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PREPARATION AND STANDARDISATION OF TRIKATU CHURNA AND ITS COMPARISION TO MARKETED FORMULATIONS

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ABSTRACT

The purpose of preparation and standardization of herbal formulations involves the safe, proper selection and handling of crude materials, ensure efficacy and stability of finished product and guiding the consumer about the product. Most of the traditional systems of medicine are effective but they lack of standardization, so required to develop a standardization technique. Central Council of Research in *Ayurveda* and *Siddha* has given preliminary guidelines for standardizing these conventional formulations. It is the demand of traditional formulation to get uniformity in production and it is necessary to develop methods for evaluation. In this research is an attempt to evaluate *Trikatu Churna*, an *Ayurvedic* formulation for its quality control parameters in comparison to the marketed formulations. In house churna preparations were compared with marketed formulations by performing physicochemical evaluation, phytochemical screening, microscopic characterization, fluorescence analysis and TLC etc. It was observed that the set parameters were sufficient to standardize the Trikatu Churna, these findings will be useful towards establishing pharmacopoeia standards for crude drugs as well as for formulation which are gaining relevance in research on traditional medicinal system.

KEYWORDS: Trikatu Churna, Standardization, Phytochemical screening, TLC.

INTRODUCTION

India has an ancient heritage of traditional herbal medicine with the emerging interest in the world to adopt to study the traditional system and to exploit their potentials based on different healthcare systems. World Health Organization estimates that about 80% of the populations living in the developing countries are rely almost exclusively on traditional medicine for their primary health care needs.

The Trikatu Churna is one of the classical Ayurvedic dosage form used in Ayurvedic system of medicine. It is official in Ayurvedic Formulary of India is combination of three reputed herbs comprised of the fruits of *Piper longum* L (Pippali), fruits of *Piper nigrum* L (Marica) and rhizomes of *Zingiber officianalis* R (Saunthi).[1]

Trikatu Churna is the digestive, tonic food for the body. It is also used as a rejuvenator and stimulant. Trikatu Churna plays an essential role in the treatment of wide variety of conditions it eliminates the aggravated Kapha in the respiratory tract and in the digestive channel. It also regulates the path for the Vata and helps in Minimizing gas formation in the abdomen being hot in nature.[2] It is being prescribed for Agnimandra (digestive impairment), Galaroga (throat diseases), Svasa (Asthma), Kushtha (skin diseases), Pinasa (sinusitis), kasa (cough) and Slipada(Filariasis).

Ayurveda is concerned with healthy living along with curative measures that synchronize an individual physically, mentally and spiritually. In this modern era, it is getting accepted as a self-care system for individual well-being. Focusing primarily towards correcting imbalances before they develop into diseases, it is a solution, for all those who acknowledge responsibility for their own health and want a healthy and long life.[3] The need of quality control for Ayurvedic drug is due to the fact that the preparation of drug according to the ancient method has been reduced due to the commercialization of Ayurvedic Pharmacy. The absence of post-market surveillance and the purity of test laboratory facilities also makes the quality control of Ayurvedic medicines exceedingly difficult at this time. Therefore, standardization of herbal formulation is essential in order to assess the quality of drug for therapeutic value.[4]

MATERIALS AND METHODS

1. Collection of powder drug

Trikatu Churna consist of three main ingredients in powder form, it consist of powder fruits of *Piper nigrum*, fruits of *Piper longum* and rhizomes of *Zingiber officinale*. All ingredients procured from local market, Pune. Ingredients were identified on the basis of morphological and microscopically chracters.

2. Collection of markeded formulations

The marketed preparations of various brands of Taritatu Churna i.e. Shet Sakharam Namechandra Shayan, Sholapur and Divya Pharmacy, Hardwar, were purchased from marketed and named as MTC 1 and MTC 2 respectively.

3. Method of preparation of Trikatu Churna

Trikatu Churna was prepared in laboratory using method described in Ayurvedic Formulary. All the ingredient passed through 80# sieve and then mixed together in equal proportion to get uniform blended churna by using spatula.[5] The In house preparation was named as TC.

4. Evaluation of Trikatu Churna

1. Organoleptic evaluation

Organoleptic evaluation refers to evaluation of formulation by appearance, colour, odour, taste, etc. The organoleptic characters of the preparations were carried out.[6] However, these characteristic are judged subjectively and substitutes or adulterants may closely resemble the genuine material, it is often necessary to substantiate the findings by microscopy and physicochemical analysis.[7]

2. Physico-Chemical Investigation

A. Determination of total ash

Total Ash determination constitutes detecting the physiological ash (ash derived from plant tissue) and non-physiological ash (ash from extrageneous matter, especially sand and soil adhering to the surface of the drug). 2g of powdered material of each formulation and the individual ingredients of the powders were placed separately in a suitable tarred crucible of silica previously ignited and weighed. The powdered drugs were spread evenly and weighed accurately. The materials were incinerated by gradually increasing the heat, not exceeding 450°C until free from carbon, cooled in a desiccator, weighed and percentage ash was calculated by taking in account the difference of empty weight of crucible & crucible with Total Ash.[8]

B. Acid insoluble ash

Total Ash obtained was boiled for 5min with 25ml of dilute hydrochloric acid. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash with reference to the air-dried drug was calculated.

C. Water soluble ash

The Total Ash obtained was boiled with 25 ml water for five minutes and then filter through an ash less filter paper. The filter paper was ignited in the silica crucible to a constant weight.[9] The water soluble ash was calculated.

D. Sulphated ash

Take 2 g of substance was taken in an accurate weighed crucible; ignited gently at first until the substance is thoroughly charred. Residues was moisten with 1ml of sulphuric acid , heated gently until white fumes were no longer evolved and ignited at 750^0C+25^0C unlit black particles disappeared. Crucible was allowed to cool; few drops of sulphuric acid was added, heated and allow to cool and reweighed. Sulphated ash was calculated.

E. Determination of water soluble extractive

5gms of each Trikatu Churna was accurately weighed and placed inside a glass stoppered conical flask. It is then macerated with 100ml of chloroform water for 18 hours. It was then filtered and about 25ml of filtrate was transferred into a china dish and was evaporated to dryness on a water bath. It was then dried to 105°C for 6 hours, cooled and finally weighed and water soluble extractive value was calculated.

F. Determination of alcohol soluble extractive

5gms of each Trikatu Churna was accurately weighed and placed inside a glass stoppered conical flask. It was then macerated with 100ml of ethanol 18 hours. It was then filtered and about 25ml of filtrate was transferred into a china dish and was evaporated to dryness on a water bath. It was then dried to 105°C for 6 hours, cooled and finally weighed and calculated.[10]

G. Loss on drying: Loss on dying is the loss of mass expressed as percent w/w. About 10g of drug samples of each formulation were weighed accurately and dried in a tarred flat weighing bottle and at 105^0C for 5 hrs. Percent w/w was calculated with reference to initial weight.

H. Determination of pH: 1% w/v 0r 10%w/v solution of samples were prepared in distilled water and pH was determined using Digital pH Meter Model EQ- 610.[11]

5. Preliminary phytochemical screening

In house and marketed formulation of Trikatu Churna were subjected to test separately for the presence of various phytoconstituents like saponins, tannins, carbohydrates, alkaloids, flavonoids glycosides, steroids, proteins and alkaloids.[12]

A. Saponins: Froth Test- 0.1 g of powders of Trikatu Churna were vigorously shaken with 5 ml of distilled water in a test tube for 30 sec and were left undisturbed for 20 min. Persistent froth indicates the presence of saponins.

B. Tannins: Test with Lead acetate-2-3 ml of aqueous extracts of the formulations, 2 ml of 10 %w/w solution of lead acetate was added. Formation of heavy dull yellowish precipitates indicates the presence of tannin.

C. Carbohydrates: Molisch's test- To the ethanol extracts of formulation, α-napthol and concentrated H_2SO_4 was added. Development of purple colour indicates the presence of carbohydrates.

Fehling's test-To 1 ml of ethanolic extract of formulation, 1 ml of the Fehling solution (Fehling A + Fehling B) was added. The mixture was heated on boiling water bath for 5-10 min. Development of yellow precipitates, changing to brick red precipitates indicated the presence of reducing sugars.[13]

D. Test for alkaloids-The alcoholic extracts added with dilute HCl and shaked and filtered .Filtrate was test for following tests following tests were performed.

Mayer's test- 2-3 ml filtrate added with few drops Mayer's reagent shown precipitation.

Wagner's test- 2-3ml of filtrate added with few drops Wagner's reagent shown yellow precipitation.

Dragendroff's test- To 2-3 ml filtrate, few drops Dragendroff's reagent was added and orange brown colour produced confirms the presence of alkaloids.

E. Test for flavonids : Shinoda test – 5ml of 95% ethanol/t-butyl alcohol , few drops. of HCL and 0.5g maganesium turning were added to extract . Orange, pink, red to purple colour appeared..

G. Steroid- Salkowaski reaction*:* 2ml of chloroform and 2ml conc.H_2SO_4 was added to extact and shaken . Chloroform layer appeared red and acid layer shown greenish yellow fluorescence.

Liebermann and burchard test- Exatract wad mixed with chloroform. 1-2ml acetic anhydride and 2 drops conc. H_2SO_4 were added from the side of test tube. It became red then blue and finally green colour appeared.[14]

6. Microscopic characteristic: Lignified tissues are to be confirmed by staining with different staining reagents. All the powders were boiled with chloral hydrate/glycerine and mounted on slide to observe under compound microscope (10 x & 40x). First all powders were stained with few drops of mixture of 1:1 Phloroglucinol + Conc. HCl, after 3 to 4 minutes, lignified cells Parenchyma cell, fibre, Cork cell, Stone cell and Oleo resins etc. were observed . Further slides were stained with iodine to observe starch grains.[15]

6. Fluorescence analysis: The powdered samples were exposed to ultraviolet and day light at wavelengths of 254 nm and 366 nm. One milligram of powdered drug was placed on a micro slide and observed under UV 366, UV 254 and in day light to observe the fluorescent characteristics of powder, if any. Same way drugs was treated with 1 ml 1N NaoH, 1 ml 50% H_2SO_4,1 ml of 50% HNO_3 , 1 ml of conc. HNO_3 and observed under UV 366 and UV 254.[16]

7. Crude fibre content determination

2 gm of drug was taken in a beaker and 50ml of 10% nitric acid was added. It was heated till boiling with stirring (30 sec).This was strained through fine cloth on a Buchner funnel. The residue was washed with boiling water and transferred to a beaker. 50ml of 2.5% v/v NaOH solution was added. It was strained and washed with hot water. The residue was transferred in a clean and dried crucible. The residue was weighed and the crude fibre content was determined.[17]

8. Physical properties of powder: Trikatu Churna were studied for the determination of physical properties, which are important for the powdered formulation for their flow property and stability.[18]

A. Bulk density: The term bulk density refers to a measure used to describe a packing of particles or granules. The equation for determining bulk density (Db) is: **Db = M/Vb**

Where, M is the mass of the particles and Vb is the total volume of the packing. The volume of the packing was determined in an apparatus consisting of a graduated cylinder mounted. 100gm of formulation was taken and carefully added to the cylinder with the aid of a funnel. Typically the initial volume was noted and the sample was then tapped until no further reduction in volume was noted. The initial volume gave the Bulk density value.

B. Tapped density

The term bulk density refers to a measure used to describe a packing of particles or granules. The equation for determining tapped density (Db) is, **Db = M/Vb**.

Where, M is the mass of the particles and Vb is the total volume of the packing. The volume of the packing was determined in an apparatus consisting of a graduated cylinder mounted on a mechanical tapping device.100gm of weighed formulation powder was taken and carefully added to the cylinder with the aid of a funnel. Typically the initial volume was noted and the sample was then tapped until no further reduction in volume was noted. The tapping the volume reduced, giving the value of tapped density.

C. Angle of repose

Angle of Repose has been used as indirect methods of quantifying powder flow ability because of its relationship with inter particle cohesion. As a general guide, powders with angle of repose greater than 50 degree have unsatisfactory flow properties, whereas minimal angle close to 25 degrees correspond to very good flow properties. The fixed funnel and the free standing cone method employs a funnel that is secured with its tip at a given height, which was taken 2.5 cm (H), above the graph paper that is place on flat horizontal surface. Powder was carefully poured through the funnel until the apex of the conical pile just touched the tip of the funnel. **Tan θ = H/ R**

Where θ is the angle of repose, R being the radius of the conical pile.

D. Hausner Ratio: It is related to interparticle friction and such can be used to predict the powder flow properties. Powders with low interparticle friction such as coarse spheres have a ratio of approximately 1.2, whereas more cohesive, less flow able powders such as flakes have a Hausner ratio greater than 1.6. The equation is as follows, **Hausner Ratio = Df / Do**

Where Df = Tapped density and Do = Bulk density

E. Carr' index: Another indirect method of measuring the powder flow from bulk density is Carr's index. The equation for measuring Carr's index is as follows,
% compressibility = (Df-Do/Do) × 100

Where Df = Tapped density and Do = Bulk density

9. Thin layer chromatography of Trikatu Churna
TLC study of methanolic extracts of the in-house formulation and marketed formulations was carried to ensure the presence of active ingredients in all the preparations. For TLC, 2gm of each sample (In house TC, MTC-1 and MTC-2) were extracted with 25ml of methanol on boiling water bath for 25 minutes consecutively three times using fresh portion of 25ml methanol, filtered and concentrated. Spots of extracted samples was done on precoated silica gel aluminium plate 60F-254. Piperine from *Piper nigrum*, *Piper longum* and Gingerol from *Zingiber officinale* are the main phytoconstituents present in the preparations, but in the present study was performed the TLC for observing the TLC pattern of phytoconstituents, for which mobile phase was developed. It was consist of **Toluene: Ethyl Acetate: Glacial Acetic Acid (8:2:0.1, v/v/v)**, subsequent to the development at 45 min. After drying the plate were examined under ultraviolet light and then in iodine chamber for 4-5 sec.[19]

RESULTS AND DISCUSSION

In house Trikatu Churna formulation was prepared by the method mentioned in Ayurvedic formulary and also collected marketed Trikatu Churna formulation were set for standardization or quality control parameters. So the quality control parameters required as per standard references were performed in comparison with the marketed sample were discussed.

Method of preparation of in house Trikatu Churna
All the ingredients were mixed in equal proportion and the composition is as given as follows.

Table- I Composition of Trikatu Churna (TC)

Sanskrit Name	Ingredients (Botanical Identify)	Quantity(gm)
Maricha	Fruit of *Piper nigrum* L.	1part
Pippali	Fruit of *Piper longum* L.	1 part
Sunthi	Rhizome of *Zingiberofficinale* R.	1 part

Organoleptic evaluation: The Trikatu Churna formulations were studied for organoleptic characteristic like the appearance, colour, taste and odour. The results are as follows.

Table– II Organolaptic Evaluation

Sr.No	Organoleptic Character	In house preparation	Marketed preparation (Divya Pharmacy)	Marketed preparation (ShetSakharam)
1	Appearance	Powder	Powder	Powder
2	Colour	Brown	Brown	Brown
3	Taste	Bitter	Bitter	Bitter
4	Odour	Pleasant	Pleasant	Pleasant

Physico-Chemical Investigation: All the Trikatu Churna were studied for their physicochemical parameters mentioned as follows.

Table- III Physico-Chemical Investigation

Sr. No.	Physico-chemical parameters	TC (% W/W)	MTC 1 (% W/W)	MTC 2 (% W/W)
1	Total Ash	14±0.5	13.5±0.5	14.2±0.3
2	Acid Insoluble ash	11.76±0.32	12±0.5	11.23±0.52
3	Water soluble ash	13±0.5	12±0.5	11.2±0.52
4	Sulphated Ash	0.54±0.04	0.43±0.01	0.38±0.005
5	Water Soluble Extractive Value	40.5±0.5	38.66±0.57	41±1
6	Alcohol Soluble Extractive Value	35±0.57	34±1	37±1
7	Loss on drying	11±1.15	10±0.57	10.5±0.5

TC- In house formulation, MTC1-In marketed formulation-1 and MTC2-In marketed formulation-2.

Determination of pH: The pH of 1% W/V or 10% W/V of Trikatu Churna and both marketed formulation was determined for different pH like 4.01, 7.0 & 9.18 and pH observed are mentioned as follows.

Table- IV pH determination of Trikatu Churna

Sr. No.	Name of the Formulation	1%W/V solution			10% W/V solution		
		pH Tablet 4.1	pH Tablet 7.0	pH Tablet 9.18	pH Tablet 4.1	pH Tablet 7.0	pH Tablet 9.18
1.	TC	5.31±0.10	6.1±0.1	6.17±0.06	5.82±0.04	5.90±0.03	6.10±0.1
2.	MTC-1	5.38±0.04	5.96±0.02	6.13±0.005	5.77±0.01	5.91±0.037	6.1±0.1
3.	MTC-2	5.74±0.02	6.17±0.064	6.20±0.1	5.77±0.01	5.78±0.01	5.89±0.005

Preliminary phytochemical Screening: The alcoholic extract of Trikatu Churna and individual ingredients were studied for the presence of various phytoconstituents. The preliminary phytochemical observations of crude extracts shown the occurrence of alkaloids, flavonoids, tannins, lignins and steroids. It indicates that the Trikatu Churna is a mixture all these phytoconstituents and interaction all these chemicals might be resulted in synergistically enhanced therapeutic efficacy of sinusitis, Asthma ,Rhinitis, tonsillitis & digestive.

Table- V Preliminary Phytochemical Screening

Sr. No	Phytochemical	Test	*Piper nigrum*	*Piper longum*	*Zingiberoffi cinalis*	TC	MTC 1	MTC2
1	Saponin	Froth test	+	-	+	+	+	+
2	Tannins	Lead acetate	+	-	+	+	+	+
3	Carbohydrates	Molisch's test	+	+	+	+	+	+
		Fehling's test	-	+	+	+	+	+
4	Alkaloids	Mayer's test	+	+	+	+	+	+
		Wagner's test	+	+	+	+	+	+
		Dragendroff's test	+	+	+	+	+	+
5	Flavonoids	Shinoda test	+	+	+	+	+	+
		Extrac+ aqueous	+	+	+	+	+	+
		NaoH+ conc.H_2SO_4	+	+	+	+	+	+
6	Steroids	Salkowski's test	-	+	+	+	+	+
		Libermann&Burchardtest	-	+	-	+	+	+

Microscopic characteristic: All the powdered samples were studied for powdered characters as shown below.

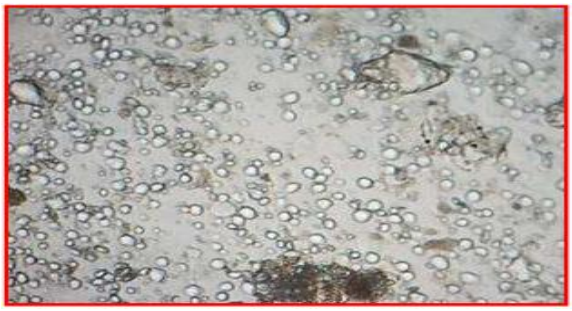


Fig. I Starch grain

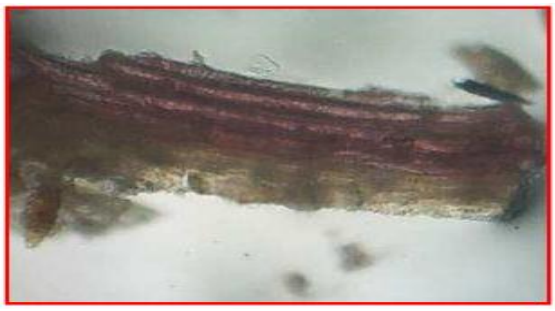


Fig. II Xylem

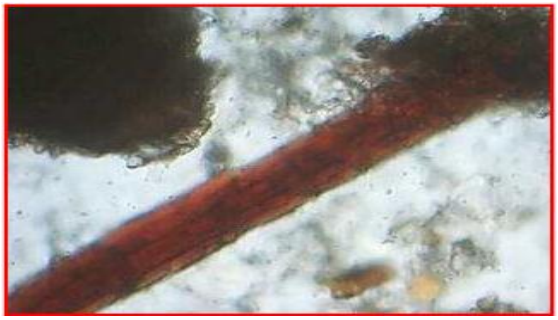


Fig. III Lignified fibers

Fig. IV Cork cell

V. Parenchymaous cells

Fig. VI Oleo resins

Fig. VII Stone cells

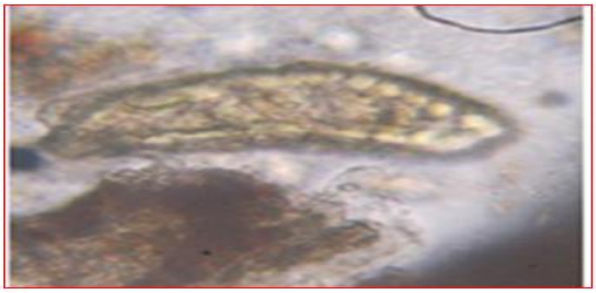


Fig.1 Study of Microscopic characters.

Fluorescence analysis: Powders were studied for Fluorescence analysis with different reagents and observed under ultra violet and day light. Results are mentioned in Table- 6.

Table- VI Fluorescence analysis

Ayurvedic Formulation	**Wavelength in nm** ↓	**Powder + Reagent** →					
		1N HCl	**1NNaoH**	**P+50%. H_2SO_4**	**P+conc. H_2SO_4**	**P+50% HNO_3**	**P+conc HNO_3**
TC	Day light	Light green	Light green	Slightly red	Brownish Black	Brown	Brown red
	U.V 254nm	Light green	Light green	Brown red	Brownish Black	Reddish brown	Brown Black
	U.V 366nm	Slightly fluroscence	Green fluroscence	Brown black	Brownish Black	Bluish black	fluroscence
MTC-1	Day light	Blackish green	Brown	Brown	Brownish Black	Brown	Brown
	U.V 254nm	Green	Brown	Green	Blackish Brown	Slightly Green	Slightly green
	U.V 366nm	Black	Brownish black	Brown	fluorescence	Slightly Black	Slightly black
MTC-2	Day light	Slightly brown	Brown	Lightly Brown	Brownish red	Brownish red	brown
	U.V 254nm	Greenish Brown	Dark brown	Blackish brown	Blackish brown	Dark brown	Dark brown
	U.V 366nm	Dark brown	Black	Blackish brown	black	brownish Black	Black

Crude fibre content determination Percentage of Crude fiber content for TC, MTC 1 and MTC 2 was determined and results obtained are as shown.

Table- VII Determination of crude fiber

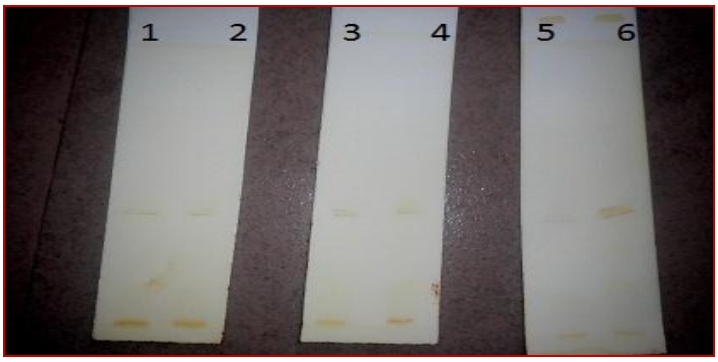
Sr.No.	Formulation	Crude fibre content %w/w
1	TC	11%
2	MTC-1	13%
3	MTC-2	10%

Physical properties of powder: Physical parameter of Trikatu Churna is included in bulk density, tap density, angle of repose, hausner ratio & Carr's index were determined and mentioned as follows.

Table-VIII Physical properties of formulations

Sr.No	Parameter	TC	MTC-1	MTC-2
1	Bulk density	0.425±0.04	0.46511±0.02	0.40±0.01
2	Tap density	0.606±0.03	0.666±0.02	0.571±0.01
3	Angle of repose	31.42±0.23	27.878±0.46	29.575±0.35
4	Hausner ratio	1.125±0.04	1.131±0.03	1.1275±0.04
5	Carr's index	12.58±0.07	14.08±0.05	12.00±0.01

9. Thin layer chromatography of Trikatu Churna
TLC was performed for all the sample to check the pattern of phytoconstituents. Results are as follows**.** TLC plates shows the bands of phytoconstituents observed in iodine chamber. Where.



"Fig.2" TLC pattern for Trikatu Churna formulations

Spot No 1*: In house Trikatu Churna,****Spot No 2:*** *MTC1,****Spot No 3:*** *MTC2,****Spot No 4:*** *Piper nigrum,****Spot No 5:*** *Piper longum,****Spot No 6:****Zingeberofficinale*

CONCLUSION

Trikatu Churna was found to possess higher the rate of phytoconstituents and promising, sinusitis, Athma, Rhinitis, tonsillitis & antibacterial activity. It is also confirmed that, these spicy products triggers natural immune system to fight against enteric bacterial infection This study would provide the preliminary scientific evidence for ethno-botanical and traditional use of this Churna for prevention of enteric bacterial infections. The developed thin layer Chromatography method for estimation of Piper longum, p.nigrum & *Zingiber officianalis* From Trikatu Churna could be used as a valuable Analytical tool in the routine analysis, to check the Batch to batch variation. Ayurvedic medicine Tikatu Churna has been standardized by intervention of modern scientific quality control measures in the traditional formulation described in classical texts. Pharmacognostic characters established for the raw materials could be employed as quality control standards for evaluating its identity and can be used for routine analysis. Purity and potency of the materials and formulations following the procedure given could be performed in quality control and assurance of pharmaceuticals.

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