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# Identity parameters on traditionally used Antiurolithiatic Herb - *Scoparia Dulcis* Linn.

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## ABSTRACT

**Introduction:** *Scoparia dulcis* Linn. locally known as *Manithumbe Gida* belongs to *Scophulariaceae* family and used in medicine by the traditional practitioners for the treatment of urinary calculi. **Materials and Methods**: Matured plants are collected from Udupi district and authenticated. Macro-microscopic features, physico-chemical standards, HPTLC and secondary metabolites were recorded as per standard guidelines. **Result**: TS of leaf has shown the presence of mesophyll and bi-collateral vascular bundles. Outer cork tissue, a layer of cortex and conjoint collateral closed vascular bundles and central pith are inclusions of stem TS. Pitted and reticulate vessels are characteristic features of plant powder. Physico-chemical standards and presence of alkaloids, carbohydrates, tannin and coumarins were indicative of its chemical nature. HPTLC fingerprints are a record of its different chemical constituents. Thus the quality monograph prepared on this drug beneficial in future research.

**Key words:** *Scoparia dulcis, Quality standards, HPTLC, Monograph.*

## INTRODUCTION

Plants are a source of food, cosmetics, medicine and many more since centuries. Though there exists innumerable number of these green sources, only few are identified, used and popularized.[1] Urbanization and simultaneous growing demand on herbal products are measure zone where one has to open his eyes towards all natural sources of drugs. It is an accepted fact that in principle all natural resources are having medicinal property, but rational utilization should be planned.[2] *Scoparia dulcis* Linn. locally known as *Manithumbe Gida* belonging to family *Scophulariaceae* is a popularly used antiurolithiatic drug in southern parts of India.[3] It is also known as *Kallurukki* i.e. stone crusher in *Malyalam* language.[4] It is an erect, usually many branched, annual plant growing 50-100cm tall, tropical plant. This weed grows in moist thickets or along sandy stream beds, commonly gathered from wild.[5]

It is indigenous to tropical America but commonly found as a weed in Bengal and Tamilnadu and in many parts of India.[6] Whole plant decoction is used in urinary calculi by traditional practitioners. An antidiabetic compound, amellin is also reported from leaves and stem of the plant, and is used in anemia, albuminuria, ketonuria and other complications associated with diabetes mellitus.[7] Such an important plant used by traditional community, but not yet recorded quality parameters. Hence an attempt to establish quality parameters of this matured medicinal weed, same presented in this paper.

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## Materials and Methods

Matured plant of *Scoparia dulcis* Linn. were collected from Udupi District, cleaned properly, authenticated using floras, and sample deposited at SDM Centre for Research in Ayurveda and Allied Sciences Udupi (Voucher No:859/17011801). Plant was washed properly in tap water and shade dried. After complete air drying the plant material was powdered and preserved for further study. Some fresh samples were preserved in FAA solution for microscopic study.

### Macroscopy

The external features of the test samples were documented using Canon IXUS digital camera. Organoleptic features of flowers like colour, taste, appearance, smell were recorded according to standard guidelines.[8]

### Microscopy

The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with saffranine. The slides were also stained with iodine in potassium iodide for detection of starch. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.[9]

### Powder microscopy

A pinch of powder was warmed with drops of chloral hydrate on a microscopic slide and mounted in glycerine. Slides were observed, photographed and characteristic features marked.[9]

### Physicochemical standards

The percentage of foreign matter, loss on drying, total ash and acid insoluble ash were determined according to the method described in Indian Pharmacopea and the WHO guidelines on quality control methods for medicinal plants materials.[10]

### Preliminary phytochemical screening

Preliminary phytochemical screening of the bark powder was performed using alcoholic extract to detect the presence of secondary metabolites.[11]

### HPTLC

1g. of *Scoparia dulcis* Linn. (whole plant) powder was extracted with 10 ml of alcohol. 3, 6 and 9µl of the above extract was applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene : Ethyl acetate (7.0 : 1.0). The developed plates were visualized in UV 254, 366, and then derivatised with vanillin sulphuric acid and scanned under UV 254nm, 366nm and 620nm. $R_f$, colour of the spots and densitometric scan were recorded.[12]

## Result

### Macroscopy

*S. dulcis* Linn. is a small branched herb found throughout India as a weed. Leaves are serrate 1.3 – 3.8 cm sub acute at apex. The leaves are dark green in colour with pubescence and petiole 9mm in length. Both the surfaces of the leaves having pinnate venation and having prominent veins.Flowers 3-6 from each whorl on slender 8-13 mm long pedicels, white colour with 4-5 sepals. It has a pungent odour. It is bitter in taste.

**Fig. 1: Macroscopy of *S. dulcis***

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## Microscopy

### TS of leaf

Transverse section of dorsiventral leaf showed thick upper epidermis, and lower epidermal cells. Between two epidermal layers, mesophyll was differentiated into palisade and spongy parenchyma. Spongy parenchyma had few layered loosely arranged cells. Mesophyll layer showed few rosette calcium oxalate crystals. Bicollateral vascular bundle was found with xylem on the inner side and phloem on the outer side. (Figure 2)

**Fig. 2: TS of leaf *S. dulcis* Linn.**

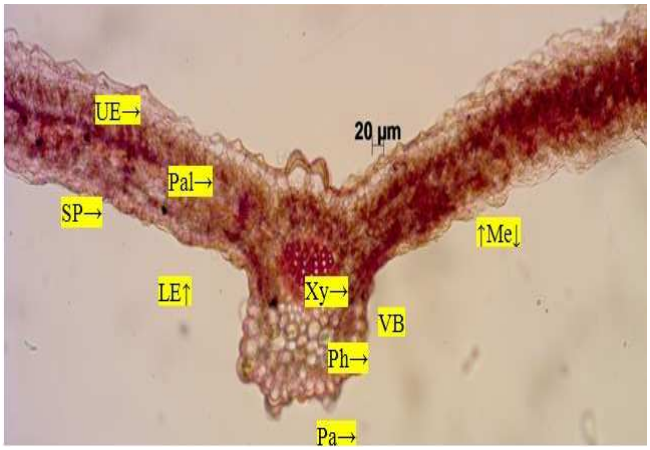


**Fig 2a. T.S of *S. dulcis* Linn.**

**LE -** lower epidermis; **Me -** mesophyll; **Pal -** palisade; **Ph -** phloem; **SP -** spongy parenchyma; **UE -** upper epidermis; **VB -** vascular bundle; **Xy -** xylem.

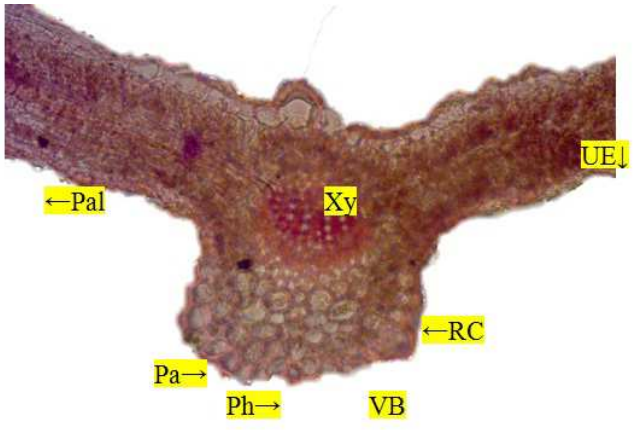


**Fig 2b. T.S of leaf enlarged**

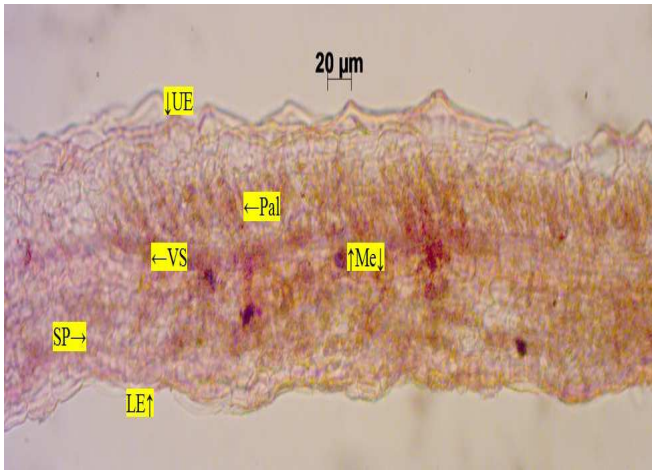


**Fig 2c. Lamina enlarged**

**LE -** lower epidermis; **Me -** mesophyll; **Pa -** parenchyma; **Pal -** palisade; **Ph -** phloem; **RC -** rosette crystal; **SP -** spongy parenchyma; **UE -** upper epidermis; **VB -** vascular bundle; **VS -** vascular strand; **Xy -** xylem.

**Fig. 3: Microscopy of matured stem of *S. dulcis* Linn.**

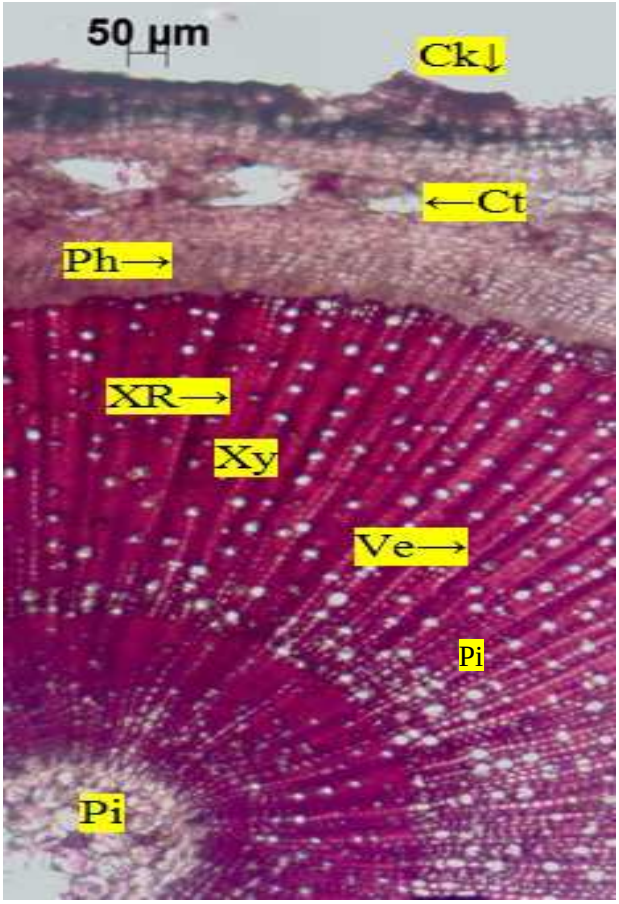


**Fig 3a. T.S of stem**

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### TS of stem

TS of stem is almost oval in outline. It shows outer cork tissue, a layer of cortex followed by phloem and rays of xylem surrounding central pith. Outer cork cells are tangentially arranged, few layered. Cells of cortex are parenchymatous and few cholenchymatous, bearing starch grains and stone cells. Few sclerenchymatous cells are also found. Phloem parenchyma surrounds inner xylem rays. Vascular bundles are conjoint collateral closed, with xylem on inner side and phloem on outerside. Central pith cells are of parenchyamtous with thick cell wall. (Figure 3,4 )

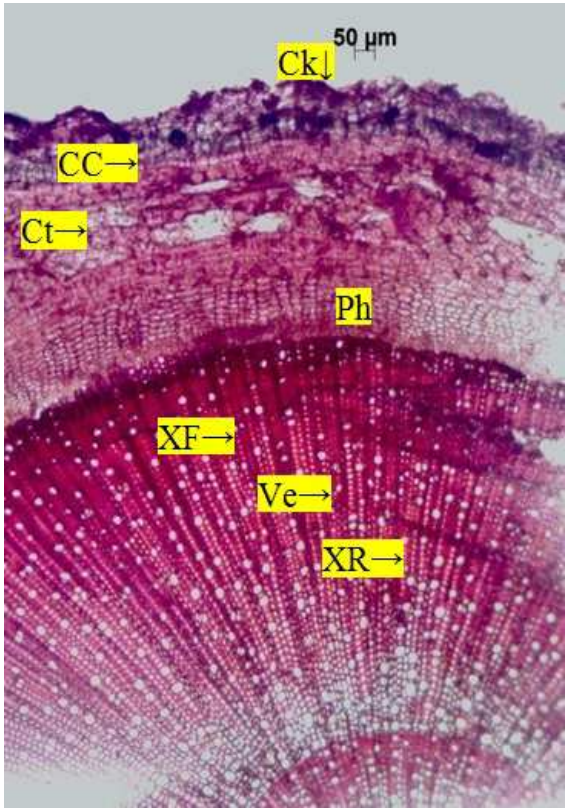


**Fig 3b. T.S of stem enlarged**
**CC -** cork cambium; **Ck -** cork; **Ct -** cortex; **Pa -** parenchyma; **PF -** phloem fibres; **Ph -** phloem; **Pi -** pith; **XF -** xylem fibres; **XR -** xylem rays; **Ve -** vessel.

### Powder microscopy

Powder shows fragments of fibres, pitted vessels, reticulate and spiral vessels. Parenchyma cells with and without starch grains are also found. Bunch of starch grains, anther, ovary and pollen grains are marked features of powder microscopy. (Figure 5)

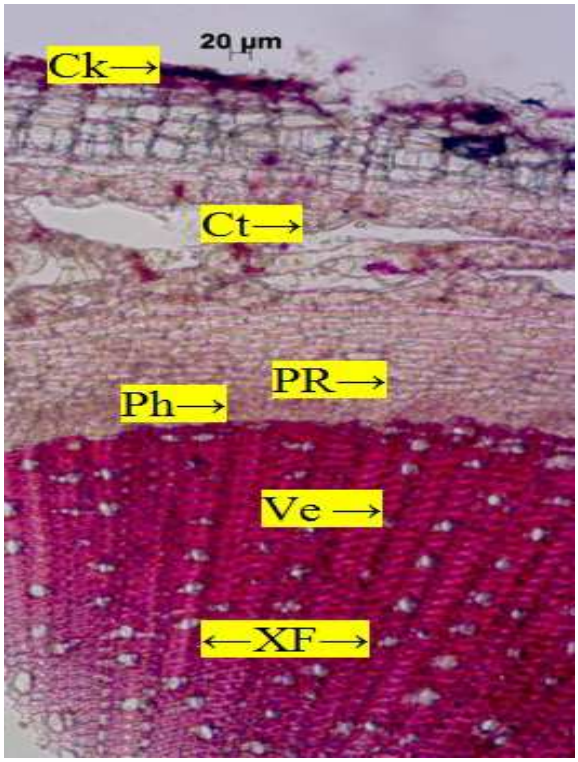


**Fig 3c. T.S of stem enlarged**

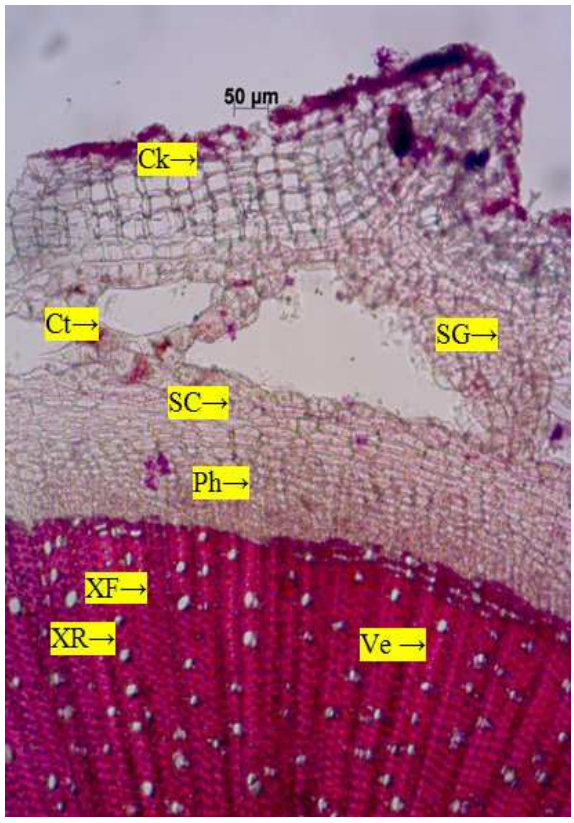


**Fig 3d. Cork, Cortex, xylem**
**Ck -** cork; **Ct -** cortex; **Pa -** parenchyma; **PF -** phloem fibres; **Ph -** phloem; **SG -** starch grains; **SC -** stone cells; **Ve -** vessel; **XF -** xylem fibres; **XR -** xylem rays.

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**Fig. 4: Microscopy of young stem of *S. dulcis* Linn.**

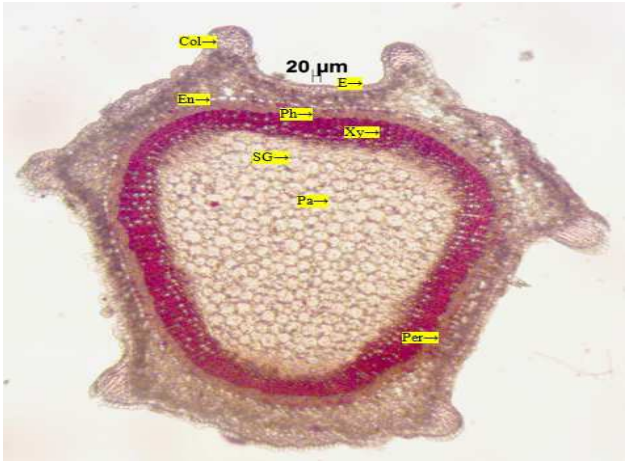



**Fig 4a. T.S of Young Stem**
**Col – Collenchyma; En – endodermis; E – epidermis;Pa – parenchyma;Per – pericyclic fibres;Ph – phloem; SG – starch grains; Xy–xylem.**

**Fig. 5a. Powder microscopy of *Scoporia dulcis* (Whole plant)**

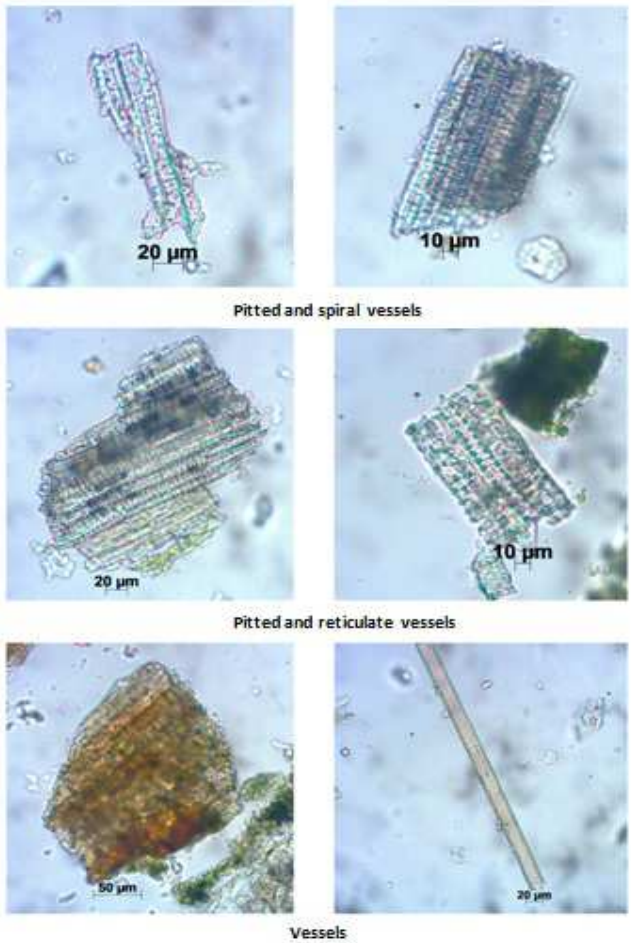
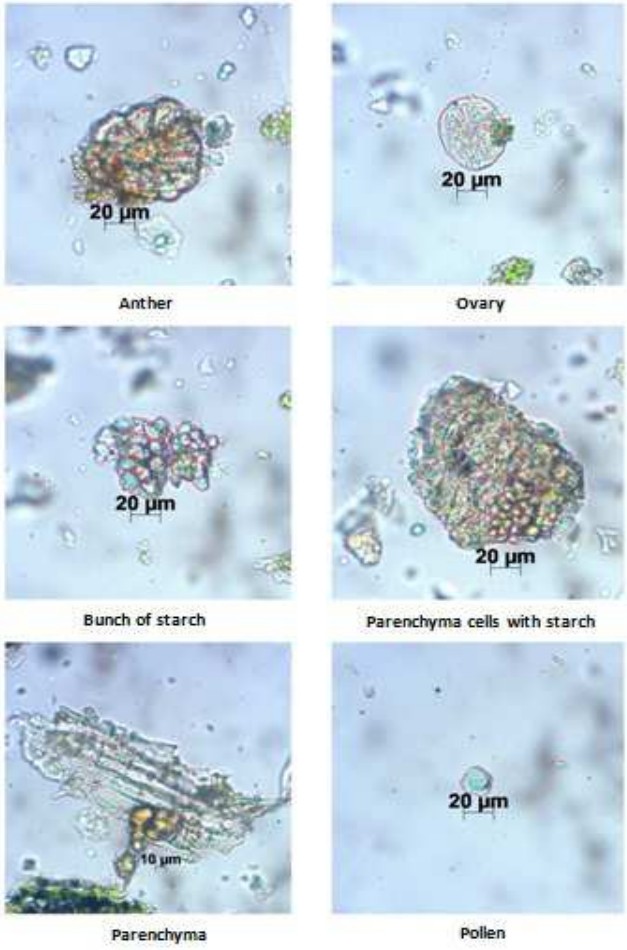



**Figure 5b. Powder microscopy of *Scoporia dulcis* (Whole plant)**




## Physico-chemical standards

*S. dulcis* Linn. whole plant powder was tested for loss on drying, total ash, acid insoluble ash, ethanol and water soluble extractive as per standard protocol. Loss on drying was 12.44 %w/w, total ash was 7.92% w/w, Acid insoluble ash 0.60%w/w, Water soluble ash3.29% w/w, alchohol soluble extractive7.97%w/w, water soluble extractive value 2.47%w/w. Physicochemical standards were a representative of its purity, physical nature and chemical composition. (Table 1)

**Table 1: Physicochemical standards of *Scoparia dulcis* Linn.**

| Parameter | Results n = 3 %w/w |
|---|---|
| Loss on drying | 12.44 |
| Total Ash | 7.92 |

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| | |
|---|---|
| Acid Insoluble Ash | 0.60 |
| Water soluble Ash | 3.29 |
| Alcohol soluble extractive value | 7.97 |
| Water soluble extractive value | 2.47 |

## Phytochemical study

Herbs are effective in particular therapeutics because of their chemical nature. Preliminary phytochemical test will decide basic chemical nature of the drug. The test drug powder has shown the presence of alkaloids, carbohydrates, tannin and coumarins.(Table 2)

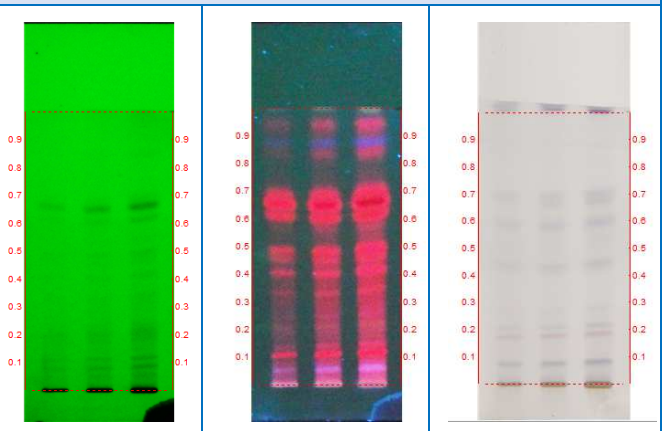
**Table 2: Preliminary phytochemical results of *Scoparia dulcis* Linn.**

| Test | Inference |
|---|---|
| Alkaloid | + |
| Steroid | - |
| Carbohydrate | + |
| Tannin | + |
| Flavanoids | - |
| Saponins | - |
| Terpenoid | - |
| Coumarins | + |
| Phenols | - |
| Carboxylic acid | - |
| Amino acids | - |
| Resin | - |
| Quinone | - |
| (+) - present; (-) – negative | |

## HPTLC

HPTLC finger print profile of ethanolic extract of S. dulcis has been obtained with Toluene: Ethyl acetate (7.0: 1.0) solvent system. The developed plates were visualized under UV light and white and then under light after derivatisation with vanillin sulphuric acid reagent. $R_f$, colour of the spots and densitometric scan at 254 and 366nm were recorded. On photodocumentation there were 11 spots under short UV, 15 at long UV, and 9 spots after post derivatisation(Figure 6, Table 3). Densitometric scan at 254 nm showed 13 peaks whereas at 366nm showed 17 peaks (Figure 7)

**Fig. 6. HPTLC photo documentation of ethanol extract of *S. dulcis* Linn.**



| Short UV | LongUV | Post derivatisation |
|---|---|---|

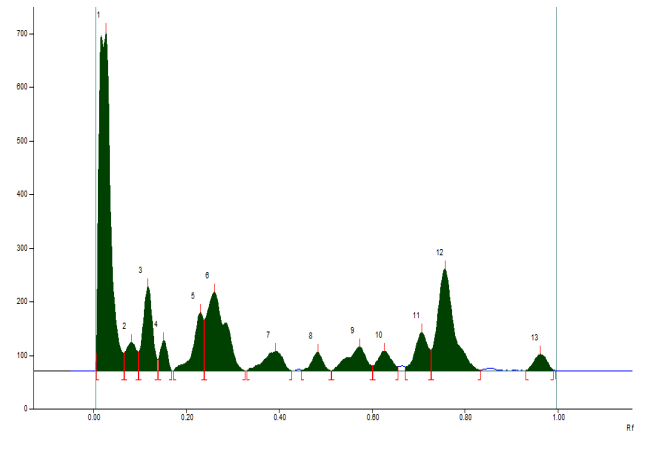
track 1-*Scoparia dulcis* – 3µl

track 2-*Scoparia dulcis* – 6µl

track 3-*Scoparia dulcis* – 9µl

Solvent system – Toluene: Ethyl Acetate (7.0: 1.0)

**Fig. 7.Densitometric scan of *S. dulcis* Linn.**



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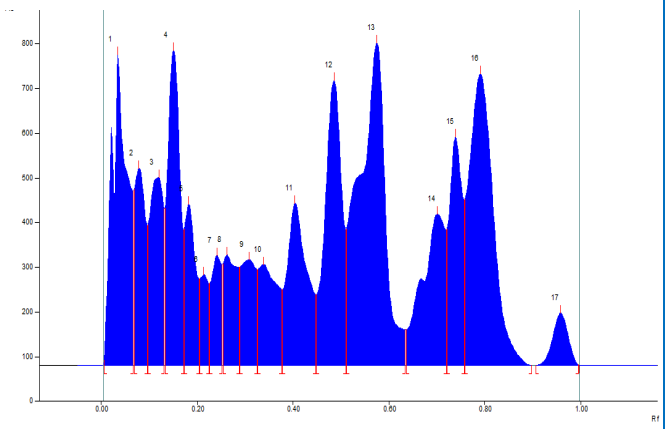
Track 3, ID: Scoparia dulcis

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.01 Rf | 33.0 AU | 0.03 Rf | 632.3 AU | 39.48 % | 0.07 Rf | 33.4 AU | 11278.0 AU | 35.95 % |
| 2 | 0.07 Rf | 34.1 AU | 0.08 Rf | 53.0 AU | 3.31 % | 0.10 Rf | 35.4 AU | 851.2 AU | 2.71 % |
| 3 | 0.10 Rf | 35.5 AU | 0.12 Rf | 157.1 AU | 9.81 % | 0.14 Rf | 21.3 AU | 2271.5 AU | 7.24 % |
| 4 | 0.14 Rf | 22.1 AU | 0.15 Rf | 56.7 AU | 3.54 % | 0.17 Rf | 0.8 AU | 628.9 AU | 2.00 % |
| 5 | 0.17 Rf | 0.2 AU | 0.23 Rf | 109.7 AU | 6.85 % | 0.24 Rf | 94.9 AU | 1717.2 AU | 5.47 % |
| 6 | 0.24 Rf | 95.0 AU | 0.26 Rf | 146.7 AU | 9.16 % | 0.33 Rf | 0.1 AU | 4196.6 AU | 13.38 % |
| 7 | 0.33 Rf | 0.3 AU | 0.39 Rf | 36.7 AU | 2.29 % | 0.43 Rf | 0.1 AU | 1074.0 AU | 3.42 % |
| 8 | 0.45 Rf | 1.6 AU | 0.48 Rf | 34.8 AU | 2.17 % | 0.51 Rf | 0.5 AU | 575.7 AU | 1.84 % |
| 9 | 0.51 Rf | 0.8 AU | 0.57 Rf | 44.5 AU | 2.78 % | 0.60 Rf | 9.3 AU | 1261.0 AU | 4.02 % |
| 10 | 0.60 Rf | 9.3 AU | 0.63 Rf | 37.2 AU | 2.32 % | 0.66 Rf | 8.2 AU | 762.9 AU | 2.43 % |
| 11 | 0.67 Rf | 7.1 AU | 0.71 Rf | 72.3 AU | 4.51 % | 0.73 Rf | 38.4 AU | 1420.3 AU | 4.53 % |
| 12 | 0.73 Rf | 38.8 AU | 0.76 Rf | 189.9 AU | 11.85 % | 0.83 Rf | 1.4 AU | 4714.0 AU | 15.03 % |
| 13 | 0.93 Rf | 1.1 AU | 0.96 Rf | 30.7 AU | 1.92 % | 0.99 Rf | 2.4 AU | 621.0 AU | 1.98 % |

**Fig 7a. At 254nm**



Track 3, ID: Scoparia dulcis

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.01 Rf | 10.8 AU | 0.04 Rf | 696.0 AU | 9.78 % | 0.07 Rf | 90.8 AU | 16002.9 AU | 9.08 % |
| 2 | 0.07 Rf | 393.7 AU | 0.08 Rf | 440.4 AU | 6.19 % | 0.10 Rf | 12.7 AU | 7083.1 AU | 4.02 % |
| 3 | 0.10 Rf | 314.8 AU | 0.12 Rf | 420.0 AU | 5.90 % | 0.13 Rf | 50.8 AU | 8468.9 AU | 4.80 % |
| 4 | 0.13 Rf | 351.3 AU | 0.15 Rf | 704.7 AU | 9.90 % | 0.17 Rf | 03.6 AU | 13003.5 AU | 7.38 % |
| 5 | 0.17 Rf | 305.4 AU | 0.18 Rf | 359.9 AU | 5.05 % | 0.20 Rf | 93.0 AU | 5724.7 AU | 3.25 % |
| 6 | 0.21 Rf | 193.1 AU | 0.21 Rf | 202.6 AU | 2.85 % | 0.23 Rf | 83.0 AU | 2536.5 AU | 1.44 % |
| 7 | 0.23 Rf | 183.4 AU | 0.24 Rf | 245.7 AU | 3.45 % | 0.25 Rf | 25.7 AU | 3779.7 AU | 2.14 % |
| 8 | 0.25 Rf | 227.5 AU | 0.26 Rf | 247.3 AU | 3.47 % | 0.29 Rf | 18.9 AU | 5061.5 AU | 2.87 % |
| 9 | 0.29 Rf | 219.4 AU | 0.31 Rf | 236.8 AU | 3.33 % | 0.33 Rf | 14.5 AU | 5234.0 AU | 2.97 % |
| 10 | 0.33 Rf | 214.7 AU | 0.34 Rf | 225.6 AU | 3.17 % | 0.38 Rf | 69.6 AU | 6426.5 AU | 3.65 % |
| 11 | 0.38 Rf | 169.8 AU | 0.40 Rf | 363.3 AU | 5.10 % | 0.45 Rf | 58.3 AU | 10874.8 AU | 6.17 % |
| 12 | 0.45 Rf | 158.9 AU | 0.49 Rf | 637.5 AU | 8.95 % | 0.51 Rf | 03.5 AU | 16004.2 AU | 9.08 % |
| 13 | 0.51 Rf | 305.8 AU | 0.58 Rf | 722.0 AU | 10.14 % | 0.64 Rf | 79.6 AU | 28457.5 AU | 16.14 % |
| 14 | 0.64 Rf | 79.7 AU | 0.70 Rf | 338.1 AU | 4.75 % | 0.72 Rf | 02.3 AU | 11778.3 AU | 6.68 % |
| 15 | 0.72 Rf | 305.8 AU | 0.74 Rf | 511.0 AU | 7.18 % | 0.76 Rf | 70.1 AU | 9687.9 AU | 5.50 % |
| 16 | 0.76 Rf | 371.3 AU | 0.79 Rf | 651.9 AU | 9.16 % | 0.90 Rf | 0.1 AU | 23470.4 AU | 13.32 % |
| 17 | 0.91 Rf | 0.2 AU | 0.96 Rf | 116.9 AU | 1.64 % | 1.00 Rf | 1.0 AU | 2672.3 AU | 1.52 % |

**Fig 7b. At 366nm**

**Table 3: $R_f$ values of samples**

| Short UV (254nm) | Long UV (366nm) | Post derivatisation |
|---|---|---|
| 0.06 (L. green) | 0.06 (FL. pink) | - |
| 0.08 (L. green) | - | 0.08 (L. purple) |
| 0.12 (L. green) | 0.12 (FD. red) | - |
| - | 0.14 (FD. red) | - |
| 0.18 (L. green) | - | 0.18 (L. purple) |
| 0.21 (L. green) | 0.21 (FD. red) | 0.21 (L. purple) |
| - | 0.25 (FD. red) | - |
| - | - | 0.28 (L. purple) |
| - | 0.30 (FD. red) | - |
| 0.35 (L. green) | 0.35 (FD. red) | 0.35 (L. purple) |
| - | 0.40 (FD. red) | - |
| 0.42 (L. green) | - | 0.42 (L. purple) |
| - | 0.44 (FD. red) | - |
| 0.48 (L. green) | 0.48 (FD. red) | - |
| 0.50 (L. green) | - | - |
| - | 0.60 (FD. red) | 0.60 (L. purple) |
| 0.62 (L. green) | - | - |
| 0.67 (D. green) | 0.67 (FD. red) | 0.67 (L. purple) |
| - | - | 0.70 (L. purple) |
| - | 0.85 (FD. red) | - |
| - | 0.88 (FD. violet) | - |
| - | 0.95 (FD. red) | - |

F - Fluorescent; L - Light; D - Dark

## DISCUSSION

*Scoparia dulcis* Linn.a member of *Scophulariaceae* family is a less known drug, but used by traditional practitioners in the treatment of urinary calculi. This is a perennial herb with woody stem commonly called as *Manitumbegida.* Scientifically recorded authentic standard parameters for herbs are an essential step in its global promotion as well as in clinical competence.[13] *S.dulci* Linn. provided with macro-microscopic illustrations, physicochemical standards, an inference of secondary metabolites and HPTLC are indicative of its quality standards.

TS of leaf has shown the presence of mesophyll which is differentiated into palisade and parenchymal layer, in between two epidermal layers. Bicollateral vascular bundles and presence of rosette calcium oxalate crystals at mesophyll layer are characteristic features of Leaf anatomy. Outer cork tissue, a layer of cortex

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consisting of parenchymatous and cholenchymatous cells possessing starch grains, stone cells are features of stem histology. Conjoint collateral closed vascular bundles and central pith are inclusions of stem TS. Powder shows fragments of fibres, pitted vessels, reticulate and spiral vessels, starch grains, anther, ovary and pollen grains. Physico-chemical standards are a measure of purity, and physical nature.[14] High loss on drying value (12.44 %w/w) indicates moisture content, whereas total ash value (7.92% w/w) shows total carbonaceous matter. Alcohol soluble extractive was much better (7.97%w/w) than water soluble extractive value (2.47%w/w). Alkaloids, carbohydrates, tannin and coumarins were main secondary metabolites detected out of test drug. HPTLC fingerprints are a record of its different chemical constituents. Thus the quality monograph prepared on this drug beneficial in future research.

## CONCLUSION

Growing herbal drug industry necessitates quality parameters for raw material for its global acceptance. *S. dulci* Linn. with scientifically recorded macro-microscopic features, along with other quality parameters forms authentic identity of the drug, thus prevent any admixture.

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