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Proximate and phytochemical analysis of methanolic extract of *Cissus quadrangularis*

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Abstract

The present work was conducted to evaluate the proximate contents, phytochemical constituents and to screen the methanolic extract of the stem of *Cissus quadrangularis* for the presence of active compounds. Methanol was the solvent used for the soxhlet extraction. The dry matter content and the total ash, crude protein, crude fat, crude fibre, acid insoluble ash, carbohydrate content were determined. The chemical composition of methanolic extract of *Cissus quadrangularis* revealed the presence of alkaloids, saponins, phytosterols, phenolic compounds, flavonoids, terpenoids, protein, amino acids, fixed oils, fats, tannins and cardiac glycosides. This work could be further useful to the structural elucidation and further quantification of bioactive compounds.

Keywords: *Cissus quadrangularis*, methanolic extract, proximate analysis, phytoconstituents

1. Introduction

Plant based products are healthier, safer and reliable than the synthetic products. Nowadays drug industries depend on plant molecules for new drugs because of the side effects of synthetic products. Approximately 80% of the people depend on traditional medicines for their primary health care according to WHO (Suhasini *et al.*, 2015) [7]. Plant constituents comprise a wide variety of organic substances namely primary metabolites and secondary metabolites. Alkaloids, phenolics, lignins, essential oils, steroids, tannins etc. belongs to the category of secondary metabolites. Secondary metabolites are also classified into nitrogen containing compounds, terpenoids, phenolics (Wink, 1987) [10]. *Cissus quadrangularis* (*C. quadrangularis*) belongs to Vitacea family is a common perennial climber which is distributed throughout in India particularly in hotter parts (Shukla, 2015) [6]. Determination of flavonoids is very important because of pharmacological effects (Tamanna *et al.*, 2017) [8]. Therefore, the present investigation was aimed to Evaluate the proximate contents, phytochemical constituents of methanolic extract of the stem of *C. quadrangularis*.

2. Materials and Methods

2.1. Chemicals: All the chemicals and reagents used in this study were procured from M/S Merck India, Ltd., India / Germany.

2.2 Plant Materials

2.2.1 Collection and identification of plant materials: The taxonomically identified *C. quadrangularis* plant stem used in this study were collected during April, 2017 from Karnataka. The plant was identified and authenticated by a botanist in the Department of botany, Calicut University. A herbarium for morphological studies was prepared and a voucher specimen deposited at Calicut University herbarium (Accession No.148208), Kozhikode, Kerala.

2.2.2. Preparation of plant materials: Plant materials collected were cleaned and dried under shade to remove water. They dried stems were powdered in an electrically driven mortar and sieved to remove the coarse particles. The fine powder obtained was stored in airtight container until use.

2.2.3. Preparation of crude methanolic extract: The powdered plant material of *C. quadrangularis* was used for extraction using methanol in

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soxhlet extraction apparatus attached with a rotary vacuum evaporator (Buchi, Switzerland). Solvents were removed using rotary vacuum evaporator at 96 mbar at a temperature in the range of 40 °C to 45 °C. The weight of the dried extract was recorded and the extractive yield was calculated as,

$$\text{Extractive value} = \frac{\text{Weight of the extract} \times 100}{\text{Weight of powered plant sample taken}}$$

Crude extracts were subjected to phytochemical analysis by dissolving the extract in methanol.



Fig 1: Whole plant *Cissus quadrangularis* in its natural habitat

2.3 Analysis of Plant Material

2.3.1. Proximate analysis of the potent crude drug powder

The proximate analysis of ash, crude fibre, crude protein, carbohydrate, crude fat, dry matter and moisture content of respective plants were carried out by using standard proximate analysis techniques as mentioned by Pathak, 1996 [5].

2.3.1.1. Determination of dry matter

The dry matter was determined using the weight difference method and estimated by deducting per cent moisture from hundred as described by James (1995) [4].

Dry matter (per cent) = 100 – per cent of moisture

The moisture content was determined by using the formula,

$$\text{Per cent of moisture content} = \frac{\text{Weight of sample} - \text{weight of dried sample} \times 100}{\text{Weight of sample}}$$

2.3.1.2 Determination of total ash contents

Ash represents the inorganic content of the sample which is determined by the method as described by Association of Official Analytical Chemists (AOAC, 2000). Total ash content was calculated by the following formula,

$$\text{Ash (per cent)} = \frac{\text{Weight of ashed sample} \times 100}{\text{Weight of sample taken}}$$

2.3.1.3. Determination of crude fat

One gram of crushed dried sample was taken in the filter paper thimble kept in a pre-weighed flask of fat extractor. Petroleum ether (80 ml) was poured into the flask and refluxed for 8 hours. The flask was cooled in desiccator and the weight of crude fat so extracted was taken. The per cent

crude fat was determined by using formula,

$$\text{Crude fat (per cent)} = \frac{\text{Weight of flask with fat} - \text{weight of empty flask} \times 100}{\text{Weight of original sample}}$$

2.3.1.4. Determination of crude fiber

One gram of the defatted plant material was taken in a sputless beaker and boiled in 200 ml of 1.25 per cent sulphuric acid for 30 minutes. The content was then filtered, washed with hot distilled water to neutralize, transferred again to the beaker and boiled in 200 ml of 1.25 per cent sodium hydroxide for 30 minutes followed by filtration and washing with hot distilled water for neutralization. The crucible was then dried in a hot air oven at 100 ± 5 °C overnight (10-12 hr), cooled in a desiccator and weighed to a constant weight. Subsequently, the desiccator with its content was put in a muffle furnace at 550-600 °C for 2-3 hour for complete burning of organic matter and cooled in a desiccator and weighed to a constant weight. The per cent of crude fibre was determined from the formula,

$$\text{Crude fibre (per cent)} = \frac{(W_1 - W_2) \times 100}{\text{Weight of sample}}$$

Where,

W₁ - The crucible having crude fibre was cooled and weighed
W₂ - The content of the crucible was ignited over a low flame until charred and then kept in a muffle furnace and weighed.

2.3.1.5. Determination of crude protein

Total nitrogen (N) content was determined with the help of Kjeldahl method described by Pearson (1976).

The per cent of protein was calculated by the formula.

$$\text{Protein (per cent)} = \frac{V \times 1.4 \times 6.25 \times 0.1N \text{ HCl} \times \text{Vol (used)} \times 100}{W \times A \times 1000}$$

Where

V = Titre value

1.4 = Weight of nitrogen expressed in gram in the formula

6.25 = Protein factor

W = Weight of sample

A = Aliquot digested sample used for distillation

d) Determination of carbohydrate

Determination of available carbohydrate in the sample was calculated from the above parameters by using the formula.

Carbohydrate (per cent) = 100 - (moisture + crude fat + ash + protein) per cent

2.3.1.6. Determination of calcium

The acid soluble mineral extract obtained in acid insoluble ash experiment is used for estimation of calcium and phosphorus. Ten ml of the soluble mineral extract was pipetted out into a beaker of 250ml capacity and 90 ml distilled water was added to increase the volume. The pH was adjusted to between 2.5 to 3 before the addition of the precipitating reagent viz. saturated ammonium oxalate.

Precipitation of calcium: After adjusting the pH, the beaker with its content was placed over the hot plate. The contents of the beaker was gently heated and then 10 to 15ml of warm

ammonium oxalate (precipitant) was added which resulted in the precipitation of calcium as calcium oxalate (CaC_2O_4) and was seen as white granular precipitate.

Barium chloride test: Barium chloride (5%) solution was used to test whether the precipitate was free from oxalate. The appearance of white precipitate between the barium chloride and filtrate solution indicated the presence of oxalate which necessitated further washing.

Titration: 0.1N KMnO_4 was used in this titration. The content of the beaker was heated till few bubbles appeared.

1 ml of 0.1N KMnO_4 = 0.002 g of calcium or 0.0028g of calcium oxide

Calculation

$$\text{Percentage of calcium} = \frac{0.002 \times D \times B \times 100}{A \times C}$$

Where

A = weight of substance

B = total volume of soluble mineral extract

C = aliquot taken

D = burette reading

2.3.1.7. Determination of phosphorus

Principle

The phosphorus was precipitated as yellow colour phosphor-ammonium-molybdate, by the addition of ammonium molybdate reagent. The precipitate was washed and dissolved in measured volume of standard alkali. The amount of alkali, which has dissolved the phospho-ammonium- molybdate precipitate, is indirectly found out by back titrating with standard 0.1N sulphuric acid.

$$\text{Calculation: \% of phosphorus} = 0.0001347 \times \text{BTV} \times \frac{B}{C} \times \frac{100}{A}$$

Where

A = Weight of sample

B = volume of soluble ash

C = Amount of aliquot taken

TV = Titration value

BTV = Back titration value = amount of NaOH added - TV

2.3.2. Phytochemical analysis of the crude methanolic stem extract

The phytochemical analysis of the methanolic stem extract was evaluated as per the procedure (Harborne, 1991) [3] described below.

2.3.2.1. Detection of alkaloids

The presence of alkaloids was detected by conducting the following tests. The extract was dissolved individually in dilute hydrochloric acid and filtered. The filtrate was further tested with following reagents for the presence of alkaloids.

a) Dragendorff's test: Filtrate was treated with potassium bismuth iodide solution (Dragendorff's reagent). Formation of an orange-yellow precipitate indicated the presence of alkaloids.

b) Hager's test: Filtrate was treated with saturated aqueous solution of picric acid (Hager's reagent). Presence of alkaloids was confirmed by the formation of yellow coloured precipitate.

c) Mayer's test: Filtrate was treated with potassium mercuric iodide solution (Mayer's reagent). Formation of a whitish yellow or cream coloured precipitate indicated the presence of alkaloids.

d) Wagner's test: Filtrate was treated with iodine in potassium iodide solution (Wagner's reagent). Formation of reddish brown precipitate indicated the presence of alkaloids.

2.3.2.2. Detection of carbohydrates

The presence of carbohydrates can be detected by conducting Molisch's test.

2.3.2.3. Detection of reducing sugars

The presence of reducing sugars was detected by conducting the following tests.

a) Benedict's test: The filtrate was treated with Benedict's reagent and boiled in a thermostatic water bath for five minutes. Formation of an orange red precipitate indicated the presence of reducing sugars.

b) Fehling's test: The filtrate was acidified with dilute hydrochloric acid, neutralized with alkali and heated with Fehling's solution A and B. Formation of red precipitate indicated the presence of reducing sugars.

2.3.2.4. Detection of saponins

The presence of saponins was detected by conducting the following tests.

a) Froth test: The methanolic stem extract was diluted with distilled water to 20 ml and shaken in a graduated test tube for 15 minutes. Formation of 1 cm layer of foam indicated the presence of saponins.

b) Foam test: A small quantity of the extract was shaken with 2 ml of water. Persistence of foam produced for ten minutes indicated the presence of saponins

2.3.2.5. Detection of phytosterols

A small quantity of extract dissolved in 5 ml of chloroform and subjected to Salkowski's and Libermann Buchard's tests for detection of phytosterols.

a) Salkowski's test: A few drops of concentrated sulphuric acid was added and allowed the solution to stand for some time. Formation of brown ring indicated the presence of phytosterols.

b) Libermann Burchard's test: The extract was dissolved in chloroform and chloroform extract solution was treated with a few drops of acetic anhydride, boiled and cooled. Then concentrated sulphuric acid was added. Formation of a bluish green colour solution confirmed the presence of phytosterols.

2.3.2.6. Detection of phenolic compounds

The presence of phenolic compounds was detected by conducting the following tests.

a) Ferric chloride test

2.3.2.7. Detection of tannins

Approximately 0.5 gram of the extract was boiled in 20 ml of water in a test tube and then filtered. Then a few drops of 0.1% ferric chloride was added. Development of a brownish green or a blue-black colouration indicated the presence of tannins.

3.1.2 Extractive values of the crude methanolic extract

The extractive values of the methanolic extracts of stem of *C. quadrangularis* was 7.09 per cent.

3.2 Analysis of Plant Material

3.2.1 Proximate analysis of crude powder of stem of *C. quadrangularis*

The proximate analysis of crude powder of the stem of *C. quadrangularis* was investigated and the per cent values obtained for crude protein, crude fat, dry matter, moisture, crude fibre, ash and carbohydrate contents are shown in table 1 and figure 4. The per cent values obtained for inorganic contents of crude powder of the stem of *C. quadrangularis* is presented in Table 2. The analysis of dry matter, moisture,

crude fibre, crude protein, ash carbohydrate and mineral contents of the stem of *C. quadrangularis* were carried out using standard proximate analysis techniques. The results obtained from the analysis are dry matter content was 90.75%, the total ash, crude protein, crude fat, crude fibre, acid insoluble ash and carbohydrate content were 19.39, 25.64, 3.33, 22.12, 14.34 and 42.65 per cent respectively. The percentage of calcium and phosphorus were 20.99% and 0.2416% respectively. Anuj *et al.* (2011) had detected the per cent of the total ash and acid insoluble ash value as 12.5% and 2.5% respectively. Krunal *et al.*, 2011 had observed the abundance of calcium oxalate. There are no further reports to support the findings.

Table 1: Biochemical contents in the crude powder stem of *C. quadrangularis*

Plant	Biochemical contents (%)						
	Dry Matter	Moisture	Crude fibre	Crude protein	Crude fat	Ash	Carbohydrate
<i>Cissus quadrangularis</i>	90.75	9.24	22.12	25.64	3.33	19.39	42.66

Table 2: Inorganic contents in the crude powder stem of *C. quadrangularis*

Plant	Biochemical contents (%)			
	Total Ash	Acid insoluble ash	calcium	Phosphorus
<i>Cissus quadrangularis</i>	19.39	14.34	20.99	0.24

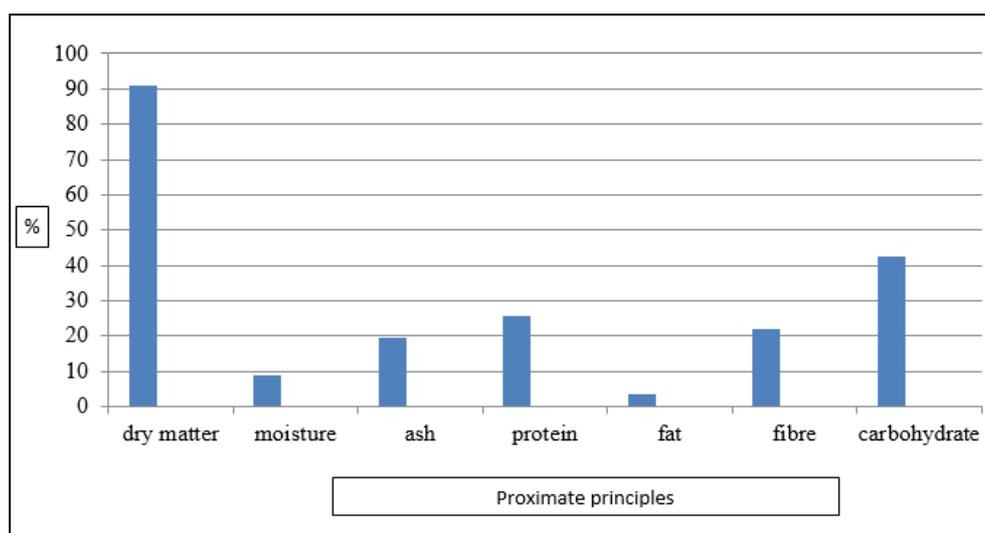


Fig 3: Figure depicting the biochemical contents in the crude powder stem of *C. Quadrangularis*

3.2.2 Phytochemical analysis of the crude methanolic stem extract

The phytochemical characteristics of methanolic extract of *C. quadrangularis* investigated are summarised in table 3. The methanolic extract indicated the presence of alkaloid, saponins, phytosterols, phenolic compounds, flavonoids, terpenoids, protein, amino acids, fixed oils, fats, tannins and cardiac glycosides. The stem extract however did not reveal the presence of carbohydrates, reducing sugars, gums and mucilages. Chidambaramurthy *et al.*, (2003) [2] had detected the presence of phytosterols and tannins in the extract of *C. quadrangularis*. In another study performed by Anitha *et al.* (2014) [1] four chemical constituents were analysed from the aqueous petroleum ether and dichloromethane extract of *C. quadrangularis*, namely, alkaloids, phenol, steroids and flavonoids. Phytochemical analysis gave a significantly high presence of saponins and phytosterols. Phytosterols are a kind of plant metabolite belonging to the triterpene family. These

compounds are essential biomolecules for human health, and so they must be taken along with foods. β -Sitosterol, campesterol, and stigmasterol are the main phytosterols found in plants. Phytosterols have beneficial effects on human health since they are able to reduce plasma cholesterol levels and have anti-inflammatory, anti-diabetic, and anticancer activities. Triterpenoid and steroidal glycosides, referred to collectively as saponins, are bioactive compounds present naturally in many plants. Kaur *et al.*, 2015 [7] reported the presence of carbohydrates, saponins, flavonoids, alkaloids, glycosides and phenols in powdered stem material of *C. quadrangularis* which indicate its high medicinal property. The chemical constituents of *C. quadrangularis* possess novel flavonoids and indanes as well as phytosterols and keto-steroids which are powerful and efficient antioxidants. They have the potential to inhibit lipase and amylase and thereby providing a mechanism for weight loss through reduced oxidative stress, dietary fat and carbohydrate blocking.

Various pharmacological activities such as bone fracture, healing and tissue repair, prevention of osteoporosis, weight loss, blood sugar regulation, digestive disorders, menstrual irregularity, cholesterol and triglyceride lowering, antimicrobial, analgesic, antipyretic, anti-inflammatory, tissue

protective, joint health have been reported and studied by many scientists (Tan Nie *et al.*, 2015) [9]. Flavanoid containing plants have many therapeutic properties like antimicrobial activity, anti-oxidant activity, free radicals scavenging activity.

Table 3: Qualitative phytochemical analysis of methanolic extract of stem of *C. quadrangularis*.

No	Phytoconstituents	Tests	Inference
1	Alkaloids	Mayer's test	-
		Dragendroff's test	-
		Hager's test	+
		Wagner's test	-
2	Carbohydrate	Molishes test	-
3	Reducing sugar	Fehling's test	-
		Benedict's test	-
4	saponin	Foam test	++
		Froth test	++
5	phytosterols	Salkowski's test	++
		Liebermann buchard's test	++
6	Phenolic compounds	Ferric chloride test	+
		Lead acetate test	+
7	Tannins	Ferric chloride	+
8	flavonoids	Lead acetate test	+
		Alkaline reagent test	+
9	Cardiac glycosides	Keller-killani test	+
10	Proteins and amino acids	Millons test	-
		Biuret test	+
		Ninhydrin test	+
11	Terpenoids	Salkowski's test	+
12	Fixed oils and fats	Spot test	+
		Saponification test	+
13	gums and mucilages	ruthenium red solution	-

Note :++ : Higher + :Lower - :Absent

4. Conclusions and the Outlook

The methanolic stem extract was analysed for various phytochemical constituents using standard procedures and revealed the presence of alkaloid, saponins, phytosterols, phenolic compounds, flavonoids, terpenoids, protein, amino acids, fixed oils, fats, tannins and cardiac glycosides. Further studies are in progress to elucidate the wide pharmacological activity of this plant and also the reported work could be further useful to the structural elucidation and further quantification of bioactive compounds.

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