



A STUDY ON PHARMACOGNOSTICAL ANALYSIS OF LAGHU BADARA (ZIZPHUS RUGOSA LAM) STEM BARK CHOORNA

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Abstract

Ziziphus rugosa is a member of Rhamnaceae family, found in the deciduous and semi-evergreen forests of Western ghats of India. It is commonly known as Suran in hindi, Chunukoli in urdu, Badara in sanskrit and Toran in marathi¹. *Z. rugosa* is traditionally used in the treatment of skin diseases, mouth ulcers, dropsy, boils, diarrhea, tachycardia, syphilis, miscarriage, misconception, flatulence, hysteria and as astringent. It has been investigated for anti-diabetic, anti- inflammatory, α -glucosidase inhibitory, cytotoxic, antioxidant, antibacterial, anthelmintic, analgesic, and insecticidal activities². In this present study, sample of stem bark of *Ziziphus rugosa* (Badara) has been standardized as per standard testing protocol. Results of powder microscopy, Physicochemical parameters, phytochemical test and HPTLC Photo documentation, Densitometric scan and R_f values have been studied. . Several constituents are seen from different parts of this plant like cyclopeptide alkaloids, pentacyclic triterpenoids (oleanolic acid, alphitolic acid, betulinaldehyde, betulin, betulinic acid, lupeol), flavonoids (kaempferol, quercetin, myricetin, apigenin, , luteolin, luteolin -7-o-glucoside), dihydroxy benzoic acid (vanilic acid), quinoline (isoquinoline)³.

Keywords: Badara, *Ziziphus rugosa*, phytochemical, Physicochemical, HPTLC.

Introduction

India contains more than 45,000 plant species and is the largest producer of medicinal plants; hence it is called the ‘Botanical garden of world’. These medicinal plants play a vital role in the development of potent therapeutic agents across the world and are immensely used traditionally. Laghu Badara⁴ (*Ziziphus rugosa*), a member of Rhamnaceae family, with taxonomical features such as Kingdome Plantae; Order Rosales ; Family Rhamnaceae; Genus *Ziziphus*; Species *rugosa*, is found in the deciduous and semi-evergreen forests of Western Ghats, India. This plant is also found in China, India, Jammu and Kashmir, Laos, Thailand, Sri Lanka, Java, Peninsular, Malaysia, Singapore, Vietnam and amans, Nicobars, Myanmar, Nepal, Pakistan, and Bangladesh. An exhaustive literature survey indicates that the bark is traditionally used as an astringent and antidiarrhoeal agent, the flowers are used in menorrhagia, stem and fruits are hypotensive⁵. Few of the Ethnomedicinal uses of *Ziziphus rugosa* are seen in the Kodava community of Kodagu region of the Western Ghats, they traditionally eat the endocarp of *Z. Rugosa* fruits in raw and ripe form as a nutrional source. This plant is food for animals such as elephants and deer. The fruit of this plant is a habitat for bee farming in Uttar Kannada district . *Z. rugosa* is reported to be used in the treatment of skin diseases, mouth ulcer, dropsy, boils, diarrhea, tachycardia, syphilis, miscarriage, misconception, flatulence, hysteria, and as an astringent⁶.

METHODOLOGY

Collection of plant material: The bark of *Ziziphus rugosa* Lam. was collected in the month of Feburary 2024 from the campus of SDM college of ayurveda udupi, Karnataka. India. It was authenticated in the Dept of dravyaguna vijyana SDMCA Udupi. The study was conducted at SDM center for research in Ayurveda and allied sciences, udupi for its Macro-microscopy, Standardisation, Phytochemical test, HPTLC.

1. PHARMACOGNOSTICAL STUDY

The sample of Badara bark and bark powder were taken for analysing Macro-Microscopic features⁷.

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

Sample (stem bark) 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 Axio Cam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.

Powder microscopy

Pinch of bark powder previously sieved was put on the slide and mounted in glycerine & powder characters were observed under the Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light.

2. PHYSICO-CHEMICAL ANALYSIS

The sample of Badara bark powder was taken for analysis of Physicochemical properties.⁸

Loss on drying at 105°C

10 g of sample was placed in tared evaporating dish. It was dried at 105°C for 5 hours in hot air oven and weighed. The drying was continued until difference between two successive weights was not more than 0.01 after cooling in desiccator. Percentage of moisture was calculated with reference to weight of the sample.

Total Ash

2 g of sample was incinerated in a tared platinum crucible at temperature not exceeding 450°C until carbon free ash is obtained. Percentage of ash was calculated with reference to weight of the sample.

Acid insoluble Ash

To the crucible containing total ash, add 25ml of dilute HCl and boil. Collect the insoluble matter on ashless filter paper (Whatmann 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccator for 30 mins and weigh without delay. Calculate the content of acid insoluble ash with reference to the air dried drug.

Water soluble ash

Boil the ash for 5 min with 25 ml of water; collect insoluble matter on an ashless filter paper, wash with hot water, and ignite for 15 min at a temperature not exceeding 450°C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water soluble ash with reference to the air-dried sample.

Alcohol soluble extractive

Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled Alcohol (approximately 95%). Shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours, cool in desiccator for 30 minutes and weigh. Calculate the percentage of Alcohol extractable matter of the sample. Repeat the experiment twice, and take the average value.

Water soluble extractive:

Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled water, shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air

oven at 105°C for 6 hours. Cool in a desiccator and weigh. Repeat the experiment twice. Take the average value.

3. PRELIMINARY PHYTOCHEMICAL TESTS

The sample of Badara bark powder was taken for analysis of Preliminary Phytochemical Tests.⁹

Tests for alkaloids

Dragendroff's test: To a few mg of extract dissolved in alcohol, a few drops of acetic acid and Dragendroff's reagent were added and shaken well. An orange red precipitate formed indicates the presence of alkaloids.

Wagners's test: To a few mg of extract dissolved in acetic acid, a few drops of Wagner's reagent was added. A reddish brown precipitate formed indicates the presence of alkaloids.

Mayer's test: To a few mg of extract dissolved in acetic acid, a few drops of Mayer's reagent was added. A dull white precipitate formed indicates the presence of alkaloids.

Hager's test: To a few mg of extract dissolved in acetic acid, 3 ml of Hager's reagent was added, the formation of yellow precipitate indicates the presence of alkaloids.

Tests for carbohydrates

Molisch's test: To the extract, 1 ml of α -naphthol solution and conc. sulphuric acid were added along the sides of test tube. Violet colour formed at the junction of the two liquids indicates the presence of carbohydrates.

Fehling's test: A few mg of extract was mixed with equal quantities of Fehling's solution A and B. The mixture was warmed on a water bath. The formation of a brick red precipitate indicates the presence of carbohydrates.

Benedict's test: To 5 ml of Benedict's reagent, a few mg of extract was added, and boiled for two minutes and cooled. Formation of a red precipitate indicates the presence of carbohydrates.

Test for steroids

Liebermann-Burchard test: To the extract was dissolved in chloroform, 1 ml of acetic acid and 1 ml of acetic anhydride were added, then heated on a water bath and cooled. Few drops of conc. Sulphuric acid were added along the sides of the test tube. Appearance of bluish green colour indicates the presence of steroids.

Salkowski test: The extract was dissolved in chloroform and equal volume of conc. Sulphuric acid was added. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer indicates the presence of steroids.

Test for saponins

To a few mg of extract, distilled water was added and shaken. Stable froth formation indicates the presence of saponin.

Test for tannins

To the extract, a few drops of dilute solution of ferric chloride was added, formation of dark blue colour shows the presence of tannins.

Test for flavonoids

Shinoda's test: To the extract in alcohol, a few magnesium turnings and few drops of conc. hydrochloric acid were added and heated on a water bath. Formation of red to pink colour indicates the presence of flavonoids.

Test for phenol

To the extract in alcohol, added two drops of alcoholic ferric chloride. Formation of blue to blue black indicates the presence of phenol.

Test for coumarins

To the extract in alcohol, a few drops of 2 N sodium hydroxide solution was added. Dark yellow colour formation indicates the presence of coumarins.

Test for triterpenoids

The extract was warmed with tin bits and few drops of thionyl chloride. Formation of pink colour indicates the presence of triterpenoids.

Test for Amino acids

The extract added few drops of Ninhydrine reagent, purple color indicates the presence of aminoacids.

Test for carboxylic acid

Extract dissolved in water is treated with sodium bicarbonate. Brisk effervescence indicates the presence of carboxylic acid.

Test for resin

Few mg of the sample was mixed with water and acetone. Turbidity indicates the presence of resins.

Test for quinone

A few mg of alcohol extract was treated with 0.5% of sodium hydroxide. Deep coloration like pink, purple or red indicates the presence of quinone.

HPTLC

One gram of powdered sample of Badara were suspended in 10 ml ethanol (99.9%) and kept for cold percolation for 24h and filtered. 4, 8 and 12 μ l of the above samples were 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 (9.0: 1.0). The developed plates were visualized in short UV, long UV and then derivatised with vanillin sulphuric acid reagent and scanned under UV 254nm, 366nm and 620nm (Post derivatisation). R_f, colour of the spots and densitometric scan were recorded¹⁰.

RESULTS

Macroscopy: The bark of *Ziziphus rugosa* is greyish-brown to dark brown, rough and uneven with longitudinal and irregular fissures. Externally it shows patches of exfoliation and a somewhat scaly surface. The outer cork layer is hard and brittle, while the inner bark is yellowish-brown, fibrous and slightly aromatic when freshly cut. On fracture, it is short and granular externally and fibrous internally. Transversely, alternating dark and light streaks of phloem rays may be visible. The bark is moderately thick and exudes little or no latex. (Fig 1)

Microscopy: The transverse section of *Ziziphus rugosa* stem bark shows a distinct arrangement of tissues. The **outermost cork layer (phellem)** is composed of several rows of tangentially arranged, brownish, suberized cells forming a protective covering. Beneath it lies the **phellogen (cork cambium)**, consisting of thin-walled, rectangular cells that give rise to new cork cells outwardly. The **cortex** is made up of several layers of parenchymatous cells containing starch grains and brown coloring matter, interspersed with **stone cells and sclerenchymatous fibers** providing rigidity. **Lenticels** are present, aiding gaseous exchange. Below the cortex, a **well-defined pericyclic region** with groups of **pericyclic fibers** is observed. The **phloem** consists of sieve tubes, companion cells, and parenchyma, while **xylem elements** with vessels, tracheids, and fibers occupy the inner region. Overall, the bark shows a compact, lignified structure typical of mature dicot stems. (fig 3)

Powder microscopy: Important microscopic characteristics of *Ziziphus rugosa* Bark choorn are vessels, sclerides, fibres containing prismatic crystals, Spiral vessel, Bundles of fibres, tracheids, Parenchyma crossing over medullary rays, Parenchyma containing starch and acicular raphides, wood elements, Vessels with bordered pits, isolated phloem fibres, parenchymatous ground tissue, stone cells. (fig 2)

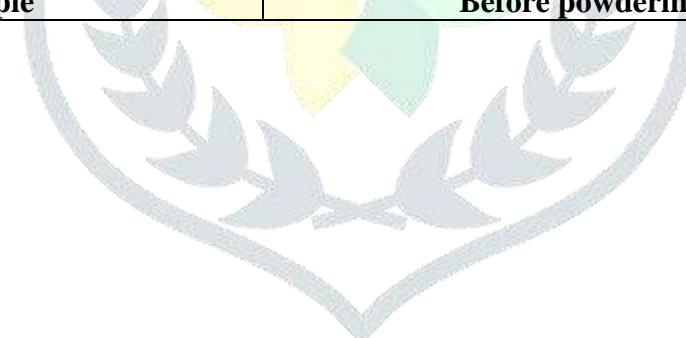
Figure1: Macroscopy of stem bark of *Ziziphus rugosa* (Badara) in different stages of drying**Figure2: Powder microscopy of stem bark of *Ziziphus rugosa* (Badara)**



Fig. 2.1 sclereids



Fig. 2.2 fibres containing prismatic crystals

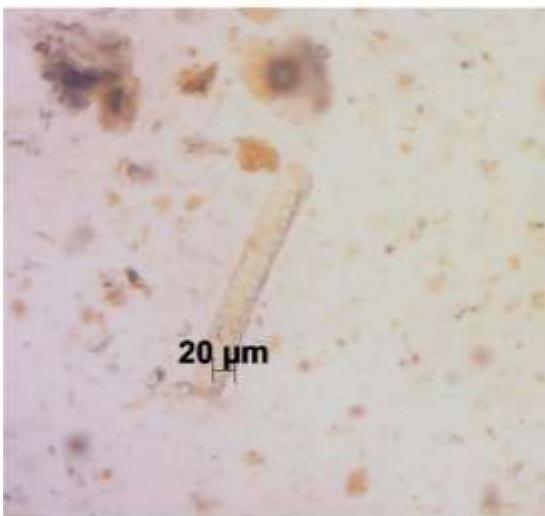


Fig.2.3 Spiral vessel



Fig.2.4 fibres containing prismatic crystals

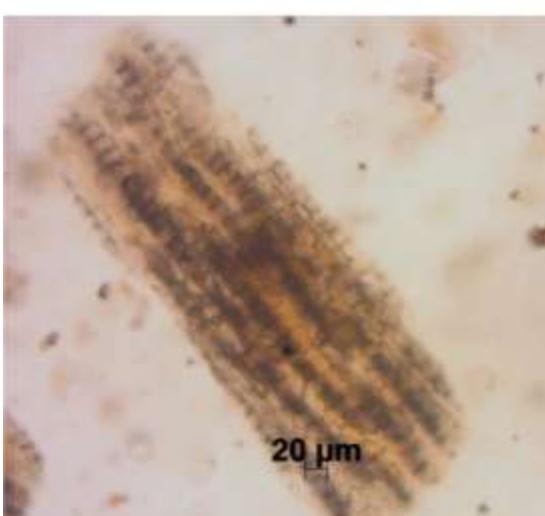


Fig.2.5 Bundles of fibres



Fig.2.6 Parenchyma crossing over medullary rays



Fig. 2.7 Parenchyma containing starch and acicular raphides



Fig. 2.8 Wood elements

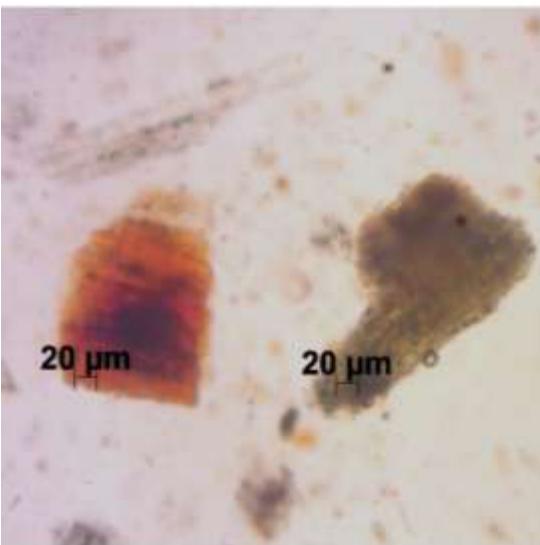


Fig.2.9 Parenchyma with brown matter



Fig.2.10 stone cells

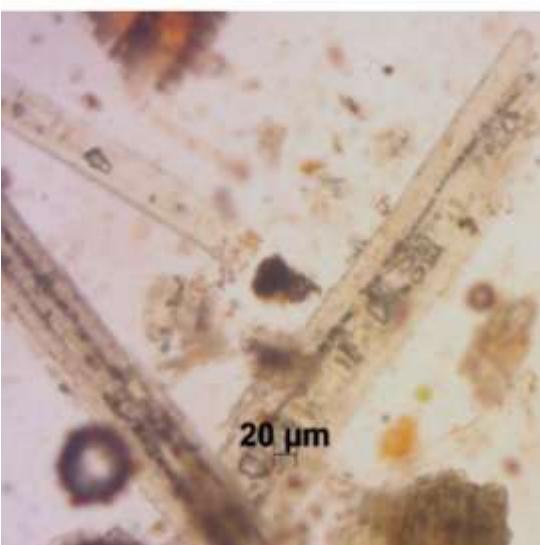


Fig.2.11 Vessels with bordered pits

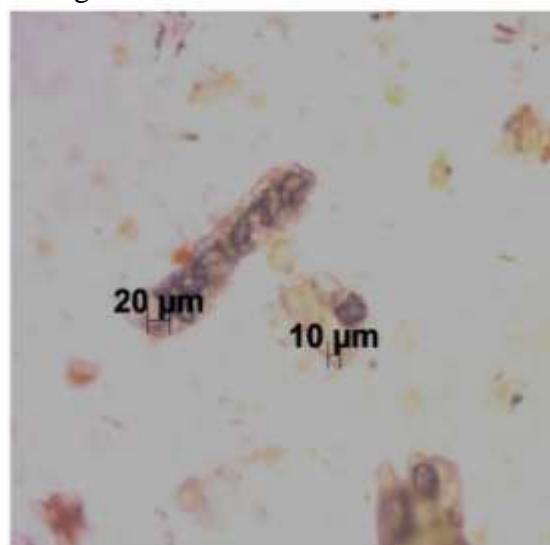
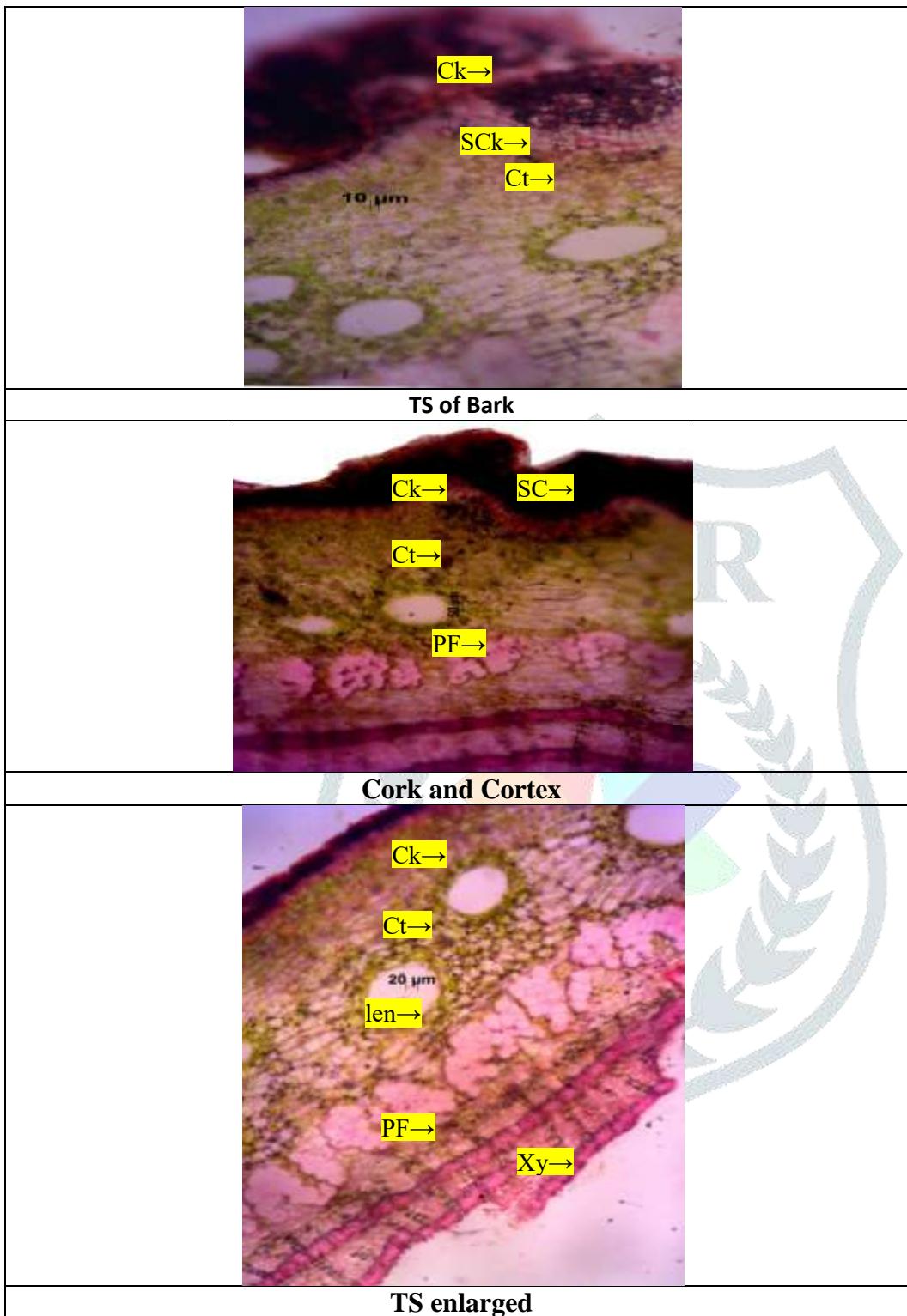
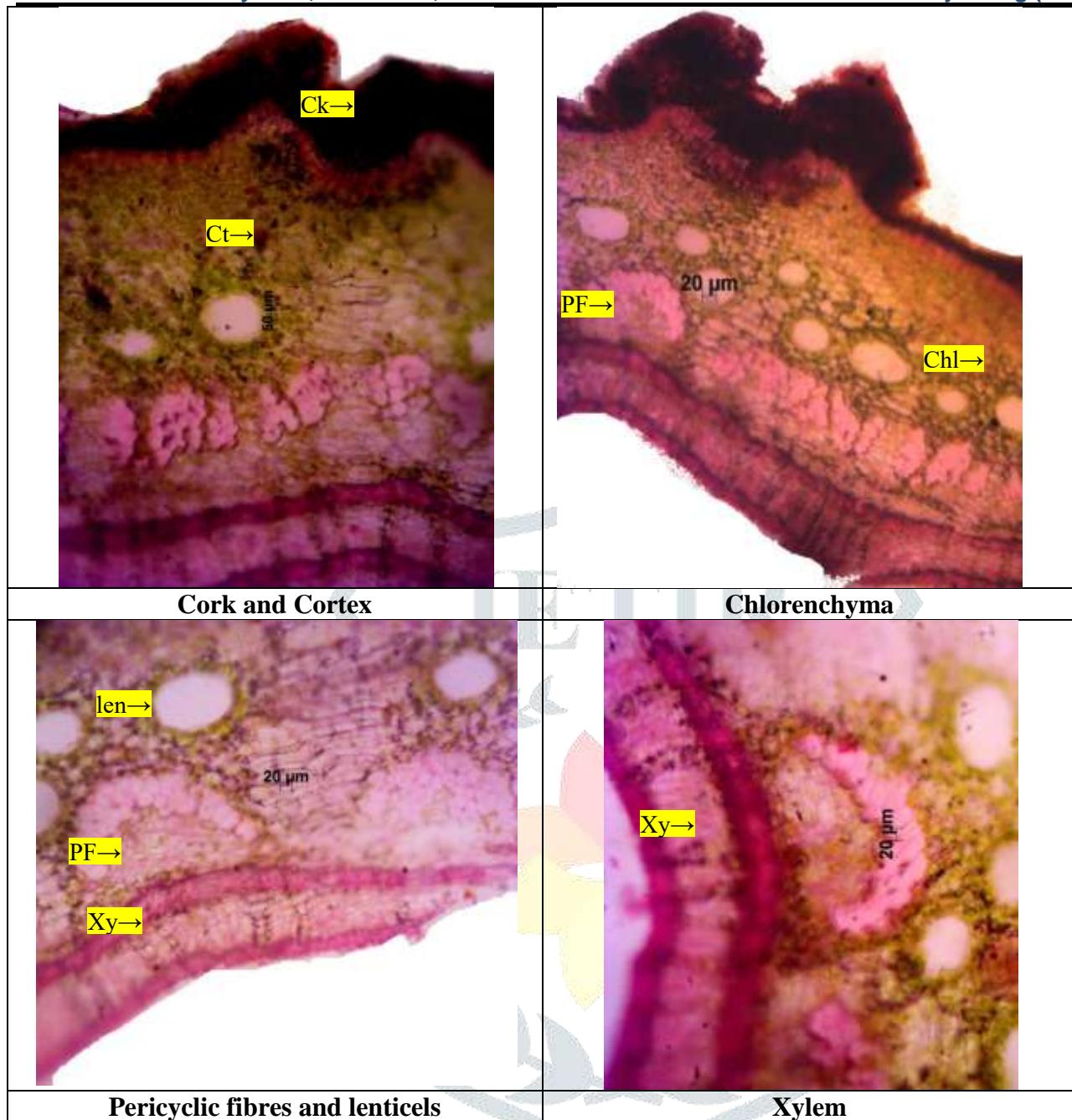


Fig.2.12 Group of stone cells having lignification

Fig 3 : Microscopy of stem bark of *Ziziphus rugosa* (Badara)

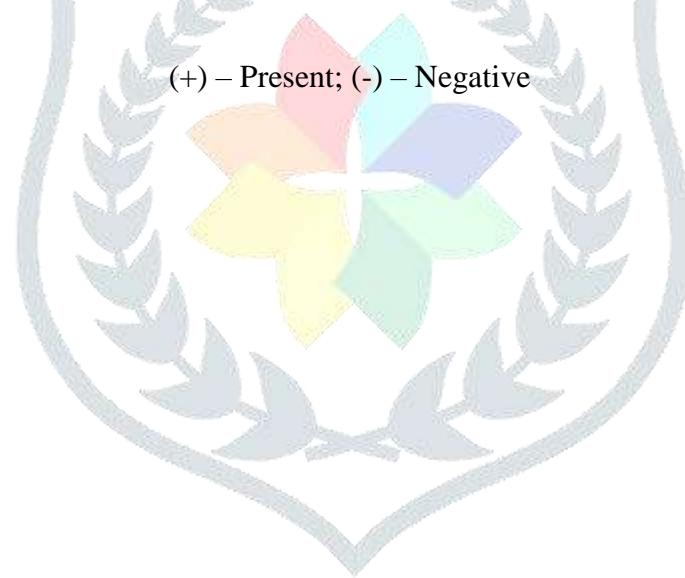
Table 1. Results of standardization parameters of stem bark of *Z.rugosa*

Parameter	Results n = 3 %w/w Avg ± SD
Loss on drying	11.86±0.02
Total Ash	7.42±0.70
Acid Insoluble Ash	0.00±0.00
Water soluble Ash	0.98±0.00
Alcohol soluble extractive value	18.16±0.02
Water soluble extractive value	36.77±0.03

Table 2: Results of preliminary phytochemical screening of *Ziziphus rugosa* (Badara) Aqueous and Alcoholic extract

Test	Inference	
	Aqueous extract of Badara bark	Alcoholic extract of Badara bark
Alkaloid	+	-
Steroid	+	+
Carbohydrate	+	+
Tannin	+	+
Flavanoids	-	+
Saponins	+	-
Terpenoid	-	+
Coumarins	+	+
Phenols	-	-
Carboxylic acid	-	-
Amino acids	-	-
Resin	+	-
Quinone	+	+

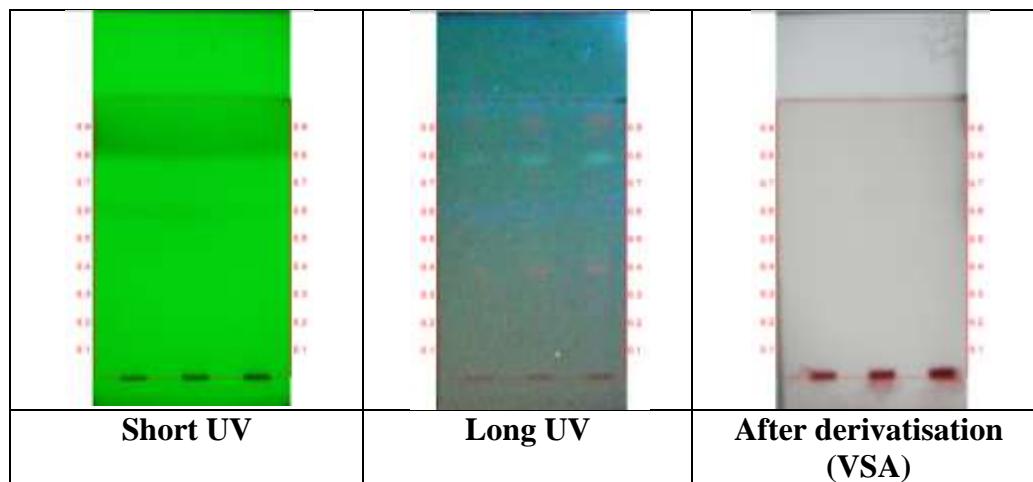
(+) – Present; (-) – Negative



Tests	Color if positive	Aqueous extract of Badara bark	Alcoholic extract of Badara bark
Alkaloids			
Dragendroff's test	Orange red precipitate	Orange red precipitate	No Orange color
Wagners test	Reddish brown precipitate	Reddish brown precipitate	No Reddish brown color
Mayers test	Dull white precipitate	Dull white precipitate	No Dull white color e
Hagers test	Yellow precipitate	Yellow precipitate	No Yellow color
Steroids			
Liebermann-buchard test	Bluish green colour	Bluish green colour	Bluish green colour
Salkowski test	Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer	Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer	Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer
Carbohydrate			
Molish test	Violet ring	Violet ring	Violet ring
Fehlings test	Brick red precipitate	Brick red precipitate	Brick red precipitate
Benedicts test	Red precipitate	Red precipitate	Red precipitate
Tannin			
With FeCl ₃	Dark blue or green or brown	Brown	Green
Flavanoids			
Shinoda's test	Red or pink	White precipitate	Red
Saponins			

With water	Stable froth	Stable froth	No Stable froth
Triterpenoids			
Tin and thionyl chloride test	Pink	Yellow precipitate	Pink
Coumarins			
With 2 N NaOH	Yellow	Yellow	Yellow
Phenols			
With alcoholic ferric chloride	Blue to blue black	No Blue to blue black	No Blue to blue black
Carboxylic acid			
With water and NaHCO ₃	Brisk effervescence	No Brisk effervescence	No Brisk effervescence
Amino acid			
With ninhydrine reagent	Purple colour	No Purple colour	No Purple colour
Resin			
With aqueous acetone	Turbidity	Slight turbidity	No turbidity
Quinone			
Conc. sulphuric acid	Pink/purple/red	Red color	Red color

Figure 4. HPTLC photo documentation of methanolic fraction of Badara bark

Track 1 - Badara bark - 3 μ lTrack 2 - Badara bark - 6 μ lTrack 3 - Badara bark - 9 μ l

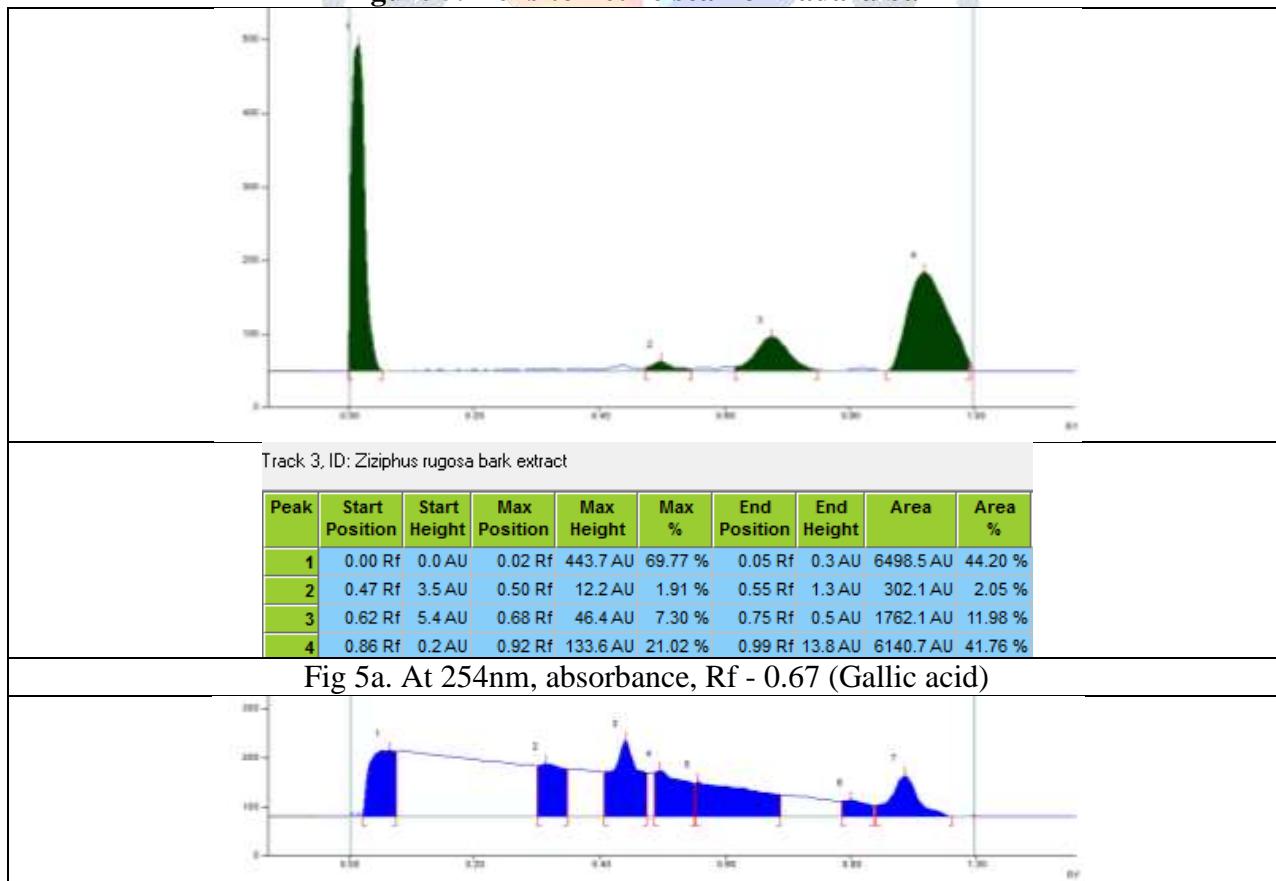
Solvent system – Toluene: Ethyl acetate: Formic acid (7.0: 3.0: 0.5)

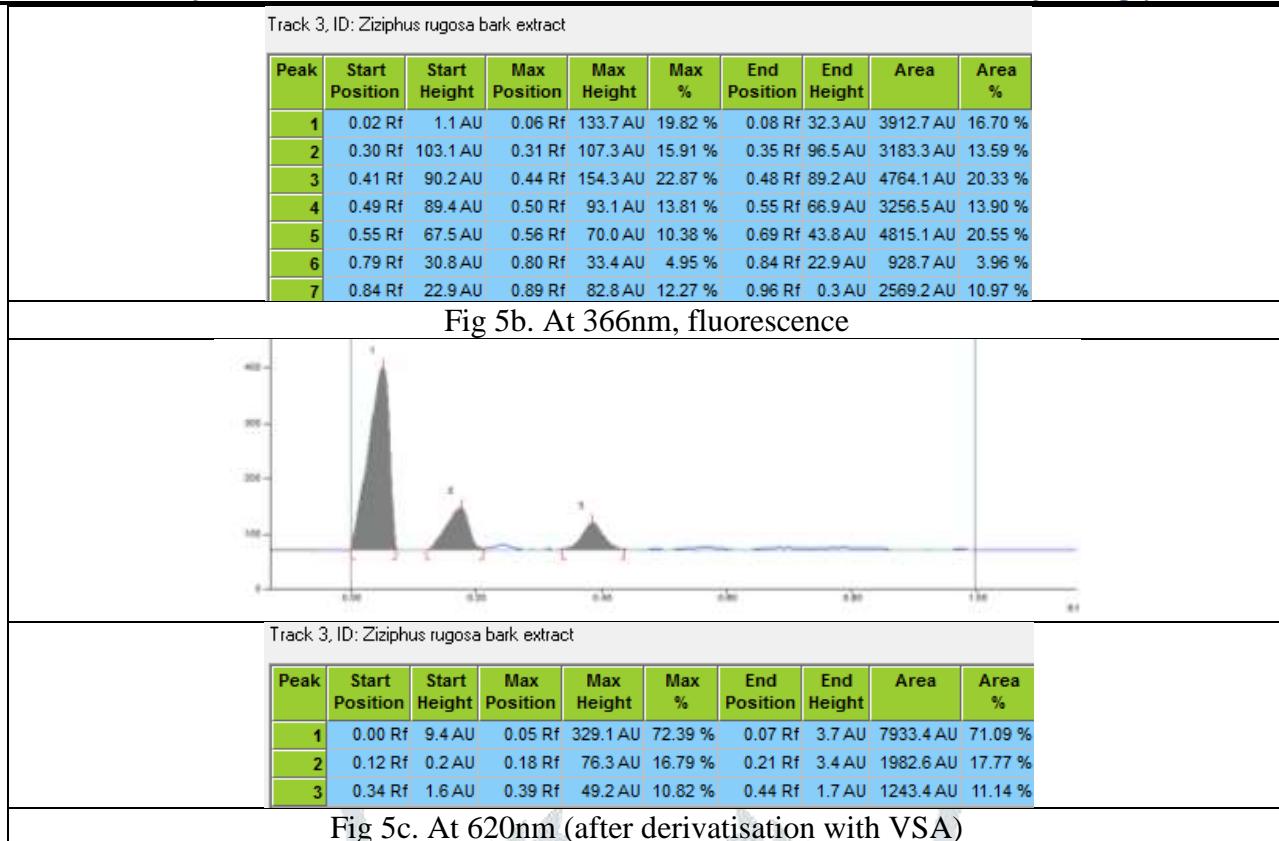
R_f - 0.67 (Gallic acid)Table 3: R_f values of sample of Badara bark

Short UV	Long UV	After derivatisation
-	0.41 (F. red)	-
-	0.80 (F. green)	-
-	0.93 (F. red)	-

*F –fluorescent; D – dark; L - light

Figure 5. Densitometric scan of Badara bark





The given sample stem bark of *Ziziphus rugosa* (Badara) has been standardized as per standard testing protocol. Results of, powder microscopy, standardization parameters, phytochemical test and HPTLC Photo documentation, Densitometric scan and R_f values for Gallic acid qualitative fingerprinting are given in respective tables and figures.

DISCUSSION & CONCLUSION

This study illustrates Pharmcognostical, Physicochemical, Preliminary Phytochemical analysis, of the plant Laghu Badara (*Ziziphus. Rugosa*). The transverse section of *Ziziphus rugosa* stem bark shows a distinct arrangement of tissues such as Phellogen, lenticels, Pericyclic fibers etc. The Microscopic characteristics of *Ziziphus rugosa* Bark powder include sclerides, fibres containg prismatic crystals, Spiral vessel. The Phytoconstituents like Tannins, Flavonoids, Saponins, Alkaloids, Terpenoids , vanillic acid, betuline, betulinic acid, kaempferol, quercetin, myricetin, apigenin and apigenin-7-O-glucoside. It is reported to contain N-formyl cyclopeptide alkaloids. The HPTLC study revealed the significant presence of Gallic acid. This plant has been evaluated for various pharmacological activities such as Antifungal, Antibacterial, Antioxidant, Anti-inflammatory, Analgesic, Anticancer, Insecticidal, and Anthelmintic activity¹¹. The presence of Gallic acid acts as Antioxidant, Anti-inflammatory, Antimicrobial and Tissue-regenerative properties. The most bioactive extracts of *Ziziphus rugosa* can be fractionated to identify new constituents, and based on the nature of constituents, they can be screened for new biological activities opening up new avenues for more intensive research on *Z. rugosa*.

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