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Phytophysico-Chemical Profile of Polyherbal Ayurvedic Formulation Trikatu Churna

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ABSTRACT
This article presents a phytochemical analysis of Trikatu Churna. Pippali (Piper longum Linn. (Fruit), Maricha (Piper nigrum Linn. (Fruit) and Sunthi (Zingiber officinale Rosc. (Rhizome) in equal proportions constitute. The first and most important stage in standardizing herbal composition is to authenticate herbs through anatomical research. Pharmacognostic research such as macroscopic, microscopic, and chemical research such as preliminary phytochemical, physico-chemical constants, and TLC/HPTLC fingerprint of Trikatu Churna were investigated in this work. Evaluation of physico-chemical and phytochemical analysis of Trikatu Churna. The current investigations deal with extraction and detection or screening of active phytochemical compounds from different extract of Trikatu Churna. Pharmacogenetic studies, physio-chemical studies carried out. The formulation's macro-microscopic, preliminary phytochemical, and TLC/HPTLC investigations have all been documented. Findings of the study is beneficial in standardization of polyherbal Ayurvedic formulation Trikatu Churna, which will increase global acceptability of the formulation and status of the Ayurveda system.
Keywords: Trikatu Churna, Pippali, Maricha, Shunti, Piper longum Linn, Piper nigrum Linn, Zingiber officinale Rosc, Phyto-chemical

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INTRODUCTION

Trikatu, an Ayurvedic polyherbal formulation is used extensively as a single compound drug and also used as an ingredient in many other formulations. It is also being used increasingly as dietary supplements to fight or prevent common disease. The dried fruits of *Piper nigrum* L. (Piperaceae), *Piper longum* L. (Piperaceae) and rhizome of *Zingiber officinale* Roscoe (Zingiberaceae) were powdered and mixed together in equal proportions to get a polyherbal formulation, Trikatu Churna. All these plant materials are used world-wide as spices. They are also used as an ingredient in folklore medicine in many Asian countries. Trikatu Churna, an Ayurvedic polyherbal formulation. Trikatu Churna is used to treat Agnimandya (digestive impairment), Gala Roga (throat diseases), Shwasa (dyspnea), Kushtha (skin diseases), Pinasa (sinusitis), Kasa (cough), and Slipada (filariasis) in Ayurveda [1]. The ingredients for Churna are collected, dried, pulverized separately, and put through sieve number 80/85 to prepare a fine powder Polyherbal formulations in powdered form with no more than 10 botanical constituents may be identified microscopically, thus the Trikatu Churna can also be analyzed [2]. The use of Trikatu has been increased important extensively for its health benefits as it contains flavonoids, sterols and triterpenoids, glycosides, saponins, flavonoids, tannins and proteins[3]. Evaluation of physico-chemical and phytochemical analysis of Trikatu Churna.

MATERIAL AND METHODS

Authentication of drug

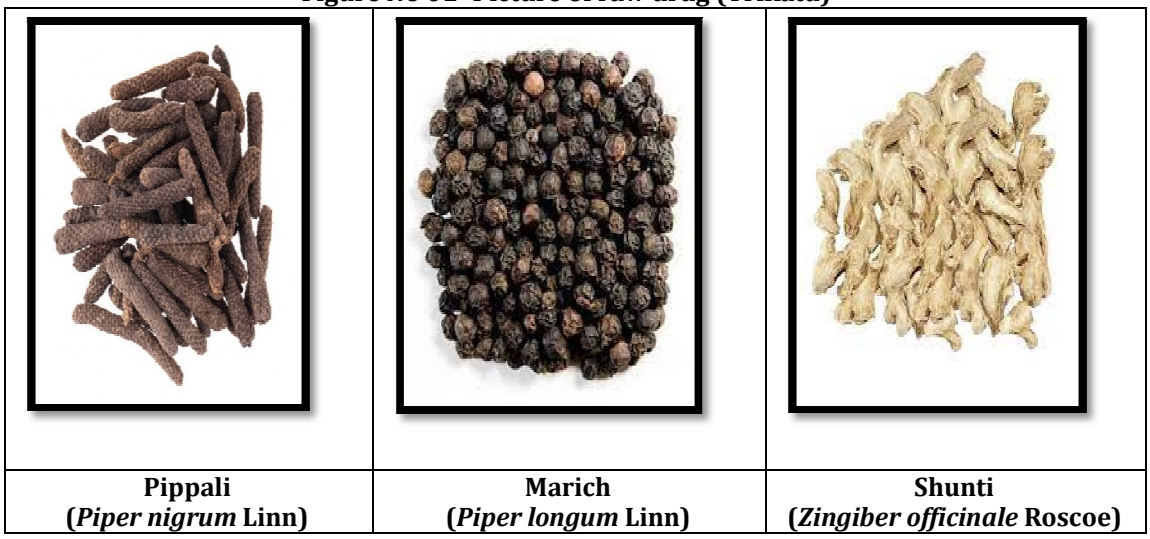
Trikatu- Standard procedures were used to collect dried fruits of *Piper nigrum* L. (Piperaceae), *Piper longum* L. (Piperaceae), and rhizome of *Zingiber officinale* Roscoe (Zingiberaceae) from Sri Dharmasthala Manjunatheshwara College of Ayurveda and hospital, Hassan.

The identity of the plant was confirmed by Department of Dravya Guna Vijnana, Sri Dharmasthala Manjunatheshwara College of Ayurveda and hospital, Hassan. No-SDMCAH-DG/2020/07, Date-18/07/2020, SDM center of research in Ayurveda and allied sciences, Kuthpady, Udupi and Biocyte institute of Research and development, Sangli, Maharashtra (Reg.No.-1831300312031566), date 05/09/2019. The microscopic properties of this plant were examined and compared to existing literature for further validation.

Table No 01- Ingredients of Trikatu Churna

Ayurvedicname	Botanicalname	Partused	Quantity
Pippali	*Piper longum* Linn.	Fruit	1 part
Marica	*Piper nigrum*Linn	Fruit	1part
Shunthi	*Zingiber officinale* Rosc.	Rhizome	1part

Figure No 01- Picture of raw drug (Trikatu)



Pippali (*Piper nigrum* Linn)	**Marich (*Piper longum* Linn)**	**Shunti (*Zingiber officinale* Roscoe)**

Foreign matter

The term "foreign matter" refers to the undesirable physical particles in a drug sample that aren't required for the investigation. Soil, stones, gravel and animal contaminates, to name a few. Before weighing and utilizing the genuine drug, these are removed. The calculation is carried out in percentages.[4]

Pharmacognostic studies

Macroscopic study- The macroscopic observation of the dried fruits of Pippali, Maricha and rhizome of Shunti was done to assess the shape, size, surface characteristics, texture, colour, consistency, odour and taste.[5]

Microscopic study-Microscopic analysis of the sample is done for both powders of individual ingredients and also the formulation. The sample is cleared in the solution of chloral hydrate and analysis is done microscopically. A few milligrams of powder treated with iodine in potassium iodide solution and mounted in glycerine for observation of starch. A few milligrams of powder treated with solution of phloroglucinol, allowed to dry, added a few drops of hydrochloric acid and mounted in glycerine to observe lignified tissues.[6]

Physico-chemical analysis- Quantitative analysis for total ash, acid insoluble ash, water and alcohol soluble extractive values and loss on drying at 105^0c were carried out in triplicate for the polyherbal Ayurvedic formulation Trikatu Churna according to the method recommended in Quality Control Methods for Medicinal Plant Materials by WHO, 1998. Preliminary phytochemical analysis was carried out. [7]

Loss on drying at 105°C- 10g of sample was placed in tarred evaporating dish. It was weighed after drying for 5 hours at 105°C in a hot air oven. The drying was continued until difference between two successive weights was not more than 0.01 after cooling in desiccator. Percentage of moisture was calculated with reference to weight of the sample. [8,9,10,11]

Total Ash- 2 gram of material was burned until carbon free ash was obtained in a tared platinum crucible at a temperature of not more than 450°C. Percentage of ash was calculated with reference to weight of the sample.[12]

Acid insoluble Ash- To the crucible containing total ash, add 25ml of dilute HCl and boil. Collect the insoluble matter on ashless filter paper and wash with hot water until the filtrate is neutral. Transfer the insoluble matter-filled filter paper to the original crucible, dry on a hot plate, and ignite to a consistent weight. Before weighing, let 30 minutes for the residue to cool in a suitable desiccator. Calculate the content of acid insoluble ash with reference to the air-dried drug. [12]

Alcohol soluble extractive- Weigh accurately 4g of the sample in a glass stoppered flask. 100 milliliters of distilled alcohol (approximately 95 percent). Shake occasionally for 6 hours. Allow to stand for 18 hours. Filter as quickly as possible, being careful not to lose any solvent. Pipette 25 mL of the filtrate into a 100 mL beaker that has been pre-weighed. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours, cool in desiccator for 30 minutes and weigh. Calculate the sample's proportion of Alcohol extractable materials. Repeat the experiment twice, and take the average value.[12]

Water soluble extractive- Weigh accurately 4g of the sample in a glass stoppered flask. Add 100 ml of distilled water and give it a good shaking for 6 hours. Allow for 18 hours of standing time. Filter as quickly as possible, being careful not to lose any solvent. Pipette 25 mL of the filtrate into a 100 mL beaker that has been pre-weighed. On a water bath, evaporate to dryness. Preheat the oven to 105°C and bake it for 6 hours. Cool in a desiccators and weigh. Repeat the experiment twice. Take the average value. [12].

RESULT AND DISCUSSION

Physicochemical studies

Physico-chemical analysis shows 10.87 % of moisture content. Ash content of the drug was 4.233 % and 0.13 % of acid in-soluble ash shows the siliceous matter in the plant. Alcohol soluble extractives 7.17 % represent the extraction of polar constituents like phenols, tannins, glycosides, alkaloids and flavonoids. The water soluble extractive 11.38 % denotes the presence of inorganic contents. (Table No 2)

Table No 2. Results of standardization parameters of *Trikatu Churna*

Parameter	Results n = 3 %w/w
	Trikatu churna
Loss on drying	10.87 ± 0.00
Total Ash	4.33 ± 0.08
Acid Insoluble Ash	0.13 ± 0.02
Water soluble Ash	3.00 ± 0.02
Alcohol soluble extractive value	7.17 ± 0.10
Water soluble extractive value	17.90 ± 0.03

Preliminary Phytochemical evaluation was done as per the standard methods.

Pharmacognostic studies- The pharmacognostic study is the most important and reliable criterion for plant drug identification. The pharmacognostic characteristics are required for confirming the identity of the crude medicine as well as determining its quality and purity. A thorough and comprehensive pharmacognostic evaluation would provide useful data for future research.

Macroscopy- Trikatu Churna is a fine yellowish green powder with a strong pungent odor and a numbing sensation on the tongue.

Microscopy- In various mounts, the following properties were seen under a microscope: Pippali — fragments of thick-walled, lignified stone cells with a large lumen in various shapes and sizes, as well as a few fragments of pointed multicellular trichome, a few fragments of perisperm embedded with aleurone grains and oil globule, a few fragments of perisperm embedded with aleurone grains and oil globule, a few yellowish–brown content cells, numerous simple, oval to rounded, starch grains measuring up to 8µ in diameter; Maricha –fragments of thick walled, lignified, in different shapes and sizes of stone cells with wide lumen, a few fragments of perisperm embedded with aleurone grains and oil globule, a few yellowish–brown content cells, numerous simple, oval to rounded starch grains measuring upto 40 µ in diameter; Sunthi-a few septate fibres, a few fragments of rectangular, thin walled, cork cells in sectional view, a few fragments of lignified vessels with spiral thickenings, a few fragments of thin walled

parenchyma with starch grains, a few yellowish–brown content cells, numerous simple, oval to rounded starch grains measuring upto 60µ in diameter.

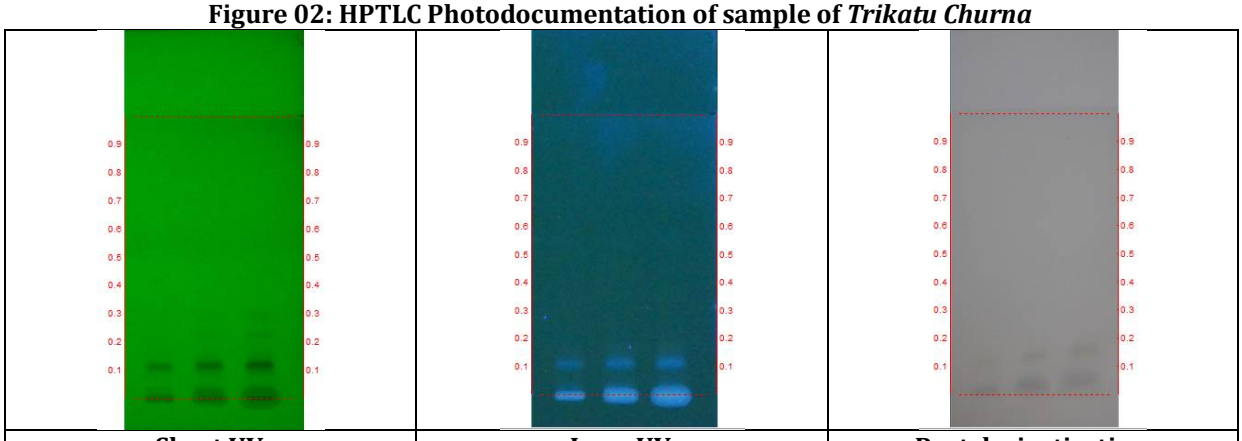
Preliminary phyto-chemical test of the aqueous alcoholic extract of Trikatu Churna- Shows presence of alkaloid, Sterols and Triterpenoids, flavonoids, saponins, tannin, proteins and the absence of Carbohydrate. (Table no 03)

Table No 03- Chemical constituents of Trikatu Churna

Chemical Constituents	Name of Test	Observed Changes	Result
Alkaloids	Mayer's Reagent	White colored turbidity	Present
	Wagner's Reagent	Reddish Brown Precipitate	Present
	Hager's Reagent	Yellow Precipitate floating	Present
	Ehrlich's Reagent	Two separate yellow and brown colored layers	Present
Sterols and Triterpenoids	Salkowaski test	Lower layer turns red	Present
Glycosides	Keller killani test	Separation between two layers, lower layer shows reddish brown and upper layer turns bluish green in colour	Present
Saponins	Foam test	Formation of foam	Present
Carbohydrates	Benedict's test	Reddish brown precipitate	Absent
Flavonoids	Alkaline reagent test	Yellow colour which becomes colorless on addition of few drops of dilute acid	Present
	Lead acetate solution test	Yellow precipitate	Present
Tannins	Ferric-chloride test	Dark colour	Present
Proteins	Biuret test	Blue colour	Present

HPTLC Photo-documentation of sample of *Trikatu Churna*- Among the various solvent systems tested, the mixture containing toluene: ethyl acetate (5:1.5) gives the best resolution. In UV 254, 366 nm, visible light and Derivatization with Dragendorff's reagent *Piper longum*, *Piper nigrum*, Trikatu Churna and *Zingiber officinale* aqueous alcoholic extracts were shown Figure 02.

Figure 02: HPTLC Photodocumentation of sample of *Trikatu Churna*



Short UV | **Long UV** | **Post derivatisation**

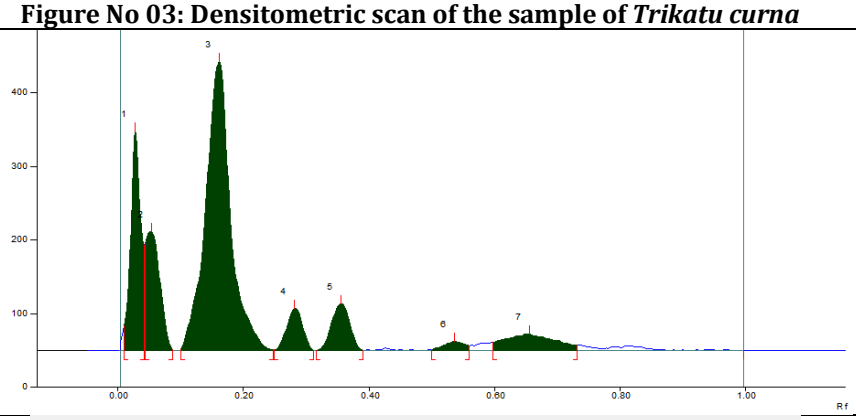
Track 1: ***Trikatu curna***- 3µl Track 2: ***Trikatu curna***- 6µl Track 3: ***Trikatu curna***- 9µl
Solvent system: Toluene: Ethyl aetate (7:1)

Table No 04: Rf values of sample of *Trikatu curna*

Short UV	Long UV	Post derivatisation
0.13 (D. green)	0.12 (F. blue)	0.12 (Purple)
0.23 (L. green)	-	-
0.30 (L. green)	-	-

***D-dark; L-light; F - fluorescent**

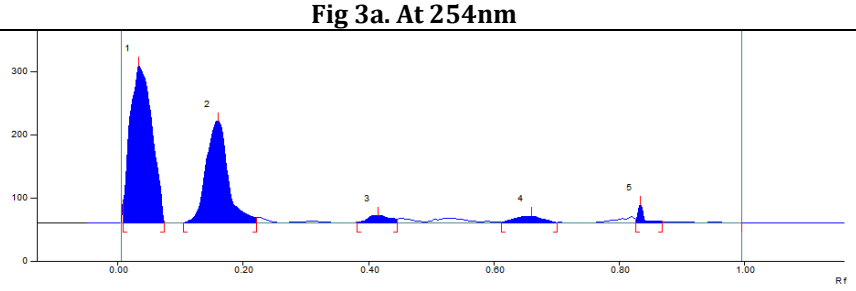
Figure No 03: Densitometric scan of the sample of *Trikatu curna*



Track 7, ID: Trikatu churna

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	33.8 AU	0.03 Rf	298.0 AU	29.62 %	0.04 Rf	42.3 AU	3521.5 AU	16.47 %
2	0.04 Rf	144.5 AU	0.05 Rf	161.4 AU	16.04 %	0.09 Rf	0.7 AU	2664.2 AU	12.46 %
3	0.10 Rf	1.2 AU	0.16 Rf	391.5 AU	38.91 %	0.25 Rf	1.2 AU	11208.4 AU	52.44 %
4	0.25 Rf	1.3 AU	0.28 Rf	57.2 AU	5.68 %	0.31 Rf	0.2 AU	1052.6 AU	4.92 %
5	0.32 Rf	0.2 AU	0.36 Rf	63.3 AU	6.29 %	0.39 Rf	0.4 AU	1328.0 AU	6.21 %
6	0.50 Rf	1.6 AU	0.54 Rf	12.6 AU	1.25 %	0.56 Rf	6.6 AU	298.1 AU	1.39 %
7	0.60 Rf	11.0 AU	0.66 Rf	22.2 AU	2.20 %	0.73 Rf	7.0 AU	1302.0 AU	6.09 %

Fig 3a. At 254nm



Track 7, ID: Trikatu churna

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	34.9 AU	0.03 Rf	248.5 AU	53.85 %	0.07 Rf	0.9 AU	6193.1 AU	55.98 %
2	0.10 Rf	0.2 AU	0.16 Rf	160.6 AU	34.81 %	0.22 Rf	8.1 AU	4004.6 AU	36.20 %
3	0.38 Rf	1.1 AU	0.42 Rf	12.3 AU	2.66 %	0.45 Rf	6.3 AU	310.4 AU	2.81 %
4	0.61 Rf	1.2 AU	0.66 Rf	10.9 AU	2.36 %	0.70 Rf	0.6 AU	351.5 AU	3.18 %
5	0.83 Rf	6.1 AU	0.84 Rf	29.2 AU	6.33 %	0.87 Rf	2.2 AU	202.9 AU	1.83 %

Fig 3b. At 366nm

Herbal drugs pharmacognostic characteristics are significant because each plant has its own macro-microscopic features. The first and most important stage in authenticating the botanical source should be a macroscopic and microscopic examination of the herbs. The TLC/HPTLC profile of aqueous alcohol extracts is a great way to keep track of the drug's identity, purity, and standardization.

CONCLUSION

The present study was carried out with an aim of authenticity of the drug along with Physico-chemical and phytochemical analysis of Trikatu Churna. The results obtained will help in standardization of Ayurvedic polyherbal formulation Trikatu Churna. The phytochemical investigation revealed the presence of various phytochemical constituents such as alkaloid, Sterols and Triterpenoids, flavonoids, saponins, tannin and proteins. The results obtained will help in standardization of Ayurvedic polyherbal formulation Trikatu Churna.

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