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Research Article

PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDY OF CHAKRANIKE (*Apama siliquosa* Lam): A FOLKLORE DRUG

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ABSTRACT

Apama siliquosa Lam is a folklore drug which is used to treat diarrhoea. It is locally known as Chakranike. The root of the drug is said to be effective in treating diarrhoea, cholera. The complete description of the drug is not available in the texts of Ayurveda. In this study an attempt is made scientifically to identify the drug *Apama siliquosa*. Lam. Pharmacognostical and phytochemical study of the drug is done to standardize the plant. The macroscopic as well as microscopic features of the plant is observed. Physico-chemical parameters such as loss on drying, total ash, acid insoluble ash, alcohol soluble extract, water soluble extract was assessed. Preliminary phytochemical screening was conducted by chloroform, benzene, methanol and petroleum ether extracts of the drug *A. siliquosa*. Lam. It revealed the presence of alkaloids, carbohydrates and glycosides. TLC, HPTLC plates of the drug were also developed and Rf value, color of the spot, densitometry scan was recorded.

KEY WORDS: *Apama siliquosa* Lam, Ayurveda, Chakranike, Pharmacognostical, Phytochemical, HPTLC, folklore.

INTRODUCTION

The knowledge about medicinally useful plants is scientifically documented and organized in Ayurveda Samhita, Nighantus and other texts. Ayurveda science is supported by a vast knowledge of drugs in the form of folklore medicine. Codified information regarding plants of folklore origin is not documented in the classical texts. The drugs which are used to treat diseases but lack references in the classical texts of Ayurveda are called as Anukta Dravya. The complete description of such drugs in the terms of their pharmacodynamics properties i.e. name, identification, morphology, rasa (taste), guna (attribute/ property), virya (potency), vipaka is not available in the classical texts. There is a need to identify these drugs on the basis of pharmacodynamics. Botanical identification and chemical evaluation of the drugs helps in development of Ayurvedic Materia Medica and also in the conservation of these endangered drugs.

Folklore practitioners are using the locally available herbs in treating the disease since ages. The effectiveness of the treatment is evident by its usage. Chakranike (*Apama siliquosa* Lam) is one such plant used by folklore practitioners in treating diarrhoea^{1,2}. It is locally known as Chakranike belongs to Aristolochiaceae family. It is an endemic plant of Western Ghats. Morphologically it is an erect shrub/ under shrub. The present study is taken for advanced research of the drug to understand the Pharmacognosy of the drug and phytochemical evaluation for the purpose of standardization of drug.

Drug review:

No reference of the drug was found in the ancient scriptures like Vedas and Puranas. It is not mentioned anywhere in the

Brihatrayee and Laghutrayee. Even the references of this drug are not found in the Nighantus.

Botanical description:

Botanical name: *A.siliquosa* Lam

Botanical synonyms: *Thottea siliquosa* (Lam) Ding Hou, *Bragantia wallichii*. Wight & Arn ⁴

Family: Aristolochiaceae

Table 1 explains the taxonomical position of *Thottea siliquosa* (Lam)

Morphological features: ⁴

It is an erect shrub or undershrub; branches swollen at the nodes with a smooth yellowish grey bark. Leaves are alternate, distichous, up to 22X7.5cm, oblong- lanceolate, acuminate at apex, acute at base, glabrous above, sparsely hairy beneath, 3-nerved at the base; petiole is very short. Slightly aromatic when bruised. Flowers are regular, in axillary shortly pedunculate irregularly umbellate cymes; bracts is small, linear; pedicels pubescent. Perianth is having lobes 3, ovate, concave, valvate, pubescent, dark purple. Stamens are 6 in number, in 3 groups of 2, adnate to style; anthers are hairy. Ovary is inferior, elongate, and 4-celled; ovules are numerous; style is short; stigma is 4 number. Fruit is a capsule, up to 10cm long, linear, torulose. Seeds are many, oblong, trigonus, pitted. Figure 1 is the photos of the test drug.

Distribution: ⁵ Peninsular India, native plant of Western Ghats, Sri Lanka.

Habitat: ^{4,5} it is commonly occurring in cool and marshy places, under shade and grows along the banks of streams. Evergreen and semi evergreen forests.

Flowering season: ⁴ January to November.

Useful parts: ⁶ Roots, Leaves, Whole plant
Vernacular names of *Apama siliquosa* Lam are listed in Table 2

Therapeutic action: ⁵
The root of the drug *Apama siliquosa* Lam is used in diarrhoea, dysentery, cholera.

Chemical composition:
The roots contain an intensely bitter nitrophenanthrene compound, isoaristolochic acid and an alkaloid. The roots also yield a small amount of a fatty oil containing palmitic, lignoceric, oleic and linoleic acids. The unsaponifiable matter contains a thick brown resinous material and P-sitosterol⁸.

MATERIALS AND METHODS

Root of the plant sources was collected from local areas surrounding Udupi district and was authenticated by the taxonomist, K. Gopalakrishna Bhat. The root was cleaned to remove the physical impurities and washed thoroughly with water. The root powder of *A. siliquosa* Lam was prepared at SDM pharmacy, Udupi. Physico chemical and phytochemical analysis was carried out at QC lab of Muniyal institute of Ayurveda medical sciences, Manipal. TLC was carried out at CARE KERALAM. HPTLC was carried out at QC lab of SDM Centre for Research in Ayurveda and Allied Sciences, Udupi.

Pharmacognostical study of root of the drug:

The study was conducted under following title

1. Macroscopical study
2. Microscopical study
3. Foreign matter

Macroscopical study

Morphological characters of the root of *A. siliquosa* Lam was studied by the observation with naked eye and with the help of magnifying lens of 10x.

Microscopical study:

Microscopical study includes transverse section of the Root of *A. siliquosa* Lam., powder microscopy.

Phytochemical study

This includes Physico-chemical parameters as: (i) Loss on drying (ii) Ash value analysis: a) Total ash b) Acid insoluble ash (iii) Extractive values ⁹ (Both alcohol and water), Preliminary phytochemical screening of Chloroform, Benzene, Methanol, Petroleum ether extracts of *Apama siliquosa* Lam, TLC, HPTLC.

Qualitative analysis for active constituents:

Phytochemical test was carried out by using different solvent extracts using standardized procedures to identify the constituents and to assess the activity of selected medicinal plant. Preliminary phytochemical analysis was carried out for the extracts namely Chloroform, Benzene, Methanol and Petroleum ether as per the standard method. ¹⁰ Chemical parameters ¹¹ include Test for alkaloids - Hager's test, Test for Carbohydrates: Fehling's test, Test for Glycosides: Keller- Killiani test, Test for Phenols, Test for Flavonoids: Shinoda test, Test for Amino acids: Ninhydrin test, Test for Tannins, Test for sterols: Salkowski test, Test for Saponin: Foam test.

Chromatographic analysis includes TLC, HPTLC.

OBSERVATION AND RESULTS:

Pharmacognostical study:

Macroscopical study: ¹²

The roots are 1 to 1.5m or more long, somewhat curved and tapering towards the ends, varying in thickness from 15-30mm across. Their external surfaces are smooth with deep longitudinal fissures giving them the appearance of islands with tapering ends; a few rootlet scars are present. They are greyish cream externally and internally yellowish white. The bark peels off easily in mature roots. While there is no characteristic smell, the taste is bitter.

Microscopical study:

The detailed microscopic feature of mature root of *A. siliquosa* Lam was done.

Transverse section of the Root of *A.siliquosa.Lam.*

It was observed in the transverse section taken that it consists of cork, cortex, phloem, xylem fibres and vessel fig (2.a). In the enlarged portion of the root of *A. siliquosa* Lam cork, cortex, phloem, xylem fibres, xylem ray, vessels were found Fig (2.b).

The outer region of root consists of cork and cortex Fig (2.c). Cork is an unorganized zone consisting thin walled cells with blackish cell walls Fig (2.c). Cortex is composed of rectangular cells some of which contain cell contents and it is represented by the colouring matter present in the cortical cells Fig (2.c).

Vascular zone of the root consists of Phloem, xylem and ground tissue Fig (2.d). A broad area of phloem parenchyma is arranged towards the periphery. Xylem is seen inner to phloem. Xylem fibres, xylem rays and xylem vessels are arranged towards the centre Fig (2.e). Secondary xylem vessels have large cavities surrounded by xylem parenchyma cells and primary xylem vessels are with small cavities. Pith is rudimentary which is in the centre consisting of thin walled cells. Fig (2.f).

Powder microscopy:

Powder analysis shows the presence of bundle of fibres view with starch, brown matter, pitted vessel, sclereids, vessel, cell with content, starch grains, tracheid's, sclerenchyma, pitted vessels, fibres crossing medullary rays, fibres. (Fig 3a to 3l)

Phytochemical study:

Table no. 3 and 5 shows the results.

TLC:

The TLC Plate shows major spots at Rf 0.07, 0.015 under 254nm and fluorescent spots at Rf value 0.03, 0.14, 0.22, 0.29, 0.34 (All blue), 0.07(purple), 0.10(Pink), 0.15(Green), 0.48(Violet), 0.59(Green), 0.73(Red). Figure 4

HPTLC: Figure 5 and Table 6 shows the results.

DISCUSSION

Medicinal plants are the important source of medicine. Proper identification of the drugs in a standardized form increases the efficacy of the medicine and reduces the side effects. Herbs which are present in the classical texts of Ayurveda are described under the Pharmacognostical and phytochemical parameters. Folklore medicine lack the parameters. There is a need for proper identification of the drugs under the standardized parameters.

Table 1: Taxonomical position of *Thottea siliquosa* (Lam) ³

| | |
|----------|---------------------------|
| Kingdom | Plantae |
| Division | Tracheophyta |
| Class | Magnoliopsida |
| Order | Piperales |
| Family | Aristolochiaceae |
| Genus | Thottea |
| Species | <i>T. siliquosa</i> (Lam) |

Table 2: Vernacular names of *Apama siliquosa* Lam ^{5, 6, 7}

| | |
|-----------|---|
| Kannada | Chakranike, Neeru vaate, Mirsaagni |
| Malayalam | Alpam, Karelvegam, Kodaashari, Kuttivayana, Thavasimuringa. |
| Sanskrit | Chakrani |
| Marathi | Chakrani |
| Telugu | Tellasyishwari |
| Tulu | Chakrani ber |

Table 3: Results of Physico chemical parameters

| Parameters | Values of the sample |
|-----------------------------|----------------------|
| Loss on drying | 11.56% w/w |
| Total ash | 4.92% w/w |
| Acid insoluble ash | 0.39% w/w |
| Alcohol -soluble extractive | 1.5% w/v |
| Water - soluble extractive | 2% w/v |

Table 4: Observations of Preliminary Phytochemical Tests

| Sl no | Tests | Colour if positive | Different extracts of <i>A. siliquosa</i> Lam | | | |
|-------|----------------------------------|---|---|--|----------------------|------------------------|
| | | | Chloroform | Benzene | Methanol | Petroleum ether |
| 1. | Alkaloids | | | | | |
| | Hager's test | Orange yellow precipitate | orange yellow colour | Orange yellow colour | Orange yellow colour | Blackish yellow colour |
| 2. | Carbohydrates | | | | | |
| | Fehling's test | Red precipitate | Red precipitate | Red precipitate | Red precipitate | Red precipitate |
| 3. | Glycosides | | | | | |
| | Keller-Killiani test | Reddish brown colour at the junction of two layers, bluish green colour in the upper layer. | Lemon yellow colour | Reddish brown colour at the junction of two layers | Brown colour | Cream yellow colour |
| 4. | Phenols | | | | | |
| | With FeCl ₃ | Green and blue colour. | Brown yellow colour | Light brown | Light brown | Yellow brown colour |
| 5. | Flavonoids | | | | | |
| | Shinoda test | Pink, crimson or magenta colour | Light orange colour | Dark yellow colour | Dark yellow colour | Light yellow colour |
| 6. | Amino acids | | | | | |
| | Ninhydrin test | violet or purple colour | Yellowish brown | Yellowish brown | Light brown | Light yellow |
| 7. | Tannins | | | | | |
| | FeCl ₃ in 90% alcohol | Dark green or deep blue colour | Transparent cream | Transparent cream | Light cream | Light cream |
| 8. | Steroids | | | | | |
| | Salkowaski test | Red colour | Transparent | Clear solution | Turbid white | Turbid white |
| 9. | Saponin | | | | | |
| | Foam test | Stable froth | No froth | No froth | No froth | No froth |

Table 5: Results of Preliminary Phytochemical Tests

| Sl no | Tests | Different extracts of <i>A. siliquosa</i> Lam | | | |
|-------|---------------|---|---------|----------|-----------------|
| | | Chloroform | Benzene | Methanol | Petroleum ether |
| 1. | Alkaloids | + | + | + | - |
| 2. | Carbohydrates | + | + | + | + |
| 3. | Glycosides | - | + | - | - |
| 4. | Phenols | - | - | - | - |
| 5. | Flavonoids | - | - | - | - |
| 6. | Amino acids | - | - | - | - |
| 7. | Tannins | - | - | - | - |
| 8. | Steroids | - | - | - | - |
| 9. | Saponin | - | - | - | - |

+ = Positive -- = Negative

Table 6: Rf value of sample of Root of *Apama siliquosa* Lam

| Short UV | Long UV | Post derivatization |
|-----------------|------------------|---------------------|
| - | 0.07 (F. Green) | - |
| - | 0.15 (F. blue) | 0.16 (Purple) |
| - | 0.20 (F. blue) | - |
| 0.30 (L. green) | - | - |
| - | - | 0.36 (Purple) |
| - | 0.51 (FL. green) | - |
| - | 0.62 (F. blue) | - |



Fig.1a Whole plant



Fig.1b Branch with swollen nodes



Fig.1c Flower of *A.siliquosa.Lam*



Fig.1d Root of *A.siliquosa.Lam*

Figure 1: Photo plates of *Apama siliquosa* Lam

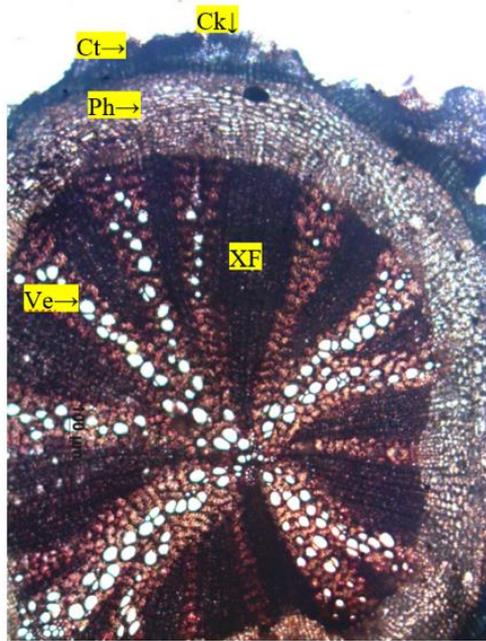


Fig 2a. Root of *A. siliquosa*

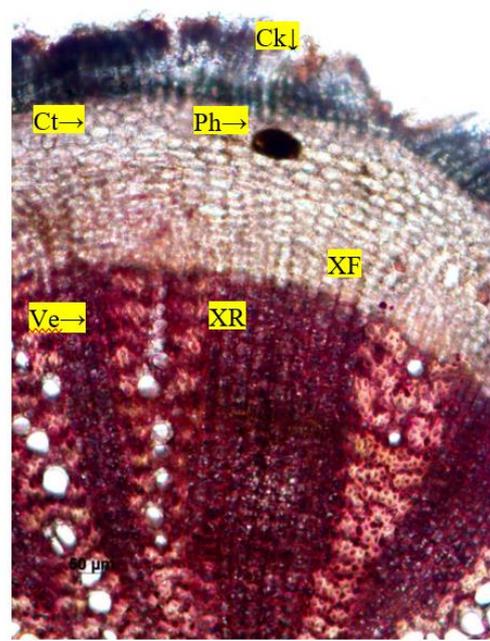


Fig 2b. A portion of root enlarged

Figure 2: T.S of root of *Apama siliquosa*

Ck – cork; Ct – cortex; Ph – phloem; XF – xylem fibres; XR – xylem ray; Ve – vessel

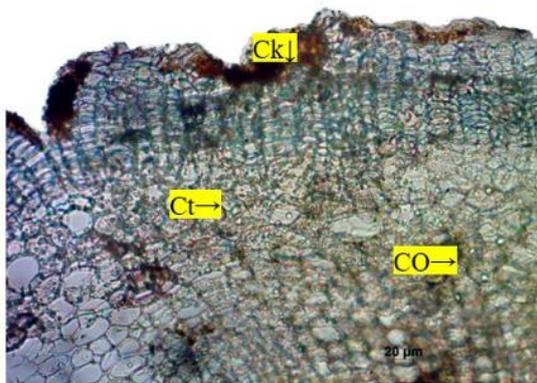


Fig 2c. Cork and cortex

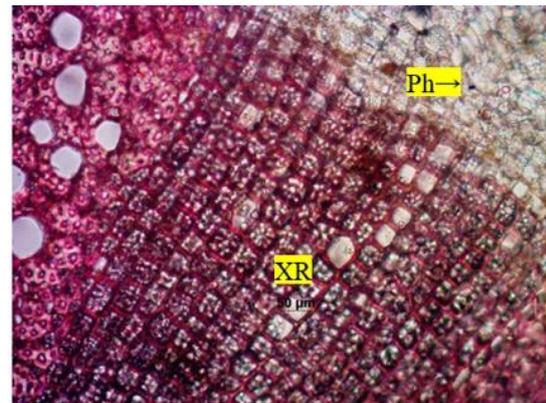


Fig 2d. Phloem and xylem

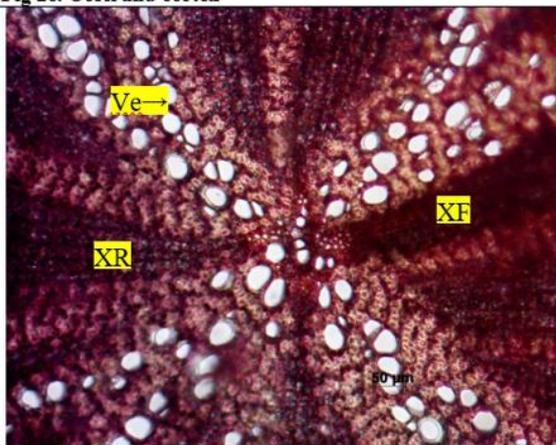


Fig 2e. Xylem rays, vessels and fibres

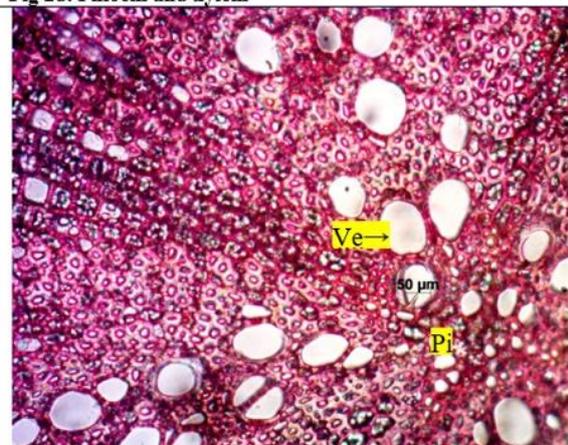


Fig 2f. Pith

Ck – cork; Ct – cortex; Ph – phloem; XF – xylem fibres; XR – xylem ray; Ve – vessel.

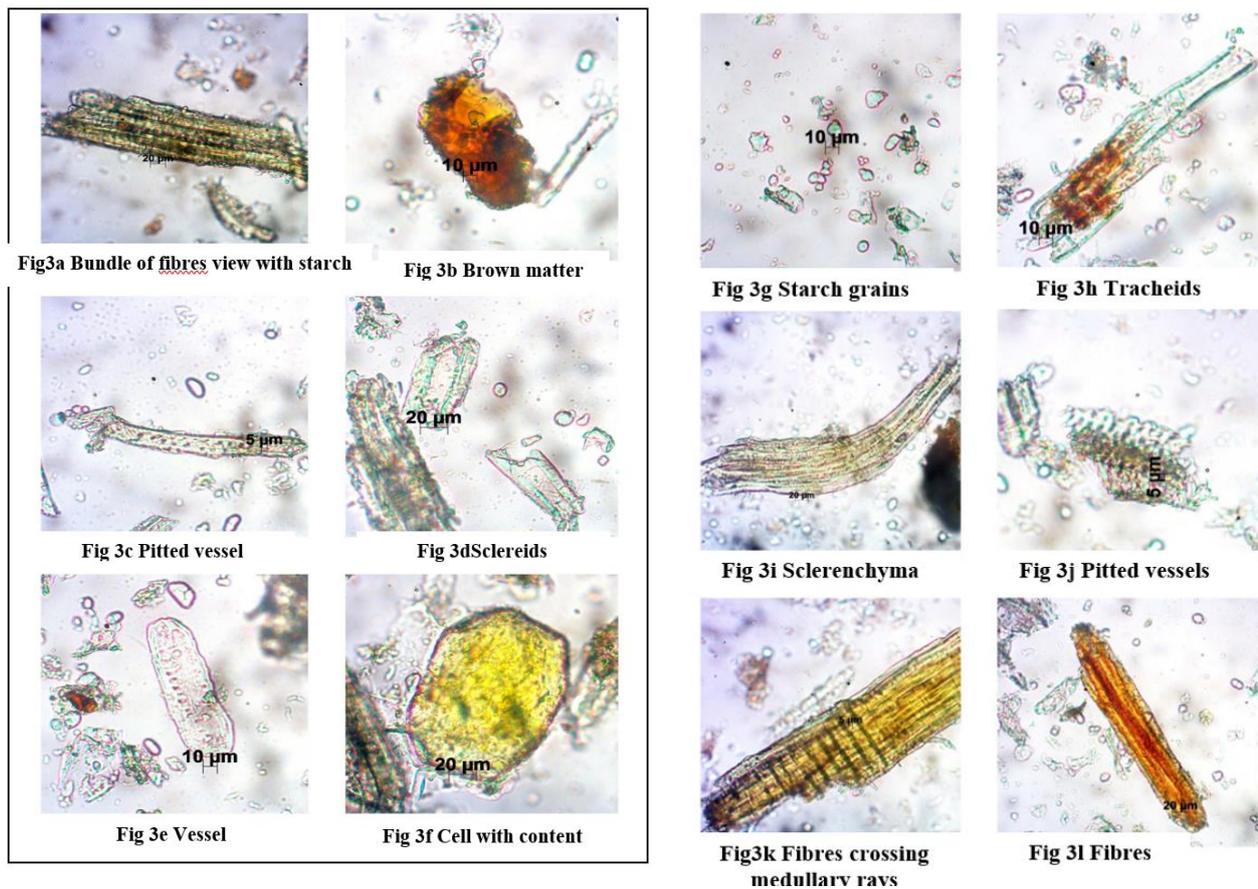


Figure 3: Powder microscopy of *Apama siliquosa* Lam

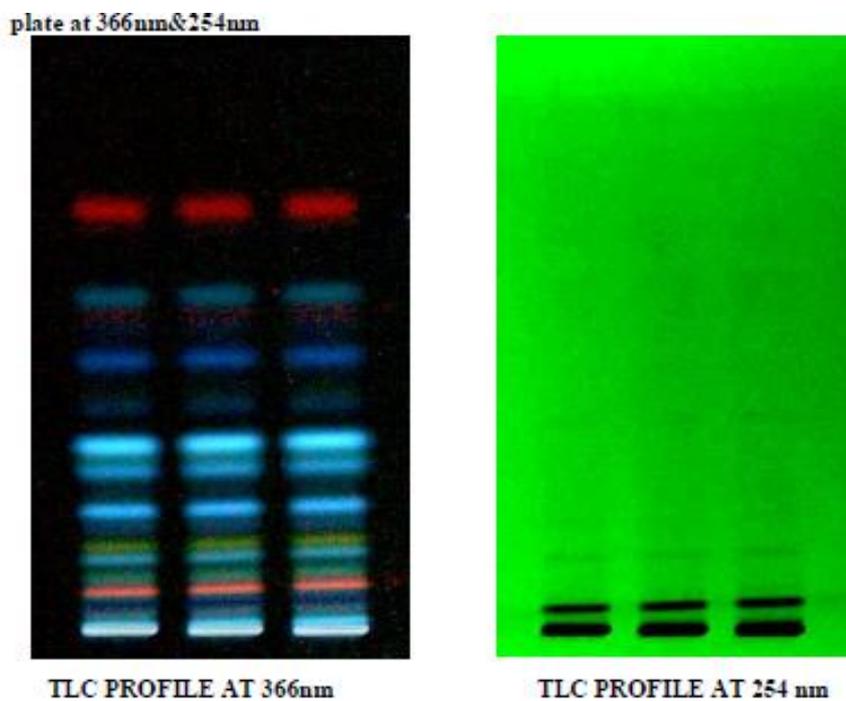


Figure 4: TLC Photo documentation of sample Root of *Apama siliquosa* Lam

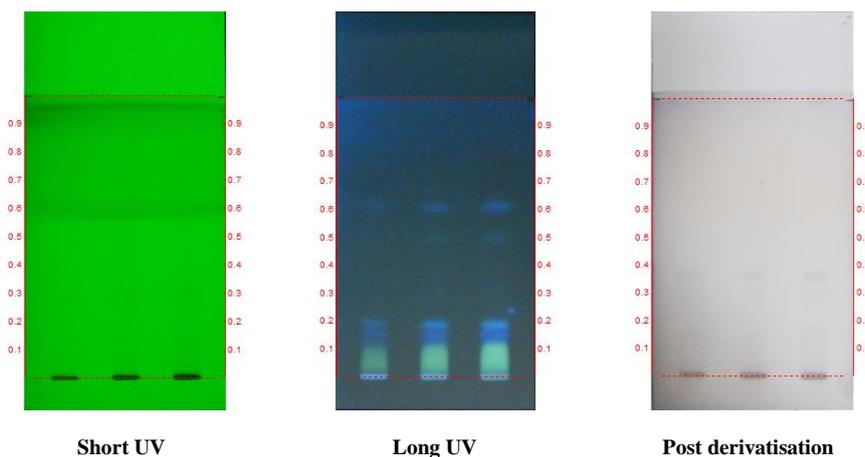
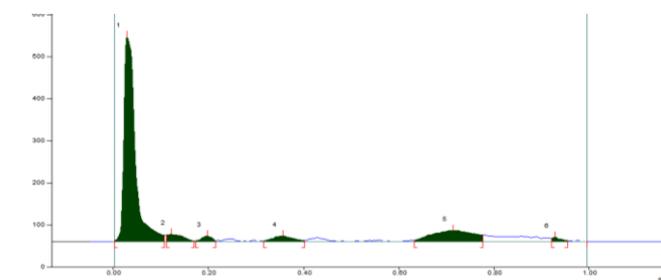


Figure 5: HPTLC photo documentation of sample Root of *Apama siliquosa* Lam

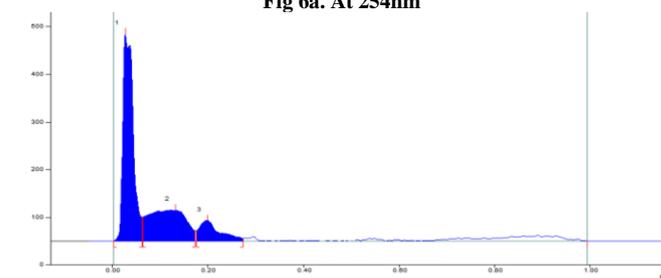
Track 1: Root of *Apama siliquosa*- 3 μ l
 Track 2: Root of *Apama siliquosa*- 6 μ l
 Track 3: Root of *Apama siliquosa*- 9 μ l
 Solvent system- Toluene: Ethyl acetate (7.0: 1.0)



Track 3, ID: *Apama siliquosa*

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|------|----------------|--------------|--------------|------------|---------|--------------|------------|-----------|---------|
| 1 | 0.00 Rf | 1.0 AU | 0.03 Rf | 485.5 AU | 85.83 % | 0.11 Rf | 15.1 AU | 8383.7 AU | 74.62 % |
| 2 | 0.11 Rf | 15.7 AU | 0.12 Rf | 17.4 AU | 3.08 % | 0.17 Rf | 1.5 AU | 418.4 AU | 3.72 % |
| 3 | 0.17 Rf | 2.1 AU | 0.20 Rf | 12.5 AU | 2.21 % | 0.22 Rf | 2.0 AU | 206.6 AU | 1.84 % |
| 4 | 0.32 Rf | 1.7 AU | 0.36 Rf | 13.0 AU | 2.29 % | 0.40 Rf | 2.5 AU | 412.2 AU | 3.67 % |
| 5 | 0.63 Rf | 2.6 AU | 0.71 Rf | 26.7 AU | 4.72 % | 0.78 Rf | 15.2 AU | 1677.5 AU | 14.93 % |
| 6 | 0.92 Rf | 7.7 AU | 0.93 Rf | 10.5 AU | 1.86 % | 0.96 Rf | 2.2 AU | 137.0 AU | 1.22 % |

Fig 6a. At 254nm



Track 3, ID: *Apama siliquosa*

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|------|----------------|--------------|--------------|------------|---------|--------------|------------|-----------|---------|
| 1 | 0.00 Rf | 0.7 AU | 0.03 Rf | 435.5 AU | 79.99 % | 0.06 Rf | 50.5 AU | 6859.9 AU | 57.05 % |
| 2 | 0.06 Rf | 50.6 AU | 0.13 Rf | 65.2 AU | 11.98 % | 0.17 Rf | 21.9 AU | 3800.8 AU | 31.61 % |
| 3 | 0.18 Rf | 21.9 AU | 0.20 Rf | 43.7 AU | 8.03 % | 0.27 Rf | 6.1 AU | 1363.5 AU | 11.34 % |

Fig 6b. At 366nm

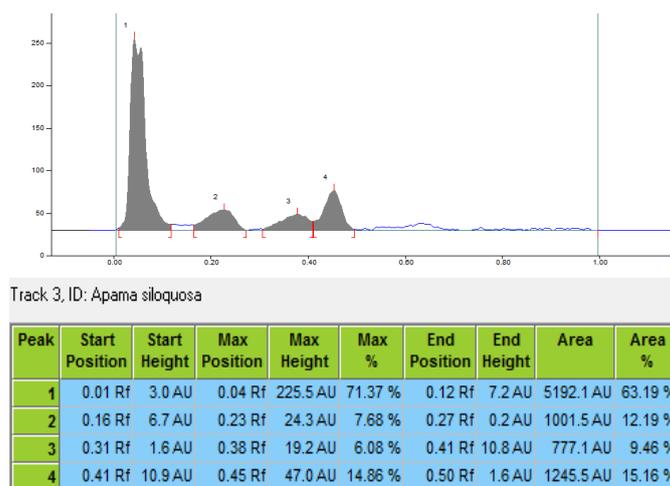


Fig 6c. At 620nm (After derivatization)

Figure 6: Densitometric scan of the sample Root of *Apama siliquosa*

Standardization of herbal medicine is the process of prescribing set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and agreeing upon technical standards.

In this study the standardization of drug was done by Pharmacognostical and phytochemical evaluation.

Pharmacognostical study:

Botanically authenticated samples of *Apama siliquosa* Lam were used for the study. The samples of *A. siliquosa* Lam root were found as genuine by performing Macroscopy and microscopy (T.S of *A. siliquosa*.Lam root, powder microscopy).Foreign matter analysis is an important factor in the quality control of the herbal drug. Percentage of foreign matter observed in the test drug was 1.02%.

Phytochemical study

Phytochemical analysis of *Apama siliquosa* Lam root powder revealed following results.

Loss on drying:

The loss on drying test is designed to measure the amount of water and volatile matters present in a sample when sample is dried under specified conditions.

Loss on drying was found to be 11.56%w/w

Total ash value:

Ash constitutes the inorganic residues obtained after complete combustion of a drug. Thus, ash value is a validity parameter to describe and to assess the degree of purity of a given drug.

Total ash is 4.92%.

Acid insoluble ash value:

Acid insoluble ash value is 0.39%.

Water soluble extractive value:

Water soluble extractive value plays an important role in the evaluation of crude drugs. Water soluble extractive value is 2%.

Alcohol soluble extractive value:

Alcohol soluble extractive value is 1.5%.

Qualitative analysis of Active Constituents:

Chloroform, Benzene, Methanol, Petroleum ether extracts of *Apama siliquosa* Lam was prepared and preliminary phytochemical tests were conducted.

In the above tests Alkaloids, Carbohydrate, Glycosides were found to be positive whereas Phenols, Flavonoids, Amino acids, Tannins, Steroids, Saponin were absent. Presence of alkaloids was detected by Hager's test in chloroform, benzene and methanol extract. Carbohydrate was positive in all the four extracts detected by Fehling's test. Benzene extract of the drug showed the presence of glycosides by Keller- Killiani test.

The TLC Plate shows major spots at Rf 0.07, 0.015 under 254nm and fluorescent spots at Rf value 0.03, 0.14, 0.22, 0.29, 0.34 (All blue), 0.07(purple), 0.10(Pink), 0.15(Green), 0.48(Violet), 0.59(Green), 0.73(Red)

CONCLUSION:

Folklore medicine lack textual reference in the classical texts. The effectiveness of the drug are evident by the use of the medicine. The detailed study of the drug helps in the standardization of herbal medicine. This study was conducted to investigate the Pharmacognostical and Phytochemical parameters. The report obtained with this study such as morphology, microscopic parameters, chemical reports can be used for the identification of the plant. TLC, HPTLC results showed the presence of certain elements with matching Rf values when visualized under UV radiations of various length.

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