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A STUDY ON PHYTOCHEMICAL ANALYSIS IN *Cissus vitiginea* LEAVES USING HPLC, UV-VIS AND FTIR TECHNIQUES

Chemistry

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ABSTRACT

The main aim of the present investigation was to study the preliminary phytochemical screening and elemental analysis of *Cissus vitiginea* leaves extract and determine the bioactive compounds with the aid of HPLC, UV-VIS and FTIR Techniques. The phytochemicals analysis of *Cissus vitiginea* leaves extract showed that the presence of tannin, saponnin, flavonoids, steroids, terpenoids, triterpenoids, carbohydrate, anthroquinone, polyphenol and glycoside. Significant quantity of tannin (43 ±0.28), phenol (168 ±2.35), flavonoids (126 ±1.32), terpenoids (52 ±0.35) and saponin (33 ±0.21) present in *Cissus vitiginea* leaves. HPLC analysis provided a good platform for identification of phenolic compounds as quercetin present in *Cissus vitiginea* leaves. The UV- VIS and FTIR the presence of phenol, alkanes, aldehyde, alcohol, carboxylic acids, aromatic and aliphatic amines compound. The results of this study offer a platform of using *Cissus vitiginea* leaves as a source of phytochemicals for nanoparticle synthesis and biological activities.

KEYWORDS

HPLC, UV-VIS, FTIR, *Cissus vitiginea*

INTRODUCTION

Phytochemical simply means plant chemicals. “Phyto” is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. In addition, these compounds may be responsible for the beneficial effects of fruits and vegetables on an array of health related measures (Dahanukar *et al*., 2000). Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments. Medicinal plants are assuming greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase of demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as alternative to allopathic medicines (Ashis, 2003). The valuable medicinal properties of different plants are due to presence of several constituents i.e. saponins, tannins, alkaloids, alkenyl phenols, glycol-alkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters (Cox and Balic, 1994). Chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs from medicinal plants (Cox, 1990). Among them some are act as synergistic and enhance the bioactivity of other compounds.

Within a decade, there were a number of dramatic advances in analytical techniques including HPLC, UV, FTIR, NMR and GC-MS that were powerful tools for separation identification and structure determination of phytochemicals (Roberts and Xia, 1995). The main aim of the present investigation was to study the preliminary phytochemical screening of *Cissus vitiginea* leaves aqueous extract and determine the bioactive compounds present in the *Cissus vitiginea* leaves extract with the aid of HPLC, UV-VIS and FTIR Techniques.

MATERIALS AND METHODS

Plant materials:

The *Cissus vitiginea* leaves were collected in March 2016 from Thanjavur, Tamil Nadu, India from a single herb. The leaves were identified and authenticated by Dr. S. John Britto, The Director, the Rapinat Herbarium and center for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen has been deposited at the Rapinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil Nadu, India.

Preparation of extracts:

The collected Cissus vitiginea leaves were washed several times with distilled water to remove the traces of impurities from the leaves. Then examined carefully old, infected and fungus damaged portion of the leaves were removed. Healthy leaves were spread out in a plain paper and shade dried at room temperature for about 10 days and ground in to fine powder using mechanical grinder. The powder was extracted with ethanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Cissus vitiginea* leaves extract (CVLE) was stored in refrigerator until used.

Preliminary phytochemical screening

Preliminary phytochemical evaluation was carried out by using standard procedure (Sofowara (1993), Trease and Evans (1989) and Harborne (1973, 1984) and Edeoga *et al*. 2006).

UV and FTIR Spectroscopic analysis

The extracts were examined under visible and UV light for proximate analysis. For UV and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 260-900 nm using spectrophotometer (Shimadzu) and the characteristic peaks were detected. FTIR analysis was performed using spectrophotometer (Shimadzu) system, which was used to detect the characteristic peaks in ranging from 400-4000 cm^{-1} and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

HPLC ANALYSIS

Sample preparation: The sample was prepared according to the procedure. The extraction was carried out using 2 ml of fermented broth with 50 mL of 95% ethanol under 80 KHz, 45°C in ultrasonic extraction device for 30 min, repeated twice. The extract was collected and filtered; the filtrate was dried at 50°C under reduced pressure in a rotary evaporator. The dried crude extract was dissolved in the 100 ml mobile phase. After filtering through a filter paper and a 0.45 mm membrane filter (Millipore), the extract was injected into HPLC.

HPLC conditions: Flavonoids were analysed using a RP-HPLC method (Weerasak Samee, 2007)[8], Shimadzu Corp., Kyoto, consisting of a LC-10ATVp pump, SCL 10A system controller and a variable Shimadzu SPD- 10ATVp UV VIS detector and a loop injector with a loop size of 20 µl. The peak area was calculated with a CLASSVP software. Reverse-phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250×4.6 mm i.d., particle size 5 µm, Luna 5µ C-18; phenomenex, Torrance, CA, USA) at 25°C. The gradient elution of solvent A [water-acetic acid (25:1 v/v)] and solvent B (methanol) had a significant effect on the resolution of compounds. As a result, solvent gradients were formed, using dual pumping system, by varying the proportion of solvent A [water-acetic acid (25:1, v/v)] to solvent B (methanol).

Solvent B was increased to 50% in 4 min and subsequently increased to 80% in 10 min at a flow rate of 1.0 mL/min. Detection wavelength was 280 nm.

RESULTS AND DISCUSSION

The pharmacological activities of any plant sample are due to the presence of metabolites, secondary metabolites and secretary products in it. These usually consist of the phenolic compounds, alkaloids, tannins, saponins, carbohydrates, glycosides, flavonoids, steroids, etc. Most phenolic compounds such as flavonoids, glycosides, triperinoids, flavonons, carbohydrates and anthraquinones are found distributed throughout the plant kingdom (Harbone, 1973). Similarly, the polyphenolic compounds most commonly found in plant extracts are the phenolic acids, flavonoids and tannins (Naik *et al*., 2006). These compounds together with other phenolic structures of plant origin have been reported as scavengers of Reactive Oxygen Species (ROS) and are seen as promising therapeutic drugs for free radical mediated pathologies including diabetic, cardiovascular diseases (Velavan, 2011). Most flavonoidic compounds exhibit antipyretic, analgesic, anti-inflammatory, anti-arthritic, antioxidant and immuno-modulatory properties (Balasundram *et al*., 2006; Gill *et al*., 2011). These activities of flavonoidic compounds may be due to the presence of gallic acid, ellagic acid, quercitin, tannin acid, vanillin, resorcinol, catechin, etc.

Phytochemical analysis

Secondary metabolites are reported to have many biological and therapeutic properties. Pharmacists are interested in these compounds because of their therapeutic performance and low toxicity (Inayatullah *et al*., 2012). On the basis of the therapeutic potential of secondary metabolites, the phytochemical characters of *Cissus vitiginea* leaves extract were investigated. The phytochemicals analysis of *Cissus vitiginea* leaves extract showed that the presence of tannin, saponnin, flavonoids, steroids, terpenoids, triterpenoids, carbohydrate, anthroquinone, polyphenol and glycoside (Table 1). Significant quantity of tannin (43 ±0.28), phenol (168 ±2.35), flavonoids (126 ±1.32), terpenoids (52 ±0.35) and saponin (33 ±0.21) present in *Cissus vitiginea* leaves (Table 2). The phytochemicals present in in *Cissus vitiginea* leaves possesses wide range of biological activity including diabetic, antipyretic, analgesic, anti-inflammatory, antioxidant and antibacterial properties reported by Balasundram *et al*. (2006) and Reddy *et al*. (2014).

Table.1: Qualitative analysis of Phytochemicals in *Cissus vitiginea* leaves extract

S.No	Name of the Test	Water Extract
1	Tannin	++
2	Phlobatannins	-
3	Saponnin	++
4	Flavonoids	++
5	Steroids	+
6	Terpenoids	++
7	Triterpenoids	++
8	Alkaloids	-
9	Carbohydrate	++
10	Protein	-
11	Anthroquinone	+
12	Polyphenol	+
13	Glycoside	+

(+) Presence, (-) Absence and (++) High Concentration

Table.2: Quantitative Analysis of Phytochemicals in *Cissus vitiginea* leaves extract

S.No	Phytochemicals	Results (mg/gm)
1	Tannin	43 ±0.28
2	Phenol	168 ±2.35
3	Flavonoids	126 ±1.32
4	Terpenoids	52 ±0.35
5	Saponin	33 ±0.21

Values are expressed as Mean ± SD for triplicates

Plants are the rich source of all the elements essential for human beings. There is a relationship between the element content of the plant and its nutritional status. Some elements are essential for growth, for structure formation, reproduction or as components of biologically active molecules while others have some other beneficial effects (NewWall *et al.,* 1996). It is clear that mineral nutrition is important to maintain good health and because of that determination of As, Ca, Fe, Mg, Na, K, Zn, Ni, Co etc. have been added to Ayurvedic Pharmacopoeia of India (The Ayurvedic Pharmacopoeia of India, 1999). From ancient times, Swarnabhasma (gold ash) has been used in several clinical manifestations including loss of memory, defective eyesight, infertility, overall body weakness and incidence of early aging. Hence, their presence is vital for the health and to cure diseases.

In the present investigation, calcium, nitrate, sodium, potassium, sulphate, phosphate, chloride, Iron were present while magnesium was absent in *Cissus vitiginea* leaves (Table 3). Mineral content indicates the nutritive value and potentially act as a cofactor for the biological activity exhibited by the plant extracts studied.

Table.3: Qualitative Analysis of Inorganic Elements in *Cissus vitiginea* leaves extract

S.No	Elements	Result
1.	Calcium	+
2.	Magnesium	+
3.	Sodium	-
4.	Potassium	+
5.	Iron	-
6.	Sulphate	-
7.	Phosphate	+
8.	Chloride	+
9.	Nitrate	-

Note: (-) Absence (+) Presence

HPLC Analysis in *Cissus vitiginea* leaves

Phenolic compounds can be defined as a large series of chemical constituents possessing at least one aromatic ring, bearing hydroxyl and other sub-constituents. HPLC analysis is the most used method for the identifcation of plant phenolic compounds. Because of the diversity and complexity of natural phenolics in medicinal plants, it is difficult to characterize every compound and elucidate its structure (Paranthaman *et al*., 2012). The chromatographic separations of Retention time (Rt), Quercetin (Rt- 2.933) shown in Figure 2. The content of favonoid was calculated from the corresponding calibration curve and presented in Table 1. The HPLC Result shows based on the Retention time Quercetin was found to be in *Cissus vitiginea* leaves. The obtained value was compared with standard. Earlier review of literature (Gupta Mradu *et al*., 2012; Nadia Alam *et al*., 2011; Michael Vagiri *et al*., 2012; Paranthaman *et al*., 2012) supported the findings of these compounds.

Fig 2 HPLC analysis of *Cissus vitiginea* leaf extract

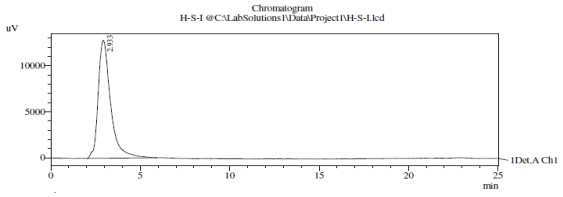

Table 2 HPLC analysis of *Cissus vitiginea* leaf extract

Peak#	Ret. Time	Area	Height	Area %	Height %	Compound identified by literature
1	2.933	618131	12812	100.000	100.000	Quercetin

Spectrophotometric analysis

The UV-VIS profile of plant extract was taken at the 200 to 800nm wavelength due to the sharpness of the peaks and proper baseline. The UV-visible spectra were performed to identify the compounds containing σ- bonds, π-bonds, and lone pair of electrons, chromophores and aromatic rings. The profile showed the peaks at 413.8nm, 467.2, 536.7, 613.6 and 664.5nm with the absorption 1.200, 0.712, 0.141, 0.200 and 0.700 respectively (Fig-3 and Table 4). Occurrence of peaks at 234-676 nm reveals the presents of phenolic and alkaloids in the *Cissus vitiginea*. On comparison of the spectra of seeds and flowers, shows that the extract has some similar alkaloid, flavonoids, and glycosides compounds reported (Jasper *et al*., 1958; Sofowora, 1993).

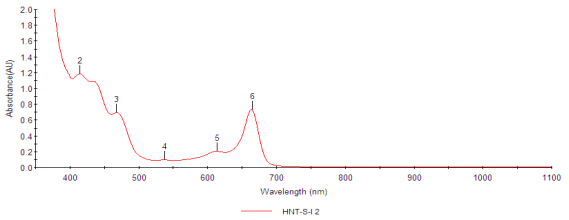

Fig 3 UV-Vis Spectral analysis of *Cissus vitiginea* leaf extract

Table 4: UV-VIS Peak Values of Extract of *Cissus vitiginea* leaf

S.No.	Wave length (nm)	Absorption Peak
1	413.8	1.200
2	467.2	0.712
3	536.7	0.141
4	613.6	0.200
5	664.5	0.700

Functional groups identification

The FTIR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of phenol, alkanes, aldehyde, alcohol, carboxylic acids, aromatic and aliphatic amines compound (Fig-4 and Table-4).

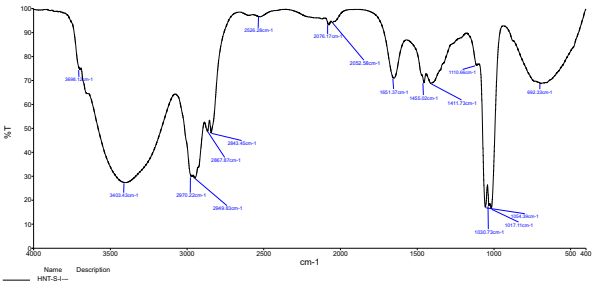

Fig 4 FTIR analysis of *Cissus vitiginea* leaf extract

Table 4: FTIR Peak Values of Extract of *Cissus vitiginea* leaf extract

S. No.	Peak Values	Bonds	Functional groups
1.	3406.43	O–H stretch, H–bonded	Alcohols, Phenols
2.	2870.22	C–H stretch	Alkanes
3.	2848.83		
4.	2867.87 2526.28	O–H stretch	Carboxylic Acids
5.	1651.37	–C=C– stretch	Alkenes
6.	1455.02	C–H bend	Alkanes
7.	1030.73.1017, 1054,	C–N stretch	Aliphatic Amines
8.	692.33	C–H “oop”	Aromatics

CONCLUSION

The qualitative and quantitative anlaysis of phytochemicals supported the active chemicals present in the *Cissus vitiginea* leaves. HPLC analysis provided a good platform for identification of phenolic compounds as quercetin present in *Cissus vitiginea* leaves. The UV-VIS and FTIR the presence of phenol, alkanes, aldehyde, alcohol, carboxylic acids, aromatic and aliphatic amines compound. The results of this study offer a platform of using *Cissus vitiginea* leaves as a source of phytochemicals for nanoparticle synthesis and biological activities.

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