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PHYTOCHEMICAL ANALYSIS AND FREE RADICAL SCAVENGING ACTIVITIES OF AQUEOUS AND ETHANOLIC EXTRACTS OF *CISSUS QUADRANGULARIS*

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ABSTRACT

Phytochemicals have been recognized as the basis for traditional herbal medicine practiced in the past and currently envogue in parts of the world. In the search for phytochemicals that may be of benefit to the pharmaceutical industry, researchers sometimes follow leads provided by local healers in a region. Following such leads, plant parts are usually screened for phytochemicals that may be present. The presence of a phytochemical of interest may lead to its further isolation, purification and characterization. Then it can be used as the basis for a new pharmaceutical product. In this view, the present study was focussed on the analysis of phytochemical components present in the aqueous and ethanolic extracts of *Cissus quadrangularis* and the free radical scavenging activity of the plant extracts. Both the extracts indicated the presence of various phytochemical components. Ethanolic extract showed the presence of maximum phyto components when compared to aqueous extract of *Cissus quadrangularis*. The free radical scavenging activity was found to be more in the ethanolic extract. Total phenolic content was found to be 51mg/g in the ethanolic extract whereas the phenolic content was found to be 45mg/g in the aqueous extract. The total antioxidant capacity was found to be more in ethanolic extract of *Cissus quadrangularis*.

Keywords: *Cissus quadrangularis* ,phytochemicals, free radicals, Total phenol, antioxidant

INTRODUCTION:

Medicinal plants consider as a rich resource of ingredients which can be used in drug development and synthesis. Besides that, these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants consider as important source of nutrition and as a result of that these plants recommended for their therapeutic values. Higher plants have been used as a source of drugs by mankind for several thousand years. In fact, ancient man was totally dependent on green plants for his day-to-day needs of medicaments **[1]**. With the development of modern medicine, synthetic drugs and antibiotics, the importance of plants as raw material for drugs decreased considerably. It has been estimated that about 13,000 species of plants have been employed for at least a century as traditional medicines by various cultures around the world. A list of over 20,000 medicinal plants has been published, and very likely a much larger number of plants **[2]**.

These days the term Alternative Medicine became very common in western culture, it focus on the idea of using the plants for medicinal purpose and medicinal plants export increased like 40% in recent years and export medicinal plant worth more than 120 billion USD annually **[3]**. However, plants were used as a basis of some of the most important drugs, even in the modern system of medicine. With the advancement of synthetic organic chemistry most of the active constituents of plants used in medicine were synthesized. At one time it was thought that ultimately all the plant drugs would be obtained from synthetic sources. Medicinal plants frequently used as raw materials for extraction of active ingredients which used in the synthesis of different drugs. Like in case of laxatives, blood thinners, antibiotics and antimalaria medications, contain ingredients from plants **[4]**. Unfortunately, many medicinal plants having a promising future and most of them their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of present or future studies.

Cissus quadrangularis is one of the important medicinal plants having potential capability to cure various illness in humans like dyspepsia, anorexia, flatulence, colic, seizures, tumors, epistaxis, asthma, abnormal menstrual disorders, inflammation, antibacterial infections and obesity **[5]**. *Cissus quadrangularis* (L). is less explored perennial climber of family vitaceae, that is widespread in tropical regions of India. In recent decade of research on *Cissus quadrangularis* shows

important properties of anticancer activity. Similarly, in vitro and in vivo studies have shown that quercetin induces cytotoxic effects on colon cancer, breast cancer, leukemia cells, and ovarian carcinoma **[6]**. Toxicological reports demonstrate that the extract of *Cissus quadrangularis* (L). does not possess any adverse toxic effect. In present study was focussed on the analysis of phytochemical components present in the aqueous and ethanolic extracts of *Cissus quadrangularis* and the free radical scavenging activity of the plant extracts.

MATERIAL AND METHODS

Collection of plant materials

The *Cissus quadrangularis* plant was collected from Government herbal garden, Tiruchirappalli, Tamil Nadu, India. Further plant crude extract plant sample was washed with the help of running tap water and dried in shade. Completely dried plant powered with grinder mixer and these sample used for all the experiment of our work. For the preparation of extract, using 5g of powder in 50ml of ethanol is added and it should be soaked for 24 hours before use. Here we are using ethanol as a solvent. The extract is then preserved by hot percolation technique.

Photochemical analysis

Phytochemical analysis was using various experiments by the presence of coloration. For Terpenoids, 1ml of extract in the test tube to this we have add few drops of chloroform. After a few minutes 1ml of concentrated sulphuric acid is added to the test tube and observed few minutes. The reddish brown color is shows the presence of terpenoids. To analysis Flavonoids, 1ml of extract and add 1 or 2 drops of concentrated sulphuric acid to the test tube. After few minutes observation the colour is noted. The yellow colour indicates the presence of flavonoids. Saponins analysis experiment 1ml of plant extract, a few drops of distilled water, and shake vigorously for stable persistent froth. Then add olive oil mixed with frothing and shaken vigorously and observe. For Tannins 1ml of extract in the test tube and add 2 or 3 drops of distilled water and 0.1 % of ferric chloride were added and after the observation. The tannins is indicate by green colour of the precipitate. For Alkaloids analysis 1ml of extract is added to the test tube. To these few drops of 10% acetic acid and stands for few minutes and a few drops of ammonium hydroxide is added to the test tube. After the observation the yellow colour precipitation shows the presence of alkaloids. For Steroids 1ml of extract is added to the test tube to these few drops of chloroform is added and 2 or 3 drops of concentrated sulphuric acid were added. After few minutes observation, the reddish brown ring is shows the presence of

steroids. For Glycosides 1ml of extract, a few drops of chloroform, and 2drops of glacial acetic acid. After observation the solution turns violet into blue into green colour is indicating the presence of glycosides. For Phlobatanins, 1ml of extract is placed in to the test tube along with s few drops of concentration hydrochloric acid. The presence of Phlobatanins can be seen in red colour precipitate after observation. For Coumarins 1ml of extract is taken in the test tube added few drops of sodium hydroxide and after few minutes observed the test tube after the observation. If the solution is yellow in colour it shows the presence of coumarins. For Emodin 1ml of the extract is taken in the test tube and few drops of ammonia solution is added and 2 or 3 drops of benzene was added to the test tube contains ammonia solution and the extract. After few minutes observed, If the solution is red in colour it shows the presence of Emodin. For Anthraquione 1ml of the extract is added to the test tube and few drops of the benzene is added to it after this the ammonia solution is added to the test tube. After the observation if the solution is pink, violet or red in colour it shows the presence of anthraquinone. For Anthocyanins 1ml of extract is added to the test tube and few drops of concentrated hydrochloric acid is added and few drops of ammonia solution is added to the test tube contain conc. HCl and leaf extract after the observation of the solution is pinkish red to bluish violet colour shows the presence of anthocyanins. For carbohydrate 1ml of extract is added to the test tube and 2ml of the distilled water is added to it and few drops of ethanolic alpha -napthol added and 1 or 2 ml of concentrated sulphuric acid is added to it. After the observation the solution contains reddish violet ring formation is shows Presence of the carbohydrate. For Leucoantholyanin 1ml of extract is taken into the test tube and 1ml of isoamyl alcohol is added to it. After the observation the solution is turned organic layer into red on colour it shows the presence of Leucoantholyanin. For Cardiac Glycosides 1ml of extract is taken in test tube and add few drops of glacial acetic acid shaken well and 1 or 2 drops of ferric chloride is added and few drops of concentrated sulphuric acid is added. After a minute, the formation of browning is happened in solution. This shows the presence of cardiac glycosides. For Xanthoprotein 1ml of extract is taken in test tube and add few drops of ferric chloride. Observe the solution. After the observation the solution is blue black in colour. This shows the presence of xanthoprotein and for total protein analysis 1ml of extract taken in the test tube and few

drops of concentrated sulphuric acid added to the test tube after the observation white precipitation is occurs due to the presence of protein**.** All the absorbance measurement was taken using UV/Vis double beam spectrophotometer **(Systronix, Model 2202)**.

Determination of total phenolics

The total phenol content in plant extracts was determined by Folin-Ciocalteu method using Gallic acid as standard with slight modification. The reaction mixture consists of 1 ml of extract and 4.5 ml of distilled water was taken and then add 1ml of Folin-Ciocalteu phenol reagent the mixture was shaken well. Then after 5 minutes 5 ml of 7% sodium carbonate solution was added to the mixture. The standard solutions of gallic acid were prepared in concentrations range of (20, 40, 40, 60, 80 and 100) µg/ml. The mixture was incubated for 90 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an Ultraviolet (UV) /visible spectrophotometer. Total phenol content was expressed as mg of GAE/gm of extract.

DPPH ASSAY METHOD

The antioxidant activity of extract was examined by stable DPPH free radical activity. Ethanolic solution of DPPH (0.05 mM) (500µl) was added to 1 ml of ethanol extract of sample with the different concentrations (20, 40, 60, 80, 100µg/ml). The freshly prepared DPPH solution was kept in the dark at 4˚C. The mixture was kept to stand for 5 minutes at 540nm, absorbance was measured spectrophotometrically. Absorbance was set to zero by using distilled water. A blank sample contains the same amount of distilled water and DPPH was prepared. The radical activity of the tested samples, expressed as percentage of inhibition was calculated.

Percent (%) inhibition of DPPH activity = [(A-B)/A] x 100.

Where A and B – absorbance values of control and test sample, respectively.

GC – MS Analysis

GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-1 fused silica capillary column (30mm×0.25mm I.D ×1 µ M 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 µ l was employed (split ratio of10:1) injector temperature 250 °C; ion-source 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 45 to 450 Da. Total GC running time is 46min.

RESULT AND DISCUSSION

In the past few years, there was an incredible growth in herbal medicines due to their protected action against many awful diseases without any harmful side effects. As a result, the herbal drugs dominated a major share of all medicinal systems like Ayurveda, Unani, Yoga, Siddha, Homeopathy and Naturopathy. Among medicinal plants listed are now are evaluated for specific diseases like infection, cancer, AIDS, Hepatitis, etc. Selection of plants for such herbal medicines with precise therapeutic values is the need of the hour. *Cissus quadrangularis* is one such species which is not only known for its nutritional value but also for various other diseases and their root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil were used for various ailments in the indigenous system of medicine for treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepatorenal disorders **[7]**. In the present study secondary metabolites were isolated from *Cissus quadrangularis using* different solvents showed the occurrence of Alkaloids, Saponins, Flavonoids, Terpenoids, Phenols, Glycosides and Phytosteroids **(Figure 1)**. Different samples of *Cissus quadrangularis* were dissolved in different solvents to find out the Phytochemical chemical compounds. The alkaloids were measured as one of the secondary metabolites which consists of heterocyclic nitrogen atom. Alkaloid in *Cissus quadrangularis* stem is one of the largest phytochemical groups of compounds which have variety of medicinal properties such as it was used as a pain killer for much kind of diseases **[8]**. The stem of *Cissus quadrangularis* was rich in Flavonoids and it also has the antidibetic activity. This type of flavones has the ability to inhibit the enzyme which regulates the glucose level in the blood **[9]**. Lignins were commonly called as phenolic substances which was polymeric in nature and it can able to precipitates the gelatin from any solution **(Figure 2) [10]**. Tannins were often called as natural polyphenols and the plant extracts with the tannins were used to treat the diseases like allergy, diarrhea, urinary infections, stomach and duodenal cancer **[11]** and it also possessed to have the anti-inflammatory effect. Tannins also have the capability to stop the replication process of the HIV virus **[12]**.

Phenols were considered as the biggest group of phytochemicals present in the different plant kingdom and also distributed extensively in the plant species. The main biochemical activity of the phenols was it served as an antioxidant **[13]**. The plant terpenes were called as Terpenoids, one of the secondary metabolite compounds present in different parts of the plant. This type of compound exists as phytoalexin in the plants and it was involved in the defense mechanism against pathogens **[14]**. Saponins were found to be acts as a defensive agent for the plants such that it was called as plant protectant **[15]**. The medicinal property of the Saponins includes antioxidant, anticancer, antifungal and antiviral. The nutritional supplement like carbohydrates, protein, fat, fiber and ash contents have been determined from the different types of samples taken from the *Cissus quandragularis*. The elevated amount of protein in the plant species will promotes the health and prevents it from the diseases. The deficiency of carbohydrates will affect the function of the body system; whereas optimum level of carbohydrate was needed for the functioning of brain, heart and immune system **[16]**. Antioxidants have a therapeutic effect by quenching free radicals which are harmful molecules generated as metabolic products by natural cells. The immune system has several pathways to combat oxidative stress by generating antioxidants that are either produced naturally *in situ* or delivered externally through foods and/or supplements. These antioxidants function as radical scavengers by reducing and restoring ROS-induced cell damage, thus enhancing immune protection and reducing the chance of cancer and degenerative diseases **(Table 1)**. In our GC-MS analysis we have identified various stress related bioactive compounds in different retention time. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in **(Table 2 and Figure 4)**. The compounds identified in GS-MS are directly involving in antioxidant and a preventive agent against epoxide-induced breast cancer carcinogenesis. It's also an efficient vaccine adjuvant with no adverse auto-immune effects **[17]**.

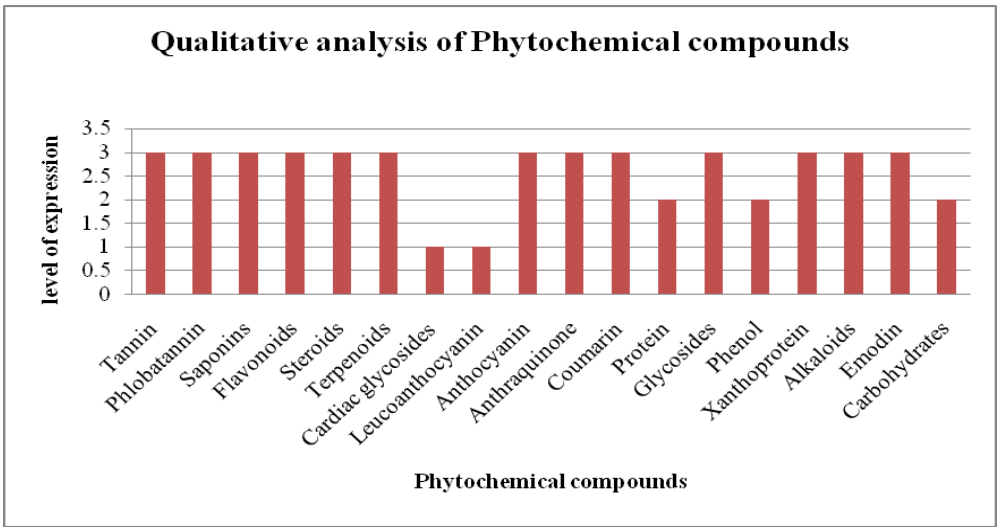


Figure 1: Determination of Phyochmicals

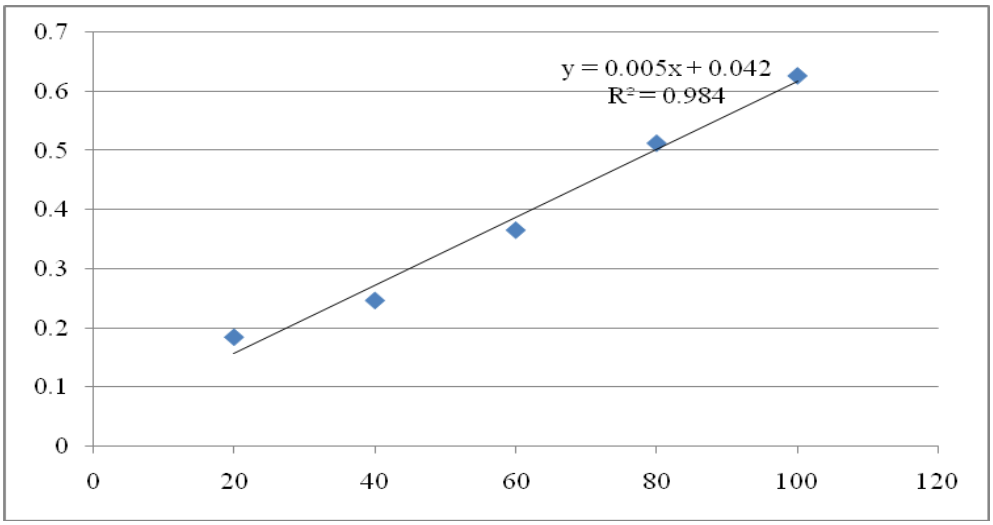


Figure 2: Standard calibration curve for total phenolic content for standard gallic acid with ethanol extract

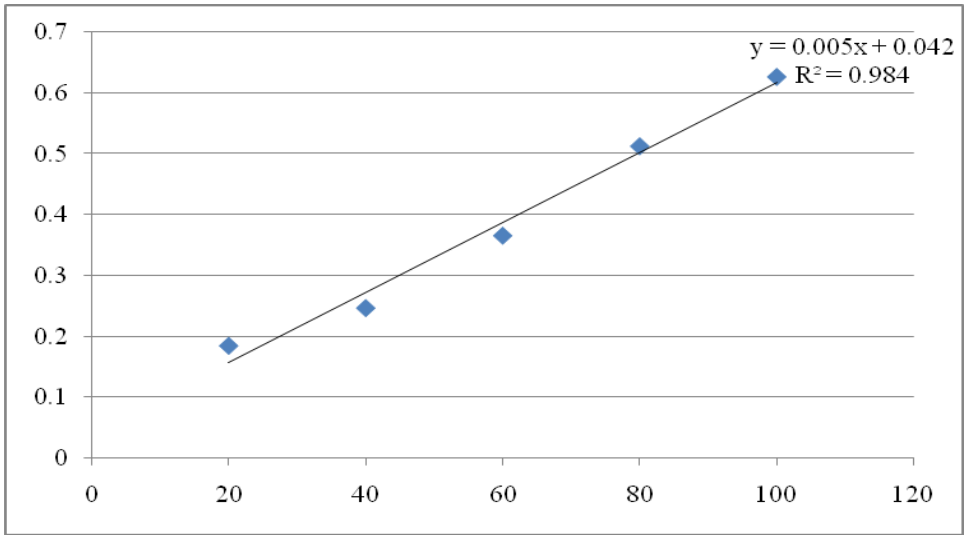


Figure 3: Standard calibration curve for total antioxidant content for standard ascorbic acid with ethanol extract

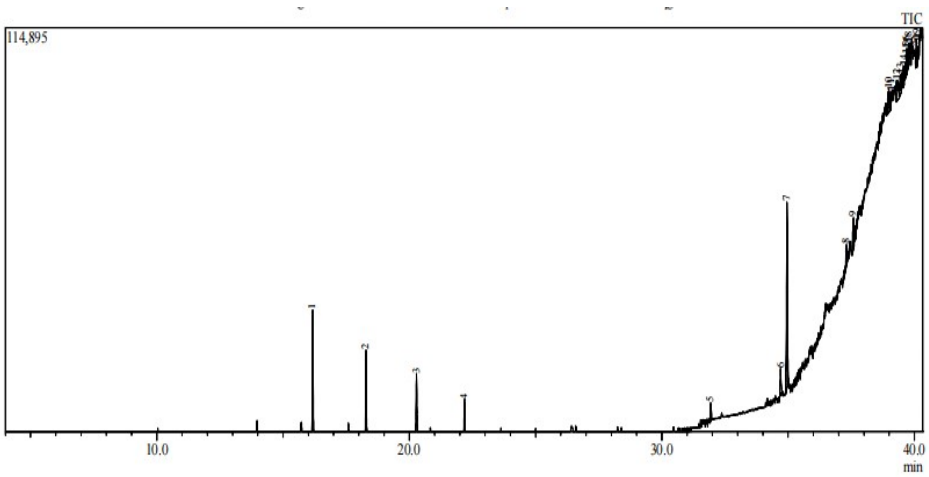
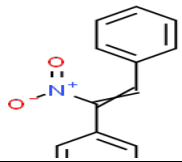
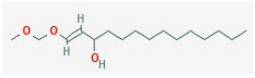
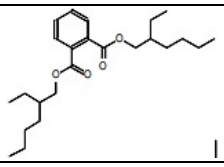


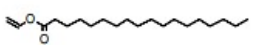
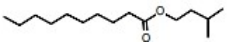
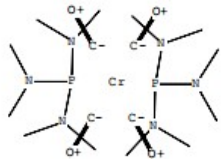
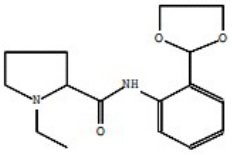
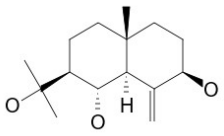
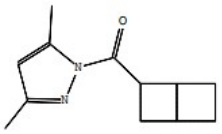
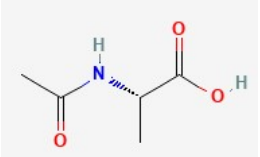
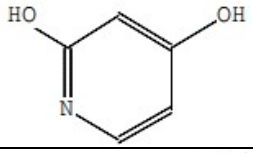
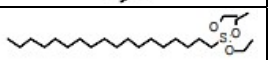
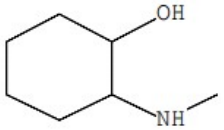
Figure 4: GC-MS analysis of *Cissus quadrangularis* leaves

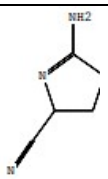
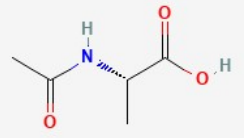
Table 1: Antioxidant activity of ethanol extract by DPPH activity

S. No.	CONCENTRATIONS	SCAVENGING EFFECT (%)	
		ETHANOL EXTRACT	ASCORBIC ACID
1	20(µg/ml)	49.53	48.33
2	40(µg/ml)	51.66	70
3	60(µg/ml)	66.66	81.66
4	80(µg/ml)	72.50	90.83
5	100(µg/ml)	83.33	98
	IC50 Value	27.14	13.81

Table 2: The Bioactive chemical composition of *Cissus quadrangularis*

Peak	Name	Retention time	Area %	Height %	MW	Molecular formulae	Structure
1	UNDECANE	16.174	13.22	14.86	156	C11H24	
2	OCTADECANE	18.284	8.4	9.96	254	C18H38	
3	PENTADECANE	20.284	6.14	7.1	212	C15H32	
4	PENTADECANE	22.184	3.36	3.96	212	C15H32	
5	1-NITRO-1-PHENYLCYCLOHEXANE	31.93	2.72	2.27	205	C12H15NO2	
6	(E)-2,3-EPOXY-1-(METHOXYMETHOXY)TETRADECANE	34.699	3.34	3.23	272	C16H32O3	
7	1,2-BENZENEDICARBOXYLIC ACID	34.953	26.57	22.67	390	C24H38O4	

8	Octadecanoic acid, ethenyl ester	37.311	4.79	2.59	310	C20H38O2	
9	3-METHYLBUTYL DECANOATE	37.585	4.89	3.86	242	C15H30O2	
10	SILICONE GREASE, SILICONFETT	38.983	4.56	2.89	9999		
11	CHROMIUM, TETRACARBONYLBIS (HEXAMETHYLPHOSPHOROUS TRIAMIDE-P)-, (OC-6-22)-	39.058	2.13	2.3	490	C16H36CrN6O4P2	
12	Pyrrolidine-2-carboxamide, 1-ethyl-, N-(2'-1,3-dioxolan-2-ylphenyl)-	39.293	5.26	2.58	290	C16H22N2O3	
13	1,5-NAPHTHALENEDIOL, DECAHYDRO-1-METHYL-4-(1-METHYLETHYL)-, (1.ALPHA.,4.BETA.,4A.BETA.,5.ALPHA.,8A.BETA.)-(.+-.)-	39.457	3.4	2.34	226	C14H26O2	
14	1-(BICYCLO[2.2.0]HEX-2-YLCARBONYL)-3,5-DIMETHYL-1H-PYRAZOLE	39.572	2.05	2.46	204	C12H16N2O	
15	L-ALANINE, N-(N-ACETYL-L-TYROSYL)-	39.68	1.66	2.62	294	C14H18N2O5	
16	2(1H)-PYRIDINONE, 4-HYDROXY-	39.7	1.44	3.12	111	C5H5NO2	
17	Octadecyltriethoxysilane	39.745	1.01	2.38	163	C24H52O3Si	
18	2-(METHYLAMINO)CYCLOHEXANOL #	39.767	1.8	2.81	129	C7H15NO	

19	5-AMINO-3,4-DIHYDRO-2H-PYRROLE-2-CARBONITRILE	40.076	2.42	3.45	109	C5H7N3	
20	L-ALANINE, N-(N-ACETYL-L-TYROSYL)-	40.109	0.85	2.53	294	C14H18N2O5	

CONCLUSION

Cissus quadrangularis a traditionally important medicinal plant used to treat various ailments. In the present view phytochemical constituents of different extracts of *Cissus quadrangularis* was studied. This plant has a very rich source of minerals useful for functioning of human body and more research is needed to study the plant to treat various ailments.

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