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A comprehensive pharmacognostic study of Trikatu Churna

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Abstract
Trikatu Churna, a classical *Ayurvedic* formulation, comprising *Piper nigrum Linn.* (Black pepper), *Piper longum Linn.* (Long Pepper), and *Zingiber officinale Roscoe.* (Ginger), is extensively utilised for its therapeutic properties, including digestive enhancement and respiratory support. This study aims to evaluate the Pharmacognostical and physicochemical properties of *Trikatu Churna* to ensure its quality, purity, and efficacy. Standardized methods were utilized for the evaluation. Pharmacognostical assessment involved macroscopic and microscopic evaluation of individual ingredients, while physicochemical studies focused on parameters such as loss on drying, pH, ash values, and extractive values. Thin-layer chromatography (TLC) was employed for qualitative analysis. The results obtained revealed the distinct organoleptic characteristics and microscopic features that confirm the authenticity of the components. Physicochemical parameters fell within acceptable limits prescribed by pharmacopeial standards. TLC profiling demonstrated the presence of active constituents, ensuring the formulation's consistency and quality. These findings validate the authenticity and standardization of *Trikatu Churna*, highlighting its potential for therapeutic applications and further research.

Keywords: Pharmacognostical, physicochemical, phytochemical, *trikatu churna*, *pippali*, *maricha*, *shunti*, TLC.

Introduction
Trikatu Churna is a combination of three pungent ingredients: *Piper nigrum Linn.*, *Piper longum Linn.*, and *Zingiber officinale Roscoe* has been an essential formulation in *Ayurvedic* medicine, renowned for its benefits in digestion, metabolism, and respiratory health. The combination of these ingredients is believed to enhance bioavailability and therapeutic efficacy. Despite its widespread use, ensuring the quality and standardization of herbal formulations remains a challenge due to variability in raw materials and preparation methods. Pharmacognostical and physicochemical studies play a crucial role in the characterization and quality assessment of herbal products. This study aims to establish comprehensive data on the Pharmacognostical features and physicochemical properties of *Trikatu Churna*, thereby supporting its therapeutic efficacy and safety.

Materials and Methods
Materials: The ingredients used in *Trikatu Churna*, including *Piper nigrum Linn.*, *Piper longum Linn.*, and *Zingiber officinale Roscoe.*, were procured from a certified Botanist and collected from Paaderu, Andhra Pradesh, from their natural habitat after proper identification.

Preparation of the sample (*Trikatu churna*)
The mixture was prepared in equal proportions, following traditional *Ayurvedic* methods. The ingredients were cleaned, dried, and powdered, and the powder was sieved through a 100-mesh sieve to ensure uniform particle size. The powders were mixed in equal proportions (1:1:1) to prepare *Trikatu Churna*.

Pharmacognostical studies
1. The Pharmacognostical study includes
2. Plant Identification
3. Drug Collection

4. Organoleptic study
5. Physicochemical study
6. Phytochemical study
7. TLC

Plant Identification: The correct identity of the species and its morphological characters were authenticated by comparing them with the characters mentioned in various *Ayurvedic* texts and the textbook of Botany. Later, the subject expert of the *Dravyaguna* Department, Dr.BRKR Govt. Ayurvedic College, Hyderabad and the botanist confirmed the identity.

Drug Collection: For this study, *Trikatu* { *Shunti* (Rhizome), *Maricha* (Fruit), *Pippali* (Fruit)} were collected from Paaderu Andhra Pradesh from their natural habitat after proper identification. After collection, the good quality material which is free from any worm infestation was cut, separated, washed, dried in the shade and stored in an airtight dried container. Churna is prepared from classical references of sufficient quantity and packed in a zip lock polythene bag and labelled. The fine powder is then used for analytical study.

Organoleptic Study: The external morphology of the whole plant is studied in terms of colour, texture, odour, and taste using sense organs and a magnifying lens where necessary.

Physicochemical Study: It includes foreign matter, moisture content, total ash, acid-insoluble ash, water-soluble extract etc.

Determination of Foreign Matter
Weigh 100-500gms of the drug sample to be examined and spread out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or by the use of the lens. Separate and weigh it and calculate the percentage.

Determination of Moisture content (Loss on Drying)
Materials: Petri plates, Physical balance, Desiccators or Oven.
Procedure: 2 g of the sample is taken in a previously weighed Petri plate. The plates are dried in an oven at 110C for 3 hours. After that, they are removed, and the weight is noted. This procedure is repeated 4 to 5 times until a constant weight is reached. When the two consecutive weights after drying for 30 minutes in a desiccator show a difference of not more than 0.01gm, the sample reaches a constant weight.

Result: Total Moisture content = Initial weight – Final weight. Where initial weight is the weight of the sample before drying and the final weight is the weight of the sample after drying.

Determination of Ash values: The ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in a residue consisting of an inorganic material (metallic salts and silica). This value varies with wide limits and is, therefore, an important parameter for the evaluation of crude drugs. In certain drugs, the percentage variation of the weight of ash from sample to sample is very small and any marked difference indicates a change in quality. Unwanted parts of the drugs, sometimes possess a character that will raise the ash value. The total ash usually consists of carbonates, phosphates, silicates and silica which is the residue of the adhering material to that plant surface (sand and soil).

Materials: Silica dish incinerator, Whatman filter paper.
Method: To avoid any moisture content, 1 g of powder or sample is taken in a heated silica crucible. This is ignited to 100C-150C in an electric ignition until the fumes appear after charring the drug. Heat until no further fumes emerge from the silica dish. Then, increase the temperature to 450C and then return to zero. Then, the sample is removed from the furnace, cooled in a desiccator to room temperature, and weighed.

Result:

$$\text{Total Ash} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

Determination of Acid Insoluble Ash
Method: The total ash that was obtained was boiled for 5 minutes with 25 ml of diluted hydrochloric acid. Collect the insoluble matter in an ashless filter paper. Washed with hot water and ignited to maintain constant weight.

Result:

$$\%\text{ of Acid Insoluble Ash} = \frac{\text{Difference in Weight}}{\text{Weight of the sample}} \times 100$$

Determination of Alcohol Soluble Extractive
Materials: Volumetric flask, Ethyl alcohol, Filter Paper, Evaporating dish, Water bath, Oven, Desiccator, Physical balance.
Method: 5 grams of powder was taken in a volumetric flask. To it, 100ml of alcohol was added and kept for 24 hours. The solution was filtered the next day and the filtrate was evaporated in a previously weighed evaporating dish in a water bath. Later it was dried in the oven at 110C to remove the traces of alcohol. Constant weights were noted.

Result

$$\%\text{ of Alcohol Soluble Ash} = \frac{\text{Difference in Weight}}{\text{Weight of Sample}} \times 100$$

Determination of Chloroform Soluble Extractive
Materials: Chloroform, Plant material (finely powdered and air-dried), Soxhlet apparatus or simple extraction setup, Desiccator, Analytical balance, conical flask or beaker, Filter paper.
Methods: Accurately weigh around 5 grams of the powdered plant material. Record the exact weight for later calculations. Place the weighed plant material in a Soxhlet extractor or in a conical flask. Add 100ml chloroform to the Soxhlet extractor (or flask), ensuring the plant material is fully immersed. Heat the Soxhlet apparatus to allow the chloroform to continuously circulate and extract soluble compounds from the plant material. Allow the extraction to continue for about 6-8 hours, or until the solvent siphons clear and plant material is thoroughly exhausted. After extraction, filter the chloroform extract through a Whatman filter paper into a clean, pre-weighed beaker. Evaporate the solvent completely using a water bath at low heat to avoid degradation of the compounds. After complete evaporation, place the beaker containing the residue in a desiccator to cool and ensure dryness. Weigh the beaker with the dry extract. Record the weight.

Result

$$\% \text{ of Chloroform-soluble extractive} = \frac{\text{Weight of extract residue}}{\text{Weight of sample}} \times 100$$

Determination of Petroleum Soluble Extractive

Materials: Petroleum ether (boiling range 40-60C, analytical grade), Finely powdered, air-dried plant material, Soxhlet apparatus, Desiccator, analytical balance, Conical flask, Whatman Filter paper, Fume hood or well-ventilated area.

Method: Weigh accurately around 5gms of the powdered plant material. Record the exact weight for calculation purposes. Place the weighed plant material in a Soxhlet extractor. Add about 100ml of petroleum ether to the Soxhlet apparatus and start heating to allow the petroleum ether to circulate through the plant material. This will continuously extract the petroleum ether-soluble constituents. Continue the extraction process for about 6-8 hours or until the solvent siphons clear, indicating that the plant material has been fully extracted. After extraction, filter the petroleum ether's high volatility and flammability. After complete evaporation, place the beaker with the dry extract residue in a desiccator to cool. Weigh the beaker with the dried residue, recording the weight.

Result

$$\% \text{ of Petroleum ether soluble extractive} = \frac{\text{Weight of extract residue}}{\text{Weight of sample}} \times 100$$

Preliminary Phytochemical Analysis: By performing different qualitative tests of a sample, we can get an idea about the type of phytoconstituent present in the sample. Hence different chemical tests of samples are performed by K.L.E's Shri.B.M.Kankanawadi Ayurveda Mahavidyalaya, Central Research Facility, Belagavi, and Karnataka.

Tests for Proteins

Biuret Test: To the aqueous extract taken in the test tube, 4% NaOH and 1% Copper sulphate solution were added, and the formation of violet or pink colour indicates the presence of proteins.

Tests for Tannins: 3ml of aqueous extract is taken in a test tube. If a few drops of 5% Ferric chloride solution are added to it, then a deep blue to black colour is observed, indicating the presence of tannins.

Tests for Steroids

Salkowski Test: To the 2ml of aqueous extract taken in the test tube, 2ml of Chloroform and 2ml of Conc.H2SO4 is added. Shake well. The chloroform layer appears red (upper layer) and the sulphuric acid layer shows greenish-yellow fluorescence.

Tests for Carbohydrates

Molish Test: To one portion of the aqueous extract taken in the test tube, a few drops of alpha naphthol solution in alcohol were added and mixed well, followed by Conc.H2SO4 from the sides. The purple ring at the junction of two liquids indicates the presence of carbohydrates.

Tests for Alkaloids

Dragendroff's Test: The aqueous extract of the drug taken in the test tube is treated with a few drops of dilute HCl and filtered. The filtrate is treated with Dragendroff's reagent (Potassium Bismuth Iodine solution). The formation of Orange brown precipitate indicates the presence of alkaloids.

Wagner's Test: The filtrate is treated with Wagner's reagent (solution of Iodine in Potassium Iodine). The formation of a reddish-brown precipitate indicates the presence of alkaloids.

Tests for Flavonoids: 1ml of aqueous extract is taken in a test tube. To it, 10% lead acetate solution was added. The formation of a yellow precipitate indicates the presence of flavonoids.

Tests for reducing sugars: Take 2ml of aqueous extract in a test tube. To it, 1ml of Fehling's solution A and 1ml of Fehling's solution B are added and kept in a boiling water bath. The formation of yellow/red colour precipitate indicates the presence of reducing sugars.

Test for Monosaccharides

Barfoed's test: Mix an equal volume of Barfoed's reagent and test solution. Heat for 1- 2 min. in a boiling water bath and cool. A red precipitate is observed.

Test for Pentose Sugar: Pentoses are components of certain gums.

Bial's Orcinol test: To boil Bial's reagent add a few drops of test solution. Green or purple colouration appears.

Aniline acetate test: Boil the test solution in the test tube. Hold filter paper soaked in aniline acetate in the vapour. Filter paper turns pink.

Mix equal amounts of the test solution and HCl. Heat this mixture. Add a crystal of phloroglucinol. Red colour appears

Test for Non-Reducing Sugar

Test solution or hydrolysed test solution does not give a response to Fehling's and Benedict's tests. Hydrolyse test solution. Fehling's and Benedict's tests are negative.

Test for Hexose Sugar

Preliminary Phytochemical Screening

Selwinoff's test (for ketohexose like fructose): Heat 3 ml Selwinoff's reagent and 1 ml test solution in a bearing water bath for 1-2 min. the red colour is formed.

Tollen's phloroglucinol test for galactose: Mix 2.5 ml conc. HCl and 4 ml 0.5% phloroglucinol. Add 1-2 ml test solution. Heat the mixture. Yellow to red colour appears.

Cobalt-chloride test: Mix 3 ml test solution with 2 ml cobalt chloride. Boil and cool. Add a few drops of NaOH solution. The solution appears greenish blue (glucose) or purplish (fructose) the upper layer is greenish blue, and the lower layer is purplish (a mixture of glucose and fructose).

Test for Glycosides

The Aqueous extract taken in the Test tube was treated with 1ml of FeCl3 reagent (mixture of 1 volume of 5% FeCl3 solution and 99 volumes of glacial acetic acid) followed by the addition of a few drops of Con H2SO4. The appearance of a greenish-blue colour within a few minutes indicates the presence of Glycosides.

Test for Cardiac Glycosides: Baljet's test: A section shows yellow to orange colour with sodium picrate.

Legal's test: To aqueous or alcoholic extract, add 1 ml pyridine and 1 ml sodium nitroprusside. Pink to red colour appears.

Test for Anthraquinone Glycosides

Borntrager's test for anthraquinone glycosides: To 3 ml extract, add dil. H_2SO4. Boil and filter. To cold filtrate, add equal volume benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammoniacal layer turns pink or red.

Modified Borntrager's test for C-glycosides: To 5 ml extract, add 5 ml 5% FeCl3 and 5 ml dil. HCl. Heat for 5 min in boiling water bath. Cool and add benzene or any organic solvent. Shake well. Separate organic layer, add equal volume dilute ammonia. Ammoniacal layer shows pinkish red colour.

Tests for Saponin Glycosides

Foam test: When the Aqueous drug extract taken in the Test tube is shaken vigorously, persistent foam is formed indicating the presence of Saponin Glycosides.

Test for Amino acids

Ninhydrin Test

The Aqueous extract taken in the Test tube is heated with 5% Ninhydrin solution in boiling water bath for 10 minutes. The development of bluish colour indicates the presence of Amino acids.

Thin later chromatography (TLC)

Thin-layer chromatography is a quantitative technique used to identify different phytochemicals present in a drug and works on the principle of separation. The separation depends on the relative affinity of the compounds towards stationary and mobile phases.

The compounds under the influence of the mobile phase (driven by capillary action) travel over the surface of the stationary phase. During the movement, the compounds with the higher affinity to the stationary phase travel slowly, while the other travel faster. Thus, the separation of components in the mixture is achieved. Once the separation occurs, individual compounds are visualized as spots.

Method: Preparation of Thin Layer Chromatography Plate Slurry was prepared from 75 grams of silica gel, 100cm3 of methanol 50cm3 of chloroform and 25cm3 of water. TLC plate was formed and activated at 110°C using oven for one hour]. TLC Separation Three grams (3g) of each ethanol, hexane, and aqueous extracts was dissolved in their respective solvent, i.e. ethanol, n-hexane and water respectively to form a sample solution. The solvent system was made from hexane and methanol 4:1 (v/v). Capillary tube was used to spot a sample solution on the silica gel TLC plate at 1cm from the edged of the plate and the drop is allowed to dry.

The plate was placed in TLC (Chromo tank) and allows ascend the TLC plate by capillary action. The plate was removed, and the solvent front was marked then allowed to dry. The iodine was used as the visualizing agent to detect the spot. A meter rule was used to measure the distance moved by the solvent and the distance moved by spot, from which the retention factor (Rf-values) of the various spots was calculated.

Rf = Distance moves by spot front/ Distance move by solvent front

Quantification

Sample and standard should be chromatographed on same plate. After development chromatogram is scanned. Camag TLC scanner scan the chromatogram in reflectance or in transmittance mode by absorbance or by flouroscent mode. Scanning speed is selectable upto100mm/s. Spectra recording is fast-36 tracks with upto 100 peak windows can be evaluated.

Calibration of single and multiple levels with linear or non-linear regressions are possible. When target values are to be verified such as stability testing and dissolution profile single level calibration is suitable.

Statistics such as RSD or CI report automatically. Concentration of analyte in the sample is calculated by considering the sample initially taken and dilution factor.

Macroscopic study

The Fruit of *Maricha* and *Pippali*, Rhizome of *Shunti*, are to be taken and inspected closely to study the macroscopic features of the plant with the naked eye.

Macroscopic Evaluation: The morphological features such as the appearance, colour, texture and odour of individual ingredients were documented.

Microscopic Study

Microscopic study was conducted as below.

T.S. of *Pippali* fruit

Materials: Sample (Fruit), Safranin stain, 70% alcohol, water, sharp razor blade, watch glass and compound microscope.

Procedure:

The Fruit of *Pippali* was taken and soaked in 70% alcohol for 24 hours and free hand sections were taken using the sharp razor blade. The sections were taken and cleared with chloral hydrate solution and water. The cleared section is then stained with safranin according to the standard prescribed methods (Johansen D.A1940 and Trease and Evans 1971). After proper staining and mounting, the slide is then studied under the compound microscope to evaluate the underlying microscopic structures of the plant.

T.S. of *Maricha* fruit

Materials: Sample (Fruit), Safranin stain, 70% alcohol, water, sharp razor blade, watch glass and compound microscope.

Procedure:

The Fruit of *Maricha* was taken and soaked in 70% alcohol for 24 hours and free hand sections were taken using the sharp razor blade. The sections were taken and cleared with chloral hydrate solution and water. The cleared section is then stained with safranin according to the standard prescribed methods (Johansen D.A1940 and Trease and Evans 1971). After proper staining and mounting, the slide is then studied under the compound microscope to evaluate the underlying microscopic structures of the plant.

T.S. of *Shunti* rhizome: Materials: Sample (Rhizome), Safranin stain, 70% alcohol, water, sharp razor blade, watch glass, and compound microscope.

Procedure: The Rhizome of *Shunti* was taken and soaked in 70% alcohol for 24 hours and free hand sections were taken using the sharp razor blade. The sections were taken and cleared with chloral hydrate solution and water. The cleared section is then stained with safranin according to the standard

prescribed methods (Johansen D.A1940 and Trease and Evans 1971). After proper staining and mounting, the slide is then studied under the compound microscope to evaluate the underlying microscopic structures of the plant.

Powder microscopic study
To examine characters of the powder, take enough powder in Chloral- hydrate solution on a slide and cover it with a cover slip, warm it over a low flame for a short time, and then the powder microscopic examination is done.

Results and Discussion
Macroscopic study of fruits and rhizome
Macroscopy of *Pippali*
The plant occurs as a Perennial climber with woody stems which are long, slender, cylindrical and pubescent. Leaves are alternate, elliptical and pointed with 5-7 nerves. It has unisexual flowers which are small and are arranged in spike inflorescence. Fruits are small, berry-like, and reddish-purple. It has fibrous and woody roots.

Macroscopy of *Maricha*
The plant occurs as a climbing shrub with woody stems which are thin, cylindrical and pubescent. Leaves are cordate-ovate, pointed and 5-7 nerved. Flowers are small, unisexual and are arranged in spike inflorescence. Fruits are small, berry-like and reddish-purple.

Macroscopy of *Shunti*
The plant occurs as a rhizomatous perennial herb. The rhizome is the underground stem, which is cylindrical, branched, and pale yellow. The Leaves are lanceolate and pointed. Flowers are yellowish-green in colour and are arranged in spike inflorescence.

Macroscopy of Trikatu Churna

Table 1: Showing the Organoleptic characters of *Trikatu Churna:*

S. No	Characteristics	*Trikatu Churna*
1	Form	*Churna*
2	Odour	Pleasant
3	Colour	Light Grey
4	Taste	Pungent

Microscopic study of fruits and rhizome
Microscopy of *Pippali*
Microscopic features of Fruit show epidermal cells which are rectangular, with thickened walls, mesocarp has parenchymatous cells with sclereids, endocarp which is fibrous with spiral vessels and seed.

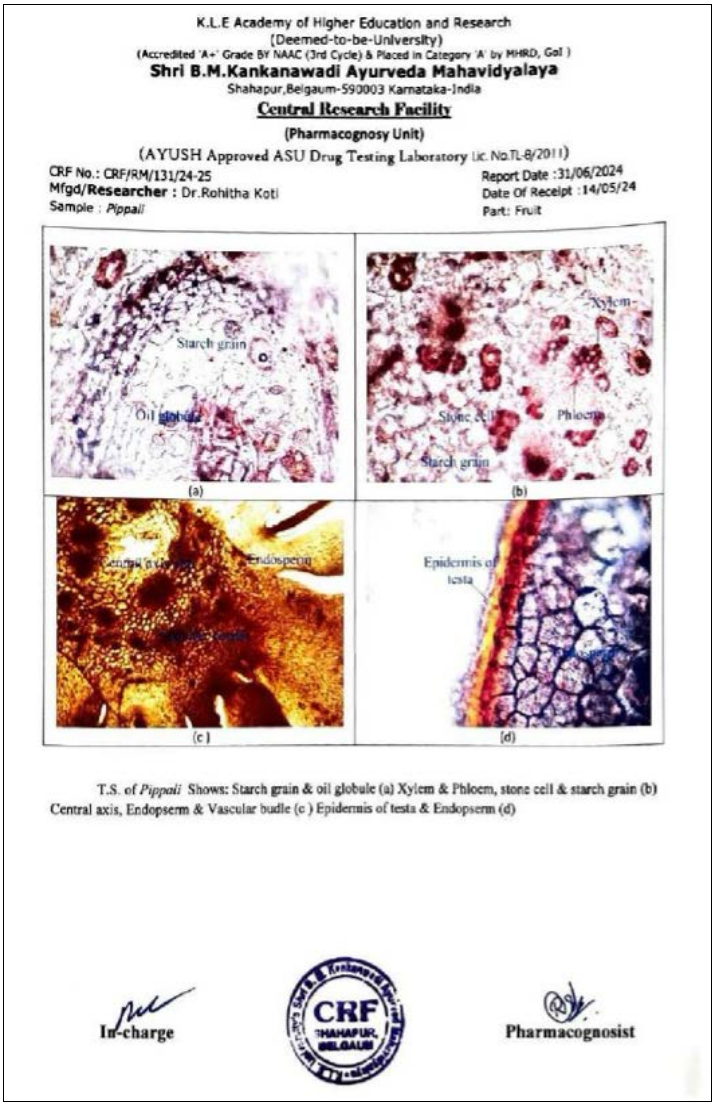


Fig 1: T.S. of *Pippali*

Microscopy of *Maricha*
Microscopic features of Fruit of *Maricha* show epidermal cells, mesocarp, endocarp and seed.

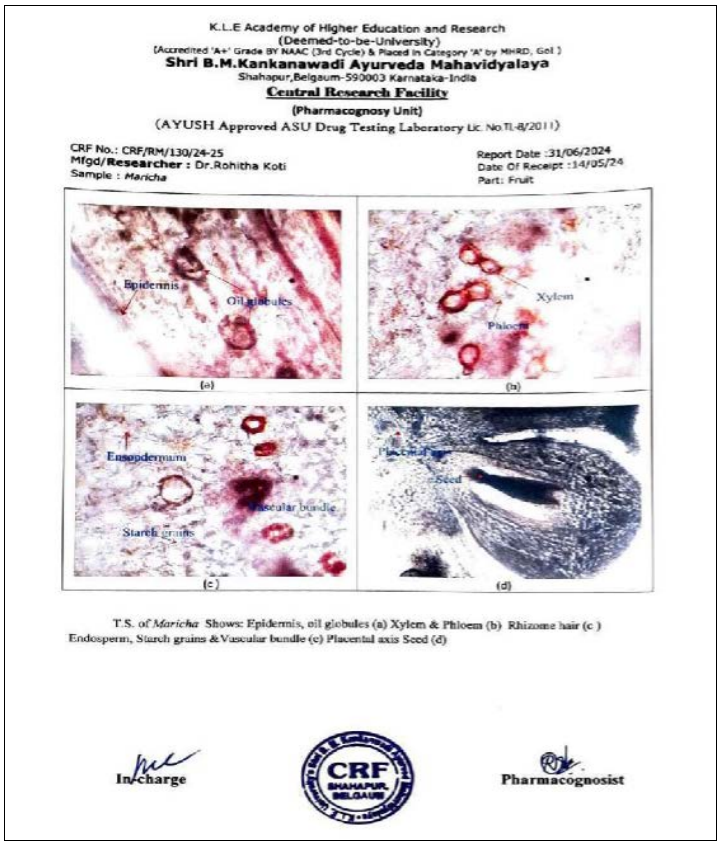


Fig 2: T.S. of *Maricha*

Microscopy of *Shunti*

Microscopic features of the rhizome of *Shunti* show epidermis, cortex, endodermis and stele.

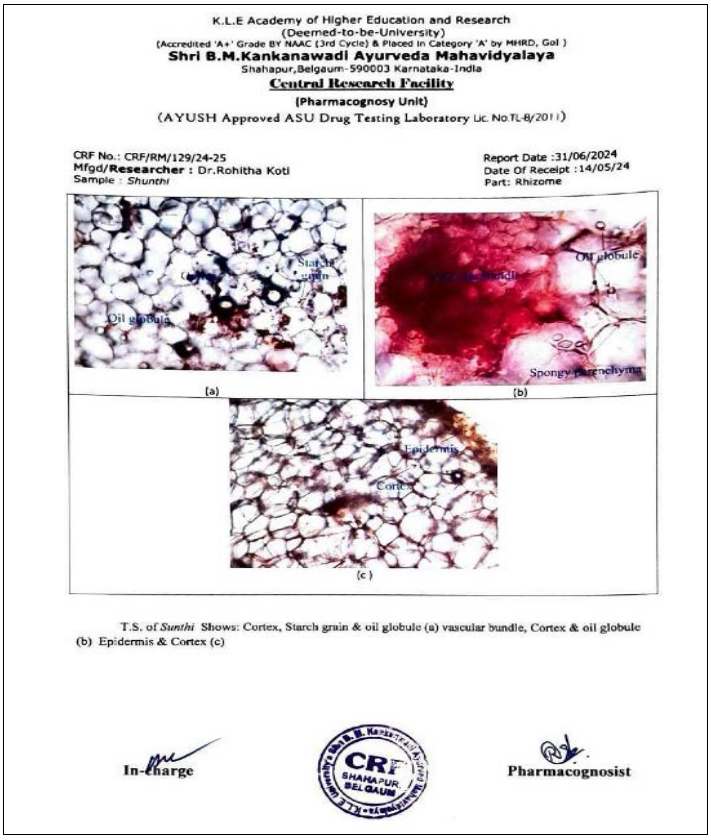


Fig 3: T.S. of *Shunti*

SHRI B.M.K. AYURVEDA MAHAVIDYALAYA
A constituent unit of KLE Academy of Higher Education & Research
Deemed–to-be-University
Central Research Facility
DRUG AUTHENTICATION REPORT

Outward: - BMK/CRF/446/2024-25

Submitted By: Dr. K. Rohitha
Submitted Date : 22/10/2024

Date of Issue: 29/10/2024

SLNo	Sample Name	Scientific Name	Family	Part submitted	CRF Code	Authenticated as			
						Common Name	Scientific Name	Family	Part Authenticated
1.	Pippali	*Piper longum* Linn.	Piperaceae	Fruit	CRF/Auth/585/2024	Pippali	*Piper longum* Linn.	Piperaceae	Fruit
2.	Maricha	*Piper nigrum* Linn.	Piperaceae	Fruit	CRF/Auth/586/2024	Maricha	*Piper nigrum* Linn.	Piperaceae	Fruit
3.	Sunthi	*Zingiber officinale* Rosc.	Zingiberaceae.	Rhizome	CRF/Auth/587/2024	Shunti	*Zingiber officinale* Rosc.	Zingiberaceae.	Rhizome

Signature:
Authentication Expert Name: Dr.Divya Khare
Date: 29/10/2024

Signature of Coordinator
ASU Drug Testing Laboratory

Report 1: Drug Authentication

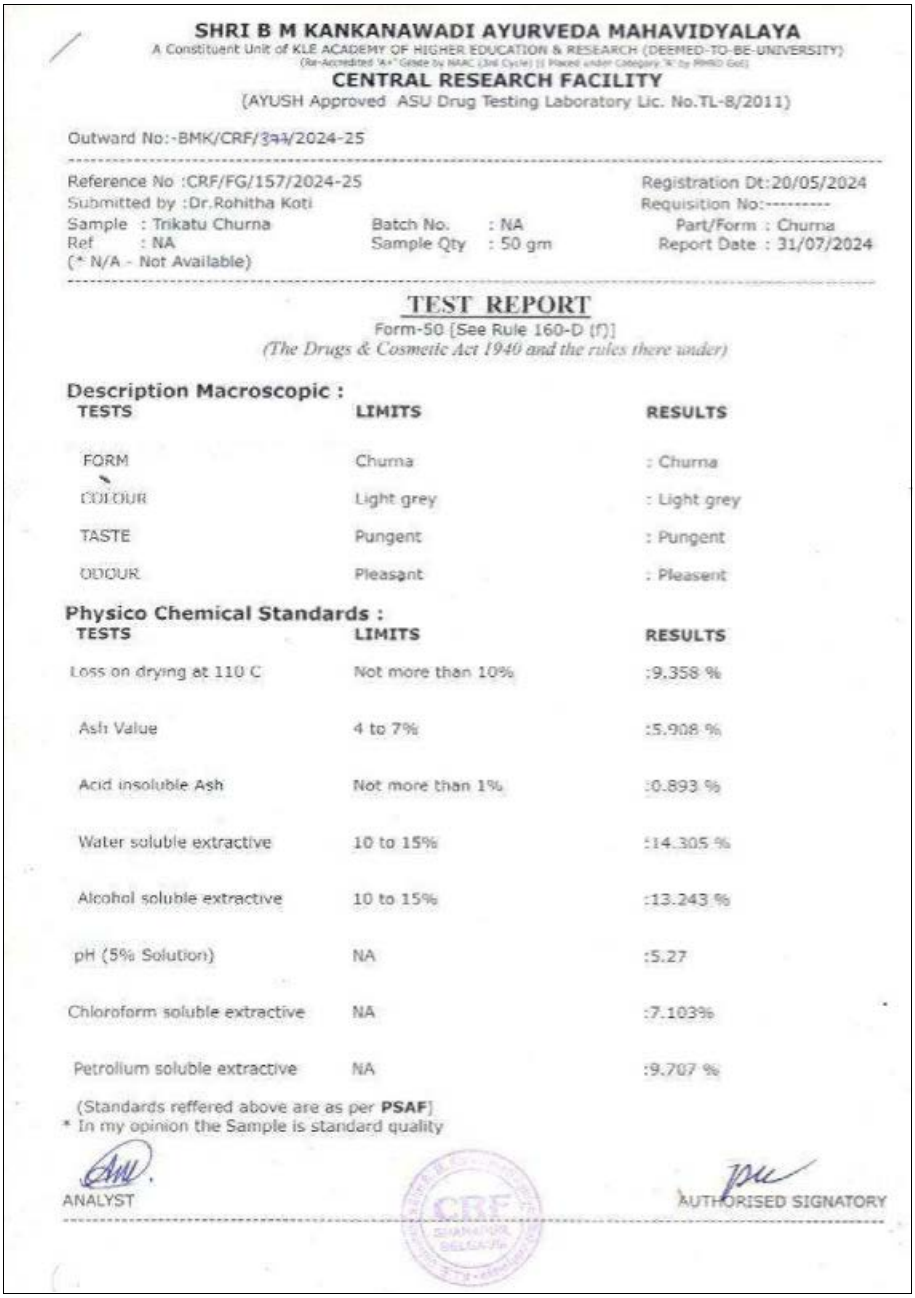
Morphological and microscopic features of Trikatu Churna: *Trikatu churna* is presented with a dark brown powder with a characteristic aroma indicative of its ingredients. Microscopic examination revealed the presence of trichomes and starch grains characteristics of the three herbs.

Table 2: Showing the Physicochemical constituents of *Trikatu Churna:*

S. No.	Constituents	*Trikatu churna*
1	Loss On Drying at 110C	9.358%
2	Ash value	5.908
3	Acid insoluble ash	0.893%
4	Water soluble extractive	14.305%
5	Alcohol soluble extractive	13.243%
6	pH	5.27
7	Chloroform soluble extractive	7.103%
8	Petroleum soluble extractive	9.707%

The Phytochemicals present in *Trikatu Churna* in various extracts

- The aqueous extract of *Trikatu churna:* Carbohydrates, Reducing sugar, Monosaccharides, Flavonoids and Saponin glycosides.
- Alcohol extracts of *Trikatu Churna:* Carbohydrates, Reducing sugar, Monosaccharides, Hexose Sugar, Steroids, Flavonoids, Cardiac glycosides and Saponin glycosides.
- Petroleum extracts of *Trikatu churna:* Carbohydrates and Cardiac Glycosides.
- The chloroform extract of *Trikatu churna:* Carbohydrates, Reducing sugar, Steroids and Cardiac glycosides.



SHRI B M KANKANAWADI AYURVEDA MAHAVIDYALAYA
A Constituent Unit of KLE ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED-TO-BE-UNIVERSITY)
CENTRAL RESEARCH FACILITY
(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Outward No:-BMK/CRF/341/2024-25

Reference No :CRF/FG/157/2024-25 Registration Dt:20/05/2024
Submitted by :Dr.Rohitha Koti Requisition No:----------
Sample : Trikatu Churna Batch No. : NA Part/Form : Churna
Ref : NA Sample Qty : 50 gm Report Date : 31/07/2024
(* N/A - Not Available)

TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Description Macroscopic :

TESTS	LIMITS	RESULTS
FORM	Churna	: Churna
COLOUR	Light grey	: Light grey
TASTE	Pungent	: Pungent
ODOUR	Pleasant	: Pleasent

Physico Chemical Standards :

TESTS	LIMITS	RESULTS
Loss on drying at 110 C	Not more than 10%	:9.358 %
Ash Value	4 to 7%	:5.908 %
Acid insoluble Ash	Not more than 1%	:0.893 %
Water soluble extractive	10 to 15%	:14.305 %
Alcohol soluble extractive	10 to 15%	:13.243 %
pH (5% Solution)	NA	:5.27
Chloroform soluble extractive	NA	:7.103%
Petrolium soluble extractive	NA	:9.707 %

(Standards reffered above are as per **PSAF**]
* In my opinion the Sample is standard quality

ANALYST AUTHORISED SIGNATORY

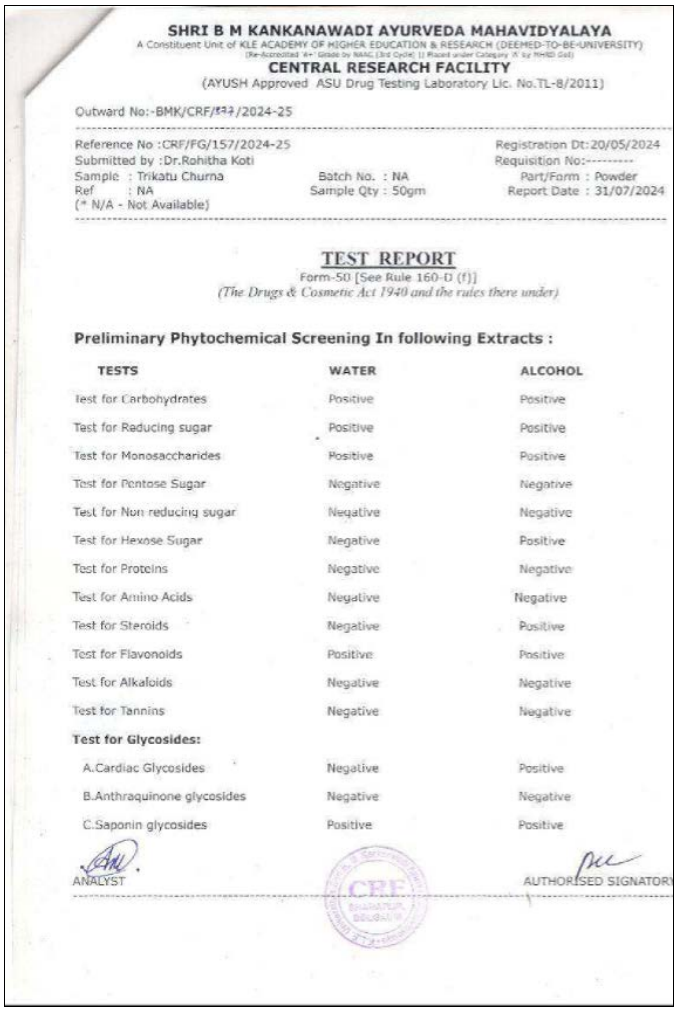
Report 2: Physicochemical Standards of *Trikatu Churna*

Table 3: Showing the Preliminary Phytochemical analysis of *Trikatu Churna:*

Tests	Aqueous extract	Alcoholic extract	Petroleum ether extract	Chloroform extract
Test for Carbohydrates	+	+	+	+
Test for Reducing Sugar	+	+	_	+
Test for Monosaccharides	+	+	_	_
Test for Pentose Sugar	_	_	_	_
Test for Hexose Sugar	_	+	_	_
Test for Non-reducing Sugar	_	_	_	_
Test for Proteins	_	_	_	_
Test for Amino Acids	_	_	_	_
Test for Steroids	_	+	_	+
Test for Flavonoids	+	+	_	_
Test for Alkaloids	_	_	_	_
Test for Tannins	_	_	_	_

Test for Glycosides

Cardiac glycosides	_	+	+	+
Anthraquinone glycosides	_	_	_	_
Saponin glycosides	+	+	_	_



SHRI B M KANKANAWADI AYURVEDA MAHAVIDYALAYA
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CENTRAL RESEARCH FACILITY
(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Outward No:-BMK/CRF/[illegible]/2024-25

Reference No :CRF/FG/157/2024-25 Registration Dt:20/05/2024
Submitted by :Dr.Rohitha Koti Requisition No:---------
Sample : Trikatu Churna Batch No. : NA Part/Form : Powder
Ref : NA Sample Qty : 50gm Report Date : 31/07/2024
(* N/A - Not Available)

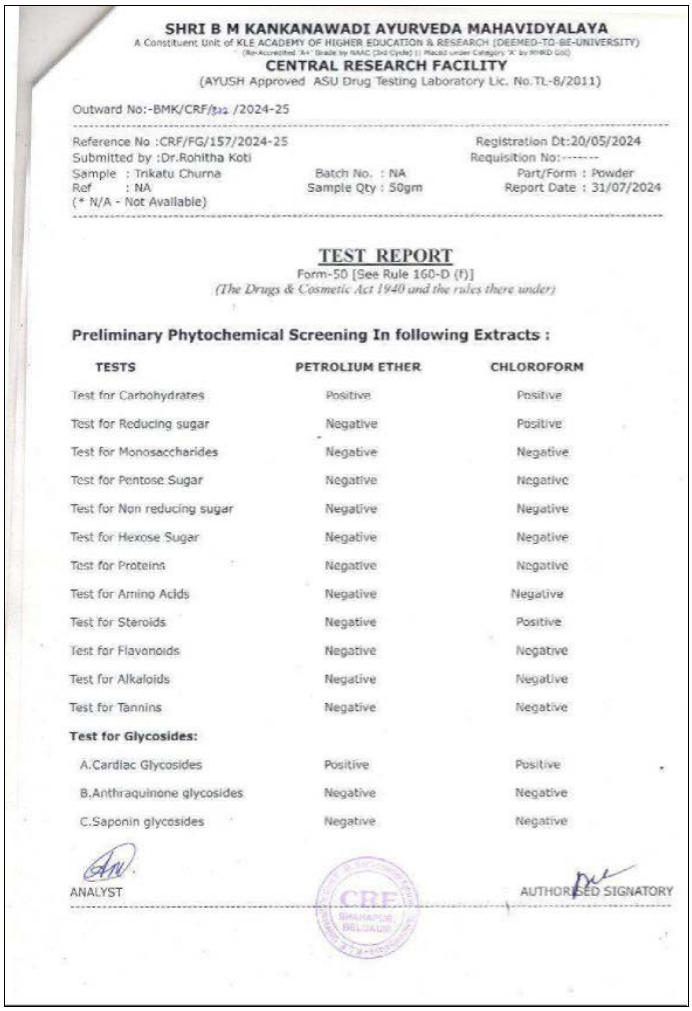
TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Preliminary Phytochemical Screening In following Extracts :

TESTS	WATER	ALCOHOL
Test for Carbohydrates	Positive	Positive
Test for Reducing sugar	Positive	Positive
Test for Monosaccharides	Positive	Positive
Test for Pentose Sugar	Negative	Negative
Test for Non reducing sugar	Negative	Negative
Test for Hexose Sugar	Negative	Positive
Test for Proteins	Negative	Negative
Test for Amino Acids	Negative	Negative
Test for Steroids	Negative	Positive
Test for Flavonoids	Positive	Positive
Test for Alkaloids	Negative	Negative
Test for Tannins	Negative	Negative
Test for Glycosides:		
A.Cardiac Glycosides	Negative	Positive
B.Anthraquinone glycosides	Negative	Negative
C.Saponin glycosides	Positive	Positive

ANALYST AUTHORISED SIGNATORY

Report 3



SHRI B M KANKANAWADI AYURVEDA MAHAVIDYALAYA
A Constituent Unit of KLE ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED-TO-BE-UNIVERSITY)
CENTRAL RESEARCH FACILITY
(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Outward No:-BMK/CRF/[illegible]/2024-25

Reference No :CRF/FG/157/2024-25 Registration Dt:20/05/2024
Submitted by :Dr.Rohitha Koti Requisition No:-------
Sample : Trikatu Churna Batch No. : NA Part/Form : Powder
Ref : NA Sample Qty : 50gm Report Date : 31/07/2024
(* N/A - Not Available)

TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Preliminary Phytochemical Screening In following Extracts :

TESTS	PETROLIUM ETHER	CHLOROFORM
Test for Carbohydrates	Positive	Positive
Test for Reducing sugar	Negative	Positive
Test for Monosaccharides	Negative	Negative
Test for Pentose Sugar	Negative	Negative
Test for Non reducing sugar	Negative	Negative
Test for Hexose Sugar	Negative	Negative
Test for Proteins	Negative	Negative
Test for Amino Acids	Negative	Negative
Test for Steroids	Negative	Positive
Test for Flavonoids	Negative	Negative
Test for Alkaloids	Negative	Negative
Test for Tannins	Negative	Negative
Test for Glycosides:		
A.Cardiac Glycosides	Positive	Positive
B.Anthraquinone glycosides	Negative	Negative
C.Saponin glycosides	Negative	Negative

ANALYST AUTHORISED SIGNATORY

Report 4

Report 3 & 4: Preliminary Phytochemical Screening Of *Trikatu Churna*

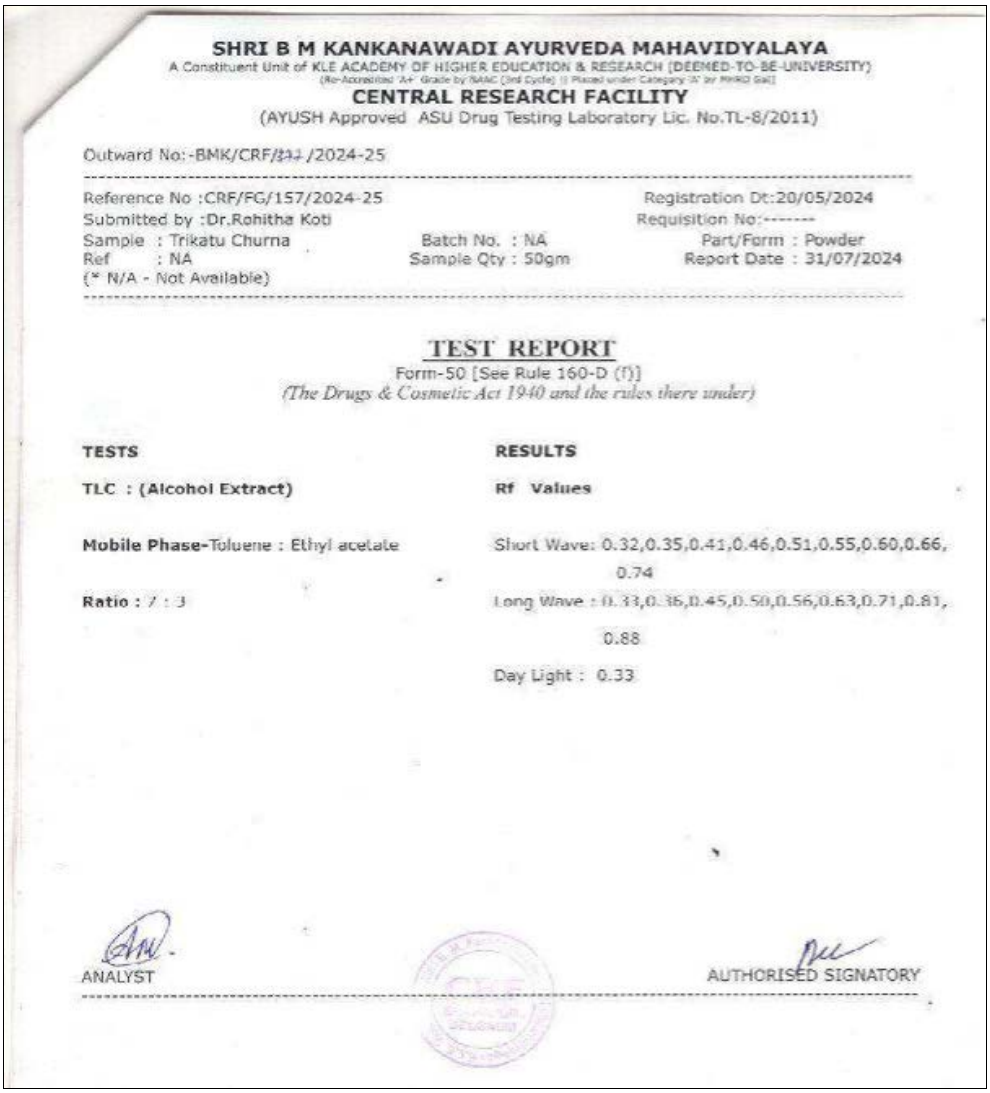
Thin layer chromatography [TLC]: TLC of the water & alcohol extract was performed using a specially developed and optimized mobile phase to avoid any variability in the pattern due to changes in the mobile phase. The TLC was performed to get the fingerprint of the crude extract and to study it qualitatively.

***Trikatu Churna* Mobile Phase:** (Alcohol extract): Toluene: ethyl acetate: 7:3

Chromatographic conditions: A well-closed CAMAG double-compartment glass chamber with a metal lid was utilised and the inside atmosphere was allowed to be saturated for half an hour before starting to run the program. Plates were allowed to run up to ¾th height of the plate or till sufficient separation was obtained and then removed from the chamber and allowed to dry.

Table 4: Showing the Rf values of alcohol extract of *Trikatu Churna*:

Type of Wave	(TLC) ALCOHOL EXTRACT
Short Wave	0.32, 0.35, 0.41, 0.46, 0.51, 0.55, 0.60, 0.66, 0.74
Long Wave	0.33, 0.36, 0.45, 0.50, 0.56, 0.63, 0.71, 0.81, 0.88
Day Light	0.33



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Outward No:-BMK/CRF/311 /2024-25

Reference No :CRF/FG/157/2024-25 Registration Dt:20/05/2024
Submitted by :Dr.Rohitha Koti Requisition No:-------
Sample : Trikatu Churna Batch No. : NA Part/Form : Powder
Ref : NA Sample Qty : 50gm Report Date : 31/07/2024
(* N/A - Not Available)

TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

TESTS	RESULTS
TLC : (Alcohol Extract)	Rf Values
Mobile Phase-Toluene : Ethyl acetate	Short Wave: 0.32,0.35,0.41,0.46,0.51,0.55,0.60,0.66, 0.74
Ratio : 7 : 3	Long Wave : 0.33,0.36,0.45,0.50,0.56,0.63,0.71,0.81, 0.88
	Day Light : 0.33

ANALYST AUTHORISED SIGNATORY

Report 5: TLC of *Trikatu Churna*

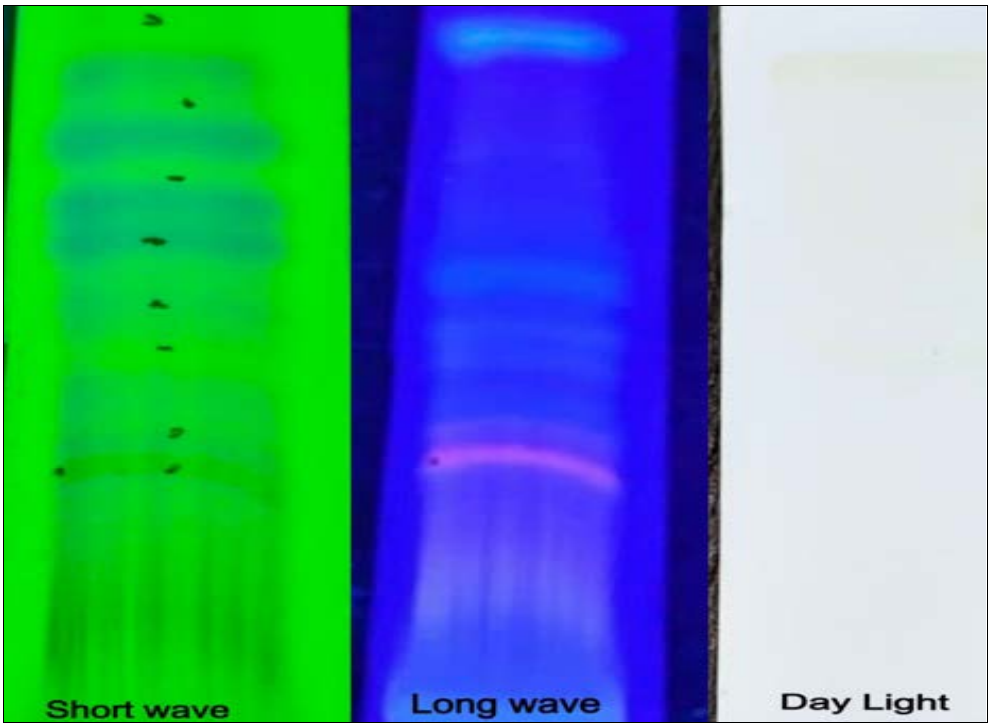

Fig 4: TLC Slides Of *Trikatu Churna*

The findings of this study reflect significant Pharmacognostical attributes in Trikatu Churna, emphasizing the interdependence of its ingredients in determining overall efficacy. The physicochemical parameters serve as benchmarks for quality control and assurance in herbal medicine.

Conclusion

The Pharmacognostical and physicochemical study of Trikatu Churna establishes a foundational understanding of its qualities. This research underscores the need for robust quality assessment protocols in herbal formulations to ensure safety and efficacy in clinical applications.

References

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