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CONCEPTUAL AND QUALITY CONTROL ANALYSIS OF *CHASSALIA CURVIFLORA* – AN EXTRAPHARMACOPEIAL PLANT

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ABSTRACT

Background: *Chassalia curviflora* (Wall.) Thwaites is a member of family Rubiaceae. It is used as a source plant for Sarpagandha by Kani tribe of Kerala. It grows as a weed and is easily available in the costal regions and has a great therapeutic potential as claimed by the folk practitioners. The leaf and root of this drug is used to treat jaundice and wounds. Decoction of root is given as a remedy in Phlegm, rheumatism and pneumonia.[1] **Objective:** Drug review along with pharmocognistic and phytochemical analysis of root of *Chassalia curviflora* (Wall.) Thwaites. **Methodology:** Review of detailed literature of the drug, macroscopy, powder microscopy, physicochemical analysis and HPTLC of the root was done. **Results and Conclusion:** Microscopy of root of *Chassalia curviflora* revealed that outermost cork region followed by cortex, phloem, xylem and innermost pith region. Cork has few layers tangentially elongated in radial rows. Loss on drying is 11.16±0.02%, Total ash value is 7.32±0.74%, Acid insoluble ash is 2.56±0.02%, Alcohol soluble extractive value is 5.47±0.02% and water soluble extractive value is 20.12±0.03%. Powder microscopy shows brown masses, cork cells, parenchyma containing stone cells, vessels, bundle of fibres, sclereids, tracheids, lichens and mosses and crystals of calcium oxalate. Phytochemical

screening of root of Chassalia curviflora shows presence of alkaloids, carbohydrates, tannins, flavonoids, triterpenoids and quinones. Along with physicochemical standards HPTLC with densitometry determination was also assessed for the root which shows different peaks at short and long UV.

KEYWORDS: *Chassalia curviflora*, folklore, *Anukta dravya*, pharmacognostical, phytochemical.

INTRODUCTION

The knowledge about a number of medicinal plants are available in Ayurvedic Literature and also been scientifically documented. Certain plants of Folklore origin have not been mentioned in the Classical texts of Ayurveda but are used traditionally to combat different diseases and are called as *Anukta Dravyas* or extra-pharmacopeial drugs. These anukta dravyas are the contribution of the various ethno medicinal survey studies. A large numbers of plants are used by tribals and folklore traditions in India for the treatment of cuts and wounds.

Chassalia curviflora as claimed by the traditional practitioners of Kani tribe of Kerala is a potent source of Sarpagandha and is effective as an antidote for snake bite, insect bites and is also used in wound healing. Since Sarpagandha is an endangered species it is important to know about the other potent sources for therapeutic purposes. It is also proven to have activities like anti-inflammatory, analgesic, hepatoprotective, sedative, anti-epileptic, anti-oxidant, anti-hypertensive, and anti-microbial.[2]

A quality standard of natural drug is necessary as referral in herbal drug research.Preliminary phytochemistry of such extra pharmacopoeial drugs will help to know about the efficacy of various parts of the plants which can further be used in treatment aspect. It also ensures safety and scientific validation of these drugs for their use in both preventive and curative aspect. Hence in present study an attempt is made to portray pharmacognostic standard parameters of *Chassalia curviflora.*

MATERIALS AND METHODS

Drug source

A research of multiple bibliographical databases and traditional Ayurvedic text books was conducted and the articles analyzed under various key themes.

Botanically identified root of *Chassalia curviflora* (Wall.) Thwaites was procured from natural habitat of Udupi. Roots were dried and stored in pharmacognosy laboratory of SDM Centre for research in Ayurveda and Allied sciences, Udupi until microscopic evaluation.

BOTANICAL NAME: *Chassalia curviflora* (Wall.) Thwaites

FAMILY: Rubiaceae.

TAXONOMICAL POSITION[3]

Kingdom- Plantae

Division- Angiosperms

Phylum- Tracheophyta

Class- Eudicots

Family- Rubiaceae

Genus- Chassalia

Species- C. curviflora

REGIONAL AND OTHER NAMES[4]

- Assamese- Titahukuta
- Kannada- Kaadu garudapatala
- Malyalam- Vellakurinji
- Marathi- Anatabi

DISTRIBUTION

It is found in Eastern Himalayas from Sikkim Eastwards toAssam at an Altitude of 600-1800m. In Penisular India from Orissa to Maharashtra Southwards and in Andaman and Nicobar Islands.[5]

HABIT

It is an erect shrub of height 60-90cm in height. Leaves are upto 18X6.5 cm, elliptic or oblong, lanceolate, acute or acuminate at apex, cuneate at base, membranous and glabrous. Flowers are sessile in trichotomously branched pedunculed terminal cymes, dimorphic, corolla pinkish-white tube 1.8cm long, slender and curved. Fruits are berry (size of a pea), dark purple to black in colour.

Flowering: more or less throughout the year

SYNONYMS[6]

Chassalia ambigua

Chassalia lurida

Chassalia ophioxyloides

Chassalia longifolia

Psychotaria ambigua

TRADITIONAL MEDICINAL USES[7]

Decoction of root is used in rheumatism, pneumonia, head disorders, ear and eye diseases and sore throat. Root and leaf are used as poultice for external applications in wounds, ulcers and headache. Whole plant is used in the Waynad district for Skin conditions. Chakma tribes of Bangladesh make use of crushed leaves to the wounds for treating snake and insect bites. It is widely used by Kani tribes of Agasthiyamalai, Kerala as an effective medicine for the treatment of jaundice.

Pharmacognostical evaluation

Collection and authentication

The plant *Chassalia curviflora* (Wall.) Thwaites was authenticated botanically by referring Flora of Udupi. Fresh root was collected from the well matured plant from Udupi district of Karnataka, India. The sample was deposited at SDM centre for Research in Ayurveda and Allied Sciences, Udupi (Voucher specimen number 1396/22072001). The root of *Chassalia curviflora* (Wall.) Thwaites dried in shade powdered and sift through sieve number 40; the powder was stored in glass vials until microscopic evaluation.

Macroscopy

The external features of the test samples were documented using Canon IXUS digital camera. Macroscopic characters like size, shape, texture and colour were noted in detail. The macroscopic features were compared to local flora for authentication.

Microscopy

Sample was preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse section using a sharp blade and

the sections were stained with safranin. 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.

Powder microscopy

A pinch of the sample was mounted on a microscopic slide with a drop of glycerin-water.Characters were observed using using Zeiss AXIO trinocular microscope attached with Zeiss Axiocam camera under bright field light. Magnifications of the figures are indicated by the pre-calibrated scale-bars using Zeiss Axio vision software.

Physico-chemical standards:

Powder of the sample tested for physico-chemical standards like loss on drying, total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive value, water soluble extractive value as per standard guidelines.

Preliminary phytochemical analysis

The preliminary phytochemical tests also included tests for alkaloids, tests for carbohydrates, tests for steroids, tests for saponins, tests for tannins, tests for flavonoids, tests for phenol, tests for coumarins, tests for triterpenoids, tests for resin, tests for carboxylic acid and tests for quinone.

HPTLC profiling

1gm of powdered sample of Root of Chassalia curviflora was dissolved in 10.0ml of alcohol kept overnight and filtered. 4, 8 and 12µl of each of the above extract was applied on a pre-coated silica gel F 254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. *Chassalia curviflora* sample plate was developed in Toluene: Ethyl acetate: Formic acid: Methanol (7:5:1:0.5). The developed plates were visualized in short UV, long UV and then derivatised with Vanillin sulphuric acid reagent subsequently scanned under UV 254nm, 366nm and 620nm (after derivatisation). RF, colour of the spots, densitometric scan and 3-D chromatograms were recorded.[8]

RESULT

Macroscopy and Microscopy

TS of root which is circular in outline with the wavy margin has outermost cork region followed by cortex, phloem, xylem and innermost pith region. Cork has few layers

tangentially elongated in radial rows. The cortical cells are thick walled parenchumatous with starch grains. Phloem in young root has a very narrow zone which is parenchymatous containing few starch grains, raphide crystals. Rosette cambium is present in matured root which is distinct 3-5 layers of small rectangular cells. Xylem has xylem rays, xylem fibres and xylem vessels. The central region contains pith which has large parenchyma cells with intercellular space.

1 (a) **1 (b)**

Figure 1: Macroscopy of Root of *Chassalia curviflora.*

Figure 2: Microscopy of Root of *Chassalia curviflora*

Fig 2b. A portion enlarged

Ca – cambium; Ck – cork; Ct – cortex; MR – medullary ray; PF – phloem fibres; Ph – phloem; SC – stone cells; XF – xylem fibres; XR – xylem ray; Ve – vessel.

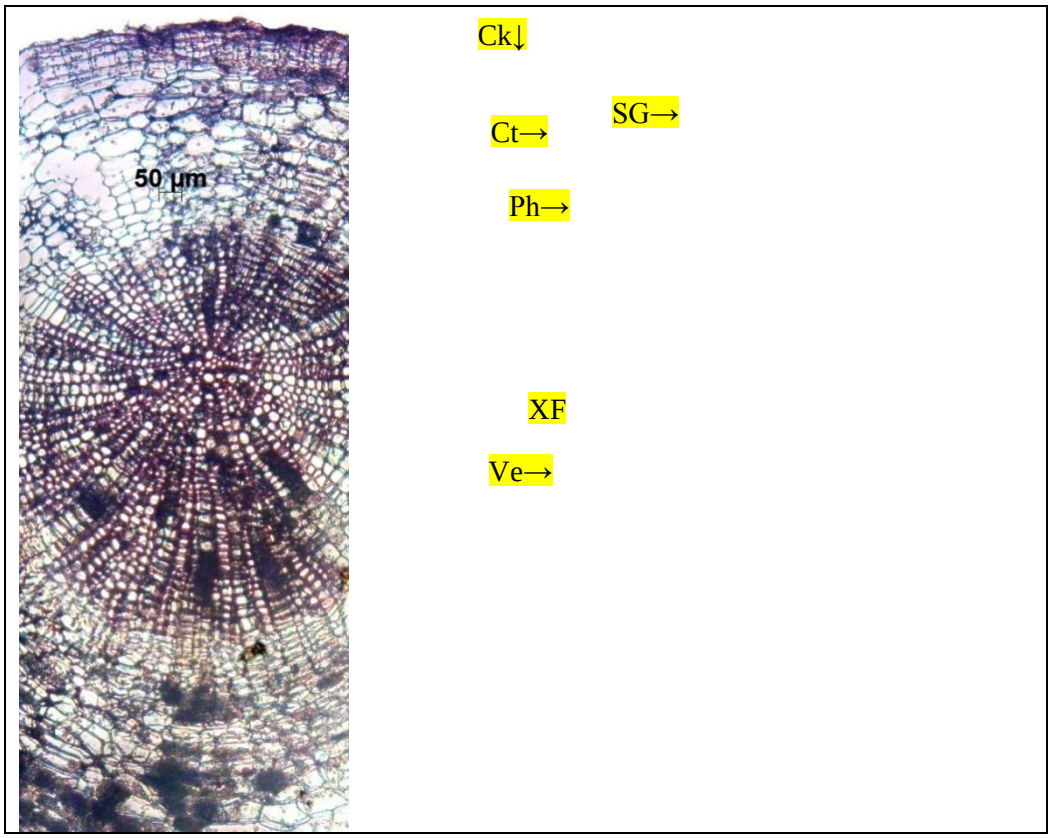


Fig 2c. T. S of root

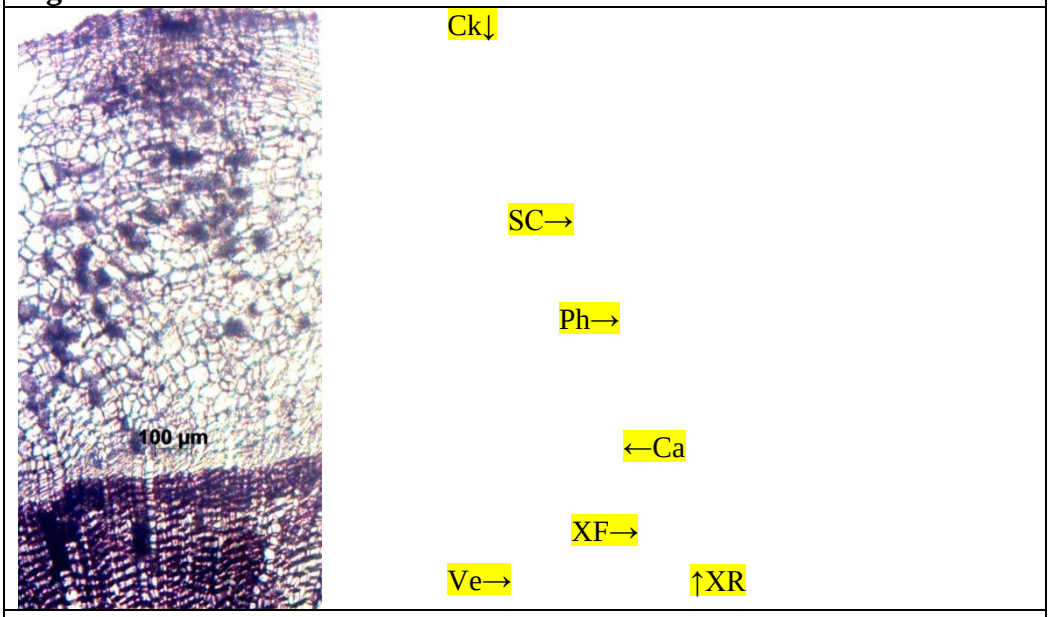


Fig 2d. Cork, cortex, phloem

Ca – cambium; Ck – cork; Ct – cortex; Ph – phloem; SC – stone cells; SG – starch grains; XF – xylem fibres; XR – xylem ray; Ve – vessel.

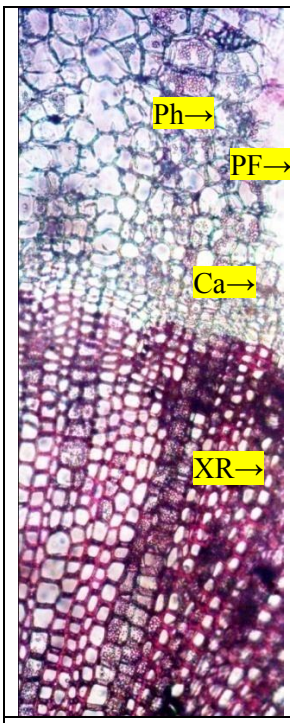


Fig 2e. Phloem, cambium and Xylem

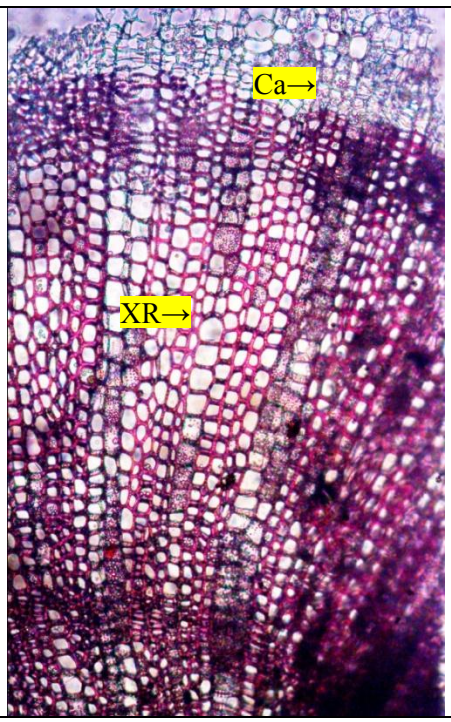


Fig 2f. cambium and xylem

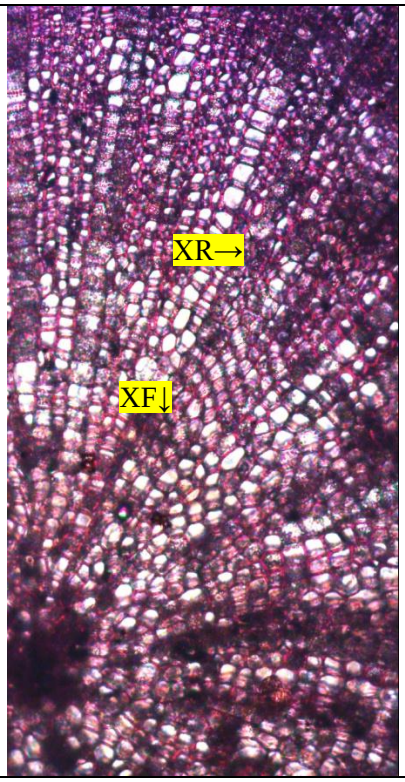


Fig 2g. Xylem rays

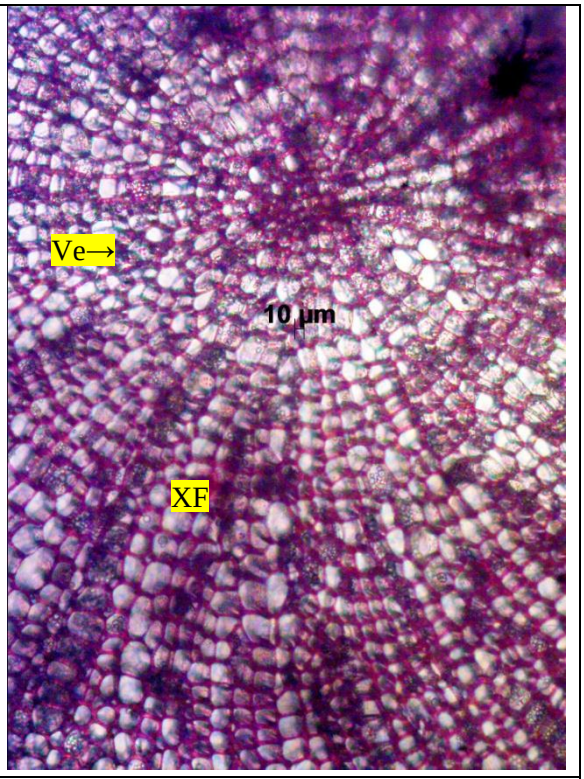


Fig 2h. Xylem

Ca – cambium; PF – phloem fibres; Ph – phloem; XF – xylem fibres; XR – xylem ray; Ve – vessel.

Powder microscopy characteristics

The root powder characteristics are brown masses, cork cells, parenchyma containing stone cells, vessels, bundle of fibres, sclereids, sclereids in group, tracheids, lichens and mosses and crystals of calcium oxalate. Parenchyma containing brown mass/ pigment cell.

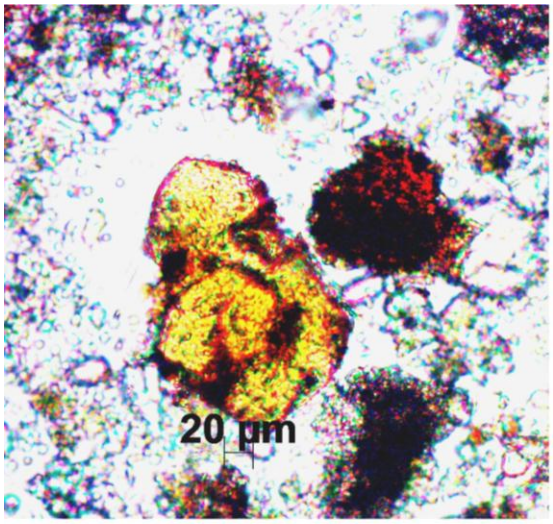


Fig 3.1 Brown masses

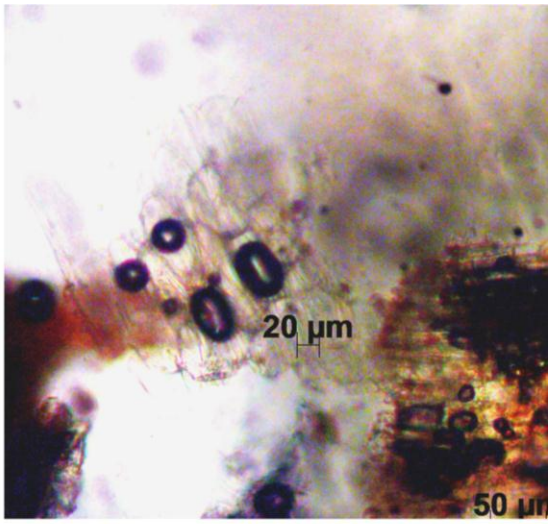


Fig 3.2 Parenchyma containing stone cells

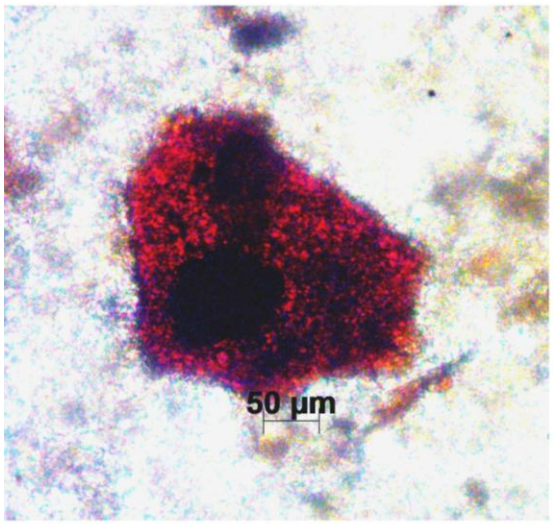


Fig 3.3 Parenchyma with pigment cells

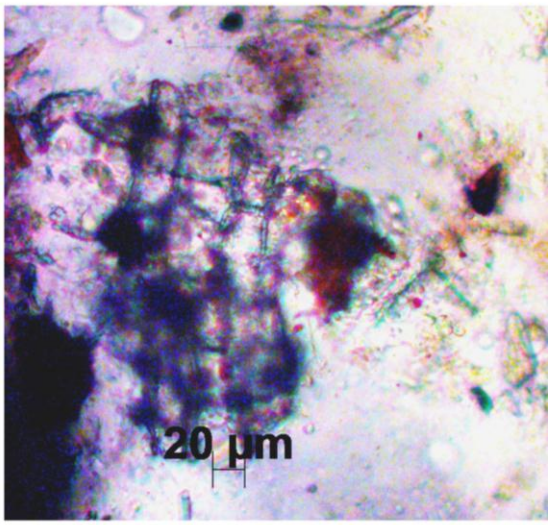


Fig 3.4 Parenchyma cells with starch

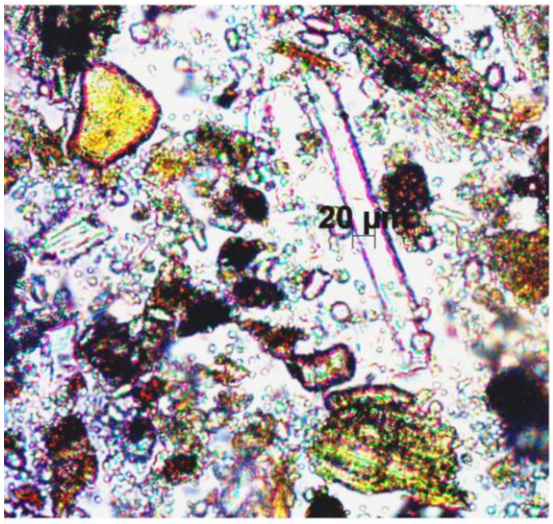


Fig 3.5 Vessel

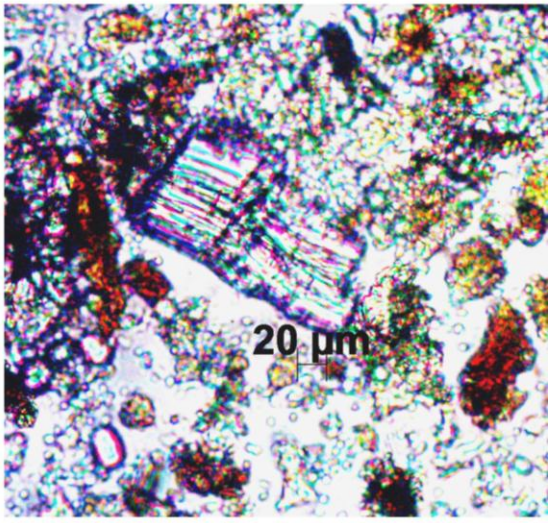


Fig 3.6 Fibres in group

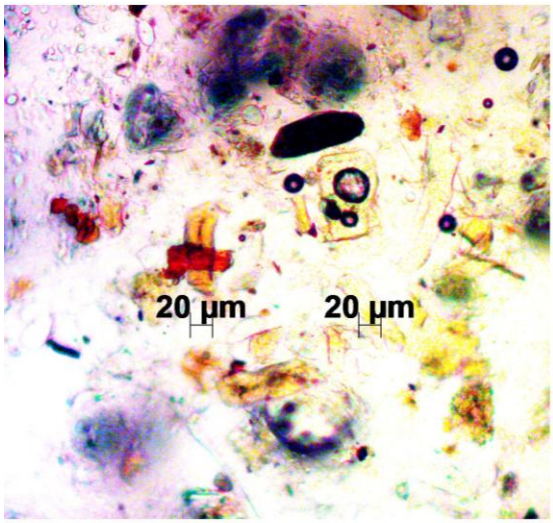


Fig 3.7 Sclereids

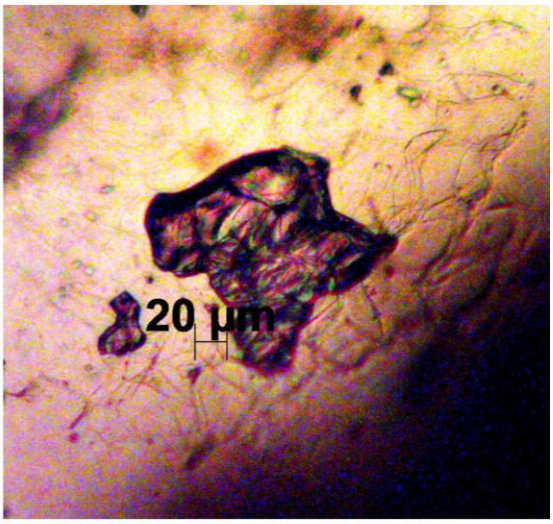


Fig 3.8 Sclereids in groups

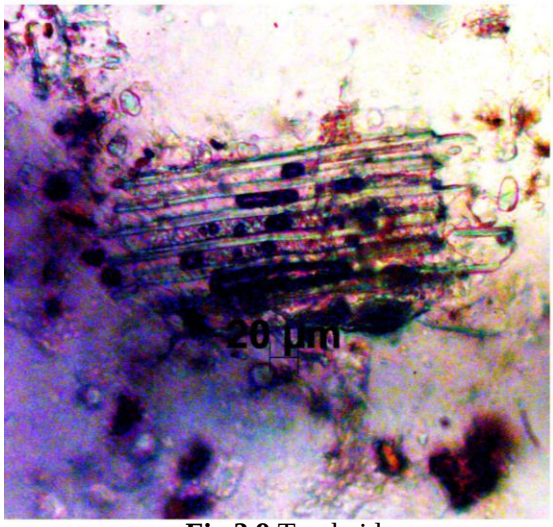


Fig 3.9 Tracheids

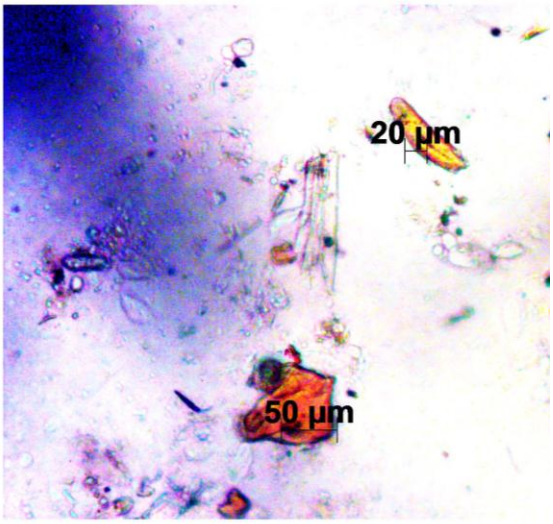


Fig 3.10 Sclereids

Fig 3.11 Likens and moss

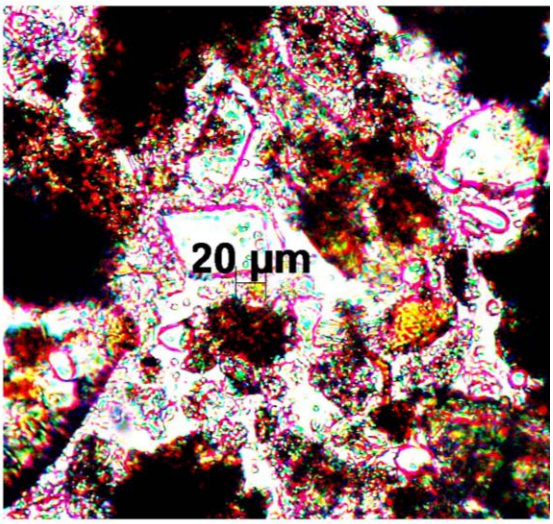


Fig 3.12 Crystals of calcium oxalate

Figure 3: Powder microscopy of Root of *Chassalia curviflora*

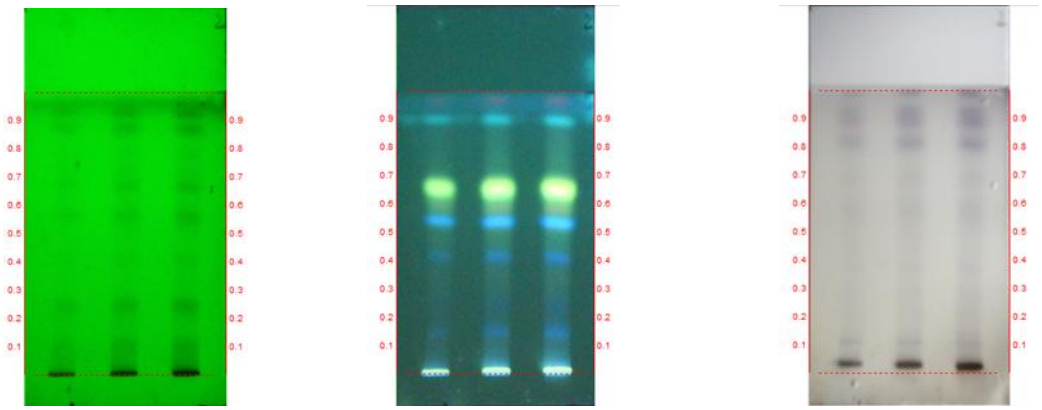
Table 1: **Results of standardization parameters of *Chassalia curviflora.***

Parameters	**Results n= 3%w/w (Avg±SD)**
Loss on drying	11.16±0.02
Total Ash	7.32±0.74
Acid Insoluble Ash	2.09±0.01
Water soluble Ash	2.56±0.02
Alcohol soluble extractive value	5.47±0.02
Water soluble extractive value	20.12±0.03

Table 2: Results of preliminary phytochemical screening of *Chassalia curviflora.*

Test	**Inference**
	Chassalia curviflora
Alkaloid	+
Steroid	-
Carbohydrate	+
Tannin	+
Flavonoids	+
Saponins	-
Triterpenoids	+
Coumarins	-
Phenols	-
Carboxylic acid	-
Amino acids	-
Resin	-
Quinone	+

Present- (+) , Negative (-)



Short UV **Long UV** **Post derivatisation**

Figure 4. HPTLC photo documentation of ethanol extract of Root of *Chassalia curviflora.*

Track 1 - Root of Chassalia curviflora – 4µl

Track 2 - Root of Chassalia curviflora – 8µl

Track 3 - Root of Chassalia curviflora – 12µl

Solvent system – Toluene: Ethyl acetate: Formic acid: Methanol (7:5:1:0.5)

Table 1: R_f values of sample of Root of *Chassalia curviflora.*

Short UV	Long UV	Post derivatisation
0.10 (Green)	-	0.11 (Purple)
-	0.15 (F. blue)	-
-	-	0.22 (Purple)
0.25 (Green)	0.24 (F. blue)	-
-	-	0.40 (Purple)
0.42 (Green)	0.42 (F. blue)	-
-	0.50 (F. blue)	-
0.56 (Green)	-	-
-	0.64 (F. green)	-
0.67 (Green)	-	-
-	-	0.70 (Purple)
0.73 (Green)	-	-
0.77 (Green)	-	-
-	-	0.82 (Purple)
0.89 (Green)	0.89 (F. blue)	0.91 (Purple)
0.92 (Green)	-	0.93 (Purple)

*F – Fluorescent; L –Light; D – Dark

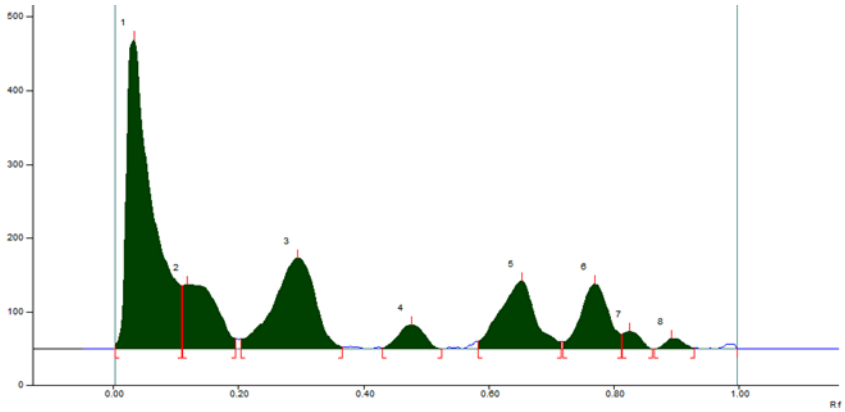


Figure 5. Densitometric scan of Root of *Chassalia curviflora.*

Track 3, ID: Root of Chassalia curviflora

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	3.4 AU	0.03 Rf	419.2 AU	47.68 %	0.11 Rf	85.5 AU	12535.5 AU	41.97 %
2	0.11 Rf	85.7 AU	0.12 Rf	87.0 AU	9.90 %	0.20 Rf	14.2 AU	3358.6 AU	11.25 %
3	0.20 Rf	13.4 AU	0.30 Rf	123.3 AU	14.02 %	0.37 Rf	2.6 AU	5771.7 AU	19.32 %
4	0.43 Rf	0.8 AU	0.48 Rf	32.7 AU	3.72 %	0.53 Rf	0.2 AU	965.8 AU	3.23 %
5	0.58 Rf	10.1 AU	0.65 Rf	92.1 AU	10.47 %	0.72 Rf	9.5 AU	3692.2 AU	12.36 %
6	0.72 Rf	9.5 AU	0.77 Rf	88.0 AU	10.01 %	0.81 Rf	19.9 AU	2791.1 AU	9.35 %
7	0.81 Rf	20.2 AU	0.83 Rf	23.2 AU	2.64 %	0.86 Rf	0.1 AU	458.8 AU	1.54 %
8	0.87 Rf	0.1 AU	0.89 Rf	13.7 AU	1.56 %	0.93 Rf	1.2 AU	293.0 AU	0.98 %

Figure (5a) at 254 nm

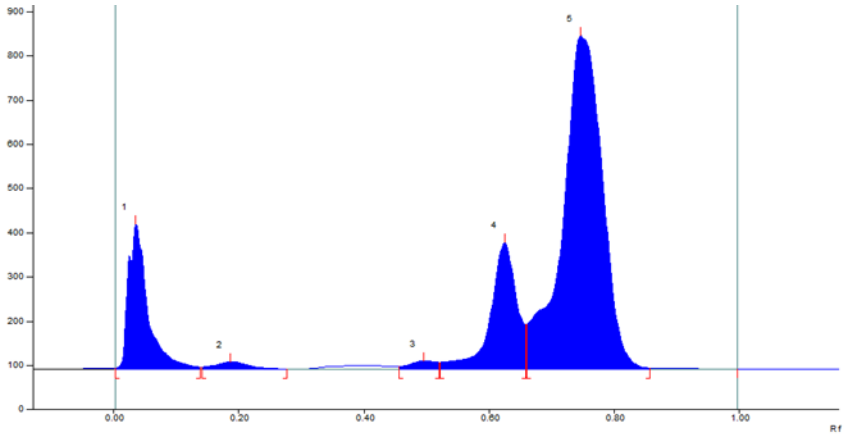
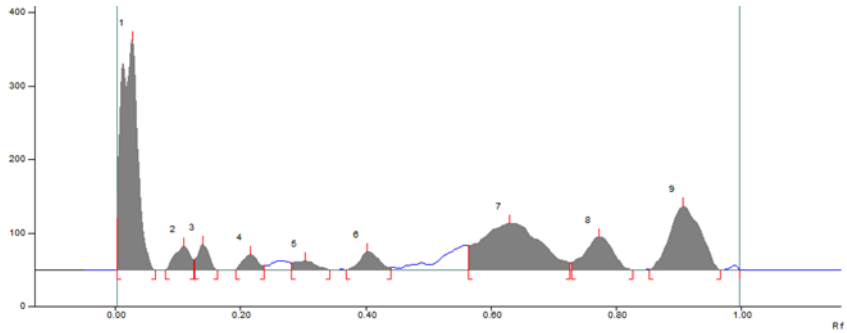


Figure 5 (b) at 366 nm



Track 3, ID: Root of Chassalia curviflora

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	69.3 AU	0.03 Rf	312.3 AU	49.33 %	0.06 Rf	0.0 AU	5875.9 AU	34.81 %
2	0.08 Rf	0.1 AU	0.11 Rf	32.4 AU	5.12 %	0.13 Rf	14.1 AU	580.9 AU	3.44 %
3	0.13 Rf	14.7 AU	0.14 Rf	34.7 AU	5.48 %	0.16 Rf	0.1 AU	451.2 AU	2.67 %
4	0.19 Rf	0.5 AU	0.22 Rf	21.2 AU	3.35 %	0.24 Rf	6.1 AU	360.0 AU	2.13 %
5	0.28 Rf	10.0 AU	0.30 Rf	12.4 AU	1.95 %	0.34 Rf	0.0 AU	309.2 AU	1.83 %
6	0.37 Rf	0.1 AU	0.40 Rf	25.0 AU	3.94 %	0.44 Rf	3.3 AU	543.8 AU	3.22 %
7	0.56 Rf	33.5 AU	0.63 Rf	63.7 AU	10.06 %	0.73 Rf	10.3 AU	4283.5 AU	25.38 %
8	0.73 Rf	10.4 AU	0.77 Rf	45.2 AU	7.14 %	0.83 Rf	0.0 AU	1437.2 AU	8.51 %
9	0.85 Rf	0.7 AU	0.91 Rf	86.2 AU	13.62 %	0.97 Rf	0.0 AU	3038.2 AU	18.00 %

Figure 5 (c) At 620nm (Post derivatisation with VSA)

DISCUSSION

Since there is an ever increasing demand for herbal drugs many of the original sources of these drugs are either extinct or on the verge of extinction and also there are more chances of adulteration so in order to avoid that it is better if the regional sources are explored for their potency and efficacy and provide better treatment.

The drug Chassalia curviflora (Wall.) has numerous medicinal properties and is used as a potent source of Sarpgandha. The decoction of root is used in rheumatism, pneumonia, head disorders, ear and eye diseases and sore throat. Root and leaf are used as poultice for external applications in wounds, ulcers and headache. Whole plant is used in the Waynad district for Skin conditions. Microscopy of root of *C. curviflora* revealed that outermost cork region followed by cortex, phloem, xylem and innermost pith region. Cork has few layers tangentially elongated in radial rows. Powder microscopy shows brown masses, cork cells, parenchyma containing stone cells, vessels, bundle of fibres, sclereids, tracheids, lichens and mosses and crystals of calcium oxalate.In physicochemical analysis loss on drying was 11.16% since the root is very rich in moisture and roots were shade dried to an optimum level 11.16% was the moisture left where no microbial growth was observed. Total ash was 7.32% which constitutes both organic and inorganic materials. Acid insoluble ash indicates inorganic elements which is 2.09% which may be due to environmental or geological factors. Water soluble ash is 2.56% which is completely organic and contributes to medicinal benefits. Alcohol soluble extractive was 5.47% which is indicative of presence of polar and non-polar compounds. Water soluble extractive was 20.12% and it cannot be strongly defended for external application though the duration of action is short but the onset of action may be fast. Phytochemical screening of root of *Chassalia curviflora* shows presence of alkaloids, carbohydrates, tannins, flavonoids, triterpenoids and quinones. HPTLC is one of the analytical tools mentioned in Quality standards for proving safety, potency and efficacy. The solvent system employed here is Toluene: Ethyl acetate: Formic acid: Methanol (7:5:1:0.5). At 366 nm in long UV at Rf 0.64 (fluorescent green 16.74%) could be gallic acid The Rf 0.64 was also evident in short UV. This was observed in Densitometric scan at 254 nm. In densitometric scan at 254 nm there were 8 peaks observed among which 0.30 (19.32%), 0.65 (Gallic acid 12.36%) At 366 nm in densitometric scan 5 peaks eluted among which 0.63 (16.74% gallic acid) was evident. At 620 nm under tungsten lamp, white light there were 9 peaks observed among which Rf- 0.63 (25.38%) was the major peak.

CONCLUSION

Ayurveda always believes in the concept of *Yukti* which can be applied by the practitioners to use innumerable available plants in the surrounding in best interest of the people both in preventive and curative aspects. *Chassalia curviflora* is one such potent drug which is proven to have medicinal properties in all the parts such as root, stem, leaves and flowers. Pharmacognostical, phytochemical standards documented in this paper signify quality out prints of this drug. Further there is more scope for clinical researches to be conducted on this drug.

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