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Research Article

Studies On Phytochemical and Mineral Analysis of *Cissus quadrangularis* Solvent Extracts

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Abstract

Dangerous illnesses are spreading worldwide and posing major threats to public health. Given the unavoidable side effects of allopathic drugs, public interest in herbal medicines to get rid of various illnesses and health issues continues to surge globally. India has an ancient history of herbal medicines. The grape family member *Cissus quadrangularis*, also referred to as "Hadjod," has a long history of usage in Indian traditional medicine because of its anti-inflammatory, antioxidant, and joint-related qualities. This study looks into how different extraction methods affect the phyto-compound makeup of *Cissus quadrangularis* and how that affects inflammation and joint-related issues. The findings show that *Cissus quadrangularis* has many bioactive chemicals that have been shown to have anti-inflammatory effects. These compounds may have potential applications in herbal formulations that address joint-related problems. As per the study of the *Cissus* plant's different parts, phytosterols are demonstrated as active compounds. B-sitosterol and stigmasterol are a derivative of phytosterols. As per the study of its aerial part, flavonoids such as quercetin and rutin are evaluated as active compounds in the range of 0.01% – 0.02% and analyzed by a high-performance liquid chromatograph and UV spectrophotometry. The qualitative analysis of *Cissus* methanolic and ethanolic extract demonstrates the existence of terpenoids, plant sterols, tannins, protein, and sugar. Methanolic extraction from plants reflects the existence of phytosterols, flavonoids, quinones, protein, vitamins, and minerals. The water extractive part was analyzed for the existence of alkaloids, vitamins, and minerals.

Keywords: *Cissus quadrangularis* extract, Joint pain, Phytochemical, HPLC, UV Spectrophotometry

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Introduction

"Hadjod," is a prominent member of the "Vitaceae family" of plants [20] and it is native to Ceylon and India. Extensive studies have been conducted on its medicinal relevance, revealing various therapeutic qualities that span various health areas. [1][10][11]. The beneficial effects of *Cissus quadrangularis* on bone

health are one of its well-researched advantages [15]. Long acknowledged for its ability to support healthy skeletons, this plant has recently drawn interest for its potential to treat bone fractures and associated conditions [21]. The active components of *Cissus quadrangularis*, known as ketosterones [16][17], are plant sterols that are essential to its efficacy. These

ketosterones are essential for strengthening bone density, supporting tendon healing, and promoting joint health [12]. The plant's accessibility as a natural therapy for those with bone and joint health issues [26] and those who are actively involved is further enhanced by its availability in the Indian tropical part. *Cissus quadrangularis* has been placed traditionally in the Ayurvedic system [18], the traditional medical system of India, to treat ailments like gout and venereal disorders.[2] Our comprehension of its chemical composition has been enhanced by phytochemical investigations. *Cissus quadrangularis* has a diverse pharmacological profile that includes antibacterial, antioxidant, antiulcer, analgesic, anti-rheumatic, and antiosteoporosis effects furthermore their impact on bone health [14][28]. These varied qualities make the plant an important natural source of therapeutic chemicals [23] with potential for use in pharmaceutical research. [3].

The growing interest in natural cures and nutritional supplements around the world makes *Cissus quadrangularis* an intriguing topic for research. Its attractiveness as a possible supplement is increased by its abundance of nutrients and trace elements [13]. The proposed work investigates the phytochemical composition and mineral content of *Cissus*

quadrangularis, a plant with traditional medicinal value in India [24]. The study aims to explore the presence of phytochemicals compounds i.e. alkaloids, flavonoids [25][27], sterols [19][29], etc. qualitatively and quantitatively. These compounds are used for addressing inflammation, obesity, hypertension, and joint-related issues.[4][22]. The plant is utilized for addressing a range of conditions including anorexia, dyspepsia, colic, flatulence, tumors, convulsions, asthma, nosebleeds, ear discharge, irregular menstruation, inflammation, pain, and syphilitic infections. Recent investigations into quercetin have suggested its potential as an anticancer agent, showing promising results regarding its ability to combat cancer. [28]

Materials and Methods

1.1. Sample Collection

Cissus quadrangularis plant components, such as stems, leaves, flowers, fruits, and roots (**fig. 1**) were collected from the vicinity of Arogyavaram, Madanapalli (tq), Chittoor (dist), Andhra Pradesh, and placed in separate sterile polythene bags. Plant components that were collected were analyzed and identified using the local flora. Specimens were further verified using herbarium sheets that were on hand at the Prathista Industries Limited department in Telangana State, India.

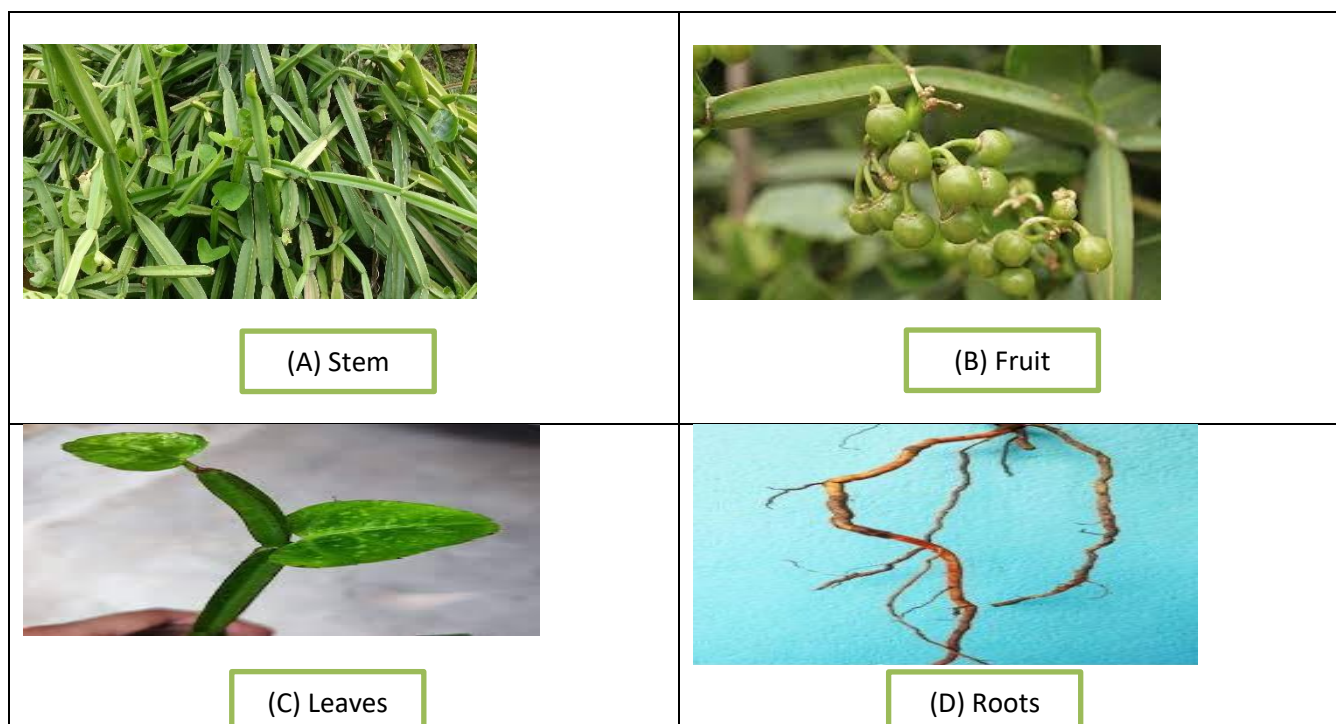


Fig. 1: *Cissus quadrangularis* plant parts: (a) Stem bearing all parts of the plant, (b) Fruit and inflorescence, (c) Typical leaf of the plant, and (d) Roots of the plant.

Preparation of Solvent Extracts

Healthy, washed plant components were divided into little pieces and allowed to dry for three to four weeks in a shaded area. The dried components were ground using an electric grinder. The resulting grounded powder was utilized for extraction and kept in a desiccator. One gram of each sample's finely powdered plant material was extracted using twenty-five milliliters of solvent, and the mixture was kept standing for 48 hours with occasional

stirring. Here, extraction was done by taking ethanol and methanol of HPLC quality. Room temperature was used for the extraction. To get filtrate as extracts, all of the solvent extracts were filtered with Whatman Number 1 filter paper. The samples were then dried to concentrate them. The leftover powder was weighed and then redissolved in the appropriate solvents to obtain a final weight. Standard protocols were employed to qualitatively analyze samples to detect carbohydrates,

terpenoids, cardiac glycoside, alkaloid, flavonoid, protein, phenol, saponin, tannin, and quinone.

Phytochemical Analysis

Test for Flavonoids

Two milliliters of each extract were taken. A small amount of sodium hydroxide (20%) was added to the extracts. After completing this stage, a vibrant yellow color was formed. There was undergoing a chemical reaction that first gave rise to the yellow color, which suggests the presence of certain chemicals, especially flavonoids. Then a small amount of 75% diluted HCL was added to the mixture. The elimination of the yellow color was the resultant impact. Since this reaction is indicative of the presence of flavonoids, the removal of the yellow color upon the addition of diluted hydrochloric acid further supports the identification of flavonoids.

Test for Alkaloids

In a 1:1 ratio, Marquis reagent was mixed with the sample extract and poured in two milliliters of sulfuric acid (concentrated). A few drops of formaldehyde (40%), were added to the mixture. This combination changed the color to an orange or purple tint when kept in the dark. This test presented here is a qualitative technique to identify the presence of alkaloids, a broad class of naturally occurring compounds present in a variety of plants, based on the distinctive color change in reaction with Marquis reagent.

Test for Saponins

Two milliliters of each extract were taken and added 5 milliliters of purified water to every extract. Given the mixture a good shake to ensure the mixing is completed, this step was essential. The bubbles and froth in the samples indicated the existence of saponins in the sample extracts. The production of foam or bubbles is a typical reaction linked to saponins, which are organic substances frequently present in plants. Based on the noticeable physical changes in the combination, this qualitative test aids in determining the existence of saponins in the mixture.

Test for Tannins

Two milliliters of each extract were taken. 10% alcoholic ferric chloride was added to every extract. Bluish brown to blackish color was observed, which indicated the existence of tannins. The capacity of tannins, a family of polyphenolic chemicals, to create complexes with ferric ions is well-known. A recognizable reaction that indicates the existence of tannins in the sample extracts is the development of a brownish-blue or black color.

Test for Phenols

Two milliliters of each extract were taken and two milliliters of 5% aqueous ferric chloride was mixed with each extract. The presence of phenol in the samples suggests the appearance of a blue color. A class of organic chemicals known as phenols can react with ferric ions to create complexes with color. Phenols may be present in the sample extracts as shown by the mixture taking on a blue hue.

Test for Proteins

Two milliliters of each extract were taken and one milliliter of 40% sodium hydroxide was added. Into this mixture, a some drops of 1% copper sulfate were added. A violet hue shows the existence of proteins and peptides. These proteins and peptides are made up of peptide bonds, which react with certain chemicals to form colored complexes. The mixture's violet coloration indicates that the sample extracts include peptide linkage molecules.

Test for Cardiac Steroids

One milliliter of all extracts was poured into a separate test tube. 0.5 milliliters of glacial acetic acid were added to each extract. To the mixture, three drops of 1% aq. ferric chloride solution were added. A brown color ring appeared near the junction of both liquids. It indicated the existence of a class of substances called cardiac glycosides.

Test for Terpenoids

1 milliliter chloroform was added in each extract followed by the addition of 2-3 ml sulfuric acid. The appearance of a reddish-brown precipitate was observed. It is a crucial symptom of the presence of terpenoids. Terpenoids are a class of naturally occurring chemicals that react to generate distinctive precipitates when combined with strong sulfuric acid and chloroform.

Test for Carbohydrates

In one milliliter of the sample extract, some drops of the alcoholic 1-naphthol solution and 1 mL of conc. sulphuric acid was added to each tube side. It was left to run down and combined with the solution. The mixture was kept for two or three minutes without stirring. Carbohydrates present in the sample extracts were dehydrated when combined with these reagents to produce a complex that caused the color shift. The dim violet color indicates the existence of carbohydrates.

Results and Discussion

The Qualitative analysis results are mentioned below:

Alkaloids: Alkaloids are present in the methanolic extract of stem, leaves, and root and ethanolic extract of root, leaves, and fruit.

Flavonoids: Flavonoids are present only in leaves in both methanolic and ethanolic extracts.

Tannins: Tannins exist in all the sections of the plant in both methanolic and ethanolic extracts.

Terpenoids: Terpenoids exist in all the sections of the plant in both methanolic and ethanolic extracts except fruit.

Saponins: Saponins are present in stem, roots, and leaves extract prepared in methanol.

Cardiac glycosides: Cardiac glycosides exist in the extract of the stem prepared in methanol.

Protein: Protein exists all the sections of the plant in both methanolic and ethanolic extracts.

Carbohydrates: Carbohydrates exist in all the sections of the plant in both methanolic and ethanolic extracts.

Phenols: Phenols exist in all the sections of the plant in both methanolic and ethanolic extracts.

Table 1: Phytochemicals present in different parts of *Cissus Quadrangularis* plant (Qualitative)

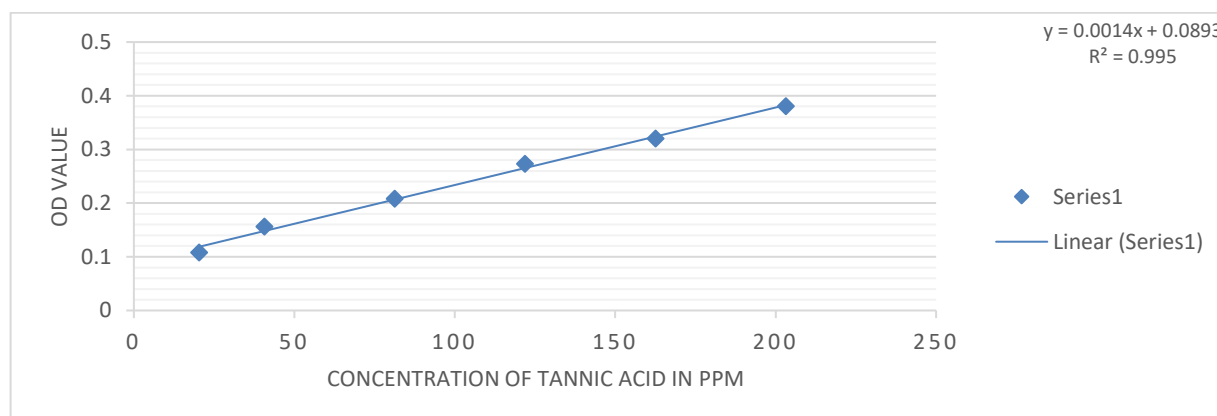
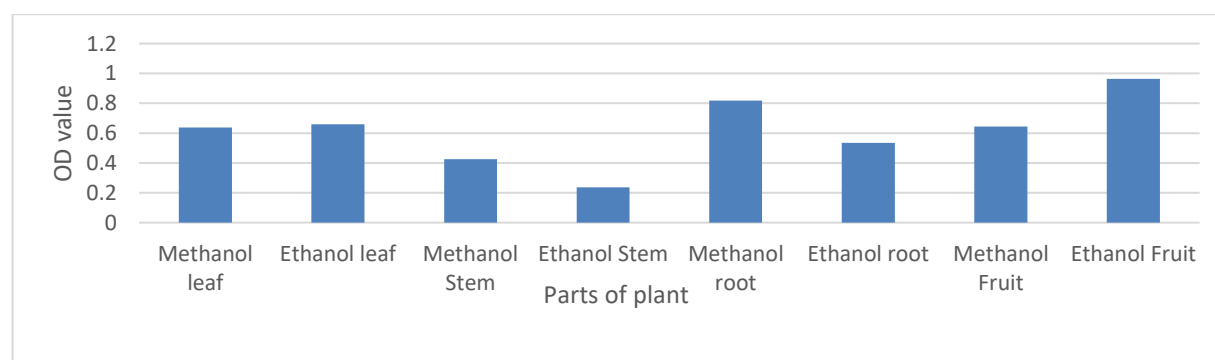
S. No.	Plant parts →	Stem		Root		Leaves		Fruit	
		Methanol	Ethanol	Methanol	Ethanol	Methanol	Ethanol	Methanol	Ethanol
1.	Alkaloid	+	-	+	+	+	+	-	+
2.	Flavonoids	-	-	-	-	+	+	-	-
3.	Tannins	+	+	+	+	+	+	+	+
4.	Terpenoids	+	+	+	+	+	+	-	-
5.	Saponins	+	-	+	-	+	-	-	-
6.	Cardiac glycosides	+	-	-	-	-	-	-	-
7.	Proteins	+	+	+	+	+	+	+	+
8.	Carbohydrates	+	+	+	+	+	+	+	+
9.	Phenols	+	+	+	+	+	+	+	+

The quantitative assay involves determining the precise concentrations of alkaloids, tannins, phenols, proteins, and carbohydrate compounds in the sample by using various analytical techniques tailored to each compound type.

Total Tannins Content

The determination of tannins was carried out using Folin and Ciocalteu's method. In a nutshell, 1.0 mL of the sample extracts were combined with 8.0 ml water. After that 1.0 mL of Folin Phenol reagent and 1.0 ml of a 35%

Na_2CO_3 solution were added. Subsequently, the absorbance was taken at 765 nm. Standard solutions were prepared by taking tannic acid solutions diluted by ranging from 0 to 1.0 milligram/mL (**Fig. 2**). The outcomes for tannin content in the plant are presented in terms of the concentration of standard, expressed in milligram/mL of the extracts. (**Fig. 3**). Methanolic and ethanolic extract of fruit part had 1.55% tannins in terms of tannic acid. Other parts had a concentration of tannic acid in the range of 0.2 – 1.0%.

**Fig. 2: Calibration curve of tannic acid on UV Spectrophotometer****Fig. 3: Maxima absorption of different parts of the plant on UV Spectrophotometer**

Total Phenol Content

The determination of phenols utilized a Folin Ciocalteu analysis method. A 0.2 mL of every sample extract was added with 0.8 mL of the Folin and Ciocalteu reagent and 2.0 mL of 7.50 % Na_2CO_3 . Then diluted the sample was to 7 mL with water and the tubes were incubated in the dark for 2.0 hours. After that absorbance was taken at

760 nm. Standard solutions are prepared using gallic acid dilutions (**Fig. 4**). The results for phenol content are conveyed in terms of the gallic acid concentration, expressed in milligrams/mL of the extracts. (**Fig. 5**). All parts with solvent extract of the plant contain phenolic content in the range of 0.01 – 0.02% as gallic acid.

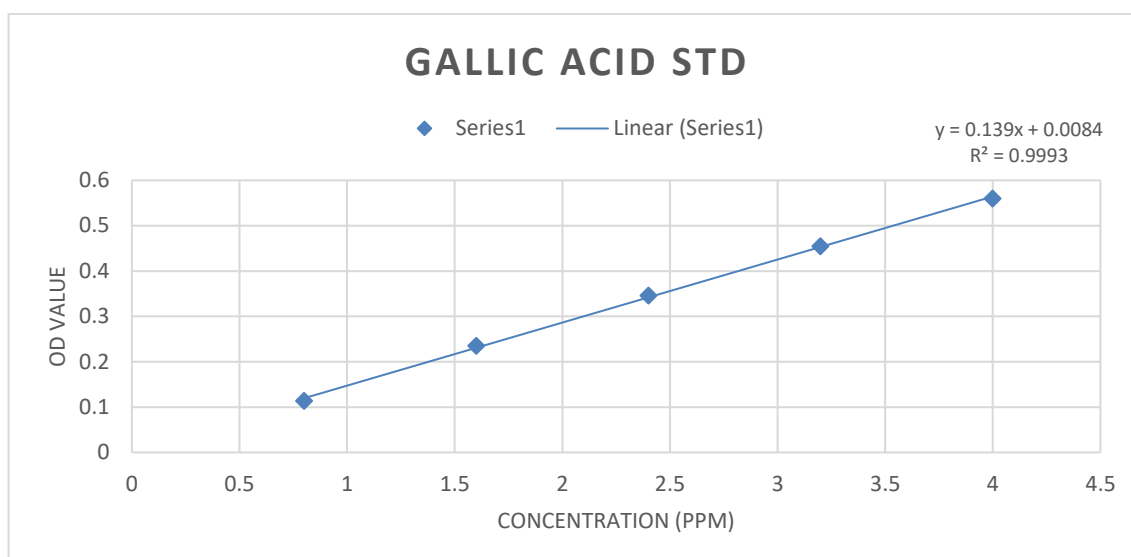


Fig. 4: Calibration curve of Gallic acid on UV Spectrophotometer

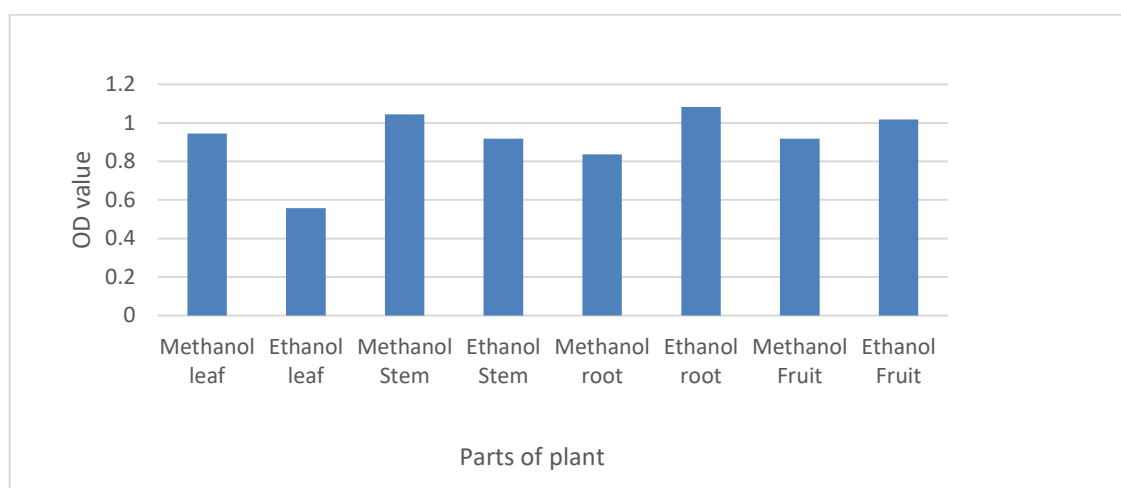


Fig. 5: Maxima absorption of different parts of the plant on UV Spectrophotometer

Total Protein Content

Bradford method was employed for the determination of total protein. In brief, 0.1 mL sample extract was added with 3.0 mL of Bradford reagent and kept in the dark for 10 minutes. Subsequently, absorbance at 595 nm was taken. Standard solutions were prepared using dilutions

of bovine serum albumin, ranging from 0.2 milligram/mL to 1.0 milligram/mL. (**Fig. 6**) and (**Fig. 7**). All parts with both solvent extracts of the plant contain protein content in the range of 7.0% – 16 % in terms of BSA standard. Maximum concentration was found in the leaf part of the plant.

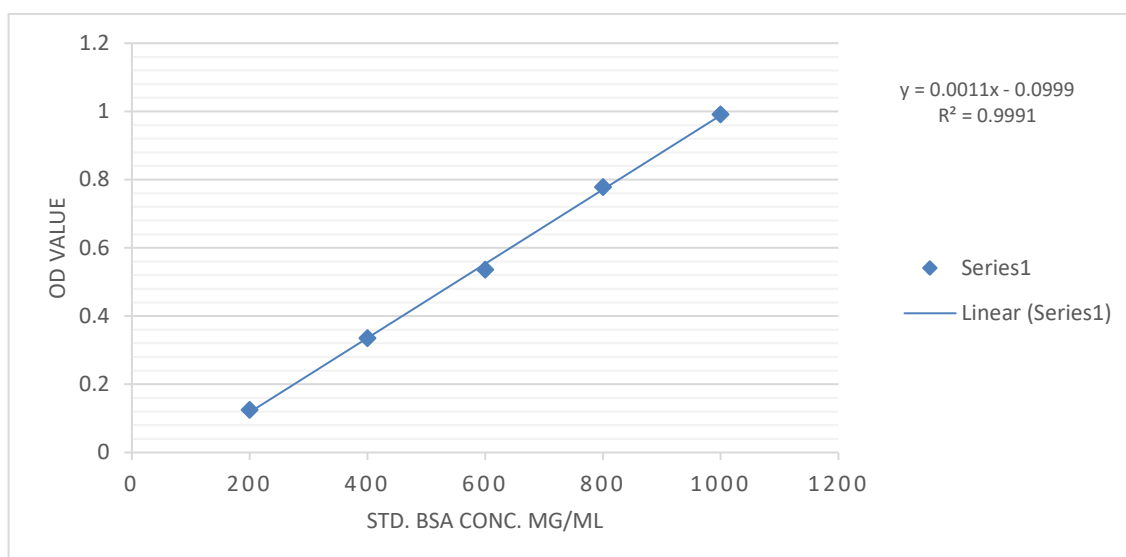


Fig. 6: Calibration curve of Bovine serum albumin on UV Spectrophotometer

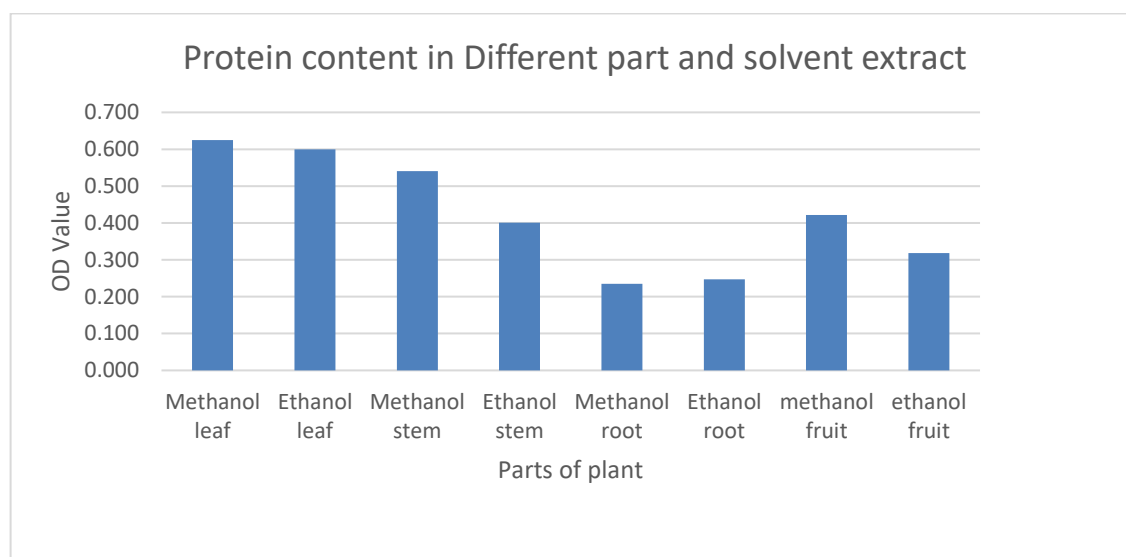


Fig. 7: Maxima absorption of different parts of the plant on UV Spectrophotometer

Total Alkaloid Content:

1.0 g powdered samples were subjected to treatment with 50 mL of 10.0 % Alcoholic Glacial Acetic acid. The mixture remained covered and kept for around 4.0 hours. The resulting filtrates were reduced by evaporation in a water bath to one-fourth of initial volume. Conc. ammonia was added dropwise in the sample extract until precipitation appeared. The solution was kept to settle down. The filtered precipitate was washed with dil. ammonia and subsequently filtered. The remaining residue was dried and weighed for final results. All parts with both solvent extracts of the plant contain Alkaloid content in the range of 0.007 – 0.1% as andrographolides. Maximum concentration was found in the stem part of the plant.

Total Carbohydrate determination

For carbohydrate estimation, the sample solution was taken (2 ml), and 3 mL DNS solution was added. Cover the test tube with paraffin film to prevent evaporation of any liquid due to heating. After that heat the mixture at 90°C for 5 minutes. Cool the mixture. Then measured the absorbance was at 575 nm (**Fig. 9**) A blank was taken for correction. The Sample solution absorbance was compared with a glucose standard solution prepared in concentrations from 100 ppb to 1 ppm (**Fig. 8**). The blank was prepared by taking water (2 ml) added to DNS solution (3 ml) and heat at 90°C for 5 minutes. All parts with solvent extracts of the plant contain carbohydrates in the range of 18% – 36 %. Maximum concentration was found in the leaves part of the plant.

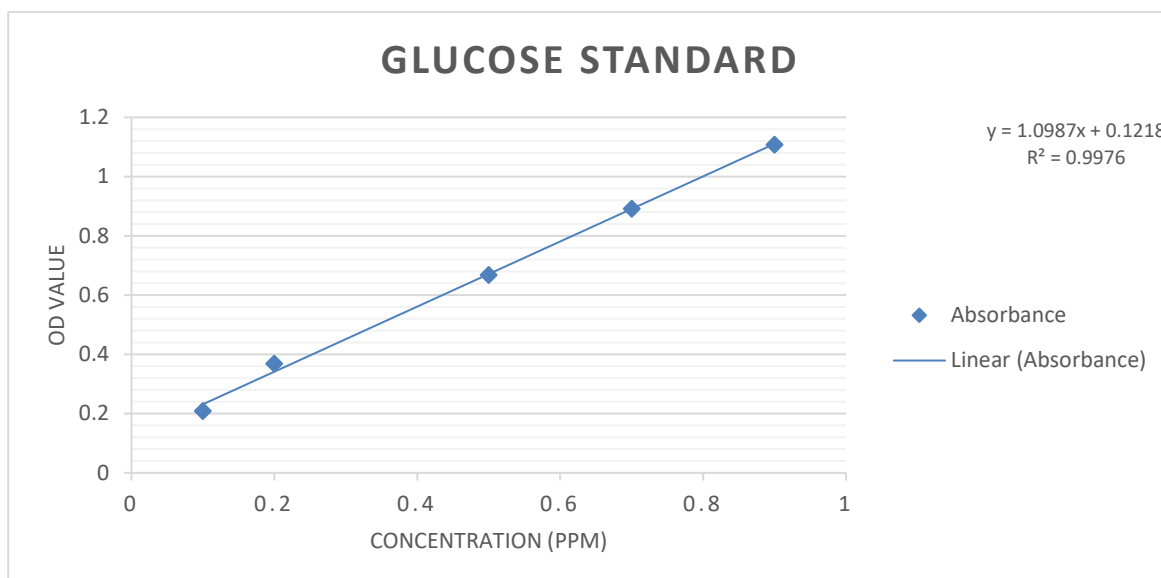


Fig. 8: Calibration curve of Glucose standard on UV spectrophotometer

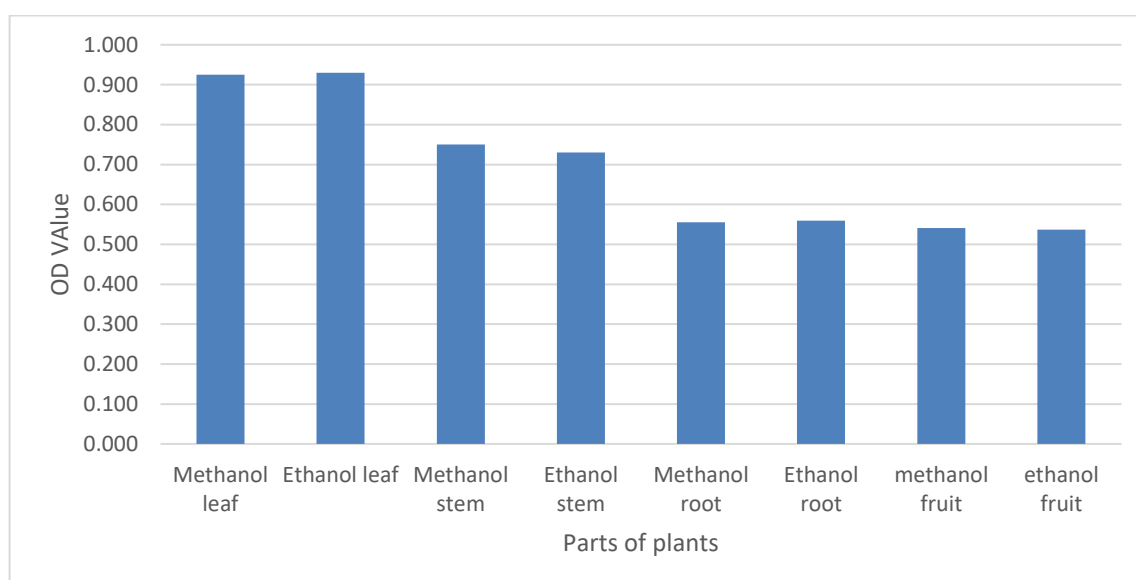


Fig.9: Maxima absorption of different parts of the plant on UV Spectrophotometer

Total Flavonoid content determination

The preparation of the quercetin & rutin stock solution (100µg/ml) involved accurately dissolving approximately 10mg of quercetin & rutin in a suitable quantity of ethanol. The volume was then adjusted to 100ml with ethanol, resulting in the desired concentration. Subsequently, a series of dilutions ranging from 1 to 20 µg/ml were created using ethanol,

providing a range of concentrations for experimental use or analysis, and then measured the absorbance at 370 nm & 256 nm. (**Fig. 10**) All the sample extracts were compared with the quercetin & rutin standard solution. (**Fig. 11**). Methanolic and ethanolic extract of the leaf part contains a maximum concentration of quercetin 0.016% & rutin 0.625% (**Fig. 12**) & (**fig 13**)

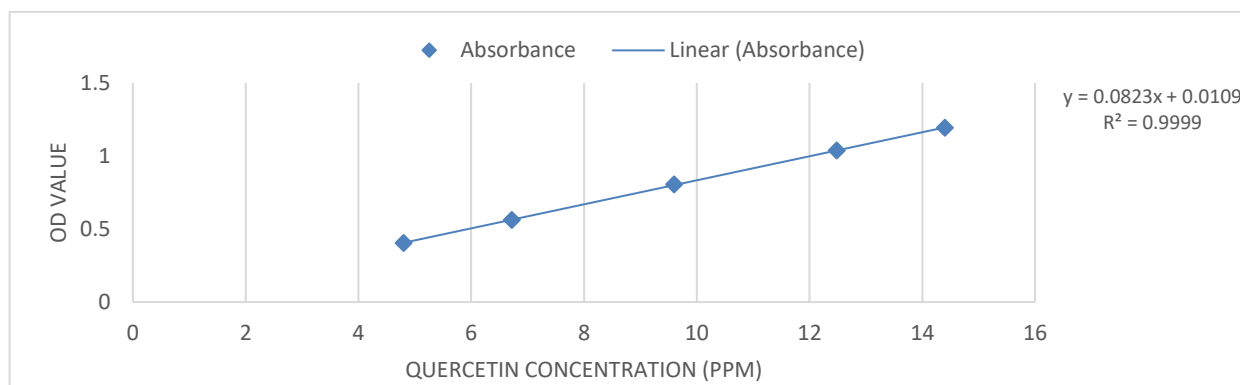


Fig. 10: Calibration curve of quercetin standard on UV Spectrophotometer

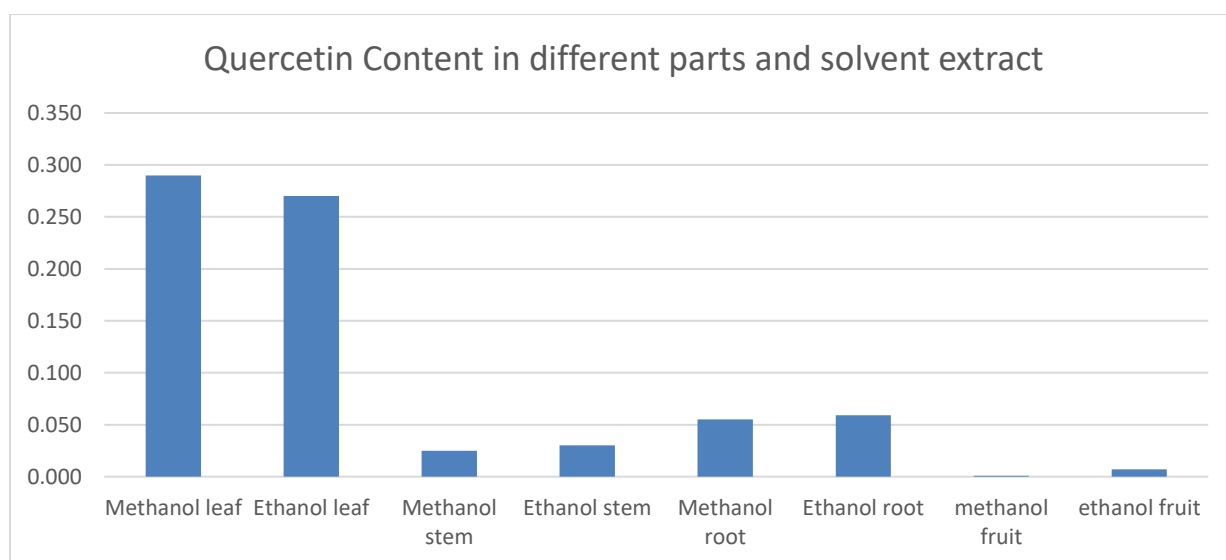


Fig. 11: Maxima absorption of different parts of the plant on UV Spectrophotometer

Respectively all the extracts were also analyzed on HPLC. Method parameters flow rate was set at 1 ml/minute & wavelength was set at 370 nm for quercetin & at 256 nm for rutin. Separation was done using HPLC

column ODS C18 250 mm x 4.6 mm, 5 microns. As a mobile phase methanol & 0.1% orthophosphoric acid were used in a ratio of 65:35

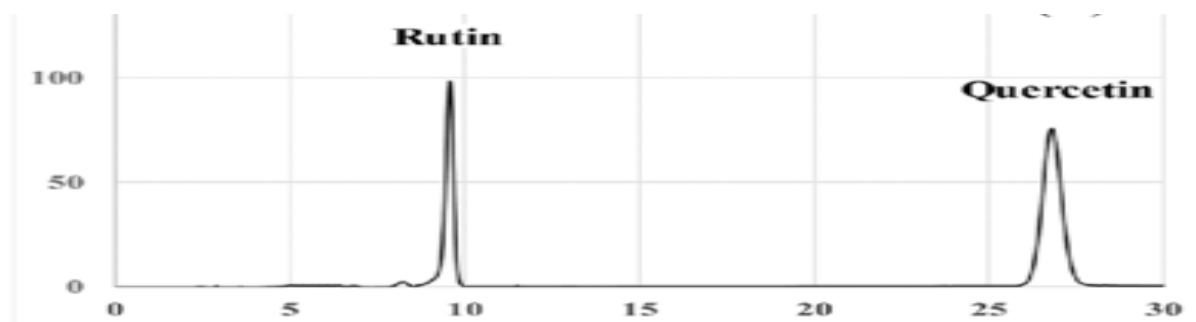


Fig. 12: HPLC chromatogram of rutin and quercetin standard

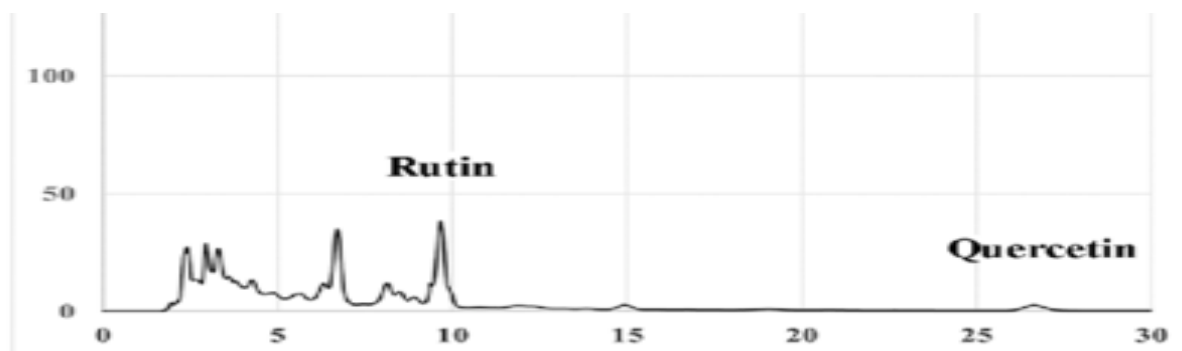


Fig. 13: HPLC chromatogram of rutin and quercetin content in *Cissus* plant methanolic & ethanolic leaf extract

Conclusion

Alkaloids are present in most samples, as proteins, carbohydrates, tannins, and phenols in all samples. Flavonoids are found exclusively in leaf extracts, and cardiac glycosides in stem extracts with methanol. Saponins are predominantly found in methanolic extracts. The conclusion emphasizes the potential use of *Cissus quadrangularis* in managing obesity and metabolic disorders, revealing antioxidant capacity and various components like flavonoids and triterpenoids. The *Cissus quadrangularis* plant is used for managing obesity and dealing with the complications associated with metabolic disorders. Vitro Antioxidant capacity and the ability to neutralize free radicals have been observed. Also, the presence of several components, such as flavonoids, triterpenoids, Ascorbic acid, and phytosterols, has been revealed through phytochemical studies. The mineral ions samples were determined by atomic absorption (Model Varian, spectra AA220). Phosphorus was determined by a UV-visible spectrophotometer, using the KH_2PO_4 standard (AOAC, 2000). Major elements, including vitamin C, Calcium, triterpene, and Ketosterones have been observed in the plant. The following percentages of components have been reported in the air-dried *Cissus quadrangularis* plant: moisture 4.02%, Total Ketosterones 10.65%, Protein 12.0%, Fiber 17.0%, carbohydrate 34.8%, and ash 15.57%, quercetin 0.016%. Additionally, the root powder is mineral abundant elements (microgram/100-gram solid content), including potassium 67, calcium 46.2, zinc 3.8, sodium 26.2, iron 8.4, Cu 0.45, and Mg 1.2. The Heavy metals i.e. Pb 0.4 ppm, Cd 0.30 ppm, As 0.3 ppm, and Hg 0.1 ppm are present in the sample and are within permissible limits.

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