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Phytochemical Analysis of Plant of *Cissus* *Quadrangularis*

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Abstract: *Cissus quadrangularis*, particularly found in tropical regions, is a popular perennial climber of the Vitaceae family and is also distributed throughout India. The plant is used to biological activities like anti-osteoporotic, antioxidant, analgesic, anti-microbial, antiobesity, antiulcer, adrogenic, antihemorrhoidal, anti-diabetic, antifungal, anti-tumour, and bone healing activities. The plant contains alkaloids, steroids, flavonoids, tannins, and saponin. For the preparation of the extract using soxhlet apparatus, powdered material was taken from *Cissus quadrangularis*. The various phytochemical tests were performed for saponins, carbohydrates, alkaloid, tannin, flavonoids, steroids and glycosides etc.

Keywords: *Cissus quadrangularis*, Materials and methods, Phytochemical analysis.

I. INTRODUCTION

Medicinal plants have been used as traditional treatment for numerous human diseases for thousand of year and in many parts of the world^[1,2]. *Cissus quadrangularis* is generally referred to as Succulent Plant the Vitaceae family, Commonly found all over the warmer parts of India^[3]. Plant blooms in the month of June. Plant material occurs as parts of different lengths, quadrangular base, 4 winged, 4-15 cm long and 1-2 cm thick internodes. Smooth, glabrous, greenish-colored buff, reddish-brown angular portion of the surface; no taste or odour. Leaves are simple, 2.5-5 cm long, broadly ovate or reniform, sometimes 3-7 lobed, denticulate, glabrous, cordate, flattened, truncate or cuneate at the base; 6-12 mm long petioles; thin, usually ovate, obtuse stipulates. Flowers are in small peduncle cymes, with spreading umbellate branches. Calyx is cup-shaped, truncated or lobed in a very obscure way. Petals are 4, short, stout, ovate-oblong. Berry is oboesic or globosic, barely Apiculate red 6 mm long, when ripe, seeded 1 Just 2^[4]. *Cissus quadrangularis* contains various bioactive substances, Like alkaloids resveratrol piceatannol pallidol parthenocissin quadrangularins ascorbic acid carotene phytosterol calcium flavinoids, the nicotinic acid enzymes, tyrosine and triterpenoids^[5].



Figure No.1: The whole plant of *Cissus quadrangularis*.

A. Traditional Uses of *Cissus Quadrangularis*

The roots and stems are particularly effective for cure of bone fracture. The bran is salty. This is supplied internally, and topically applied in broken bones. It is also seen in rear and spine complaints. A stem paste is good for muscle pains. In Ayurved^[6,7] Plants for the treatment of osteoarthritis, rheumatoid arthritis and osteoporosis have been reported. For the prevention of scurvy, menstrual disorders, otorrhoea and epistaxis^[8] plant stem juice is used. To stimulate milk production, the herb is fed to cattle.

Plant ash is useful as a supplement to baking powder. A stem paste is treated in asthma and burns, poisonous insect bites and horses and camels for saddle sores. Shoots of dry ginger and black pepper are decocted for body pain and herb infusion is anthelmintic. Leaves and young shoots are strong, dried and powdered alternatives. They treat indigestion-related diseases in some intestines. The plant is useful for Anorexia helminthiasis dyspepsia colic flatulence skin disease leprosy, bleeding, epilepsy, hallucinations, Chronic ulcers in tumours with haemoptysis, swelling^[9].

II. MATERIALS AND METHODS

A. Collection And Drying Of The Plant Material

The *Cissus quadrangularis* plant was collected from the sangli district. The collected plant material was washed thoroughly under running tap water, air-dried at room temperature under the shade for 8-10 days. Dried plant were stored in tightly sealed polyethylene bags.

B. Authentication Of Plant Species

The plant was authenticated by Prof. D. G. Jagtap, Head of Department of Botany Principal Shri. Vijaysinha Yadav Arts and Science College, Peth Vadgaon, Dist.- Kolhapur.

C. Extraction of Plant Material

Plant of *Cissus quadrangularis* were extracted in a soxhlet extractor, successively with Petroleum ether (60°-80°), Chloroform, and Ethanol (95%) for 24-36 hrs for each solvent Figure No. 2. After extraction with each solvent, the solvent was evaporated and residue was air dried. The residues from each extract were dried and the resultant extracts were stored in an air tight container for further use. The extract was subjected to preliminary phytochemical testing.



Figure No. 2: Soxhlet extraction of *Cissus quadrangularis*.

III. PHYTOCHEMICAL INVESTIGATION OF PLANT^[10]

A. Test for Proteins

- 1) *Biuret Test*: Test solution was treated with equal volume of 10% sodium hydroxide solution and two drops of 1% copper sulphate solution, mixed well and observed for the formulation of violet/pink colour. If it is so, presence of proteins was detected.
- 2) *Xanthoproteic Test*: Few drops of conc. Nitric Acid add two ml of extracts and mixed well. It resulting the formation of light to dark yellow colour which indicates the presence of proteins.
- 3) *Millon's Test*: Two ml of crude extract when mixed with 2ml of Millon's reagent, if a white precipitate appeared which turned red upon gentle heating and disappeared on cooling confirmed the presence of protein.

B. Test for Saponins

- 1) *Foam Test*: In this test 0.5gm of extract was added in 10-20 ml of water, shaken for few minutes formation of frothing which persisted for 60-120seconds, shows presence of saponins.

C. Test for Carbohydrates

- 1) *Benedict's Test*: Test solution was mixed with one or two drops of Benedict's reagent and it is boiled in water bath, wait for few minutes and observe the formation of reddish-brown precipitate which indicates the positive result for the presence of carbohydrates.
- 2) *Molisch's Test*: Filtrate was treated with one or two drops of alcoholic α -naphthol solution in a test tube. It resulting the formation of the violet ring which is present at the junction which is indicates the presence of carbohydrates.
- 3) *Fehling's Test*: Filtrates were hydrolysed with dil. HCL, neutralized with alkali and heated Fehling's A and B solutions. Formation of red precipitate which shows the presence of reducing sugar.

D. Test for Alkaloids

- 1) *Wagner's Test*: A fraction of extract was treated with three to 3-5 drops of Wagner's reagent it resulting the formation of reddish-brown precipitate. This shows the presence of alkaloid.
- 2) *Mayer's Test*: Filtrates were treated with Mayer's reagent. Formation of a yellow coloured precipitate which indicates the presence of alkaloid.

E. Test for Phenols

- 1) *Ferric Chloride Test*: Extracts was treated with 3-4 drops of ferric chloride solution. It resulting the formation of bluish black colour which indicates the presence of phenols.

F. Test for Tannins

- 1) *Gelatin Test*: To the extract add 1% Gelatin solution which containing sodium chloride. It resulting the formation of white precipitate which indicates the presence of tannins.
- 2) *Braymer's Test*: 2 ml of extract was treated with 10% alcoholic ferric chloride solution. Which resulting the formation of blue or greenish colour solution which indicates the presence of tannins.

G. Test for Flavonoids

- 1) *Shinoda Test*: Crude extract was mixed with few fragments of magnesium ribbon and add drop wise con. HCL in that mixture. Wait for few minutes, then it shows the pink scarlet colour which is indicates the flavonoids.
- 2) *Alkaline Reagent Test*: Crude extract was mixed with 2 ml of 2 % solution of NaOH. An intense yellow colour was formed which turn colourless on addition of few drop of dilute acid which indicates the presence of flavonoids.

H. Test for Steroids

- 1) *Liebermann Burchard Test*: Extract was mixed with 1-2 drops of acetic anhydride, boil it and cool it. After the cooling add concentrated sulphuric acid from the side of test tube the observed the formation of a brown ring at the junction of two layers. Green coloration of the upper layer which indicates positive test for steroids.

I. Test for Glycosides

- 1) **Liebermann’s Test:** Crude extract was mixed with each of 2 ml chloroform and 2 ml acetic acid. The mixture was cooled in ice. Carefully concentrated H₂SO₄ was added. A colour change from violet to blue to green indicated the presence of glycoside.
- 2) **Salkowski’s Test:** Crude extract was mixed with 2 ml chloroform, then 2ml of concentrated H₂SO₄ was added carefully and shaken gently. A reddish-brown colour indicates the presence of steroidal ring. i.e., glycone portion of the glycoside.
- 3) **Keller-Killiani Test:** Test solution was treated with 1-2 drops of glacial acetic acid and ferric chloride solution mixed well. Then add few concentrated sulphuric acid, it resulting the formation of two layers. Lower layer is reddish-brown layer and upper layer is acetic acid layer which turns in bluish green which indicates the positive test for glycosides.

IV. RESULTS AND DISCUSSION

Cissus quadrangularis was successively extracted by using the Soxhlet assembly taking the different solvents such as Petroleum ether, Chloroform and Methanol based on the increasing polarity. All the extracts were evaporated to remove excess of solvent in a water bath. These extracts were then stored in air tight container at cold temperature (approx. 15°C). These extracts were then used for further chemical test for phytochemical investigation for protein, amino acid, glycoside, alkaloid, phenolic compounds, flavonoids, steroids and tannins etc. the results of phytochemical screening of *Cissus quadrangularis* plant extracts are mentioned in the following Table No. 01

Sr.No.	Test	Petroleum ether	Chloroform	Methanol
1.	Test for Proteins			
	1)Biuret test	-	+	-
	2)Xanthoproteic test	-	-	-
2.	3)Millon’s test	+	-	+
	Test for Saponins			
	1)Foam test	+	-	+
3.	Test for Carbohydrates			
	1)Benedict’s test	+	-	+
	2) Molisch test	-	+	-
4.	3) Fehling’s test	-	-	+
	Test for Alkaloid			
	1)Wagner’s test	+	+	+
5.	2)Mayer’s test	-	-	+
	Test for Phenol			
6.	1)Ferric chloride test	-	-	+
	Test for Tannins			
7.	1)Gelatin test	-	+	+
	2)Braymers test	+	-	-
8.	Test for Flavonoids			
	1) Shinoda test	+	-	+
9.	2)Alkaline Reagent test	+	-	+
	Test for Steroids			
10.	1)Liebermann Burchard test	+	+	+
	Test for Glycosides			
	1)Liebermann’s test	-	-	+
11.	2)Salkowski’s test	+	+	+
	3)Keller-Killiani test	-	+	+

Note: + indicates presence and - indicates absence of phytoconstituent

V. CONCLUSION

The whole plant of *cissus quadrangularis* was selected for pharmacological evaluation. *cissus quadrangularis* belonging to family vitaceae is used as a traditional medicine for the treatment of various diseases and disorders. Whole plant of *cissus quadrangularis* have been procured from the local market, Islampur, Sangli, evaluated according to WHO guidelines. Pharmacognostical and physicochemical parameters assure the authenticity, quality and purity of the crude drug. Pharmacognostical parameters mentioned in this work would be helpful for identification and authentication of whole plant. Physicochemical evaluation of powdered whole plant revealed that the standard quality and purity of the herbal drug. Phytochemical studies on the various extracts of *cissus quadrangularis* showed presence alkaloids, steroids, flavonoids, tannins, protein, glycosides, and saponin compound.

Considering the above facts, isolation, purification and characterization of the phytochemicals found in this species may introduce a future medicine that will change the life of mankind. Furthermore, a detailed study needs to ascertain their antioxidant, antiobesity, antiulcer, antifungal activities.

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