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Phytochemical Investigation and Pharmacognistic Studies of Plant *Cissus Quadrangularis* (Hadjod)

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Abstract

The stem and leaves of Hadjod (*Cissus quadrangularis*) is reported to have a great medicinal value. It has been traditionally used by common man in India for healing of fractured bones. Pharmaceutically, it is an important herb used in drugs meant for treating diseases like anorexia, indigestion, piles, chronic ulcers,and in healing of bone fractures.Cissus quadrangularis is a common perennial climber, particularly found in tropical regions. The aim of the present study was to determine a chemical profile of the extract of the plant, highlighting the phytochemicals present in it, which will have various uses when therapeutically or medicinally used.The coarsely powdered product is used for the preparation of extract,which is then used for the identification of various chemical constituents. The phytochemical tests indicate the presence of carbohydrates, saponins, flavonoids, alkaloids, glycosides, tannins and other active chemical components of significant pharmacological values.

Keywords: herb, phytochemical, pharmacological, antioxidant

Introduction

Cissus quadrangularis Linneaus belongs to the family Vitaceae, an edible plant found in India, Sri Lanka, Malaya, Java, West Africa and throughout Thailand. It is commonly known as “bone setter”; the plant is referred to as “Asthisamadhani” in Sanskrit and “Hadjod” in Hindi because of its ability to join bones. Chanda Sumitra in her work, ‘Spectral Analysis of Methanolic Extract of *Cissus quadrangularis’* reports that this plant has been documented in Ayurveda for its various medicinal uses like venereal diseases, piles, tumours, anorexia, indigestion, chronic ulcers, wounds and augmenting fracture healing process.

C. Quadrangularis is often used as medicinal plant because it contains some important bioactive compounds. The present study reveals the presence of various secondary

metabolites such as flavonoids, terpenoids, glycosides alkaloids, vitamin C, carbohydrates, saponins, and many others. Preliminary qualitative tests of the extracts were found positive for phytosterols, flavonoids and terpenoids. Chromatographic analysis also supports the presence of flavonoids and glycosides. Antioxidant activities were also traced in this plant extract. Thus, this study reveals that this plant has important pharmaceutical properties.

Details of the Plant

Fig 1: Leaves and stems of *C. quadrangularis*

Fig 2: Dry stems of *C. quadrangularis*

C. quadrangularis L. is a common perennial climber of family vitaceae, particularly found in tropical regions. The plant is a fleshy, cactus-like vine used as a common food item in India. It can be cultivated in plains coastal areas, jungles, and wastelands up to 500m elevation. Plant material occurs as pieces of varying lengths; stem quadrangular, 4-winged, internodes 4-15cm long and 1-2cm thick. The surface is smooth, glabrous, buff coloured with greenish tinge, angular portion reddish-brown, no taste and odour. Chanda Sumitra and their group in their work, 'Spectral Analysis of Methanolic Extract of *Cissus quadrangularis'* report that the whole plant including all parts such as stems, leaves, roots are documented to possess medicinal properties by ethnobotanists in traditional system of medicine.

Traditional Uses

The roots and stems are quite useful for healing of fractured bones. The stem is bitter; it is given internally and applied topically in broken bones, used in complaints of the back and spine.A paste of stem is useful for muscular pains.The stem juice of plant is used to treat scurvy, menstrual disorders, otorrhoea and epistaxis.A paste of stem is given in asthma, burns and wounds, bites of poisonous insects and for saddle sores of horses and camels.The stout fleshy quadrangular stem is traditionally used for treatment of gastritis constipation, eye diseases, piles and anaemia.

Pharmacological Uses

Following the folk and traditional uses of the plant, it has been investigated scientifically in animals to validate the potential of the plant in cure of variety of ailments, such as anti-ulcer activity, bone healing activity, antimicrobial and antibacterial activity, central nervous system activity and toxicology. It is one of the most widely used ingredients in alternative medicine for the treatment of piles, anorexia, indigestion, otorrhoea and in fracture healing.

Aims and Objective

Plants have always served human beings as a natural source of treatments and therapies; amongst them medicinal herbs have gain attention because of its wide uses and less side effects. *C. quadrangularis*Linn. has been used in India for promotion of fracture healing. The aim of the present study was to determine a chemical profile of the extract of the plant and its fractions, which will be useful when therapeutically used. Through preliminary phytochemical screening, pharmacological studies, TLC, and antioxidant activity the present study aimed towards phytochemical evaluation and qualitative identification of constituents on the plant extracts. It was also focused that the pharmaceutical importance of the plant and its active constituents be well understood, identified and promoted for the practical uses.

Materials and Methods

The powder of *Cissus Quadrangularis,* both stem and leaves werecollected and used for this study. Various chemical experiments were performed to find out the chemical constituents present in it. The coarsely powdered product was used for the preparation of extract, which was further used for the preliminary pharmacognistic studies.

Qualitative Determination for Mineral Constituents

For detection of various inorganic elements in the plant ash such as Fe, Cl, P, S etc., one gram ash material was dissolved in 25 ml of 50% HCl for 12 hours and then filtered through filter paper. The filtrate was treated with suitable reagents to identify the presence of elements quantitatively. When the test solution of the ash was treated with 2 drops of dil. HNO□, 3 ml of dist. water and a few drops of AgNO□, a white precipitate was formed, which indicated the presence of Chlorine. Similarly, when the test solution was mixed with 2 drops of HCl and 10 drops of BaCl□, it formed a white precipitate of the sample showing the presence of Sulphur.

Preparation of the Extract

Maceration with Petroleum Ether:As the process followed by Teware Kalpana in her work, 'HPLC Analysis in Vivo Medicinally Important Climber *Cissus quadrangulari*,' the dried powdered plant material was used for extraction. About 50 gm of the sample taken was homogenized or macerated for 48 hours with 400 ml of petroleum ether. In the process, the plant material was dissolved in petroleum ether and kept in a mechanical shaker at 40°c for 6 hours. It was then kept aside for 48 hours. After extraction it was filtered through Whatman filter paper. The filtrate was poured in an evaporating disk and evaporated at 40°c to dryness and was stored in a desiccator for further use. The extract was sticky in nature, dark green in colour and percentage yield was 3.154%.

Soxhlet Extraction with Ethyl Acetate: Another procedure was used for the preparation of the extract using Soxhlet apparatus. 25 g of the powdered sample was extracted against 400 ml of Ethyl Acetate for 6 hours. The extracts obtained were dried at room temperature and used for the further study.The percentage yield of the extract was 7.712%.

Preliminary Phytochemical Screening

The extract was subjected to qualitative tests for detection of phytoconstituents present in it.Wagner's test and Mayer's test for alkaloids gave reddish-brown and creamy white precipitate respectively, indicating the presence of Alkaloids. Keller-Killani test and Baljet test for glycosides gave positive results showing its presence. Salkowski test for Terpenoids resulted in the formation of yellow ring which turned reddish brown after two minutes, which indicated the presence of terpenoids. Fehling's test and Molisch's test for carbohydrates resulted in the formation of red precipitate and violet ring respectively. It shows the presence of carbohydrates in the plant sample. However, Petroleum extract did not show the presence of flavonoids. Similarly, Libermann Burchard test for Steroids and tests for tannins and saponins did not give positive results.

Estimation of Phytochemical Constituents

Estimation of Alkaloids

A Standard procedure was followed for the estimation of Alkaloids, and the product obtained spectrophotometrically measured at λ 630 nm. Then the graph was drawn by plotting the

concentration of theophylline along the X- axis and the optical density reading along Y-axis. From the standard curve on the graph, unknown sample concentration was calculated.

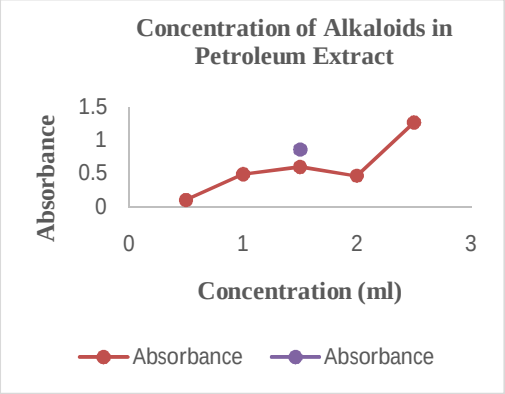

Fig 3: Conc. of Alkaloids and its absorbance under UV

Result:

Since 1.5 ml of unknown sample contains 0.863 µg of theophylline

So, 100 ml of unknown sample contains = (0.863 × 100)/ (1.5 × 1000) mg/g of theophylline

= 0.057 mg

So, the amount of alkaloid present in the sample is 0.057 mg/g

Estimation of Alkaloids from Ethyl Acetate Extract

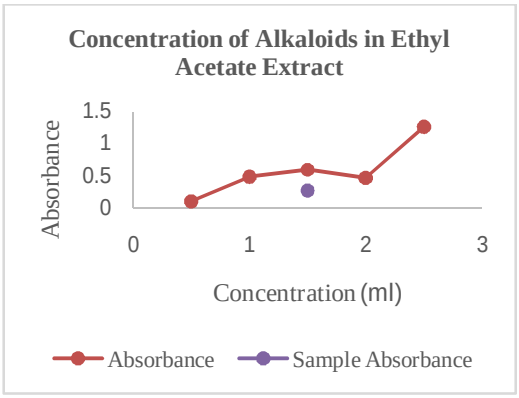

Fig 4: Conc. of Alkaloids and its absorbance under UV

Result:

Since 1.5 ml of unknown sample contains 0.273 µg of theophylline

So, 100 ml of unknown sample contains = (0.273 × 100)/ (1.5 × 1000) mg/g of theophylline = 0.0182 mg

So, the amount of alkaloid present in the sample is 0.0182 mg/g

Estimation of Flavonoids

Aluminium Chloride Method was used for this estimation. Flavonoids present in the extract from a charge transfer complex with several heavy metals to give a characteristic colour.

The high electro-positive nature of AlCl□ attracts the atomic nuclei of the aromatic rings in the flavonoids through the μ-electrons and creates a charge transfer resonance hybrid. This hybrid is highly stable in the aq. medium, which then interacted with the sodium nitrite in an alkaline medium to form a pink colour complex that is spectrophotometrically measured at λ 510 nm.

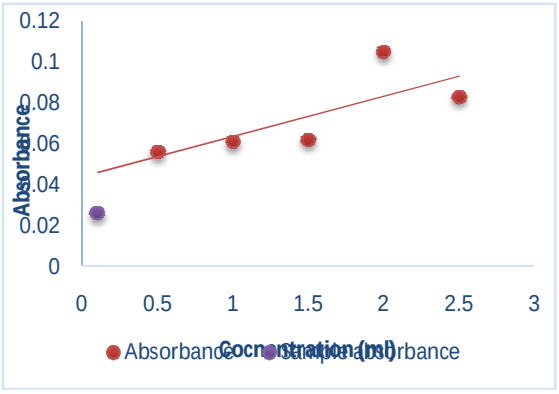

Fig 5: Concentration of Flavonoids and its absorbance under UV

Result:

Since 0.1 ml of unknown sample contains 0.0261 μg of rutin

So, 100 ml of unknown sample contains = (0.0261 × 100)/ (0.1 × 1000) mg/g of rutin

= 0.0261 mg

So, the amount of Flavonoids present in the sample is 0.057 mg/g

Estimation of Antioxidant Activity

To 0.1 ml of the extract (100 -1000 μg/ml), added 0.9 ml 96% ethanol, 5ml of distilled water, 1.5ml of 1M HCl, 1.5ml of 1% K□[Fe(CN)□], 0.5ml of 1% SDS and 0.2% FeCl□. Boiled the mixture in a water bath at 50°c for 20 minutes. Then cooled it rapidly and mixed well. The increase in the absorbance at λ 750 nm is used to measure the reducing power of the plant extract.

Ascorbic acid was used as a standard positive control.

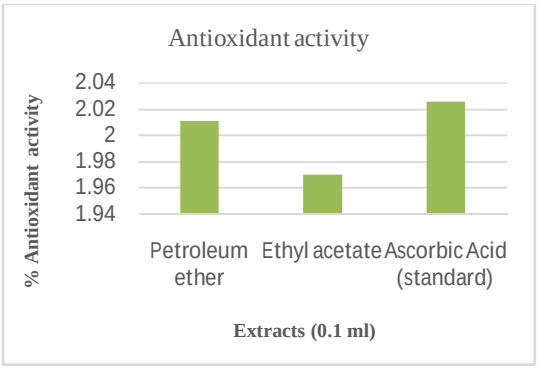

Fig 6: Antioxidant activity of the plant compared with Ascorbic acid

Thin Layer Chromatography (TLC) Study of Ethyl Acetate Extract

Ethyl acetate extract was subjected to thin layer chromatography by the following solvent combination. Each plate was kept in the beakers containing methanol and water in the ratio of 8:2. Afterwards the run plates were dried and kept in the iodine chamber to detect the bands on the TLC plates. The movement of the active compounds was expressed by its retention factor (Rf) values.The same procedure was repeated with solvent combination of chloroform and methanol in the ratio of 95:5.

Table 1: Data showing the TLC of alcoholic combined extracts of *Cissus quadrangularis*

Stationary phase	Mobile phase	No. of spots	Rf values	Possible phytoconstituents
Silica gel G	Methanol : water (8:2)	1	0.85	Glycosides, Alkaloids, Flavonoids
	Chloroform : methanol (95:5)	4	0.08, 0.33, 0.75, 0.95	

TLC profiling of the extract indicated the presence of number of phytochemicals. Different phytochemicals gave different Rf values. This variation in Rf values of the phytochemicals provide a very important clue about their polarity and also helps in selection of appropriate solvent system. The selection of appropriate solvent system for a particular plant extracts can only be achieved by analysing the Rf values of compounds in different solvent system.

Result and Discussion

The pharmacological activity of any plant is useful due to the presence of chemical constituents. In consideration to the importance of phytochemical investigation on endemic medicinal plants, the present work carries the identification and extraction of medicinal constituents, phytochemical screening, and spectral analysis of plant *Cissusquadrangularis* L. Preliminary phytochemical screening of the extract show positive results for alkaloids, flavonoids, glycosides, phytosterols, terpenoidsand carbohydrates, and they may be responsible for the pharmacological activities of the plant. Due to medium polarity of ethyl acetate nearly all the polar constituents (glycosides, saponins and flavonoids) were extracted in ethyl acetate along with phytosterols. It was also exposed qualitatively that minerals are abundantly present in the plant.

The thin layer chromatography of the extracts was carried out using different solvent system, and the numbers of spots with its Rf values were calculated. The extracted compounds were identified as glycosides, flavonoids and alkaloids.Murthy and Vanitha in their work, "Antioxidant and Antimicrobial Activity of *Cissus quadrangularis,*" report that antioxidants are believed to interpret the free radical chain of oxidation and to donate hydrogen from the phenolic hydroxyl groups, thereby forming a stable end-product, that does not initiate or propagate further oxidation of lipids. The conclusion of the study directs towards some diagnostic guides for the identification and preparation of an essay of the plant. This could make it useful for treating different ailments and having a potential of providing useful drugs of human use. However more Clinical and Pathological studies should be conducted to investigate the active potential of bioactive compounds present in this plant.

Traditional medicine is an important source for the development of chemotherapeutic agents. The present investigation may help to proper identification and to ensures the quality of the drug and also help this medicinal plant, *C. quadrangularis,* grown on commercial basis for better pharmaceutical use. The spectral analysis would help the manufacturer for quality control and standardization of herbal formulations. Such analysis is useful in differentiating the species from contaminants and other sub species and the result of this study can become the finger print of this plant and it may act as a biochemical marker which may be useful for pharma industry and systematic studies.

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