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Research

Physicochemical Evaluation and Pharmacognostic Study of Panibel *(Ampelocissus latifolia)* (Roxb.) -Stem

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Abstract

Ampelocissus latifolia, belonging to the Vitaceae family, is a climber plant, mostly found in the Sub-Himalayan region of India, ranging from the Sutlej eastward to Kumaon up to 4000 feet, as well as in Aasam, Konkan, Western Ghats from Bombay to Nilgiris and Anamallis Deccan. Various parts of this plant is using to treatment of the several human diseases like leaf and stem bark is useful for wound healing**,** whole plant is used Kustha (leprosy) and Sotha (swelling).The stem bark is used in stomach pain and bone fracture. The roots are used in skin diseases, wound healing, rheumatic affections, fractures, diuretic, gonorrhoea, syphillis, eye diseases, menstrual troubles and also as a tonic. Ethno-medicinal and therapeutic uses of *Ampelocissus latifolia* various parts study was undertaken. In present investigation various tools and techniques included like that pharmacognostic studies, physicochemical tests, preliminary phytochemical analysis and development of HPTLC fingerprints profile. The established parameters can be used as standards for further study and also preparation of a monograph of *Ampelocissus latifolia* plant stem.

Keywords: *Ampelocissus latifolia*, Pharmacognosy, HPTLC fingerprints, Physicochemical.

INTRODUCTION

Natural products have been used since the beginning of medicine, particularly those made from plants, to support human health[1]. Throughout history, traditional medicine has been widely accepted and used by people, having existed since the beginning of time. Plants have been used as a model for medicinal purposes since ancient times. Because they have few adverse effects and improve human health, plant-derived pharmaceuticals have long caught the interest of scientists worldwide.[2,3]

Ampelocissus latifolia, family Vitaceae is commonly known as Panibel. Plant is an annual, herbaceous climber, terrestrial, large twining, wild, commonly found in open waste lands, hedges and shrubberies. Roots are fasciculate with many tuberous roots in cluster having irregular shape and size arising from root stock. Roots are 6.5cm - 16 cm long and 1cm - 5 cm thick. Roots are taped at both the end with bulging in middle. Leaves are broadly heart-shaped or circular, 6-16 x 7-15cm, length, broad, 3-7 lobed. Lobes are acute. Leaf stalk 3.5-6cm

long, crenate-serrulate or dentate margin, with pubescent nerves, often tinged with pink or purple beneath, base 5-7 nerved. Petiole varying in length up to 20cm deeply grooved above. Flowers are deep reddish colour, numerous, 2.5 mm diameter, Petals - 5, oblong, in pyramidal panicle pubescent cymes borne on a very stout peduncle together with a forked tendril. Petals not cohering at the apex recurved glabrous on both surfaces. Stamens bright yellow colour. Inflorescence is a compact cyme with a stalk 6-7 cm long, ending in a long bifurcate tendril. Flowers appearing in the month of May- August. Fruits are black colour, globose, spherical, succulent, 5-8mm shape, 2 seeded, rarely 3 seeded. Whole plant of *Ampelocissus latifolia* used for wound healing[4], Infusion, anorexia, muscular pain, sores, pneumonia, bone fracture and also used as tonic by aged person. Decoction of the stem bark is given in stomach pain. Root used in skin diseases, in fracture, as a tonic[8], menstrual troubles, wound healing, diuretic, eye disease, gonorrhoea, syphilis, rheumatic affection, dental pains, ulcers and dysentery[5]. Root also fed to domestic pigs after delivery to increase lactation and are chewed or a half glass of infusion is administered orally for easy delivery. Crushed root with water is given to animal with the help of drenching tube to cure fractured bone. The extract of tuber is given orally to cure dyspepsia and indigestion and to cure tuberculosis.[6] Root powder is mixed in water, after about one hour this paste is applied on body for inflammation[7]. Root and stem bark of plant are used in treatment of bone fracture, stomach pain, dysentery, fever and in menstrual complaints. The roots are also employed in treatment of pain in stomach, snake bites, sores, skin disease and wounds[5]. Root paste is applied to wounds heal, decoction is given in cases of chronic dysentery. *Ampelocissus latifolia* leaf juice poured into eyes in 1-2 drops twice a day for 3 days in Opthalmia[7].

MATERIAL AND METHODS

Collection and authentication of plant

Ampelocissus latifolia (Roxb.) whole mature plant was collected from Arogyadham campus, Deendayal Research Institute, Chitrakoot, Satna (M.P.) India, in the month of October, 2022. Plants material were identified and authenticated by Dr. Manoj Tripathi, Senior Scientist & Head (R&D Department), Arogyadham, Deendayal Research Institute, Chitrakoot, Satna (M.P.), India. Prepared the herbarium of *Ampelocissus latifolia* plant and deposited (voucher specimen no. Govt./PGC/551), in the herbarium department of Government, Autonomous, Post Graduate, Collage, Satna (M.P.).[9]

Preparation of sample

Fresh stem of *Ampelocissus latifolia* was used for pharmacognostical investigation such as preparation of herbarium, macroscopic and microscopic studies, While under tray dryer dried stem of *Ampelocissus latifolia* was powdered and stored in airtight containers for further studies**.**[10]

Macroscopic and microscopic study

Organoleptic study colour, odour and taste were evaluated. Fresh stem section was cut by free hand sectioning and numerous sections examined microscopically. Photographs of the microscopical sections were captured with the help of Olympus Trinocular Research Microscope CX- 21*I* with Digi-eye camera using Caliper plus version 4.2 software.[11]

Powder microscopic study

About 5 gm of powder washed thoroughly with potable water, pour out the water without loss of material. Mounted a small portion in glycerine, warmed a few mg with chloral hydrate solution, wash and mounted in glycerin. Treat a few mg with iodine solution and mount in glycerine, about 1 g of powder warmed over water bath with Chloral hydrate solution till brown fumes appear, cool and wash with water thoroughly and mount a small portion in glycerin and seen under microscope at 40X x 10X magnification of the Trinocular Research Microscope.[12]

Physico-chemical parameters

Physico-chemical tests were performed and set up the certain standards for *Ampelocissus latifolia* stem. Physicochemical tests were includes moisture content (loss on drying at 105^0C), water soluble extractive value, alcohol soluble extractive value, total ash value and acid insoluble ash value[13].

Heavy metals tests

Heavy metals are toxic and generally occur through earth in plants. Mainly four types of heavy metals harmful for us they are Pb, Cd, As and Hg. These heavy metals detected through Atomic Absorption Spectrophotometer as per standard method.[14,15,16]

Preliminary phytochemical studies

Phytochemical tests for screening and identification of bioactive chemical constituents present in the *Ampelocissus latifolia* stem, various tests were performed in ethanol and water extracts for the preliminary photochemical screening[17].

High performance thin layer chromatography (HPTLC)

For High-performance thin layer chromatography, about 5 gm accurately weighed *Ampelocissus latifolia* samples (root, stem and leaf) with 100 ml of methanol (3X100) in a Soxhlet apparatus for 6 hours separately. Filtered and concentrated the extracts under a vacuum oven to get the residue. Dissolved 100 mg of *Ampelocissus latifolia* extracts residue (root, stem and leaf) in a 10ml (10mg/ml) volumetric flask and make up the volume with methanol to get the working test solution separately.[18,19,20]

Preparation of standard solution- (Ferullic acid and Quercetin)

For preparation of the standard marker working solutions, 10mg of Ferullic acid and Quercetin were dissolved in a 10 ml volumetric flask and made up the volume with methanol separately. Then transferred 1 ml of stock solution to a 10 ml volumetric flask and made up the volume with methanol separately (0.1mg/ml). From the solution, prepared standard solutions by transferring aliquots (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml) corresponding to (1, 2, 3, 4, 5 and 6 ug/ml) of stock solution to 10ml volumetric flasks and made up the volume in each case to 10 ml with methanol.

Calibration curve

Applied 8 µl each of the standard solution (80 to 480 mg per spot) on the HPTLC plate. Develop the plate in the solvent system till the solvent rises to a distance of 8 cm. Dry the plate and scan at 358nm. Record the peak areas. Prepared a calibration curve of Ferulic acid and standard by plotting peak areas against concentration of Ferulic acid and Quercetin standard.

High performance thin layer chromatography (HPTLC) study of the methanolic extracts of *Ampelocissus latifolia* root, stem and leaf with Ferulic acid and Quercetin standard marker spots applied in precoated TLC plate. Samples (root, stem and leaf) as well as standard markers (Ferulic acid, and Quercetin) were applied by spotting test solution 8 µl (each test solution root, stem and leaf) on pre-coated silica-gel aluminum plate 60 F_{254} (10x20 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe. The samples, in the form of bands of length 6 mm were spotted 15 mm from the bottom, 15 mm from the left margin of the plate, and 10 mm part. And apply 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0, µl standard markers Ferullic acid and Quercetin, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 µl on pre-coated silica-gel aluminum plate 60 F_{254} (10x20 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe and standard markers, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from the left margin the plate and 10 mm part.

RESULT AND DISCUSSION

Macroscopic characters

Ampelocissus latifolia, family vitaceae is mostly found in the Sub-Himalayan region of India, ranging from the Sutlej eastward to Kumaon up to 4000 feet. Root colour is reddish brown. Roots are fasciculated with many tuberous roots in cluster having irregular shape and size arising from root stock. Roots are 6.5cm - 16 cm long and 1cm - 5 cm thick. Roots are taped at both the end with bulging in middle. Stem bark colour is green, surface smooth, often tinged purple especially at the nodes, covered with thin glabbrous bloom. Young shoots glabrous and hollow, Tendrils forked. Plant stem is cylindrical shape 3.5-6.5 meter long, 8-14mm diameter which are scarcely woody. Leaves are broadly heart-shaped or circular, 6-16 x 7-15cm, length broad, 3-7 lobed. Lobes are acute, toothed. Leaf stalk 3.5-6cm long, crenate-serrulate or dentate margin, cordate mealy when very young and glabrescent and with pubescent nerves when mature, often tinged with pink or purple beneath, base 5-7 nerved. Petiole varying in length up to 20cm deeply grooved above (Fig.1&2).

Fig 1*: Ampelocissus latifolia* plant

Fig 2*: Ampelocissus latifolia* stem

Microscopic study

Transverse section of *Ampelocissus latifolia* stem found oval/circular in outline. Transverse section of *Ampelocissus latifolia* stem in outer most region was found to contain 2-5 layers of closely packed cells in cork. Cortex contains 3-6 layers of collenchyamatous cells and 3-8 layers of loosely arranged parenchymatous cells. Pericyclic fibres containing sclerencymatous cells were found in form of cap over vascular bundle. Collateral vascular bundle was arranged in circular. Phloem was on outer side and xylem was inner side in vascular bundle. Pith is the central largest region of stem contained polygonal parenchymatous cells. Unstained transverse section of *Ampelocissus latifolia* showed presence of acicular crystals of calcium oxalate (Fig.3a&3b).

Fig 3a: Diagrammatic TS of *Ampelocissus latifolia* stem

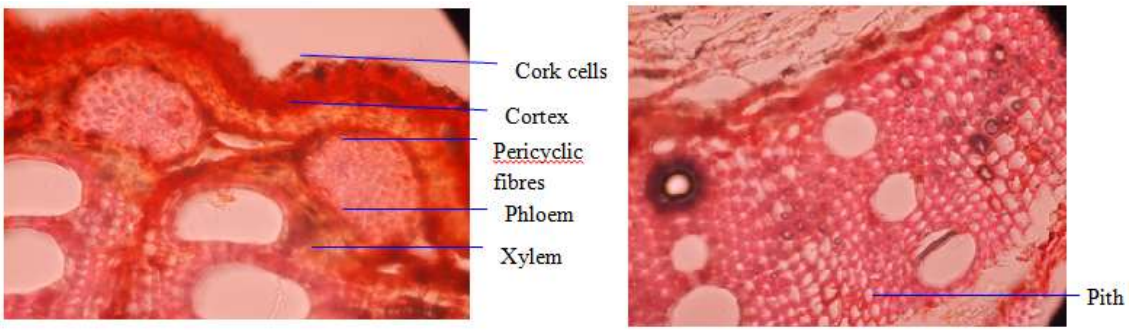

Fig 3b: Detailed TS of *Ampelocissus latifolia* stem

Powder microscopic characters

Ampelocissus latifolia stem powder shows various types of structure like that different types of sclerids and thickenings, cork cells, parenchyma filled with rosette crystals of calcium oxalate and lignified parenchyma (Fig.4a-4d).

Fig 4a- Various types of pitted vessels

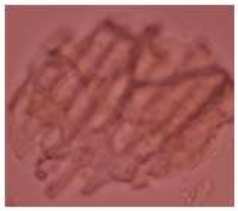

Fig 4b: Cork cells

Fig 4c: Parenchyma filled with rosette crystals of calcium oxalate

Fig 4d: Lignified parenchyma

Physico-chemical analysis

The physico-chemical tests such as Loss on drying at 105^0C, extractive values such as water and alcohol soluble extractive value, total ash value and acid insoluble ash value were performed. The results are expressed as mean (n=3) ± standard deviation in w/w. *Ampelocissus latifolia*, stem. The Loss on drying was found 4.97% w/w, total ash value 5.6% w/w, acid insoluble ash value 2.38% w/w, alcohol soluble extractive value 11.13% w/w and water soluble extractive value 20.12%w/w.

Preliminary phyto-chemical investigation

Preliminary phytochemical analysis was performed methanol and water extracts of *Ampelocissus latifolia* stem was carried out. Alkaloids, flavonoid, saponins, tannins, phenols, carbohydrates and proteins were present.

HPTLC finger print profile

Calibration curve

Applied 8 µl each of the standard solution (80 to 480 mg per spot) on the HPTLC plate. Develop the plate in the solvent system till the solvent rises to a distance of 8 cm. Dry the plate and scan at 358nm. Record the peak areas. Prepared a calibration curve of Ferulic acid and Quercetin standard by spotting peak areas against concentration of Ferulic acid and Quercetin standard.

High performance thin layer chromatography (HPTLC) study of the methanolic extracts of *Ampelocissus latifolia* root, stem and leaf with Ferulic acid and Quercetin standard marker spots applied in precoated TLC plate. Samples (root, stem and leaf) as well as standard markers (Ferulic acid, and Quercetin) were applied by spotting test solution 8 µl (each test solution root, stem and leaf) on pre-coated silica-gel aluminum plate 60 F_{254} (10x20 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe. The samples, in the form of bands of length 6 mm were spotted 15 mm from the bottom, 15 mm from the left margin of the plate and 10 mm part. And apply 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 µl, standard markers Ferullic acid and Quercetin, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 µl on pre-coated silica-gel aluminum plate 60 F_{254} (10x20 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe and standard markers, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from the left margin the plate and 10 mm part. The plate was developed using a mobile phase consisting of *toluene: ethyl acetate* (7:5v/v). Linear ascending development was carried out in a 20x20cm twin through glass chamber equilibrated with the mobile phase. The optimized chamber saturation time for the mobile phase (20 ml) was 30 min at room temperature. The length of the chromatogram run was 85 cm. Subsequent to the development, a thin layer of chromatography plate was dried at room temperature. The peak area for samples and standards were recorded with the camera photo documentation system Camag Reprostar 3 and the plate was scanned densitometrically with the help of Scanner- 4. Record the respective areas and prepare a calibration curve by plotting peak area *vs* concentration of standard markers Ferullic acid and Quercetin. Major spots R_f values with colour were recorded before derivatization at 254nm. Major spots of R_f values before derivatization at 254nm major spots R_f values are 0.32 light black, *Ampelocissus latifolia* root, stem and leaf with Ferulic acid standard marker, 0.26 light black *Ampelocissus latifolia* root with quercetin standard marker. It is observed that the Ferulic

acid is higher present in root than the stem and leaf, while Quercetin higher present in root but absent in stem and leaf (Fig.5).

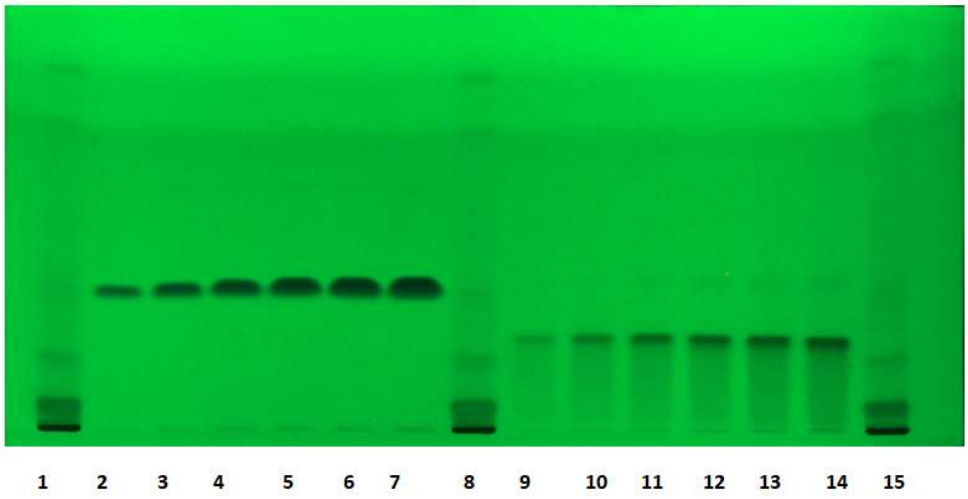

Fig 5: HPTLC fingerprints profile of *Ampelocissus latifolia*

Abbreviation: **Track 1:** test solution of *Ampelocissus latifolia* root*;* **Track 2-7** Ferulic acid standard; **Track 8:** test solution of *Ampelocissus latifolia* stem; **Track 9-14:** Quercetin standard; **Track 15:** test solution of *Ampelocissus latifolia* leaf.

Heavy metals tests

Heavy metals detected through Atomic Absorption Spectrophotometer in *Ampelocissus latifolia* root, stem and leaf as per described standard method. The results obtained in ppm and ppb level and found within limits as per guideline of WHO/API for heavy metals. As per obtained results of heavy metals, it was observed that the screened metals Pb, Cd, As and Hg are detected in very low values, means samples are safe and not harmful for the health.

The macroscopic, microscopic and powder microscopic distinguished characters have been established to identify *Ampelocissus latifolia* stem. Physicochemical tests were performed and results obtained. The loss on drying value obtained is an indicative of amount of moisture content could prevent bacteria, fungal or yeast growth.Water soluble extractive value is higher than the alcohol soluble extractive value. The extractive values, indicates the amount of active constituents in given amount of plant material when extracted with respective solvent and useful for the determination of exhausted or adulterated drug. Ash values of the drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Total ash value is an indicative of total amount of inorganic material after complete incineration and the acid insoluble ash value is an indicative of silicate impurities, which might have arisen due to improper washing of the ingredients. Ash value is useful in determining authenticity and purity of the drug and also these values are important quantitative standards. HPTLC finger print profile helps in identification of various phytochemical constituents present in the crude drug thereby substantiating and authenticating of crude drug. The TLC profile also helps to identify and isolate's important phyto-constituents. It is observed that the Ferulic acid is higher present in root than the stem and leaf, while Quercetin higher present in root but absent in stem and leaf. Heavy metal elements are found under limits as per guideline WHO. All findings are indicating samples are genuine and free from any adulterations. The finding could be helpful in identification and authentication of *Ampelocissus latifolia* stem.

CONCLUSION

Ampelocissus latifolia, mostly found in the Sub-Himalayan region of India, ranging from the Sutlej eastward to Kumaon up to 4000 feet, as well as in Aasam, Konkan, Western Ghats from Bombay to Nilgiris and Anamallis Deccan. Various parts of this plant is using to treatment of the several human diseases like leaf and stem bark is useful for wound healing**,** whole plant is used Kustha (leprosy) and Sotha (swelling). Therefore, the current investigation of the plant stem was undertaken. Study includes anatomical characters, physico-chemical, tests, heavy metals test and high performance thin layer chromatography. The established parameters can be used as standards for further study and also preparation of a monograph of *Ampelocissus latifolia* plant stem.

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