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RESEARCH PAPER

Pharmacognosy and Chromatography Screening of *Cissus quadrangularis*

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ABSTRACT

Cissus quadrangularis (bone-setter plant, Vitaceae) was subjected to comprehensive pharmacognosy and HPTLC screening to establish quality standards for Ayurvedic applications in bone healing and inflammation. Physicochemical analysis revealed acceptable loss on drying (6.59% w/w), pH 4.88, high aqueous extractives (11.98% w/w), and substantial mineral ash (11.97% w/w); microscopy confirmed identity via raphides, oxalate crystals, vessels, and fibers. Phytochemical screening detected carbohydrates, alkaloids, phenolics, and tannins (positive in both extracts), while HPTLC fingerprints at 254/366/540 nm displayed reproducible R_f bands (e.g., 0.708, 0.739) indicative of flavonoids/stilbenes like resveratrol. These findings validate authenticity, support traditional uses, and provide standardization markers for therapeutic formulations.

Keywords: - Pharmacognosy, Invitro anti-inflammatory assay, *Cissus quadrangularis*, HPTLC, Phytochemical, Physiochemical, Microscopy.

INTRODUCTION

The study of bioactive natural products derived from plants, animals, and minerals, plays a vital role in drug discovery and the development of novel therapeutic agents. Among medicinal plants, "*Cissus quadrangularis*", a perennial plant from the family Vitaceae, has attracted widespread attention in traditional and modern medicine for its diverse pharmacological activities. Commonly known as the "bone setter" plant, it has been extensively used in Ayurveda and other traditional healing systems for the treatment of bone fractures, joint disorders, and inflammatory conditions.

Cissus quadrangularis is a succulent, perennial climber in the grape family, known for its widespread distribution in warm, dry habitats and notable medicinal uses [1]. *Cissus quadrangularis* is native to India but is now found throughout drier regions of Africa, Arabia, and Southeast Asia, including Sri Lanka, Malaysia, and as far as the Philippines. It typically grows in scrub, thicket, Acacia woodland, grasslands with scattered trees, riverine thickets, coastal forest edges, and sandy riverbanks. The plant thrives in areas with low rainfall, well-drained soils, and full sun, from sea level up to 2250 meters altitude [2].

Major bioactive compounds identified from *Cissus quadrangularis* include:

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- Flavonoids: quercetin, daidzein, genistein
- Triterpenoids: friedelin, α-amyrin, β-amyrin
- Phytosterols: β-sitosterol, ketosetosterol
- Stilbene derivatives: quadrangularin-A, resveratrol, piceatannol
- Iridoids: 6-O-meta-methoxy-benzozyl catapol, picroside, pallidol
- Other: Vitamin C, carotene, calcium oxalate, taraxeryl acetate, taraxeroliso-pentadecanoic acid, calcium ions, phosphorus.
- The leaves contain resveratrol, piceatanon, pallidol, parthenocissus, and alicyclic lipids. The roots provide minerals like potassium, calcium, zinc, sodium, iron, lead, cadmium, copper, and magnesium.

Cissus quadrangularis exhibits a broad spectrum of pharmacological activities:

- Bone healing: Promotes fracture healing due to its anabolic steroids, triterpenoids, and vitamins.
- Anti-obesity: Reduces waist circumference, BMI, and serum lipid levels in clinical studies.
- Anti-ulcer: Inhibits gastric acid secretion and protects against ulceration.
- Anti-diabetic: Lowers blood glucose, as shown in animal models.
- Antioxidant: Scavenges free radicals due to its high β-carotene and flavonoid content.
- Gastroprotective: Reduces gastrointestinal toxicity, especially from medications like aspirin.
- Central nervous system: Root extract has stimulant and sedative actions,reducing spontaneous activity in experimental animals.
- Analgesic, anti-inflammatory: Demonstrates pain relief and anti-inflammatory effects attributed to flavonoids and β-sitosterol.

These properties underscore its importance in traditional medicine, especially for bone health, metabolic disorders, gastrointestinal protection, and inflammation [3].

Material and Method

Macroscopic study: The collected sample was studied organoleptically, with naked eye & magnifying lens, with the help of Pharmacognostical procedure i.e. Appearance, size, shape, colour, and odour and findings were recorded.

Powder microscopy: Powder microscopic inspection of medicinal plant materials is indispensable for the identification of broken or powdered materials; the specimen has to be treated with chemical reagents. An examination by microscopy alone cannot always provide complete identification, though when used in association with other analytical methods it can frequently supply invaluable supporting evidence. Comparison with a reference material will often reveal characteristics not described in the requirements which might otherwise have been attributed to foreign matter rather than normal constituents.

For examining the characters of the powder take sufficient amount of powder in different chemical reagents on a slide and warm over a low flame for a short time. Put drop of glycerine on the slide, cover it with the cover slip and observe under the microscope.

Chemical reagents used for staining of the powder samples were Safranin, Dilute Ferric chloride , Iodine, *Sudan III,* Hcl and phloroglucinol [4].

Loss on drying

The test sample that had not been dried before was weighed at around 10 g and put in a tared evaporating dish. I used a hot air oven set to 105°C for five hours to dry it. Drying and weighing should be done every hour until the discrepancy between two successive weight readings is less than 0.25%. The weight was deemed constant if there was a variation of no more than 0.01 g between two successive weigh-ins after a 30-minute drying and 30-minute cooling interval in a desiccator. Ascertain the percentage of loss concerning 10 grams of the specimen under examination.

pH: Tablets of different pH were taken and each tablet was dissolved in 100 ml of distilled water to prepare solutions of different pH. The instrument was switched on and left for some time until required different pH solutions appeared. Buffer solution was taken in the beaker and the electrode was dipped in it. Same procedure was repeated for the other buffer solution after washing the electrode thoroughly with distilled water. The sample was taken (10% aqueous solution) and electrode was dipped in it and the value of pH was noted [5].

Alcoholic Extractive Value

For 24 hours, macerate 5 g of the coarsely ground, air-dried test sample in a closed flask with 100 ml of alcohol. Shake the mixture frequently for the first 6 hours, then leave it alone for the remaining 18 hours. Filter as soon as possible, taking care to avoid solvent loss. Next, evaporate twenty-five milliliters of the filtrate in a shallow dish with a flat bottom until it achieves a constant weight, then weigh it. Cried at a temperature of 105°C. Calculate the extractive % of alcohol in respect to the test sample that has been dried. The methanol-soluble extractive was found by substituting methanol for alcohol.

Aqueous Extractive Value

For 24 hours, macerate 5 g of the coarsely ground, air-dried test sample in a closed flask with 100 ml of distilled water. Shake the mixture frequently for the first 6 hours, then leave it alone for the remaining 18 hours. Filter as soon as possible, taking care to avoid solvent loss. Next, evaporate twenty-five milliliters of the filtrate in a shallow dish with a flat bottom until it achieves a constant weight, then weigh it. Dried at a temperature of 105°C. Calculate the extractive percentage that is soluble in water relative to the test sample that is dried.

Total ash

2 to 3 g of the powdered sample should be burned in a silica crucible at a temperature no higher than 600°C until the sample is carbon-free. After cooling, weigh it. In the event that carbon-free ash could not be produced using this procedure, the burned mass was put out, the residue was collected on ashless filter paper, the residue and filter paper were burned together, the filtrate was added, the evaporated material was dried, and the mixture was ignited at a temperature not to exceed 600°C. The proportion of ash was found in the test sample that has been dried.

Acid-insoluble ash

The crucible was filled with filled all of the ashes and then added 25 milliliters of diluted hydrochloric acid. Whatman 41 ashless filter paper was used to collect the insoluble particles, and the filtrate washed in hot water until it reached a neutral pH. After relocating the filter paper with the insoluble material into the original crucible and allowing it to dry on a hot plate, I burned it until it reached a constant weight. Immediately the residue was weighted after allowing it to cool for thirty minutes in the proper desiccator. Ascertain the reference dry test sample's acid-insoluble ash %.

Water-Soluble Ash

The crucible was filled with all of the ash and added 25 milliliters of distilled water. Whatman 41 ashless filter paper was used to collect the insoluble particles, and the filtrate washed in hot water until it reached a neutral pH. After relocating the filter paper with the insoluble material into the original crucible and allowing it to dry on a hot plate it was burned until it reached a constant weight. Immediately weighed the residue was weighted after allowing it to cool for thirty minutes in the proper desiccator. Ascertain the reference dried test sample's water-soluble ash percentage.

Analysis of Primary and Secondary Metabolite Qualitatively

Phytochemical testing is used to identify the primary (carbohydrate, protein, and amino acid) and secondary (alkaloids, glycosides, tannin, saponin, and phenolic compounds) metabolites. The alcoholic and aqueous extracts were used to look for primary and secondary metabolites in test samples.

Molisch's Test: After adding two milliliters of test solution and two milliliters of Molisch's reagent, and giving it a gentle shake, one milliliter of concentrated H_2SO_4 was poured out the test tube's side and allowed to stand for a minute. A purple ring at the junction of the two layers indicated the presence of carbs.

Benedict's test: Sugars was reduced using this technique, which mainly used copper sulfate and sodium hydroxide. Four milliliters of the drug's aqueous solution were combined with one milliliter of Benedict's solution, and the mixture was heated to almost boiling. As the concentration of simple sugar rose, colors such as green, yellow, orange, red, or brown were generated in the test solution as a result of the production of cuprous oxide.

Fehling solution test: Usually used to lower sugar levels, this test consists of two solutions blended in-situ. Fehling solution A includes 0.5% copper sulfate, and Fehling solution B is composed of sodium potassium tartrate. Two milliliters of the drug's aqueous solution were added after one milliliter each of Fehling A and Fehling B solutions were blended. The mixture was next brought to boil in a water bath for five to ten minutes.

Dragondroff's Reagent: Two milliliters of the test solution and two milliliters of bismuth subnitrate and potassium iodide solution, or Dragondroff's reagent, were added to a test tube. The presence of alkaloids was demonstrated by the materials' capacity to produce an orange precipitate.

Wagner Test: The presence of alkaloids was indicated by the production of a reddish-brown precipitate after a few drops of Wagner's reagent (diluted iodine solution) were added to two milliliters of test solution.

Hager test: Picric acid was dissolved in a saturated aqueous solution. After the test filtrate was treated with this reagent, an orange-yellow precipitate was formed, indicating the presence of alkaloids.

Ninhydrin test: This test was used to identify proteins with free amino groups and alpha-amino acids. The characteristic deep blue or light blue color is caused by the synthesis of a mixture between two ninhydrin molecules and the nitrogen of free amino acids.

Biuret test: The mixture was mixed with one milliliter of 4% sodium hydroxide solution, five milligrams of residue, and a drop of 1% copper sulfate solution. The proteins presence was indicated by the emergence of a violet or pink tint.

Xanthoprotic test: A small portion of the test material was treated with two milliliters of water and five milliliters of strong nitric acid. The presence of proteins was shown by the emergence of a yellow hue.

Foam test: A tiny amount of the sample was added to a test tube together with a small amount of water and sodium bicarbonate, and the mixture was shaken vigorously. Saponins were identified by a continuous, identifiable froth that looked like honeycomb.

Borntragor's Test: The ethanolic extract was mixed with 1 milliliter of benzene and 0.5 milliliter of diluted ammonia solution. Reddish-pink hue, which indicated the presence of glycoside.

Phenolic compound test: Two milliliters of ferric chloride solution were added to the sample extract after it was heated with water. After that, the mixture was left to be observed to see if any green or blue hue developed, which would suggest the presence of phenolic chemicals.

Salkoweski reaction: 5 mg of extract, 2 ml of chloroform, and 2 ml of concentrated sulfuricacid were introduced from the side of the test tube. The test tube was shaken for a few minutes. Rednesswas an indication that steroids were present.

Lead acetate: The test filtrate was mixed with a 10% w/v solution of basic lead acetate in distilled water. Precipitate formation suggested the presence of tannins.

Potassium dichromate: The filtrate was put to a solution of potassium dichromate for this test. The presence of tannins was indicated by the black tint.

Thin-Layer Chromatography is a technique for determining and separating the various chemical components present in a test sample. The stationary phase, mobile phase, and visualization phase of the chromatography process were all followed. Phase in station: 10 x 20 cm, silica gel 60F254.Toluene (8 ml): Ethyl acetate (2 ml) is the mobile phase.

Rf Value: The difference in the travel distances from the origin line of the solvent and solute. Visualized under 366, 540, and 254 nm. Derivatization anisaldehyde sulphuric acid are used [6-8].

Result and Discussion

Table 1: Physiochemical Tests result of Cissus quadrangularis

S. No	Macroscopic study	Observation
1	Color	Creamish brown
2	Odor	Characteristic
3	Taste	Bitter

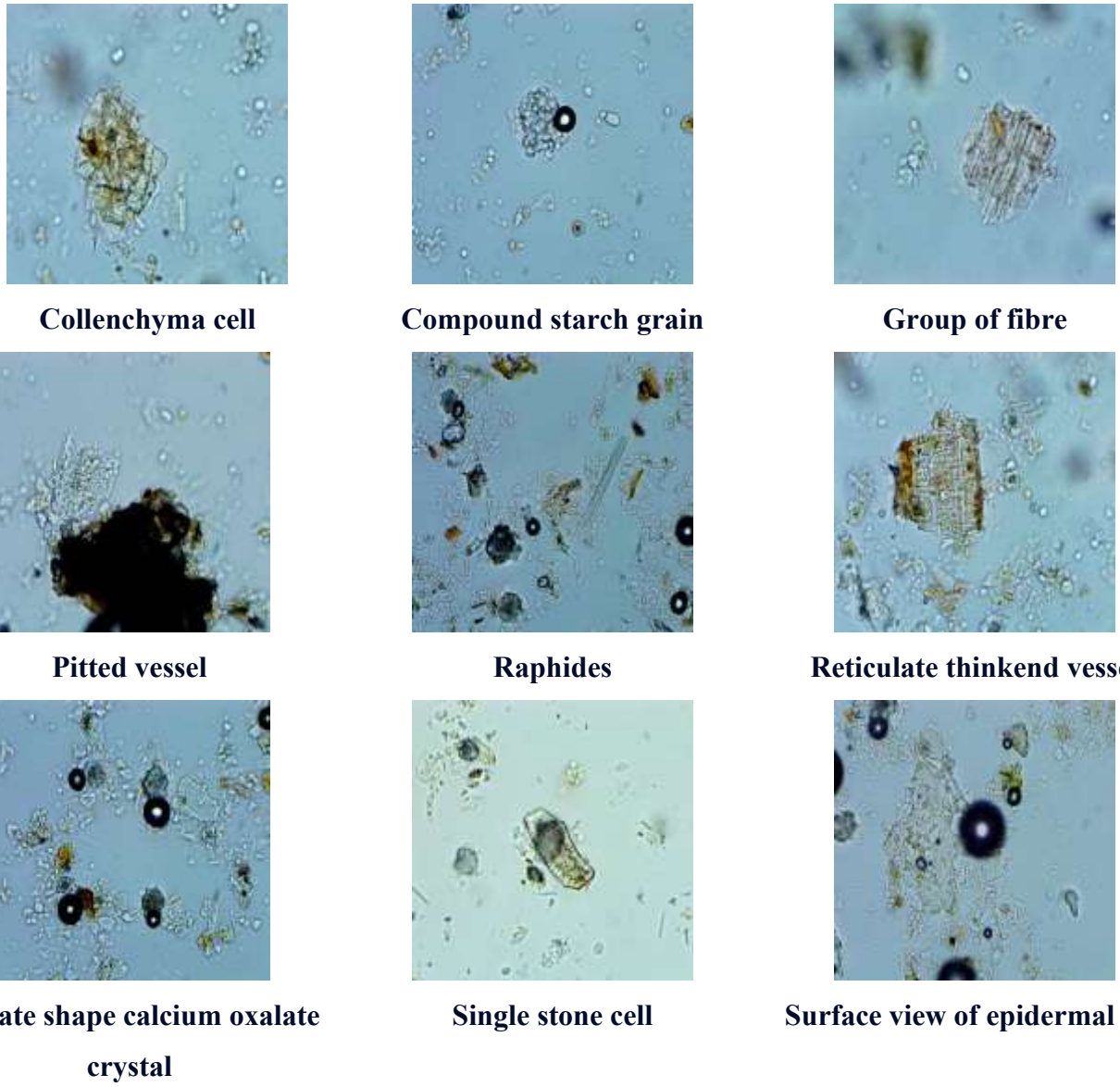


Figure 1: Powder Microscopyof Cissus quadrangularis

Table 2: Physiochemical Tests result of Cissus quadrangularis

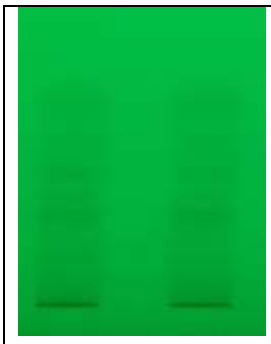
S. NO	Tests	Observation
1	Loss on Drying (% v/w)	6.59
2	pH	4.88
3	Alcoholic Extractive Value (% w/w)	8.62
4	Aqueous extractive Value (% w/w)	11.98
5	Total Ash (% w/w)	11.97
6	Acid Insoluble Ash (% w/w)	1.86
7	Water Soluble Ash (% w/w)	2.47

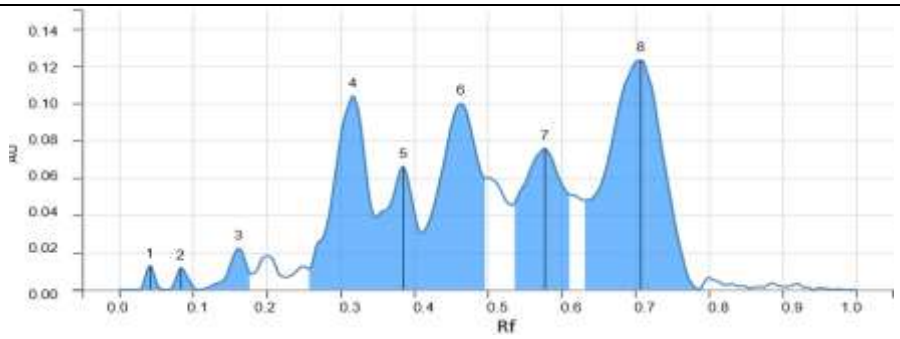
Table 3: Phytochemical Tests resultsof Cissus quadrangularis

Tests	Aq. Ext.	Alco. Ext.
Carbohydrate		
Molish test	***+ve***	***+ve***
Benedict test	***+ve***	***+ve***
Fehling test	***+ve***	***+ve***
Alkaloids		
Dragendorff test	***+ve***	***-ve***
Wagner's test	***+ve***	***+ve***
Hager's test	***+ve***	***+ve***
Amino acids		
Ninhydrine	**+ ve**	**-ve**
Protein		
Biuret test	-ve	-ve
Xenthoprotic test	+ve	+ve
Millon test	+ve	+ve
Saponin		
Foam test	+ve	-ve
Glycosides		
Borntrager's test	-ve	-ve
Phenolic compound		
Phenolic test	+ve	+ve
Steroids		
Salkowaski	-ve	-ve
Tannins		
Fecl3	+ve	+ve
Lead acetate	+ve	+ve

Pot. Dichromate	+ve	+ve

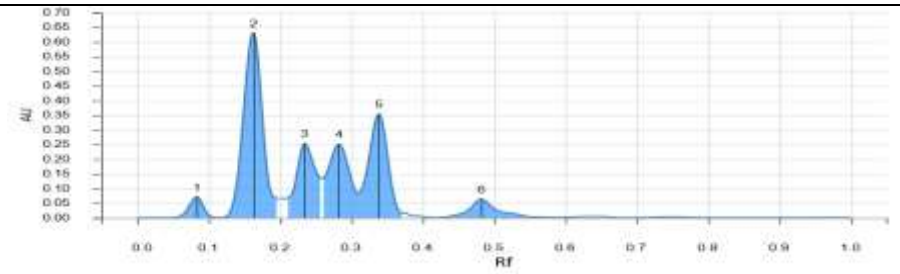
Table 4. Thin Layer Chromatography screening at 254 nm wavelengthof Cissus quadrangularis





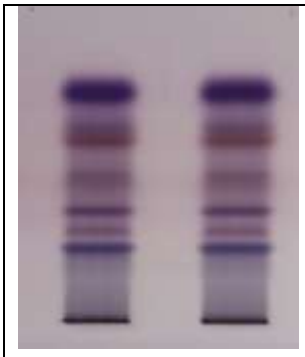
R_f Value (S1)	R_f Value(S2)	Area %	Band colour
0.085	0.042	0.45	Faded Brown
0.160	0.084	1.53	Brown
0.200	0.163	1.62	Faded Brown
0.315	0.318	15.81	Brown
0.384	0.385	8.90	Brown
0.461	0.465	23.83	Brown
0.573	0.577	17.62	Brown
0.708	0.708	30.23	Brown

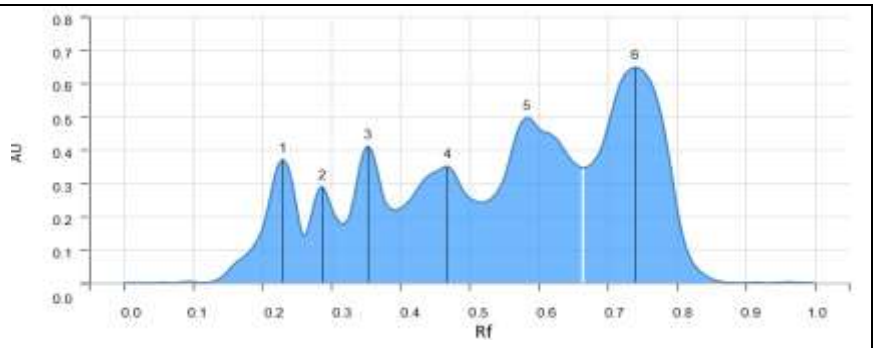
Table 5. Thin Layer Chromatography screening at 366 nm wavelengthof Cissus quadrangularis



R_f Value (S1)	R_f Value (S2)	Area %	Band colour
0.081	0.084	3.14	Light Pink
0.160	0.163	37.33	Fluorescent Peach
0.232	0.234	15.33	Fluorescent Peach
0.282	0.282	17.13	Fluorescent Peach
0.337	0.339	21.51	Blue
0.482	0.482	5.57	Light Pink

Table 6. Thin Layer Chromatography screening at 540 nm wavelength Derivatized**of Cissus quadrangularis**





R_f Value (S1)	R_f Value (S2)	Area %	Band colour
0.227	0.231	9.74	Deep Blue
0.287	0.287	5.72	Purplishpink
0.352	0.353	9.74	Violet
0.466	0.468	16.82	Violet
0.582	0.584	25.88	Brown
0.739	0.740	32.11	Violet

Discussion and Conclusion

Powder microscopy reveals diagnostic traits: collenchyma cells, compound starch grains, fiber groups, pitted/reticulate vessels, raphides, rosette calcium oxalate crystals, stone cells, and epidermal cells—confirming Cissus identity per pharmacognostic standards. Calcium crystals and minerals correlate with ash data, reinforcing bone-healing claims; starch/fibers indicate structural polysaccharides.

Loss on drying was 6.59% w/w, indicating moderate moisture content suitable for storage stability in herbal materials. The pH of 4.88 suggests mild acidity, consistent with plant acids and extractives, while alcoholic extractive value (8.62% w/w) was lower than aqueous (11.98% w/w), implying higher polar compound solubility—aligning with the plant's flavonoid and tannin profile. Ash values (total 11.97% w/w, acid-insoluble 1.86% w/w, water-soluble 2.47% w/w) reveal substantial mineral content (e.g., calcium, phosphorus noted in introduction), low silica impurities, and good physiological ash, supporting its traditional bone-healing use.

Qualitative tests confirm carbohydrates (positive Molisch, Benedict, Fehling in both extracts), alkaloids (Dragendorff, Wagner, Hager positive, varying by extract), amino acids/proteins (Ninhydrin, Xanthoproteic, Millon positive), phenolics/tannins (FeCl3, lead acetate, K2Cr2O7 positive), but negative for saponins (foam in aq. only), glycosides, and steroids. These align with literature on C. quadrangularis flavonoids (quercetin), stilbenes (resveratrol), and triterpenoids, explaining anti-inflammatory and antioxidant effects via phenolics and alkaloids. Absence of steroids/glycosides in extracts suggests method-specific detection limits or concentration issues.

- Carbohydrates support energy metabolism roles.
- Alkaloids/phenolics drive analgesic-anti-inflammatory activity.
- Tannins contribute gastroprotective effects.

HPTLC at 254 nm shows multiple brown bands (Rf 0.085–0.708, prominent at 0.708/30.23 area, 0.461/23.83), 366 nm fluorescent peach/blue/pink (Rf 0.081–0.482, max 0.160/37.33), and 540 nm derivatized violet/brown/blue (Rf 0.227–0.739, max 0.739/32.11). These multi-wavelength profiles indicate diverse polyphenols/flavonoids; high R_f bands likely non-polar stilbenes/triterpenoids, low Rf polar phenolics—consistent with known compounds like resveratrol (expected Rf ~0.5–0.7). Band consistency across tracks (S1/S2) validates reproducibility for standardization.

The pharmacognosy and HPTLC screening of Cissus quadrangularis establishes its macroscopic (creamish-brown, bitter), microscopic (raphides, oxalate crystals), physicochemical (high ash/minerals), and chemical (phenolics/alkaloids positive) standards. These affirm its safety, identity, and potency for bone/joint therapies, bridging traditional claims with modern validation. Future studies should quantify markers (e.g., via HPLC) and assess in vivo anti-inflammatory activity to advance drug development.

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