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**Advances in Applied Science Research, 2016, 7(4):11-17**

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**ISSN: 0976-8610**
**CODEN (USA): AASRFC**

# Studies on Qualitative and Quantitative Phytochemical Analysis of *Cissus quadrangularis*

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**ABSTRACT**

*Plants have served human beings as a natural source for treatments and therapies from ancient times, amongst them medicinal herbs have gain attention because of its wide use and less side effects. In the recent years plant research has increased throughout the world and a huge amount of evidences have been collected to show immense potential of medicinal plants used in various traditional systems, thus in the present investigation the phytochemical analysis of Cissus quadrangularis was carried out as these plants have been proved to be one of the important medicine for treatment of bone fractures. The phytochemical analysis was carried out for the different parts of the plant extracted with methanol and ethanol solvents. The qualitative analysis showed that alkaloids were mainly seen in most of the samples except methanolic extract of stem and fruit. Tannins, proteins, carbohydrate and phenol were present in all the 4 samples extracted by both methanol and ethanol. Flavonoids were seen only in leaf samples where as cardiac glycosides were seen only in stem samples extracted with methanol. Saponins were mainly present in the samples extracted from methanolic solvent. The qualitative analysis carried out for the determination of phenols, carbohydrates, tannins, alkaloids and proteins. Tannins were seen mainly in leaf samples of both the extracts, ethanol extract of the fruit shown the maximum amount of phenol and proteins. The leaf samples extracted by the solvents possessed contained high carbohydrate content. The presence of high amount of phytochemical compounds suggest that the Cissus quadrangularis plant has higher medicinal value and can be extensively studied to extract the natural compounds which are beneficial to human beings and that could be commercialized for higher production than using synthetic drugs with side effects.*

**Key words:** *Cissus quadrangularis,* Photochemical, Qualitative analysis, Tannins.

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## INTRODUCTION

Medicinal plants and herbs have been proved to be of great importance to the health of the individuals and communities. In recent years, many scientific investigations of traditional herbal remedies for several diseases have been carried out and this has lead in the development of alternative drug and therapeutic strategies. Since the consumption of medicinal plants is increasing, it is interesting to use these plants as a supplement in food taking into account that these plants can present a significant amount of trace elements [1, 2, 3, 4] and other nutrients.

*Cissus quadrangularis* is one such plant which is been studied for its medicinal properties like its useful in bone fractures [5, 6, 7], obesity [8] and neuropharmacological effects [9]

*Cissus quadrangularis* is the most common species, belonging to the family Vitaceae, commonly known as "Hadjod". It is an ancient medicinal plant native to the hotter parts of India and Ceylon. It is said to be also present in some parts of Srilanka, Malaya, Java and West Africa [10]. In Ayurveda, the plant has been documented for its medicinal uses in gout, syphilis, venereal diseases, piles, leucorrhoea [11]. The Phytochemical studies of *Cissus quadrangularis* using different solvent extracts revealed that the plant contains a high amount of ascorbic acid, carotene, phytosterol substances, and calcium [12], and there are a very chemical compounds like β-sitosterol, δ-

amyrin, and δ-amyrone [13] which are of great importance. All of these components have potentially different metabolic and physiological effects.

The other properties from the extract of *Cissus quadrangularis* have been investigated such as the antibacterial activity [14], antioxidant activity [15, 16], antiulcer activity[16], analgesic and anti-inflammatory [17, 18, 19] antiosteoporotic [15], proteolytic [20,21], mutagenetic and genotoxic activity [22] which makes the plant a natural source of chemical compounds with medicinal value that can be used as a drug to cure diseases and making it a plant with greater commercial value.

## MATERIALS AND METHODS

***Sample Collection:***
Fresh & healthy plant parts of *Cissus quadrangularis* like stem, leaf, flower, fruit & root were collected in a separate sterile polythene bags from the area in and around Arogyavaram, Madanapalli (tq), Chittor (dist), Andhra Pradesh. Collected plant parts were examined and identified with the help of regional floras. Specimens were further confirmed with reference to Herbarium sheets available in the department of Botany, Tumkur University, Tumkur, Karnataka, India.

***Preparation of Solvent Extracts***
The cleaned, healthy plant materials are cut in to small sections and dried under shade for three to four weeks. The dried material was ground into fine powder in an electric grinder. Powder so obtained was stored in desiccators setup and used for extraction. Extraction was carried out using 1gm of each sample coarsely powdered plant material with 25 ml of solvent and kept for 48 hrs with slight shaking. Here, ethanol and methanol (HPLC grade) was used for extraction. The extraction was done at room temperature. All the extracts were filtered through Whatmann No.1 paper to get filtrate as extracts and were dried to concentrate the samples. The residual power was weighed and was re dissolved in the respective solvents to get a final concentration 1mg/ml. The powder was stored in airtight containers under refrigeration condition

**PHYTOCHEMICAL ANALYSIS**
***QUALITATIVE ANALYSIS***
Following standard protocols were used for qualitative analysis of samples to check for the presence of Alkaloids, Carbohydrates, Cardiac glycosides, Flavonoids, Phenols, Saponins, Tannins, Terpenoids, Quinones and Proteins.

***Test for Flavonoids:***
2 ml of each extract was added with few drops of 20% sodium hydroxide, formation of intense yellow colour is observed. To this, few drops of 70% dilute hydrochloric acid were added and yellow colour was disappeared. Formation and disappearance of yellow colour indicates the presence of flavonoids in the sample extract.

***Test for Alkaloids*:** To 1 ml of each extract, 1 ml of marquis reagent, 2ml of concentrated sulphuric acid and few drops of 40% formaldehyde were added and mixed, appearance of dark orange or purple colour indicates the presence of alkaloids.

***Test for Saponins:***
To 2 ml of each extract, 6 ml of distilled water were added and shaken vigorously; formation of bubbles or persistent foam indicates the presence of saponins.

***Test for Tannins:***
To 2 ml of each extract, 10% of alcoholic ferric chloride was added; formation of brownish blue or black colour indicates the presence of tannins.

***Test for Phenols:***
To 2 ml of each extract, 2 ml of 5% aqueous ferric chloride were added; formation of blue colour indicates the presence of phenols in the sample extract.

***Test for Proteins:***
To 2 ml of each extract, 1 ml of 40% sodium hydroxide and few drops of 1% copper sulphate were added; formation of violet colour indicates the presence of peptide linkage molecules in the sample extract.

***Test for Cardiac Glycosides:***
To 1 ml of each extract, 0.5ml of glacial acetic acid and 3 drops of 1% aqueous ferric chloride solution were added, formation of brown ring at the interface indicates the presence of cardiac glycosides in the sample extract.

***Test for Terpenoids:***
Take 1 ml of extract of each solvent and add 0.5 ml of chloroform followed by a few drops of concentrated sulphuric acid, formation of reddish brown precipitate indicates the presence of terpenoids in the extract.

***Test for Carbohydrates:***
Take 1 ml of extract, add few drops of Molisch's reagent and then add 1 ml of concentrated sulphuric acid at the side of the tubes. The mixture was then allowed to stand for 2 to 3 minutes. Formation of red or dull violet colour indicates the presence of carbohydrates in the sample extract.

***QUANTITATIVE ANALYSIS***
Depending on the above qualitative results the quantitative assay is carried out for Alkaloids, Tannins, Phenols, Proteins and Carbohydrates.

***Total Tannins Content Determination:***
The tannins were determined by slightly modified Folin and Ciocalteu method. Briefly, 0.5 ml of sample extract is added with 3.75 ml of distilled water and added 0.25 ml of Folin Phenol reagent, 0.5 ml of 35% sodium carbonate solution. The absorbance was measured at 725 nm. Tannic acid dilutions (0 to 0.5mg/ml) were used as standard solutions. The results of tannins are expressed in terms of tannic acid in mg/ml of extract.

***Total Phenol Content Determination:***
The phenols were determined by slightly modified Folin and Ciocalteu method. Briefly, to the 200µl of the sample extract, 800 µl of Folin Ciocalteu reagent mixture and 2 ml of 7.5% sodium carbonate added. The total content is diluted to 7 volumes with distilled water and finally kept the tubes for 2 hrs incubation in dark. The absorbance was measured at 765 nm. Gallic acid dilutions were used as standard solutions. The results of phenols are expressed in terms of Gallic acid in mg/ml of extract.

***Total Protein Content Determination:***
The total proteins content was determined by using Bradford's method. Briefly, to the 100 µl of the sample extract add 3 ml of Bradford's reagent and incubate in dark for 5 minutes. The absorbance was measured at 595nm. Bovine serum albumin dilutions (0.1mg/ml to 0.5mg/ml) are used as standard solutions.

***Total Alkaloid Content Determination:***
40 ml of 10% acetic acid in ethanol was added to 1g of powdered sample, covered and allowed to stand for 4 hours. The filtrate was then concentrated on a water bath to get 1/4th of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and collected precipitate was washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed.

***Total Carbohydrate determination:***
For estimating the polysaccharide content, take 1ml of sample solution and add 1 ml of 5% phenol and then add 5 ml of concentrated sulphuric acid mix well and leave for 10 minutes. Measure the absorbance at 488 nm against blank. Then compare it with standard solution of glucose. To prepare blank, 1 ml of distilled water added to1 ml of 5% phenol followed by 5 ml of concentrated sulphuric acid.

## RESULTS AND DISCUSSION

***QUALITATIVE ANALYSIS***
Alkaloids were mainly seen in most of the samples except methanolic extract of stem and methanolic extract of fruit. Tannins, proteins, carbohydrate and phenol were present in all the 4 samples extracted by both methanol and ethanol. Flavonoids were seen only in leaf samples where as cardiac glycosides was seen only in stem extracted when extracted with methanol. Saponins were mainly present in the samples extracted from methanolic solvent.

**Table 1 - Qualitative Analysis of Phytochemicals present in CQ Plant Parts**

| | PLANT PARTS | STEM | | ROOT | | LEAVES | | FRUIT | |
|---|---|---|---|---|---|---|---|---|---|
| | SOLVENTS | M | E | M | E | M | E | M | E |
| 1 | Alkaloids | + | - | + | + | + | + | - | + |
| 2 | Flavonoids | - | - | - | - | + | + | - | - |
| 3 | Tannins | + | + | + | + | + | + | + | + |
| 4 | Terpenoids | + | + | + | + | + | + | - | - |
| 5 | Saponins | + | - | + | - | + | - | - | - |
| 6 | Cardiac glycosides | + | - | - | - | - | - | - | - |
| 7 | Proteins | + | + | + | + | + | + | + | + |
| 8 | Carbohydrates | + | + | + | + | + | + | + | + |
| 9 | Phenols | + | + | + | + | + | + | + | + |

*(+ Present, - Absent of the particular compound)*

## *QUANTITATIVE ANALYSIS*

### *Total Tannin Content*

Tannins are present in all the samples extracted by both the solvents, the tannins are present majorly in leaf samples extracted by both the solvents, tannins are highly present in ethanolic extract from fruit sample

**Figure 1: Tannic acid concentration in fruit sample**

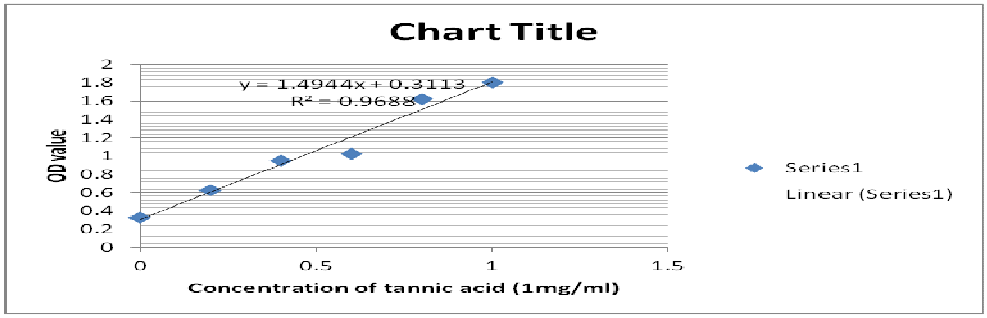
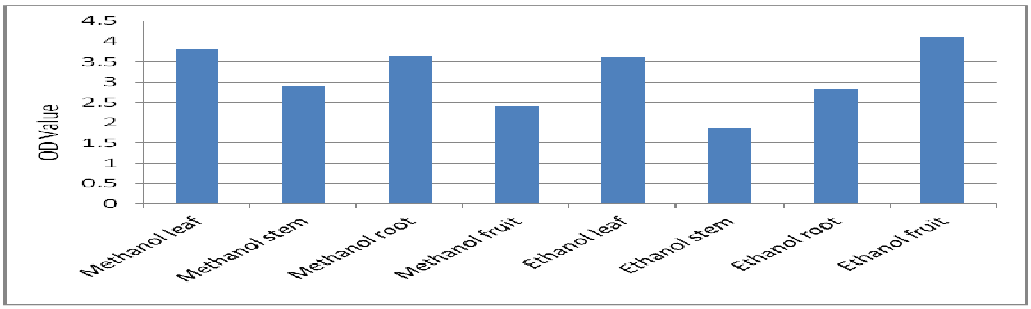



**Figure 2: Tannic acid concentration in different sample extracts**




### *Total Phenol Content*

Phenolic compounds are present in all stem, root, leaf and fruit samples extracted by both methanol and ethanol. The ethanol extract of the fruit showed maximum amount of phenol when compared with a standard of Gallic acid.

**Figure 3: Total Phenol Content**

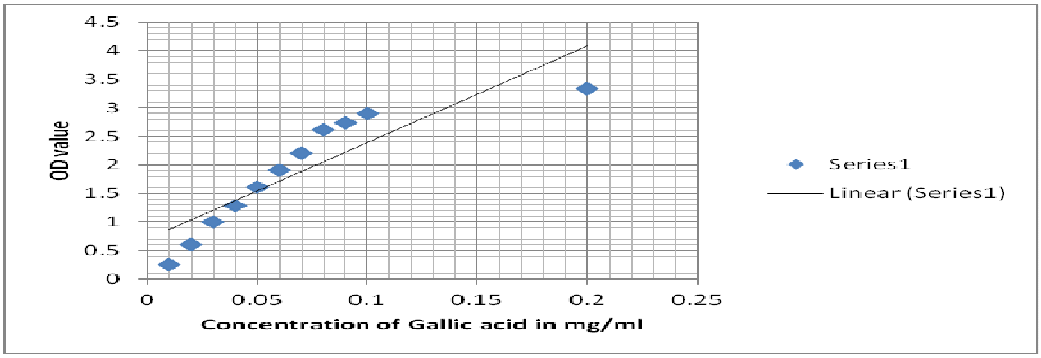
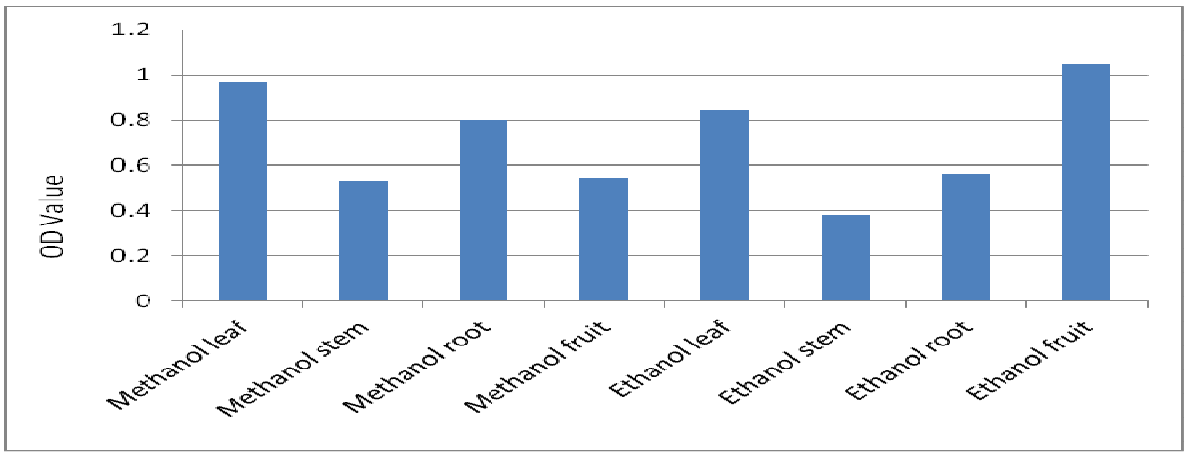


**Figure 4: Total Phenol Content in different sample extracts**




***Total Protein Content***

The proteins were detected by a standard of BSA, the ethanolic extract of fruit showed maximum content of total protein. The root samples showed least protein content when extracted by both the solvents.

**Figure 5 : Toal Protein content**

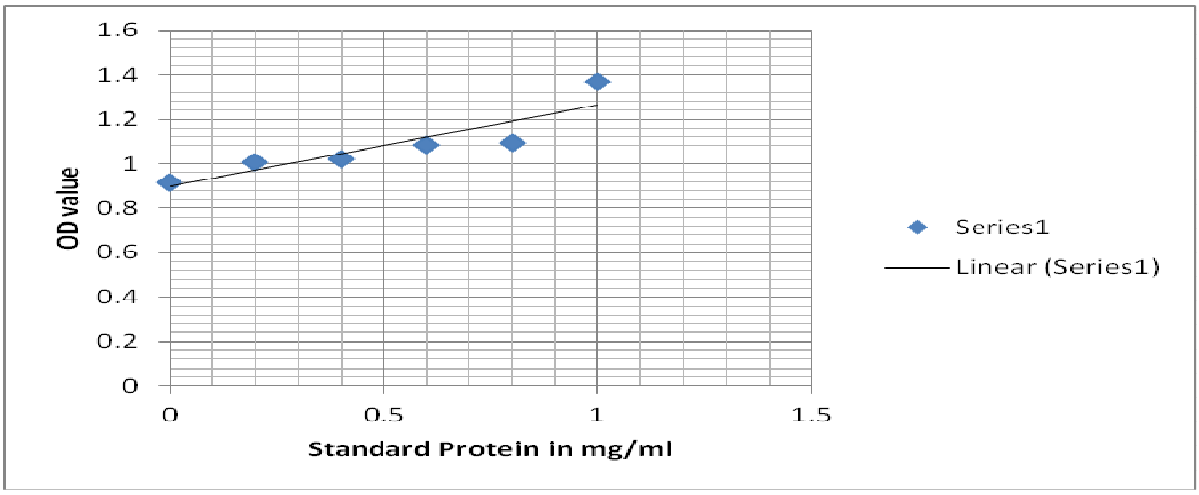
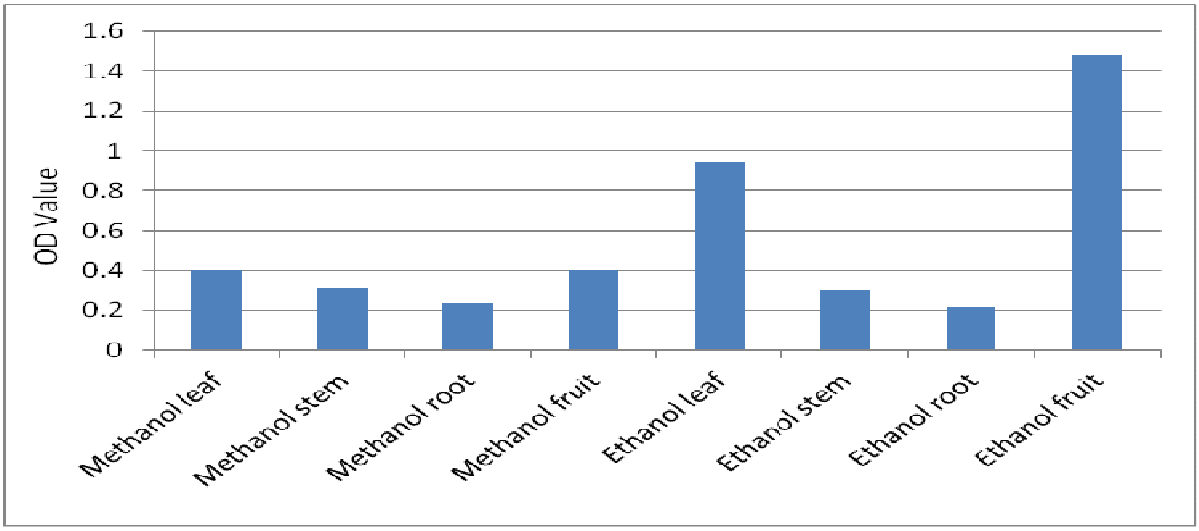



**Figure 6 : Total Protein Concentrations in different sample extracts**



***Total Carbohydrate Content***

The proteins were detected by a standard of BSA, the ethanolic extract of fruit showed maximum content of total protein. The root samples showed least protein content when extracted by both the solvents.

**Figure 7: Total Crbohydrate concentrations**

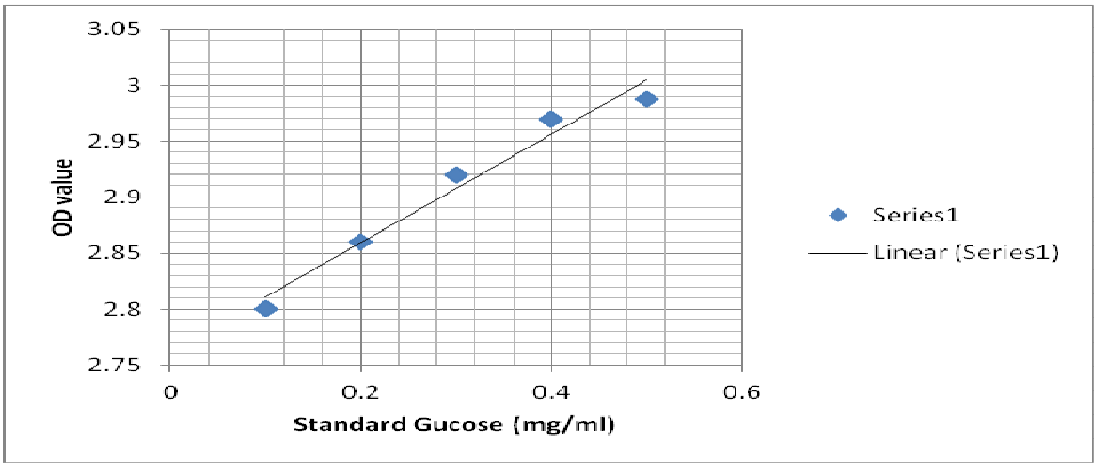
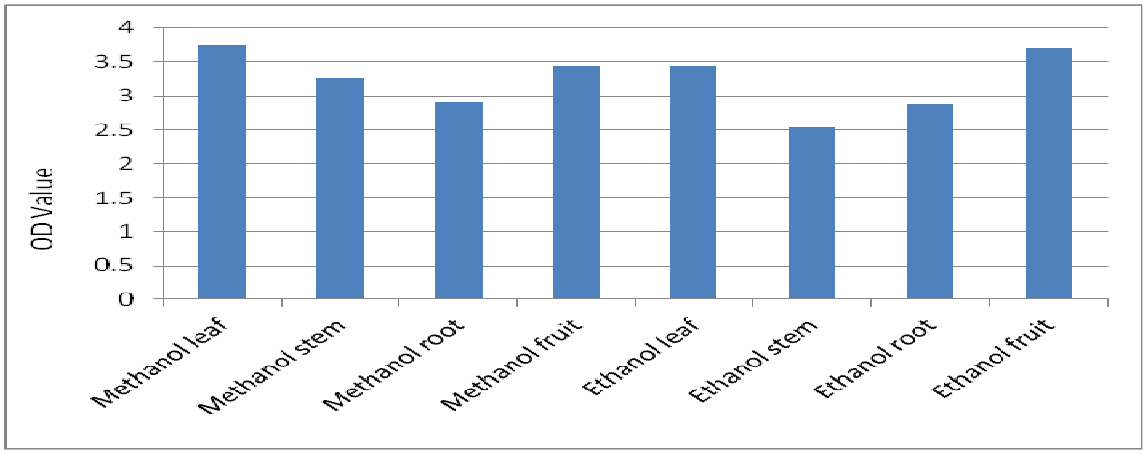



**Figure 8 : Total Carbohydrate Concentrations on different solvent extracts**




## CONCLUSION

*Cissus quadrangularis* has been proved to be effective in management of obesity and complications associated with metabolic disorders [23], as well as its antioxidant and free radical scavenging activity *in vitro* [24, 25].

Phytochemical studies of *Cissus quadrangularis* have shown the presence of various versatile constituents such as flavanoids, triterpenoids, vitamin C, stilbene derivatives and many others like resveratrol, piceatannol, pallidol perthenocissin and phytosterols. Out of which ascorbic acid, triterpene, β-sitosterol, ketosteroid, two asymmetrical tetracyclic triterpenoids and calcium were identified as major constituents of this plant [26, 27, 28].

*Cissus quadrangularis* contains high amount of carotene A, anabolic steroidal substances and calcium. The aerial parts of *Cissus quadrangularis* are found to contain a new asymmetric tetracyclic triterpenoid, 7-Oxo-Onocer 8-ene-3 β 21 α diol. The stem of the plant contains two asymmetric tetracyclic triterpenoids, onocer – 7 ene 3 α, 21 β diol and onocer - 7 ene – 3 β, 21 α diol [29, 30, 31, 32, 33].

The air-dried *Cissus quadrangularis* plant reported to contains (%) moisture 13.1, protein 12.8, wax 1.0, fiber 15.6, carbohydrate 36.6, mucilage and pectin 1.2 and ash 18.2%. The root powder also contain a rich source of mineral elements (mg/100g dry matter): potassium 67.5, calcium 39.5, zinc 3.0, sodium 22.5, iron 7.5, lead 3.5, cadmium 0.25, copper 0.5 and magnesium 1.15 [34, 35, 36, 37].

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