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Original Article

Microscopic Standardization and Bioactive Profiling of *Cissampelos pareira* Roots

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ABSTRACT

The present study focuses on the pharmacognostic characterization and phytochemical profiling of *Cissampelos pareira* roots to establish reliable diagnostic features for identification and standardization. *C. pareira*, a medicinal plant from the family Menispermaceae, is often erroneously identified as *Cyclea peltata* due to morphological similarities, highlighting the need for detailed microscopic evaluation. Macroscopic and microscopic analyses revealed distinctive features such as a multilayered cork, lignified stone cells, medullary rays, vascular strands, starch grains, and calcium oxalate crystals. Histochemical tests confirmed the presence of alkaloids, phenolics, mucilage, lignin, starch, and oils in specific tissues. Preliminary phytochemical screening of ethanolic root extracts indicated the presence of alkaloids, flavonoids, glycosides, steroids, sterols, tannins, terpenoids, essential oils, and amino acids, while saponins and anthraquinones were absent. Physicochemical parameters such as total ash (6.5% w/w) and water-soluble extractive (16.8% w/w) supported quality evaluation. These findings validate the ethnomedicinal uses of *C. pareira* in treating inflammatory, febrile, and reproductive disorders and provide essential pharmacognostic markers for authentication and prevention of adulteration. The study further underscores the plant's phytochemical richness, warranting advanced analytical and pharmacological investigations to substantiate its therapeutic potential.

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INTRODUCTION

The Menispermaceae family comprises several medicinally important plants that are rich in alkaloids, flavonoids, and other secondary metabolites[1,2]. Velvet leaf, that is, *Cissampelos pareira* Linn., has been reported to have uterotonic as well as antimalarial, antipyretic, and anti-inflammatory properties, as it is a widely used ethnomedicinal plant[1,3,4]. The roots of *Cissampelos pareira* are traditionally used for their **diuretic** and **febrifuge** properties and are employed as a remedy for various ailments, including **heart conditions, dysentery, and skin sores**. In Ayurvedic medicine, the roots are commonly incorporated into formulations prescribed for the treatment of **rheumatism, ulcers, and fevers**.

C. pareira is known for its broad spectrum of therapeutic actions, including **antibacterial, anticonvulsant**, and **antiulcer** effects. It is also traditionally used to manage **indigestion, skin irritations, cough, fever, and intestinal worms**, and is valued for its roles as a **purgative, stomachic, wound healer**, and **antiperiodic agent**. Additionally, the root is believed to be effective in treating snake bites and other toxic conditions. However, its root's phytochemical composition remains scientifically incompletely understood, despite people usually employing it in Ayurveda and other healing systems.

As preliminary pharmacological studies exist already, detailed phytochemical profiling remains scarce, particularly regarding the root part. This highlights a critical gap, as no comprehensive chemical investigation of *Cissampelos pareira* root has been reported within the Menispermaceae family.

In addition, histochemical studies and powder microscopy have not been carried out on this plant root, and the available microscopical descriptions are not recent, with no high-quality photographic documentation. Since *C. pareira* is often erroneously identified as *Cyclea peltata* due to similarities in morphology, microscopic evaluation becomes essential for its correct identification and authentication. To complement this, standard qualitative phytochemical tests for major secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and terpenoids, are also required, along with physicochemical standardization (Table 2). The present study therefore, aims to conduct an integrated approach involving macroscopic and microscopic characterization supported by high-quality imaging, preliminary phytochemical screening, and physicochemical parameter analysis of *C. pareira* roots. Such an approach not only helps establish reliable Pharmacognostic markers but also provides chemical evidence for standardization, thereby preventing adulteration and validating the plant's traditional uses.

GEOGRAPHICAL DISTRIBUTION AND ETHANOBOTANICAL USES

Kingdom – Plantae

Division – Magnoliophyta

Class – Magnoliopsida

Order – Ranunculales

Family – Menispermaceae

Genus – *Cissampelos*

Species – *pareira*

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Table 1. Phytochemical studies

TEST	OBSERVATION
Amino Acid	+
Alkaloids	+
Flavonoids	+
Glycosides	+
Anthraquinone	-
Saponins	-
Sterols	+
Steroids	+
Tannins	+
Terpenoids	+
Essential Oil	+

Table 2. Physicochemical standardization of Cissampelos pareira

Parameter	Observed Value (%)
Loss on Drying (LOD)	6.78 % w/w
Total Ash	6.5 % w/w
Acid-Insoluble Ash	1.5 % w/w
Water-Soluble Extractive	16.8 % w/w
Alcohol-Soluble Extractive	10.32 % w/w

Cissampelos pareira Linn., commonly known as Patha, is a well-documented medicinal plant in Ayurveda, dating back to 6000 BCE[5]. According to Charaka Samhita[6], it is traditionally used for treating fever, asthma, vomiting, diarrhoea, itching, leprosy, heart disorders, poisoning, worm infections, and abdominal tumours. It is a key ingredient in classical Ayurvedic formulations such as Agnimukh Churna, Pusyanug Churna, Mahayograj Guggulu, and Pathadi Kwath[7,8]. The *Ayurvedic Pharmacopoeia of India* prescribes its root for abdominal pain, fever, cough, blood purification, and breast milk secretion issues[9-12]. Traditionally, the root paste is applied for inflammation, wounds, piles, skin rashes, and snake bites[13], and decoctions are used for pneumonia, malaria, and even birth control[14-16]. In other Asian countries, it is used to treat ulcers, nephritis, anorexia, and promote lactation[17,18]. African ethnomedicine employs it for cough, sore throat, pregnancy-related pain, and helminthiasis[19,20], while in South America, known as "midwives herb," it is used to manage menstrual cramps, prevent miscarriage, and control uterine bleeding post-delivery[21,22] Figure 1.

MATERIALS AND METHODS

Plant material

The roots of *Cissampelos pareira* were collected in the Fields of Gujarat. The plant material was identified and authenticated taxonomically at the Siddha Central Research Institute, Chennai (Authentication certificate - 586.03082313 CODE: C03082313P).

Microscopical evaluation

The microscopical features of the test specimen were documented using a Nikon D-5600 digital camera. The sample was preserved in FAA (formalin-acetic acid-alcohol) fixative for over 48 hours. Thin transverse sections were obtained using a sharp blade and stained with safranin. These sections were then examined and photographed under bright-field illumination using an Axiolab 5 trinocular microscope fitted with an Axiocam 208 color digital camera, with magnification indicated by a scale bar.

Powder microscopy

A small quantity of the dried, powdered sample was cleared with saturated chloral hydrate solution and mounted in 50% glycerol on a glass slide. Microscopic features were observed under bright-field light using a Nikon ECLIPSE E200 trinocular microscope equipped with a Zeiss ERc5s digital camera. Photomicrographs of the diagnostic characteristics were captured and documented [23-25].

Histochemical testing

Histochemical tests (SOP No. PCOG-008-SOP): Plant sections were treated following the standard procedures:

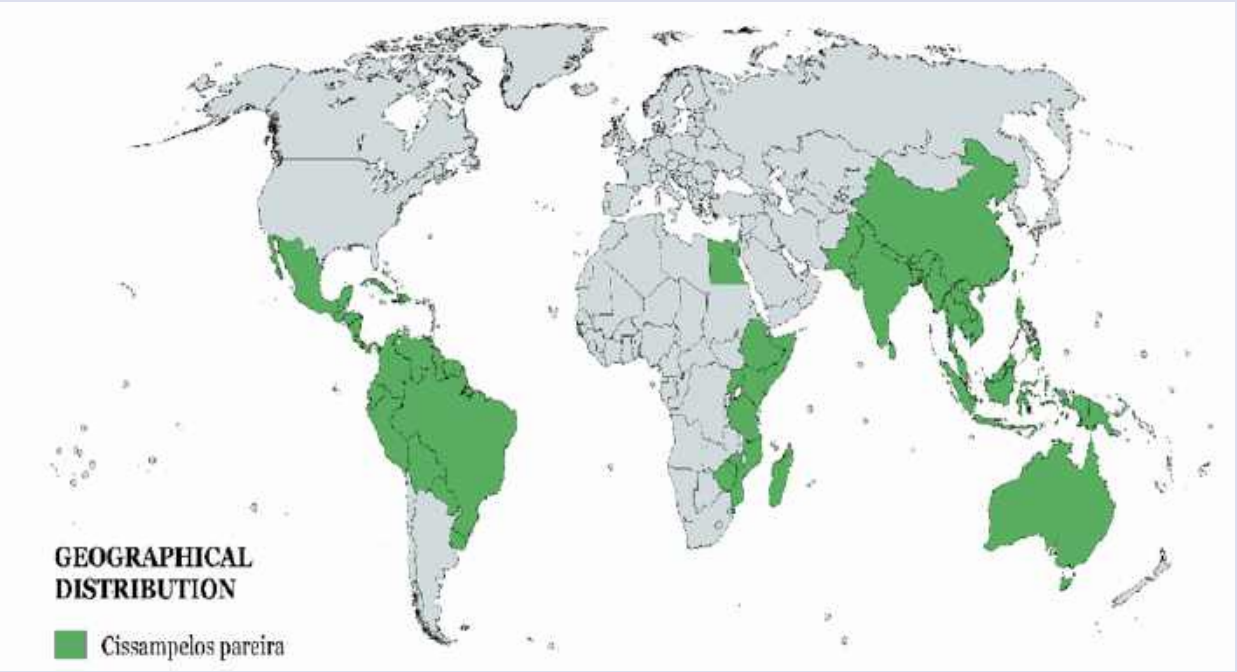


Figure 1. Geographical distribution of *Cissampelos pareira*

Figure 2. Root of *Cissampelos pareira*

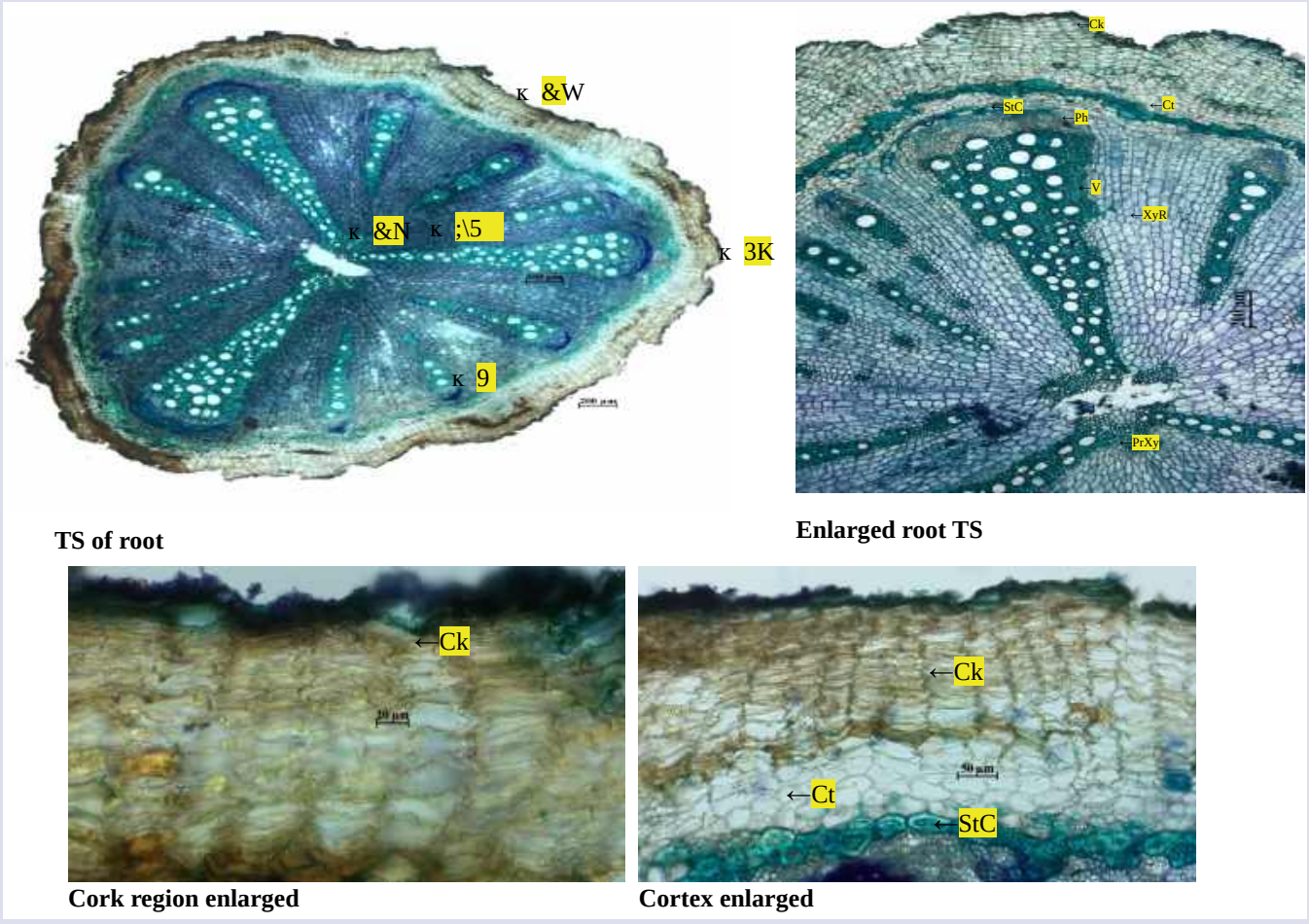
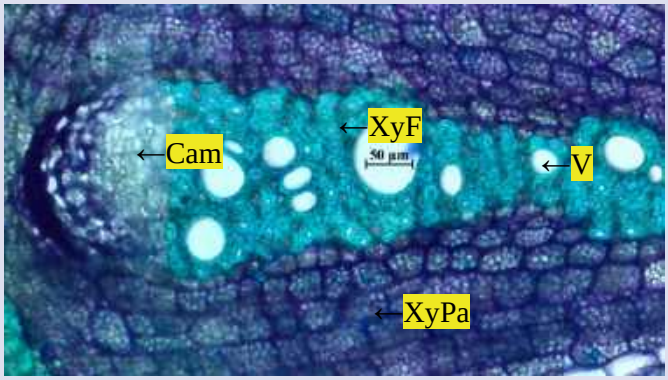
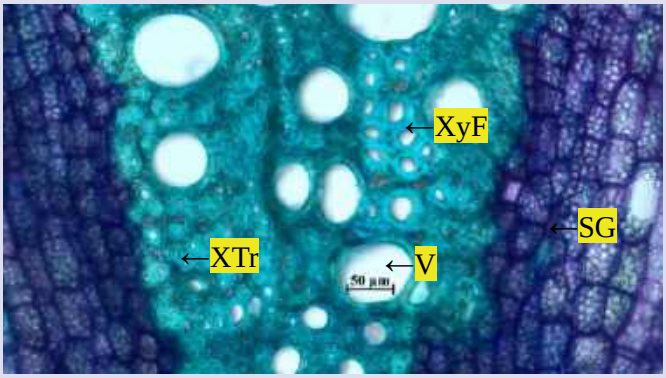
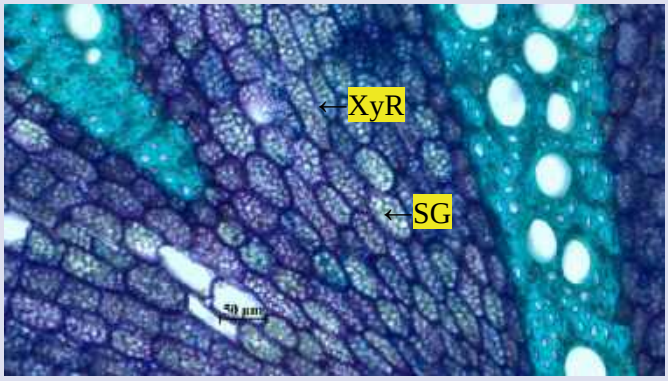


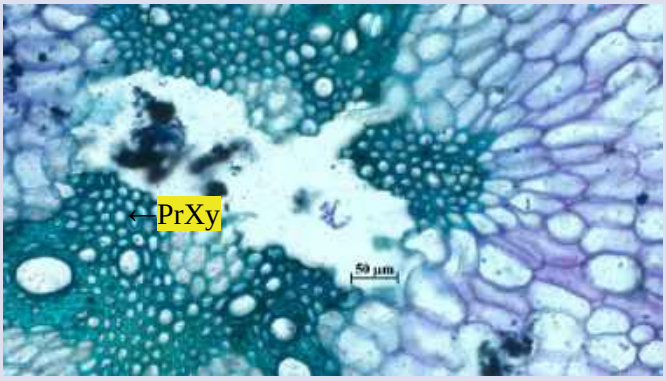
Figure 3. Microscopy of Cissampelos pareira root





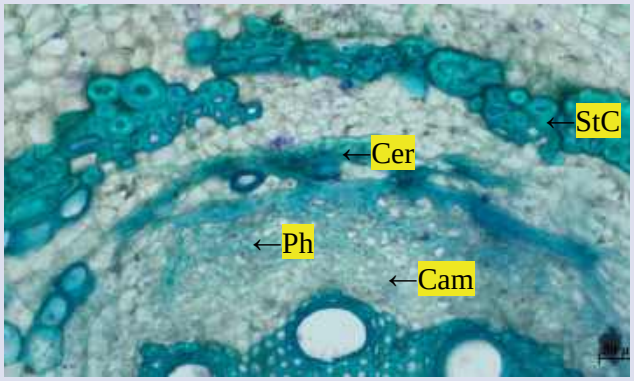
Xylem portion enlarged

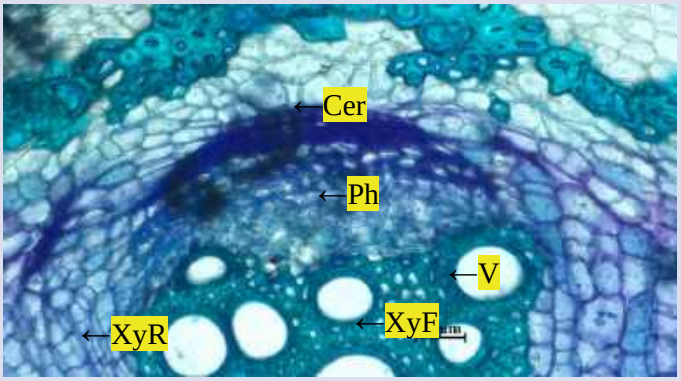




Xylem ray enlarged

Central portion enlarged

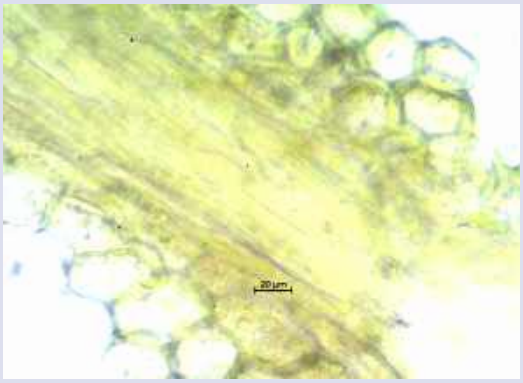




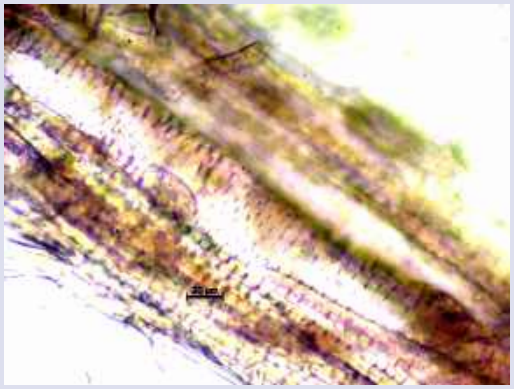
Vascular region

Figure 4. Cam - cambium; **Cer -** ceratenchyma; **Ck** - cork; **Ct** - cortex; **Pa** - parenchyma; **Per** - pericycle; **Ph** - phloem; **PrXy** - protoxylem; **SG** - starch grain; **StC** - stone cell; **V** - vessel; **XyF** - xylem fibre; **XyPa -** xylem parenchyma; **XTr -** xylem tracheid; **XyR** - xylem ray

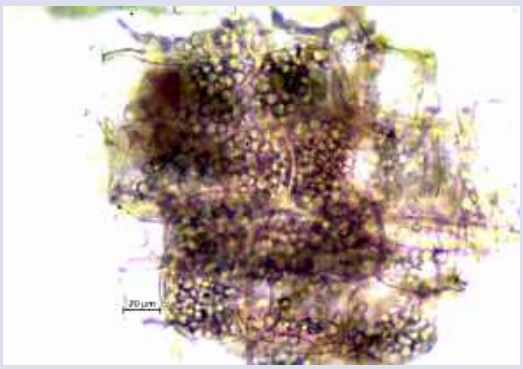
Cork fragment



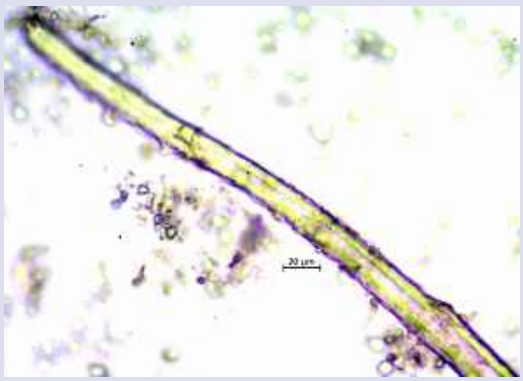
Medullary ray



Pitted vessels



Parenchyma with starch



Fibre

Tracheids

Figure 5. Powder Microscopy of Cissampelos pareira root

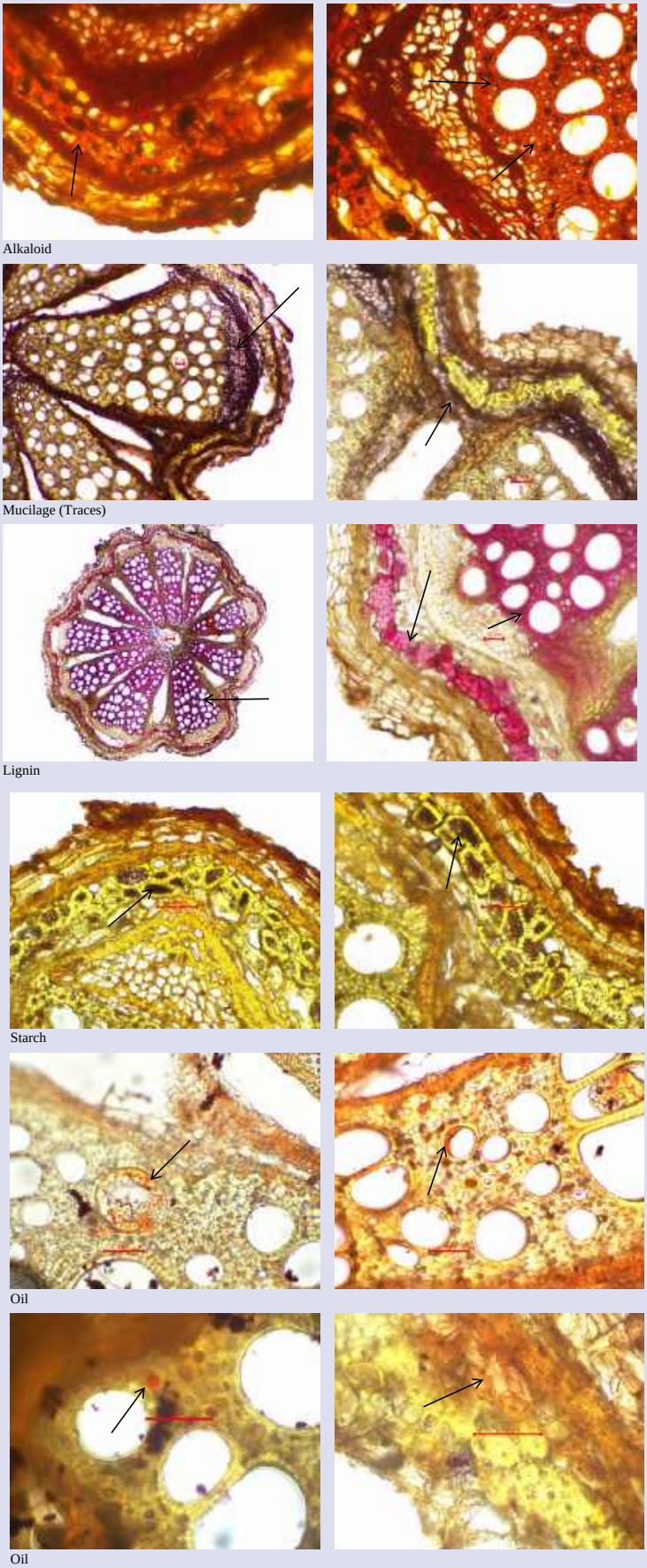


Figure 6. Histochemistry of *Cissampelos pareira* root

1. **Crystals**: The section was mounted in water, and one end of the cover slip was irrigated with acetic acid. While looking through the microscope, the water within the cover slip was replaced using a piece of filter paper at the opposite end of the cover slip

- Formation of air bubbles indicated Calcium carbonate crystals

- If no air bubbles were formed, the experiment was repeated with Conc. HCl, wherein dissolution of the crystal and formation of needles of Calcium sulphate indicated the presence of Calcium oxalate crystals

2. Fats, Fatty oils, volatile oils, and resins: About 1 to 2 drops of Sudan-IV were added to the section and allowed to stand for a few minutes. Presence of fatty oil substances was indicated by orange-red/pink/red coloured globules, while red coloured irregular contents indicated resin.

3. **Starch**: A drop of 2% iodine water solution was added - blue colour indicated starch.

4. **Phenolic compounds:** A drop of alcoholic ferric chloride was added - bluish black coloured contents indicated phenolic compounds like flavonoids/tannins, etc.

5. **Mucilage**: A drop of ruthenium red was added - pink to red coloured contents indicated mucilage.

6**. Lignified cell walls**: A drop of phloroglucinol was added to the section and allowed to stand for about 2 min or until almost dry. A drop of 50% HCl was added and observed over a cover-glass - cell walls-stained pink to cherry red, indicating the presence of lignin.

7**. Suberized or cuticular cell walls**: A drop of Sudan red III was added and allowed to stand for a few minutes, warmed gently if necessary - cell walls-stained orange-red or red indicated suberin or cutin deposition over the cell wall.

8. **Alkaloids**: A drop of Wagner's reagent was added - the presence of yellow to reddish brown coloured contents confirmed alkaloids.

Extract preparation

The fresh roots of *Cissampelos pareira* were thoroughly washed with distilled water to remove any soil and adhering soil particles, and then shade-dried. The dried roots were ground into a coarse powder and passed through a 24-mesh sieve. The powder so obtained was then extracted three times with a 50% solution of ethanol (v/v). The filtered extracts were then combined, shaken, and concentrated by a rotary evaporator at a lowered temperature of –5 °C.

Phytochemical screening

The samples (Extracts) were analysed to detect the presence of phytochemicals such as alkaloids (Wagner's reagents), saponins (Froth test), steroids and terpenoids (Liebermann Burchard's tests), Quinones, and flavonoids (Shinoda tests).

RESULTS

Macroscopical and microscopical study

Cylindrical pieces, measuring 0.5 to 1 cm in diameter and 5 to 11 cm in length; longitudinally wrinkled; woody internally; dark brown outside and pale yellowish inside; odour characteristic with initial sweet taste changing bitter eventually (Figure 2).

TS of the root is irregularly oval to triangular in shape with few ridges and furrows; a wide central xylem and distinct medullary rays.

The TS shows an outermost layer of cork composed of 8 to 20 layers of non-lignified, tangentially elongated, rectangular cells with few exfoliating outer layers; phellogen is distinct; parenchymatous cortex made up of 4 to 8 layers followed by 1 to 2 layered pericycle made up of a discontinuous layer of stone cells embedded with a group of lignified fibres; the vascular region is composed of radially elongated 8 to 10 vascular strands of xylem with patches of phloem on the outer side; alternating with xylem rays; xylem is composed of vessels, tracheids, fibres and parenchyma; few ceratenchyma cells are seen above phloem region; phloem is composed of sieve tubes and parenchyma; cambium made up of 2 to 3 layers of parenchyma cells are seen in between xylem and phloem; medullary rays are uni to multiseriate made up of parenchyma cells; abundant simple and compound starch grains and few rosette and prismatic crystals are seen scattered throughout the parenchymatous cells (Figure 3 and Figure 4).

Powder is brown in colour with characteristic odour and sweet turning into bitter taste and shows fragments of cork, medullary ray, pitted vessels, parenchyma with starch grains, fibre, tracheid, stone cell and prismatic crystals (Figure 5).

Histochemical studies

Phenolic compounds and alkaloids were observed in the outer cortical cells; traces of mucilage were seen along the phloem cells; lignin deposition was observed in the cortical stone cells and the walls of xylem vessels; starch grains were seen in the stone cells, and oil droplets were detected in xylem parenchyma cells Figure 6.

Phytochemical investigation

Phytochemical studies: Phytochemical screening of the ethanolic root extract of *C. pareira* showed the presence of alkaloids, flavonoids, glycosides, steroids, sterols, tannins, and essential oils Table 1

Physicochemical standardization

See Table 2

DISCUSSION

Macroscopy and Microscopy

The pharmacognostic study of the root reveals important features useful for its identification and standardization. Macroscopically, the root is cylindrical, wrinkled, woody inside, with a dark brown outer surface and pale-yellow interior. Its unique odour and a taste that changes from sweet to bitter are key organoleptic markers. These traits help distinguish genuine material from adulterants in raw drug markets.

Microscopically, the transverse section shows an irregular shape with ridges, furrows, and a well-defined cork layer. The cortex and pericycle contain stone cells and lignified fibres, providing structural support. The vascular region has organized xylem and phloem with alternating medullary rays. Abundant starch grains and calcium oxalate crystals are seen in parenchymatous cells. These microscopic markers support the plant's medicinal use. Together, the macro- and micro-features ensure reliable identification and quality control of the crude drug.

Powder microscopy

The powder of the root is brown in colour with a characteristic odour and a taste that changes from sweet to bitter, which serves as a useful organoleptic marker for identification. Powder microscopy revealed key diagnostic features, including fragments of cork and medullary rays, which are indicative of secondary growth. The presence of pitted vessels, fibres, and tracheids confirms the origin from lignified vascular tissues. Parenchyma cells containing abundant starch grains suggest the presence of storage tissues. Stone cells and lignified fibres provide mechanical support and are typical of mature root tissues. The detection of prismatic calcium oxalate crystals adds another layer of authenticity, as these are common in many medicinal plants. These

microscopic characteristics are essential for the identification of powdered crude drugs. Such features help differentiate the genuine drug from possible adulterants. Altogether, the observations support the proper authentication and quality control of the root in powdered form.

Histochemical test

Histochemical analysis revealed the presence of key phytoconstituents distributed across different tissues of the root. Phenolic compounds and alkaloids were localized in the outer cortical cells, indicating their role in defence and possible therapeutic activity. Traces of mucilage along the phloem cells suggest protective and water-retention functions, which may contribute to the plant's medicinal properties. Lignin deposition in the cortical stone cells and xylem vessel walls confirms the presence of rigid, supportive tissues necessary for structural integrity. Starch grains identified within stone cells point to energy storage components of the plant. Additionally, oil droplets detected in the xylem parenchyma may represent lipid-based secondary metabolites. The specific localization of these compounds highlights the complexity of tissue function and supports the pharmacological relevance of the root. These findings also provide important markers for the standardization and quality assessment of the crude drug.

Preliminary phytochemical screening

The phytochemical screening of the ethanolic root extract of *Cissampelos pareira* revealed the presence of alkaloids, flavonoids, glycosides, steroids, sterols, tannins, terpenoids, essential oils, and amino acids. In contrast, saponins and anthraquinones were absent (Table 1). Such a profile reflects a rich diversity of bioactive secondary metabolites, which may contribute to the traditional uses of this plant in Ayurveda, Siddha, and folk medicine for treating fever, respiratory disorders, inflammation, and reproductive health issues.

The consistent detection of alkaloids in the root agrees with earlier reports emphasizing the abundance of bisbenzylisoquinoline alkaloids such as hayatinine, curine, hayatidine, and pelosine, which are known to possess antimalarial, anti-inflammatory, and immunomodulatory properties[26,27]. Similarly, the occurrence of flavonoids and tannins is of particular interest due to their well-documented antioxidant and hepatoprotective potential, which substantiates the use of *C. pareira* in managing oxidative stress–related disorders[28].

The presence of glycosides, steroids, and sterols suggests cardioprotective and endocrine-modulating effects, adding pharmacological relevance to the plant's ethnomedicinal claims. In addition, the identification of terpenoids and essential oils aligns with previous reports on their antimicrobial and antifungal activities, thereby broadening the therapeutic profile of the root extract[29].

Detection of amino acids is also consistent with earlier pharmacognostic findings, which reported proteins and amino acids in the root powder. Interestingly, saponins and anthraquinones were not detected in the present analysis. Although some studies on leaf and aerial parts have reported saponins, their absence in the root extract suggests a possible organ-specific distribution of phytoconstituents[23]. Anthraquinones, on the other hand, are rarely reported in *C. pareira*, confirming that these metabolites are not characteristic markers for this species.

Overall, the phytochemical profile observed in the present study is largely in agreement with earlier findings, yet it also highlights certain differences, particularly the absence of saponins in root extracts. These variations may be attributed to differences in plant part analyzed, solvent polarity, geographical source, or seasonal collection. From a pharmacognostic perspective, the results strengthen the evidence base for the therapeutic applications of *C. pareira*, while also underscoring the need for further bioassay-guided isolation, chromatographic profiling, and structure elucidation to correlate specific compounds with pharmacological activities.

Pharmacological and Medicinal Relevance

Cissampelos pareira has a long history of use in Ayurveda, Siddha, and folk medicine, and its pharmacological profile has been increasingly supported by modern research. The plant exhibits a broad spectrum of activities, including antimalarial, antiviral, antimicrobial, anti-inflammatory, antioxidant, hepatoprotective, cardioprotective, immunomodulatory, and anticancer effects[26-29]. The bisbenzylisoquinoline alkaloids such as curine, hayatinine, and isoliensinine have been recognized as potent antimalarial agents, effective even against resistant strains of *Plasmodium falciparum*[30,31].

Recent studies have expanded its therapeutic potential. Ethanolic extracts of *C. pareira* have shown broad-spectrum antiviral activity, including inhibition of all four dengue virus serotypes and downregulation of pro-inflammatory cytokines[32]. Flavonoids and tannins contribute significantly to the antioxidant and hepatoprotective activities, while glycosides, steroids, and sterols are implicated in antidiabetic and cardioprotective effects[33]. Extracts have also demonstrated antilithiatic activity, reducing calcium oxalate deposition in experimental urolithiasis[34].

Traditional claims related to women's health, such as regulating menstruation, promoting lactation, and controlling uterine bleeding, correlate with estrogenic and uterotonic activities confirmed in experimental models[35]. More recently, leaf and stem extracts have exhibited cytotoxicity against breast cancer cell lines, highlighting the anticancer potential of phenolic and flavonoid-rich fractions[36]. These findings reinforce the medicinal relevance of *C. pareira* and justify further studies to isolate active compounds and evaluate clinical efficacy.

CONCLUSION

The present study provides an integrated pharmacognostic, histochemical, and phytochemical evaluation of *Cissampelos pareira* roots. Diagnostic features such as cork layers, stone cells, medullary rays, vascular strands, starch grains, and calcium oxalate crystals were documented for reliable identification and quality control. Histochemical testing confirmed the presence of alkaloids, phenolics, mucilage, lignin, starch, and oil droplets, while phytochemical screening demonstrated a wide array of bioactive secondary metabolites, including alkaloids, flavonoids, glycosides, steroids, sterols, tannins, terpenoids, and essential oils.

These observations validate the ethnomedicinal applications of *C. pareira* in treating fever, inflammation, infections, metabolic disorders, and reproductive health issues. The pharmacognostic markers reported here will aid in preventing adulteration with closely related species such as *Cyclea peltata*. Furthermore, the phytochemical richness underscores its pharmacological importance and provides a basis for future bioassay-guided isolation, structural elucidation, mechanistic studies, and clinical validation. Such efforts will be critical in translating traditional claims into evidence-based therapeutic applications.

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