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PHARMACOGNOSTICAL AND PRELIMINARY PHYTOCHEMICAL EVALUATION OF *SALIX TETRASPERMA ROXB.*

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ABSTRACT

Background: *Salix tetrasperma* Roxb. (Jalavetasa) family-Salicaceae is one among the traditionally used medicinal plant being used by the traditional healers for its multiple therapeutic indications. Present study reports a details pharmacognostic and preliminary physiochemical and phytochemical aspects of *Salix tetrasperma* stem bark. **Material & Methods:** The stem bark sample was collected after proper authentication from Dehradun, Uttarakhand India. Macroscopic observations were made with naked eyes, microscopy, powder microscopy, physicochemical and qualitative analysis and HPTLC studies of stem bark were carried out by following API (Ayurvedic Pharmacopoeia of India) recommended standard protocols. **Results and Discussion:** Stem bark was cylindrical, striated, greenish brown in color, measures 15-20 x 1.5-2 cm, odor aromatic, fracture fibrous with irregular surface. In microscopic study of *Salix tetrasperma* bark commonly known as the four-seeded willow, would typically involve examining thin sections of the bark under a microscope to observe its structural features**.** Diagrammatic T.S. of stem bark shows periderm, cortex, phloem, vascular cambium and xylem. In powder microscopy of *Salix tetrasperma* stem bark powder colour was light greenish yellow, odour aromatic; taste astringent & bitter; texture fibrous. Diagnostic powder character of

stem bark shows vessels, tracheid, calcium oxalate, simple & compound starch grains. Qualitative phytochemical analysis of stem bark was carried out in ethanolic and aqueous extracts to identify the major chemical constituents. Which shows the presence of amino acid, protein phenolic compound, tannins in both aqueous and ethanolic extract. The TLC study of *Salix tetrasperma* ethanolic stem bark extract was scanned and R_f Value: 0.25, 0.54, 0.67, 0.78, 0.89 observed. HPTLC study of stem bark was done at 256 nm,366nm,512nm and was observed respectively 16 R_f value, 14 of R_f value and 24 of R_f value. **Conclusion**: The results obtained from pharmacognostical, phytochemical, physicochemical characteristic along with TLC & HPTLC studies are the diagnostic tool of *S. tetrasperma* plant and will help in standardization of the raw drug in terms of its quality, strength and purity.

KEYWORDS: HPTLC, Pharmacognostical, Phytochemical, TLC.

INTRODUCTION: *Salix tetrasperma* Roxb. (*Jalavetasa*) family- Salicaceae, deciduous shrub or small tree up to 9 m high, flowering after leafing. Bark is greyish-brown, rough with vertical fissures and branchlets often pubescent. Leaves 2-5 by 5/8 – 11/2 in., petiole narrowly or broadly ovate-lanceolate acuminate serrulate rarely entire, lanceolate or ovate-lanceolate, upper surface green and glabrous and lower surface covered with white blooms, glabrous or the young as well as the branchlets more or less softly tomentose or silky. Flower appearing after the leaves; catkins hairy, 2-5 in. long; peduncles silky-villous, leafy at the base. Fruits: hard and 7mm long, each fruit bears 4-6 seeds. Seeds 4-6. Flowering and fruiting are during September-December. It is commonly known as *Indian willow* (English); *Neeruvanji* (Kannada); *Jalavetasa* (Sanskrit). It is commonly found along the banks of rivers and streams up to 1200 m., throughout tropical and subtropical India from the Punjab eastwards to Mishmi, Assam and Munnipore, ascending the Himalaya to 7000ft., and southwards to Travancore, Sumatra, Java and Singapore[1,2].

Genetic studies summarized by the Angiosperm Pylogeny Group (APG) have greatly expanded the circumscription of the family to contain 56 genera and about 1220 species, Genera 2; species about 180, chiefly in N. temperate regions. In India most of the species are found in the Himalaya from Jammu& Kashmir to Arunachal Pradesh[3].

Various ethnomedicinal claims in different diseases such rheumatism, epilepsy, venereal diseases, bladder stone, piles, swellings, etc[4,5,6,7], bark decoction in fever[8], Leaves externally as poultice in wound[9], Leaf juice externally in ear pain[9], Root in cold and cough[9], Root internally in type 2 diabetes[10]. Stem & flower in wound[11] have been reported. Various analytical[12] and pharmacological studies of different parts such as leaf, stem bark and root has been reported.

The present study was designed to study the unexplored pharmacognostic morphology, powder microscopic, physiochemical parameters and phytochemical screening including TLC, HPTLC of *Salix tetrasperma* stem bark.

MATERIALS AND METHODS:

Collection, authentication and preservation

Available literature pertaining the plant *Salix tetrasperma* were compiled from 10 different e- floras and 6 floras in printed format (Flora of Presidency of Madras, Flora of British India, Flora of Davanagere District, Flora of Shimoga district, Forest Flora of the Dehradun, Forest Flora of The Bombay Presidency and Sind) and flora in e-version, different books (45), journals, web-based search engines and presented in systematic manner.[13,14]

For future reference, herbarium sheet was submitted to pharmacognosy laboratory of this institute & authentified, provided with herbarium reference no. dsrrau/pgia/dg/08-a/2023.

For authentication and preservation, fresh stem bark of *S. tetrasperma* sample were preserved both in wet (Ethyl alcohol, Glacial Acetic acid, Formalin - 90:5:5) and dry condition for further study[15, 16]. (Fig.1A-F)

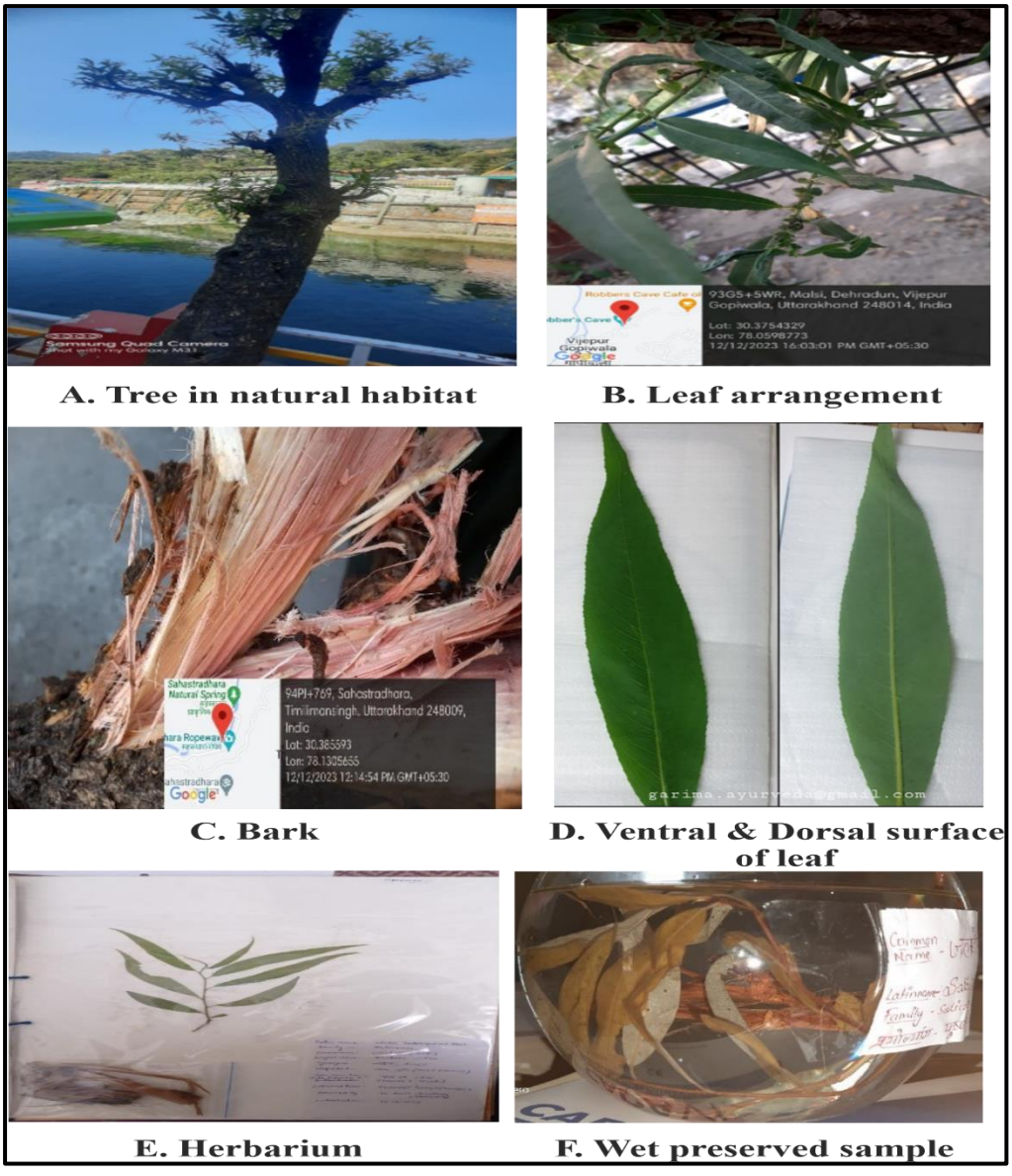

Fig.1: Collection, authentication and preservation of *Salix tetrasperma*

Processing and preservation of drug: For pharmacognostical and analytical evaluation, shade dried stem bark was grinded by mechanical grinder and sieved through 80# mesh and powder were kept in air tight glass jar.

Organoleptic study: Test drug *Salix tetrasperma* Roxb. Stem bark was subjected to various organoleptic characters like taste, odour, colour, touch[17].

Macroscopic study: The fresh stem bark was subjected to observe various macroscopic features i.e. shape, venations, margin, apex, base, surface and texture were studied as per various flora and texts[18]. Macroscopic observation was made with naked eyes and centimetre scale was used to measure the leaf size.

Microscopic study:

Transverse sections (TS): Free hand thin transverse section of stem bark was taken and cleared with choral hydrate to observe the anatomy and microscopical characteristic of leaf with help of Quasmo binocular compound microscope. Free hand thin transverse section of root and stem were taken, cleared with choral hydrate and with help of Quasmo binocular compound microscope to observe the anatomy and microscopical characteristic of root and stem. Thick transverse sections of the root and stem were exposed to Phloroglucinol + Conc. HCl, Iodine and Ferric chloride solution for observation of lignin, starch grains and tannin respectively for histochemical test[19].

Powder microscopy: Microscopically, the stem bark powder of *Salix tetrasperma* were studied and photographs were taken with the help of a Quasmo binocular compound microscope.

Physicochemical analysis: Stem bark powder of *S. tetrasperma* was analysed for physicochemical parameters such as moisture content, ash value, acid-insoluble ash, pH, water-soluble and alcohol-soluble extractive value following standard methods[20].

Preliminary phytochemical screening: The ethanolic and aqueous extracts stem bark obtained following standard methods were analysed to know presence or absence of the major class of compound for a qualitative analysis[21].

HPTLC Study: Samples of root and stem were prepared according to standard methods described by Wagner and Bladt (1984)[13] for different the plate as 8-mm band length using the CAMAG Linomat 5 TLC (Hamilton, Bonaduz, Switzerland 100 µL) sample applicator equipped with syringe on pre-coated silica gel glass plate 60F-254 (20 cm x 10 cm x 250 µm (E. Merck, Darmstadt, Germany. After the application root and stem of *A. monophylla*, plates were developed vertically ascending in a glass twin-trough chamber (CAMAG, Switzerland) pre-saturated for 20 min at room temperature, with Toluene: Ethyl acetate (9:1) V/V was used as mobile phase. The chromatographic run length was 80 mm from the bottom edge of the plate. After development, the plate was air-dried for complete removal of mobile phase and derivatized by dipping the developed plate in methanol agent for 2 sec. The plate was then air-dried and heated at 110 °C on TLC plate heater for 10 min. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and

images were taken. Densitometric scanning was then performed at 254 nm and 366 nm with win CATS software version 1.4.6. The retention factor (Rf) value for each spot found on plate was recorded[22,23,24].

OBSERVATION AND RESULTS:

Macroscopic Study: Stem bark: Cylindrical, striated, greenish brown in colour, measures 15-20 x 1.5-2 cm, odor aromatic, fracture fibrous with irregular surface (Fig.2A, 2B).

Organoleptic characters: (Fresh sample): The details of the organoleptic characters of stem bark of *Salix tetrasperma* described in (Table 1).

Table 1: Macroscopic organoleptic characters of stem bark

Average length of procured stem	15 cm to 20 cm
Touch	Rough
Colour	Greenish brown
Odour	Aromatic
Taste	Astringent, bitter
Fracture	Fibrous with irregular surface

Microscopic study:

Stem Bark: Diagrammatic T.S. of stem bark microscopy of Salix tetrasperma, commonly known as the four-seeded willow, would typically involve examining thin sections of the bark under a microscope to observe its structural features. (Fig.2A-E)

Outer Bark (Periderm): The outermost layer, consisting of dead cells and primarily serving a protective function. May show remnants of lenticels (small pores for gas exchange) depending on the maturity of the bark.

Cortex: Beneath the outer bark, consisting of several layers of living cells responsible for storage and transport.

Phloem: The innermost layer of the cortex, comprising sieve tubes, companion cells, phloem parenchyma and fibres. Sieve tubes are involved in transporting organic nutrients throughout the plant

Vascular Cambium: A thin layer of meristematic tissue responsible for secondary growth (thickness) of the stem. Generally seen between the phloem and the xylem

Xylem: Located inward from the cambium, consisting of various types of cells including vessels, tracheids, fibers and xylem parenchyma. Provides structural support and conducts water and minerals. (Fig.2C)

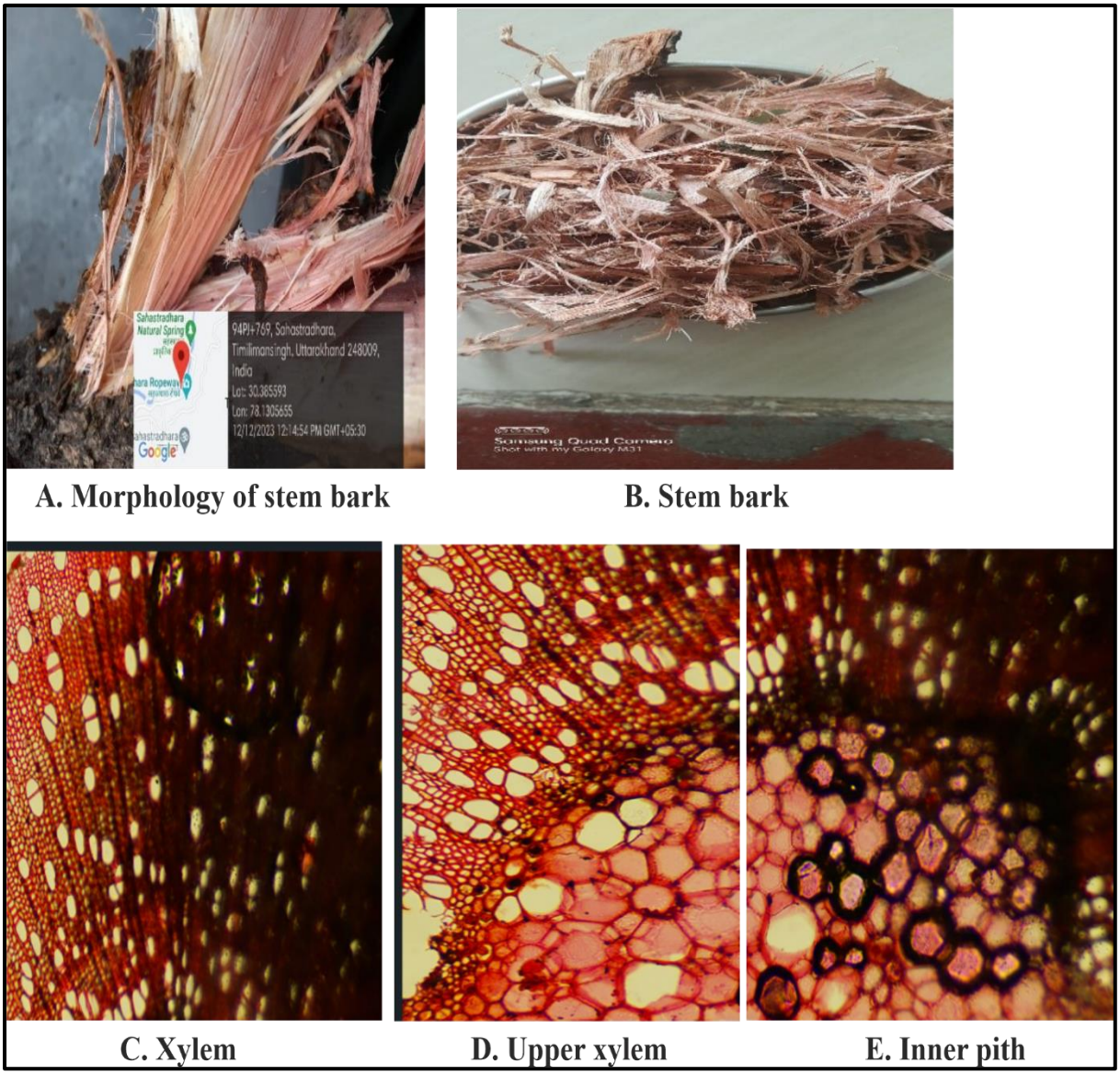

Fig.2: Morphology and microscopic characters of *Salix tetrasperma* stem bark.

Powder microscopy:

Stem bark: Stem bark powder colour was light greenish yellow, odour aromatic; taste astringent & bitter; texture fibrous. Diagnostic powder character of stem bark shows vessels, tracheid, calcium oxalate, simple & compound starch grains (Fig. 3A-F).

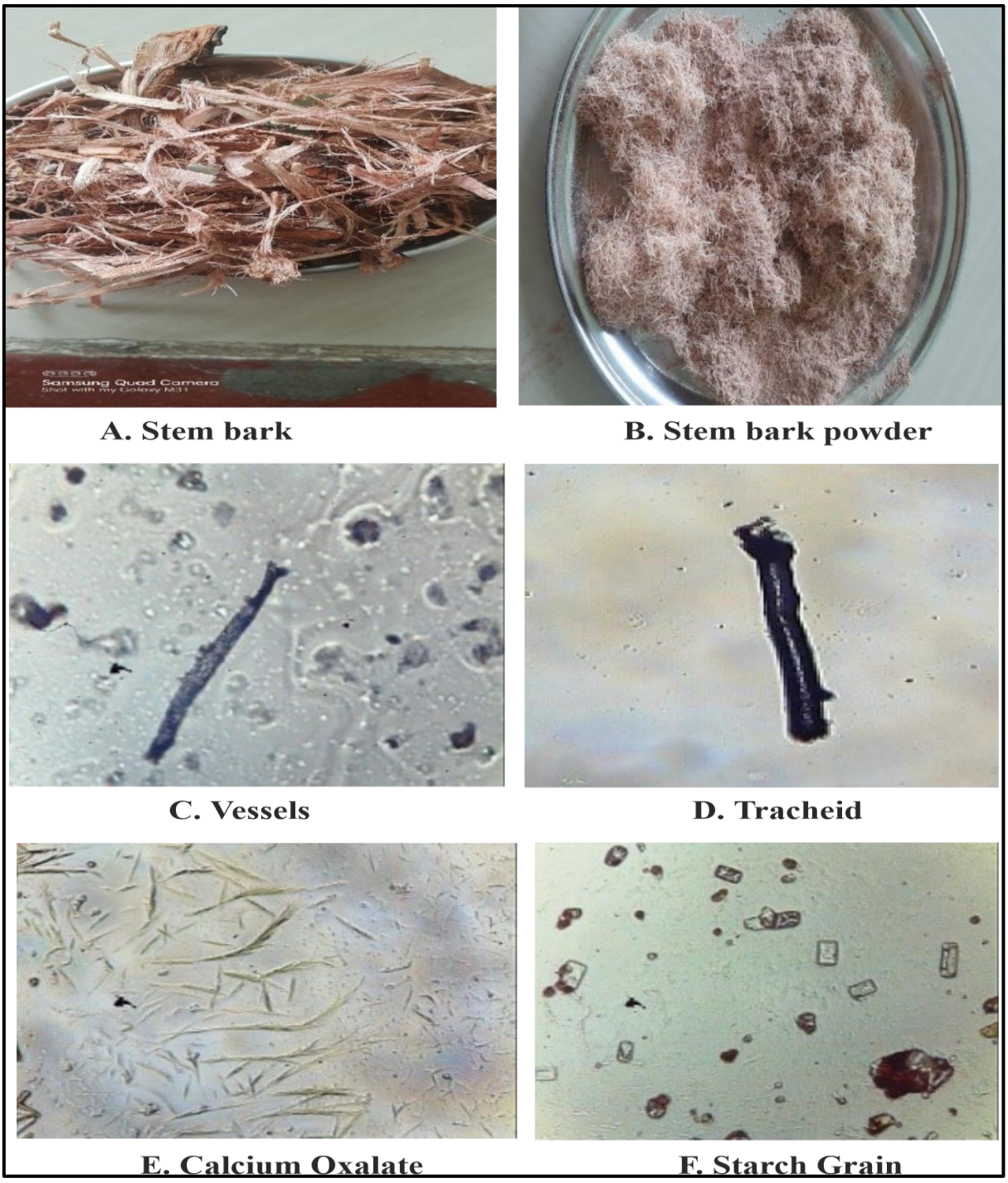

Fig.3: Powder microscopy of *Salix tetrasperma* stem bark

Physicochemical parameters: The values of physicochemical parameters of stem bark are expressed in percentage. *S. tetrasperma* stem bark loss on drying value 5.65% w/w, total ash value 7.15% w/w, acid insoluble ash 2.48% w/w, water soluble ash 5.28% w/w, water extractive value 18.65%w/w and alcoholic extractive value 14.65% w/w. The details result of physicochemical analysis has been depicted in Table 2.

Table 2: Physicochemical parameters of stem bark of *Salix tetrasperma*

Sr. No.	Physico-chemical parameters	Stem bark
1	Loss on Drying (% w/v)	5.65

2	Aqueous Extractive Value (w/w)	18.65
3	Alcoholic Extract Value (w/w)	14.65
4	Total Ash (w/w)	7.15
5	Acid Insoluble Ash (w/w)	2.48
6	Water Soluble Ash (w/w)	5.28

Preliminary phytochemical parameters: Preliminary phytochemical screening of *Salix tetrasperma* stem bark were carried out in ethanolic and aqueous extracts to identify the major chemical constituents. Primary (carbohydrate, protein, and amino acid) and secondary (alkaloids, glycosides, tannin, saponin, and phenolic molecule) metabolites are identified by phytochemical testing. Test samples were examined for the presence of primary and secondary metabolites using the alcoholic and aqueous extract.

The result of stem bark revealed the presence of amino acid, protein (Biuret test), phenolic compound, tannins ($Fecl_3$) both in aqueous and ethanolic extract whereas carbohydrate (Molish test, Benedict test), alkaloids (Dragendorff test), protein (Millon test), saponin (Foam test), glycosides **(**Borntrager's test), tannins (Lead acetate) in aqueous extract and carbohydrate (Fehling test), alkaloids (Wagner's test, Hager's test), protein (Xenthoprotic test) in ethanolic extract .The details results are as quoted in Table 3.

Table 3: Preliminary phytochemical investigation of Ethanolic & aqueous extract of *S. tetrasperma* stem bark

Sr. No.	Phytochemical	Ethanolic Extract	Aqueous Extract
A.	**Carbohydrate**		
1	Molish test	Absent	Present
2	Benedict test	Absent	Present
3	Fehling test	Present	Absent
B.	**Alkaloids**		
1.	Dragendorff test	Absent	Present
2.	Wagner's test	Present	Absent
3.	Hager's test	Present	Absent
C.	**Amino Acid**		
1.	Ninhydrine test	Present	Present

D.	**Protein**		
1.	Biuret test	Present	Present
2.	Xenthoprotic test	Present	Absent
3.	Millon test	Absent	Present
E.	**Saponin**		
1.	Foam test	Absent	Present
F.	**Glycosides**		
1.	Borntrager's test	Absent	Present
G.	**Phenolic compound**		
1.	Phenolic test	Present	Present
H.	**Steroids**		
1.	Salkowski	Absent	Absent
I.	**Tannins**		
1.	Fecl3	Present	Present
2.	Lead acetate	Absent	Present
3.	Pot. Dichromate	Absent	Absent

TLC study: The TLC study of *Salix tetrasperma* ethanolic stem bark extract was scanned and R_f Value: 0.25, 0.54, 0.67, 0.78, 0.89 observed. (Fig. 4D).

HPTLC study: The HPTLC study of *Salix tetrasperma* ethanolic stem bark extract was scanned under 254 nm, 366 nm and 540 nm obtained R_f values are mentioned in Table 4. The HPTLC plate, 3D graphs and peak displays are depicted in (Fig. 4.A-C).

HPTLC and TLC of ethanol extract of powdered *S. tetrasperma* stem bark was evaluated using Toluene: Ethyl Acetate (6:4 v/v) and Toluene: Ethyl Acetate (12:8 v/v) solvent system respectively. The ethanol extracts of stem bark observed with spots of 16 R_f value at 256 nm, while 14 of R_f value at 366 nm and 24 of R_f value at 540 nm. The TLC study of *Salix tetrasperma* ethanolic stem bark extract was scanned and R_f Value: 0.25, 0.54, 0.67, 0.78, 0.89 observed.

Table 4: R_f values obtained at Underivatized, UV light (254nm), Underivatized UV light (366nm) and Derivatized, UV light (540nm), of *Salix tetrasperma* stem bark.

Sample	Underivatized, UV light (254nm)		Underivatized UV light (366nm)		Derivatized, UV light (540nm)	
	No. of spot	R_f Value	No. of spot	R_f value	No. of spot	R_f value
Stem bark	16	0.050, 0.052, 0.056, 0.131, 0.134, 0.142, 0.335, 0.340, 0.339, 0.344, 0.421, 0.424, 0.429, 0.768, 0.769, 0.771	14	0.005, 0.006, 0.095, 0.100, 0.102, 0.416, 0.419, 0.421, 0.424, 0.682, 0.684, 0.685, 0.777, 0.982	24	0.050, 0.052, 0.055, 0.129, 0.131, 0.132, 0.140, 0.285, 0.287, 0.289, 0.398, 0.402, 0.405, 0.600, 0.602, 0.605, 0.671, 0.673 0.674, 0.756, 0.758 0.760, 0.882, 0.884

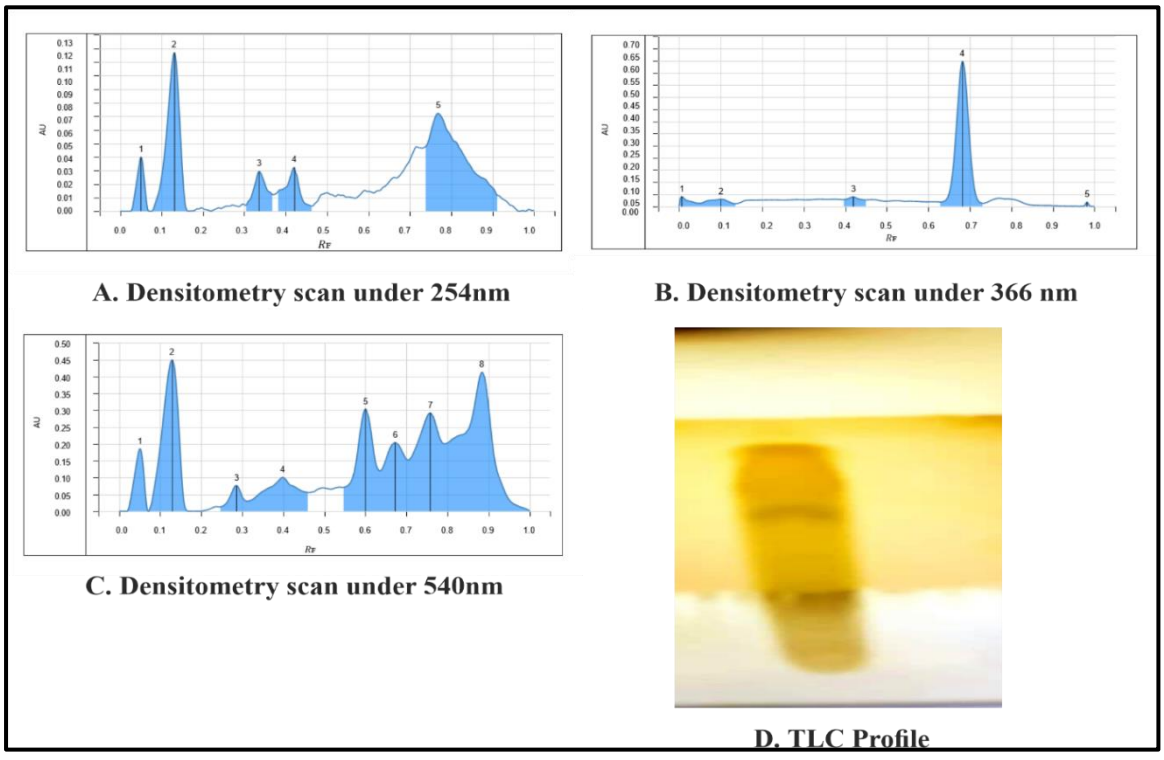

Fig.4: HPTLC Densitometry chromatogram of *Salix tetrasperma* at 254 nm and 366 nm and TLC profile

DISCUSSION:

For the purpose of quality control, assessment of purity and identification of any sample, standardization is very much essential[25]. Standardization of a crude drug and correct identification of plant is crucial to maintain efficacy. Standardization parameters include physicochemical, phytochemical, macroscopic, microscopic and powder study of the plant. Evaluating all these parameters will ensure and help in maintaining quality, purity and efficacy of the plant drug for its various uses and prevent the plant drug from adulteration and

substitution[26]. Macroscopically, it was observed that Stem bark of *Salix tetrasperma* was cylindrical, striated, greenish brown in colour, measures 15-20 x 1.5-2 cm, odour aromatic, fracture fibrous with irregular surface. In organoleptic characters the fresh stem bark color was greenish brown, rough in touch; aromatic odour; astringent & bitter taste, fracture fibrous with irregular surface. Transverse section of stem bark shows the outermost layer, consisting of dead cells. The innermost layer of the cortex, comprising sieve tubes, companion cells, phloem parenchyma, and fibers. Inward from the cambium, consisting of various types of cells including vessels, tracheids, fibers and xylem parenchyma. Macroscopical evaluation is a qualitative evaluation based on the study of morphological and sensory profiles of drugs and serve as diagnostic parameters[27]. Morphoanatomical features will help in the identification of the crude drug[28].Microscopic powder character of stem bark shows vessels, tracheid, calcium oxalate, simple & compound starch grains respectively help in identification, authentication of the plant material and also will be useful in making a monograph of the plant. Further present observations will help in maintaining the quality, reproducibility, efficacy of natural drugs[28] and to detect the adulteration[27].

The result of loss on drying, *S. tetrasperma* stem bark loss on drying value is 5.65% w/w. Therefore, this method is important for determining the water content of plant material encourages microbial growth and deterioration following hydrolysis[30].

The total ash value was7.15% w/w, acid insoluble ash 2.48% w/w, water soluble ash 5.28% w/w the low ash value indicates the low amount of inorganic salts of carbonates, phosphates, silicates of sodium, potassium, calcium and magnesium. The acid insoluble ash was very low that support the fact that a very small amount of the inorganic component is insoluble in acid and the ash values determined in the present study is a key diagnostic tool which may be useful in stabilizing standards of purity and quality of drug[21].

Water soluble extractive value represents the percentage of water-soluble active constituents which was found water extractive value 18.65%w/w and alcoholic extractive value was 14.65% w/w.

Extractives values are primarily useful for the determination of exhausted and adulterated drugs. Extractive values are also useful to evaluate the chemical constituents present in crude drug and help in estimation of specific constituents soluble in particular solvent[22].

Preliminary qualitative analysis of The result of stem bark revealed the presence of amino acid, protein (Biuret test), phenolic compound, tannins ($Fecl_3$) both in aqueous and ethanolic extract whereas carbohydrate (Molish test, Benedict test), alkaloids (Dragendorff test), protein (Millon test), saponin (Foam test), glycosides **(**Borntrager's test), tannins (Lead acetate) in aqueous extract and carbohydrate (Fehling test), alkaloids (Wagner's test, Hager's test), protein (Xenthoprotic test) in ethanolic extract.

S. tetrasperma Roxb. plant stem bark extract screening revealed the presence of phytochemicals such as carbohydrates, steroid, glycosides, saponins, alkaloid, tannin and flavonoids which are responsible for most of the pharmacological activities. Carbohydrates reported for neuroprotective, anti-inflammatory and immunomodulatory activities[23]. Steroid have been reported to have antibacterial[24] and anti-inflammatory[25]

activities. Alkaloids for analgesic, antispasmodic, antibacterial[24] and anti-inflammatory[26]. Flavonoids have been found to be antimicrobial, immunomodulatory, antioxidant, anticancer activities and anti-inflammatory[277]. Glycosides and tannins also reported anti-inflammatory activity[25, 28].

It was found that the most suitable solvent system for HPTLC of ethanol extract of powdered stem bark was Toluene: Ethyl Acetate (6:4 v/v). HPTLC and TLC of ethanol extract of powdered *S. tetrasperma* stem bark was evaluated using Toluene: Ethyl Acetate (6:4 v/v) and Toluene: Ethyl Acetate (12:8 v/v) solvent system respectively. The ethanol extracts of stem bark observed with spots of 16 R_f value at 256 nm, while 14 of R_f value at 366 nm and 24 of R_f value at 540 nm. The TLC study of *Salix tetrasperma* ethanolic stem bark extract was scanned and R_f Value: 0.25, 0.54, 0.67, 0.78, 0.89 observed. HPTLC fingerprinting is proved to be linear, precise, accurate method for herbal identification, help the manufacturer for quality control and standardization of herbal formulations, in differentiating the species from the adulterant and act as biochemical markers[18].

CONCLUSION:

S. tetrasperma, deciduous shrub or small tree Upto 9 m high flowering after leafing. Macroscopically stem bark are cylindrical, striated, greenish brown in colour. Microscopic powder character of stem bark shows vessels, tracheid, calcium oxalate, simple & compound starch grains are diagnostic key feature for identification of plant. The Pharmacognostic, phytochemical screening, physicochemical characterization parameters and HPTLC study can serve as criteria for the evaluation of the identity and authenticity quality characters of the plant which can be used to detect adulteration in raw materials and also will help in maintain of quality, strength, purity and efficacy of the drug.

CONFLICT OF INTEREST:

Authors have declared no conflict of interest exist.

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ABBREVIATION USED:

T.S.: Transverse section; cm: centimetre; **w/w:** weight by weight; **v/v:** volume by volume; **nm:** nanometre; **HPTLC:** high performance thin layer chromatography; **R_f:** retardation factor

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