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Phytochemical Evaluation and Anti Diabetic Effect of *Cissus quadrangularis*

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ABSTARCT: The present study aims to investigate the phytochemical screening and *In-vitro* anti-diabetic activity in stem of *Cissus quadrangularis* commonly known as Veldt Grape. Phytochemical analysis showed the presence of secondary metabolites like flavonoids, phenols, alkaloids, tannins, glycosides and terpenoids in different solvents extracts from which anti diabetic study was carried out in 70 % of ethanolic extract stem of *Cissus quadrangularis* by α -amylase inhibitory activity and glucose uptake assay. The findings shows the potential benefits of the plant as described in traditional medicine. *Cissus quadrangularis* may be used in the long term management of diabetes.

KEYWORDS : *Cissus quadrangularis*, antidiabetic activity, α -amylase inhibitory activity, glucose uptake assay

I. INTRODUCTION

Traditional medicines are still used as part of the habitual treatment of various diseases [1]. About 60 % of the total world population remains dependent on traditional medicines for their health. There are thousands of species of plants known to have medicinal values [2]. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate diseases. The most important of these biologically active constituents of plants are alkaloids, flavonoids, tannins and phenolic compounds.

Cissus quadrangularis, is a perennial tendril climber which belongs to family Vitaceae which is commonly known as "Veldt grape" has been used for thousands of years in Indian System of Medicine for its diverse healing properties [3]. Extracts of this plant particularly stem and leaves have been traditionally used for various ailments in the traditional Ayurvedic and Siddha system of medicine [4]. It possesses various pharmacological activities such as anti-oxidant [5], anti-diabetic [6], antiosteoporotic [7], anti-inflammatory [8], analgesic [9], immunomodulatory [10], hepatoprotective [11], neuroprotective [12], antipyretic effects [13], gastroprotective effect [14].

Considering all these facts the present study was designed to evaluate various phytochemicals present in stem of *Cissus quadrangularis* a plant which evokes numerous therapeutic effects.

II. MATERIALS AND METHODS

PLANT COLLECTION AND AUTHENTICATION The stem material of the *Cissus quadrangularis* was collected from maraimalai nagar, Chennai, Tamil Nadu. The specimen was identified by expertise of the Anna Siddha medical college, Anna nagar, Chennai – 600 040. The stem part were separated from the other parts of the plant, washed, cleaned and air dried for further use.

PREPARATION OF PLANT EXTRACTS :The stem of *Cissus quadrangularis* was air-dried at room temperature (26°C) for 2 weeks, after which it was ground to a uniform powder. The extracts of the stem samples were prepared in a sequential procedure by soaking 2 g of dried powder in 20 ml of different solvents (Ethanol, chloroform and aqueous

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solution) for 48 hours. At the end of respective extraction, the extracts were filtered using Whatman filter paper. The filtrate was concentrated under reduced pressure in vacuum at 40°C for 25 minutes using a rotary evaporator. The yield of extracts was calculated. The filtrate was collected. It was stored at 4°C for further use.

Phytochemical Screening

Each extract was then tested for the presence of Phenols, flavonoids, alkaloids, Saponins, Terpenoids, Glycosides, Reducing sugars and Proteins. Tests were carried out on the 70 % ethanol extract to identify the phyto constituents using standard procedures [15-19].

1. Detection of alkaloids (Evans, 1997)

Solvent free extract (50mg) was stirred with 2 ml of dilute hydrochloric acid (1ml of hydrochloric acid and 1ml of distilled water) and filtered. The filtrate was tested carefully used for various alkaloidal reagents as follows:

A) Mayer's test (Evans, 1997) : To a 2 ml of filtrate, two drops of Mayer's reagent were added to the side of the test tube. The positive result was obtained by Creamy color precipitate

B) Wagner's test (Wagner, 1993) To a 2 mL of filtrate, 3-5 drops of wagner's reagent was added [2 g of potassium iodide and 1.27 g of iodine in 100 ml of distilled water, A reddish- brown precipitate indicated the test as positive.

2. Detection of phenolic compound

i) Ferric chloride test (Mace, 1963)

50 mg of extract was dissolved in 5ml of distilled water and then few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of Phenol.

3. Detection of glycosides (Evans, 1997)

5ml of concentrated hydrochloric acid was added to 50 mg of the extract which was hydrolysed for 2hours on a water bath which was filtered and the hydrolysate .

To 2 ml of filtrated hydrolysate, 3 ml of chloroform was added which was shaken . The chloroform layer was separated . To the test tube 10% ammonia solution was added. Pink colours indicate the presence of glycosides.

4. Detection of terpenoids

Salkowski test

0.5 g of the extract was added in 2 ml of chloroform. Concentrated H₂SO₄ (3ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

5. Detection of flavonoids

Alkaline reagent test (Evans, 1997): 0.5g of extract was dissolved in 5mL of Distilled water. An aqueous solution of the extract was treated with 10 % ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

6. Detection of tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration

7. Detection of reducing sugars (Ramakrishnana et al., 1994)

The extract (100mg) was dissolved in 5ml of water and filtered. The filtrate was subjected to the following test.

i) Molish's test

To 2 ml of filtrate, two drops of alcoholic solution of α -naphthol were added, the mixture was shaken well and 1 ml of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. Violet rings indicate the presence of carbohydrates.

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ii) Fehling's test

One mL of filtrate was boiled on water bath with 1ml each of fehling's solution A and fehling's solution B . A red precipitate indicated the presence of sugar.

iii) Barfoed's test

To 1 ml of filtrate, 1 ml of Barfoed's reagent was added and heated on a boiling water bath for 2 min. Red precipitate indicated presence of sugar.

iv) Benedict's test

To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 min. Characteristic coloured precipitates indicate the presence of sugar.

8. Detection of saponins

Foam test (Kokate, 1999)

The extract (50 mg) was diluted with 5mL distilled water. The suspension was shaken in a graduated cyclinder for 15 min. A 2cm layer of foam indicated the presence of saponins.

9. Detection of proteins (Ruthmann, 1970)

The extract (100 mg) was dissolved in 5mL of distilled water and filtered through Whatmann No.1 filter paper and the filtrate was subjected to tests of proteins and amino acids. 0.2% Ninhydrin solution was added (5 drops). Violet/Purple colour indicates positive for Proteins and aminoacids.

III. QUANTITATIVE ANALYSIS OF THE PHYTOCHEMICALS

Tests were carried out on the 70 % ethanol extract of stem of *Cissus quadrangularis* to identify the phyto constituents using standard procedures [20-24] .

Estimation of Alkaloids (Harborne and Baxter, 1983)

5 g of the sample was weighed taken in 250 ml beaker. 200 ml of 10% acetic acid in ethanol was added and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop by drop to the extract to get precipitation. The whole solution was allowed to settle down and the precipitate was collected and washed with diluted ammonium hydroxide and filtered. The residue is the alkaloid that is dried and weighed.

Estimation of Saponins (Obadoni and Ochuko, 2001)

Take 20 g of sample in a conical flask and 100 ml of 20% aqueous ethanol. The sample was heated over a hot water bath for four hours with continuous stirring at about 55°C. The mixture was filtered and the residue was re-extracted with another 200 ml of 20% ethanol. The combined extract was reduced to 40 ml over water bath at about 90°C. The concentrated was transferred into a 250 ml of separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded, the purification process was repeated. Then 60 ml of n-butanol extract was added. The combined n-butanol extracted was washed twice with 10 ml of aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample was dried in the oven to a constant weight. The saponins content was calculated as percentage.

Estimation of Phenols (Malick and Singh, 1980)

0.5 ml of freshly prepared was taken in the test tubes. 8 ml of distilled water was added to all the tubes. 0.5 ml of Folin's Ciocalteau reagent added to all tubes. All the tubes were kept in B.O.D for incubation at 40°C for 10 minutes. Then, 1 ml of sodium carbonate solution was added to all the test tubes. Then, the tubes were kept in the dark for incubation for one hour. The colour so developed was read spectrophotometrically at 660 nm. Standard curve was drawn using tannic acid as standard. Different concentrations of tannic acid were prepared and O.D was read at 660 nm in a shimadzu UV-1650 spectrophotometer. The concentrations of sample werecalculated based on the standard curve.

Estimation of Total Flavonoids (Chun, 2005)

100 mg of tannic acid is dissolved in little amount of distilled water and the volume is made up to 100 ml with distilled water. Different concentrations of the standard were obtained by appropriate dilution with distilled water. The

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concentration of the solution will be 100 mg / ml. 0.5 ml of aqueous extract sample is dilute with 3.5 ml of distilled water at zero time. 0.3 ml of 5% sodium nitrate was added to the tubes. After five minutes, 0.3 ml of 10% aluminium chloride was added to all the tubes. which was incubated for 6 minutes . 2 ml of 1M sodium hydroxide was added to the mixture. Immediately, the content of the reaction mixture were diluted with 2.4 ml of distilled water and mixed thoroughly. Absorbance of the mixture was determined at 510 nm versus a prepared blank immediately. Tannic acid was used as standard compound for quantification of total flavonoids as mg / 100g of edible portion.

Estimation of Tannins (Robert, 1971)

Dissolve 100 mg tannic acid in 100 ml of distilled water. Dilute 5 ml of stock solution to 100 ml with distilled water. 1 ml containing 50 µg tannic acid. Extraction of Tannin: Weigh 0.5 gm of the powdered material and transfer to a 250 ml conical flask add 75 ml water. Heat the flask gently and boil for 30 min centrifuge at 2,000 rpm for 20 min and collect the supernatant in 100 ml volumetric flask and make up the volume. Transfer 1 ml of the sample extract to 100 ml volumetric flask containing 75 ml water. Add 5 ml of folin-denis reagent, 10 ml of sodium carbonate solution and dilute to 100 ml with water. Shake well. Read the absorbance at 700 nm after 30 min.

Antidiabetic Activity

Inhibition of α -amylase activity

Alpha-Amylase inhibitory assay Alpha-amylase inhibitory activity of extracts was carried out [25] with slight modification. The reaction mixture containing 500µl phosphate buffer (50mM, pH= 6.8), 100µl alpha-amylase (10U/ml) and varying concentrations of extracts from stock solution (1mg/ml) was pre-incubated at 37°C for 10 min. Then 200µl soluble starch (0.05%) was added as a substrate and incubated further at 37°C for 15 min. The reaction was stopped by adding 200µl 1N HCl, followed by addition of 1000µl iodine reagent (5mM I₂ and 5mM KI, stored in amber colored bottle). The absorbance was read at 620nm. Control without sample was set up in parallel. The α -amylase inhibitory activity was calculated by using following formula:

$$\text{Inhibition \%} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Glucose uptake by Yeast cells

Yeast cells were prepared according to the method of Cirillo [26]. Commercial baker's yeast was washed by using repeated centrifugation (3,000×g; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of sample (100–300µg/ml) from stock solution (1mg/ml) were added to 1ml of glucose solution (5, 15 and 25mM) and incubated together for 10 min at 37°C. 100µl of yeast suspension was added, vortexed and further incubated at 37°C for 60min. After 60 minutes the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant [27]. The absorbance was read at 540nm. The percentage of increase in glucose uptake by yeast cells was calculated using the following formula

$$\text{Increase in glucose uptake (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Where, Abs control is the absorbance of the control reaction (which contains all reagents except the test sample), and Abs sample is the absorbance of the test sample

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IV. RESULTS

The qualitative analysis of phytochemicals of the three extracts has been analysed in the present study (Table 1). Ethanolic extract was found to have a wide range of secondary metabolites like alkaloids, flavonoids, saponins, proteins, phenols, reducing sugars, terpenoids and tannins. The chloroform extract was found to have bioactive constituent except reducing sugars and proteins. The aqueous extract was found to have phytochemicals except proteins. The presence of bioactive constituents indicates that the *Cissus quadrangularis* stem extract can be used in a multitude ways for the beneficiary of population.

The quantitative estimation of secondary metabolites revealed that the various phytochemical constituents present in the ethanol stem extract (Fig 1). the alkaloid content was 10.01 mg/100g , phenol content was 43.28 mg/100g, flavonoids content was 10.98 mg/100 g ,saponin content was about 4.35 mg/100 g, Tannin content was 3.2 mg/100g . The active constituents in stem are better extracted out by ethanol extract, having a high % yield of *Cissus quadrangularis* and may be responsible for its pharmacological activities.

Fig. 2 shows the results of *In vitro* Antidiabetic activity of ethanolic stem extracts of *Cissus quadrangularis* by the use of alpha amylase enzyme activity. There was a significant increase of percentage of inhibitory activity was noticed in a concentration dependent manner. At a concentration of 10 µg /ml of ethanolic extract have the inhibitory activity of 6.63% . The maximum concentration of 60 µg /ml of the ethanolic extract have 27.3 % of inhibitory effect against the alpha amylase enzyme.

Fig. 3 shows Glucose Uptake by Yeast Cells. This experiment was studied that the glucose uptake rate was increased with the increasing concentration of the ethanolic stem extracts of *Cissus quadrangularis* stem extract and decreased with the increasing extracellular glucose concentration .

Table :1The preliminary phytochemical screening of different solvent extract of *Cissus quadrangularis*

Phytochemicals	Ethanol Extract	Chloroform extract	Aqueous extract
Phenols	+++	++	++
Flavonoids	+++	++	++
Tannins	+++	++	++
Alkaloids	++	++	++
Saponins	+++	+	+++
Terpenoids	++	+	+++
Glycosides	+	+++	+++
Reducing sugars	++	-	+++
Proteins	++	-	-

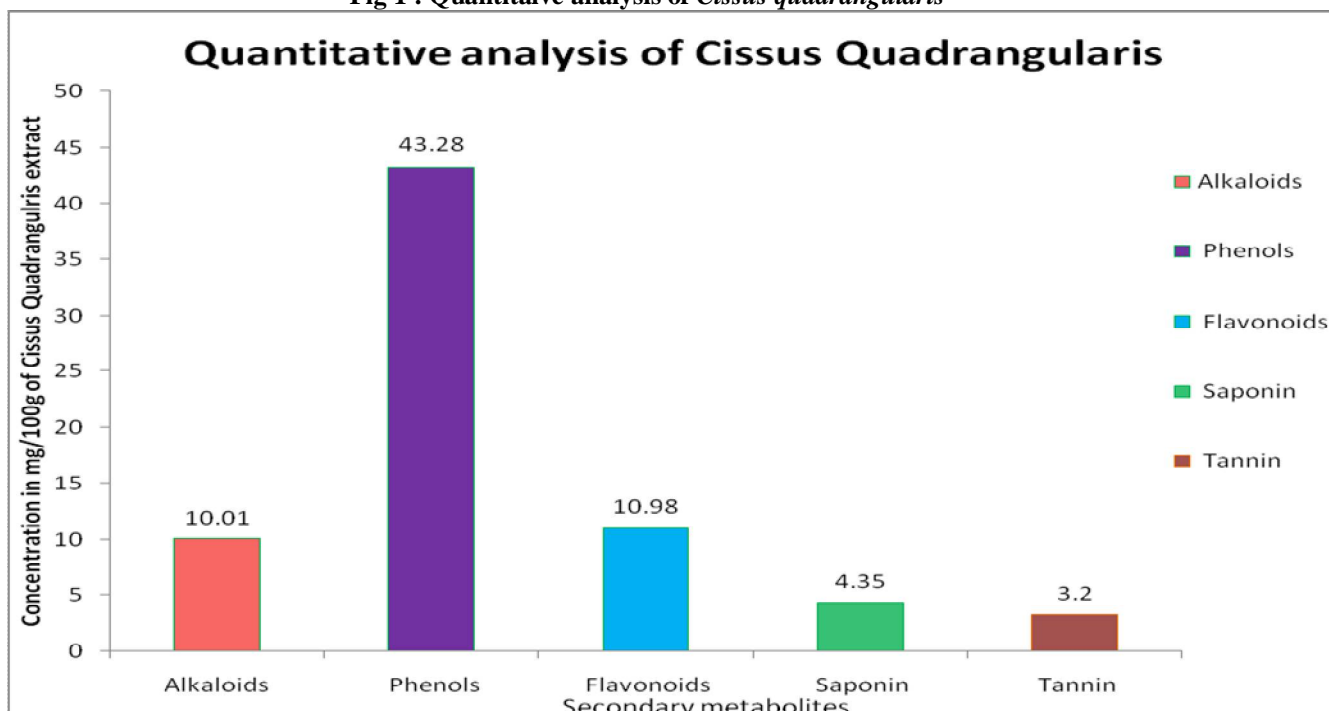
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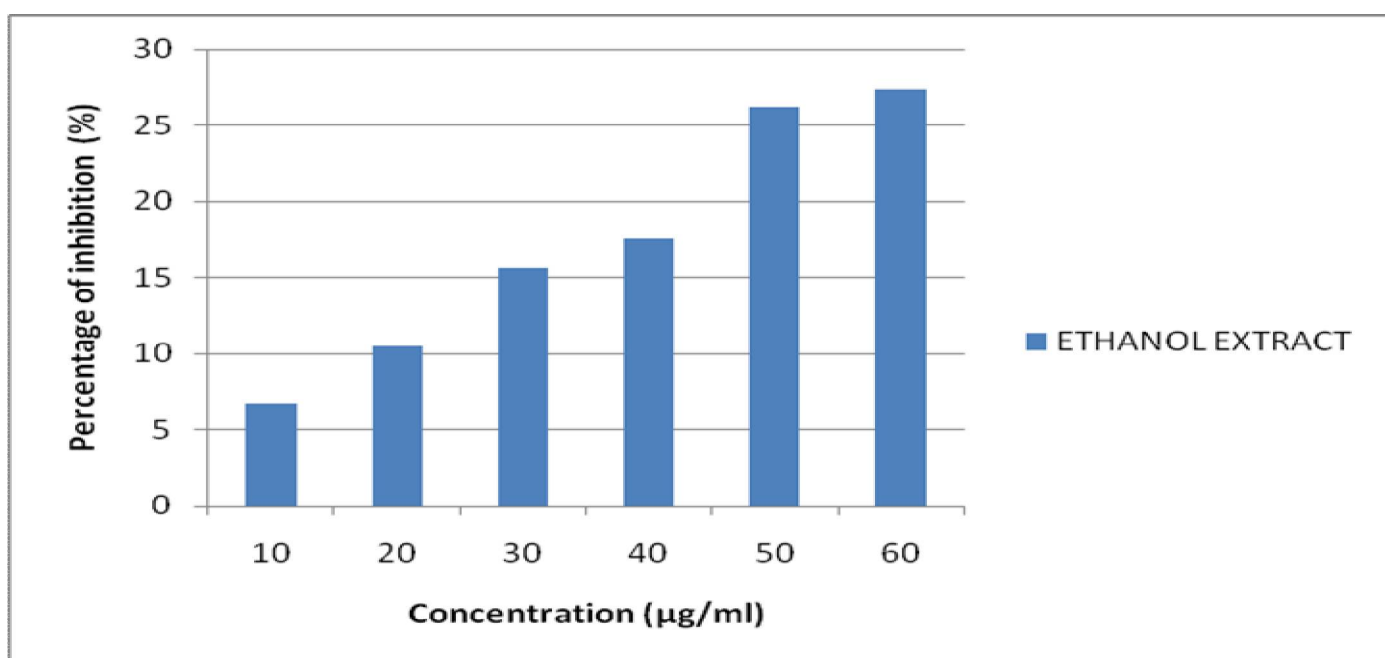
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Fig 1 : Quantitative analysis of *Cissus quadrangularis*



Antidiabetic Activity

Fig 2 : Inhibition of α -amylase activity



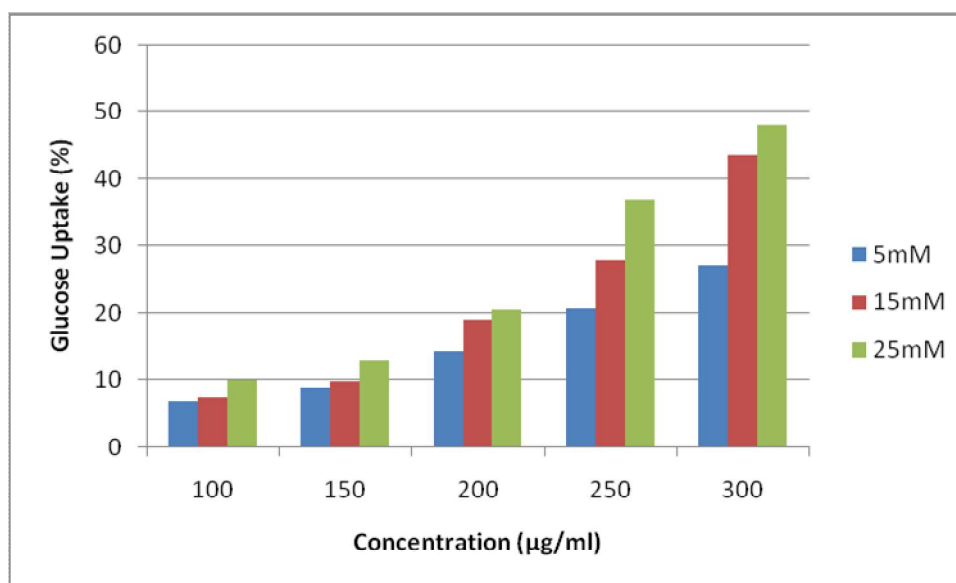
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Fig 3 : Glucose Uptake Assay By Yeast Cells



V. DISCUSSION

The medicinal values of plants lies in some phytochemical substances that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of actions, which may help in protection against chronic diseases [28]. Flavonoids have been used against the cancer and it inhibits the promotion of growth and progression of tumors [29]. Phenols and flavonoids compounds in plants are reported to show multiple activities like antioxidant, anticarcinogenic, anti-inflammatory etc. [30]. Plants with tannins are used for healing of wounds, gastrointestinal protective, haemorrhoids, frost-bite and burns [31, 32]. Terpenoids are reported to have antidiabetic, anti-inflammatory, anti-viral, anti-malarial, inhibition of cholesterol synthesis and anti-bacterial activity [33, 34].

The carbohydrate metabolic disorder may causes various health problems such as diabetes, oral diseases, obesity etc., in worldwide. Diabetes mellitus is a metabolic disorder, life threatening disease which is increasing day by day. Now a days management of diabetes without side effects is still challenge to the medical community. It is caused mainly due to the lack of insulin secretion or action. Insulin is key player hormone to regulate carbohydrate, fat and protein metabolisms, which control of glucose homeostasis. Insulin deficiency may affect the above important metabolisms. The treatment of type-II diabetes includes the stimulation of insulin secretion, inhibition of breakdown of polysaccharides and disaccharides etc. Alpha amylase is an enzyme which is responsible for hydrolysing the starch, which breaks down into glucose before absorption [35]. The inhibition of this enzyme increases the carbohydrate digestion time and reduces the glucose absorption [36] can leads to reduction in post prandial hyperglycemia [37]. Various plant extracts were reported that the *in vitro* antidiabetic activity of plants due to the inhibitory effect of alpha amylase. In our findings, antidiabetic activity of ethanolic stem extract of *Cissus quadrangularis* was effective against alpha amylase enzyme. Inhibitory action of stem extractsof *Cissus quadrangularis* increases with increased concentration. Most of the herbal plants and its parts have the tendency to reduce the blood glucose level. This is due to

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the availability of tannins, terpenoids and flavonoids [38]. Tannins and flavonoids have potential inhibitory effects on alpha amylase and alpha glucosidase [39]. The alpha amylase inhibitory action was due to the availability of terpenoids [40]. The antidiabetic potential was mainly due to the presence of tannins and terpenoids [41]. Alpha amylase inhibitory effects of tannins were due to its ability to bind carbohydrates and proteins. The above studies proved that, the secondary metabolites like flavonoids, tannins and terpenoids were effectively inhibit alpha amylase. The natural chemical constituents may attribute this activity. The ethanolic stem extract of *Cissus quadrangularis* have potential amount of flavonoids, tannins and terpenoids were found may responsible for antidiabetic activity.

It is stated that the transport of glucose across yeast cell membrane occurs by facilitated diffusion which down the concentration gradient [42]. Hence glucose transport occurs only if the intracellular glucose is effectively reduced. The experiment suggests that the ethanol extract of *Cissus quadrangularis* is capable of effectively enhancing glucose uptake which in turn suggests that it is capable of enhancing the effective glucose utilization, thereby controlling blood glucose level.

VI. CONCLUSION

The present study was carried out to analyse the secondary metabolites and *In vitro* antidiabetic activity of stem of *Cissus quadrangularis*. In the present study the evaluation of secondary metabolites revealed the presence of alkaloids, flavonoids, saponins, proteins, phenols, reducing sugars, terpenoids and tannins. These phytochemicals are used to treat various ailments. The stem of *Cissus quadrangularis* extract showed significant level of antidiabetic activity. Further concentration of extract isolation, purification and characterization of compounds from the plant sample responsible for antidiabetic activity has to be carry out.

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