ORIGINAL ARTICLE

Phytochemical investigation and pharmacognostic standardization of *Cissampelos pareira* stem

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Background: Cissampelos Linn (family -Manispermaceae) is perennial climbing herbs with small greenish-yellow flower. It belongs to the genus Cissampelos, of which thirty to forty species are distributed in the tropical and subtropical world. One species occur in India. In the market three plants *Cissampelos pareira*, Cyclea peltata and Stephania japonica (Fam. Menispermaceae) are being used as source of Patha. **Aim:** Therefore, an establishment of pharmacognostical standards on identification, purity, quality and classification of the herbal plant is required. **Materials and Methods:** Microscopic characteristics were observed under a light microscope. Physicochemical properties - including loss on drying, total ash, acid-insoluble ash, and water alcohol and ether extractive values - were determined. Phytochemical screening for major groups of compounds was performed, and a thin-layer chromatography of methanolic extract of the air dried powdered stem of this plant was performed. **Results:** The microscopic characteristics showed the wavy epidermis with unicellular trichomes. Lignified xylem vessels, biseriate radial medullary rays had also been found. Phytochemical screening revealed that *Cissampelos pareira* stem extract contains flavonoids, terpenoid, alkaloid, tannins, amino acid protein and carbohydrate. Alkaloids were detected in TLC of *Cissampelos pareira* stem extract developed using blends of methanol: concentrated ammonia (200:3) and n-Butanol:Acetone:Water (3:1:1). Flavonoids and essential oil were detected in TLC of *Cissampelos pareira* flower extract developed using blends of n-Butanol:Acetone:Water (4:1:5) and Benzene: Ethyl acetate: Formic acid (9:7:4) as solvent systems for flavonid where as chloroform(100%), Benzene(100%), Chloroform:Benzene (1:1) and Ether:Benzene (1:1) as solvent systems for essential oil. **Conclusion:** These findings will be useful towards establishing pharmacognostic standards on identification, purity, quality and classification of the plant-drug research.

Key words: *Cissampelos pareira* stem, phytochemical investigation, pharmacognostic standardization

INTRODUCTION

Cissampelos pareira was first described from Latin America, but actually occurs throughout the tropics. The plant is common in orchards, hedges, park and gardens of moist soils, either creeping or twining around other plants, also common on the hilly tracts along water courses. *C. pareira*, commonly known as Bhatindupat in Punjab, is a perennial climbing shrub with small greenish-yellow flowers, peltate or orbicular-reniform, ovate-sub-reniform leaves with truncated cordate base, glabrous or hairy above up to 3-2 cm long. Flowers are unisexual; the pedicel is up to 2 mm long; male flowers are with 4 (–5) sepals, ovate to obovate, c. 1.5 × c. 0.5 mm, keeled, hairy outside, greenish or yellowish, corolla cup-shaped, c. 1 mm long, filaments of stamens are completely fused; female flowers are with one sepal c. 1.5 mm long, 1 obtriangular to kidney-shaped petal c. 1.5×2 mm, ovary is superior, hairy, 1-celled, style thick with spreading, 3-lobed stigma. The fruit is short-hairy, orange to red drupe c. 5 mm long, and is curved with style-scar near base; stone is with two rows of very prominent transverse ridges, and is 1-seeded. Seeds are horseshoe-shaped; the embryo is elongated, narrow, and is embedded in endosperm. The flowers of *C. pareira* are pollinated probably by small insects.[1] It can also be propagated from root cuttings, planted at the beginning of monsoon. Sometimes it dies in hot weather. *C. pareira* is mostly collected from the wild. *C. pareira* is very widespread and commonly found. It is used locally to cure gastro-intestinal complaints such as diarrhoea, dysentery, ulcers, colic, intestinal worms and digestive complaints, and also in urogenital problems such as menstrual problems, venereal diseases, infertility, uterine bleeding and life-threatening miscarriages.[2] A rhizome decoction or the leaves are also widely used orally or externally applied as a febrifuge and stomachic, against cough, heart problems, rheumatism, jaundice, snake bites and skin infections such as sores, boils, scabies and childhood eczema.[3,4] Tribal people in India use the plant to prevent pregnancy.[5-7]

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Commercially there are three varieties of plants – *C. pareira, Cyclea peltata* and *Stephania japonica* (Family – Menispermaceae) that are being used as source of Patha. Standardization problem of *C. pareira* is encountered in proper identification of the source plant. Therefore, an establishment of pharmacognostical standards on identification, purity, quality and classification of the *C. pareira* is required. It was, therefore, planned to investigate phytochemical characteristics and pharmacognostic properties of the *C. pareira* stem in the territory of Chandigarh, India. Microscopic characteristics were observed under a light microscope. Physicochemical properties – including loss on drying, total ash, acid-insoluble ash and water alcohol and ether extractive values – were determined. Phytochemical screening for major groups of compounds were performed, and thin-layer chromatography of methanolic extract of the air dried powdered stem of *C. pareira* were performed.

MATERIALS AND METHODS

Collection of Plant Material

Samples of *C. pareira* were collected from the Panchkula district of Haryana. Botanical identification was performed in the Botany Department of Panjab University, Chandigarh, India by comparing it with the existing specimen number PB/6954. Plant materials were dried in shade and ground to a coarse powder. All reagents used in quantitative and chemical investigation were of analytical grade and purchased from E. Merck, Ranbaxy and S. D. Fine Chemicals.

Qualitative Investigation

The macroscopic features of a cross-section of *C. pareira* stem and its dried powder were determined using the method of Evans.[8] Photographs of the macroscopic features were taken using a high-resolution camera (SONY, 14.1 megapixel Digital Camera), as seen in Figures 1-5.

Quantitative Investigation

The moisture content, ash and extractive values of the powdered samples were determined by the method as described in WHO guide line. Results are tabulated in Table 1.[9]

Table 1: Pharmacognostic evaluation of the dried powdered *Cissampelos pareira* stem

Evaluation parameters	Stem value (% w/w)
Moisture content	11.01±0.38
Total ash value	9.95±0.86
Water-soluble ash value	5.34±0.54
Acid-insoluble ash value	1.86±0.61
Pet ether soluble extractive value	0.67±0.39
Alcohol soluble extractive value	8.09±0.29

*Mean value of five counts

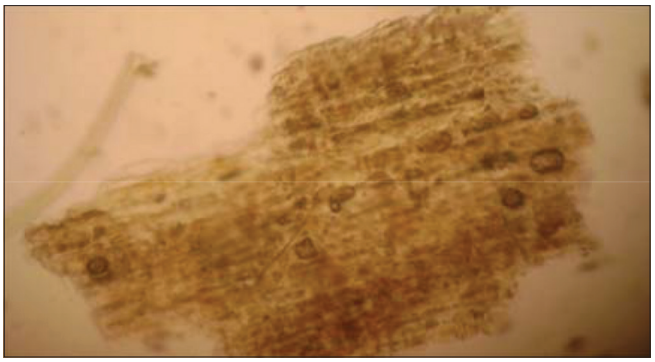

Figure 1: Microscopy of *Cissampelos pareira* stem. Radial medullary rays

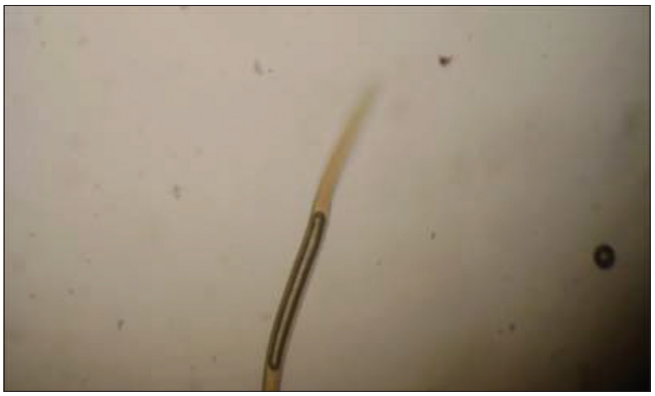

Figure 2: Microscopy of *Cissampelos pareira* stem. Unicellular trichome

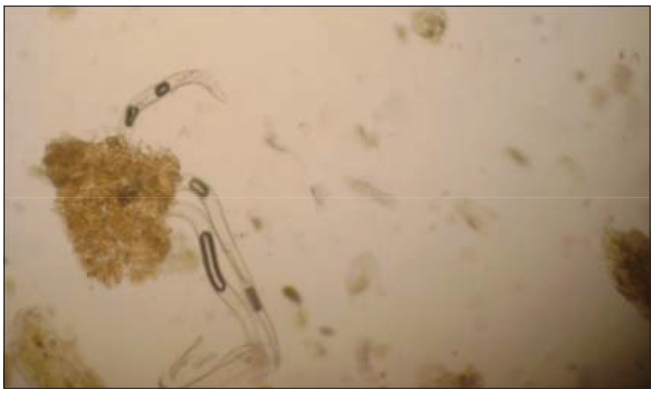

Figure 3: Microscopy of *Cissampelos pareira* stem. Wavy epidermis with trichomes

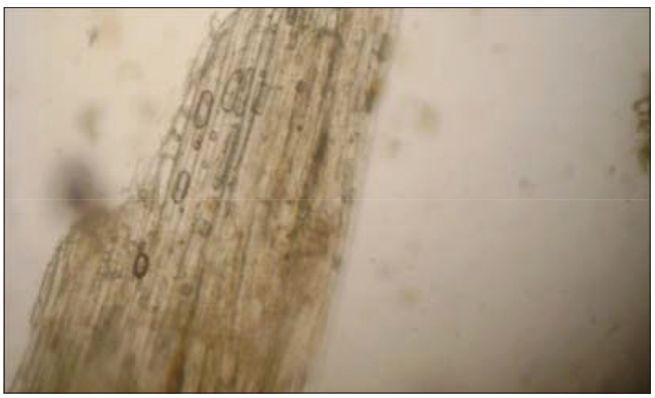

Figure 4: Microscopy of *Cissampelos pareira* stem. Biseriate radial medullary rays

Chemical Investigation

About 500 g of the air-dried powdered plant stem of *Cissampelos pareira* was extracted by the maceration method using different solvents, starting with petroleum ether followed by light petroleum, cyclohexane, benzene, chloroform, acetone, ethanol, methanol and water. Before each extraction, with the next solvent, the powdered material was air dried at a temperature below 50°C; each extract was concentrated by distilling the solvent and evaporating it on a water bath to obtain a dry extract. The colour and pH of different methanolic extracts of the dried powder of the stem of *C. pareira* was checked and the results are represented in Table 2. The extract was subjected to phytochemical screening and the results are represented in Table 3. The phytochemical investigation was done by the standard chemical tests described in The Practical Evaluation of Phytopharmaceuticals by Brain and Turner.[10] All TLCs had been performed according to methods proposed by Harborne.[11] Methanolic extract of *C. pareira* stem was examined by TLC on silica gel G using the following solvent systems mentioned in Table 4. and corresponding R_f (×100) were computed. Pictures for TLC of methanolic extract of *C. pareira* stem with different solvents are shown in Figures 6a-h.

Table 2: Colour, pH and extractive values of *Cissampelos pareira* stem methanolic extracts

Strength (% v/v)	Evaluation parameter		
	Colour	pH	Extractive value (% w/w)
50	Brown	6.8	5.01
70	Pale brown	6.5	7.10
100	Green	5.9	8.09

RESULTS AND DISCUSSION

Cross-section of *C. pareira* stem had shown the presence of trichome, epidermis, cortex, phloem, xylem and parenchyma with pith in the stem. The microscopic characteristics of dried powder of *C. pareira* stem showed the wavy epidermis with unicellular trichomes, lignified xylem vessels and biseriate radial medullary rays. The quantitative determinations of some pharmacognostic parameters are

Table 3: Phytochemical investigation of *Cissampelos pareira* stem methanolic extract

Test for active constituents	Results
Triterpenes	+
Saponine	–
Alkaloids	+
Tannins	+
Flavonoids	+
Glycosides	–
Coumarin	+
Amino acids	+
Sugar	+

(+) sign indicates that identification tests gave positive result, (-) sign indicates that identification tests gave negative result

Table 4: TLC of *Cissampelos pareira* stem methanolic extract

Solvent medium	No. of spot	R_f (×100)	Inference
Methanol:Conc. ammonia (200:3)	1	6.7±0.3	Alkaloid[#]
Butanol:Acetone:Water (3:1:1)	1	32.0±0.1	Alkaloid[#]
Butanol:Acetone:Water (4:1:5)	3	(54.8±0.5, 74±0.5, 77±0.6)	Flavonoid[Δ]
Benzene:et. acetate:Formic acid (9:7:4)	2	51.2±0.3	Chalcones[Δ]
		63.4±0.6	Aurones[Δ]
Chloroform	1	43.3±0.8	Essential oil[◊]
Benzene	1	14.8±0.2	Essential oil[◊]
Chloroform:Benzene (1:1)	1	17.7±0.3	Essential oil[◊]
Ether:Benzene (1:1)	1	14.5±0.2	Essential oil[◊]

Conc.: Concentrated; Et.: Ethyl;*Mean value of three counts taken for determining R^*_f (×100); [#]Spray reagent used for identification of alkaloid was Dragendroff's Reagent; [Δ]Colour development had been seen under UV light; [◊]Spray reagent used for identification of essential oil was vanillin-sulphuric acid and then heated. at 100-105°C for development of colour

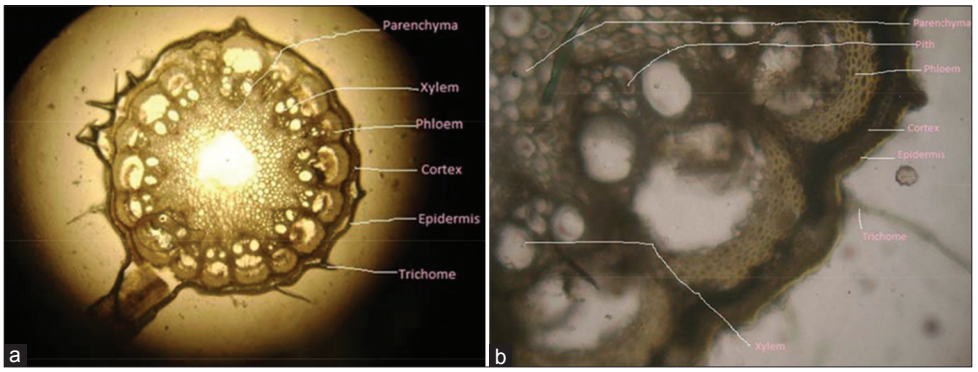

Figure 5: Microscopy of *Cissampelos pareira* stem. Cross-section of *Cissampelos pareira* stem (a) ×10 (b) ×40

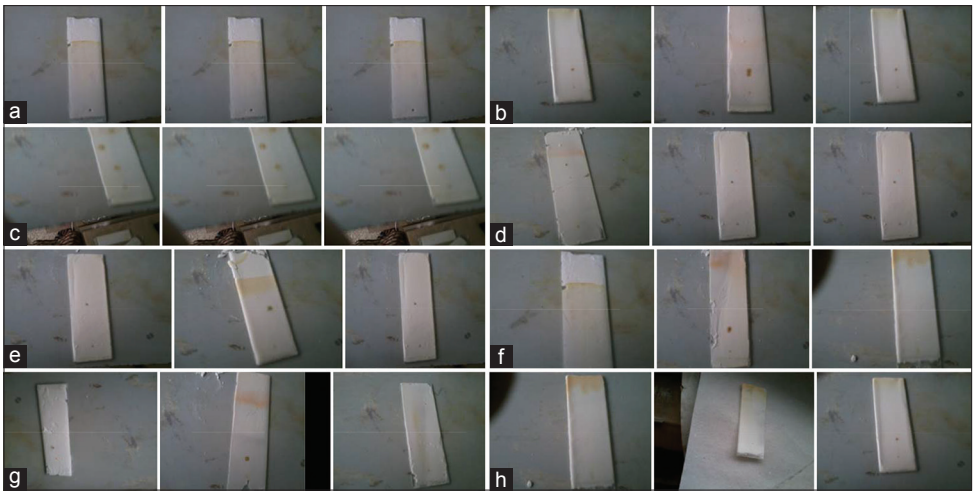

Figure 6: TLC of *Cissampelos pareira* methanolic stem extracts. TLC with (a) methanol:conc. ammonia (200:3); (b) butanol:acetone:water (3:1:1); (c) butanol:acetone:water (4:1:5); (d) benzene:ethyl acetate:formic acid (9:7:4); (e) Chloroform; (f) benzene; (g) chloroform:benzene (1:1); (h) ether:benzene (1:1)

useful for setting standards for crude drugs. The physical constant evaluation is an important parameter in detecting adulteration or improper handling of the drug. Various ash values are important to determine the purity of the drug, i.e., the presence or absence of foreign inorganic matter. Since the plant *C. pareira is* useful in traditional medicine for the treatment of various ailments, it is important to standardize it for use as a drug. The pharmacognostic constants for the stem of this plant, the diagnostic microscopic features and the numerical standards reported in this work could be useful for the compilation of a suitable monograph. The quantity of extract was found to be maximum in case of methanolic extract and minimum in case of benzene and chloroform extract. The quantity of extract was found to be higher in case of 100% v/v methanolic extract as compared with 70% v/v and 50% v/v methanolic extract. Results of TLC indicate that the stem contains alkaloids, flavonoids and essential oils, which is established by the results of phytochemical investigation and chemical tests of the stem extract. Different chemical compounds such as triterpene, flavonoids, glycosides, alkaloids and carbohydrates were detected in the plant which could make it useful for treating different ailments and has the potential of providing useful drugs for human use. Traditionally and experimentally it has been found that the leaf is a potential antifertility agent.[12] But the active constituent (constituents) is (are) not yet identified. Generally, it had been found that some flavonoids, alkaloids and terpenoids are potential antifertility agents. Hence, stem of *Cissampelos pareira* should be explored scientifically as antifertility agent.

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