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Research Article

HPLC ANALYSIS OF EXTRACT OF IN *VIVO* MEDICINALLY IMPORTANT CLIMBER *CISSUS QUADRANGULARIS* L. (HADJOD)

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ABSTRACT

Cissus qudrangularis L. (Hadjod) belongs to family Vitaceae is an indigenous medicinal plant of India. It has been prescribed in ancient Ayurvedic texts as a general tonic especially for the fractured patient. The stem of *Cissus quadrangularis* L. is also reputed in Ayurveda as alterative, anthelmintic, dyspeptic, digestive, tonic, analgesic in eye and ear diseases, in the treatment of irregular menstruation and asthma, and in complaints of the back and spine. The plant extracts also exhibit cardio tonic property. In present study reveals the presence of various secondary metabolites as Alkaloids, Carbohydrates, Flavones and flavonoids, Saponins, Steroids and sterols, in various types of extracts. Preliminary qualitative chemical tests of extracts were found positive for Phytosterols, flavonoids and triterpenoids in Ethyl acetate fraction. Two important flavonoids were reported in the extract of this highly important medicinal plant *Cissus quadrangularis* L. HPLC analysis of the extract of *in vivo* plant give support to the presence of important flavonoids. This piece of work reveals that this climber has important pharmacological properties. The results of the study could be useful for further researches in the world of pharmacology and serve society.

Key words: Callus, *Cissus quadrangularis* L., flavonoids, quarcetine, kaempherol

INTRODUCTION

Cissus quadrangularis (Vitaceae), a climbing shrub, characterized by a thick quadrangular fleshy stem, is an edible plant found in hotter parts of India, Sri Lanka, Malaya, Java and West Africa. Commonly known as the "bone setter," the plant is referred to as "Asthisamdhani" in Sanskrit and "Hadjod" in Hindi because of its ability to join bones. The plant has been documented in Ayurveda for its medicinal uses in gout, syphilis, venereal disease, piles, leucorrhoea and as an aphrodisiac. The Siddha system of medicine illustrates its administration for the treatment of piles, diarrhea and dysentery as well as in kapham[1]. Previous studies reported the presence of triterpenoids, steroids, lipids, stilbenes, flavonoids and iridoids [2, 3, 4, 5, 6] in this study two important flavanoid are reported and identified.

Botany: Hadjod Climbing herb, tendrils simple, opposite to the leaves, leaves simple or lobbed, sometimes 3-folialate, dentate. Flowers bisexual, tetramerous, in umbellate cymes, opposite to the leaves, Calyx cup-shaped, obscurely 4-lobed. Fruit globose or obovoid fleshy berries, one seeded, dark purple to black; seeds ellipsoid or pyriform.Flowering and fruiting time May-June[7]. Chemical Constituents: Stem isolates include 3- keto steroids, onocer-7-en-3a, 21b-diol (I) and onecer-7-en-3a, 21a-diol (II).

MATERIAL AND METHODS

Collection of the plant: The plant material was collected from MFP park (SANJEEVANI) Barkheda Pathani, Bhopal, India, a govt. organization.

Drying and size reduction

After identification and authentication stems were subjected to drying in normal environmental condition under shade with some change in temperature in oven. The dried stems were powdered by pulverization and were stored in air tight container[8]

Fluorescence Characteristic of the Different Extracts of the Stem of *Cissus quadrangularis* L. (Hadjod)

The fluorescence characteristic of different extracts was studied by observing them under UV Light at 365nm. The tests and observations are recorded in the given table.

Extraction

The dried powdered plant material is generally used for extraction. The fresh plant parts when used are homogenized or macerated with a solvent such as alcohol or water. Several plant constituents including chlorophyll and resins are generally interfering in the isolation process. The precise mode of extraction naturally depends on the texture and water content of the plant material. A water immiscible solvent such as petroleum ether is used for the separation of alkaloids and quinines. Extraction itself may be performed by repeated maceration with agitation percolation or by continuous extraction by soxhlet extraction.

Extraction by fractionation

(a) Petroleum Ether (60°_ 80°) Extract -About 1.5 kg of shade dried powder of stems of *Cissus quadrangularis L.* was extracted with petroleum ether (60°- 80°) for 24 hrs by Using soxhlet apparatus. After completion of extraction the solvent was removed under reduced pressure and the extractive was determined.[10]

(b) Methanolic Extract: - The marc left after petroleum ether extraction was dried and extracted with methanol for 24hrs. After completion of extraction, the solvent was removed under reduced pressure and the extractive value was determined. The crude methanol extract, after removal of the solvent, was dissolved in 10% sulfuric acid solution and partitioned with chloroform, ethyl acetate and n-buatnol successively to give chloroform , Ethyl acetate , n-Butanol and water soluble fractions respectively.

Different extracts obtained from the above extraction processes and this extract were analyzed for different phyto constituents present in these by the method of qualitative photochemical analysis. The following chemical tests were carried out and the results were tabulated. In this test for carbohydrate, alkaloids, glycosides, gums and mucilage, proteins and amino acids[10], tannins and saponins, phenolic compounds, steroids and sterols, triterpenoids, were carried out result are displaced in table2. [8]

Phytochemical investigation:

Preliminary Phytochemical screening of *Cissus quadrangularis* Linn.

Test for carbohydrate, gum and mucilage, protein and amino acid, alkaloid, glycoside, phytosterol, flavonoide, tannin and phenolic compound are carried out and obtained results are placed in table 2.

Air dried powdered whole plant of *Cissus quadrangularis* Linn. was exhaustively extracted with various solvents like n-hexane, chloroform, ethyl acetate, ethanol and methanol using soxhlet apparatus. Aqueous extract was obtained by maceration. These extracts were dried and dissolved in water. The dissolution was facilitated by sufficient quantity of Dimethyl sulph-oxide (DMSO). As the principal active constituents of the plant are saponins, phytosterols and phenolic compounds, which are polar in nature, so extracted best in the solvent of the highest polarity along with other polar constituents.

HPLC analysis of fraction (*in vivo* condition)

The flavanoides were well resolved within 10 min, and quantification was achieved, on an end capped C18 column at 370 nm with acetonitrile-phosphate buffer (pH 3.4, adjusted with glacial acetic acid) 60:40 (v/v) as isocratic mobile phase at a flow rate of 1.0 mlmin−1 .It shows two distinct peak of 5.821 and 6.245 average retention time and each peak got baseline separation which shows area 173 and 362 respectively are is comparable with standard retention time of quercetine and kaempherol respectively.

Table 1: Fluorescence characteristic of the different extracts of the stem of *cissus quadrangularis L.*[9]

Extracts	Fluorescence under UV light (365nm)
Petroleum Ether Extract (600C – 800C)	No Fluorescence
Methanol Extract	No Fluorescence
Chloroform Extract	Light Orange
Ethyl Acetate Extract	No Fluorescence
n-Butanol Extract	Light yellowish orange

Table 2: Preliminary phytochemical tests for identification of phytoconstituents in *cissus qudrangularis L.*

Test for	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Butanol extract
Alkaloids	+	+	+	-
Carbohydrate	-	-	-	-
Glycosides	+	-	+	+
Tannin-phenolic compounds	++	++	-	++
Protein and amino acid	++	++	+	+
Gum and mucilage	+	-	-	-
Flavones and flavonoids	-	++	++	++
Saponins	+	--	++	+
Steroids and sterols	++	+	-	++

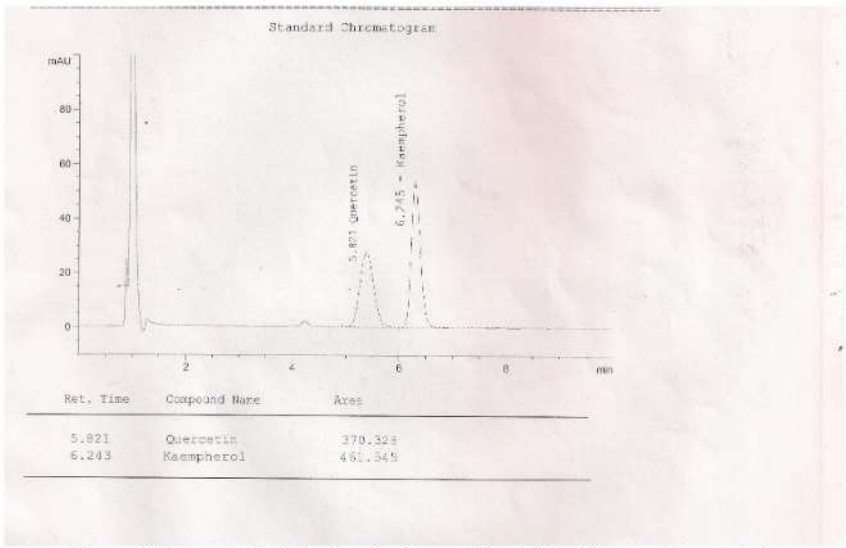

Figure: HPLC analysis of fraction (*in vivo* condition) of the *Cissus qudrangularis L.*

RESULTS AND DISCUSSION

In this proposed work Fluorescence characteristic of the different extracts of the stems of *cissus quadrangularis* L(Hadjod) shows that chloroform extract and n-butanol extract shown the Fluorescence under UV light (365nm)as Light yellowish orange and Light Orange colour. Before HPLC preliminary Phytochemical investigation shows the presence of phytoconstituents in different extract of *cissus qudrangularis* L.(Hadjod) these test shows that alkaloids present in all pet.- ether, ethyl acetate, chloroform extract.Tenin and phenolic compounds are found in all extracts except ethyl acetate. Flavones and flavonoids are reported in all extract except pet. Ether. Steroids were reported in all except ethyl acetate extract. HPLC analysis of fraction shows the presence of quercetine and Kaempherol shown in form of peaks of chromatogram of this pharmacologically important plant *Cissus qudrangularis* L.

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