Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(6): 1043-1048
Received: 19-09-2018
Accepted: 21-10-2018

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Preliminary pharmacognostical and phytochemical analysis of *Chassalia curviflora* (wall.) Thwaites roots

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Abstract
Background: *Chassalia curviflora* (Wall.) Thwaites occurs throughout the India. It is mainly used in folklore practise. It is widely used in diseased conditions like jaundice, malaria, coughs, wounds, and ulcers. Preliminary phytochemistry of leaves, stem, and flowers has already done and revealed the presence alkaloid, carbohydrate, saponins, phyto-steroids, triterpenoids, phenolic compounds, flavonoids, fixed oils and fats.
Aim: But so far the pharmacognostic standardization of root has not been reported for its proper identification. Hence the present study was carried out for the establishment of Pharmacognostical and phytochemical standardization of root.
Methods: Macroscopy, powder analysis (Organoleptic and powder microscopy), physiochemical properties like ash values, extractive values and HPTLC Profile of whole plant was done.
Results and Conclusion: Microscopy shows normal root structure with outer 5-7 layered cork, inner cortex, xylem and phloem. Medullary rays are uni to biseriate. Starch grains are present. HPTLC finger print suggested the presence of maximum number of peaks and maximum total area obtained in cold water extract. Qualitative phytochemical analysis showed the presence of alkaloids, flavanoids, tannins, saponins, steroids and glycosides. Maximum phytoconstituents was obtained for ethanolic extract.

Keywords: Preliminary pharmacognostical, *Chassalia curviflora* (wall.)

Introduction
Multiple exotic plants are existent in India which are not referred to either in classical literature of *Āyurveda. Chassalia curviflora* (Wall.) Thwaites is one such plant which is mainly used in folklore practice. It is a small shrub or tree upto 2m tall of rubiaceae family. It is mainly used in jaundice by the kani tribes of Wayanad [1]. The juice of leaves boiled with oil is used for ear and eye diseases, ulcer and sore throat. Whole plant is used for skin diseases by tribes of Mananthavady, Wayanad district. Decoction of root is given as a remedy in phlegm, rheumatism, and pneumonia [2]. Leaves of tree boiled in ordinary water and the decoction is given in colic pain.[3]Root is an antidote against the sting or bite of serpents [3]. It is used in the Malay traditional medicine for treatment of malaria, coughs, wounds, and ulcers [4]. Chakma tribes of Bangladesh make use of crushed leaves to the wounds for treating snake and insect bites [5].
Preliminary phytochemistry of leaves, stem, and flowers has already done. The phytochemical screening carried out on the ethanolic leaf extract of *C. curviflora* revealed the presence of pharmacologically active constituents such as alkaloid, carbohydrate, saponins, phyto-steroids, triterpenoids, phenolic compounds, flavonoids, fixed oils and fats [6]. Physico-chemical and biochemical analysis of the crude drug powder of the stem of *Chassalia curviflora* (Wall. ex Kurz.) showed foreign content 0.313%, moisture content 11.333 %, total ash content 11.416%, acid soluble ash 56.833%, water soluble ash 15.054 % and alcohol soluble ash 10.595. The result of biochemical contents showed that, the highest value of dry matter was 88.666 ± 0.166%, followed by carbohydrate 63.027 ± 0.023%, crude fibre 14.693 ±0.170 %, crude protein 13.125 ± 0.004%, total ash 11.416 ± 0.289%, moisture 11.333 ± 0.166% and crude fat 1.099 ± 0.062% [7].
It is also proven for antimicrobial, antihypertensive, antioxidant and acaricidal activity.

Materials and Method
The plant specimen for the proposed study was collected from natural habitat of Kerala. It was identified and authenticated by Department of Dravyaguna Vijnana, V.P.S.V Ayurveda College, Kottakkal. The pharmacognostical and phytochemical work carried out in Department of Dravyaguna Vijnana, V.P.S.V Ayurveda College, Kottakkal.

Macroscopy
The external features of the test samples were documented using Canon IXUS digital camera. The macroscopic features were compared to local flora for authentication.

Microscopy
A cylindrical portion of almost straight and sufficient length to hold the sample is selected. The blade was moved back and forth from one end to other for obtaining fine slices. Enough number of sections were taken. The sections were carefully transferred to a Petri dish containing water. A few thin sections floated in water were selected and moved to a watch glass containing Safranin stain using a thin brush. Sections were kept there for 2-3 minutes. The sections were then transferred to pure water to remove the excess stain and thus made ready for mounting on a slide. A stained section was carefully transferred on a clean glass micro slide. The Photographs of the sections were taken using digital camera.

Powder microscopy
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 AxioCam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.

HPTLC profiling
A. Sample Solutions
1- 1 g *Chassalia curviflora* sample is weighed. Add 20ml water and kept for overnight under cold maceration process. Then it is filtered, evaporated to dryness, extracted with 10ml methanol, and spotted as 30 µl.
2- 1 g *Chassalia curviflora* sample is weighed. Add 20ml alcohol and boil well. Then it is filtered, evaporated to dryness, extracted with 10ml methanol, and spotted as 30 µl.
3- 1 g *Chassalia curviflora* sample is weighed. Add 20ml water and boil well. Then it is filtered, evaporated to dryness, extracted with 10ml methanol, and spotted as 30 µl.
4- 1 g *Chassalia curviflora* sample is weighed, extracted with 10ml methanol, and spotted as 30 µl.

B. Stationary phase
Merk, 1.05554.0007, TLC Silica gel 60 $F_{254,}$ 20x10 cm Aluminium sheet.

C. Mobile phase
Toluene: Ethyl acetate: Formic acid: Methanol (14:10:2:1)

D. Development
CAMAG 20 x 10 cm Twin trough chamber.

E. HPTLC Instrumentation
CAMAG Linomat 5, CAMAG TLC Scanner 3, CAMAG Reprostar 3.

F. Derivatization
Iodine vapour.

Preliminary phytochemical analysis
The phytochemical analysis included total ash, water insoluble ash, acid insoluble ash, moisture, volatile oil content, sugar content, fibre content. Cold water soluble extract, hot water soluble extract, cold alcohol soluble extract and successive solvent extraction in petroleum ether, cyclohexane, acetone and ethanol was also done.
Qualitative analysis
The extracts obtained were subjected to qualitative tests for the identification of various plant constituents which include detection of alkaloids, steroids, phenols, flavonoids, tannins, saponins, anthraquinones and glycosides.

Result
Macroscopy
Pieces of root mostly about 15 – 30 cm long and 0.5 – 1 cm in thickness, sub cylindrical, slightly curved, stout, thick and branched. Outer surface is greyish white in colour and inner whitish.

Fig 1: Macroscopy of *Chassalia curviflora* (Wall.) Thwaites roots

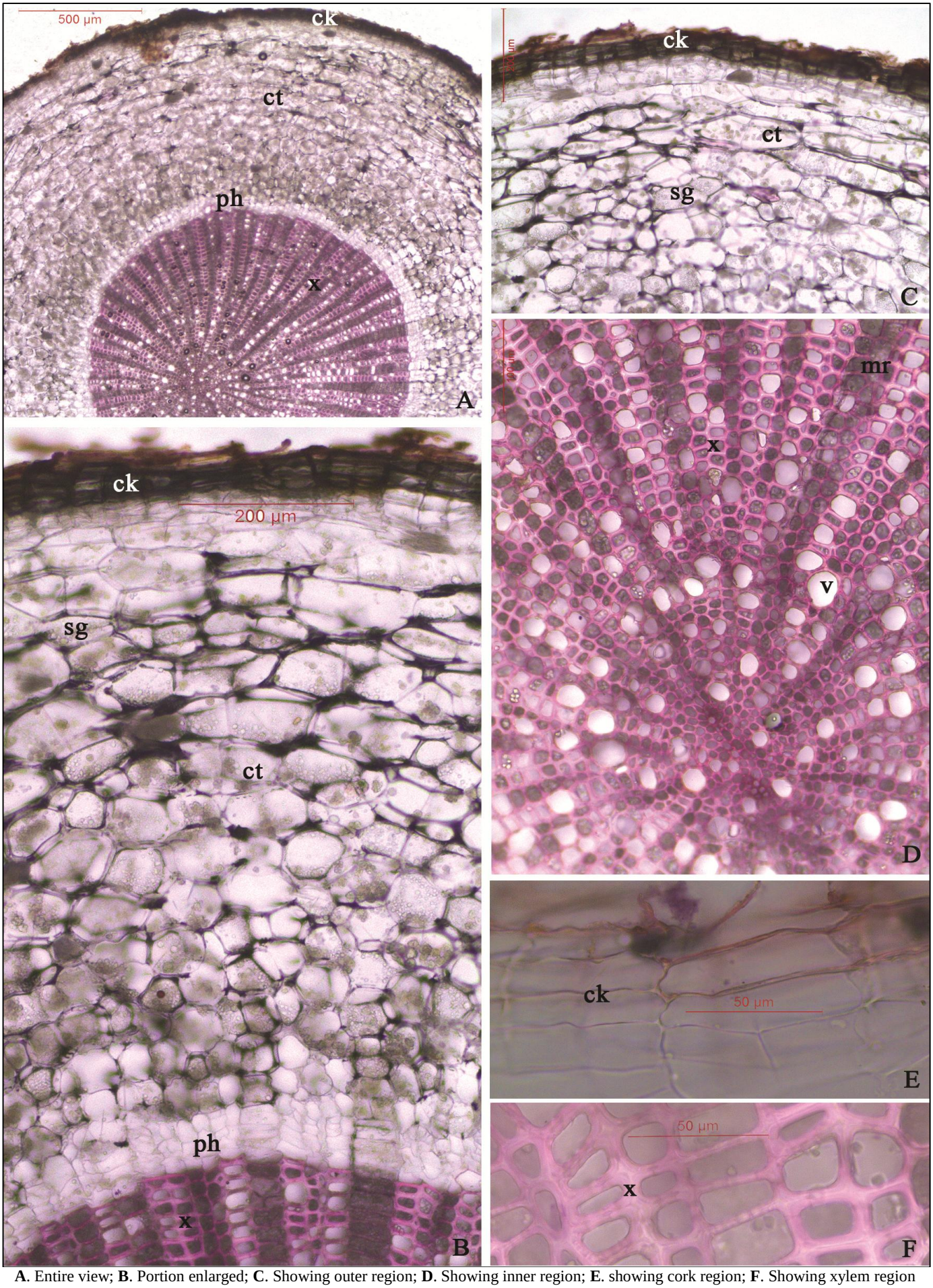
Organoleptic evaluation of root powder
Organoleptic evaluation refers to evaluation of formulation by color, odor, taste, texture etc. It means conclusions drawn from studies resulted due to impressions on organs of senses.

Table 1. Organoleptic evaluation of *Chassalia curviflora* (Wall.) Thwaites root powder

1	Touch	Course and powdered
2	Colour	Whitish
3	Taste	Pungent
4	Odour	Irritating
5	Consistency	Course

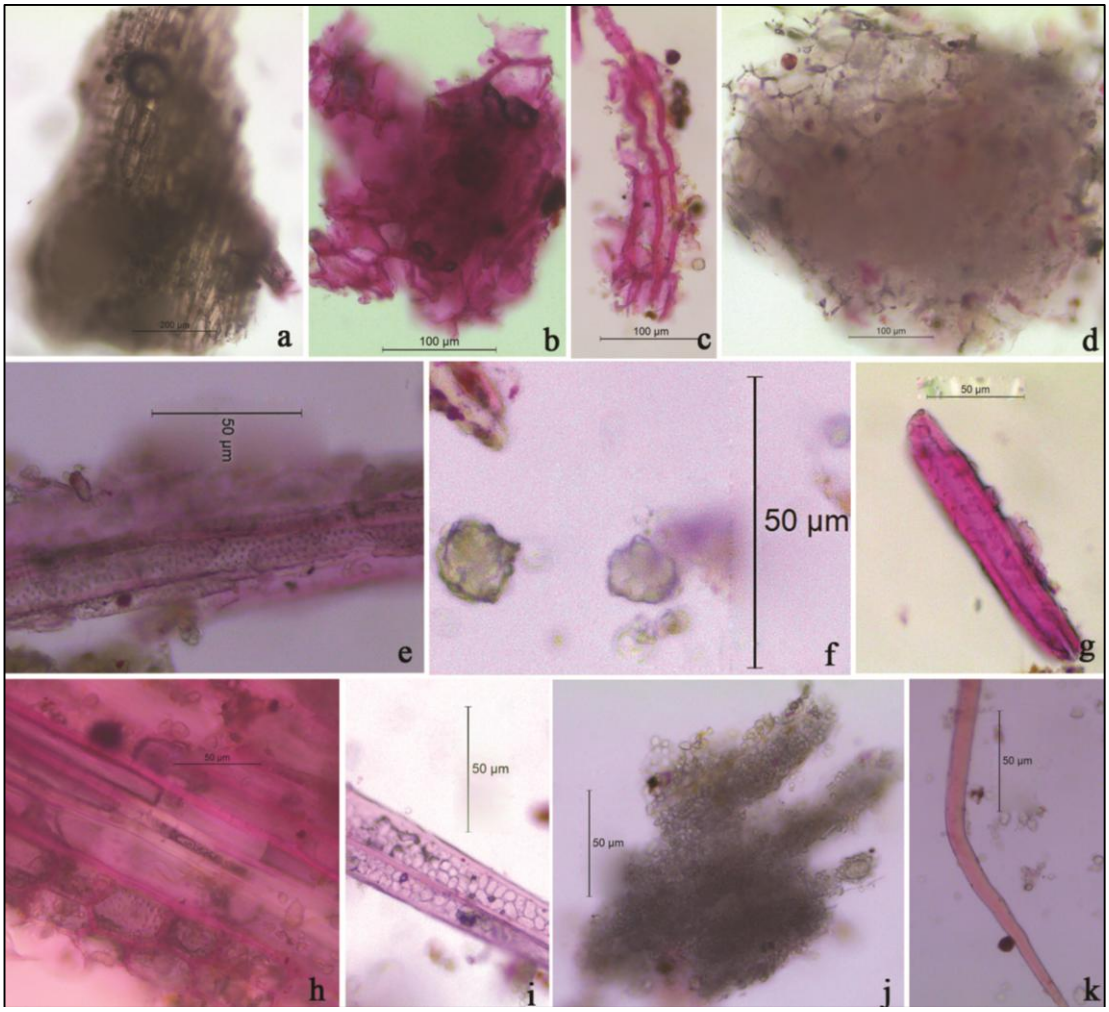
Microscopy of *Chassalia curviflora* (Wall.) Thwaites roots
Transverse section of root shows: outer cork – 5-7 layered tangentially elongated, thick walled cells. Cortex consist of 12-15 layered loosely arranged parenchymatous cells. Cells are filled with starch grains. Phloem consist of 5-10 layered tightly packed cells. Xylem consist of xylem vessels and other xylem elements. Xylem vessels are solitary. Tracheids are almost equal size and arranged radially. Medullary rays are uni to biseriate. Starch grains are seen extended to the phloem.

Powder microscopy of *Chassalia curviflora* (Wall.) Thwaites roots
Microscopic studies of powdered root of *Chassalia curviflora* (Wall.) Thwaites showed the presence of xylem vessels with cluster crystals of calcium oxalate, fragment of pitted fibre, pitted vessel, fragment of fibres attached with pitted parenchyma and cells containing starch grains.



A. Entire view; **B**. Portion enlarged; **C**. Showing outer region; **D**. Showing inner region; **E**. showing cork region; **F**. Showing xylem region enlarged. **ck**. cork; **ct**. cortex; **mr**. medullary ray; **ph**. phloem; **sg**. Starch grains; **x.** xylem.

Fig 2: Microscopy of *Chassalia curviflora* (Wall.) Thwaites root



a. cork cells with underlying cortical cells **b**. cork cells in surface view **c.** cork **d.** cortical cells with starch grains **e.** pitted vessel **f.** cluster crystals **g.** fragment of pitted fibre **h.** fragment of fibres attached with pitted parenchyma and cells containing starch grains **i.** fibre **j.** starch grains **k.** fragment of fibre

Fig 3: Powder Microscopy of *Chassalia curviflora* (Wall.) Thwaites. Root

Phytochemical analysis

Table 2. Quantitative phytochemical analysis

S. no	Experiments	Percentage
1	Total ash	1.75%
2	Water insoluble ash	1.7%
3	Acid insoluble ash	0.3%
4	Moisture content	20%
5	Volatile oil content	Nil
6	Sugar content	
7	a. Total Sugar b. Reducing Sugar	a.23.35% b.15.45%
8	Fibre content	47.32%

Table 3. Percentage of water soluble and alcohol soluble extractives

No.	Name of extract	Percentage of extract
1.	Hot water soluble	9.8%
2.	Cold alcohol soluble	2.9%
3.	Cold water soluble	10.75%

Table 4. Successive solvent extractives

No.	Experiments	Percentage
1.	Petroleum ether	0.56%
2.	Cyclohexane	0.83%
3.	Acetone	0.86%
4.	Ethanol	3.66%

Table 5. Qualitative Phytochemical analysis of the extractives

	Alkaloids	Flavanoids	Phenol	Tannin	Saponin	Steroids	Anthraquinones	Glycosides
Petroleum ether	_	_	_	+	+	_	_	_
Cyclohexane	_	_	_	_	+	_	_	_
Acetone	_	_	_	+	+	+	_	+
Ethanol	+	+	_	+	+	+	_	+
Hot water extract	+	_	_	_	+	+	_	_
Cold water extract	+	_	_	+	+	+	_	+
Cold alcohol extract	+	+	_	+	_	+	_	+

HPTLC results

Rf value of sample 1 at 254 nm: 0.16, 0.18, 0.28, 0.34, 0.39, 0.43, 0.46, 0.54, 0.65, 0.77 {total area – 31858.1(AU)}

Rf value of sample 1 at 440 nm: 0.18, 0.28, 0.33, 0.39, 0.44, 0.53, 0.65, 0.70, 0.79 {total area – 28528.1(AU)}

Rf value of sample 2 at 254 nm: 0.23, 0.34, 0.39, 0.42, 0.48,

0.54, 0.61, 0.72, 0.79 {total area – 14126.8(AU)}
Rf value of sample 2 at 440 nm: 0.08, 0.23, 0.35, 0.39, 0.42, 0.53, 0.64, 0.71, 0.79 {total area – 20831.2(AU)}
Rf value of sample 3 at 254 nm: 0.34, 0.43, 0.46, 0.55, 0.62, 0.73, 0.79 {total area – 3112.1(AU)}
Rf value of sample 3 at 440 nm: 0.08, 0.36, 0.43, 0.46, 0.63, 0.69, 0.82, 0.83 {total area – 7400.9(AU)}
Rf value of sample 4 at 254 nm: 0.37, 0.46, 0.49, 0.65, 0.75, 0.81 {total area – 2199.3(AU)}
Rf value of sample 4 at 440 nm: 0.09, 0.27, 0.40, 0.44, 0.47, 0.65, 0.72, 0.85 {total area – 6417.2(AU)}

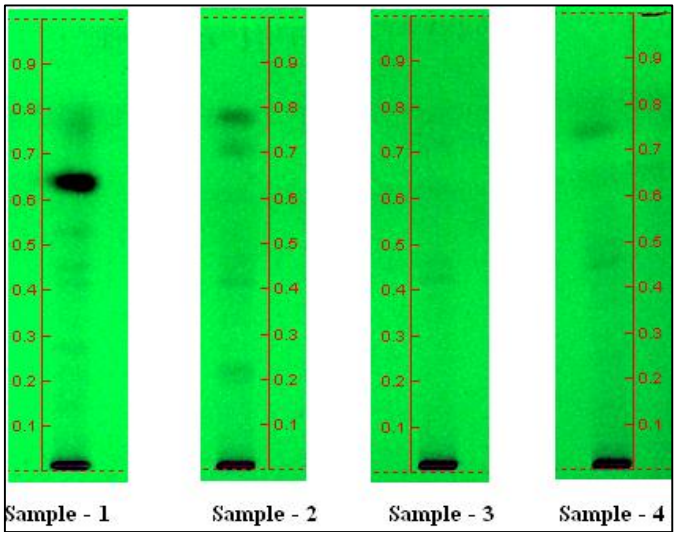


Fig 2: TLC plate views of *Chassalia Curviflora* samples at 254nm

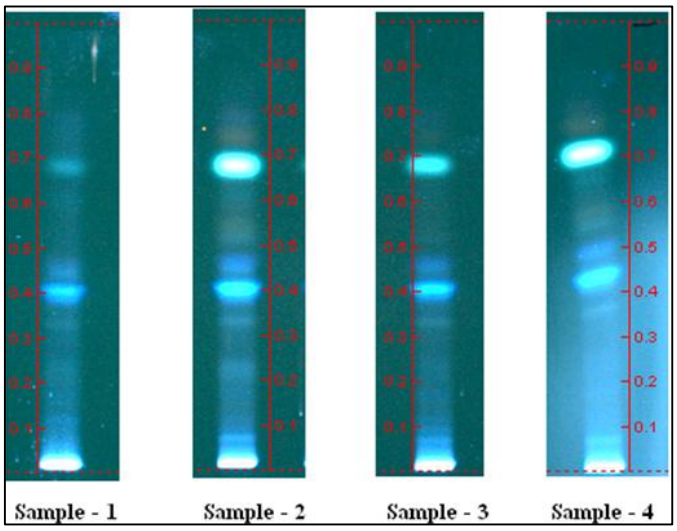


Fig 3: TLC plate views of *Chassalia Curviflora* samples at 366 nm

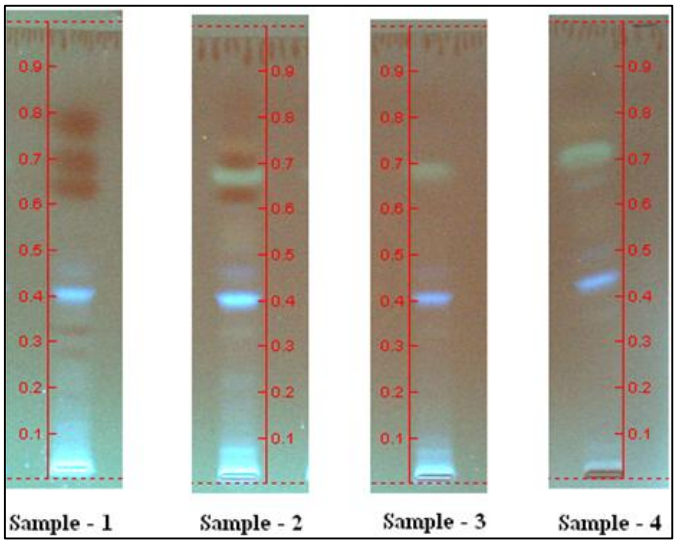


Fig 4: Derivatized TLC plate view of *Chassalia Curviflora* samples at 366nm

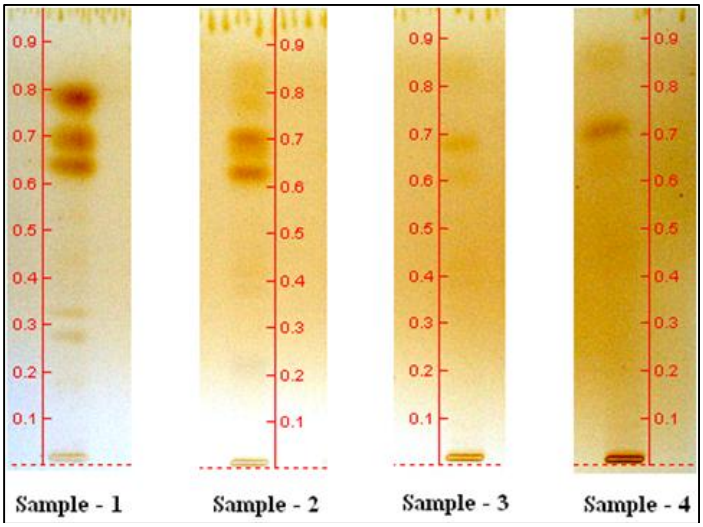


Fig 5: Derivatized TLC Plate view of *Chassalia Curviflora* at white light

Discussion

Microscopy

Microscopy shows normal root structure with outer 5-7 layered cork, inner cortex, xylem and phloem. Medullary rays are uni to biseriate. Starch grains are present.

HPTLC

TLC photo documentation revealed presence of many phytoconstituents with different Rf values. Densitometric scan of the plates showed numerous bands under 254 nm and 366 nm after derivatization. The area under graph gives an idea about the quantitative analysis of test drug. The result suggested that the presence of maximum number of peaks and maximum total area was obtained for cold water extract of the drug.

Phytochemical analysis

The highest percentage of extract was obtained by the extraction with ethanol (3.66%) and least with solvent petroleum ether (0.56%). Successive solvent extractives represent the fraction of phytoconstituents of drug. Alkaloids were present in ethanol, hot water, cold water and cold alcohol extract. Flavanoids were present in ethanol and cold alcohol extract. Tannins were present in petroleum ether, acetone, ethanol, cold water and cold alcohol extract. Saponins were present in all extracts except cold alcohol extract. Steroids were present in acetone, ethanol, hot water, cold water and cold alcohol extract. Glycosides were present in acetone, ethanol, cold water and cold alcohol extract. Anthraquinones and phenols were absent in all the extracts. Maximum number of secondary metabolites was obtained in ethanol extract.

Conclusion

Preliminary pharmacognostic and phytochemical analysis of *Chassalia curviflora* (Wall.)Thwaites was conducted. HPTLC finger print suggested the presence of maximum number of peaks and maximum total area obtained in cold water extract. Qualitative phytochemical analysis showed the presence of alkaloids, flavanoids, tannins, saponins, steroids and glycosides. Maximum phytoconstituents was obtained for ethanolic extract.

Acknowledgement

It's my duty to give a sincere vote of thanks to them who helped me to complete this work. I express my profound sense of gratitude, sincere regards and humble thanks to respected professor, my guide & head of the department Dr. N Manoj Kumar for his guidance, critical scrutiny and support throughout the work. I express my gratitude with respect to my coguide Dr. Jyolsna G Krishna, Assistant professor, Dep of Dravya guna vijnana, VPSV Ayurveda College, Kottakkal for her suggestions and discussions during the study.

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