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Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

ISSN 2278-4136
ISSN 2349-8234
JPP 2014; 2 (5): 27-31

Received: 18-11-2013
Accepted: 19-11-2013

Vandana G. Patel
Department of Pharmacognosy, A R College of Pharmacy, Mota Bazaar, Vallabh Vidhyanagar, Gujarat, India
Email: vandugp@yahoo.co.in

Sangita H. Shukla
Department of Pharmacognosy, Indukaka Ipcowala College of Pharmacy, Near GIDC, New VallabhVidhyanagar, Gujarat, India.
Email: shshukla@rediffmail.com

Bhavika V. Jogi
Department of Pharmacognosy, A R College of Pharmacy, Mota Bazaar, Vallabh Vidhyanagar, Gujarat, India
Email: jogi.bhavika@yahoo.com

Hemali A. Mistry
Department of Pharmacognosy, A R College of Pharmacy, Mota Bazaar, Vallabh Vidhyanagar, Gujarat, India
Email: hemali_mystery@yahoo.co.in

Correspondence
Vandana G. Patel
Department of Pharmacognosy, A R College of Pharmacy, Mota Bazaar, Vallabh Vidhyanagar, Gujarat, India
Email: vandugp@yahoo.co.in
Tel: +91-9723537532

Pharmacognostical standardization and preliminary phytochemical screening of *Cissampelos pareira* Linn. var. Hirsuta Roots

Vandana G. Patel, Sangita H. Shukla, Bhavika V. Jogi, Hemali A. Mistry

ABSTRACT

Cissampelos pareira Linn. var. hirsuta belongs to family Menispermaceae. It is commonly known as venivel. The present study was undertaken to carry out pharmacognostical and phytochemical investigation of the roots of the plant. Different pharmacognostical parameters studied were macroscopy and microscopy, fluorescence analysis, physicochemical analysis and elemental analysis. Phytochemical analysis included preliminary screening and qualitative chemical tests for identification of the chemical constituents present in the roots. In the light of the above factors, the purpose of the study was to set up the standardization parameters which can be of immense value for the identification of the genuine drug.

Keywords: *Cissampelos pareira* Linn., pharmacognostical parameters, physicochemical studies, standardization.

1. Introduction

Cissampelos pareira Linn. var. hirsuta belongs to the family Menispermaceae. It is commonly known as venivel. The plant is found throughout tropical and sub-tropical India from Sind and Punjab to South India and Ceylon. It is a very variable, lofty, slender, dioecious, perennial climber, commonly distributed throughout tropical and subtropical India, ascending upto an altitude of 2000 m. The plant flowers during the rainy season as well as autumn and fruits during winter[1, 2]. The roots are astringent, antispasmodic (used for cramps, painful menstruation), analgesic, diuretic, antilithic and emmenagogue. It is prescribed for diarrhea, dysentery, piles, urogenital affections (cystitis, nephritis, and menorrhagia). Root paste is applied topically on scabies and eruptions on the body. Also used for preventing miscarriage. Traditionally the roots are prescribed in combination with other drugs for the treatment of snake bite and scorpion sting[3, 4, 5]. The present investigation deals with qualitative and quantitative pharmacognostical and phytochemical evaluation of roots of *Cissampelos pareira* Linn. var. hirsuta.

2. Materials and methods

2.1 Collection and authentication of plant material

Fresh roots were collected from fully grown flowering plants of *Cissampelos pareira* Linn. var. hirsuta from New V V Nagar, Gujarat, India. The plants were identified and authenticated by Dr. Geetha K A, Senior Scientist, Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand, Gujarat. A voucher specimen of plant (VGP/CP-1/5/ARGH-09) was deposited in the herbarium of the Department of Pharmacognosy, A R College of Pharmacy, V V Nagar, and Gujarat. The roots were dried under shade, powdered with the aid of an electrical grinder, stored in an airtight container and used for further studies. All the chemicals and reagents used were of analytical grade and were procured from S.D. fine chemicals, Mumbai, India.

2.2 Pharmacognostical studies

2.2.1 Macroscopic and microscopic examination

The macroscopic examination was carried out with the help of naked eyes and simple hand lens for evaluation of shape, size, color, and fracture. For microscopic evaluation, free hands sections were taken of the fresh roots.

The resulting sections were boiled in chloral hydrate for 10 minutes to clear of interfering pigments in the tissues. The sections were then treated with phloroglucinol and concentrated hydrochloric acid for 10 min. and were observed under binocular microscope. By following the same procedure the microscopic studies of the powdered roots was also carried out [6, 7].

2.2.2 Quantitative microscopy for determination of fibre size

2 gm of powder (No. 60 powder) was mixed with 50 ml of 10% nitric acid in a casserole. It was boiled and maintained at boiling point for 30 seconds diluted with water and strained through a fine cloth held over the mouth of filter funnel. The washed residue was transferred to casserole and boiled further with 50 ml of 2.5% sodium hydroxide for 30s. The residue was washed and used for quantitative analysis. The values for 25 fibres were calculated and multiplied by the factor. The average value was calculated and the range for width and length of fibres was calculated [8].

2.2.3 Fluorescence analysis

The fluorescence and general behavior of the powdered roots was studied by treating separately with different reagents and exposed to visible, ultraviolet light. The color that developed was observed within one minute since the color may change due to the evaporation of the methanol [9, 10].

2.3 Physicochemical analysis

Different physicochemical constants were assessed such as moisture content, total solid content, solvent extractive values (water, alcohol, and ether), ash values (total ash, acid insoluble ash and water soluble ash), foaming index, total phenolic content and total flavonoid content [11-16].

2.4 Elemental analysis

2 gm of dried powdered roots were weighed and subjected to dry-ashing in a well-cleaned porcelain crucible at 550 °C in a muffle furnace. The resultant ash was dissolved in 5 ml of HNO_3/HCl/H2O (1:2:3) and heated gently on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of deionized water was added and heated until a colorless solution was obtained. The mineral solution in each crucible was transferred to a 100 ml volumetric flask by filtration through a Whatman No 42 filter paper and the volume was made to the mark with deionized water. This solution was used for elemental analysis by inductively coupled plasma atomic emission spectrophotometer (Perkin Elmer, USA, 3300RL) and concentration of element in the sample was calculated as the percentage of dry matter [17,18].

2.5 Phytochemical screening

20 gm of the powdered plant material was extracted successively with petroleum ether (60-80º C), toluene, chloroform, acetone, methanol and water in a Soxhlet extractor. Each time before extracting with the next solvent, the powdered material was dried in air-oven below 50 ºC. The completion of the extraction was confirmed by evaporating a few drops of extract from thimble on watch glass to observed that no residue remain after evaporation of the solvent. Finally, the marc was macerated with water for 24 hours to obtain the aqueous extract. All the extracts were concentrated by distilling off the solvent and then evaporated to dryness on the water-bath. The extract obtained with each solvent was weighed and their percentage was calculated in terms of the air-dried weight of the plant material. The color, consistency and fluorescence of the extract were also noted. The extracts were subjected to various qualitative tests using reported methods to determine the presence of various phytoconstituents such as alkaloids, glycosides, flavonoids, carbohydrates, amino acid, sterols, fixed oil and fats, saponins, phenolic compounds and tannins [19-22].

3. Results and discussion

3.1 Macroscopic characters of roots

The roots were cylindrical, tortuous in shape; 1–1.5 cm in diameter; had rough surface and at places rugged due to transverse wrinkles, cracks and fissures; fracture was short and splintery; had faint aromatic odor; bitter in taste and light brown to yellowish brown in color. [Figure 1].

Fig 1: Roots of *Cissampelos pareira* Linn. var. *hirsute*

3.2 Microscopic examination of root

Transverse section of root showed non-lignified cork, composed of 7 to 15 layers of tangentially elongated, rectangular cells, outermost layer was obliterated, cortex parenchymatous 3 to 10 cells wide; pericycle characterized by 1 to 3 celled thick continuous ring of stone cells embedded with group of lignified fibres [figure 2a]; vascular zone was composed of radially arranged discrete vascular strands with 6 to 8 narrow streaks of xylem with some reaching upto the centre, externally capped with semi- circular patch of phloem on the outer side, alternating with medullary rays. Xylem was composed of vessels, tracheids, fibres and parenchyma; phloem was separated from xylem by 2 to 3 layers of cambium; sieve tubes and parenchyma were distinct but got obliterated towards the peripheral zone of cap; medullary rays parenchymatous; rod shaped prismatic crystals of calcium oxalate and simple and compound starch grains were present throughout the parenchymatous cells[23, 24] [Figure 2].

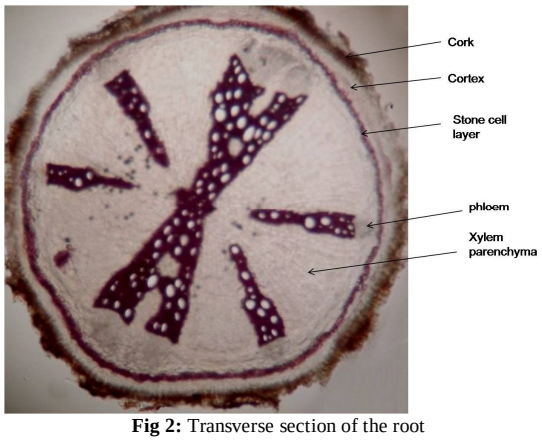

Fig 2: Transverse section of the root

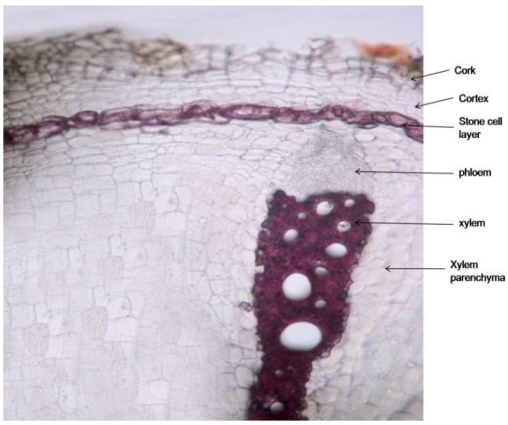

Fig 2a: Transverse section of outer part of the root.

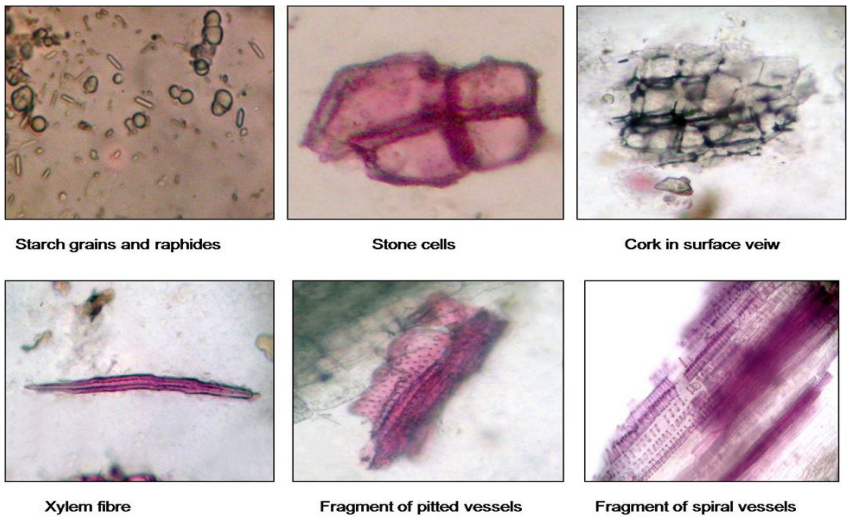

Fig 3: Powder characteristics of the roots of Cissampelos pareira Linn.

3.3 Microscopic examination of powdered roots

The powdered roots were fibrous and fine in appearance, light brown in color with faint aromatic odor and bitter taste. On microscopic examination the powdered roots showed the presence of scattered simple and compound starch grains and raphides of calcium oxalate; stone cells; cork in surface view; thin walled xylem fibres and fragment of spiral and pitted vessels [Figure 3].

3.4 Quantitative microscopy

The width and length of fibres of powdered roots were as indicated in Table 1.

Table 1: Quantitative microscopy of the powdered roots of the *Cissampelos pareira*

Fibre	Size
length	405.45-632.1-915.9 µ
width	25.8-41.93-46.5 µ

3.5 Fluorescence analysis

The fluorescence analysis of the powdered roots of *Cissampelos pareira* with various reagents were performed under normal and UV light. The results are as summarized in Table 2.

3.6 Physicochemical analysis

The results of different standardization parameters such as total solids, moisture content, ash value, extractive values, foaming index, total phenolic content and total flavonoid content were as indicated in the Table 3. Total ash values were high indicative of the presence of more silicaceous matter and calcium oxalate crystal. Water soluble extractive value was high indicating presence of more water soluble constituents. High foaming index indicated the presence of good amount of saponins in the powdered roots.

3.7 Elemental analysis

The elemental contents arsenic, cadmium, lead, and selenium were analysed in the powdered roots and the results were as indicated in the Table 4. It showed absence of the heavy metals.

3.8 Phytochemical screening

Powdered root were separately extracted with petroleum ether (40–60 °C), toluene, chloroform, acetone, methanol and water in soxhlet apparatus respectively. The results of colour, consistency, extractive value and fluorescence of extract were as presented in Table 5. Highest yield

was found in water extract followed by methanol extract indicating presence of more polar constituents. Preliminary qualitative chemical tests were performed on the extracts which showed that the roots of *Cissampelos pareira* Linn. was credited with alkaloids, flavonoids, saponins, carbohydrates and fats. The results are summarized in Table 6.

Table 2: Fluorescence analysis of the powdered roots of *Cissampelos pareira*

Reagent	Day light	UV 254	UV 365
1M sodium hydroxide	Brown	Greenish brown	Brown
1% picric acid	Brown	Greenish brown	Brown
Acetic acid	Brown	Brown	Brown
1M Hydrochloric acid	Brown	Yellowish brown	Brown
Dilute nitric acid	Brown	Creamish brown	Brown
5% iodine	Creamish black	Creamish brown	Brown
5% ferric chloride	Black	Creamish brown	Black
Methanol	Brown	Brown	Brownish black
50% nitric acid	Brown	Green	Brown
1M sulphuric acid	Brown	Greenish brown	Brown
Dil. Ammonia	Creamish brown	Brown	Brown
10% potassium dichromate	Brown	Dark green	Black
Sodium hydroxide in methanol	Brown	Creamish black	Creamish black

Table 3: Physicochemical analysis of the powdered roots of *Cissampelos pareira*

Standardisation parameter	%w/w
Moisture content	6.78
Total solids	93.22
Total ash	6.5
Acid insoluble ash	1.5
Water soluble ash	1
Water soluble extractive value	16.8
Alcohol soluble extractive value	10.32
Ether soluble extractive value	3.12
Foaming index	111.11
Total phenolic content	0.046
Total flavonoid content	0.032

Table 4: Elemental analysis of the powdered roots of *Cissampelos pareira*

Element	Wavelength	Instrument Detection Limit ppm (mg/l)	Sample Results ppm (mg/kg)
Arsenic (As)	188.979	-	Not Detected
Cadmium (Cd)	228.802	0.0027	Not Detected
Lead (Pb)	220.353	0.0420	Not Detected
Selenium (Se)	196.026	0.0750	Not Detected

Table 5: Phytochemical analysis of the powdered roots of *Cissampelos pareira*

Solvent	% w/w	Consistency	Day light	UV 264	UV 365
Pet ether (60-80º)	2.25	semisolid	yellow	cream	yellow
Toluene	1.05	semisolid	brownish green	yellowish green	dark green
Chloroform	1.6	solid	brownish green	yellowish green	dark green
Acetone	2.15	semisolid	brown	light green	dark green
Methanol	8.8	semisolid	dark brown	greenish black	greenish black
Water	10.85	solid	brown	light green	dark green

Table 6: Qualitative evaluation of extracts of powdered roots of *Cissampelos pareira* obtained by soxhlet extraction

Phytoconstituent	Pet ether (60-80º)	Toluene	Chloroform	Acetone	Methanol	Water
Alkaloid	-	-	+	-	+	+
Carbohydrates	-	-	-	-	+	+
Anthracene glycosides	-	-	-	-	-	-
Cardiac glycosides	-	-	-	-	-	-
Saponin	-	-	-	-	+	+
Steroids and triterpenoids glycosides	-	-	-	-	+	+
Flavonoid	-	-	-	-	+	+
Phenolic compounds and tannins	-	-	-	-	+	+
Fixed oils and fats	+	+	-	-	-	-
Proteins and amino acids	-	-	-	-	-	-

*+ present; - absent

4. Conclusion

The study was undertaken to develop the standardization parameter of the roots of the *Cissampelos pareira* Linn. var. hirsuta. The results obtained from the pharmacognostical and phytochemical investigation of the roots can be used for the identification of the genuine drug. The parameters can also be

used for the further scientific investigation of the roots of *Cissampelos pareira* Linn. var. hirsuta.

5. Acknowledgements
Authors are grateful to Dr. Geetha K A, Senior Scientist, Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand, Gujarat for authenticating the plant sample. The authors express sincere thanks to Sophisticated Instrumentation Centre for Applied Research and Testing (SICART) for providing with the facility of Inductively Coupled Plasma Atomic Emission Spectrophotometer instrument.

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