NUTRITIONAL COMPOSITION AND QUALITATIVE PHYTOCHEMICAL ANALYSIS OF *CISSUS QUADRANGULARIS* STEM EXTRACTS

[1]Sruthi. A, [2]Dr. Karpagapandi. L

[1]Department of Food Science and Technology, Palar Agricultural College, Vellore – 635805
[2]Department of Family Resource Management, Tamilnadu Agricultural University, Madurai – 625 104

***Abstract- Cissus quadrangularis* is an ancient medicinal and multipurpose plant used traditionally for different healing treatments. The stem of *Cissus quadrangularis* is also reputed in Ayurveda as alterative, anthelmintic, dyspeptic, digestive, tonic, analgesic in eye and ear diseases in the treatment of irregular menstruation and asthma in complaints of the back and spine. On the account of its traditional usage, there is no precise scientific evidences; Hence, this study was planned to find out nutritional values and preliminary screening of phytochemicals of *Cissus quadrangularis* stem extracts with various solvents viz., Petroleum ether, Acetone, Hexane, Ethanol, Methanol, Ethyl acetate, Chloroform, and Aqueous. The results revealed that the composition of chemical constituents in 100 g of powdered air dried sample were as moisture 85.54 g, pH 5.04, acidity 8.96, crude fibre 17.01 g, protein 11.4 g, fat 5.83 g, ascorbic acid 295 mg and β-carotene 237 mg. Results indicated that *Cissus quadrangularis* was acidic in nature and had high amount of crude fibre and protein with less amount of fat. It had substantial amounts of ascorbic acid and β-carotene. The seven phytochemicals were screened for, and all were found present in one solvent extract or the other. It manifested that the presence of different phytochemicals in the *Cissus quadrangularis* stem extracts confirmed the ability to serve a source of natural medicines.**

***Keywords*: *Cissus quadrangularis*, stem, phytochemical, hadjod, proximate, vitaceae**

INTRODUCTION

Since ancient times, plants, animals and minerals are natural products that have been the basis for treatment of human ailments. Holistic medicine has been practiced in different parts of the world from time immemorial. Indigenous people obtained therapeutic materials from various plants which served as medicine for curing and healing diseases. In past decades, many studies have been conducted with respect to traditional herbal plants in treating diseases which led to the advancement in drug and pharmacological industries. Nowadays, medicinal plants has been used as food source due to its substantial nutraceutical properties (Thalkari *et al.*, 2019).

Cissus quadrangularis belongs to the family Vitaceae. The stem of *C. quadrangularis* Linn plays significant role in treating piles, bone fracture, joints pain, swelling, scurvy, gout, asthma, diseases of ear and nose bleeding (Nawghare *et al.*, 2017). Recent analysis elucidated ability of Cissus to hasten bone healing due to its glucocorticoid antagonistic properties. The stem along with sesame oil has been used to medicate Sandhivata induced by chromosome aberrations and sperm deformation. The stem juice of the plant is used to treat scurvy and irregular menstruation, otorrhoea and epitasis. Phytoestrogenic steroids from *Cissus quadrangularis* plant led to early regeneration and quick mineralization of bone fracture healing process. Dry shoots powder is used for digestive troubles, stomach ache, colonopalhy, scurvy, and asthma. Fresh shoot paste is used in burns and wounds. Stem and leaf is used in labour pain. Decoction of shoot along with dry ginger and black pepper is used in body pain. Shoot is used as aphrodisiac, carminative, laxative, digestive and decoction is used as blood purifier and immune modulator (Sadhana *et al.*, 2018). *Cissus quadrangularis* is an ancient medicinal plant used in various regions of the world due to its tremendous medicinal properties. In Cameroon, the whole plant is used in oral re-hydration, while the leaf, stem, and root extracts of this plant used for treating various ailments (Mohanambal *et al.*, 2011).Scientific studies revealed that Cissus extract possessed cardiotonic and androgenic property (Chopra *et al.*, 1986). The plant contained 3-ketosteroids which imparted anti-glucocorticoid properties useful in healing tendons and bones. *Cissus quadrangularis* contained water–soluble glycoside that controls blood pressure (Kavitha *et al.*, 2015). Phytochemical studies of *Cissus quadrangularis* found several phytochemical constituents such as ascorbic acid, carotene, anabolic steroidal substances, calcium, β-sitosterol, δ-amyrin, δ- amyrone, flavonoids, triterpenoids and various secondary metabolites. Atram Seena, (2015) reported that dried *Cissus quadrangularis* plant contained 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 air-dried *Cissus quadrangularis* root powder also constituted good source of mineral elements (mg/100 g 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 (Rasale, 2014).

MATERIALS AND METHODS

Collection of plant material

The plant namely *Cissus quadrangularis* was collected at Simmakkal, Madurai district, Tamil Nadu. It was identified and authenticated by botanically. Then the plant was washed thoroughly and subjected to drying in cabinet drier at 40˚C. This dried plant was powdered finally using a grinder. The sample was transferred into air tight container

Estimation of Chemical composition of *Cissus quadrangularis*

Moisture

The moisture content of the samples was estimated by hot air oven method as per the procedure given by AOAC (1995). The sample was dried at 105°C. Oven drying was continued till we obtain concordant values. Dried samples were cooled in a dessicator and further used for measurement of weight.

$$\text{Moisture (\%)} = \frac{\text{Loss in weight}}{\text{Weight of the sample}} \times 100 = \frac{(W_2 - W_3)}{(W_2 - W_1)} \times 100$$

W1 = Initial weight of empty plate
W2 = Weight of empty plate + sample before drying
W3 = Final weight of empty plate + sample after drying

pH

The pH of the samples was estimated by the method described by Saini *et al*. (2000). Five gram of the sample was mixed well by stirring with 50 ml of distilled water using a glass rod and the pH of the suspension was determined in the pH meter.

Acidity

Acidity of the sample was estimated by the method described by Ranganna (1995). About 10 g of the sample was weighed and dissolved in a known amount of water and made up to 100 ml and filtered. From the filtrate an aliquot of the sample was taken and titrated against 0.01N NaOH using phenolphthalein as an indicator till the appearance of the pale pink colour. The titration was repeated to obtain concordant values. The result was expressed as percent.

Fat

The fat content of the sample was estimated by the method described by Cohen (1917). The lipid in the sample was extracted with petroleum ether (60-80℃) in Sox plus apparatus for two hours. Then the solvent was evaporated and the remaining fat residue was weighed. The fat content was expressed as percentage.

$$\text{Fat (\%)} = \frac{(W_3 - W_2)}{W_1} \times 100$$

W1= Weight of sample used
W2 = Weight of flask
W3 = Weight of flask with fat residue

Protein

Protein was estimated by Lowry's method. 500 mg of the sample was weighed and grinded well with a pestle and mortar in 5-10 ml of the phosphate/ Tris-HCl buffer (pH 7.0). It was then centrifuged and the supernatant was used for the estimation. 0.2, 0.4, 0.6, 0.8, and 1 ml of the working standard was pipetted out into a series of test tubes. 0.2 ml and 0.4 ml of the sample extract was pipetted out into two other test tubes and all the test tubes were made to the volume of 1 ml. These were mixed well and allowed to stand for 10 minutes. A tube with 1 ml of water served as the blank. 5 ml of reagent C was added to each test tube including the blank. It was mixed well and allowed to stand for 10 minutes. Then 0.5 ml of reagent 'D' was added and mixed well and incubated at room temperature in the dark for 30 minutes. Blue colour developed and then the reading was taken at 660nm (if the protein concentration of the sample is high i.e., above 500 µg/ml, measure the colour intensity at 550 nm). A standard graph was drawed and the protein in the sample was calculated.

$$\text{Amount of protein present in 100g of sample} = \frac{\text{Graph value}}{\text{vol. of test}} \times \frac{\text{Total value of extract}}{\text{weight of sample(g)}} \times 100$$

Crude fiber

Crude fiber content was determined as per the method described by Maynard (1970). The dried sample (fat free) was taken in the pre weighed glass crucible (W1) and was placed in crucible holder with the glass extractor. 150 ml of pre heated 1.25% H_2SO_4 was added in the extractor and the contents are boiled for 30 mins at 500°C and 30 mins for 400°C. The acid residue was drained out from the extractor through fibra flow system. The residue was washed with distilled water. Then 150 ml of pre heated 1.25% NaOH was added and digested for 30 mins at 500°C and 30 mins at 400°C. Then the residue was washed with distilled water and dried for two to four hours at 100°C, cooled and weighted.

$$\text{Crude fiber (\%)} = \frac{(W_3 - W_2)}{W_1} \times 100$$

W1= Weight of sample used
W2 = Weight of crucible

W3 = Weight of residue with crucible

Beta Carotene

The sample was weighed (5-10 g) and the sample was repeatedly extracted with acetone using pestle and mortar until the residue was colourless. The acetone extracts was pooled and transferred to a separating funnel containing about 20 ml petroleum ether and 20 ml of distilled water, and it was mixed gently. Carotenoid pigments get transferred from the lower aqueous layer to upper petroleum ether layer. The upper layer (petroleum ether layer) was collected by using separating funnel and was kept aside. The bottom layer was poured again in the separating funnel and then petroleum ether (20 ml) was added and mixed well to extract the β-carotene.

The procedure was repeated three or four times until the colour of the extract became colourless. The petroleum ether extracts was pooled and washed once with 20 ml distilled water in order to remove alkalinity. It was filtered into a conical flask through cotton wool over which anhydrous sodium sulphate was sprinkled to get water free solution. The sodium sulphate slurry was washed with petroleum ether until it was colourless. The final volume was made up to 50 ml with petroleum ether and the absorbance of the solution was determined at 450 nm in a UV-Visual Spectrophotometer using petroleum ether as blank.

$$\text{mg Carotenoids}/100\text{gm} = \frac{\text{OD value} \times \text{Total volume of the extract} \times 100}{0.2592 \times \text{Weight of the sample} \times 1000}$$

Ascorbic acid

The ascorbic acid content was estimated by following the procedure of Sadasivam and Manickam (2008). Hundred mg of ascorbic acid was accurately weighed and made up to 100 ml with 4 per cent oxalic acid (stock standard). Working standard was prepared with 10 ml of the stock standard and diluted to 100 ml in a volumetric flask giving a concentration of 0.1 mg of ascorbic acid per ml. 52 mg of dichlorophenol indophenol dye and 42 mg of sodium bicarbonate was weighed accurately and diluted to 200 ml with distilled water. A sample of 5 g was extracted with 4 per cent oxalic acid and was made up to 100 ml. An aliquot was titrated against 2, 6 dichlorophenol indophenol dye. The amount of ascorbic acid present in the sample was calculated and expressed as mg of ascorbic acid per 100 g of sample on fresh weight basis (FWB).

$$\text{Dye factor} = \frac{1\text{ mg}}{v_1}$$

$$\text{Amount of ascorbic acid present in the 100g of the fruit sample} = \frac{\text{Dye factor} \times v_2 \times \text{Total volume of fruit juice}}{\text{Volume of fruit juice taken for titration} \times \text{weight of fruit(g)}} \times 100\text{ mg}$$

Phytochemical screening of *Cissus quadrangularis*

Solvent extraction (Maceration)

Twenty five grams of dried powdered samples was saturated with 250ml of different solvents like petroleum ether, acetone, hexane, ethanol, methanol, ethyl acetate, chloroform and aqueous. It was allowed to stand for 24hrs which was shaken frequently. It was filtered rapidly taking precautions against loss of solvents, initially it was filtered with muslin cloth and then it was filtered through Whatman No.1 filter paper and the filtrate obtained was evaporated by using Flash Evaporator to dryness and then it was further used for qualitative analysis of phytochemicals. Percentage yield of all the extracts (w/w gm) were determined and documented.

Qualitative analysis of phytochemicals

Detection of Alkaloids

About 50 mg of solvent – free extract was stirred with little quantity of dilute Hydrochloric acid and filtered and the filtrate obtained was taken for below test.

Wagner's test

To a few mL of the filtrate, few drops of Wagner's reagent were added along with the sides of the test tube. Formation of reddish brown precipitate indicates test as positive.

Detection of Glycosides

Keller-Killani test

To 5ml of the extract is treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution and 1ml of concentrated sulphuric acid. A brown ring at the interface indicates the presence of cardiac glycosides.

Detection of Saponins

Foam or Froth Test

A small quantity of the extract was diluted with distilled water to 20 mL. The suspension was shaken in a graduated cylinder for 15 minutes. A two centimetre layer of foam or froth which is stable for 10 minutes indicates the presence of saponins.

Detection of Phenols

Ferric chloride test

About 50 mg of extract was dissolved in distilled water and to this few drops of neutral 5% ferric chloride solution was added. Formation of blue, green, black and violet colour indicates the presence of phenols.

Detection of Tannins

Folin Dannis reagent (0.5ml) was added to the extract and then maintained for 5min at room temperature. Then, 2ml of 20% sodium carbonate was added and left for 5 minutes. Formation of blue colour indicates the presence of tannins.

Detection of Flavonoids

Alkaline Reagent Test

Addition of 5 drops of 5% Sodium hydroxide to 1 ml of the test solution resulted an increase in the intensity of the yellow colour which became colourless on addition of a few drops of 2 M hydrochloric acid which indicated the presence of flavonoids**.**

Detection of Phytosterols

Salkowski's test

The extract was dissolved in 2ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence, indicating the presence of the phytosterols and sterols compound, in the extract.

RESULT AND DISCUSSION

Chemical composition of *Cissus quadrangularis* stem

The chemical constituents like moisture, pH, acidity, fat, protein, crude fiber, β- carotene and ascorbic acid content of *Cissus quadrangularis* were analysed and given in the table 1.

Table 1. Chemical composition of *Cissus quadrangularis* stem

S.No.	Parameters	Quantity (per 100 g)
1.	Moisture (gm)	85.54 ± 0.47
2.	pH	5.04 ± 0.31
3.	Acidity (%)	8.96 ± 0.32
4.	Fat (gm)	5.83 ± 0.32
5.	Protein (gm)	11.4 ± 1.12
6.	Crude Fibre (gm)	17.01 ± 1.98
7.	β- carotene (mg)	237 ± 7.21
8.	Ascorbic acid (mg)	295 ± 9.12

The moisture content of the fresh sample of *Cissus quadrangularis* stem was 85.54%. Manikandan *et al.* (2013) and Seema (2015) reported that moisture content of air dried *Cissus quadrangularis* were 20.10% and 13.1% . The pH of the dried powdered sample of *Cissus quadrangularis* stem was 5.04 which are slightly acidic in nature and the acidity of the sample was found to be 8.96%. The fat and protein content of the powdered sample were found to be 5.83 and 11.4 gm %. Manikandan *et al.* (2013) also revealed that fat and protein content of the air dried powdered sample were 12.31 and 4.35% respectively. The fibre content of the powdered fat free sample was 17.01gm %. Similar studies investigated on the proximate analysis of *Cissus quadrangularis* implied that it had fibre content of 15.6 gm % (Mishra *et al*., 2010 ; Seema, 2015; Samaranayake *et al.*, 2015; and Rasale, 2014). The β-carotene content of the fresh sample of *Cissus quadrangularis* stem was 237 mg. It has been reported that content of the air dried sample of *Cissus quadrangularis* was 267 mg (Mishra *et al*., 2010 ; Seema, 2015; Samaranayake *et al.*, 2015; and Rasale, 2014).The Ascorbic acid content of the fresh sample of *Cissus quadrangularis* stem was 295 mg. It has been reported that Ascorbic acid content of the air dried sample of *Cissus quadrangularis* was 479 mg (Mishra *et al*., 2010 ; Seema, 2015; Samaranayake *et al.*, 2015; and Rasale, 2014).

Phytochemical screening of *Cissus quadrangularis*

The dried powder of stem of *Cissus quadrangularis* was extracted with solvent extraction (Maceration) using various solvents such as Petroleum ether, Acetone, Hexane, Ethanol, Methanol, Aqueous, Ethyl acetate, and Chloroform. Phytochemical tests determined that yield percentage (w/w gm) of extract of plant was high in methanol extract (11.50 %) and lowest in petroleum ether extract (0.86 %). The results of the yield (per cent) in different extracts were mentioned in table 2.

Table 2. Percentage yield of the extracts

S.No.	Name of extract	% Yield (w/w gm)
1.	Petroleum ether	0.86
2.	Acetone	3.76
3.	Hexane	1.32
4.	Ethanol	8.70
5.	Methanol	11.50
6.	Ethyl acetate	2.72
7.	Chloroform	2.02
8.	Aqueous	5.20

The various stem extract of the plant of *Cissus quadrangularis* were subjected to phytochemical screening which reveal the presence of various bioactive components. The results of extracts are presented table 3 and 4.Yadav, (2016) also reported that, the presence of various pharmacological active constituents in various stem extract of *Cissus quadrangularis*.

Table 3. Phytochemical compounds in different extracts of *Cissus quadrangularis* stem

S.No.	Name of extract	Phytochemicals
1.	Petroleum ether	Alkaloids, Glycosides, Phytosterols, Tannins
2.	Acetone	Alkaloids, Saponins, Phenolics, Tannins
3.	Hexane	Alkaloids, Flavonoids, Phytosterols, Tannins
4.	Ethanol	Alkaloids, Glycosides, Saponins, Phenolics
5.	Methanol	Alkaloids, Glycosides, Saponins, Flavonoids, Phenolics, Tannins
6.	Ethyl acetate	Alkaloids, Glycosides, Phenolics, Tannins
7.	Chloroform	Alkaloids, Glycosides, Phenolics, Tannins
8.	Aqueous	Alkaloids, Glycosides, Flavonoids, Phenolics

All the eight extracts taken showed positive results indicating the presence of alkaloids. Kakade *et al.* (2014) also reported that petroleum ether, chloroform, methanol extracts of *Cissus quadrangularis* showed positive results indicating presence of alkaloids. All the extracts showed positive results of the test indicating the presence of glycosides except hexane extract which showed negative results indicating the absence of glycosides. Mohanambal (2011) reported that ethanolic extract of *Cissus quadrangularis* showed positive result in glycosides. Ethanol, methanol, acetone extracts showed the positive result that, indicating presence of saponin but other extracts showed negative results which showed absence of saponins. Yadav (2016) reported that ethanolic extract of *Cissus quadrangularis* showed positive result in foam test indicating presence of saponins.

All other extracts except petroleum ether and hexane extracts showed the presence of phenolic compounds. Kakade *et al.* (2014) also reported that methanol and ethanol extracts of *Cissus quadrangularis* showed positive results indicating the presence of phenolic compounds. Hexane, methanol and aqueous extracts showed the presence of flavonoids whereas other extracts showed absence of flavonoids. Talreja *et al*. (2016) reported that ethanol extracts of *Cissus quadrangularis* showed negative results indicating the absence of flavonoids. All extracts other than ethanol and aqueous extracts showed the presence of tannins. Talreja *et al.* (2016) also reported that ethyl acetate extract of *Cissus quadrangularis* showed negative results indicating the presence of tannins. Petroleum ether and hexane extract showed the presence of phytosterols whereas other extracts indicated absence of phytosterols. Talreja *et al*. (2016) reported that methanol, ethanol, aqueous, chloroform, acetone extracts of *Cissus quadrangularis* showed negative results indicating the absence of phytosterols.

Table 4. Qualitative Analysis of Phytochemicals presents in *Cissus quadrangularis* stem

Tests for	Petroleum ether extract	Ethanol extract	Methanol extract	Acetone extract	Chloroform extract	Hexane extract	Ethyl acetate extract	Aqueous extract
Alkaloids	++	++	++	++	++	++	++	++
Glycosides	++	++	++	_	++	_	++	++
Saponins	_	+	+	+	_	_	_	_
Phenolic compounds	_	++	++	++	++	_	++	++
Flavonoids	_	_	+	_	_	++	_	+
Tannins	+	_	+	+	+	+	+	_
Phytosterols	++	_	_	_	_	++	_	_

Note: ++ (Much Abundant), + (Less), - (Absent)

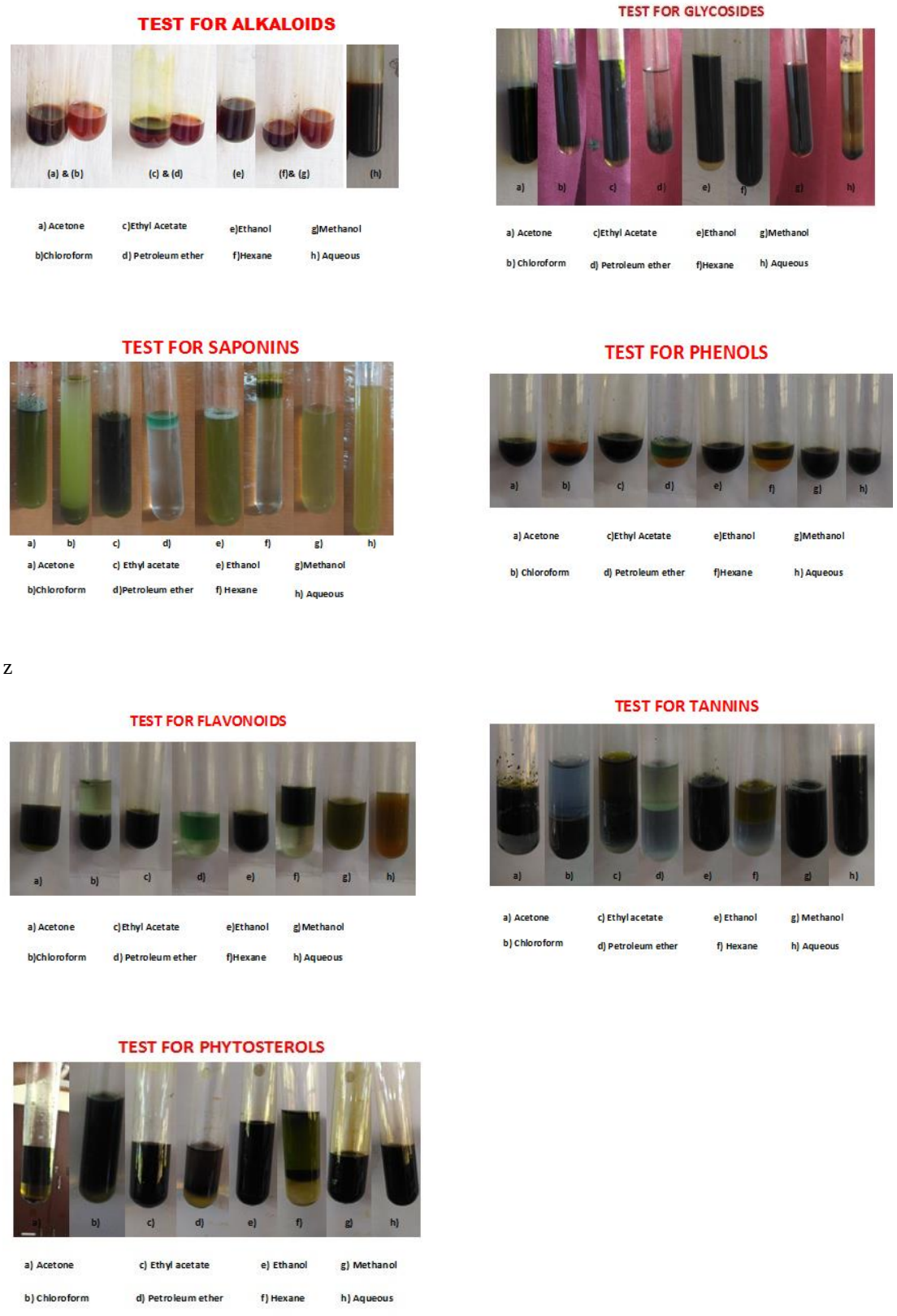

Plate 1. Presence of phytochemicals in *Cissus quadrangularis* stem (qualitative test)

Conclusion

Correlation between the phyto-constituents and bioactivity of plant is desirable to know for the synthesis of compounds with specific activities treats various health ailments and chronic diseases as well. Therefore, the basic phytochemical investigation is vital. The identification and isolation of such active compounds makes it more effective therapeutic applications (Nandagoapalan *et al.*, 2015). Successful determination of biological active compounds from plant material is largely dependent on the type of

solvent used in the extraction procedure. This therefore underscores the need to try as much solvents as possible in screening plants parts for phytochemicals. It was found that *Cissus quadrangularis* has high amounts of ascorbic acid and β-carotene. It is acidic in nature. It has high amount of crude fibre and protein and less amount of fat. The Phytochemical qualitative analysis showed the presence of alkaloids in all the extracts, the presence of glycosides in all except acetone and hexane, and flavonoids are present in hexane, aqueous and absence in all other extracts. It was noted that phenols are present in methanol, ethanol, acetone, chloroform, ethyl acetate and aqueous medium extracts, where as tannins are present less in petroleum ether, acetone, chloroform, methanol, ethyl acetate and hexane extracts. Saponins are abundantly present in acetone, ethanol and less in methanol; Phytosterols are present strongly in acetone, petroleum ether, hexane, than in chloroform extract. Of the seven phytochemicals screened for, all were found present in one solvent extract or the other. It indicates that the presence of different phytochemicals in the *Cissus quadrangularis* stem extracts that confirms the ability to serve a source of natural medicines.

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