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Madhira Geetha
Department of Pharmacognosy,
C.L. Baid Metha College of Pharmacy,
Thoraipakkam,
Chennai -97, Tamil Nadu, India.

Vijayalakshmi A.
Department of Pharmacognosy,
C.L. Baid Metha College of Pharmacy,
Thoraipakkam,
Chennai -97, Tamil Nadu, India.

Correspondence:
Vijayalakshmi A.
Department of Pharmacognosy,
C.L. Baid Metha College of Pharmacy,
Thoraipakkam,
Chennai -97, Tamil Nadu, India.
Tell: +91-44-22662150
Fax: +91-44-66881010
E-Mail: aviji_1975@rediffmail.com

Pharmacognostic and Phytochemical Investigation of *Givotia rottleriformis* Griff. Ex Wight Bark

Madhira Geetha and Vijayalakshmi A.*

ABSTRACT

The aim of the present study is to investigate the pharmacognostic and phytochemical investigation of the bark of *Givotia rottleriformis* Griff. EX Wight. belonging to the family *Euphorbiaceae*, which is greatly valued in Ayurveda for setting of bone fractures. In microscopic studies, transverse section (TS) of bark and its powder characters were studied and characteristic features were established. Physicochemical parameters such as total ash value, acid insoluble ash value and water soluble ash value were determined. The alcohol soluble extractive and water soluble extractive was also determined. Preliminary phytochemical analysis of alcoholic extract was carried out. The results of preliminary phytochemical screening were positive for flavonoids, carbohydrates, alkaloids, glycosides, proteins and saponins. The results obtained from standardization of bark of *Givotia rottleriformis* established the macro- and microscopical parameters, physicochemical parameters, TLC profiles that characterize the genuine plant drug. The present study provides pharmacognostical, physicochemical and phytochemical details of the bark of *Givotia rottleriformis* which are useful in laying down standardization and pharmacopoeia parameters.

Keywords: *Givotia rottleriformis*, Flavonoids, Pharmacognostical, Phytochemical.

1. Introduction

Givotia rottleriformis Griff. Ex Wight moderately sized tree of the family Euphorbiaceae distributed in limited areas of the forests of Tamil Nadu, Andhra, Karnataka, West Bengal and coastal Sri Lanka [1]. The bark and seeds of the tree are used in indigenous medicine in the treatment of rheumatism, dandruff and psoriasis [2]. The literature survey revealed that the systemic evaluation including pharmacognostical study of this plant is still lacking. There is a need for documentation of research work carried out on traditional medicines. With this backdrop, it becomes extremely important to make an effort towards standardization of the material to be used as medicine. The main aim of the present work is to study the macro, microscopic, physico-chemical standards and phytochemical analysis of the bark of *Givotia rottleriformis*, which could be used for the proper identification of this drug [1].

2. Materials and Methods

2.1. Plant material

Givotia rottleriformis is a moderately sized tree of the family Euphorbiaceae distributed in limited areas of the forests of Tamil Nadu, Andhra, Karnataka, West Bengal and Coastal Sri Lanka. The plants specimen for the proposed study was collected in the forest of Attur, Salem district, Tamilnadu. It was identified and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Tambaram, Chennai. A voucher specimen No. PARC/2011/2140 has been deposited for further references.

2.2. Microscopical characterization

The qualitative studies were performed. Free hand transverse sections of bark were studied for different microscopic characters and photographs of different magnifications of the sections were taken with Nikon Lab Photo 2 (Two) Microscopic unit. For normal observations, bright field was used. For the study of crystal, starch grains and lignified cells, polarized light was employed [3].

2.3. Powder analysis
The shade dried aerial parts of the plant were powdered and powder was passed through 100 # sieve. A small amount of powder was taken onto a microscopic slide, cleared from chlorophyll by heating with chloral hydrate solution and was mounted in 50% v/v glycerol in water. This was then observed under microscope to study the characteristic features [4].

2.4. Physico-chemical evaluations
The ash values, extractive values and loss on drying were performed according to the officinal methods prescribed in Indian pharmacopeia and the WHO guidelines on quality control methods for medicinal plants materials [5]. Fluorescence analysis was carried out according to the method of Kokoski [6].

2.5. Inorganic Mineral Analysis
The amount of sodium and potassium present in 1 gm of plant material was estimated by flame photometry. The amount of other metals present was estimated by Atomic absorption spectroscopy.

2.6. Extraction
The collected aerials parts of plant was made thoroughly free from any foreign organic matter, dried under shade and powdered. The ethanol extract was prepared using 70% aqueous ethanol by triple maceration process for 48 h each time. The extract was filtered and concentrated under vacuum. The concentrated extract was used for phytochemical screening and establishment of TLC profile.

2.7. Preliminary Phytochemical Screening
The ethanol extract (70%v/v) was subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as alkaloids, glycosides, tannins and phenolic compounds, flavonoids, steroids, saponins, proteins, amino acids, carbohydrates and triterpenoids [7].

2.8. Thin Layer Chromatography
The ethanol extract was subjected to thin layer chromatography. Number of solvent system was tried. The solvent system which shows good resolution was used. The visualization of spot was done by exposing the plate to iodine vapour using the solvent system Benzene: Methanol: Ammonia (9:0.5:0.5) [8].

2.9. High performance thin layer chromatography
Chromatograph was performed on 10x10 cm aluminum packed TLC plate coated with 0.2 mm layer of silica gel $60F_{254}$ (E. Merck Ltd, Darmstadt, Germany) stored in a dessicator, application was done by Hamilton microsyringe (Switzerland), mounted on a Linomat V applicator. Spotting was done on the TLC plate, ascending development of the plate, migration distance 80 mm (distance to the lower edge was 10 mm) was performed at 25±20 °C with Benzene: Methanol: Ammonia as a mobile phase in a camag chamber previously saturated for 30 min. After development the plate was dried at 60 °C in an oven for 5 minutes. Densitometric scanning was then performed with a Camag TLC Scanner 3 equipped with win CATS Software and the chromatograms were recorded [9].

3. Results and Discussion
3.1. Macroscopical Study
The Leaves are alternate haracterous, broadly ovate or orbicular, coarsely dentate, acuminate, glabrous above yellowish tomentose below 5-nerved. The flowers are in sub-terminal pendulous panicles, flowering from April-July. Fruits are a drupe, subglobose or ellipsoid fulvous-tomentose. Seeds are globose or ellipsoid with a bony testa, fruiting from May-June. Bark smooth brown, peeling off in circular scales. Bruised bark yields a blood red sap. (Fig. 1)

Fig 1: Entire plant of *Givotia rottleriformis*

3.2. Microscopical Study
3.2.1. Transverse section of Bark
The bark is very thick and extremely hard and brittle. In cross sectional view, the bark exhibits outer periderm and inner secondary phloem.

Periderm: The periderm includes two or more narrow tangential bands of phloem cells; the phellem is undulate forming lens shaped gaps in between the inner and outer periderm layers. These gaps have non-peridermous tissue, which is mostly cortical tissue and sclereids. This type of periderm which comprises periderm layers with lens shaped nonperiderm tissue located in between the periderm layers is called rhytidome. (Fig.2.1)

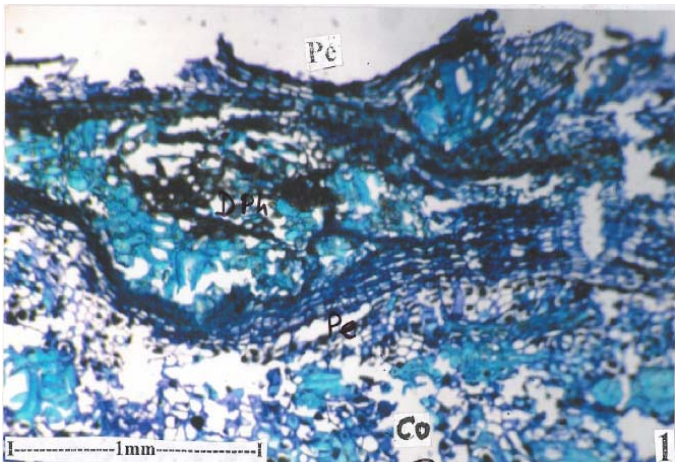


Fig 2.1: TS of Bark: Periderm (Rhytidome) – Portion enlarged (Co: Cortex; DPh: Dead Phloem tissue; Pe: Periderm)

Secondary Phloem: It is the major part of the bark and it extends from the inner border of the rhytidome upto the cambial zone. The secondary phloem can be divided into two zones, namely outer wider collapsed phloem and inner narrow noncollapsed phloem. (Fig. 2.2)

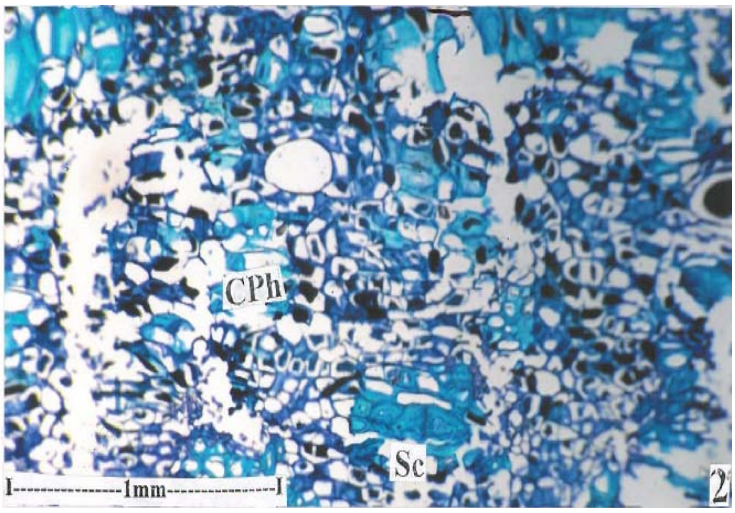


Fig 2.2: TS of Bark: Collapsed phloem with scattered Sclerenchyma cells (CPh: Collapsed phloem; Sc: Sclereids)

The collapsed phloem includes highly dilated phloem rays, scattered irregular masses of sclerenchyma cells and dark thick tangential streaks of crushed sieve elements.

Non Collapsed Phloem: The noncollapsed (intact) phloem zone is much narrow and it is 700µm thick. It includes wide rectangular sieve tubes which occur in radial parallel rows. The companion cells are prominent and they are located at lateral part of the sieve tube members. (Fig.2.3)

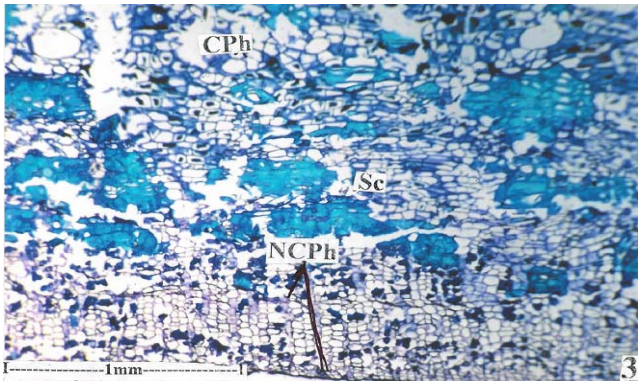


Fig 2.3: TS of Bark: Outer Collapsed Phloem and inner Non Collapsed Phloem (CPh: Collapsed phloem; NCPh: Non-Collapsed Phloem; Sc: Sclereids)

The phloem parenchyma cells are mixed within sieve tubes; the parenchyma cells are smaller than the sieve elements. The phloem rays are wavy and run straight penetrating the phloem tissue.

3.2.2. Tangential Longitudinal View of Bark

In tangential longitudinal sections of the bark, the features such as phloem rays height, thickness and frequency can be studied. The rays are exclusively uniseriate, narrow and heterocellular with terminal upright cells and middle procumbent cells. The upright cells are much elongated and conical in shape. The middle procumbent cells are rectangular and vertically elongated. The range is 250-650 µm in height and 30 µm in thick. Ray frequency is 13-15/mm.

The sieve tubes are long and thick walled straight. They have very oblique sieve plate which is nodulated due to the deposition of cellulose on the pores of the sieve plate. (Fig. 3)

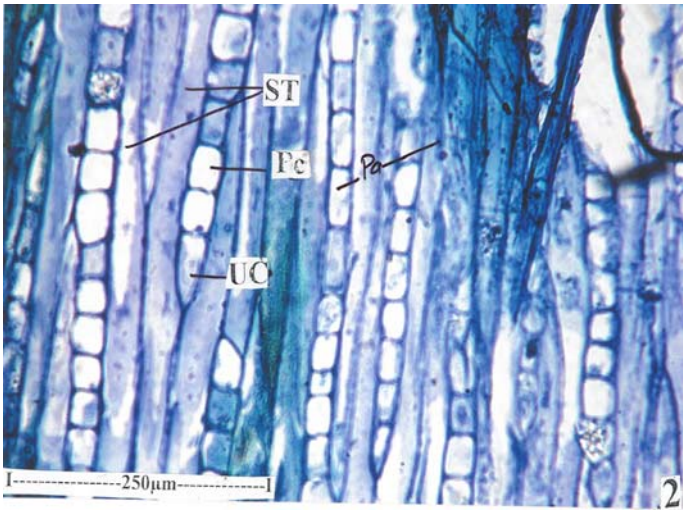


Fig 3: Tangential Longitudinal Section of the bark of *Givotia rottleriformis*
(Pa: Parenchyma cells; PC: Procumbent Cell; ST: Sieve Tube; UC: Upright Cells)

Phloem parenchyma cells are vertically rectangular and they occur in vertical strands.

3.2.3. Crystal distribution

Calcium oxalate crystals are sparsely seen in the bark. The crystals are of two types: prismatic crystals and sphaero crystals or druses. The crystals located in the phloem rays are druses. (Fig.4.1)

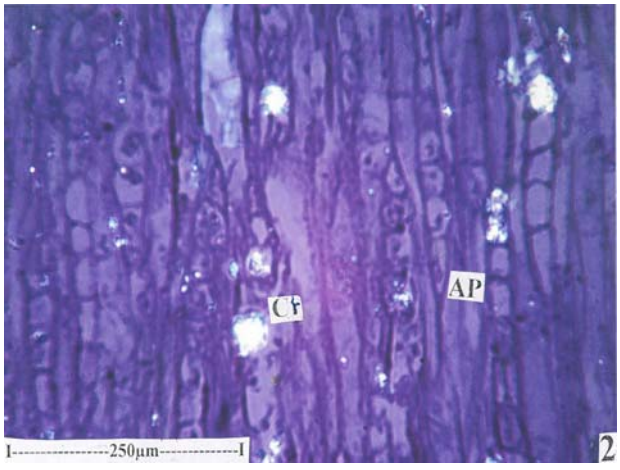


Fig 4.1: Crystal Distribution: Druses in the Phloem ray cells (Cr: Crystals; AP: Axial Parenchyma)

The crystals associated with sclerenchyma elements of the collapsed phloem are only prismatic type. (Fig. 4.2)

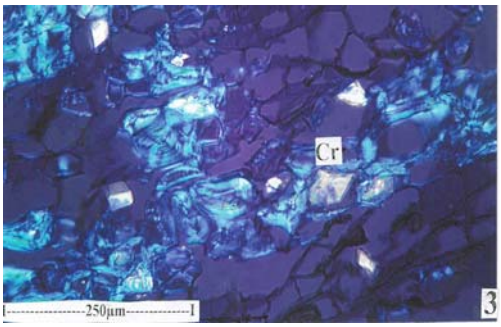


Fig 4.2: Crystal Distribution: Prismatic Crystals in the Phloem Sclerenchyma cells (Cr: Crystals)

3.2.4. Powder microscopy

The following elements were observed in the powder. (Fig. 5**)**

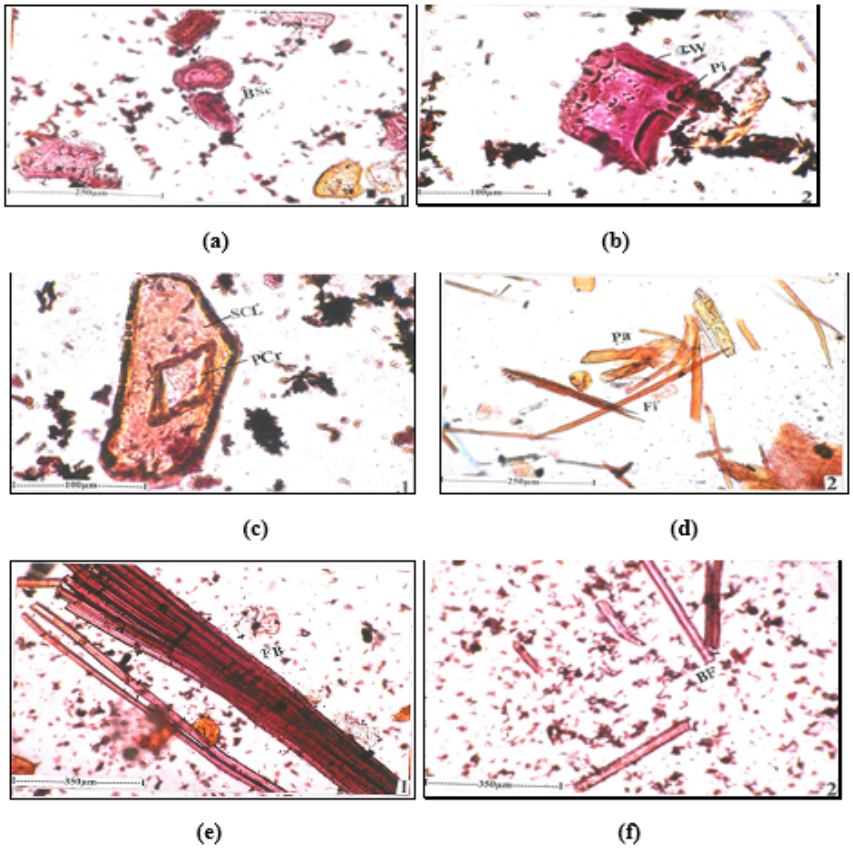


Fig 5: Powder microscopy: (a) Brachy sclereids; (b) A sclereids enlarged; (c) Sclereids with prismatic crystals; (d) Fibres and parenchyma cells; (e) Bundle of fibres; (f) Broken fibres.
(BSc: Brachy sclereids; LW: Lignified wall; Pi: Pit; Fi: Fibre; Pa: Parenchyma; PCr: Prismatic crystal; SCL: Sclereid; FB: Fibre bundle; BF: Broken fragments of fibres)

Sclereids: Brachy sclereids of different shape and size were seen in the powder. The brachy sclereids are generally isodiametric in shape, but they are elongated, rectangular and cubical. The sclereids have very thick secondary lignified walls with long narrow canal like pits. The cell lumen is wide. The sclereids also have thin walls with short pits. Prismatic calcium oxalate crystals are sometimes seen with in the sclereids. This is unique features for the sclereids.

Fibres: Short, narrow thick walled libriform fibres are common in powder. They have thick walls and narrow lumen. They are 250 - 370 µm long. The fibres are also seen in thick bundles or in small broken pieces.

Parenchyma cells: Rectangular thin walled parenchyma cells are seen mixed with other elements. The parenchyma cells have some storage products.

3.3. Physicochemical Parameters

The physico chemical constants are important parameters for detecting adulteration or improper handling of drugs. Various physicochemical parameters *viz*., ash, extractive values and loss on drying were determined. The results were summarized in Table 1.

Table 1: Physico chemical analysis of the bark of *Givotia rottleriformis*

S. No	Parameters	Values (%w/w)
1.	**Ash values**	
	Total ash	9.87
	Water soluble ash	2.43
	Acid insoluble ash	1.82
	Sulphated ash	15.72
2.	**Extractive values**	
	Petroleum ether extractives	1.26
	Chloroform extractives	2.70
	Alcohol soluble extractives	10.25
	Water soluble extractives	8.20
3.	**Loss on drying**	3.84
4	**Crude fibre content**	12.46

Table 2: Fluorescence analysis of powder of the bark of *Givotia rottleriformis*

Particulars	White light	UV light
Powdered drug	Reddish brown	Light brown
Powder + 1 N HCl	Brown	Black
Powder + 1 N H_2SO_4	Brown	Black
Powder + 1 N NaOH	Reddish Brown	Black
Powder + 50% HCl	Orange	Dark brown
Powder + 50% H_2SO_4	Brown	Black
Powder +50% HNO_3	Reddish brown	Dark brown
Powder + Methanol	Reddish brown	Greenish yellow
Powder + 5 % KOH	Dark brown	Black
Powder + Con HNO_3	Reddish orange	Black

Table 3: Inorganic mineral analysis of *G. rottleriformis* bark

S. No	Parameters	Concentration/ g sample
1	Cadmium	0.0002 µg
2	Calcium	0.452 µg
3	Chromium	0.0011 µg
4	Copper	0.804 µg
5	Iron	2.54 µg
6	Lead	0.0026 µg
8	Magnesium	0.145 µg
9	Nickel	0.0006 µg
10	Zinc	0.0043 mg
11	Phosphorus	Nil
12	Potassium	1.0158 mg
13	Sodium	0.0231 mg

The fluorescence analysis of the powder was also done and results were given in Table 2. The powder was treated with various reagents and the mixture was observed under UV light (366 nm) to see the type of fluorescence. Fluorescence studies revealed specific fluorescence in visible light and white light with different chemicals treatment; which helps in identification and standardization of the plant within the species. The moisture content of dry powder of bark was 3.84 % which was not very high, hence it would discourage bacteria fungi or yeast growth. The crude fibre content which was studied can be implied to determine the nutritive value of the *Givotia rottleriformis* bark. These data's were helpful for identifying and ascertaining the quality of the collected crude drug. (Table 1)

3.4. Inorganic Mineral Analysis

In nutrition, minerals are those elements for which the body requirements is at least 100 mg/kg and the trace elements that are needed in smaller quantities. The minerals include calcium,

chloride, magnesium, phosphorus, potassium, sodium and sulphur. The trace elements are chromium, cobalt, copper, iodine, zinc, molybdenum, nickel, selenium etc.

In this study, inorganic analysis of *Givotia rottleriformis* bark showed trace quantity in microgram level of toxic metals (lead, chromium, copper, cadmium, nickel) when compared to beneficial elements such as zinc, manganese, iron. So the plant was absolutely safe to consume medicinally which is an indicative of nutritional potential of the plant for the treatment of various ailments. The results were tabulated in Table 2.

3.5. Preliminary Phytochemical Screening

The hydro-alcoholic extract of plant aerial parts shows the presence of carbohydrates, alkaloids, flavonoids, glycosides, saponins, steroids, tannins, triterpenoids.

3.6. Thin Layer chromatography (TLC)

To support phytochemical screening, the ethanol extract was subjected to thin layer chromatography. Number of solvent system was tried. The solvent system which shows good resolution was used. The ethanol extract showed seven spot with R_f value 0.11, 0.18, 0.23, 0.26, 0.36, 0.62 and 0.74 using the solvent system Benzene: Methanol: Ammonia (9:0.5:0.5). TLC findings were in agreement with the data of qualitative chemical tests and reported in Fig. 6.

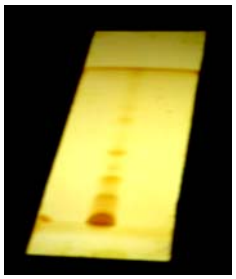


Fig 6: TLC of ethanolic extract of the bark of *Givotia rottleriformis*

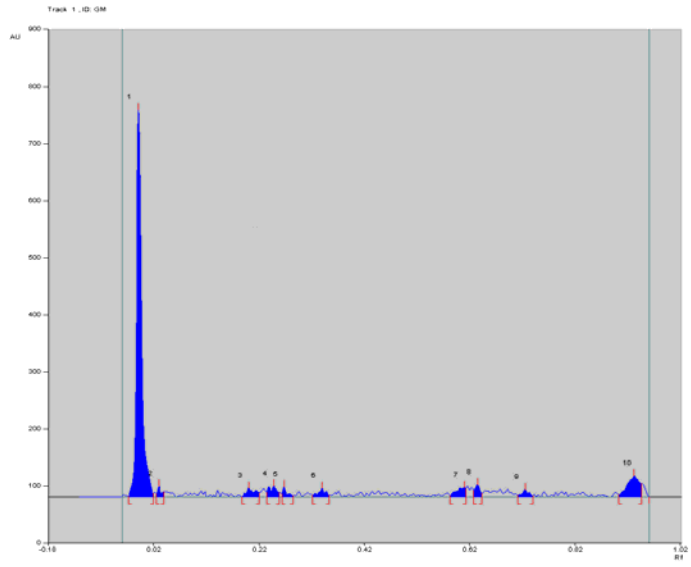
3.7. High performance thin layer chromatography

The ethanolic extract was further subjected to HPTLC for the conformation of the active constituents. The ethanolic extract showed nine resolutions of spot with the solvent system Benzene: Methanol: Ammonia (9:0.5:0.5). Out of 9 components, the component with R_f values 0.04, 0.16, 0.30, 0.53 and 0.77 were found to be more predominant as the percentage area was more with 15.06%, 20.55%, 22.04%, 15.08% respectively. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 9.0%. The R_f values were correspondingly depicted in Fig. 7**.**

Thus the developed chromatogram will be specific with selected solvent system Benzene: Methanol: Ammonia (9:0.5:0.5), R_f value and serve the better tool for standardization of the drug. Characteristic TLC/HPTLC fingerprinting of particular plant species will not only help in the identification and quality control of a particular species but also provide basic information useful for the isolation, purification, characterization and identification of marker chemical compounds of the species.

4. Conclusion

In the present investigations, the pharmacognostical and physicochemical characteristics of *Givotia rottleriformis* Griff. Ex Wight (bark) were studied. Various parameters established in the present study will help in controlling the standards and quality of the raw material of *Givotia rottleriformis*. Moreover, the plant has been traditionally used for the treatment of inflammatory disorders such as rheumatism and psoriasis. The preliminary phytochemical analysis showed the presence of various phytoconstituents which may contribute to the anti-inflammatory activity of this plant. All the pharmacognostical characters and physico-chemical parameters have been reported for the first time. The present investigation adds to the existing knowledge of *Givotia rottleriformis* and will be quite useful to pharmaceutical industries for quality control, ensuring batch to batch consistency of raw drug and in the field of medical, pharmacological evaluation and development of a formulation for treating various ailments.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.03	0.8	-0.01	678.5	83.02	0.02	7.1	6414.1	78.90	unknown *
2	0.03	6.2	0.03	18.7	2.28	0.04	9.3	138.2	1.70	unknown *
3	0.19	0.5	0.20	15.7	1.92	0.22	7.6	257.4	3.17	unknown *
4	0.23	8.7	0.25	19.3	2.37	0.26	8.6	287.3	3.53	unknown *
5	0.26	6.1	0.27	17.5	2.14	0.29	2.2	144.3	1.77	unknown *
6	0.32	3.5	0.34	15.8	1.94	0.35	3.2	213.0	2.62	unknown *
7	0.58	2.3	0.61	16.5	2.02	0.61	14.6	303.6	3.73	unknown *
8	0.63	12.4	0.64	21.5	2.63	0.64	6.9	199.1	2.45	unknown *
9	0.71	3.3	0.73	13.8	1.68	0.74	2.4	172.9	2.13	unknown *

Fig 7: Peak densitogram display of ethanolic extract of the *Givotia rottleriformis* bark

5. Acknowledgements

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6. References

1. Madhava chetty K, Sivaji K, Tulasi rao K. Flowering plants of Chitoor district, 1st edition. Andhra Pradesh, 2008, p, 185, 317.
2. Pulliah T, Encyclopaedia of World Medicinal Plants, Volume 1, New Delhi, Regency publication, 2006, p. 1007.
3. Sass JE, Elements of botanicals of microtechnique, New York, Mc Graw Hill and co, 1998, 222 – 225.
4. Johansen DA, Plant Mirotechnique, New York, Mc Graw Hill book and Co, 1940, 523- 525.

5. Quality control methods for medicinal plant materials WHO, Indian edition, Geneva, 2004, 28-30.
6. Kokoshi CJ, Kokoshi RJ, Sharma FT, Fluorescence of powdered vegetable drugs under ultraviolet radiation, J Pharm Assos, 1958, 47, 715-717.
7. Harborne JB, Phytochemical methods, A guide to modern techniques of plant analysis, London, Chapman and Hall, 1984, 84-274.
8. Wagner H, Bladt S, Plant Drug Analysis, A Thin Layer Chromatography Atlas, 2nd edn, New Delhi, Springer private limited, 1996, 3-235.
9. Rich E, Schibli A, HPTLC for the Analysis of Medicinal Plants, Thieme Medical Publisher, Inc, ISBN 13:978-1-58890-409-6(US)