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Original Article
Standardisation and Quality Control – Single drug

Pharmacognostic and Phytochemical Analysis of Agnimantha (*Premna corymbosa* Rottl.) Leaf

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ABSTRACT

Introduction: *Agnimantha* (*Premna corymbosa* Rottl.) Family Verbinaceae, a very important plant has been in use since Vedic times. It occurs throughout India, in the plains, as a large perennial shrub, growing to a height of 9 to 10 m. It is one among the combination drugs i.e. *Brhatpanchamoola* (Great 5 root drugs) and of *Dasamoola* (10 root drugs). Its leaves are used as expectorant in asthma, bronchitis, cold, catarrh and fever. In this work an attempt was done to establish pharmacognostic and preliminary phytochemical standards of leaves including HPTLC. ***Methods*:** Pharmacognostical parameters for the leaves of *P. corymbosa* was performed using parameters like macromorphology, microscopy, physico-chemical constants and phytochemical screening were done using standard methodology. ***Results*:** The leaves showed presence of tannins and flavanoids. HPTLC densitometric scan showed twelve chemical components at 254nm, nine at 366nm and ten at 620nm. ***Conclusion*:** The present study will serve as a standard for the identity of *Premna corymbosa* Rottl leaves.

KEYWORDS

Agnimantha, Brhatpanchamoola, Dasamoola, Premna corymbosa Rottl., Standardisation.

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Agnimantha, Premna corymbosa Rottl.(Verbinaceae) is very important plant since *Vedic* period.[1] It is called as *Agnimathanaha*,[2,3,4] *Arani*,[5,6,7] and *Nadeyi*[4,5,7,8,9] in Sanskrit, Munja in Malayalam[7,10]and Taggiberu[7,10] in Kannada. The twigs of this plant used to be rubbed together to light fire[1] during ceremonial sacrifices, which is evident from the Sanskrit name, *Agnimantha*. It possesses *Kapha Vatahara*[11] karma and *Sothahara* (anti-inflammatory) property. In Ayurveda, the usage of *Agnimantha* leaves[12,13] is mentioned, as stomachic[3] and against dyspepsia.[3] Leaves are reported to have anti-inflammatory[15], antioxidant[16], hepatoprotective[17], antidiabetic actions[18]. The acute and chronic anti-inflammatory activity of leaf ethanolic extract of *Agnimantha* at the doses of 200 and 400mg/Kg body weight showed inhibition in odema formation significantly in acute models, and reduction in the granuloma formation with percentage of 35.17% and 50.38% inhibition in chronic models.[15] Pledging antioxidant action was found from the methanol leaf extract of *P. corymbosa*.[16] Evident hepatoprotective activity and reduction in the liver weight was seen in *P. corymbosa* leaves against carbon tetrachloride (CCl_4) induced hepatic damage.[17]Antidiabetic action of the methanol extract of leaves of *P. corymbosa* was tested by subjecting it with, ethyl acetate and hexane, and the results proves to manage hyperglycemia.[18] Literature on pharmacognostic standards for *P. corymbosa* Rottl. leaf is not available. Considering its wide availability and therapeutic utility, the present work was taken up to establish standardization of *P. corymbosa* Rottl. leaf with the main objective to evaluate pharmacognostical parameters such as macroscopic, microscopy, physicochemical, phytochemical and chromatographic studies of the leaves.

MATERIALS AND METHODS

The leaves of *P. corymbosa* were collected from the herbal garden of Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan. Authentication was done at the Department of Dravyaguna, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan. Authenticated samples were subjected for pharmacognostic, physico-chemical, phytochemical analysis and HPTLC profiling at Department of Pharmacognosy, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi.

Pharmacognostic Study: Macroscopic / organoleptic evaluation of leaves was done for evaluation of external morphology, its shape and size, colour, odour and taste of the drug.[10] A pinch of powder was warmed with drops of chloral hydrate on a microscopic slide and mounted in glycerine. Slides observed under microscope and diagnostic characters were observed and photographed using Zeiss AXIO trinocular microscope attached with Zeiss Axiocamera under bright field light. Magnifications of the figures are indicated by the scale-bars.[10]

Physico-Chemical Constants: Physico-chemical constants of leaves were determined to elicit loss on drying, total ash, acid Insoluble ash, water soluble ash, alcohol soluble extractive value and water soluble extractive value.[10]

Phytochemical Screening: Phytochemical analysis of leaves were determined to see the presence of alkaloids, steroids, carbohydrate, tannin, flavanoids, saponins, triterpenoids, coumarins, phenols, carboxylic acid, amino acid, resins and quinone.[19]

HPTLC Profile: One gram of powdered samples were dissolved in 10 ml of ethanol and kept for cold percolation for 24h and filtered. 3, 6 and 9 µl of the leaf samples of the drug *P. corymbosa* Rottl. were applied on a pre-coated silica gel F254 on aluminium plates to a band width of 7 mm using CAMAG (Muttenz, Switzerland) Linomat 5 TLC applicator. The plate was developed in the solvent system of toluene ethylacteate and formic acid (7.0: 3.0: 0.3) in CAMAG twin trough chamber. The developed plates were visualized in under short and long UV and then derivatised with vanillin sulphuric acid reagent, prior to derivatisation in CAMAG Photo documentation unit. The plate was scanned under UV 254 and 366 nm using CAMAG Scanner 4. R_f, colour of the spots and densitometric scan were recorded.[20]

RESULTS AND DISCUSSION

The study on the pharmacognostic features of medicinal plants is a process to know its quality, purity and also to check the presence of adulterants and substitutes.

Macroscopical / Organoleptic: Macroscopically the leaves were simple, oblong to obovate in shape, opposite/ whorly arranged; its surface was coriaceous, dark green above and below dull colour, with strong characteristic odour and astringent taste.

Powder microscopy: Leaf powder was rough, light green, strongly aromatic and astringent in taste. In microscopic view, the powder showed the presence of fragment of epidermis with stomata, upper epidermis with palisade, lower epidermis, vein islet, glandular trichome, vasculature in the lamina, thick walled trichome, parenchyma from petiole, thin-walled multicellular trichome, spiral vessels, bundle of fibres and fragment of vasculature (Figure 1).

Physico- chemical evaluation: Determination of loss on drying helps to evaluate the moisture content of a drug. It aids to prevent the decomposition of the drugs either due to chemical change or microbial contamination. From the results obtained for loss on drying, *Agnimantha* leaves have 9.01% of moisture content.

Determination of ash values is the criterion to judge purity of crude drugs. The residue remaining after incineration is the ash content of the drug. These could be inorganic salts such as carbonates, sulphates, phosphates, silicates etc. naturally occurring in the drug or adhered to it or deliberately added to it in order to adulterate the drug. Since the drugs were collected personally from their natural habitat there was no scope for adulteration. Acid insoluble ash or water soluble ash content is the residue obtained after boiling the total ash either with dilute hydrochloric acid or water which measures the amount of sand and silica matter present in the drug. From the above results the total ash value for leaves of *Agnimantha* showed 8.01 %, acid insoluble ash was 0.60 %, while water soluble ash content was found to be 3.89 %.Determination of extractive value measures the nature of the chemical constituents present in a crude drug. The results of ethanol soluble and water soluble extractive values of *Agnimantha* leaves showed 6.11% and 19.18% respectively (Table 1).

Table 1. Physical analysis of leaves of *Agnimantha*

Parameter	Result
Loss on drying	9.01
Total Ash	8.01
Acid Insoluble Ash	0.60
Water soluble Ash	3.89
Alcohol soluble extractive value	6.11
Water soluble extractive value	19.18

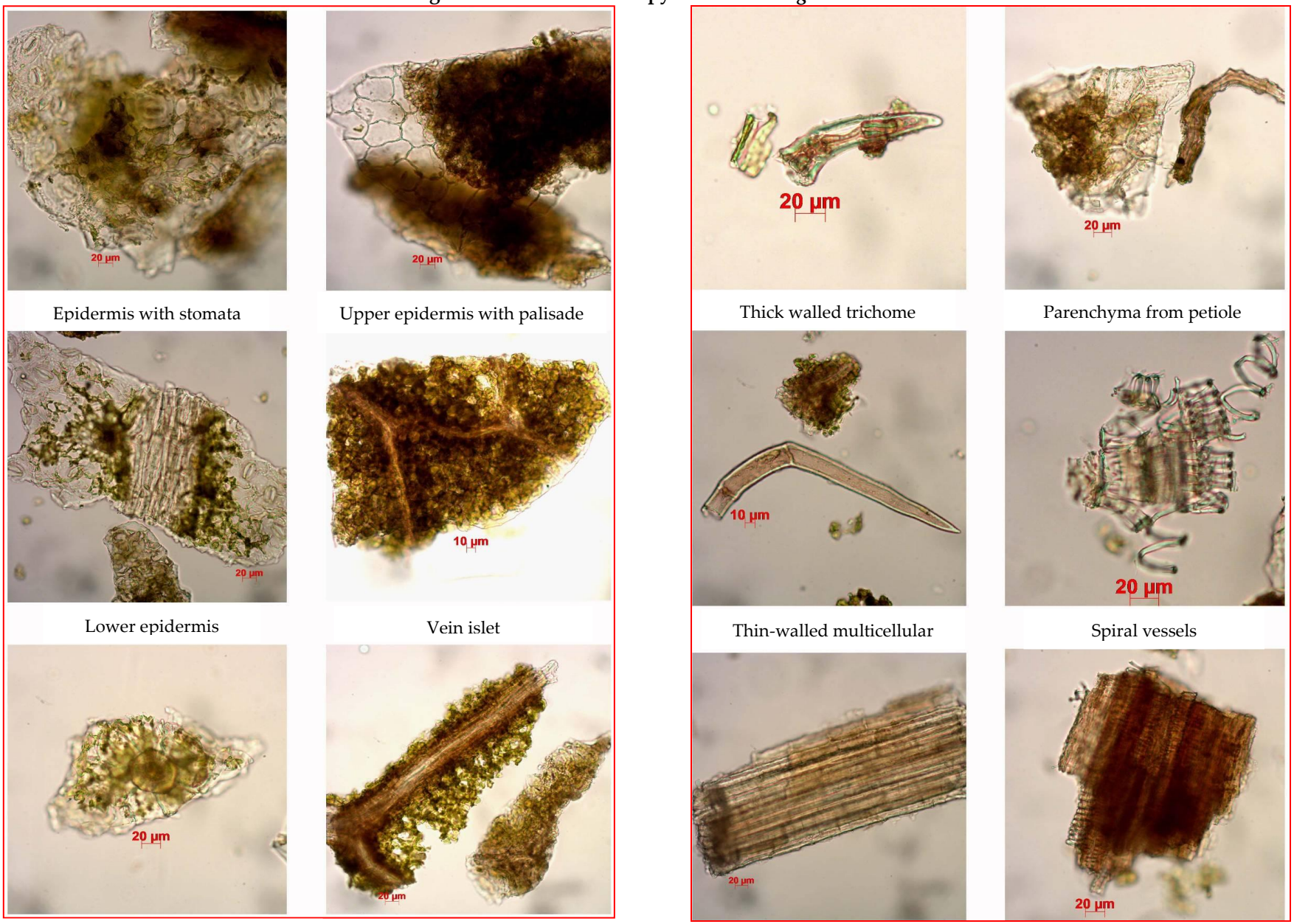
Phyto-chemical evaluation

The aqueous extract of *P. corymbosa* leaves showed the presence of Tannins, Flavanoids, Coumarins and Phenols (Table 2).

Table 2. Preliminary phytochemical screening of leaves of *Agnimantha*

Class	Result
Alkaloid	-
Steroid	-
Carbohydrate	-
Tannin	+
Flavanoids	+
Saponins	-
Terpenoid	-
Coumarins	+
Phenol	+
Carboxylic acid	-
Amino acids	-
Resins	-
Quinone	-

Figure 1. Powder microscopy of leaves of *Agnimatha*



Epidermis with stomata | Upper epidermis with palisade | Thick walled trichome | Parenchyma from petiole

Lower epidermis | Vein islet | Thin-walled multicellular | Spiral vessels

Glandular trichome | Vasculature in the lamina | Bundle of fibres | Fragment of vasculature

HPTLC

Retention factors (R_f) values of bands obtained were calculated by exposing the plates to different wavelengths of light. Under short UV, leaves of *P. corymbosa* showed green coloured spots at 0.30, 0.50, 0.59, 0.84 and 0.91.The R_f values in long UV showed red coloured spots at 0.06, 0.27, 0.33, 0.44, 0.55, 0.71, 0.76, 0.82 and 0.91. After derivatisation, when the plate was observed under white light, it showed violet and blue coloured spots for leaves. R_f values in derivatised plate were0.05, 0.19, 0.27, 0.31, 0.40, 0.44, 0.48, 0.54, 0.59, 0.66, 0.76, 0.84, in that the R_f value 0.84 showed blue coloured spot and the rest was violet coloured (Table 3).

Figure 2. HPTLC of ethanoilc extract of leaves of *Agnimatha*

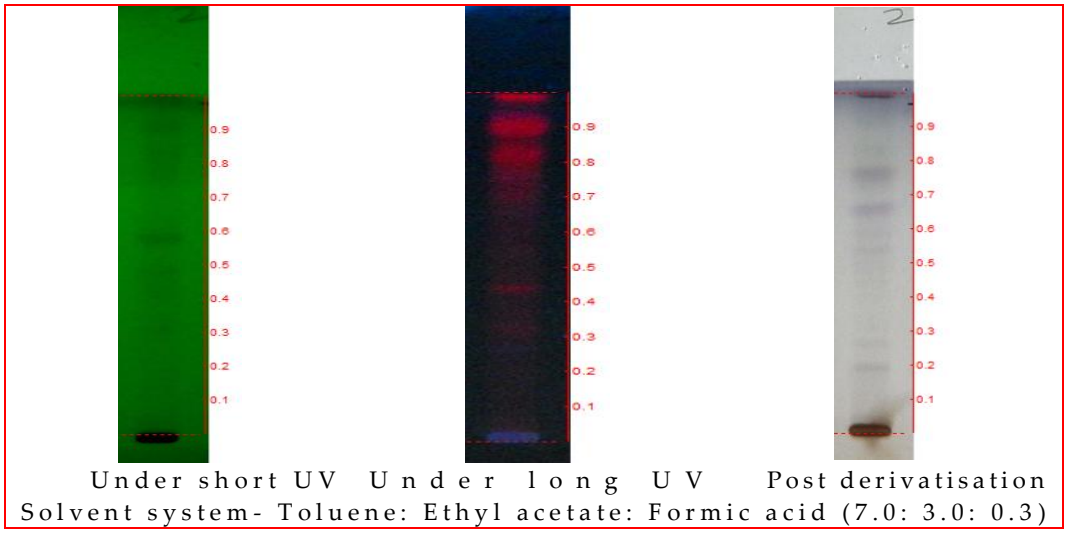
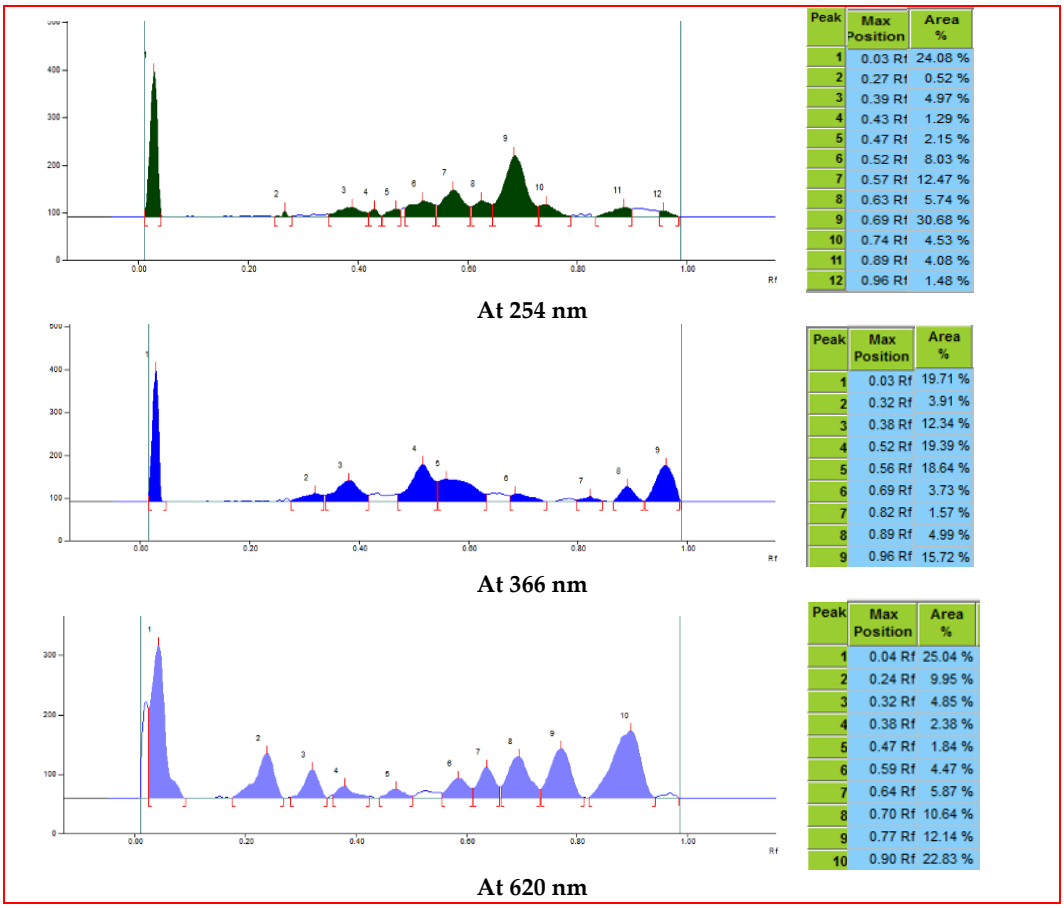


Figure 3.Densitometric scan of leaves of *Agnimatha*



Peak	Max Position	Area %
1	0.03 Rf	24.08 %
2	0.27 Rf	0.52 %
3	0.39 Rf	4.97 %
4	0.43 Rf	1.29 %
5	0.47 Rf	2.15 %
6	0.52 Rf	8.03 %
7	0.57 Rf	12.47 %
8	0.63 Rf	5.74 %
9	0.69 Rf	30.68 %
10	0.74 Rf	4.53 %
11	0.89 Rf	4.08 %
12	0.96 Rf	1.48 %

Peak	Max Position	Area %
1	0.03 Rf	19.71 %
2	0.32 Rf	3.91 %
3	0.38 Rf	12.34 %
4	0.52 Rf	19.39 %
5	0.56 Rf	18.64 %
6	0.69 Rf	3.73 %
7	0.82 Rf	1.57 %
8	0.89 Rf	4.99 %
9	0.96 Rf	15.72 %

Peak	Max Position	Area %
1	0.04 Rf	25.04 %
2	0.24 Rf	9.95 %
3	0.32 Rf	4.85 %
4	0.38 Rf	2.38 %
5	0.47 Rf	1.84 %
6	0.59 Rf	4.47 %
7	0.64 Rf	5.87 %
8	0.70 Rf	10.64 %
9	0.77 Rf	12.14 %
10	0.90 Rf	22.83 %

Table 3. R_f values of leaves of *Agnimatha*

Short UV	Long UV	Post derivatisation
-	-	0.05 (L. violet)
-	0.06 (F. red)	-
-	-	0.19 (L. violet)
-	0.27 (F. red)	0.27 (L. violet)
0.30 (L. green)	-	0.31 (L. violet)
-	0.33 (F. red)	-
-	-	0.40 (L. violet)
-	0.44 (FD. red)	0.44 (L. violet)
-	-	0.48 (L. violet)
0.50 (L. green)	-	-
-	0.55 (FL. red)	0.54 (L. violet)
0.59 (D. green)	-	0.59 (L. violet)
-	-	0.66 (D. violet)
-	0.71 (FD. red)	-
-	0.76 (FD. red)	0.76 (D. violet)
-	0.82 (FD. red)	-
0.84 (L. green)	-	0.84 (L. blue)
0.91 (D. green)	0.91 (FD. red)	-

D – dark; F – fluorescent; L - light

From the results of HPTLC densitometric scan, twelve chemical components at 254nm, nine chemical components at 366nm and ten chemical compounds at 620 nm were found in leaves of *P. corymbosa* (Figure 2 and 3).

CONCLUSION

In the present study, the presence of fragment of epidermis with stomata, glandular trichome, thick walled trichome, spiral vessels, bundle of fibres and fragment of vasculature serve as a microscopic reference standard for leaf identification. The aqueous extract of *P. corymbosa* leaves showed the presence of tannins, flavanoids, coumarins and phenols. The parameters thus obtained from the present pharmacognostic study and HPTLC analysis of the leaves of *Agnimantha* serves as a reference standard for its identity and authenticity.

CONFLICTS OF INTEREST

Nil

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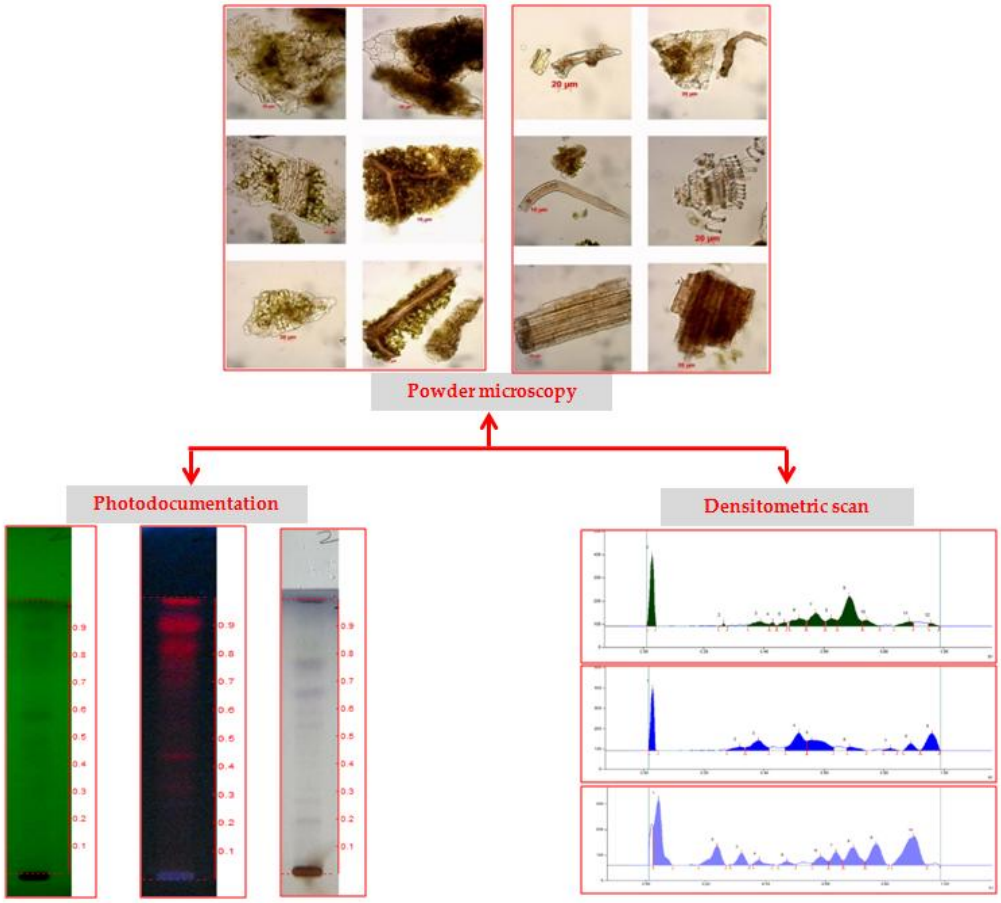
GRAPHICAL ABSTRACT



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