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Original Article
Standardisation and Quality Control - Formulation

Pharmacognostical and Phytochemical Evaluation of a Polyherbal Ayurvedic Formulation *Trikatu Churna*

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ABSTRACT

***Introduction*:** *Trikatu Churna* is an Ayurvedic polyherbal formulation useful in wide range of diseases and disorders. Efficacy of formulation depends on their genuineness of herbs used. Authentication of herbs by anatomical studies is first and fundamental step for standardization of herbal formulation. In this paper Pharmacognostic investigations like macroscopic, microscopic and chemical studies like preliminary phyto-chemical, physico- chemical constants and TLC/HPTLC fingerprint of *Trikatu Churna* were studied. ***Methods*:** The standard methods recommended in Quality Control Methods for Medicinal Plant Materials by WHO, 1998 was followed. ***Results*:** Macro-microscopic, preliminary phyto-chemical studies and TLC/HPTLC studies of the formulation has been documented. ***Conclusion*:** Findings of the study is helpful in standardization of polyherbal Ayurvedic formulation Trikatu Churna, which will promote global acceptance of the formulation and reputation of the Ayurveda system.

KEYWORDS

Trikatu Churna, Pharmacognosy, Phytochemistry, Standardization, HPTLC fingerprint

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*T*rikatu *Churna* is an Ayurvedic polyherbal formulation consisting of fine powders of Pippali (*Piper longum* Linn (Fruit), Marica (*Piper nigrum* Linn. (Fruit) and Sunthi (*Zingiber officinale* Rosc. (Rhizome) in equal proportion. [1] In preparation of *Churna* the ingredients are collected, dried, powdered individually and passed through sieve number 80/85 to prepare a fine powder.[1,2] In Ayurveda, *Trikatu Churna* is used in the treatment of *Agnimandya* (digestive impairment), *Gala roga* (throat diseases), *Svasa* (dyspnea), *Kushtha* (skin diseases), *Pinasa* (sinusitis), *Kasa* (cough) and *Slipada* (filariasis).[1] In Siddha, *Tirikatugu Churanam* having same composition is used in the treatment of loss of appetite, rumbling in the abdomen, stomach pain, cough and fever.[3] A small quantity of *Trikatu Churna* is mixed with water and dropped into the nostrils in coma and drowsiness.[4] Polyherbal formulation in powdered form where the botanical ingredients are not more than ten can be identified microscopically.[5] Pharmacognostic characters of herbal drugs play an important role since particular macro-microscopic features are unique for each plant. The macroscopic and microscopic studies of the herbs should be the first and fundamental step to authenticate the botanical source. Proceeding for chemical methods of standardization, preclinical and clinical evaluations will bear no value if authentic drugs are not used. Macro-microscopic evaluation is simple and cost effective. TLC/HPTLC is one of the most effective and common chromatographic technique because of its simplicity of use and cost effectiveness. The accuracy and precision of HPTLC with low uncertainty emerge this technique as simple powerful separation technique and widely adopted in many Pharmacopoeias as an identification method. Another advantage of HPTLC is being used by personnel with minimum of technical training and under reasonable laboratory facility.[9] Preliminary phytochemical evaluation provides information about presence of phytoconstituents in the extract. Physico-chemical constants indicate the purity and identity of the formulation. In the present investigation macro-microscopic, preliminary phyto-chemical, physico-chemical constants and TLC/HPTLC fingerprint of the formulation were carried out.

MATERIALS AND METHODS

The ingredients of *Trikatu Churna* were purchased from local raw material traders and the raw materials were authenticated by comparing with the in-house standards of Botany/Pharmacognosy department, Captain Srinivasa Murthy Regional Ayurveda Drug Development Institute, Arignar Anna Government Hospital Campus, Arumbakkam, Chennai – 600 106. The dried cleaned samples

were powdered and passed through sieve No. 80. Each one of the powder is weighed separately and equal parts of each powder are mixed together. Powders of individual ingredients of *Piper longum* (Fruit), *Piper nigrum* (Fruit), *Zingiber officinale* (Rhizome) (Table 1 and Figure 1.1-1.3), and the compound formulation (Figure 1.4) were analysed microscopically after clearing them in chloral hydrate solution. A few milligram of powder treated with iodine in potassium iodide solution and mounted in glycerine for observation of starch. A few milligram 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. Quantitative analysis for total ash, acid insoluble ash, water and alcohol soluble extractive values and loss on drying at 105^{0}c 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.[6] Preliminary phytochemical analysis, Fluorescence analysis and TLC/HPTLC fingerprint were also carried out.[7,8]

Table 1. Ingredients of *Trikatu Churna*

Ayurvedic names	Botanical names	Part used	Quantity
Pippali	*Piper longum* Linn.	Fruit	All ingredients in equal parts
Marica	*Piper nigrum* Linn.	Fruit	
Sunthi	*Zingiber officinale* Rosc.	Rhizome	

Figure 1. Ingredients of *Trikatu Churna*

1.1 *Pippali*

1.2 *Marica*

1.3 *Sunthi*

1.4 *Trikatu Churna*

Preparation of extracts for TLC/HPTLC

4 g of the each drug sample were soaked in aqueous alcohol (10%) for overnight, refluxed for 30 minutes on water bath and filtered. The filtrates were concentrated on water bath and made up to 10 ml in a standard flask separately.

Method for developing TLC/HPTLC

Chromatographic separation was achieved on TLC/HPTLC fs pre-coated with silica gel 60 F_{254} TLC plate (E-Merck) of 0.2 mm thickness with aluminium sheet support. Samples were spotted using CAMAG Linomat IV Automatic Sample Spotter (Camag Muttenz, Switzerland) equipped with syringe (Hamilton, 100µL). Plates were developed in a glass twin trough chamber (CAMAG) pre-saturated with mobile phase. Scanning device used was CAMAG TLC scanner II equipped with CATS 3 software. The

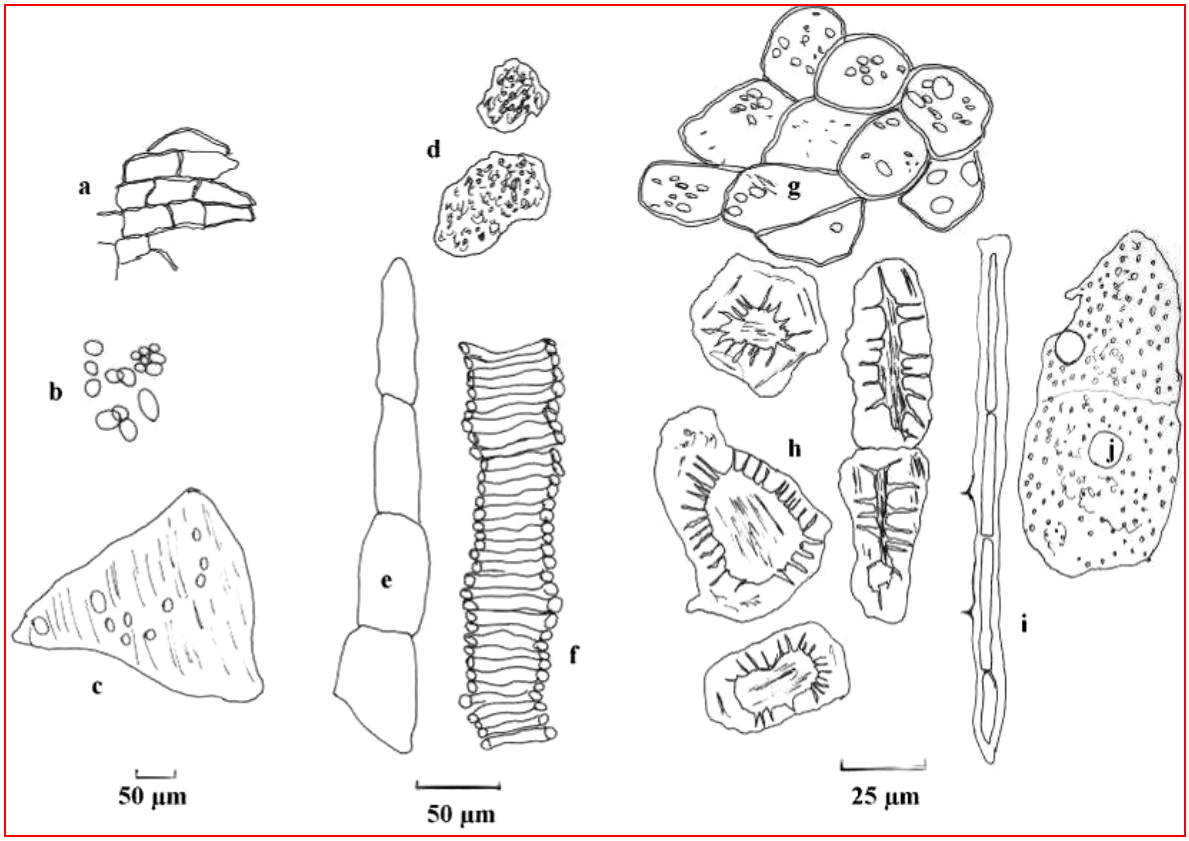
experimental condition was maintained at 20± 2⁰C. The aqueous alcoholic extract was applied on pre-coated silica gel 60 F_{254} TLC plate (E-merck) as absorbent and the plate was developed using solvent system toluene: ethyl acetate (5: 1.5). After developing, the plates were dried and observed the colour spots at UV-254, UV-366 nm and vanillin-sulphuric acid spraying reagent and Dragendorff's reagent.[9,10]

RESULTS AND DISCUSSION

Macroscopy: *Trikatu churna* is a yellowish green fine powder with aromatic odour, taste is pungent producing numbness on the tongue (Figure 1.4).

Microscopy: Under microscope the following characteristics were observed in various mounts; *Pippali* – fragments of thick walled, lignified, in different shapes and sizes of stone cells with wide lumen, a few fragments of pointed multicellular trichome, 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 8µ in diameter; *Marica* – 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 40µ 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 (Figure 2).

Figure 2. Microscopy of *Trikatu Churna*



a. Cork cells (*Sunthi*); b. Starch grains (*Sunthi, Marica and Pippali*); c. Yellowish-brown content (*Sunthi, Marica* and *Pippali*); d. Perisperm with aleurone grains (*Marica* and *Pippali*); e. Multi-cellular pointed trichome (*Pippali*); f. Vessel with spiral thickenings (*Sunthi*); g. Parenchyma cells with starch (*Sunthi*); h. Stone cells (*Pippali* and *Marica*); i. Septate fibre (*Sunthi*); j.Perisperm embedded with oil globules (*Marica* and *Pippali*).

Physico-chemical analysis

Physico-chemical analysis shows 11.36 % of moisture content. Ash content of the drug was 4.22 % and 0.72 % of acid in-soluble ash shows the siliceous matter in the plant. Alcohol soluble extractives 9.59 % 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 2).

Table 2. Physico-chemical analysis of *Trikatu Churna*

Parameters	Mean Value (n=3) ± S.D
Ash value	
a. Total ash (%)	4.22 ± 0.18
b. Acid-insoluble ash (%)	0.72 ± 0.20
Extractive value	
a. Water soluble extractive (%)	11.38 ± 0.24
b. Alcohol soluble extractive (%)	9.59 ± 0.21
Loss on drying at 105^{0}C (%)	11.36 ± 0.10

Preliminary Phyto-chemcial test

Preliminary phyto-chemical test of the aqueous alcoholic extract of *Trikatu churna* shows presence of alkaloid, sugar, phenol, quinone, tannin and triterpenoids and the absence of coumarin, flavanoid, steroid, saponin and acid (Table 3).

Table 3. Preliminary phyto-chemical analysis of *Trikatu Churna*

Test	Presence/Absence in aqueous alcoholic extract
Alkaloid	+
Coumarin	-
Flavonoid	-
Sugar	+
Phenol	+
Quinone	+
Steroid	-
Tannin	+
Triterpenoid	+
Saponin	-
Acid	-

Fluorescence Analysis

Fluorescence analysis of the *Trikatu churna* with different chemical reagents shown in Table 4.

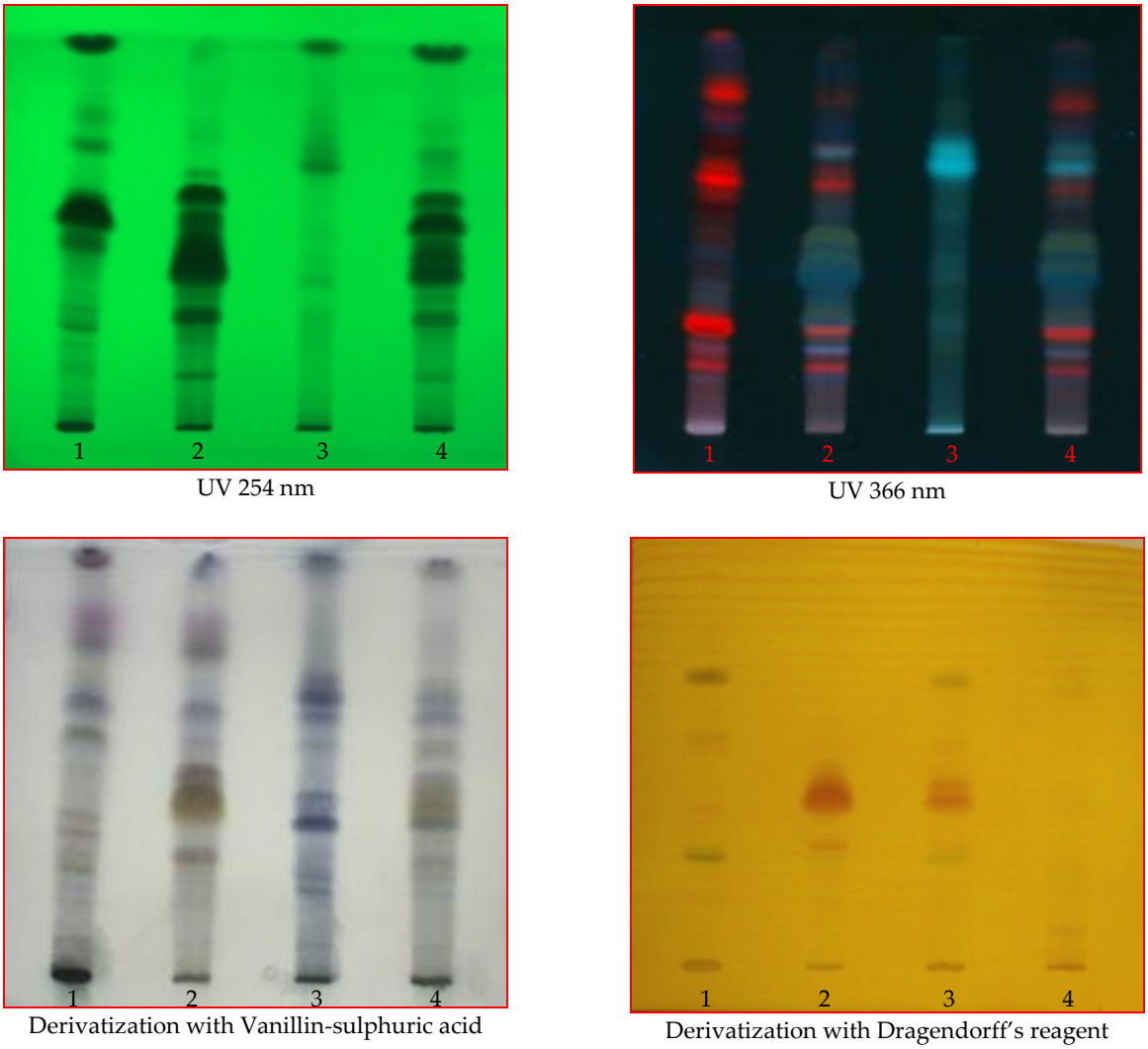
Table 4. Fluorescence Analysis of *Trikatu Churna*

Reagents with powder	UV-254 nm	UV- 366 nm	Visible light
Powder as such	Black	Pale greyish colour	Yellowish brown
n-hexane	Black	Pale greyish colour	Dark brown
Chloroform	Black	Pale greyish colour	Black
Ethyl acetate	Black	Pale greyish colour	Yellowish brown
Ethanol	Black	Pale greyish colour	Brown
Acetone	Black	Pale greyish colour	Yellowish brown
Water	Brown	Pale greyish colour	Brown
1N Sodium hydroxide (Aqueous)	Black	Yellowish green	Yellowish brown
1N Sodium hydroxide (Alcohol)	Black	Yellowish green	Yellowish brown
1N Hydrochloric acid	Black	Yellowish green	Yellowish brown
50 % Nitric acid	Black	Black	Yellowish brown
50 % Sulphuric acid	Brown	Yellow	Yellowish brown
Conc. Sulphuric acid	Black	Yellowish green	Black

TLC

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 3.

Figure 3. TLC Photo-documentation of *Trikatu Churna*



Track 1: *Piper longum* 2: *Piper nigrum* 3: *Trikatu churna* 4: *Zingiber officinale*

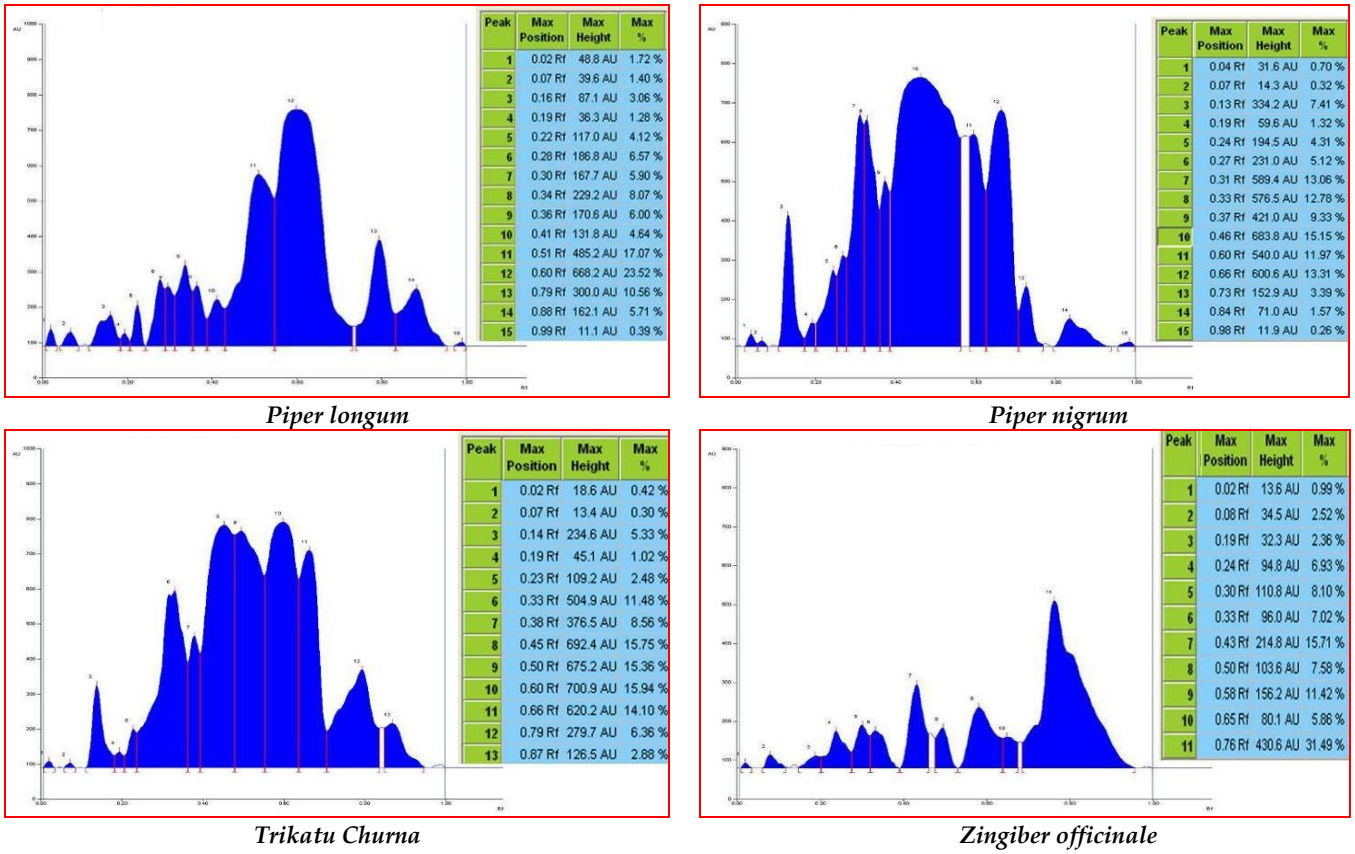
HPTLC

The HPTLC densitometric scan of *Piper longum* at 366 nm showed 15 peaks. The R_f values 0.51, 0.60 and 0.79 were major peaks and the R_f values 0.02, 0.07, 0.16, 0.19, 0.22, 0.28, 0.30, 0.34, 0.36, 0.41, 0.88 and 0.99 were smaller peaks. *Piper nigrum* showed 15 peaks with the R_f values 0.04, 0.07, 0.13, 0.19, 0.24, 0.27, 0.31, 0.33, 0.37, 0.46, 0.60, 0.66, 0.73, 0.84 and 0.98 of which R_f values 0.13, 0.31, 0.33, 0.37, 0.46, 0.60 & 0.66 were the major peaks (Figure 4).

The HPTLC fingerprint profile of *Trikatu Churna* showed 13 peaks. The R_f values 0.33, 0.38, 0.45, 0.50, 0.60 and 0.66 were major peaks whereas the R_f values 0.02, 0.07, 0.19, 0.23, 0.79 and 0.87 were smaller peaks. HPTLC fingerprint of *Zingiber officinale* showed 11 peaks with the R_f values 0.02, 0.08, 0.19, 0.24, 0.30, 0.33, 0.43, 0.50, 0.58, 0.65 and 0.76 of which R_f values 0.43 and 0.76 were the major peaks (Figure 4).

Pharmacognostic characters of herbal drugs play an important role since particular macro-microscopic features are unique for each plant. The macroscopic and microscopic studies of the herbs should be the first and fundamental step to authenticate the botanical source. TLC/HPTLC profile of aqueous alcohol extracts provides a suitable method for monitoring the identity, purity and also standardization of the drug.

Figure 4. HPTLC fingerprint of *Trikatu Churna* at 366 nm



CONCLUSION

The present study analyzed the macro-microscopic characters, preliminary phyto-chemical, physico-chemical constants and TLC/HPTLC finger print of the formulation. The results obtained will help in standardization of Ayurvedic polyherbal formulation *Trikatu churna*.

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CONFLICT OF INTEREST

Nil

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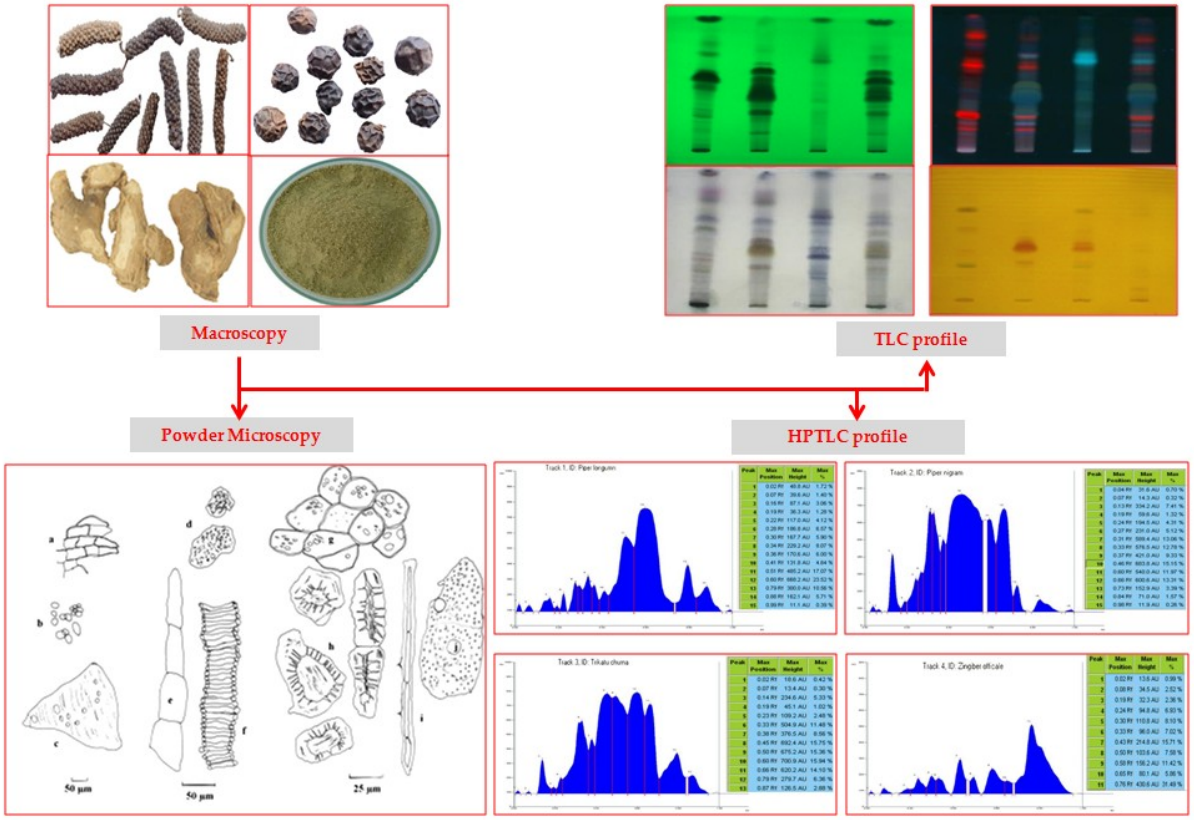
GRAPHICAL ABSTRACT



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