Original Article

Characterization and antimicrobial study of *Trinakantamani (Amber) Pishti*

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Abstract

Background: *Trinakantamani Pishti* (TMP) is a cardio-tonic (*Hridya*), styptic (*Rakta Stambhaka*), astringent (*Kashaya*) formulation frequently used in varieties of bleeding disorders such as bloody diarrhea (*Raktatisaara*), *Raktarsha* (bleeding piles), and disorders of excessive menstruation (*Atyartava*). Still, no published data is available regarding its characterization. **Aim:** To generate a fingerprint for raw and processed TMP using sophisticated instrumental techniques to assess antimicrobial activity of TMP. **Materials and methods:** Three samples of TMP were prepared using the standard reference method. Characterization of TMP was carried out by Fourier-transform infrared spectroscopy (FTIR), energy dispersive X-ray analysis (EDEX) with scanning electron microscopy, powder X-ray diffraction (XRD)**.** Antibacterial activity was carried out by the well-diffusion method. **Results:** Analysis by scanning electron microscope revealed maximum particle size <5 µm and <3 µm in the raw sample and TMP, respectively. Minimum particle size in TMP ranges from 1 to 2 µm and 701 nm. EDEX analysis shows carbon and oxygen as major constituents while Na, Mg, Ca, Si, Fe, and S were present in traces. XRD pattern indicates the amorphous nature of the drug, while FTIR analysis reveals the presence of functional groups such as O–H, CO2, C = O, C-N, N–H. Heavy metals, total microbial count, and microbial limit test were found to be under permissible limits. Anti-microbial study against tested pathogens *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhimurium* did not show any effect of TMP. **Conclusion:** The results of EDEX study showed that *Pishti* samples have the small particle size i.e., 701nm than the raw i.e., 1-2 µm, which may facillitate absobtion of drug into the body. All heavy metals in the samples were within the permissible limit. Carbon, hydrogen and oxygen are the chief elements of drug which confirms similarity to the *Amber*, Since the present work is the first published literature on characterization and anti-microbial study on TMP, the outcome can be considered as fingerprint for the drug prepared using the mentioned reference method.

Keywords: *Amber*, Fourier-transform infrared spectroscopy, quality, scanning electron microscope, *Trinakantamani Pishti*, X-ray diffraction

Introduction

Rasaushadhies, Bhasma; Pishti or other herbo-mineral formulations, are used in clinical practice since hundreds of years. However, the regulatory agencies in most of the countries have banned the sales of these herbo-mineral products in their respective countries quoting safety issues and lack of clinical evidence for their efficacy. Hence, for consumer prospective, scientific validation of Ayurveda, Siddha and Unani systems of medicine in terms of drug standardization is the first and foremost requirement to validate these medicines by using modern tools and techniques.

In the present work, *Trinakantamani Pishti* (TMP), one of the commonly used formulation was screened for the purpose of standardization and quality control. *Trinakanta* is a fossil resin which belongs to araucariaceae (conifers/gymnosperms) and Fabaceae/Leguminoase (flowering plants/angiosperms).[1] *Amber* (Latin-*Succinum*) is generally taken as *Trinakantamani,* that is used since prehistory period in the manufacture of jewelry and ornaments, and

Access this article online

Quick Response Code:

Website: www.ayujournal.org

DOI: 10.4103/ayu.AYU_155_19

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How to cite this article: Joshi N, Ahuja MR, Rastogi GK, Dash MK. Characterization and antimicrobial study of *Trinakantamani (Amber) Pishti*. AYU 2020;41:225-34.

Submitted: 28-Jun-2019 **Revised:** 23-Sep-2019

Accepted: 23-Nov-2021 **Published:** 03-Jun-2022

also in folk medicine and for amulets.[2,3] In *Ayurveda* it is mentioned under various groups like *Ratna* of herbal origin[4] *Upratnavarga*[5,6] & *Parthiva Aushadha Varga*.[7] *Trinakantamani* is a powerful cardio-tonic (*Hridya),* soothing for senses (*Indriya-Prasadana)*, anti-diarrheal (*Grahi),* styptic (*Rakta-Stambhaka*) that pacifies *Pitta* (*Pitta-Shamaka). Trinakanta* is good for strengthening the heart.[8] It is useful in bloody diarrhea (*Raktaatisara*), bleeding piles (*Pittarsh*), and disorders of excessive menstrual flow (*Asrigdara*).[9]

In *Unani* medicine, it is known as *Kaharuba*– "the straw magnetizer"[5] and comes under group of *Mukabbimeda* and *Habishdum* drugs,[10] used in the treatment of bleeding disorders and also in certain infectious conditions such as bacillary dysentery, ulcer, and wounds.[11] Since no research work has been done on characteristic study *Trinakantamani*, the present research work was conducted with the following objectives- (1) To set a standard for preparing good quality of TMP. (2) To know the chemical composition and the microbial contamination (if any) in the finished product (*Trinakantamani Pishti*). (3) To determine the antimicrobial property of the finished product (*Trinakantamani Pishti*).

Materials and methods

Three samples of TMP were prepared using the standard operating procedure mentioned in Rasamritam.[12]

Pharmaceutical processing of *Trinakantamani Pishti*

Trinakantamani was purchased from the local market of Delhi. The raw sample was identified under UV light (366 nm) at Shriram institute of industrial research, Delhi (JO no307-132-3575, dated 13/08/2013) which was found to be identical with the reference standard of *Kahruba* (*Trinakantamani*) *Mani*. Analysis was done at Shriram institute of industrial research, Delhi.

Shodhana of Trinakantamani

One thousand milliliters normal-saline solution was taken in stainless steel vessel and heated with the help of LPG gas stove. The vessel was removed from the fire and *Trinakantamani* stones were put in warm saline water and rubbed with hand till its external impurity was removed.[12] The material was then washed with plain water and then dried under sunlight. The same process was adopted for *Shodhana* of all three samples named as TM-1, TM-2 and TM-3.

Powdering of Shodhita Trinakantamani

This process is an intermediate process between *Shodhana* and *Pishtikarana*. Although there is no direct reference for this process it is applied in almost all the *Pishti* formation processes to bring the material in a powder state to make the processes easier [Table 1].

Preparation of Gulab Arka

Roses were collected from the herbal garden of the college premises and cleaned properly. 7500 ml of water was added to the 750 g rose and was kept overnight for soaking. Next day, it was crushed gently with hand and it was kept in traditional distillation apparatus with mild heating. In the beginning, the vapors consist of only steam and therefore were discarded. The process was completed in approximately 6 h and finally 2500 ml *Gulab Arka* was obtained.[13,14]

Impregnation (Bhavana) in Gulab Arka

Powder of processed *Trinakantamani* obtained after *Shodhana* was taken in a mortar and subjected to impregnation (*Bhavana*) in *Gulab Arka*. The material was subjected to continuous trituration for 8 h a day. In between the process, required quantity of *Gulab Arka* was added to make the material wet for proper trituration. The material was subjected to continuous trituration for 8 h a day. The same process was repeated for seven times. By the same method, three samples of TMP were prepared named as TMP-1, TMP-2 and TMP-3. Table 2 shows batch-wise observation in the processing of three samples of TMP. Pharmaceutical processing in preparation of TMP is shown in Figure 1.

Organoleptic and analytical study

All samples were subjected to organoleptic tests, *Bhasma Pariksha* like *Rekhapurnta, Varitaratva,*[15] as well as Physico-chemical tests, i.e., loss on drying, total ash value, acid insoluble ash, specific gravity, pH value according to "Protocol of testing of Ayurvedic, Siddha and Unani Medicines"[16] Sophisticated techniques such as elemental analysis with energy dispersive X-ray analysis (EDAX), structural study with powder X-ray diffraction (XRD), particle size with the scanning electron microscope (SEM) were also conducted. Elemental analysis with EDAX,[17] was carried out in Carl Zeiss AG-Supra 40 WDS, manufactured by Zeiss Gemini, Carl Zeiss SMT, Oberkochen (Germany). For XRD[18]-Model-D8 advance, manufactured by Bruker Corporation Pvt. Ltd.(Germany) was used. Surface analysis of particles was done using SEM. All tests were conducted at State Ayurvedic Drug Testing Laboratory, Haridwar, Devansh Testing and Research Laboratory Pvt. Ltd., Bhagwanpur, Roorkee and in Institute Instrumental Centre, Indian Institute of Technology (IIT), Roorkee. Fourier-transform infrared spectroscopy (FTIR), analysis was done in Department of Material Science, Indian Institute of Technology (IIT), Banaras Hindu University.

Microbial enumeration test

Total aerobic microbial count

Enrichment technique

Dissolved 10 g or dilute 10 ml which of the preparation was examined in fluid lactose medium showed to have no antimicrobial activity under the conditions of test and adjusted the volume to 100 ml with the same medium.[19]

Examination of the sample by plate count for bacteria by pour-plate technique

Using Petri dishes 90-100 mm in diameter, and about 15 ml of liquefied soyabean casein digest agar (SCDA) at not more than 45°C was added to each dish. If necessary, pre-treated preparation was diluted as described above so that a colony

Figure 1: Pharmaceutical processing of *Trinakamani Pishti*

Table 1: Observations during *Shodhana* and powdering of *Trinakantamani*

Sample number	Weight before *Shodhana* (g)	Weight after *Shodhana* (g)	Loss in weight (g)	Weight after powdering (g)	Loss in weight (g)
TM 1	200	198	2	195	3
TM 2	200	196	4	194	2
TM 3	200	197	3	194	3

TM: *Trinakantamani*

Table 2: Observations during preparation of samples of *Trinakantamani Pishti*

Sample number	Initial weight (g)	Final weight (g)	Weight gain (g)
TMP 1	195	204	9
TMP 2	194	203	9
TMP 3	194	204	10

TMP: *Trinakantamani Pishti*

count of not more than 300 may be expected. At least two such Petri dishes were prepared using the same dilution and incubated at 30°C to 35°C for 5 days, unless a more reliable count was obtained in a shorter time. The number of colonies that are formed were counted. The results was based on using of colony counter considering the greatest number of colonies but taking 300 colonies per plate for the maximum consistent with good evaluation.

Plate count for fungi by pour-plate technique

As described in the test for bacteria using SCDA and the plates were incubated at 25°C for 5 days, unless a more reliable count were obtained in a shorter time. The result calculated using Colony Counter with not more than 100 colonies.

Validity of the tests for total aerobic microbial count

The following test strains were grown separately in tubes containing soyabean-casein digest medium at 30°C to 35°C for 18–24 h or, for *Candida albicans*, at 20°C for 48 h. *Staphylococcus aureus* (ATCC 6538), *Salmonella typhimurium* (ATCC 14028), *E. coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (ATCC 2091).

Anti-microbial study

The *in vitro* antibacterial activities of test samples were determined by the agar-well plate diffusion method.[20] Stock solutions of samples were prepared in ethanol and were filtered using 0.45 um sterile filters. A well was made in the plates amended with test pathogens namely, *S. aureus*, *E. coli, P. aeruginosa* and *S. typhimurium,* with sterile borer (5 mm). The test sample (50 µl) was introduced into the well and plates were incubated at 37°C for 72 h. *Ampicilln* was used as a standard drug for comparison as a positive control. Mueller Hinton agar plate without amended bacteria was taken as negative control. After incubation plates were observed for the carried out at zone of inhibition around the wells. The study has been Gurukul Kangri University, Haridwar and Himalayan Institute of Medical Sciences (HIMS), Jollygrant.

Results

Raw *Trinakantamani* was tasteless, yellowish-brown color fossil resin which produces lemon odor on rubbing with silk or woolen cloth. Processed *Trinakantamani* had similar organoleptic characteristics as that of unprocessed *Trinakantamani*. The *Shodhana* process marginally increased its luster only because of the removal of dust or foreign particles. TMP was tasteless, odorless, soft to touch and solemn yellow [Table 3].

Table 3: Organoleptic characters of raw sample and *Trinakantamani Pishti*

Character	TM	TMP-1	TMP-2	TMP-3
Colour	Yellowish Brown	Solemn yellow	Solemn yellow	Solemn yellow
Taste	Lemon odour on rubbing	Tasteless	Tasteless	Tasteless
Odour	Not specific	Odourless	Odourless	Odourless
Touch	Hard	Soft	Soft	Soft
Rekhapurnatva	Negative	Positive	Positive	Positive
Varitartva	Negative	Positive	Positive	Positive
Shlakshnatva	Negative	Positive	Positive	Positive

TM: *Trinakantamani*, TMP: *Trinakantamani Pishti*

Evaluation of classical parameters

All samples of TMP passed through classical parameters as namely, *Rekhapurnatva*, *Mriudutva* and *Varitaratva*. The floating of *Pishti* on stagnant water surface justifies the *Varitaratva* as well as its light weight, whereas smoothness on touch reveals the *Mriudutva* nature of *Pishti*. Micro fineness of the *Pishti* is depicted by its *Rekhapurnatva* [Table 3].

Physio-chemical parameters

The pH of raw *Trinakanta* (RT) and *Trinakantamani Pishti* (TMP) was 6.08 and 6.65 respectively, predicting its weak acidic nature. *Gulab Arka,* the levigating *Dravya* for TMP with pH 4.5 (moderate acidic) did not show any significant effect on the pH of the formulation. Loss of drying of both the raw sample (1.38) as well as prepared TMP (1.19) was found to be low. Lower the moisture content better will be the shelf life of the substance. The total ash value for RT and TMP was 0.54 and 0.663 respectively indicative of the presence of less inorganic contents in the drug.

Low value of acid-insoluble ash i.e., 0.035 and 0.037 for raw and prepared sample, may be postulated as probably good dissolution in gastric secretions, leading to better bioavailability. Water soluble extract indicates that the bioavailability of the drug would be more in a media other than water. The value of water-soluble extractives for RT and TMP sample was 0.83 and 2.90 respectively. It shows that the raw sample as well *Pishti*, both are less soluble in water which proves the resinous nature of the sample.

Alcohol soluble extractives

Alcohol soluble extract indicates that the bioavailability of the drug would be more in a media other than alcohol. The value of alcohol-soluble extractives for RT, TMP were 10.47 and 14.79, respectively [Table 4].

Phase identification of *Trinakantamani Pishti* by X-ray powder diffraction

XRD is an essential tool for the rapid identification and quantification of minerals, compounds and other crystalline phases. As *Trinakantamani* comes under group of either precious or semiprecious gems (*Ratna* or *Upratna Varga*) XRD study was carried out. XRD of raw *Trinakantamani* and TMP, is shown in Figures 2 and 3. The XRD patterns exhibited definite amorphous character. ll XRD patterns indicates amorphous humps between 2 θ =10 and 70°, reaching maximum height at around 15° with RT showing peaks at d = 5.82829, θ = 15.190° d = 6.21016, 2 θ = 14.250° d = 3.39351, 2 θ = 26.240° and TMP at d = 5.85957, 2 θ = 15.108° d = 2.19605 2 θ = 41.068° d = 2.41803 2 θ = 37.152°. This pattern indicates the amorphous nature of the drug which means it does not have an ordered structure. Comparison of diffraction pattern 2theta, space of lattice parameters were studied by version 4.9 of High Score X'Pert software analysis. XRD pattern showed the presence of a structure containing elements silica, calcium, sodium, oxygen, aluminum of the chemical name wairakite (98-009-8200), mesolite (98-016-8088) with tetragonal, orthorhombic crystal system in both the samples. Impregnation (*Bhavana*) of RT in *Gulab Arka* 7 times trituration makes additional peaks in TMP. Average crystallite size was found to be RT (90.20 nm) and TMP (79.53 nm) as calculated by Scherer's formula. Each sample contains maximum concentration of carbon and oxygen. Amorphous nature and presence of a high concentration of carbon and oxygen strongly correlate it with *Amber*.

Table 4: Value of physico-chemical parameters of samples of raw sample and *Trinakantamani Pishti*

Parameter	TMP1	TMP2	TMP3
Loss on drying (%w/w)	1.20	1.12	1.24
Total ash value (%w/w)	0.64	0.68	0.67
Water soluble ash (%w/w)			
Acid insoluble ash (%w/w)	0.031	0.041	0.039
Water soluble extractive (%w/w)	2.63	2.79	3.29
Alcohol soluble extractive (%w/w)	15.12	14.37	14.89
Petroleum ether soluble extractive value	12.16	13.56	13.27
pH	6.67	6.60	6.69

TMP: *Trinakantamani Pishti*

Particle size with scanning electron microscopy

Scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM/EDX) is the best known and most widely used of surface analytical techniques. SEM/EDX can be used to simultaneously determine a particle's morphology and elemental composition.[21] SEM photographs of raw *Trinkanta* and TMP at different magnifications are shown in Figures 4 and 5, respectively. SEM photographs of the raw and *Pishti* samples show the heterogeneous structure of the sample, i.e., all the particles are not of equal size and shape. The SEM images of RT and TMP sample reveal the maximum particle size <5 μm and <3 μm respectively whereas the minimum particle size ranges 1–2

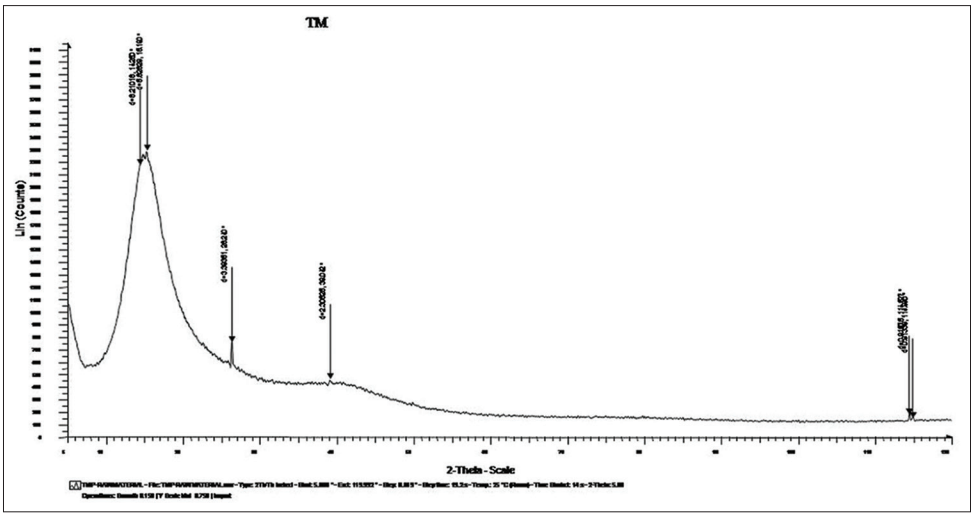


Figure 2: X-ray diffraction pattern *Trinakantamani*

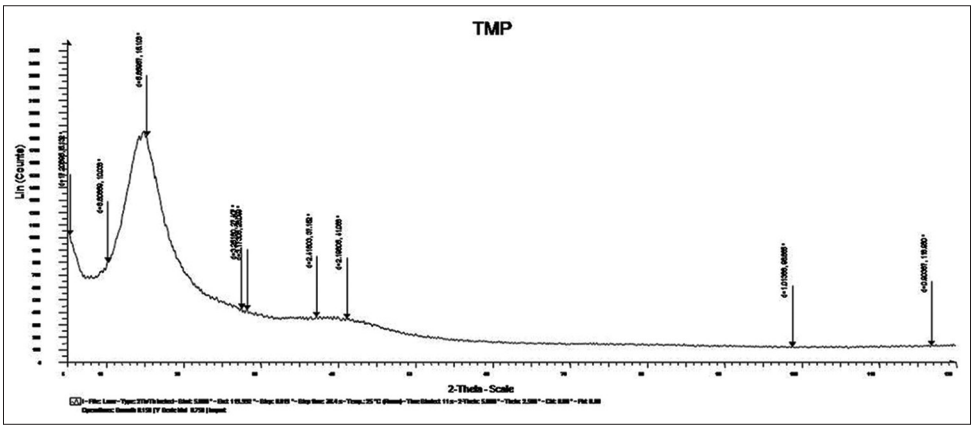


Figure 3: X-ray diffraction pattern of *Trinakantamani Pishti*

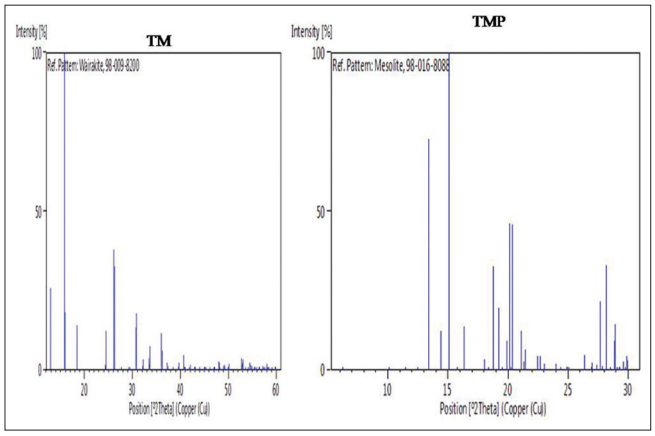


Figure 4: Stick pattern of TM and *Trinakantamani Pishti*

μm and 701 nm, shown in figure 6. The particle size range is shown in Table 5. It is also true that when the particles are broken down into very smaller size, strong cohesive forces act between them. Hence due to this cohesive force and binding nature of the *Bhavana Dravya,* nanoparticles aggregate and give the particle size in micron range. The aggregation of particles is clear in all the SEM images. From the image, it is clear that several crystallites are agglomerated in a particle giving rise to microcrystalline structure as shown in Figures 4 and 5. It suggests that *Pishti* shows the presence of few nanoparticles. However, individual particle size cannot be estimated due to the aggregation of particles. *Pishti* containing nanoparticles in comparison to the raw are very easily absorbed through ion channels into the cells of the body making it very fast effective absorption without taking large doses.[22] It is significant reduction of size, that allows the phenomenon of *Rekhapurnatva* and *Varitaratva*[23] to develop in particle size that facilitates absorption and assimilation of the *Bhasma* in the system.[24] Hence, it becomes clear that particles become finest by extensive grinding and pounding in the wet state with *Gulab-Arka* using mortar and pestle to increase the dissolution as well as the absorption of drug. This trituration process significantly reduced the particle size from 10 microns to <1 microns. Reduction in particle size influences bioavailability, being one of the key factors for the same. The rate of diffusion is proportional to the surface area and the particle size is inversely proportional to the rate of absorption.

Chemical composition analysis by energy dispersive X-ray analysis

With the help EDAX, information about the distribution of different elements was carried out. On analysis, it was found that TMP is chiefly composed of carbon and oxygen while other elements such as Na, Mg, Ca, Si, Fe, S were found to be in traces as shown in Tables 6 and 7. The organic structure of the drug confirms its similarity to *Amber* which also have the same elemental composition in major part while trace elements are supposed to be trapped in the drug during the process of fossilization of *Trinakanta*. Heavy metals such as As, Hg, Cd, Pd were detected below the permissible limits[13] [Table 8], The Government of India, Department of Health and Family Welfare, Ministry of Ayush, has issued new safety standards for the Ayurvedic drugs. The permissible limits of the heavy metals in Ayurvedic drugs with herbal ingredients as per WHO (World Health Organization) and FDA (Federal Drug Organization) are shown in Table 9. So, *Pishti* prepared by the method followed in the study is not only quite safe but also a natural source that provides essential micronutrients on internal administration.

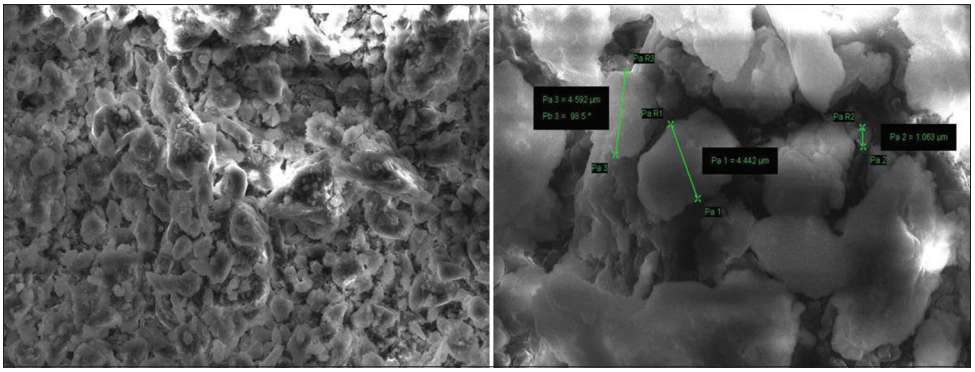


Figure 5: Scanning electron microscope photographs of *Trinakantamani*

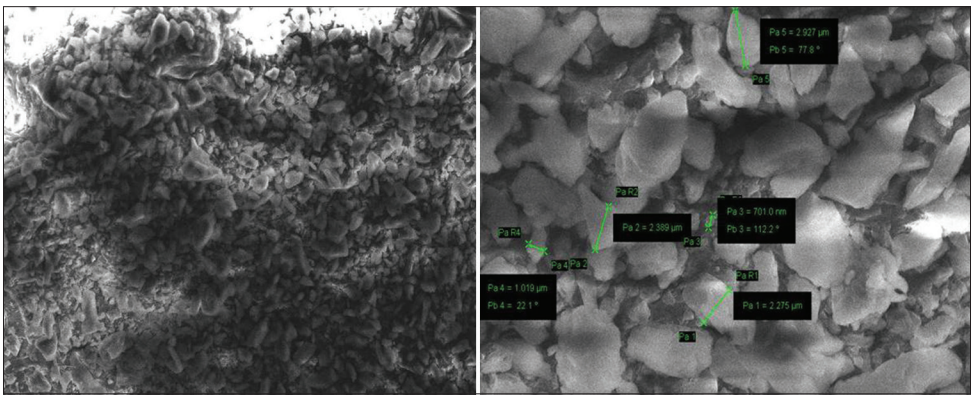


Figure 6: Scanning electron microscope photographs of *Trinakantamani Pishti*

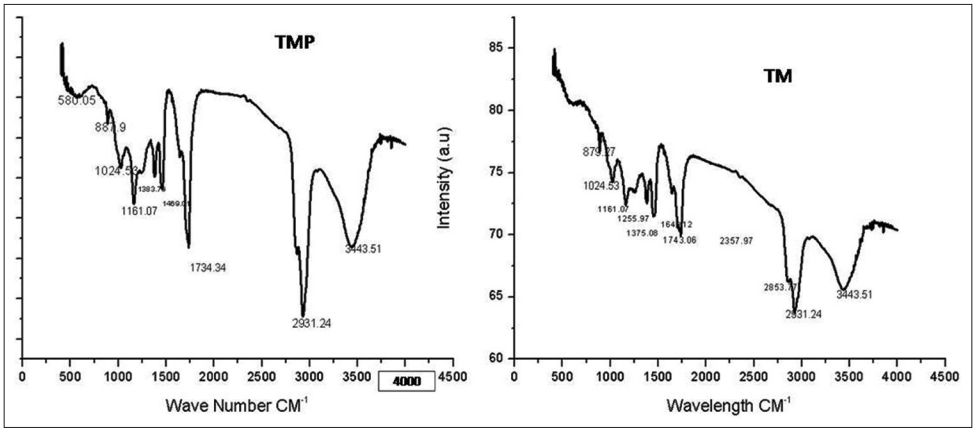


Figure 7: Fourier-transform infrared spectroscopy pattern

Fourier-transform infrared spectroscopy for identification of functional groups

The board peak around 3500-3000 cm^{-1}, 3300-2500 cm^{-1}, 2500-2000 cm^{-1}, 1710-1665 cm^{-1}, 1400-1000 cm^{-1}, 1250-1020 cm^{-1}, 1000-650 cm^{-1}, 995-985 cm^{-1}, 840-790 cm^{-1},730-665 cm^{-1}, 600-500 cm^{-1} corresponds to O–H, O–H, CO2, C = O, O-H, C-N, N–H, stretching vibrations [Figure 7]. Peak at 3443 cm^{-1}, 2931 cm^{-1} assigned to carboxylic acids, Alkyl have role in the pathogenesis of inflammatory disorders within the CNS and possibly other organs. A sharp peak at 1649 cm^{-1} assigned to vibrations of the Alpha, beta–unsaturated aldehydes, ketones (C = O), while another peak at 1452 cm^{-1}isrecognized as stretching vibration of carboxylic acid group (O-H). Peaks lying in 1161 are identified as aliphatic amines (C-N) stretching vibrations. A peak around in 879 cm^{-1} is identified as Alkene stretching vibrations [Table 10]. Although the FTIR spectra of RT and TMP are identical, a well-defined carboxylic acids, alkyl peak around 3300-2500 cm^{-1}, 2500-2000, cm^{-1} was not observed in TMP spectra may be due to the weak intermolecular bonding between alpha carbon and an-OH or hydroxyl group. Alpha carbon is aromatic may be due to the addition of *Gulab Arka*, the carboxylic acid forms an aromatic ring.

Table 5: Average particle size range in different samples

Sample	Particle size range
TM	4.592 µm-1.069 µm
TMP	2.275 µm-701 nm

TM: *Trinakantamani*, TMP: *Trinakantamani Pishti*

Microbial limit test

Microbial and fungal contamination not only affects the chemical composition but also decreases the therapeutic potency of herbal drugs. Microbial contamination of herbal drugs is a major impediment that prevents India from becoming an herbal giant. Therefore, fungal contamination of drugs, especially raw materials, should be prevented during the manufacture of these preparations. Under Microbial limit test, all samples of *Trinakantamani* processed for the quantitative determination of microbial load, in which total bacterial count and total fungal count of sample I having 28 colonies forming unit (cfu) followed by sample II and III having 27 and 26 cfu/ml/gm, respectively [Table 11 and Graph 1]. Above microbial count satisfies the microbiological quality of given samples and defined the least probability of contamination during processing shown in Figure 8. Conclusively the samples are having microbial count under the limits and samples are microbiological quality assured. The microbial limit confirmatory test for

Table 6: Major elements in all the samples of *Trinakantamani Pishti* in energy dispersive x-ray analysis

Element	TMP-1		TMP-2		TMP-3	
	Weight %	At %	Weight %	At %	Weight %	At %
C	78.78	81.62	84.65	87.99	78.27	82.02
O	15.25	11.86	15.47	12.07	15.63	12.30

TMP: *Trinakantamani Pishti,* Carbon, oxygen

Table 7: Minor elements in all the samples of *Trinakantamani Pishti* in Energy dispersive x-ray analysis

Element	TMP-1		TMP-2		TMP-3	
	Weight %	At %	Weight %	At %	Weight %	At %
Na	0.16	0.09	0.07	0.04	-0.04	-0.02
Mg	0.11	0.06	0.04	0.02	0.04	0.02
Ca	0.05	0.02	0.16	0.05	0.12	0.04
Si	0.16	0.07	0.18	0.08	0.14	0.06
Fe	0.18	0.02	0.17	0.04	0.20	0.05
S	0.15	0.06	0.44	0.17	0.22	0.05

TMP: *Trinakantamani Pishti*

Table 8: Heavy elements in all the samples of *Trinakantamani Pishti*

Element	TMP-1		TMP-2		TMP-3	
	Weight %	At %	Weight %	At %	Weight %	At %
Hg	−0.14	−0.01	−0.23	−0.01	0.01	0.00
As	−0.06	−0.01	−0.17	−0.03	0.00	0.00
Cd	−0.19	−0.02	−0.54	−0.06	0.10	0.01
Pb	−0.49	−0.03	0.04	0.00	−0.08	0.00

TMP: *Trinakantamani Pishti,* Hg: Mercury, As: Arsenic, Cd: Cadmium, Pb: Lead

pathogens confirmed the absence of any of the pathogens in the test samples. None of the tested mediums showed the characteristic features for any pathogens. The result of the present study of microbial limit count test in TMP shows that microbial load of the preparation was under acceptance limit [Table 12]. *E. coli*, *S. typhimurium*, *S. aureus* and *P. aeruginosa,* all were absent. Total aerobic microbial count and total yeast and mould count of TMP was within the limit [Table 13] and indicating that TMP is safe Ayurvedic preparation for oral administration.

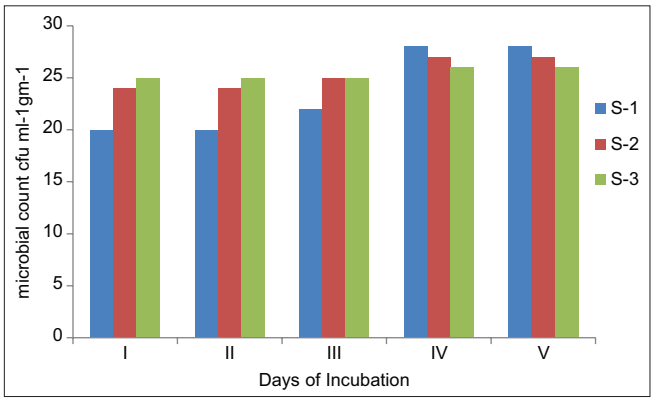
Antimicrobial activity of test sample

According to *Ayurveda*, TMP is efficacious in the treatment of bloody diarrhea (*Raktatisara*), diarrhea (*Atisara*), and infected chronic wounds (*Dushtavrana*).[24,25] Unani medicine too recommends *Kaharuba Pishti* in the treatment of infective conditions like wound, ulcer, etc. From the modern point of view, *Amber* is also thought to inhibit bacterial infection.[26] It is used as snuff to treat the flu and in the tender gums of infants. So in addition to standardization, three samples of TMP had also undergone anti-microbial study against *S. aureus*, *Escherichia coli, P. aeruginosa* and *S. typhimurium*. All three samples were found to be ineffective as evident by no inhibition zone around the wells as compared to that of control [Figure 7a and b]. Hence, to establish role of TMP in infective conditions more advanced studies are still required.

Table 9: Permissible limits of heavy metals in Ayurvedic drugs

Heavy metal	Maximum permissible limit
As	10^3 ng/g
Cd	0.3 µg/g
Pb	10 µg/g
Hg	1 µg/g

Hg: Mercury, As: Arsenic, Cd: Cadmium, Pb: Lead



Graph 1: Showing result after microbial study

Table 10: Functional group

Peak	Actual peak in RT	Actual peak in TMP	Bond	Functional group	Appearance
3500-3000	3443.51	3443.51	O-H	Carboxylic acids, alkyl	Medium to strong
3300-2500	2931.24	2931.24	O-H	Carboxylic acids, alkyl	Medium to strong
3300-2500	2853.77	-	O-H	Carboxylic acids, alkyl	Short and broad
2500-2000	2357.97	-	CO2	Alkyl	Short and broad
1710-1665	1743.06	1734.34	C=O	Alpha, beta-unsaturated Aldehydes, ketones	Strong
1710-1665	1649.12	Peak not observed	C=O	Alpha, beta-unsaturated Aldehydes, ketones	Short and broad
1400-1000	1452.55	1469.01	O-H	Carboxylic acid	Short and broad
1400-1000	1375.08	1383.79	O-H	Carboxylic acid	Short and broad
1250-1020	1161.07	1161.07	C- N	Aliphatic amines	Short and broad
1250-1020	1161.07	1161.07	C- N	Aliphatic amines	Short and broad
1250-1020	1024.53	1015.81	C- N	Aliphatic amines	Short and broad
1000-650	879.27	887.99	N-H	Primary Amines, secondary amines	Short and broad

TMP: *Trinakantamani Pishti*. RT: Raw *Trinakantamani*

Table 11: Drug samples give the following results after microbial study

Days	TMP-1		TMP-2		TMP-3	
	TFC (cfu/ml)	TBC (cfu/ml)	TFC (cfu/ml)	TBC (cfu/ml)	TFC (cfu/ml)	TBC (cfu/ml)
1	Nil	20	Nil	24	Nil	25
2	Nil	20	Nil	24	Nil	25
3	Nil	22	Nil	25	Nil	25
4	Nil	28	Nil	27	Nil	26
5	Nil	28	Nil	27	Nil	26

TMP: *Trinakantamani Pishti,* TBC: Total bacterial count, TFC: Total fungal count

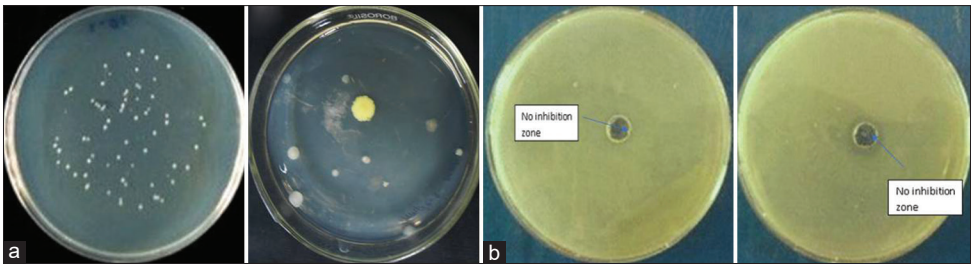


Figure 8: (a) Bacterial colony forming units in microbial limit test. (b) No inhibition zone against test pathogens in antimicrobial activity test

Table 12: Microbial contamination limits according to World Health Organization

Parameters	Permissible limits
Staphylococcus aureus/g.	Absent
Salmonella typhimurium/g.	Absent
Pseudomonas aeruginosa/g	Absent
Escherichia coli	Absent
TPC	105/g*
Total yeast and mould	103/g

TPC: Total microbial plate count

Table 13: Microbial contamination limits in samples of *Trinakantamani Pishti*

Product name	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Salmonella typhimurium*	*Aspergillus brasilliensis*
TMP-1	Absent	Absent	Absent	Absent	Absent
TMP-2	Absent	Absent	Absent	Absent	Absent
TMP-3	Absent	Absent	Absent	Absent	Absent

TMP: *Trinakantamani Pishti*

Conclusion

The adopted method for preparation of *Trinakantamani Pishti* (TMP) can be considered standardized procedure for preparation of *Pishti*. It may be considered as bio-medicine that contains carbon and oxygen as major constituents in similarity with *Amber*. It also contains certain trace elements such as, sodium, magnesium, calcium, silica, iron, sulphur which act as micronutrients and thereby help in the therapeutic applicability of *Trinakantamani Pishti* (TMP) in variety of disorders.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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