

Analysis of Bio-Active Compounds and Elements in *Cissus Vitiginea* Leaf Extract



Chemistry

KEYWORDS : *Cissus vitiginea*, Phytochemical, Inorganic elements

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ABSTRACT

The aim of the study to investigate the phytochemicals and inorganic elements in *Cissus vitiginea*. The results of this study clearly indicate that the preliminary phytochemical analysis of *Cissus vitiginea* revealed the presence of flavonoids, polyphenol, steroids, tannin, saponins, glycosides, polyphenol, terpenoids, triterpenoids and carbohydrate were present while alkaloids was absent in methanol, ethanol and aqueous extract. Anthraquinone, flavonoids and saponin were present only in petroleum extract. Quantitative analysis revealed that *Cissus vitiginea* rich amount of Total Phenols (37.70mg/gm), Steroids (27.60mg/gm), Terpenoids (22.50mg/gm) and Flavonoids (30.50mg/gm) were presented. The histochemical analysis of *Cissus vitiginea* leaf powder further confirmed the presence of phytochemicals. The inorganic elements of *Cissus vitiginea* showed that the presence of calcium, sodium, potassium, sulphate, phosphate, chloride, Nitrate, magnesium and iron. The results of the present study concluded that *Cissus vitiginea* may be a good source of phytochemicals and minerals. Supplementation of this *Cissus vitiginea* leaves may be useful for human health associated emerging diseases such as cardiovascular diseases, diabetes, hypertension and cancer

INTRODUCTION

Phytochemicals are a field of increasing attention, both in science and in commerce. As is now generally recognized, many plant compounds and pigments have effects on animals and human beings. There is a great effort now to study and understand at a fundamental level and significant health effects of these compounds. This field is maturing and the health effects of these compounds are now getting the careful study they warrant at both a chemical and a molecular biological level. Identifying bioactive compounds and establishing their health effects are active areas of scientific inquiry. Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans⁽¹⁾. They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals^(2,3). Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals fruits and herbs and each works differently⁽⁴⁾. The plant kingdom is a rich source of potential drugs. In India, medicinal plants are widely used by all sections of the population, either directly in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicine. Research on natural resources has been encouraged by the World Health Organization since 1978. Most of the medicinal plants contain a number of chemical constituents such as flavonoids, alkaloids, tannins, saponins, steroids, terpenoids, rotenoids etc. The phytochemical screening of the plants is a preliminary for verification and then these plants may be utilized as new sources of herbal drugs⁽⁵⁾.

The mineral elements are separate entities from the other essential nutrients like (proteins, fats, carbohydrates, and vitamins. Animal husbandry had demonstrated the need for minerals in the diet. In this century, biological assay methods clarified the significance and importance of mineral elements for human and animal nutrition and modern analytical techniques led to the detection of trace elements as essential nutrients and this is still an active area of cur-

rent research. Micronutrient deficiencies are a major public health problem in many developing countries, with infants and pregnant women especially at risk⁽⁶⁾. Infants deserve extra concern because they need adequate micronutrients to maintain normal growth and development⁽⁷⁾. The micronutrient deficiencies which are of greatest public health significance are iron deficiency, causing varying degrees of impairment in cognitive performance, lowered work capacity, lowered immunity to infections, pregnancy complications e.g. babies with low birth weight, poor learning capacity and reduced psychomotor skills⁽⁶⁾.

Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics and About 150 phytochemicals have been studied in detail. Since there is no report on the phytoconstituents of methanolic fraction of *Cissus vitiginea* leaves extract it was chosen as the subject of this study. Keeping in view, in the preset study to investigate the phytochemical and inorganic elements in *Cissus vitiginea* (Family: Vitaceae).

MATERIALS AND METHODS

Plant materials:

The leaf of *Cissus vitiginea* was collected in January 2015 from Tamil University, Thanjavur District, Tamil Nadu, India from a single herb. The leaf was identified and authenticated by Dr. S. John Britto, The Director, the Rabiant Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of alcoholic extract

The leaf of *Cissus vitiginea* was first washed well and dust was removed from the plant. Leaf was washed several times with distilled water to remove the traces of impurities from the plant. The leaf plant was dried at room temperature and coarsely powdered. The powder was extracted with Different extracts (Ethanol, Petroleum ether, methanol and aqueous) for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used.

Preliminary phytochemicals screening

Chemical tests were carried out on the alcoholic extract using standard procedures to identify the preliminary phytochemical screening following the methodology of Harborne⁽⁸⁾, Trease and Evans⁽⁹⁾ and Sofowara⁽¹⁰⁾.

Quantitative analysis of phytochemicals

Determination of total phenols⁽¹¹⁾. Tannin was determined by the method of Van-Burden and Robinson⁽¹²⁾. Saponin was determined by the method of Obdoni and Ochuko⁽¹³⁾. Flavonoid was determined by the method of Bohm and Kocipai-Abyazan⁽¹⁴⁾.

Histochemical analysis of phytochemicals and Qualitative analysis of inorganic elements

Histochemical analysis carried out by the method of Rao⁽¹⁵⁾. Ash of drug material (500 mg) was prepared and treated with HNO₃ and HCl (3:1 v/v) for 1hour. After the filtration, the filtrate was used to perform the following tests Khandelwal⁽¹⁶⁾.

RESULTS AND DISCUSSION

Phyto is the Greek word for plant. There are many families of phytochemicals and they help the human body in the variety of ways. Phytochemicals may protect human from a host of diseases. Phytochemicals are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. There are more than thousand known and many unknown phytochemicals. It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases⁽¹⁷⁾.

An assessment of the previous trends and impact of research into the phytochemistry on medicinal plants of the world is quite desirable before considering recent trends. After centuries of empirical use of herbal preparation, the first isolation of active principles alkaloids such as morphine, strychnine, quinine etc. in the early 19th century marked a new era in the use of medicinal plants and the beginning of modern medicinal plants research. Emphasis shifted away from plant derived drugs with the tremendous development of synthetic pharmaceutical chemistry and microbial fermentation after 1945. Plant metabolites were mainly investigated from a phytochemical and chemotaxonomic viewpoint during this period. Over the last decade, however, interest in drugs of plant and probably animal origin has grown steadily⁽¹⁸⁾.

Utilization of medicinal plants has almost doubled in Western Europe during that period. Ecological awareness, the efficacy of a good number of phytopharmaceutical preparations, such as ginkgo, garlic or valerian and increased interest of major pharmaceutical companies in higher medicinal plants as sources for new lead structures has been the main reasons for this renewal of interest. With the development of chemical science and pharmacognosy physicians began to extract chemical products from medicinal plants. A few examples of the products extracted from medicinal plants are - in 1920, quinine was isolated from Cinchona by the French pharmacist, Peletier & Caventou. In the mid-nineteenth century, a German chemist, Hoffmann obtained Aspirin from the bark of the willow. With the active principles in medicinal plants identified and isolated, plant-based prescriptions began to be substituted more and more with pure substances, which were more powerful and easier to prescribe and administer⁽¹⁹⁾.

Phytomedicine almost went into extinction during the first half of the 21st century due to the use of the 'more powerful and potent synthetic drug'. However, because of the numerous side effects of these drugs, the value of medicinal plants is being rediscovered as some of them have proved to be as effective as synthetic medicines with fewer or no side effects and contraindications. It has been proved that although the effects of natural remedies may seem slower, the results are sometimes better on the long run especially in chronic diseases.

In the present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *Cissus vitiginea* leaf investigated and summarized in Table-1. the presence of flavonoids, polyphenol, steroids, tannin, saponins, glycosides, polyphenol, terpenoids, triterpenoids and carbohydrate were present while alkaloids was absent in methanol, ethanol and aqueous extract. Anthroquinone, flavonoids and saponin were present only in petroleum extract.

Table 1: Phytochemical screening of *Cissus vitiginea*

S.No	Group	Methanol extract	Ethanol Extract	P.E Extract	Aqueous extract
1	Tannin	+	+	-	+
2	Phlobatanin	+	-	-	+
3	Saponin	+	+	+	+
4	Flavonoids	++	++	+	+
5	steroids	+	+	-	+
6	Terpenoids	++	+	-	++
7	Triterponids	+	+	-	++
8	Alkaloids	-	-	-	-
9	Carbohydrate	+	+	-	+
10	Protein	+	-	-	-
11	Anthroquinone	+	+	+	+
12	Polyphenol	++	+	-	+
13	Glycoside	+	+	-	+

(+) Presence (-) Absence (++) High concentrations

P.E = Petrolum ether

Quantitative analysis

Quantitative analysis revealed that the *Cissus vitiginea* contain significant amount of phenols, alkaloids, saponin and terpenoids. Quantitative analysis revealed that *Cissus vitiginea* rich amount of Total Phenols (37.70mg/gm), Steroids (27.60mg/gm), Terpenoids (22.50mg/gm) and Flavonoids (30.50mg/gm) were presented. (Table 2). The above phytoconstituents were tested as per the standard methods. This is because of the pharmacological activity of this plant is used to trace the particular compound.

Table 2: Qualitative analysis of *Cissus vitiginea* leaf

S.No	Test	Result (mg/gm)
1	Terponoids	22.50
2	Steroid	27.60
3	Flavoinds	30.50
4	Total phenol	37.70

Histochemical analysis

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues. Histochemical study has been carried out to detect various phytochemicals groups localized in different tissue zones of the leaf. Different phytochemical groups like tannins, steroids, alkaloids, lignin, polyphenol etc. have found localised in different tissue zones of the stem. It has been found that vascular bundles and cortical zone are main active sites

for synthesis of high content of different phytochemical groups. Starch deposition occurs widely in the plant body, but the particularly common places of its accumulation are seeds, the parenchyma of the secondary vascular tissues in the stem and root, tubers, rhizomes and corn. Starch and proteins are the principal ergastic substances of the protoplast. Tannin is the heterogeneous group of phenol derivatives, usually related to glucosides. Tannins are particularly abundant in the leaves (xylem) of many plants. Saponins are the rare occurrence. Fats are widely distributed in the plant body and they probably occur in small amount in every plant cell. Fats are common reserve material in seeds, spores and embryos in meristematic cells. Glucosides are the degradation product of the carbohydrates. Alkaloids are the degradation product of protein⁽²⁰⁾. (Table-3).

Table 3: Histochemical analysis of *Cissus vitiginea*

S.No	Test Reagent	Observation	Characterization	Present/absent
1	Phloroglucinol + Conc.HCl	Red/Pink	Lignin	+
2	Dilute Ammonia + H ₂ SO ₄	Yellow	Flavonoids	+
3	Meyers/Dragnet reagent (Few drop)	Reddish brown	Alkaloids	-
4	FeCl ₃ solution	Dark Blue to Black	Tannin/ Phenol cells	+
5	Conc. HCl	Drak Black	Crystal	+
6	Iodine	Blue	Starch grain	+
7	Lieberman(5 drop of acetic acid + 5 drop of H ₂ SO ₄)	Violet to Blue or Green	Steroids	+
8	Toludine	Blue Green/ Red	Polyphenol	+

Minerals

All human beings require a number of complex organic/inorganic compounds in diet to meet the need for their activities. The important constituents of diet are carbohydrates, fats, proteins, vitamins, minerals and water⁽²¹⁾. Every constituent plays an important role and deficiency of any one constituent may lead to abnormal developments in the body. 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⁽²²⁾. The elements of the *Cissus vitiginea* leaves were investigated and summarized in Table-4. The following elements were found in *Cissus vitiginea* leaves. They are calcium, sodium, potassium, sulphate, phosphate, chloride, Nitrate, magnesium and iron in *Cissus vitiginea* leaves extract.

Table 4: Qualitative analysis of inorganic element in *Cissus vitiginea*

S.No	Inorganic Element	Observation	Remarks
1	Calcium	White precipitate	Presence of calcium
2	Magnesium	White Crystal	Presence of Magnesium
3	Sodium	Yellow precipitate	Presence of Sodium
4	Potassium	Yellow precipitate	Presence of Potassium
5	Iron	Blue Color	Presence of Iron
6	Sulphate	White Precipitate	Presence of Sulphate

7	Phosphate	Yellow precipitate	Presence of Phosphate
8	Chloride	White Precipitate	Presence of Chloride
9	Nitrate	Brown Color ring	Presence of nitrate

(+) Presence (-) Absence (++) High concentrations

Minerals are inorganic substances, present in all body tissues and fluids and their presence is necessary for the maintenance of certain physicochemical processes which are essential to life. Minerals are chemical constituents used by the body in many ways. Although they yield no energy, they have important roles to play in many activities in the body. Every form of living matter requires these inorganic elements or minerals for their normal life processes. Minerals may be broadly classified as macro (major) or micro (trace) elements. The third category is the ultra trace elements. The macro-minerals include calcium, phosphorus, sodium and chloride, while the micro-elements include iron, copper, cobalt, potassium, magnesium, iodine, zinc, manganese, molybdenum, fluoride, chromium, selenium and sulfur. The macro-minerals are required in amounts greater than 100 mg/dl and the micro-minerals are required in amounts less than 100 mg/dl. The ultra trace elements include boron, silicon, arsenic and nickel which have been found in animals and are believed to be essential for these animals. Evidence for requirements and essentialness of others like cadmium, lead, tin, lithium and vanadium is weak⁽²³⁾.

CONCLUSION

The results of this study clearly indicate that preliminary phytochemical analysis of *Cissus vitiginea* revealed presence of the presence of flavonoids, polyphenol, steroids, tannin, saponins, glycosides, polyphenol, terpenoids, triterpenoids and carbohydrate were present while alkaloids was absent in methanol, ethanol and aqueous extract. Anthroquinone, flavonoids and saponin were present only in petroleum extract. Quantitative analysis revealed that *Cissus vitiginea* rich amount of Total Phenols (324.0 mg/gm), Alkaloids (35.0 mg/gm), Tannin (98.4 mg/gm), Saponin (121.0 mg/gm), Flavonoids 165.0mg/gm) were presented. The histochemical analysis of *Cissus vitiginea* leaf powder further confirmed the presence of phytochemicals. The inorganic elements of *Cissus vitiginea* showed that the presence of calcium, sodium, potassium, sulphate, phosphate, chloride, Nitrate, magnesium and iron. The results of the present study concluded that *Cissus vitiginea* leaves may be a good source of phytochemicals and minerals. Supplementation of this *Cissus vitiginea* leaves may be useful for human health associated emerging diseases such as cardiovascular diseases, diabetes, hypertension and cancer.

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