead (Pb)	mg/kg	0.69	NMT 10
14.	Mercury (Hg)	mg/kg	BLQ (LOQ 0.1)	NMT 1
C.	**Microbiological Analysis**			
15.	Total Bacterial Count	Cfu/g	<10	100000
16.	Total Fungal Count	Cfu/g	<10	1000

Table 5: TT Paste parameters

A.	**Physiochemical Analysis**			
S.N.	**Parameters**	**Unit**	**TT Paste**	
1.	Moisture content	%w/w	13.35	
2.	Homogenecity	-	Homogenize	
3.	Spreading ability	Sec.	6.0	
4.	Fineness (#150µm)	-	0.07	
5.	**Foaming Power** Primary Height Secondary Height	 ml ml	 Nil Nil	
6.	Thermal Stability (At 5, 25, 45°C for 28 days)	-	Stable	
7.	Volatile Content	%w/w	13.54	
8.	Viscosity	Cps	96400	
B.	**Heavy Metal Analysis**			
9.	Lead (Pb)	mg/kg	1.74	NMT 10
10.	Arsenic (As)	mg/kg	BLQ (LOQ 0.1)	NMT 1

Anti-microbial Study[6-9]

The antimicrobial study is performed for the sample *TT* Paste. The two oral cavity microorganisms is carried out for this study-

Table 6: Details of Microorganisms

S.N.	Micro-organism	Gram Positive/ Negative	MTCC No.	Media used
1.	Porphyromonas gingivalis	Gram Negative	33277	Mueller Hinton Agar
2.	Streptococcus Mutans	Gram Positive	890	Mueller Hinton Agar

RESULTS

Table 7: Result of Antimicrobial Study

S.N.	Microbial Strains	*Standard (Positive control)	TT Toothpaste		DMSO (Negative control)
			5mg/ml	10mg/ml	-
1	*Porphyromonas gingivalis*	18	8	10	8
2	*Streptococcus mutans*	20	8	11	8

Activity Index = Zone of inhibition by sample/Zone of inhibition by standard

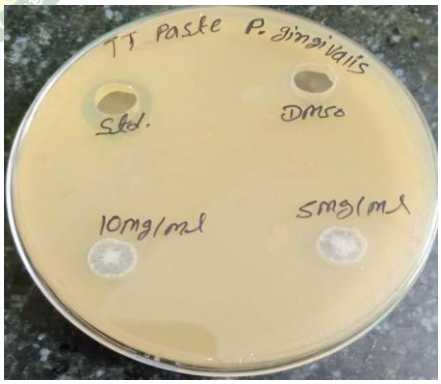
Table 8: Activity index of TT Paste at 5mg/ml

S.N.	Sample	Activity Index
1.	*Porphyromonas gingivalis*	0.444
2.	*Streptococcus mutans*	0.4

Table 9: Activity index of TT Paste at 10mg/ml

S.N.	Sample	Activity Index
1.	*Porphyromonas gingivalis*	0.555
2.	*Streptococcus mutans*	0.55

Table 10: Images of Antimicrobial Activity of TT toothpaste

S.N.	Name of Microbe	Image
1.	*Porphyromonas gingivalis*	
2.	*Streptococcus mutans*	

Antimicrobial study was done on TT toothpaste with compared to standard drug chlorhexidine gluconate 2% solution on two selected microbes. As these formulation are mentioned in *Ashthang Sangraha* in *Dantadhawana*, all the microbes selected are common responsible bacteria for the condition.

The results shows positive against all the microbes found to be susceptible against the sample of TT toothpaste. Moderate zone of inhibition (8) was observed against Porphyromonas gingivalis MTCC no. 33277, and Streptococcus mutans MTCC no. 890 in 5mg/ml concentration. Zone of inhibition was there against Porphyromonas gingivalis MTCC no. 33277 and Streptococcus mutans MTCC no. 890 in 10mg/ml concentration was as good as standard drug. Comparatively, almost same zone of inhibition found seen as compare to standard drug. It is presumed that quiet effective anti-microbial activity was reported.

DISCUSSION

Pharmaceutical study involved two formulation i.e., *TT Churna* and TT Paste were formulated according to Ayurveda and modern concept and prepared as per the active ingredients (*Triphala, Trikatu, Trijata, Kuth* and honey) taken from classical reference. By this way a standardized process as well as standardized finished product can be obtained.

Concept of Geometrical dilution was adopted for uniform distribution of *TT Churna* ingredients. Geometric dilution process of addition of one ingredient to another in doubling portion of drugs. It ensures randomness and homogeneity of ingredients and presence of all the components in required ratio in even every small quantity of compound formulation.

In present study paste was prepared as per the modern concept. The extraction of *Pipalli, Maricha, Sunthi*, *Tvak, Sukshmaila, Patra* and *Kwatha* drugs were prepared by alcohol soluble extraction method and water soluble extraction method as per mentioned in API. Decoction method is also used for extraction of *Triphala* (*Hariatki, Bibhitak, Amalaki*) because water is a good solvent for many of the active ingredients in herbs, whereas the high efficiency and complete extraction is obtained.

Decoction method is not used for *Trikatu. Trijata* and *Kutha* drug because it contains aromatic property and essential oil.

Triphala Masi is a novel therapeutic agent in management of dental disorders so initially *Triphala Masi*, a classical Ayurvedic pattern was applied in the formation of paste, but it did not matched the standards of proper formulation. Particles of *Triphala Masi* delivered an unpleasant taste in addition with coarse size particles and it finally converted in charcoal type formulation. So the *Triphala Masi* was eventually discarded for the paste formation.

While evaluating analytical parameters, it is found that in *TT churna* the value of foreign matter is 0.1% which is considered as normal. So this formulation is safe without any harmful foreign matter. The pH of TT *churna* and TT paste is 4.41 and 6.66, has respectively acidic in nature. Appropriate pH enhances the drug absorption and distribution and the pH is less than 4 is healthy environment for bacterial growth in oral cavity. The acidic and approx neutral pH shows moderate bacteriostatic activity of the formulations. Loss on drying should be minimum to prevent degradation of product. Excess of water in drug encourage microbial growth, presence of fungi or insects and deterioration following hydrolysis. *TT Churna* contained 10.80% w/w and TT paste 13.35% w/w. TT paste showed more moisture content than *TT Churna* may be due to water content was used during the preparation of paste. The total ash method is designed to measure the total amount of inorganic material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "non-physiological" ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface. TT *Churna* showed comparatively low amount of total ash (4.63%) may be due to it contained mainly organic material. Acid Insoluble Ash is a part of total ash insoluble in dil. HCL It is recommended for certain drugs because of presence of siliceous materials are determined by it and was 1.02%w/w for TT *Churna.* Extractive values are primarily useful for the determination of adulterated drugs, and determines the purity as well as quality of the drug. Alcohol soluble extract and water soluble extract of all the raw drugs are within limits when matched with API standard monographs. The alcohol soluble extract and water soluble extract of TT *Churna* was 27.90% and 28.29%. All the raw drugs show approx similar solubility in water soluble extractive and alcohol soluble extractive. Particle size is one of the factors which will affect dissolution and absorption of drug. Particle size and surface area of a solid drug are inversely related to each other. Smaller the drug particle greater will be the surface area available for chemical reaction and thus more will be the activity of drug. TT *Churna* coarse powder was throughout 80 no. Sieve. The bulk density of a sample is the ratio of the mass to the volume of an untapped powder sample. Tap density of powders is an increased bulk density attained after mechanically tapping a cylinder containing the sample. The bulk density of TT *Churna* is 0.4290 and Tap density is 0.6129.

Heavy metals presence is a serious concern today because it affects the safety of drug. According to WHO heavy metal content in the herbal drug as follows- mercury- 1 ppm, arsenic- 3 ppm, lead 10 ppm. For TT *Churna* the value of Lead is 0.69 and mercury is BLQ (LOQ 0.1). For TT paste the value of Lead is 1.74 and Arsenic is BLQ (LOQ 0.1). However in order to ensure that the test sample is free from heavy metal contamination. Analysis of mercury, lead and arsenic

were done which revealed that the samples were free from heavy metal contamination.

Microbiological Analysis- Total bacterial count and total fungal count was within normal limits as per Ayurvedic Pharmacopoeia of India, so the sample was free from bacterial and fungal infection. Hence can be said that it is completely safe and have been prepared under Good Manufacturing Practices (GMP).

Thermal Stability test of the TT Paste was carried out for four weeks at various temperature conditions like 5°C, 25°C and 45°C and relative humidity from 45% to 60% in a closed container. The ointment was found to be physically stable by its texture, colour and consistency with no phase separation seen. Thus it is inferred that the TT paste is thermally stable and it is not altered by the varying temperature at different seasons and different regions.

While analyzing antimicrobial study, it was done on TT toothpaste with compared to standard drug chlorhexidine gluconate 2% solution on two selected microbes. As these formulations are mentioned in Ashthang Sangraha in *Dantadhawana*, all the microbes selected are common responsible bacteria for the condition. The results shows positive against all the microbes found to be susceptible against the sample of TT toothpaste. Moderate zone of inhibition (8) was observed against Porphyromonas gingivalis MTCC no. 33277, and Streptococcus mutans MTCC no. 890 in 5mg/ml concentration. Zone of inhibition was there against Porphyromonas gingivalis MTCC no. 33277 and Streptococcus mutans MTCC no. 890 in 10mg/ml concentration was as good as standard drug. Comparatively, almost same zone of inhibition found seen as compare to standard drug. It is presumed that quiet effective anti-microbial activity was reported.

CONCLUSION

The pharmaceutical process reveals that a good quality TT *Churna* and TT Paste can be easily prepared by taking caution about various stages occurring during the grinding, sieving, extraction etc. During the preparation the characteristics such as color, odor, appearance etc, were mentioned and the quantity and quality of the ingredients as for the formula were also mentioned. The total quantity of TT *Churna* obtained was 4.460kg and loss was 540gm whereas TT paste was 8.9kg. Analytical test results were within limits of TT *Churna* and TT paste samples. Also anti-bacterial susceptibility shows against both bacteria in TT paste sample in each concentration.

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Cite this article as:
Sharma Ravi, Shukla Govind Sahay, Agarwal Rajaram, Goyal Manisha, Tyagi Vijay Pal. A Pharmaceutico-Analytical and Antimicrobial Study of Trivargtrityadi Yoga (TTY). International Journal of Ayurveda and Pharma Research. 2022;10(11):16-22. https://doi.org/10.47070/ijapr.v10i11.2605

Source of support: Nil, Conflict of interest: None Declared

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