ISSN: 2455-281X

Contents lists available at http://www.albertscience.com

ASIO Journal of Pharmaceutical & Herbal Medicines Research
(ASIO-JPHMR)

Volume 3, Issue 2; 2017; 01-07

TRIKATU CHURNA: COMPARATIVE STUDY OF POLYHERBAL AYURVEDIC FORMULATIONS

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ABSTRACT

India is a land mark for the traditional system of medicine from the past few centuries. 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. Marketed and laboratory prepared Trikatuchurna were subjected to phytochemical screening, physical characteristics, physicochemical properties and HPLC. The standardized herbal formulation is essential in order to assess the quality, purity, safety & efficacy of the drug. The laboratory prepared trikatuchurna were compared with marketed formulations by performing physic-chemical evaluation, phytochemical screening, microscopic characterization, fluorescence analysis and HPLC etc. The various parameters were sufficient to standardize the Trikatuchurna and their findings will be useful 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, Formulation, Phytochemicals, Piperine, HPLC

INTRODUCTION

Herbal formulations have been used by the majority of Indians since ancient times with emerging interest in the world to adopt to study the traditional system different healthcare system. Churna is one such 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* (hippali), fruits of *Piper nigrum* (menasu) , and rhizome of *Zingeber officinale* (shunti) [1].

Trikatuchurna is the digestive, tonic food for body. Trikatuchurna plays an essential role in the treatment of wide variety of conditions it eliminates the aggravated kapha in the respiratory tract and in digestive channel. It is being prescribed for Agnimandra (digestive impatient), Galroga (throat disease), Svasa (asthma), Kusta (skin disease), Pinasa (sinusitis), Kasa (cough) and Slipada (Filariasis). The WHO has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and by applying suitable standards and parameters [2]. Standardization and quality control depends upon the nature of drug and compound drugs, its source i.e. factors associated with raw materials which are beyond of human control like seasonal geographical, age of the plant, time of collection, type of drying etc. due to this natural conditions. The percentage of chemical constituents of the drug does no remain uniform as our expectations [3].

Ayurvedic is concerned with healthy living along with curative measures that synchronize an individually physically, mentally and spiritually. It is getting accepted as a self-care system for individual well being. Focusing primarily acknowledge towards correcting imbalances before they develop into diseases. It is a solution for all those who responsibility for their own health and want a healthy and long life. The need of quality control for ayurvedic drug is due to the ancient method has been reduced due to the commercialization of ayurvedic pharmacy. The development of these traditional systems of medicine with the perspective of safety and quality will help not only to preserve the traditional heritage but also rationalize the use of natural products.

MATERIALS AND METHOD

MATERIAL

Collection of Powder Drug

Trikatuchurna consists of three main ingredients in powder form, it consists of fruits of *Piper longum* (hippali), fruits of *Piper nigrum* (marica) and rhizomes of *Zingiber officinalis* (shunti).

Collection of marketed formulations

The marketed preparations of Trikatuchurna, Divya Pharmacy, Tumkur were purchased from market and named as TC-2.

Trikatuchurna (TC-1) was prepared in departmental laboratory using method described in ayurvedic formulary in equal proportion of each powdered drugs. Each drugs dried and cleaned by hand sorting drugs then crushed using pestle and mortar and then weighed as per the quantity required and mixed geometrically. This mixed formulation was weighed and packed in polythene covers.

DOI Link :: http://doi-ds.org/doilink/10.2016-19146535/ ; DOI Link :: http://doi-ds.org/doilink/10.2017-25963529/

Evaluation of Trikatuchurna

Organoleptic evaluation

Organoleptic evaluations were studied with their appearance, taste, odour, colour etc. The organoleptic characteristics are judged subjectively and or adulterants may closely resemble the material.

Physico-Chemical Investigation

Determination of foreign matters: About one gm of sample was weighed and matter differing in colour and texture were considered as foreign matters. The separated matter was weighed and subtracted from one gram and percentage was calculated.

Determination of moisture content: One gm of powder was weighed and dried at 80 ^{o}C for 24 hrs in air oven. After 24h, the powder was weighed again and the difference in the weight was determined. The percentage of moisture was calculated.

Determination of pH: The 5% (5 grm in 100 ml of water) powder was kept on shaker for 5 h with 140 rpm and filtered. The filtrate was analyzed for the pH using pH meter.(Elico, India) [3].

Determination of water soluble extractive: 5 grms of powder was weighed and added into a 100 ml conical flask. About 25ml of distilled water added into it and kept on a rotator shaker (140 rpm) for 24 hrs. After 24hrs it was filtered and dried in hot air oven set at 80 ^{o}C for 24hr and weighed again, the difference in the weight was determined and water soluble extractive was calculated [4-5].

Determination of alcohol soluble extractive: 5grm of powder was weighed and taken into 100ml of conical flask. 25ml of absolute alcohol was into it and kept on a rotator shaker (140 rpm) for 24 hrs. After 24 hrs it was filtered and dried in hot air oven set at 80 ^{o}C for 24 h and weighed again. The difference in weight was determined and percent of soluble extractive was calculated [4-5].

Determination of total ash content: The clean and dry crucible (Silica) was weighed and 10grm of powder was weighed in crucible and powder was turns into ash. The crucible was cooled and weighed again. The difference in the weighed was noted and percentage of total ash was calculated. [6-7].

Determination of water soluble ash: One gram of ash was weighed and 10ml of distilled water was added into it. The mixture was kept on a shaker with 140 rpm for 8h and filtered through filter paper. The ash remained in the paper was kept in a crucible (silica) and burned to ash again in a muffle furnace for 3-4 h. The weight of ash obtained was noted and percent of water soluble ash was determined [8].

Determination of acid insoluble ash: One gm of ash was weighed and 10ml of concentrated H_2SO_4 was added into it. The mixture was kept on a shaker with 140 rpm for 8h and filtered through filter paper. The ash remained in the paper was kept in a crucible (silica) and burnt ash again in a muffle furnace for 3-4h.The weight of ash obtained was noted and percent of acid insoluble ash was determined [8].

Preliminary phytochemical analysis: Samples of trikatuchurna were subjected to test separately for the presence of various phytoconstituents like saponins, flavonoids, glycosides, steroids, proteins, alkaloids and carbohydrates by standard procedure [9].

Microscopic characters : Slightly heated the powders with chloral hydrate / glycerine and mounted on glass slide and then powders were stained with few drops of mixture of 1:1 phloroglucinol + Conc. HCl after 3 to 4 minutes and observed under compound microscope (10 X and 40 X).

Fluorescence analysis: The powdered samples were exposed to day light and ultraviolet. One milligrams of powdered drug was placed on a microslide and observed under UV (366 nm and 254 nm) and day light to observe the fluorescent characteristics of powder [10].

Thin layer chromatography of trikatuchurna: TLC showed that the extracts of the both samples formulations were carried to ensure the presence of active ingredients in all the preparations. For TLC, 2 gm of each samples (TC–1 and TC–2) were extracted with 25 ml of methanol and chloroform on boiling water for 25 minutes consecutively, then filtered and concentrated spots of extracted samples were done on percolated with silica gel aluminum plate, main piperine with developed mobile phase, it was consists of Butanol : Water : Acetic acid (60:10:10 v/v/v). After drying the plate were examined under UV light and then in iodine chamber.

HPLC Analysis : The alcohol and chloroform extracts of solvent used for HPLC analysis of important drug piperine in the extracts was performed using Shimadzu (Japan) equipped with detector UV – SPD 10 A detection at 344 nm on C-18 column (Phenomenex, USA) 5 mm, 250 X 4.6 mm i.d at 27 ^{o}C was used for analysis. Tolune : Ethyl acetate : Glacial acetic acid (8:2:0.1) was used as mobile phase in isocratic mode at a flow rate of 1ml /min and the injection volume was 10-15 ml.

RESULTS AND DISCUSSION

Herbal medicine are not a simple task since many factor influence the biological efficacy and reproducible therapeutic effect, pharmacological properties of any herbal formulation depend on phytochemical composition [11]. The laboratory prepared Trikatu churna done by the method mentioned in ayurvedic formulary and marketed Trikatu churna formulation were set for standardization or quality control parameters. The standardized trikatuchurna compared with marketed formulations. In this preparation the herbal formulations involves the safe, proper selection and handling of crude materials. Most of the traditional systems of medicine are effective because they lack of standardization [12] and same way comparative standardization of a polyherbal ayurvedic formulations of trikatuchurna is essential in order to assess the quality, purity, safety and efficacy of drug [13]. Therefore, the methods of quality controlled parameters for both samples were discussed.

Method of preparation of Trikatu churna in laboratory: All the ingredients were mixed in equal proportion and the composition is as followed in Table 1.

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Table 1: Composition of Trikatu churna

Sanskrit Name	Ingredients (botanical identify)	Quantity (gm)
Maricha	Fruit of *Piper nigrum* L.	20
Hippali	Fruit of *Piper longum* L.	15
Shunti	Rhizome of *Zingeber officinale* R.	15

Table 2: Organoleptic evaluation

Sl.No.	Organoleptic character	In laboratory preparation	Marketed preparation
1	Appearance	Powder	Powder
2	Colour	Brown	Brown
3	Taste	Bitter	Bitter
4	Odour	Pleasant	Pleasant

Organoleptic evaluation: The trikatuchurna formulations were studied for organoleptic characteristics showed in Table 2.

Phytochemical investigations: The preliminary phytochemical observations of crude extracts shown the occurrence of alkaloids, fiavonoids, tannins, steroids carbohydrates (Table 3) which indicates, Trikatuchurna is a mixture of all the constituents and interactions all these chemical might result in enhanced therapeutic efficacy of sinusitis, asthama, rhinitis, tonsillitis and digestive [12].

Table 3: Phytochemical investigations of TC – 1 and TC -2

Sl. No.	Tests	TC-1		TC-2	
		Methanolic extract	**Chloroformic extract**	**Methanolic extract**	**Chloroformic extract**
1	Carbohydrates	+	+	+	+
2	Glycosides	+	+	+	+
3	Polysaccharides	-	-	-	-
4	Test for proteins Free amino acids	+	-	-	-
	Bradford test	+	+	+	+
5	Test for alkaloids				
	Dragendroff's test	+	+	+	+
	Mayer's test	+	+	+	+
6	Tests for steroids				
	Libermann Burchard Test	-	+	-	-
	Salkowski's test	-	-	-	+
	Triterpenoids	+	+	+	+
7	Tests for Flavonoids Test 1	-	-	-	-
	Shinoda test	+	+	-	-
	With Sodium Hydroxide	+	+	+	+
8	Tests for Tannins				
	Fecl3 test	-	-	-	-
	Dilute HNO3 test	-	+	+	+
9	Test for lipid	-	+	+	+
10	Tests for oils	+	+	+	+
11	Tests for Saponins	+	-	+	+

Note: TC-1=Trikatu churna 1; TC-2=Trikatu churna 2; + = Present; - = Absent

Physico-chemical investigation: Trikatuchurna were studied for their physio–chemical parameters mentioned in Table 4.

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Table 4: Physioco–chemical investigation

Sl. No.	Parameters	TC-1	TC-2
1	Foreign matter	1.2%	1.2%
2	Moisture content	34.8%	32.40%
3	pH	6.66	7.02
4	Determination of Water soluble extractive	21.2%	17.4%
5	Alcohol soluble extractive	17.8%	28.8%
6	Determination of total ash content	18.0%	9.0%
7	Determination of water soluble ash	11.8%	24.0%
8	Determination of acid insoluble ash content	69%	69%

Microscopic characteristics: All the powdered samples were studied for powdered characters as shown in Plate 1 and Table 5.

Table 5: Fluorescence Analysis

Formulations	TC-1		TC-2	
Wavelength	Day light	UV light	Day light	UV light
Powder + H_2O	Creemish light	White	Dark creemish	Creemish
Powder + HCl	Yellow	Light green	Golden yellow	Light green
Powder + HNO_3	Light brown	Brown	Reddish brown	Brown
Powder + $FeCl_3$	Dark brown	Light brown	Brown	Light brown
Powder + NaOH	Pale yellow	Light green	Light creemish	Dark Creemish
Powder + H_2SO_4	Blakish brown	Black	Blakish brown	Black
Powder +NH_4Cl	Creemish	Yellowish green	Light green	Creemish white
Powder+Glacial acetic acid	Yellow	Light green	Light yellow	Light yellow

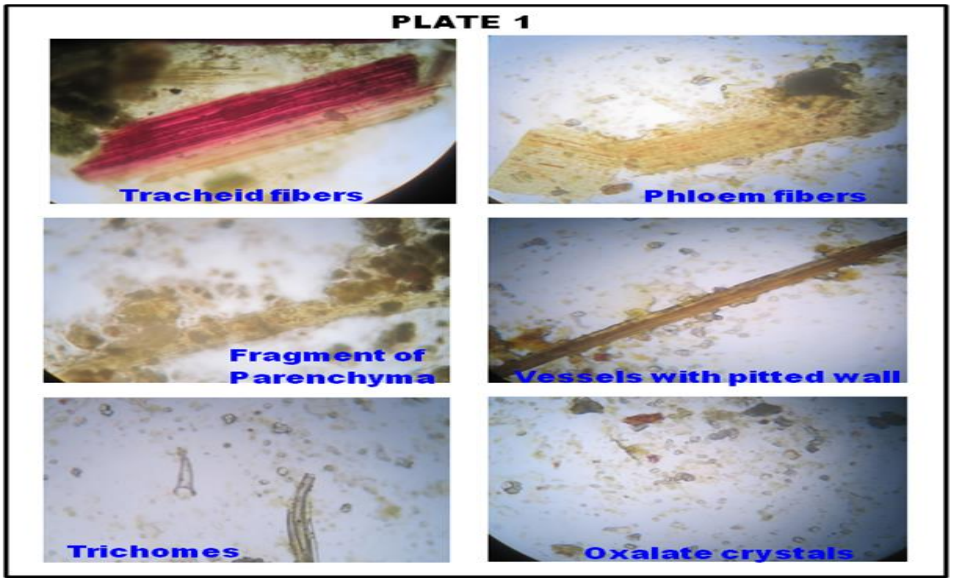

Figure 1: Study of microscopic characters

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Thin layer chromatography of Trikatu churna:
The standardized protocol for the formulations of Trikatu churna, ayurvedic preparation prescribed for a wide range of disorders of an age old formulations with references on its quality control and standardization. The drug piperine content of TC was determined using HPLC [14]. Hence, TLC was performed for samples to check the pattern of phytoconstituents results are as follows.

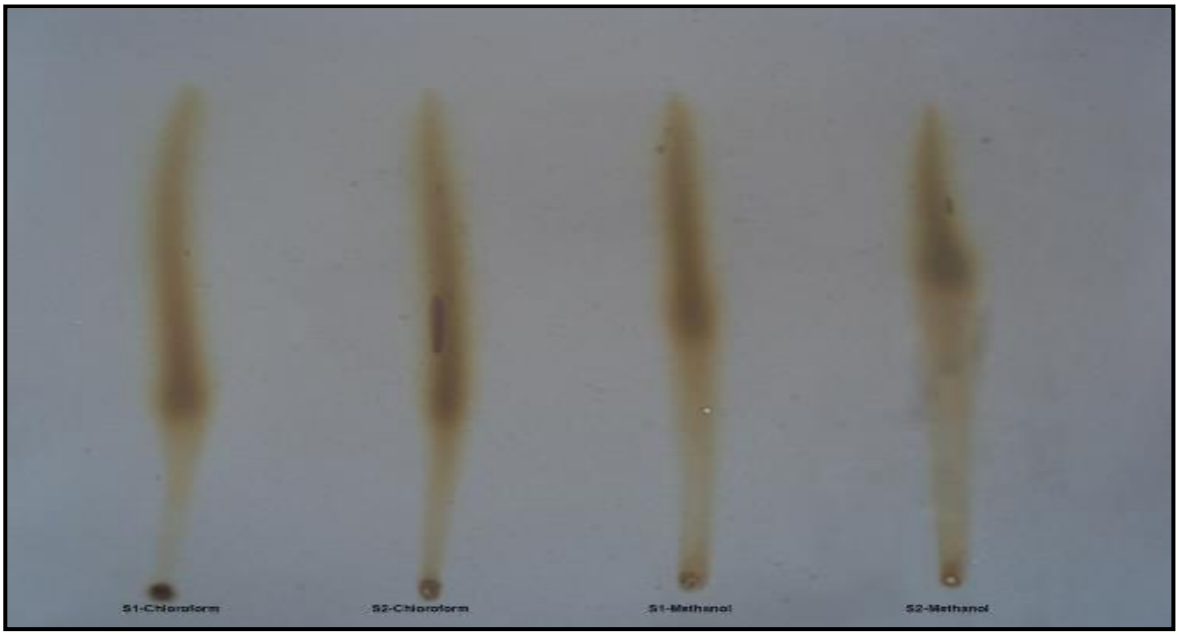

Figure 2A: TLC plates showing the bands of phytoconstituents in TC-1

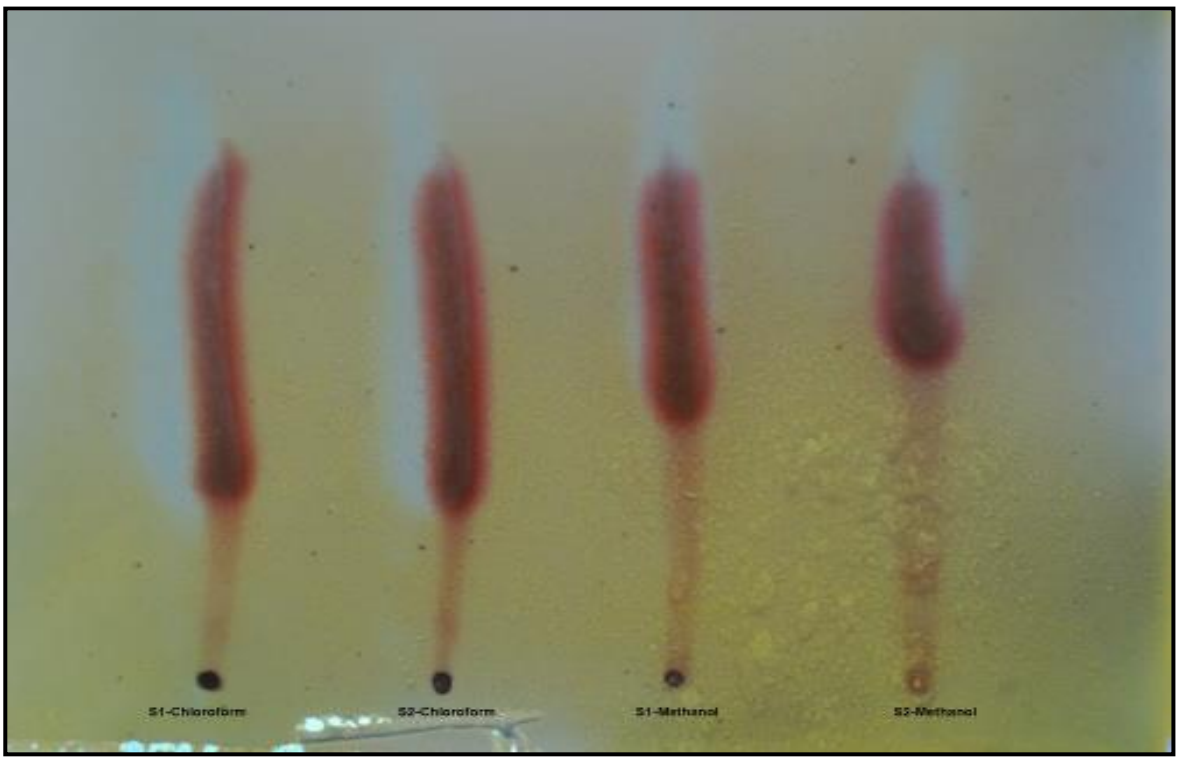

Figure 2B: TLC plates showing the bands of phytoconstituents in TC-2

HPLC analysis of Trikatu churna:
The HPLC method was validated by defining the linearity, peak purity, limit of quantification and detection, precision, accuracy, specificity and robustness. For the qualitative purposes, the method was evaluated by taking into account the precision in the retention time, peak purity, and selectivity of Piperine. Therefore, Trikatu churna is important group of formulated by ayurvedic and siddha physicians to treat various types of diseases. Trikatuchurna were estimated for their piperine content against standard piperene solution by HPLC at λ_{max} 342-343nm with mean value 99.35±0.25% of piperine [15].

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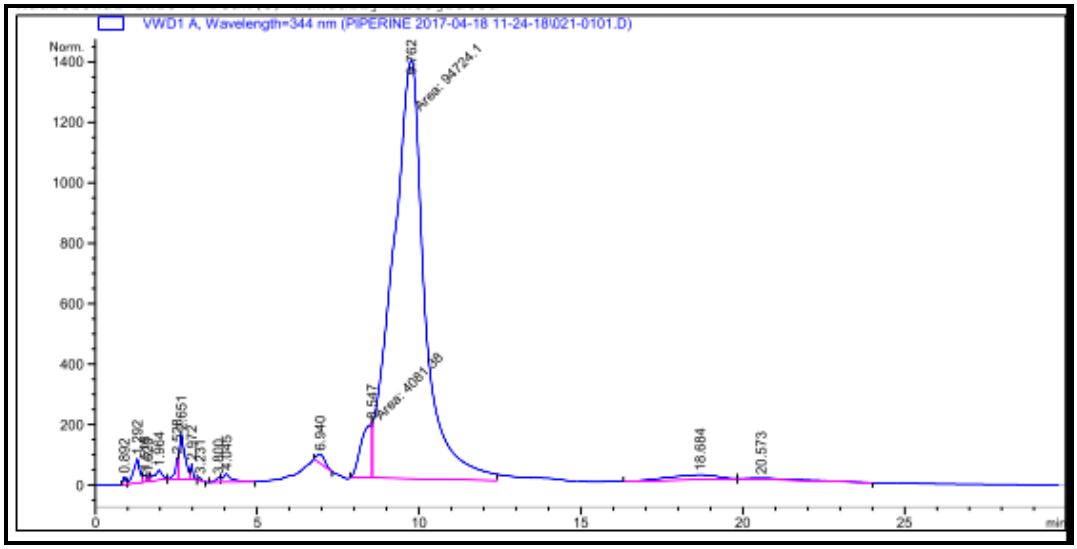

Figure 3: HPLC Analysis: Chromatogram of chloroform extracted sample showing content of Piperine (TC-1)

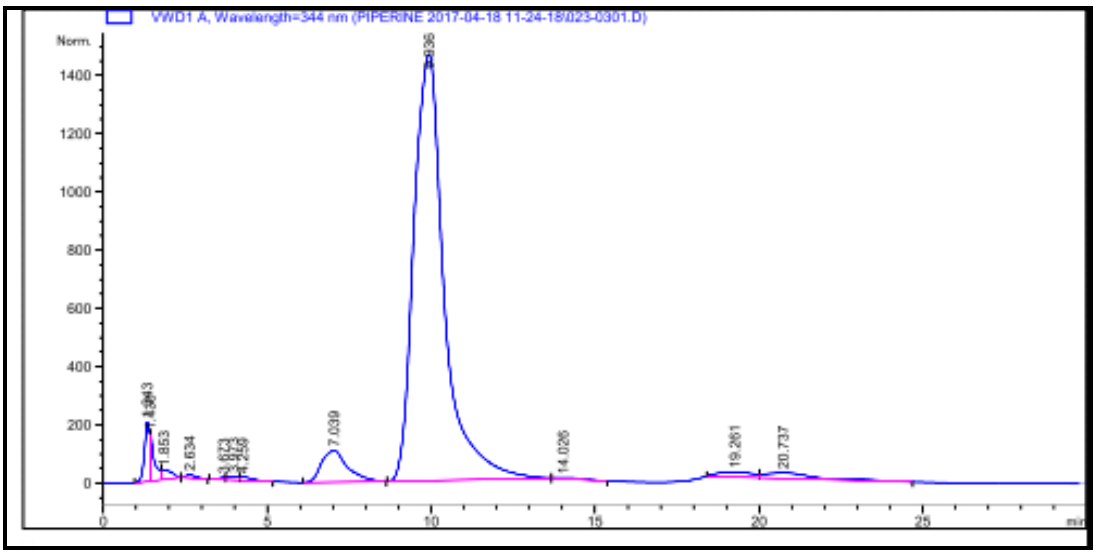

Figure 4: HPLC Analysis: Chromatogram of Methanol extracted sample showing content of Piperine (TC-1)

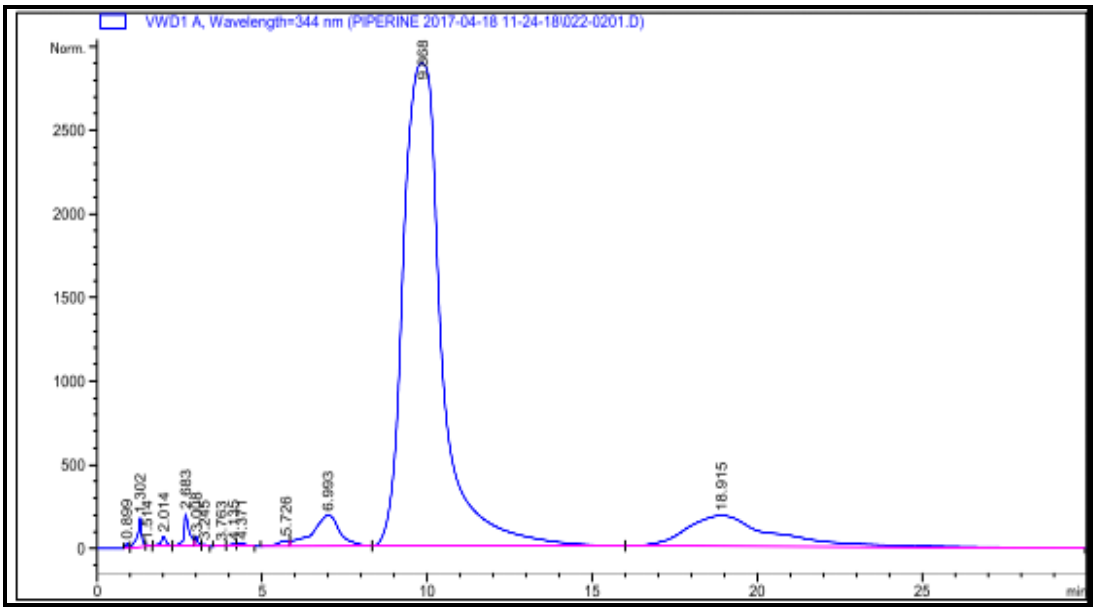

Figure 5: HPLC Analysis: Chromatogram of Chloroform extracted sample showing content of Piperine (TC-2)

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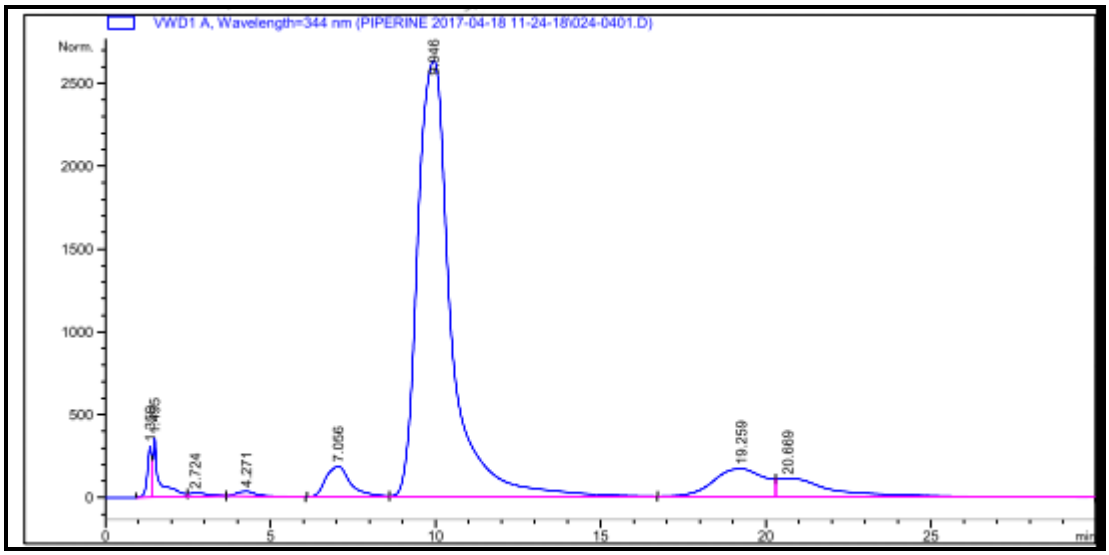

Figure 6: HPLC Analysis: Chromatogram of Methanol extracted sample showing content of Piperine (TC-2)

CONCLUSION

This evaluation study was carried out to determine quality, purity, integrity of Trikatuchurna with due aid of comparative analysis of laboratory and marketed product. Trikatuchurna was found to possess higher amount of phytoconstituents. This study may 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 Trikatuchurna could be used as a valuable analytical tool in the routine analysis to check the variation among them. These can be standardization by investigation of modern scientific quality control measure in the traditional formulations.

ACKNOWLEDGEMENT

Authors acknowledge the Co-ordinator, Department of Botany, Davangere University, Davangere for the facilities extended.

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DOI Link :: http://doi-ds.org/doilink/10.2016-19146535/ ; DOI Link :: http://doi-ds.org/doilink/10.2017-25963529/