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Pharmacognostic Phytochemical Investigation and Medicinal Uses of *Ficusvirens* (Leaf and Stem Bark) for Treatment of Various Diseases by Tribal People of Chitrakoot

Abhishek Kumar Pandey*
Department of Botany, Kalinga University,NayaRaipur, Chhattisgarh
*Email: abhishek.pandey@kalingauniversity.ac.in

Abstract

Plants contain variety of secondary metabolite and phytochemicals. These phytochemicals are source of medicinal drug. For the extraction of these phytochemical it is necessary and compulsory step to identify plant by microscopy, macroscopy, phyto chemical analysis and HPTLC technique. Present study provide monograph study of *Ficusvirens*(leaf and stem bark) which will be helpful in Identification of plant. Monograph study of plant includes microscopy macroscopy, powder microscopy, fluorescence study physiochemical, phytochemical and HPTLC study. Present research also summarizes ethnobotanical uses of plant.

Keywords: Medicinal importance, *Ficusvirens*, physiochemical screening, HPTLC, tribal use

INTRODUCTION

In Indian Methodology *Ficus* tree is dwelling place of god Vishnu. In India many *Ficus* species such as *Ficusvirens, Ficusbenghalensis, Ficusreligiosa, Ficus racemose* are worshipped. These plants also have great medicinal properties. From ancient time we are using number of herbal&iikoplant product for treatment of various diseases. In Ayurveda *Ficusvirens* is used for treatment of frequent urination, filtering of blood and healing of wound. This plant has been used in various medicinal treatments. It is large spreading tree with aerial root. Leaves are petiolate; alternate; simple; stipulate and Inflorescence is catkin type. Flower is small inconspicuous; bracteates and bracteolate; actinomorphic; incomplete; unisexual; hypogynous. Plant contains four perianth which are arranged in two whorls. Stamens are present in four to five in numbers. Male flowers are opposite to the perianth. Tow carpel are present in female flowers which are simple, ovary is superior type; apocarpous and basal placentation is found. Fruit is endospermic contain number of amino acids.

Traditionally various part of plant has been used by tribal of Chhattisgarh and Madhya Pradesh. Recent study of plant shows that bark of *Ficusvirens* has wound healing capacity [1] and antioxidant properties [2]. Review of literatures shows that no systematic data related to medicinal properties of Ficusvirens are present. Therefore, the present work was planned to study the medicinal properties including pharmacognostic properties. The pharmacognostic study includes microscopic and macroscopic, powdermicroscopical, physiochemical,fluorescence study and chromatographic characteristics of the leaf and bark of this plant. This study will be helpful for formulation of medicinal drugs and serve as a standard reference for identification, authentication and for distinguishing the plant from its adulterants.

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MATERIAL AND METHODS

Plant Material: The fresh stem bark and leaf were collected from the Raghuveer Temple JankikundChitrakoot Satna M.P. India in the month of March. For future reference, three plants specimen were also collected and placed in Department of Botany Kalinga University, Department of Pharmacognosy Ayurveda Sadan and Department of Botany Mahatma Gandhi ChitrakootGramoday University. The plant species was identified by Dr. R.L.S Sikarwar and Dr.Manoj Tripathi Director of Ayurveda Sadan and Dr. Ravindra Singh, Associate professor of Botany. Anatomical studies was carried out by fresh material whereas shade dried material was powdered in electric grinder for physio-chemical, phytochemicals and HPTLC studies.

Macroscopy and Microscopy: Macroscopy relates to morphology and appearance of plant body.*Ficusvirens* is a large spreading tree having aerial roots. Leaves are petiolate; alternate; simple; stipulate; Inflorescence is catkin type. Flower is small inconspicuous; bracteates and bracteolate; actinomorphic; incomplete; unisexual; hypogynous. Perianth is four and arranged in two whorls. Four to five Stamens are present in male flowers and opposite to the perianth. Leaf and bark section was cut out by free hand sectioning using blade. Good section of leaf shows epidermis, cortex, endodermis. Vascular bundle is endarch, collateral, and conjoint. Histochemical studies were also carried out using hydrochloric acid-phloroglucinol to reveal lignified elements, Nile blue stain to discover presence of fat, oils, fatty acid and phospholipids, Ferric chloride test for phenols, bromophenol blue method for protein, Dragendroff's reagent for alkaloidal substance, ruthenium red for mucilage, iodine-iodide for starch. Photomicrograph of the microscopical sections were captured with the help of binocular microscope with image analysis software KXL-2001.

Powder microscopy: Plant material was kept under drier so that all moisture content is evaporated. After it plant material was grind with the help of Bajaj mixer. Dried powder of leaf and stem bark was pass through 355um IS sieve and not less than 50% pass on through 180 um IS sieve. About 2g of powder was washed thoroughly with potable water; pour out the water without the loss of material. Eosins, Safranine, Crystal violet, Haemotoxylin, Sudan, Methylene blue, fast green reagents were used for powder microscopy. Mount a small portion in glycerin. Warmed a few mg of bark powder with chloral hydrate solution and mounted in glycerin. Treated a few mg of bark powder with iodine solution and mounted in glycerin, about 1g of powder, warmed over water bath with 50% conc. Nitric acid till brown fumes appear, cooled and washed with water thoroughly and mounted a small portion in glycerin and seen under microscope at 40 x10x magnification of binocular research microscope.

Physiochemical Test: Physiochemical test provide data related to solubility of plant part in alcohol and water, solubility in acid. We performed many test for knowing moisture content, water soluble extractive value, alcohol soluble extractive value, total ash value, acid insoluble ash value and water soluble ash were calculated.

Preliminary Phytochemical Test: Phytochemical test was performed for knowing presence/absence of phyto-constituents like alkaloids, flavonoids, tannins, resins, carbohydrates, proteins and saponins.

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Fluorescence Study: Fluorescence study has been performed with the help of UV cabinet. Plant powder kept under UV light. First of all plant powder treated with different reagent then fixed in slide. We kept slide under UV cabinet. Observe impact of UV light and noted colour of emissions.

High Performance Thin Layer Chromatography (HPTLC): For HPTLC, the powdered stem bark and leaf 5grams sample were dissolved in 100ml of ethanol and was kept overnight, filtered and concentrated. It was applied by spotting extracted sample on pre-coated silica gel aluminium plate 60 F254 (5X10 cm with 0.2mm layer thickness Merk Germany using camag Linomat-5 sample applicator and a 100µl Hamilton syringe. The samples, in the form of bands of length 6mm, were spotted 15mm from the bottom, 15mm from the left margin the plate and 10mm part. Plates were developed using mobile phase consisting of toluene: Ethyl acetate (7:3 v/v). Linear ascending development was carried out in 10 x 10 cm twin through glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 30min. at room temperature. Subsequent to the development TLC plates were dried with the help of Hot air oven. The peak area for samples and standard were recorded with Camera photo documentation system CamagReproster 3. Visualization of spot was made before and after derivatization (with 5% methanolic-sulphuric acid reagent) at 254 nm and day light with win cat software and RF values noted.

RESULTS AND DISCUSSION

Macroscopy and microscopy: The stem bark is purple pinkish in colour, extremely rough, fibrous longitudinally striated. Leaves are petiolates, and green in colour. At immaturity they are reddish brown in colour but when they mature they acquire green colour. Leaves are simple alternate spiral. Aerial root are also present to support main trunk of plant body but they are not so hard like banyan tree. Aerial roots attached to main stem and give strengthen to main stem. Milky latex are also present which are very nutritious in nature. They attract lots of insect and ants during bleeding. *Ficusvirens* is large spreading tree like other plant of Fabacecae family. Size of leaves of *Ficusvirens* are start from 8cm to 19cm long and 3 to 6 cm wide. Midrib is whitish in colour. Stipules are less than 1cm long. The pea-sized figs are in pairs and greenish-white to brown with spots. It is a deciduous tree. Plant shade their leaves in the month of February and new leaves emerge in march with colour of purple and red and bronze, giving the tree a wonderful look. The colour transformation goes on till the month of April. It acquires height up to 30meters.Flowers unisexual; inflorescence a syconia, axillary, paired, 1-1.5 cm across, globose, often obconical; peduncle 1-6 mm long, slender, pubescent; basal bracts 3, ovate, acute, persistent; orifice plane, closed by 3 flat apical bracts in a disc 1 mm wide, internal bristles abundant, white, chaffy-vesicular; flowers of 4 kinds; male flowers ostiolar, sessile, in 2-3 rings; tepals 2-3, ovate, acute or shortly gamophyllous; stamen 1; filament 0.5 mm; anther oblong, parallel; female flowers sessile; tepals 3-4, free; ovary superior, 1.2 mm, obovoid, sessile or stalked, red-brown; style filiform, tapering; gall flowers sessile or shortly pedicellate; tepals 3-4, reddish, spathulate to linear-lanceolate, free, ovary sessile or stalked, red-brown. Syconium white and fleshed with pink when ripe; achenes smooth. The plant often begins life as an epiphyte, growing in the branch of another tree; as it grows older it sends down aerial roots which, when they reach the ground quickly form roots and become much thicker and more vigorous. They supply nutrients to the fig, allowing it to grow faster than the host tree. The aerial roots gradually encircle the host tree, preventing its main trunk from expanding, whilst at the

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same time the foliage smothers the foliage of the host. Eventually the host dies, leaving the fig to carry on growing without competition.

The transverse section of stem bark showed cells of the dark coloured rhytidoma, 6-10 rows of rectangular radially arranged suberized cork cells, a continuous band of 3 to 5 layered, stone cells, embedded with few fibrous sclereids, the remaining cortical cells being parenchymatous, filled with tannins and prismatic crystals of calcium oxalate, outer cortical region cells gate obliterated and tangentially elongated bands .band of ceratenchyma running throughout the phloem tissue divides it into 2distinct zones. Outer zone is devoid of resin canals and inner zone with resin canals. Medullary rays are muitiseriate; the outer phloem runs straight and almost parallel in the inner zone .lignified phloem fibres, thin walled rectangular a few phloem parenchyma containing prismatic crystal is of calcium oxalate.

Powder Microscopy: The powder of stem bark is pinkish brown in colour whereas leaf powder shows dark green in colour, not characteristics odor and astringent taste. Microscopic view of stem bark powder showed abundant prismatic crystals of calcium oxalate scattered as such filled in the parenchymatous cells, the fragments of laticiferous canal filled with light brown granular contents often associated with stone cells, fragments of lignified cork cells in surface view, stone cells various shape and sizes, few containing prisms, elongated shape and sizes, few containing prisms, elongated phloem fibres with wide lumen and pointed ends, tangential- longitudinally cut medullary rays embedded with prisms, associated with phloem parenchyma and sieve tissue, radially-longitudinally cut sclerosed medullary rays crossing the fibres. Sclerenchymatousfibres are also present in powder of stem bark.

The powder of leaf shows the presence of collenchymatous and parenchymatous cells. Presence of calcium oxalate crystal is a characteristic of Moraceae family. Leaf section also shows crystal of calcium oxalate. Rupture epidermis with stomata were observed in the section. Thick cuticle is present in natural condition.

Physiochemical Analysis

The physiochemical parameters provide certain useful information such as moisture content, extractive values are useful for the determination of exhausted or adulterated drug, ash value of drug gave an idea of the inorganic content which are present in powder whereas acid insoluble ash provide percentage of inorganic content which is dissolved in acid. In leaf of *Ficusvirens* total percentage of moisture content is 7.63 whereas stem bark contain 6.07% moisture content. To evaluate moisture content, powder is treated with 105°C temperature to evaporate moisture from the sample.

Alcohol soluble extractive value of leaf of *Ficusvirens* is 7.33% where as 8.5% reported in case of stem bark. Water soluble extractive value of leaf of *Ficusvirens* is 7.28% whereas 6.07 % reported in stem bark.

The total ash is the residue remaining after incineration. Total ash values provide inorganic content which is present in sample of plant. In case of *Ficusvirens* total ash of leaf is 15.27% and total ash of stem bark of *Ficusvirens* 11.46 %.

The acid insoluble ash is the part of the total ash which is insoluble in diluted hydrochloric acid. It is reported 12.11% in case of leaves and 10.32 % reported in case of stem bark.

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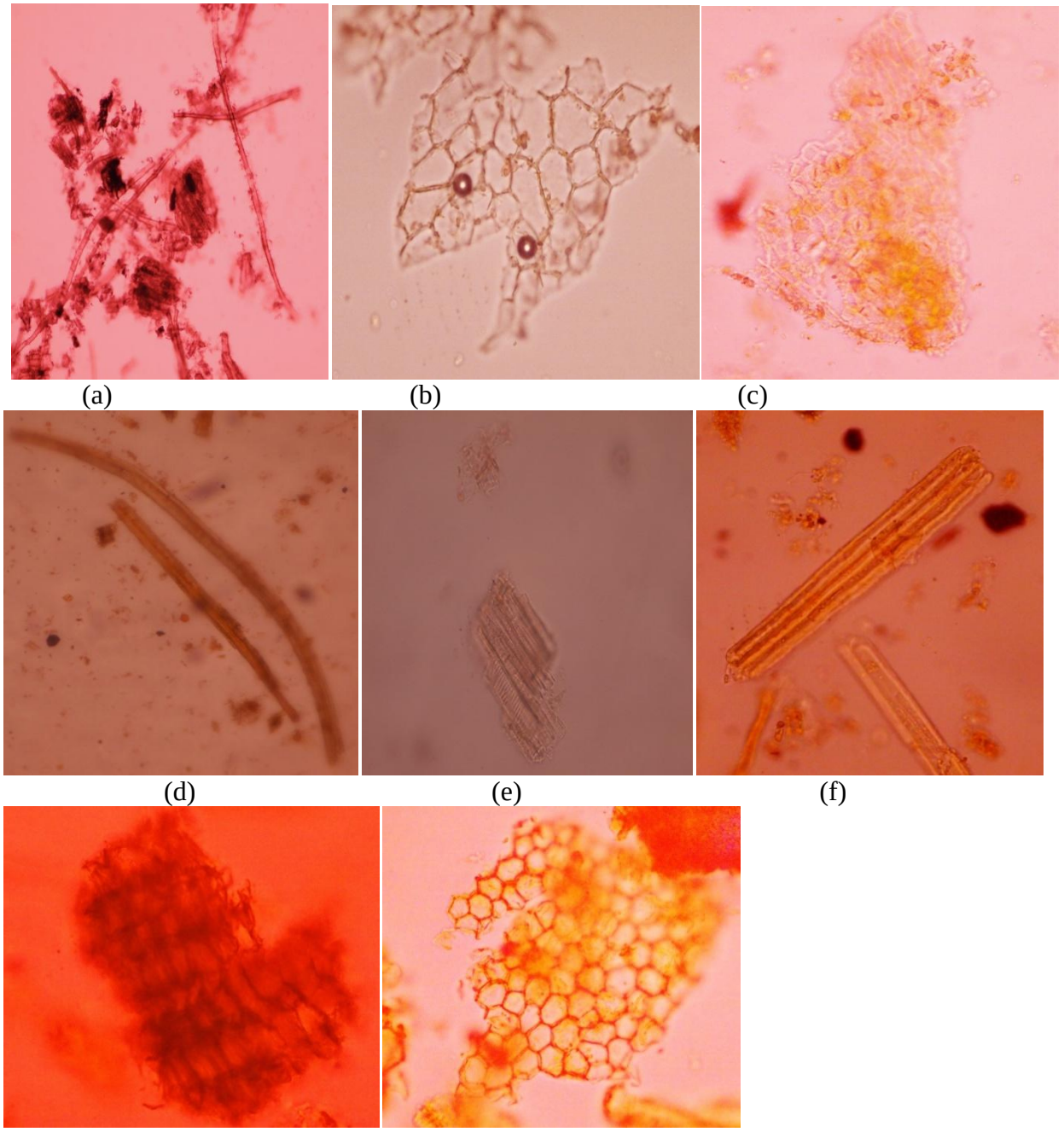

Figure1: Powder Microscopy (a) Sclerenchyma Fibre (b)Upper Epidermis (c) Lower Epidermis with Stomata(d)Xylem Vessels (e) Spiral Thickening (f) Fibres with Starch Particle(g) Cork Cell(h) Parenchymatous Cell

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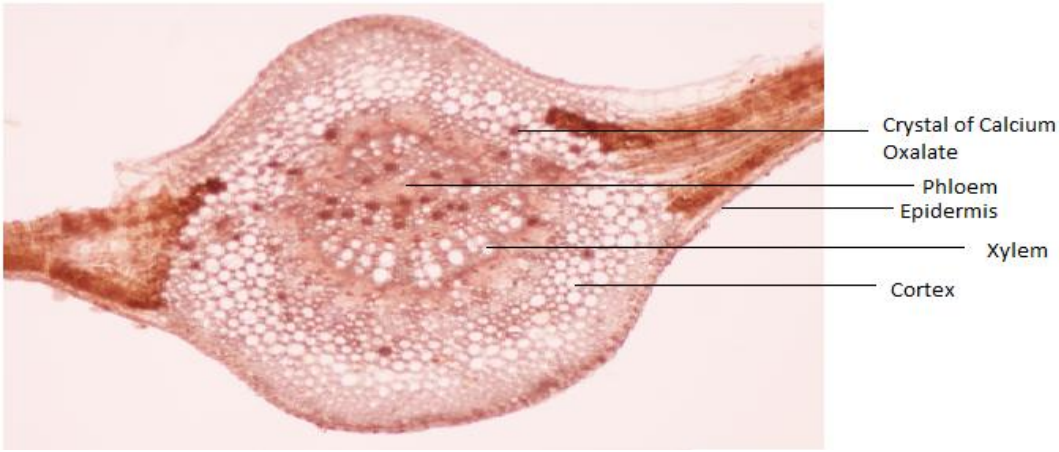

Figure 2: Transverse Section of leaf of *Ficusvirens*

Table 1: Fluorescence Study of Stem Bark of *Ficusvirens*

Sr. No.	Drug Powder+ Chemical	Colour at 254 nm	Colour at 366 nm
1	Powder	Violet	Brown
2	NaOH in Water	Brown	Blue
3	NaOH in Methanol	Brown	Dark brown
4	50% HNO3	Light brown	Brown
5	50%H2SO4	White red	Red
6	CH3COOH	Brown	Brown
7	1NHCL	Dark brown	Greenish brown
8	50%KOH	Brown	Brown
9	Aqueous H2SO4	Brown	Brown
10	Powder +Iodine	Brown	Brown

Table 2:Fluorescence Study of Stem Leaves of *Ficusvirens*

Sr. No.	Drug Powder+ Chemical	Colour A 254 nm	Colour at 366 nm
1	Powder	Green	Green
2	NaOH in Water	Green	Blue
3	NaOH in Methanol	Bluish and blackish	Dark brown
4	50% HNO3	Light blackish	Grey
5	50%H2SO4	Dark olive green	bluish
6	CH3COOH	Greenish	Green
7	1NHCL	Dark Green	Greenish brown
8	50%KOH	Green	Brown
9	Aqueous H2SO4	Blackish Green	Brown
10	Powder +Iodine	Blackish Green	Green

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Table 3: Phytochemical result of leaf of *Ficusvirens*

Sr.No	Constituents	Observation	Appearance	Result
1	**Tannin**	White Brown Colour	Brown Colour	Positive
2	**Saponin**	Foam appear after 10 minute	Foam appear	Positive
3	**Flavonoids**	Pink and Brown colour	Brown Colour	Positive
4	**Starch**	Brown Colour is produced	Green &Blue	Negative
5	**Terpinoid**	Radish Brown Ring appear	Radish Brown	Positive
7	**Wagner test Alkaloid**	White Colour appear	White Yellowish	Negative
8	**Anthraquinones**	Like Rose Pink Colour	Light pink Colour	Positive
9	**Resin**	Turbidity	Turbidity	Positive
10	**Steroids**	Red Colour	Light White	Negative
17	**Carbohydrate**			
	(a)Anthrone test	Blue or Green Colour	Dark Green Colour	Positive
	(b)Fehling test	Brick Colour appear	Blue Colour	Negative
	(c)Molish test	On adding excess of alkali red violet Colour disappear	red violet disappear	Positive
18	**Proteins**	Violet red Colour appear	Red Colour	Positive
	Million's test	Brick red Colour appear	Red Colour	Positive

HPTLC Finger print profile: The High Performance Thin Layer Chromatography (HPTLC)was examined under 254nm, 366nm. After derivatization 366nm. Chromatogram profiles are given in plate-1 and plant-2. The RF value and colour of the bands were recorded. Followingtable will demonstrate RF value.

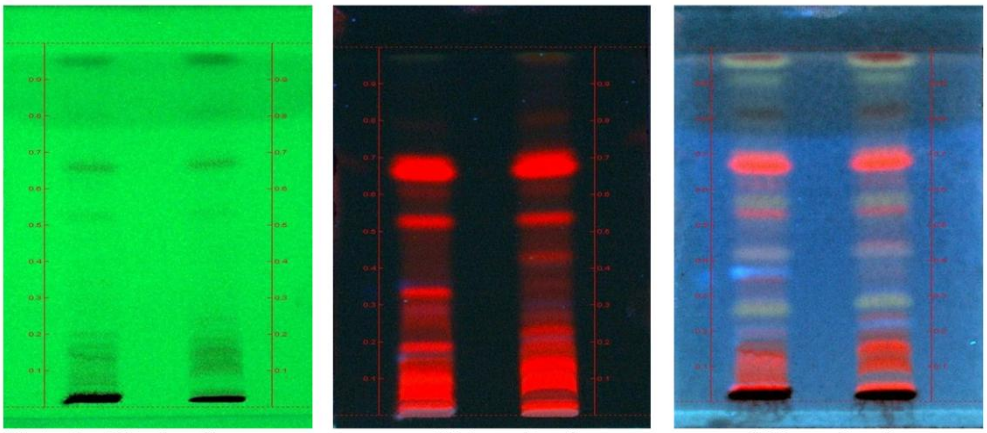

Plate 1: DL- Leaf 254nm- Leaf 366nm- Leaf

Table 4.1: *Rf* value of 254nm (Day Light*) Ficusvirens* Leaf

S.No.	Ethanol	colour	Methanol	colour
RF_1	0.29	Light brown	0.23	Light brown
RF_2	0.42	Light brown	0.32	Light brown

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RF_3	0.56	Light brown	0.39	Light brown
RF_4	0.67	Light brown	0.46	Light brown
RF_5	0.91	Light Blue	0.71	light blue

Table 4.2: ***Rf*** **value of 366nm (Day light)** ***Ficusvirens*** **Leaf**

S.No.	Ethanol	colour	Methanol	colour
RF_1	0.29	Brown	0.27	Brown
RF_2	0.42	Violet	0.35	Violet
RF_3	0.56	Light Brown	0.58	Light Brown
RF_4	0.67	Light Green	0.63	Light Green
RF5	0.91	Grey	0.89	Grey

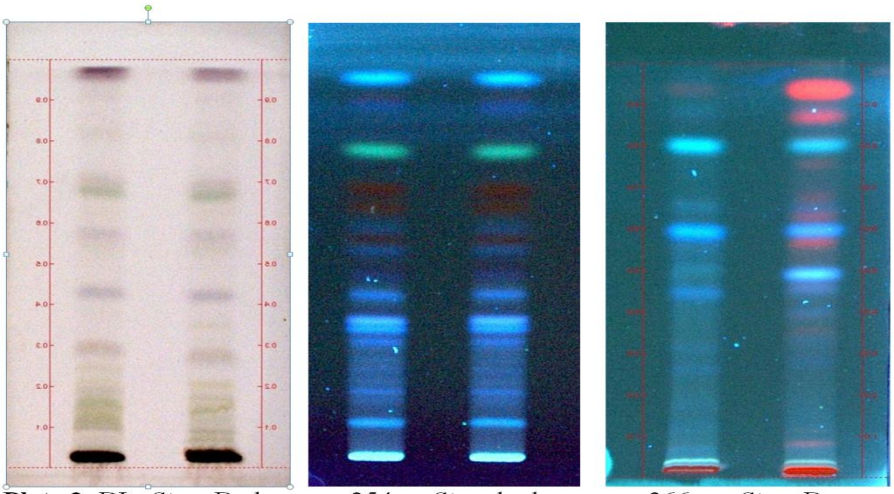

Plate 2: DL- Stem Bark 254nm Stem bark 366nm- Stem Bar

Table 5.1: ***Rf*** **value*Ficusvirens*Stem Bark at 254 nm**

S.No.	Ethanol	colour	Methanol	colour
RF_1	0.18	Red	0.18	Red
RF_2	0.33	Red	0.33	Red
RF_3	0.53	Red	0.53	Red
RF_4	0.66	Red	0.66	Red

Table5.2: ***Rf*** **value of 366nm** ***Ficusvirens*** **Stem Bark at Blue Light**

S.No.	Ethanol	colour	Methanol	colour
RF_1	0.15	Red	0.15	Red
RF_2	0.27	Greenish	0.27	Greenish
RF_3	0.42	Yellow	0.42	Yellow
RF_4	0.54	Brown	0.54	Brown
RF_5	0.58	Red	0.58	Red
RF_6	0.67	Brown	0.67	Brown

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RF7	0.91	Light Yellow	0.91	Light yellow

Medicinal Uses of *Ficusvirens* by Tribal PeopleofChitrakoot:

Tribes of Chitrakootuse the leaves of this plant for the treatment of excessive and frequent urination. Leaves quath are used by tribes for frequent urination and filtering of blood. Stem bark of plant(Powder) are used for treatment of gastric ulcer. Fruit of *Ficusvirens* are taken as a tonic. Milky sap is used for cleaning of wounds.Dry flower of *Ficusvirens*with *Lawsoniainermis* (mehandi) and onion leaves are taken by people when they suffer from heat strokes. There are many traditional uses of this tree. Decoction of the bark was used in the treatment of leucorrhoea, as a wash on skin ulcers, and as a gargle, while a bark extract mixed with other plants was given for bone fractures.

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