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A STUDY ON ANTIOXIDANT, PROXIMATE ANALYSIS, ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *CISSUS QUADRANGULARIS* BY GC-MS

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ABSTRACT

Plants have been an important source of medicine with qualities for thousands of years. Mainly on traditional remedies such as herbs for their history it has been used as a popular folk medicine. *Cissus quadrangularis* is used as medicinal values. Screening of photochemical (Qualitative and Quantitative) analysis of *Cissus quadrangularis* shows that almost of the chemical constituents are present Tannin, Phlobatannins, Saponin, Flavonoids, Steroids, Terpenoids, and Cardiac glycosides Anthroquinones which are used in medicinal purpose. *Cissus quadrangularis* are having the antimicrobial activity against human pathogens. In 100% concentration of extraction zone of inhibition is high.But 25%, 50% and 75% shows the lowest inhibition activity. Proximate analysis indicates the nutrients efficacy. In GC-MS analysis some of the Phytocomponents are screened as Eugenol, n-Hexadecanoic acid, 1, 2-Benzenedicarboxylic acid, diisooctyl ester, Phenol, 2, 4-bis (1-phenylethyl)- are present in *Cissus quadrangularis.*

Keywords: *Cissus Quadrangularis*, Analysis, Antioxidant Activity, Proximate Analysis, Antimicrobial Activity and Phytochemical

INTRODUCTION

Plants are used medicinally in different countries and are a source of many potent and powerful drugs. *Cissus quadrangularis* is more responsive to nutrient, grows in arid

climates and widely distributed in India and other arid areas and it is used in folk medicine **[1]**. The fresh stem and leave of *Cissus quadrangularis* is used for the treatment of hemorrhoid, menstrual disorder, scurvy and as anti-flatulence. In India it is used for many diseases. Phytochemical studies of *Cissus quadrangularis* found several phytochemical constituents such as flavonoids, Triterpenoids **[2]** reported the antibacterial and antioxidant activities of the extract from *Cissus quadrangularis*.

MATERIALS AND METHODS

Collection of Plant Materials

Fresh, Healthy and non infected *Cissus quadrangularis* stem were collected in Thanjavur. Collected stems was washed with distilled water and kept it in room temperature for air dried. Dried stems was powdered and kept it in polythene bags for further uses.

Sample Preparation

Aqueous extract of *Cissus quadrangularis* samples were used to carry out the Qualitative and Quantitative analysis using standard procedures to identify the phyto constituents as described by **[2-4]**.

Phytochemical Screening

Test for Tannins [5]

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for browrish green or a blue-black coloration.

Test for Phlobatannins [6]

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatinins.

Test for Saponin [1]

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for Flavonoids [6]

5ml of the diluted ammonia solution was added to a portion of aqueous filterate of plant extract followed by the addition of concentrated sulphuric acid formation of yellow color.

Test for Steroids

Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H2S04. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for Terpenoids (Salkowski test)

Five ml of each extract was mixed in 2 ml of chloroform, and concentrated

H2S04 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for Cardiac glycosides (Keller-Killani test)

2 ml of glacial acetic acid containing one drop of ferric chloride solution was add to 5ml of the aloe vera extract. This was sunder layer with 1ml of concentrated sulphuric acid. Formation of a brown ring at the interface indicates the presence of cardiac glycosides.

Test for Anthroquinones

0.5gm of the extract was boiling with 10ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette out into another test tube & 1ml of diluted ammonia was added. The resulting solution was observed for color change.

Proximate Analysis

Proximate analysis for *Cissus quadrangularis* i.e., Moisture, Protein, Fat, Fiber, Ash and Insoluble Ash.

Antioxidant Activity

Cissus quadrangularis air dried powdered samples (10g) were extracted with 250ml of methanol using the soxhlet extractor for 72 hrs at a temperature not exceeding the boiling point of the solvent. From the extracted sample antioxidant activities was carried out by DPPH Method and FRAP Method **[7]**. Results are shown in **Table 4**.

Antimicrobial Activity

The antibacterial and antifungal activity studies were carried out by disc diffusion technique. The sterile nutrient agar plates and potato dextrose agar plates were prepared. The bacterial test organisms like, *Pseudomonas aeroginosa* and *Xanthomonas citri* were spread over the nutrient agar plates by using separate sterile cotton buds. Then the fungal test organism like *Aspergillus flavus* and *Aspergillus niger* were spread over the potato dextrose agar plates After the microbial lawn preparation three different extracts of plant disc were placed on the organism inoculated plates with equal distance control discs were also prepared. All bacterial plates were incubated at 27°C for 24 hrs and fungal plates at 24°C for 72hrs. The diameter of the minimum zone inhibition was measured in mm. For each test, three replicates were performed.

RESULTS AND DISCUSSION

Cissus quadrangularis shows that the Tannin, Saponin, Flavonoids, Terpenoids, Cardiac glycosides and Anthroquinones are present while Phlobatannins and Steroids

were absent **(Table 1)**. Phytochemical studies of *Cissus quadrangularis* found several phytochemical constituents such as flavonoids, Triterpenoids **[2]**.

Proximate Analysis

In proximate analysis *Cissus quadrangularis* showed the high fat, protein and fiber **(Table 2)**.

Determination of Antioxidant activity in *Cissus quadrangularis*

Antioxidant activity is high in *Cissus qudrangularis* about 30.60% in DPPH Method **(Table 3)**.

Antimicrobial Activity

The antibacterial activities of the ethanol and chloroform stem extract of *Cissus quadrangularis* were evaluated by disc diffusion method against two pathogenic bacteria. The ethanolic and chloroform stem extract of *Cissus quadrangularis* is ineffective to inhibit the growth of the tested bacteria. The diameter of inhibition zones for each solvent extracts were compared with the standard antibiotic chloramphenicol (30 µg/disc) **(Table 4)**. Ethanol and chloroform stem extract showed nil inhibition on the all tested fungi **(Table 5)**. The diameter of inhibition zones for each solvent extracts were compared with the standard antibiotic nystatin (30 µg/disc).

GC-MS Analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30mm×0.25mm ID ×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 El was employed (split ratio of10:1) injector temperature 250 °C; ionsource temperature 280 °C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da. **(Figure 1)**.

Identification of Components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the

components of the test materials were ascertained **Table 6**.

CONCLUSION

According to the results *Cissus quadrangularis* shows that presence of Tannin, Saponin, Flavonoids, Anthroquinones and Terpenoids are present. Proximate analysis indicates that Nutrients are present in *Cissus quadrangularis.* It contains more fat, protein, and Crude fiber. In quantitative analysis of Phytochemical content *Cissus quadrangularis* shows the maximum amount of Phytocomponents . Antioxidant activity was high in *Cissus quadran gularis* in DPPH Method in FRAP Method it shows the lowest activity. *Cissus quadrangularis* (Ethanolic Extract) shows the highest zone of inhibition in *Pseudomonas aeroginosa. Cissus quadrangularis* (Ethanolic Extract) shows the highest zone of inhibition in *Aspergillus niger. Cissus quadrangularis* Ethanolic Extracts used to screen all phytocomponents by using Gas Chromatography method. According to the results some of the medicinal value components are present in the plant extracts such as Asarone, Phytol, Phenol, 2,4-bis(1-phenylethyl)- which are all have medicinal properties. Squalene is used in cosmetics as a natural moisturizer. Oleic acid is used as an emulsifying or solubilizing agent in aerosol products. Stearic acid is useful as an ingredient in making candles, plastics, dietary supplements, oil pastels and cosmetics, and for softening rubber. The essential fatty acids are all omega-3 and - 6 methylene-interrupted fatty acids. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K1. It is used along with simple sugar or corn syrup as a hardener in candies.

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S. No.	Secondary Metabolites	Results	Inference
1.	Tannin	Brownish green colour	+
2.	Phlobatannins	No Red colour precipitation	-
3.	Saponin	Emulsion formed on the top	+
4.	Flavonoids	Yellow colour change	+
5.	Steroids	No colour change	-
6.	Terpenoids	Reddish brown colour	+
7.	Cardic glycosides	No Brown ring formed	+
8.	Anthroquinones	Formation of cloudiness	+

Table 1: Qualitative analysis of the Phytochemicals in *Cissus quadrangularis*

Table 2: Proximate Analysis of *Cissus quadrangularis*

S. No.	Parameters	Amount in Percentage (%)
		Cissus quadrangularis
1.	Moisture	20.10
2.	Ash	12.59
3.	Insoluble Ash	00.10
4.	Fat	12.31
5.	Crude Fibre	60.71
6.	Protein	04.35

Table 3: Antioxidant Activity by DPPH and FRAP Method

S. No.	Test for Antioxidant activity	*Cissus qudrangularis*
1	DPPH Method (Inhibition %)	130.608
2	FRAP Method (mM/100g)	1.96

Table 4: Antibacterial activity

S. No.	Extract	Zone of Inhibition (mm in diameter)	
		Pseudomonas aeruginosa	*Xanthomonas citri*
1.	Aqueous	-	-
2.	Ethanol	0.30 ± 0.10	1.23 ± 0.05
3.	Chloroform	-	-

Table 5: Antifungal Activity

S. No.	Extract	Zone of Inhibition (mm in diameter)	
		Aspergillus flavus	*Aspergillus orizae*
1.	Aqueous	-	-
2.	Ethanol	1.26 ± 0.25	0.70 ± 0.26
3.	Chloroform	1.13 ±	-

Table 6: Phyto Components Identified in *Cissus qudrangularis*

S. No.	RT	Name of the compound	Molecular formula	MW	Peak area %
1	4.69	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176	4.23
2	8.68	Eugenol	$C_{10}H_{12}O_2$	164	4.92
3	11.32	Undecanoic acid	$C_{11}H_{22}O_2$	186	0.87
4	11.87	Asarone	$C_{12}H_{16}O_3$	208	0.78
5	13.53	Azulene, 1,4-demethyl-7-(1-methylethyl)-	$C_{15}H_{18}$	198	2.33
6	14.00	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.91
7	15.13	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.65
8	15.34	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278	1.31
9	16.33	Pentadecanoic acid, 1,4-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	0.60
10	17.30	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	12.59
11	17.45	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	4.38
12	19.65	Phytol	$C_{20}H_{40}O$	296	2.93
13	20.40	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	27.17
14	20.74	Octadecanoic acid	$C_{18}H_{36}O_2$	284	5.52
15	24.41	Methanone, (1,4-dimethyl-7-(1-methylethyl)-2-azulenyl) phenyl-	$C_{22}H_{22}O$	302	1.36
16	25.94	Phenol, 2,4-bis (1-phenylethyl)-	$C_{22}H_{22}O$	302	3.03
17	27.26	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	26.41

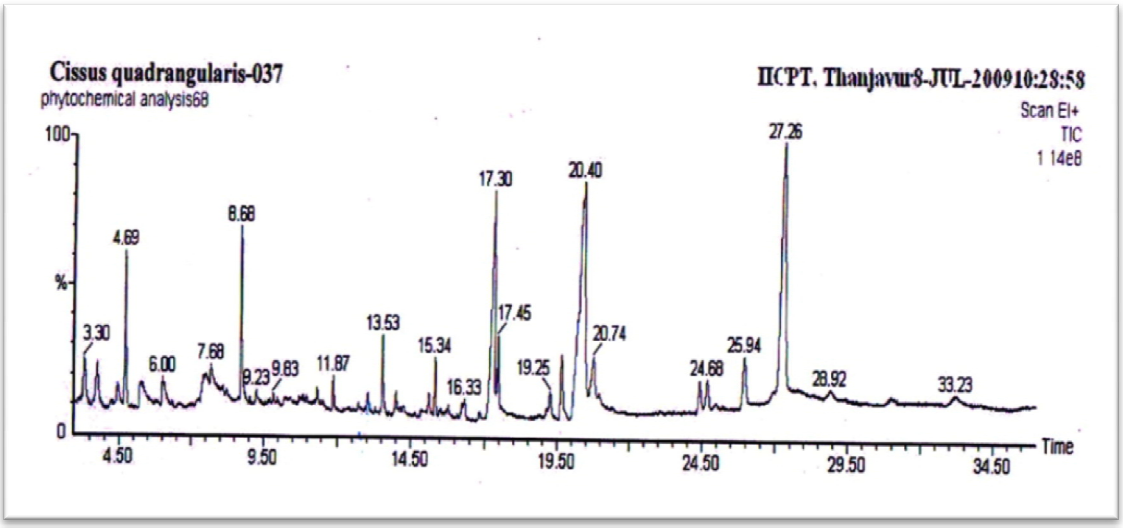

Figure 1: Phytochemical Analysis of *Cissus quadrangularis*