



Preliminary Phytochemical Study of Different Extracts of One Medicinal Plant *Cissus quadrangularis*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Herbal plants are the most valuable resources to prevent from many illness and to treat the many disorders in humans. *Cissus quadrangularis* is a one of the traditional medicine and belonging to vitaceae family. In Ayurvedha medicinal system this plant is used to treat many diseases like diabetes, snake bites, rheumatic pain, cardiovascular diseases. *Cissus quadrangularis* used as anticancer properties against MG63 human osteocarcinoma cells. Stem of the *Cissus quadrangularis* is an edible vegetable and the plant commonly called as 'bone setter'. Our present study was analyzed the primary phyto constituents present the different solvents of *Cissus quadrangularis* and antioxidant activity. Three different extracts aqueous, methanol and ethanol were used to find out the phytoconstituents and antioxidant properties. The results were reveals that the plant *Cissus quadrangularis* consists of numerous primary and secondary phytoconstituents. Flavonoids and tannins were found to be higher in ethanol extracts, legnings, saponins was high in methanol extracts. Antioxidant study also reveals ethanol extracts shows more antioxidant activity than other two solvents.

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1. INTRODUCTION

Plants are the valuable sources of food and medicine for the prevention and treatment of human ailments [1]. Since from ancient times, *Cissus quadrangularis* is easily available medicinal plant. It is a perennial climber, belonging to vitaceae family. It is commonly called as veldt grape or Devil's Backbone and 'bone setter'. This plant considered as a native plant for many tropical countries and found all over the world. The distribution of the plant more in tropical countries like india, srilanka, South Africa as 13 genera and 800 species. Especially in India 8 genera and 63 species have been identified [2]. Appearance of the plant stem resembles the shape of the bones and joints in our body. The stem of the plant is edible vegetable. All parts of this plant have important medicinal properties, and used to cure piles, bone fracture, weight loss, muscular pains, antiulcer, antimicrobial, anti hemorrhoidal, swelling, scurvy, gout, disease in ear and nose bleeding, diabetes [3]. Fresh shoot paste is used in burns and wounds. *Cissus quadrangularis* stem extract have the high impact on loss of body weight by inhibiting the oxidation of LDL cholesterol and by lowering the blood glucose on obese patients [4]. Ethanol extract of *Cissus quadrangularis* possess more antioxidant and anti cancer activity against bone cancer [5]. The medicinal properties of flavonoids, Tannins, proteins, phenolics, lignins, carbohydrates and saponins are found important secondary metabolites in *Cissus quadrangularis* extracts. Ethanol extract of CQ act as antioxidant, anticancer, antifungal and antiviral [6]. The current study was analyzed for phytochemical analysis and antioxidant activity of *Cissus quadrangularis*.

2. MATERIALS AND METHODS

2.1 Sample Collection

Cissus quadrangularis stem were collected from erode. The collected plant material was identified and authenticated by Botanical survey of India, southern regional centre, Coimbatore. Voucher No. BSI/SRC/5/23/2020/Tech/799. The plant was identified and authenticated by agricultural university. Coimbatore. The voucher no. The stems bars were isolated from the whole plant and the separated stems were air dried under the shade. About 2 kg of dried samples was powdered

by using maceration method. The powdered samples were stored for this study.

2.2 Preparation of Extracts

The powder samples were subjected to extraction by hot percolation method with aqueous methanol and ethanol solvents in Soxhlet apparatus. Each solvent extraction was carried out for 24 hrs. After the process the extracts were concentrated by rotary evaporator and stored at 4°C. The stored extracts were used for further study [6].

2.3 Phytochemical Analysis

Phytochemical screening was carried out for the aqueous, methanol, and ethanol extracts of *Cissus quadrangularis* to evaluate the presence of phytochemicals such as phenolic groups, alkaloids groups, proteins, carbohydrates, fat and oils, lignins, tannins, quercetin.

2.4 Test for Alkaloids

Mayer's test was done to find out the alkaloids. In brief, 5 mg samples were taken in the fresh test tube, 1% HCL were added into sample. The solution was heated. Appearance of slight Red colour indicate the presence of alkaloids [7].

Wagners test – 5 mg samples were added in the test tube and 0.5 ml of wagner's reagent were mixed with that sample and shaken gently. Appearance of slight brown colour will indicate the presence of alkaloid [7].

2.5 Test for Flavonoids

Shinoda test – 5 mg extracts were added into the test tube and minimal quantity of magnesium were top up with in the solution and add few drops of concentrated H₂SO₄. The solution in the test tube pink in colour it indicate the presence of flavonoids [8].

Lead ethanoate test – Take 5mg of extract in the test tube, add lead ethanoate, 1-2 ml. The obtained solution gives buff coloured, it will indicate the flavonoid content (Test for Sodium hydroxide – 1 ml of 10% of H₂O₂ were added with 5 mg of extract. The colour appearance of yellow colour after addition of 1 ml of diluted HCl, if the presence of alkaloids the colour should be changed from yellow to colourless after adding 2 ml of diluted HCL [8].

2.6 Test for Tannins

Test for Ferric chloride – Minimal amount (5 mg) of extract were mixed with 0.5 ml of ferric chloride. Appearance of black colour precipitate indicate the presence of tannins.

Gelatine test one of the main test done to identify tannins. 5 mg of sample were added into gelatin. After that 1 ml of H₂O were poured into it. Presence of white precipitate will indicate the tannins [9].

2.7 Test for Oil

Test for Stain – Minimal amount of sample were taken in the whatman filter paper. Roll the paper with that sample. Deposition of oil on the paper will indicate the presence of oil [9].

2.8 Test for Saponins

Saponins were identified with the help of Form test. 5 ml of distilled water were added with minimal amount of sample. The test tube were rotated well for proper mixing till foam was observed [10].

2.9 Test for Glycoside

Liebermann's test – 2 ml of chloroform and 2 ml of acetic acid were mixed with minimal amount of sample in the test tube. After get cooled, 1 ml of concentrated sulfuric acid were mixed with that solution. Colour changes were observed from violet to green. Green colour indicate the presence of glycoside [8].

Salkowski's test – 2 ml of chloroform were mixed with 1 ml of extract. Additionally 2 ml of concentrated H₂SO₄ were added and shaken gently. A reddish brown colour will indicate the presence of glycoside [8].

2.10 Steroid Test

About 5 ml of extract were added with 1 ml of chloroform then one or two drops of concentrated sulfuric acid and acetic acid was poured in it. The green colour indicate the presence of steroids [8].

2.11 Test for Proteins

Biuret's test – about 5 ml of extract were mixed with some drops of biuret's reagent. The solution were mixed well and allowed to heat 1-5 minutes.

Appearance violet colour will indicate the presence of proteins.

Million's test – 2 ml of Million's reagent were added with 5 ml of extract. The solution was warmed for 5 min. Appearance of red colour precipitation will indicate the presence of proteins [8].

2.12 Test for Lignins

Extracted sample materials was oven dried at 24 h before acid hydrolysis. 350 g of extracts were taken in a glass beaker along with 72% sulfuric acid 3 ml were added. The mixer were shaken at room climate for 1 h. After this pre-hydrolysis stage, the medium were diluted by adding 84 ml of distilled water. The reaction mixer was heated to boiling the reaction was maintained under reflux for 4 h. At the end of the process, the flask cooled to room temperature and the content was filtered by using whatman filter paper no1. Lignin retained with 100 ml of distilled water and dried in an oven at 103°C for 48 h [9]. Same procedure was done for all the three solvents.

2.13 Test for Phenolic Compounds

The sample were dissolved in the mixture of alcohol and 1 drop of neutral ferric chloride. The obtained solution intense colour indicates the presence of phenolic compounds [8].

2.14 Carbohydrates Test

Benedict's test – few drops of benedict's reagent were taken in a test tube and 5 mg extract were added and obtained solution was heated. Reddish brown precipitate will indicate the presence of the carbohydrates [11].

Molisch's test – Minimal amount of extract were taken in test tube, 1 ml of Molisch's reagent were added into it. The solution were shaken well. 2 ml of concentrated H₂SO₄ were poured carefully along the sides of the test tube. Appearance of a violet ring at the interface indicated the presence of carbohydrates [11].

2.15 Test for Antioxidant

The antioxidant activity of the all the three extracts were evaluated according to the procedure by prieto et al. In brief, 2 ml of extract was taken at dissimilar concentrations (100,200,300,400 and 500 µg) and mixed with 1 ml of standard reagent 0.6 M sulfuric acid, 28

mm sodium phosphate and 4 mm ammonium molybdate. Then reaction mixture was incubated at 95° C for 90 min. Absorbance of all the samples was measured at 635 nm [12]. The same test was repeated for all the three solvents.

3. RESULTS AND DISCUSSION

3.1 Phytoconstituents Screening

Phytochemical analysis discovered the presence of many important phytochemical constituents such as glycosides, flavonoids, saponins, tannins, aminoacids, proteins, fat and oil, lignins (Table 1). The plant *Cissus quadrangularis* extracts shows valuable phytochemical compounds that has been reported to have greater potential health benefits in humans. Tannins and phenolics have more antioxidant properties. Tannins also have the ability to deactivate the replication process of HIV virus. Aqueous, methanol, ethanol extracts reveals the presence of flavonoids more. Flavonoids plays major role in oxidative stress. No source of alkaloids and carbohydrates are found in ethonal extract.

3.2 Antioxidant Assay

3.2.1 DPPH free radical scavenging

NazninAra and HasanNur method were used for the present study. The effects of these extract to

scavenge DPPH radical were determined by this method. The absorbance were read at 518 nm.

Antioxidant activity (%)= $\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$

Abs control- Optimal density of control

Abs sample- Optimal density of sample extract.

3.2.2 FRAP assay

Total antioxidant activity for this study were measured by ferric reducing antioxidant power assay of Benzie and stain. The FRAP assay, is presented as a novel method for analyzing antioxidant power. Absorbance at 593 nm were measured. FRAP value of sample (μM)= $\frac{\text{change in absorbance of sample from 0 to 5 mints}}{\text{change in absorbance of standard from 0 to 5 mints}} \times \text{FRAP value of standard (1000 } \mu\text{M)}$ [12].

3.2.3 Antioxidant study

Cissus quadrangularis have been identified to possess a lot of antioxidant properties which enable their extracts and active principles such as oxidative stress, free radical scavenging activities [11]. In this study three different solvents extracts were investigated for their radical scavenging activity and their results were shows in Fig. 1. Methanol extracts contains more

Table 1. phytochemical screening of *Cissus quadrangularis*

| S. No. | Phytochemical Test | Test Name | Solvents | | |
|--------|--------------------|-----------------------|----------|---------|----------|
| | | | Aqueous | Ethanol | Methanol |
| 1. | Alkaloids | Meyer's test | + | - | + |
| | | Wager's test | + | - | + |
| | | Shinoda test | + | ++ | ++ |
| 2. | Flavonoids | Lead ethanoate test | + | ++ | ++ |
| | | Sodium hydroxide test | + | + | + |
| | | Ferric chloride test | - | + | + |
| 3. | Taninns | Gelatin test | - | + | + |
| 4. | Fat and oil | Stain test | + | + | + |
| 5. | Saponins | Form test | - | + | + |
| 6. | Glycosides | Libermann's test | + | + | + |
| | | Salkowski's test | + | + | + |
| 7. | Steroids | Steroids test | + | + | + |
| 8. | Proteins | Biuret's test | + | + | + |
| | | Million's test | + | + | + |
| | | Benedict's test | - | - | - |
| 9. | Carbohydrates | Molisch's test | - | - | - |
| | | Phenolics test | ++ | ++ | ++ |
| 10. | Phenolics | Phenolics test | ++ | ++ | ++ |
| 11. | Lignins | | + | + | + |

(+) - present; (-) - absent

phenolics and flavonoids. This secondary phytochemicals have more antioxidant properties. Ethanol and aqueous extracts shows relatively low antioxidant activity when compare with the methanol extracts. Saponins act as a protective agent for the plant such that it is called plant protector, it also contains more antioxidant properties [13]. *Cissus quadrangularis* can act on the lipid peroxidation to lower their levels of enzymes having antioxidant properties.

3.3 Discussion

Cissus quadrangularis extracts contains more useful and powerful phytoconstituents, they are

used to treat bone related many diseases and anti diabetic drug [14]. Recently the same extracts were used for infertility study purpose [15]. Due to high amount of antioxidant properties, the plant *Cissus quadrangularis* extracts were used for infertility study [16]. The exact mechanism behind the extracts need to be explored.

Fig. 1 shows the antioxidant activity of different extracts of stem of *Cissus quadrangularis*. Ethanol extracts shows high values, when compare with the methanol and aqueous extracts. The values will increases according to the concentration.

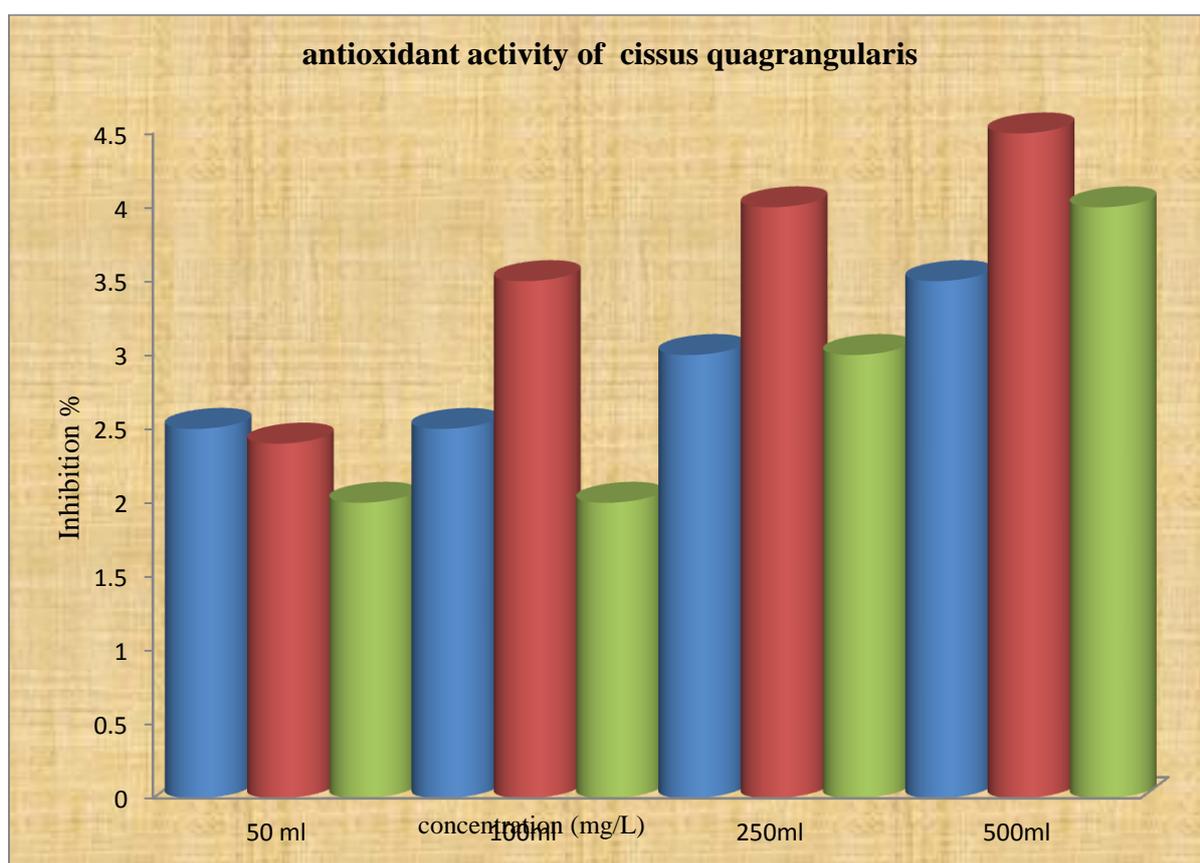


Fig. 1. Antioxidant activity of *Cissus quadrangularis*
Blue - Methanol; Red - Ethanol; Green - Aqueous

Table. 2. Antioxidant activity

| Conc of Extract (in µg) | % Inhibition of AECQ | % Inhibition of EECQ | % Inhibition of MECQ |
|-------------------------|----------------------|----------------------|----------------------|
| 50 | 29.8 ± 3.41 a** | 14.26 ± 0.9 b** | 65.3 ± 0.7 |
| 100 | 44.1 ± 5.4 a** | 56.9 ± 1.76 b** | 98.0 ± 1.2 |
| 200 | 20.5 ± 1.00 a** | 56.2 ± 0.85 b** | 34.2 ± 1.76 |
| 500 | 53.9 ± 7.6 a ns | 35.5 ± 6.45 b** | 61.2 ± 5.65 |
| 1000 | 61.53 ± 6.95 a** | 45.9 ± 1.2 b** | 34.76 ± 1.79 |

4. CONCLUSION

Natural products are the important sources of bioactive properties *Cissus quadrangularis* was used since ancient times. This medicinal plant have more protective effects on antidiabetic, antimicrobial, anticancer, antifungal, and antiinferility activity [17]. The present study suggests the bioactive compounds and anti oxidant properties such as phenolics and flavanoids are found abundant, can be used for future studies. The stem of *Cissus quadrangularis* may be consider as powerful antioxidant and further studies are needed to explore the exact mechanism responsible for the infertility activity. Presence of Antioxidant properties of stem of *Cissus quadrangularis* are used as good herbal food supplements for the stress management and infertility.

RESEARCH SIGNIFICANCE

The study highlights the efficacy of "Ayurvedha" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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Saranya Arunagiri gathered the data, designed and edited the manuscript. Dr. Srinivasan gave the inputs and wrote the topic of "preliminary study". All authors discussed the methodology and results and contributed to the final manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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