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Extraction of *Cissus quadrangularis* L. Stem Extracts Using Various Solvents via Soxhlet Method and Preliminary In-Vitro Profiling of Secondary Metabolites.

[1]Vinay Bhushan Kumar, [2]Tanuja and [3*]Arvind Kumar
[1]Department Of Botany, T.P.S. College, Patliputra University, Patna-800001
[2]Centre For Interdisciplinary Research In Basic Sciences, Jamia Millia Islamia, New Delhi-110025
[3]University Department Of Botany, Patliputra University, Patna-800001
*(*Corresponding Author-Arvind Kumar)*

Abstract
Medicinal plants are valuable sources of pharmacologically active compounds. Cissus quadrangularis L., a member of the Vitaceae family, is well-known for its medicinal properties. It is a succulent plant recognized for its ability to synthesize a variety of bioactive molecules. The purpose of this study was to conduct a preliminary phytochemical profiling of the stem extract of Cissus quadrangularis L. using different solvents. Using the Soxhlet extraction method, plant extracts were isolated with various solvents, and a preliminary phytochemical profiling was performed following different protocols. The percentage yields of the plant extracts were as follows: 4% for acetone, 6.5% for aqueous, 6% for chloroform, 4.5% for dichloromethane, 6% for hexane, and 5.5% for methanol. Alkaloids were found in all solvent extracts, whereas tannins were present only in the aqueous extract. Phenols and flavonoids were present in all extracts except for acetone. Steroids were detected in all extracts except chloroform, and saponins were present in all extracts except dichloromethane and chloroform. On average, the stem of Cissus quadrangularis L. contained all the tested phytochemicals.

Keywords: *Bioactive Components, Cissus Quadrangularis, Phytochemical, Soxhlet, And Stem Extract.*

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I. Introduction

The exploration of medicinal plants as abundant sources of pharmacologically active compounds has gained considerable interest among researchers. There is a growing emphasis on investigating the potential of phytochemicals for managing and treating various human diseases. Over half of today's clinical drugs are derived from natural products, highlighting the crucial role these compounds play in the development of modern pharmaceuticals [1]. Ayurveda, Unani, Siddha, and modern medicine all make extensive use of various plants for treating a wide range of illnesses [2]. In the Indian subcontinent, a wide variety of medicinal plants are commonly used as treatments for numerous health conditions [3]. Herbal medicine, often referred to as phytomedicine, involves the use of plants for therapeutic and medicinal purposes, with the goal of treating illnesses and improving health. Plants produce a variety of secondary metabolites known as phytochemicals—derived from the Greek word 'phyto,' meaning 'plant'—which contribute to their healing properties. These phytochemicals demonstrate a range of biological activities and play a vital role in supporting overall health. The holistic nature of herbal medicine leverages the combined effects of these plant compounds, providing a traditional form of healthcare that has been practiced for centuries in many cultures around the world. Additionally, these compounds act as a natural defense system for plants, helping to protect them from microbial infections and pest infestations. Phytochemicals are bioactive substances recognized for their therapeutic benefits and medicinal qualities. Phytochemicals can be classified on the basis of their chemical composition as follow Alkaloids (e.g. - morphine, caffeine, berberin, codeine) [4], Polyphenoles (Flavonoids, Phenolics Tannins) (e.g. - Quercetin, reservaratrol kaempferol and quercitrin, caffeic acid, flavones, rutin, naringin, hesperidin and chlorogenic, tannic acid, gallic acid and ellagic acid) [5] and Saponins (e.g. - Diosgenin and hecogenin) [6]. *Cissus quadrangularis* L. is a notable plant within the botanical realm, recognized for its numerous benefits. This perennial herb, part of the grape family, is characterized by its sturdy, fleshy stems that have a unique quadrangular shape. This plant is found widely in tropical regions around the globe and is well-regarded for its significant medicinal properties [7]. *Cissus quadrangularis*, also known by its synonym Cissus succulent, is referred to as "horjora" in Hindi and "pirandai" in Tamil. As a member of the Vitaceae family, this plant plays an important role in traditional medicine practices in India. While it is believed to be native to regions such as India, Sri Lanka, Malaysia, Java, and West Africa, it is commonly found in the tropical forests of Asia and Africa [7]. *Cissus quadrangularis* is an evergreen climbing plant noted for its fast growth. Extensive

research has been conducted on its phytochemical composition, pharmacological properties, and safety assessments. Scientists have investigated its various chemical constituents and potential health benefits while evaluating its safety for use in traditional medicine.

Cissus quadrangularis extract contains a variety of phytochemicals, including alkaloids, tannins, lignin, suberin, phenols, flavonoids, resveratrol, piceatannol, pallidol, perthenocissin, phytosterols, and other compounds [8,9]. Among the phytochemicals found in Cissus quadrangularis, key compounds include ascorbic acid (vitamin C), triterpenes, beta-sitosterol, ketosterol, two asymmetrical tetracyclic triterpenoids, and calcium. These constituents are believed to enhance the plant's medicinal properties and potential health benefits [2,10]. *Cissus quadrangularis* offers a wide array of beneficial properties, including antimicrobial, antioxidant, anti-inflammatory, anti-cancer, and cytotoxic effects [11,12,13]. Additionally, *Cissus quadrangularis* is recognized for its role in promoting bone healing, highlighting its versatility and potential therapeutic benefits for various health conditions [14].

II. Materials And Methods

Preparation of stem extract

Fresh stems of *Cissus quadrangularis* L. were collected from the Supaul district in Bihar, situated at a latitude of 26.5520640 and a longitude of 87.0555330 (Figure 1). The authentication of the plant and its stems was performed to confirm their identity by Professor Rimjhim Sheel, Principal GDM College, Patliputra University, Patna. A voucher specimen has been preserved for future reference at the University Department of Botany, Patliputra University, Patna, Bihar, India. To prepare the plant material, the stems were thoroughly washed with tap water and rinsed with distilled water. They were then shade-dried and ground into a fine powder. The resulting powder was stored in a clean, sealed container for future use.

Fig 1: - Plant of *Cissus quadrangularis* L.

Soxhlet extraction

The extraction of the stems of *Cissus quadrangularis* L. was performed using the Soxhlet extraction method [15,16]. Dried powder of *Cissus quadrangularis* L. stems (50 g) was placed in the thimble of a Soxhlet apparatus. A total of 350 ml of various solvents—methanol, acetone, chloroform, dichloromethane, hexane, and aqueous (MACDHA)—were used sequentially. The solvents dissolved the active biomolecules, while the stems remained as a precipitate. Extraction continued until the solvent in the thimble was clear, which typically took around 8 hours. Afterward, the extract was dried in a water bath until a dark orange residue was obtained.

Phytochemical screening

Phytochemical screening was conducted on the various solvent extracts (methanol, acetone, chloroform, dichloromethane, hexane, and water) of *Cissus quadrangularis* L. stems using standard protocols to identify the secondary metabolites [17,18] .

Test for alkaloids: -

The alkaloid test was conducted using Wagner's test. In this procedure, a small amount of Wagner's reagent was added along the sides of a test tube containing 10 mg of the plant extract. The sample was then closely observed for any signs of turbidity or precipitation.

Test for flavonoids:-

The presence of flavonoids was assessed using an alkaline reagent test. In this procedure, the plant extract was treated with a 10% NaOH solution. The formation of an intense yellow color indicated the presence of flavonoids, which then turned colorless upon the addition of two drops of diluted acid.

Test for phenolic compounds and tannins:-

To detect phenolic compounds and tannins, a lead acetate test was performed. In this procedure, 5 mg of the plant extract was dissolved in 5 ml of distilled water, and then 1 ml of a 10% lead acetate solution was added. The sample was observed for any signs of turbidity or precipitation.

Test for tannin:-

The presence of condensed tannins was assessed using Braymer's test. In this procedure, 5 mg of the plant extract was mixed with 4 ml of ferric chloride (FeCl). The development of a blue-black or green color in the sample indicated the presence of condensed tannins.

Test for steroids:-

The presence of steroids was evaluated using the Salkowski test. In this procedure, 5 mg of the plant extract was dissolved in 10 ml of chloroform, and an equal volume of concentrated H SO was carefully added along the side of the test tube. The appearance of a red color in the upper layer and a yellow color with green fluorescence in the H SO layer indicated the presence of steroids.

Test for saponins:-

A suspension of the plant extract was prepared by diluting 2 mg of the extract in distilled water to a total volume of 20 ml. The mixture was shaken in a graduated cylinder for 15 minutes. The formation of persistent froth in the suspension indicated the presence of saponins.

III. Result And Discussion

The results of phytochemical screening are vital for understanding the medicinal properties of plants and provide an empirical foundation for their traditional therapeutic applications. Phytochemical constituents such as alkaloids, flavonoids, phenolic compounds, tannins, saponins, and steroids play significant roles in the pharmacological effects of the plant. Each of these compounds may demonstrate various biological activities, contributing to the plant's potential therapeutic benefits.

Estimation of plant extracts

The percentage yield for MACDHAq extracts were tabulated in the table (Table: - 2). The percentage yields were 5.50, 4.00, 6.00, 4.50, 6.00 and 5.50 for methanol, acetone, chloroform, dichloromethane, hexane and aqueous respectively. The extract were kept at -20^0 C till further use. All the process of Soxhlet extraction was completed in University Department of botany and Department of Botany TPS College, Patliputra University.

Table 2: - Dry form of stem extract of different solvents (i.e. MACDHAq) of the plant *Cissus quadrangularis* L.

S.N.	Name of the solvent	Weight of powder of stem (in gms)	Volume of solvent (in mL)	Dry weight of plant extract (in gms)	% of plant extract
01	Methanol	50.00	350	2.75	5.50
O2	Acetone	50.00	350	2.00	4.00
O3	Chloroform	50.00	350	3.00	6.00
O4	Dichloromethane	50.00	350	2.25	4.50
05	Hexane	50.00	350	3.00	6.00
06	Aqueous	50.00	350	3.25	6.50

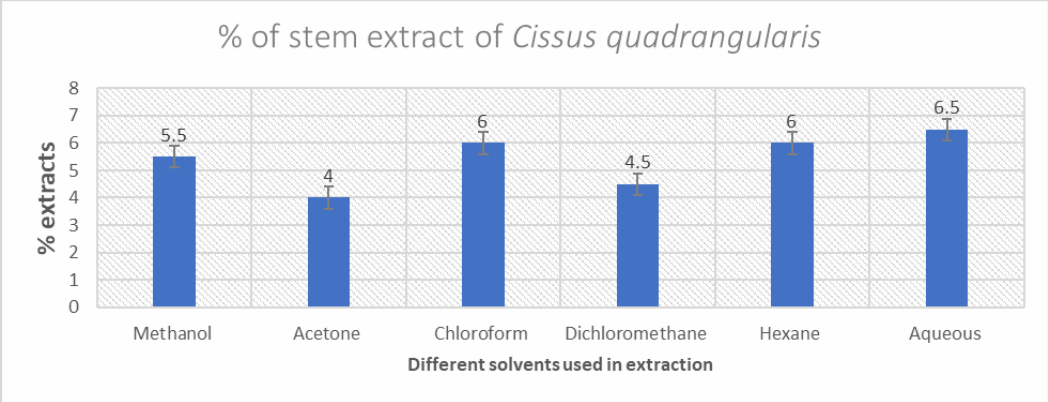


Fig: - 3 Dry form of stem extract of different solvents (i.e. MACDHAq) of the plant *Cissus quadrangularis* L.

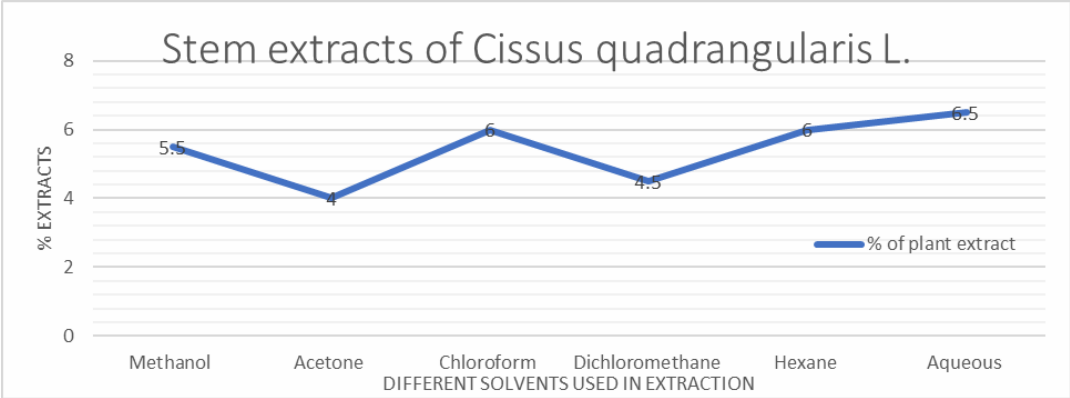
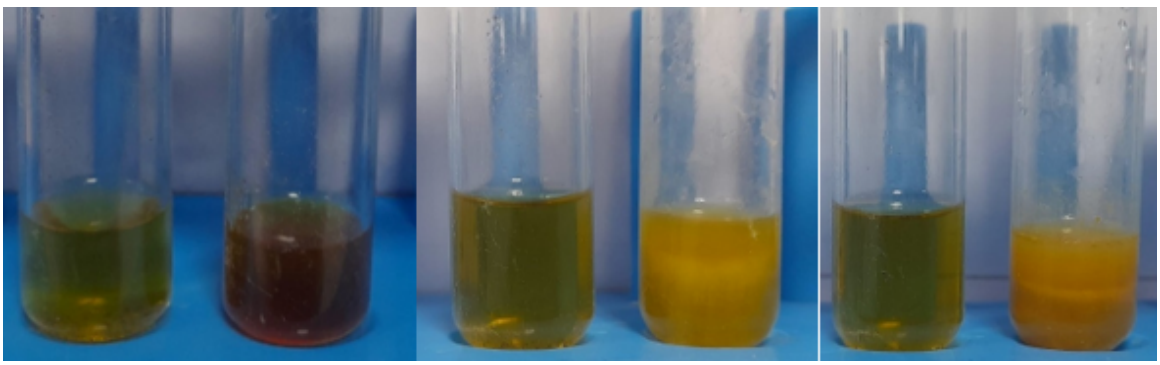
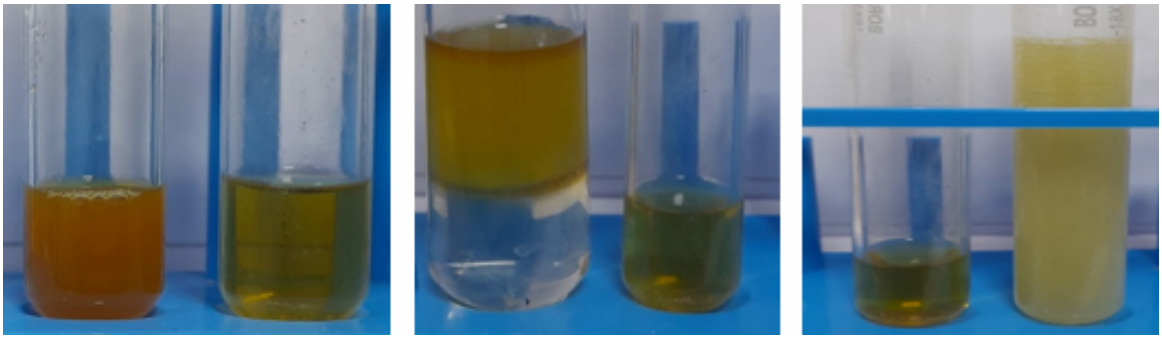


Fig: - 4 Graphical presentation of dry form of stem extract of different solvents (i.e. MACDHAq) of the plant *Cissus quadrangularis* L.

Screening of secondary metabolites (alkaloids, flavonoids, phenols, tannins, steroids and saponins) in Methanol extract.



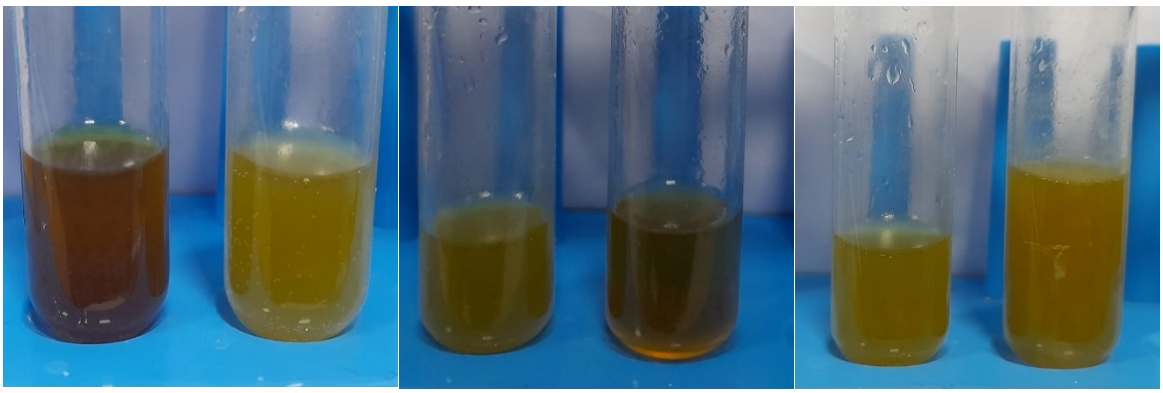
(3.1.1) **(3.1.2)** **(3.1.3)**



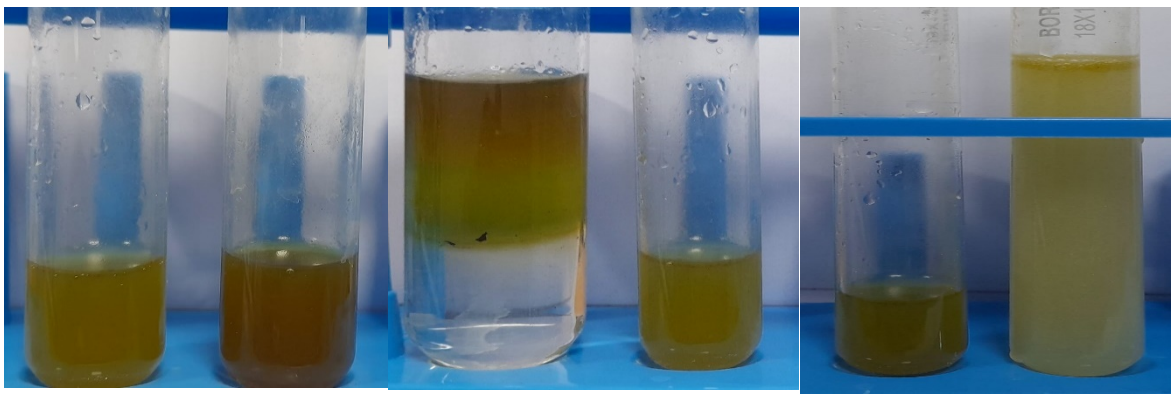
(3.1.4) **(3.1.5)** **(3.1.6)**

Fig 5: - Phytochemical analysis of methanol extract (3.1.1) Wagner's reagent test. (3.1.2) Alkaline reagent test. (3.1.3) Lead acetate test. (3.1.4) Ferric chloride test. (3.1.5) Salkowaski's test (3.1.6) Saponins forth test.

Screening of secondary metabolites in Acetone extract.



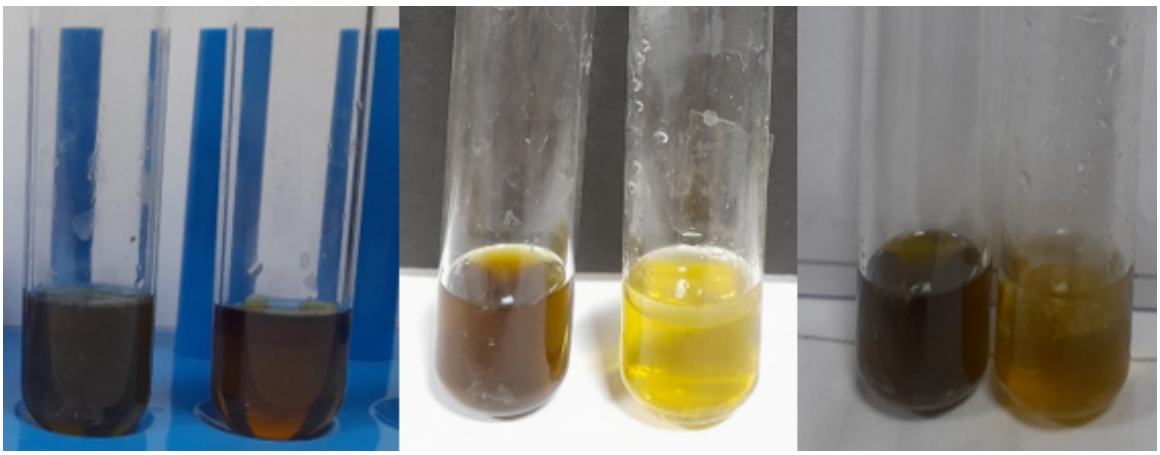
(3.2.1) **(3.2.2)** **(3.2.3)**



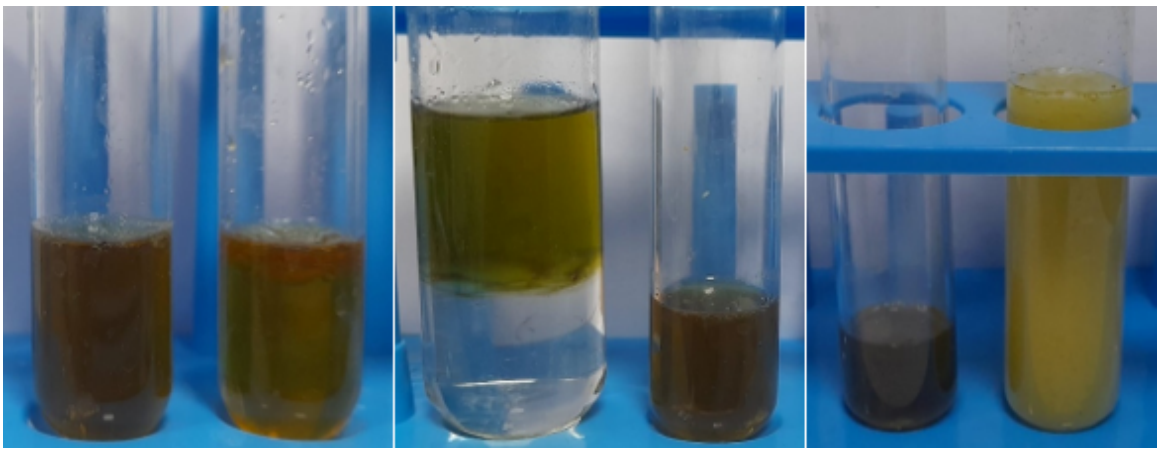
(3.2.4) **(3.2.5)** **(3.2.6)**

Fig 6: - Phytochemical analysis of acetone extract of *Cissus quadrangularis* L. (3.2.1) Wagner's reagent test. (3.2.2) Alkaline reagent test. (3.2.3) Lead acetate test. (3.2.4) Ferric chloride test. (3.2.5) Salkowaski's test (3.2.6) Saponins forth test.

Screening of secondary metabolites in Chloroform extract.



(3.3.1) (3.3.2) (3.3.3)



(3.3.4) (3.3.5) (3.3.6)

Fig 7: - Phytochemical analysis of chloroform extract of *Cissus quadrangularis* L. (3.3.1) Wagner's reagent test. (3.3.2) Alkaline reagent test. (3.3.3) Lead acetate test. (3.3.4) Ferric chloride test. (3.3.5) Salkowaski's test (3.3.6) Saponins forth test.

Screening of secondary metabolites in Dichloromethane extract.

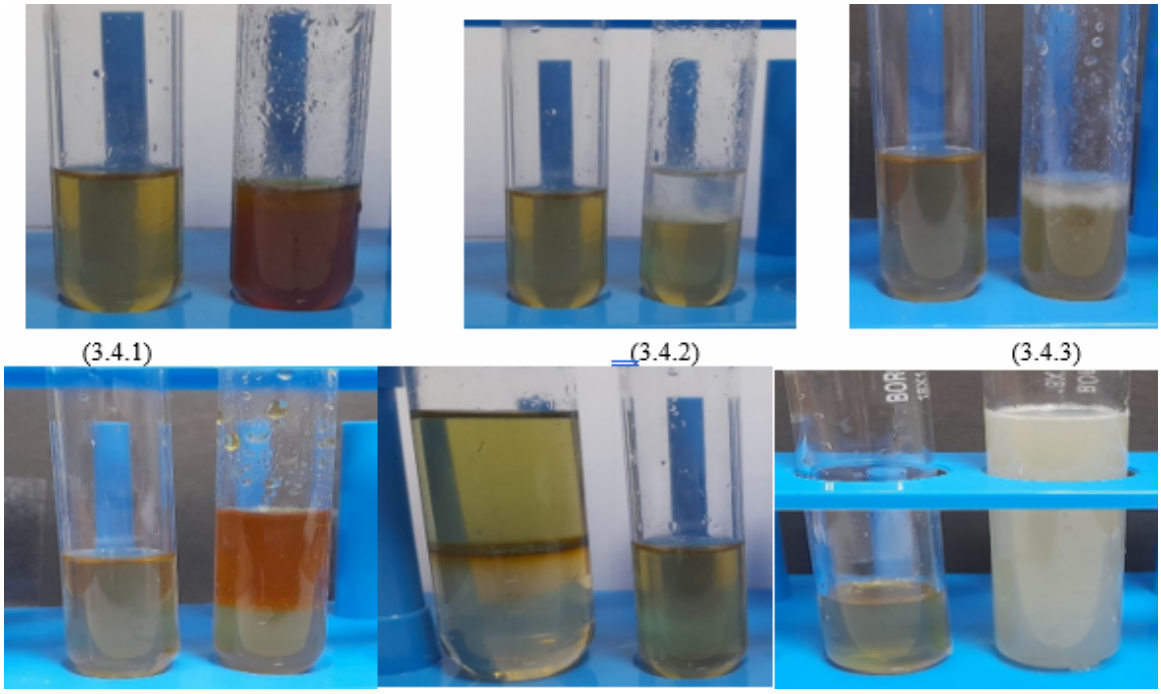


Fig 8: - Phytochemical analysis of dichloromethane extract of *Cissus quadrangularis* L. (3.4.1) Wagner's reagent test. (3.4.2) Alkaline reagent test. (3.5.3) Lead acetate test. (3.4.4) Ferric chloride test. (3.4.5) Salkowaski's test (3.4.6) Saponins forth test.

Screening of secondary metabolites in Hexane extract.

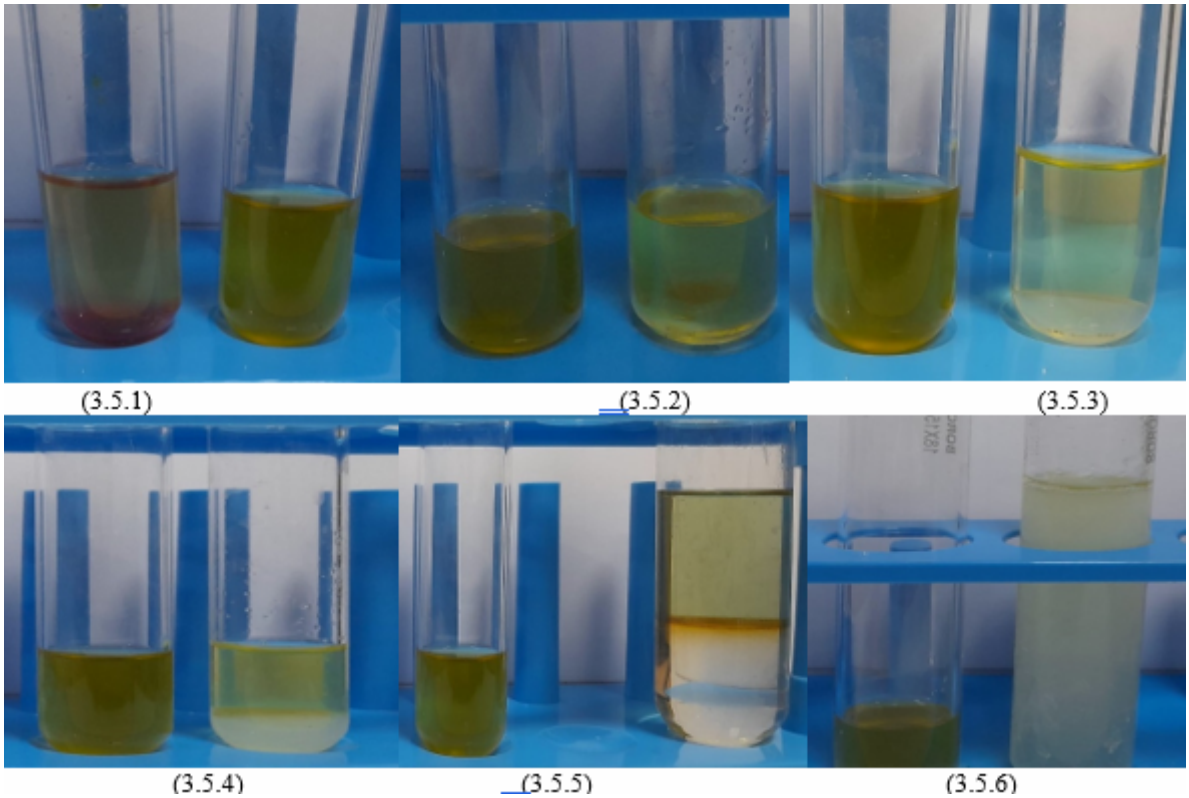


Fig 9: - Phytochemical analysis of hexane extract of *Cissus quadrangularis* L. (3.5.1) Wagner's reagent test. (3.5.2) Alkaline reagent test. (3.5.3) Lead acetate test. (3.5.4) Ferric chloride test. (3.5.5) Salkowaski's test (3.5.6) Saponins forth test.

Screening of secondary metabolites in Aqueous extract.

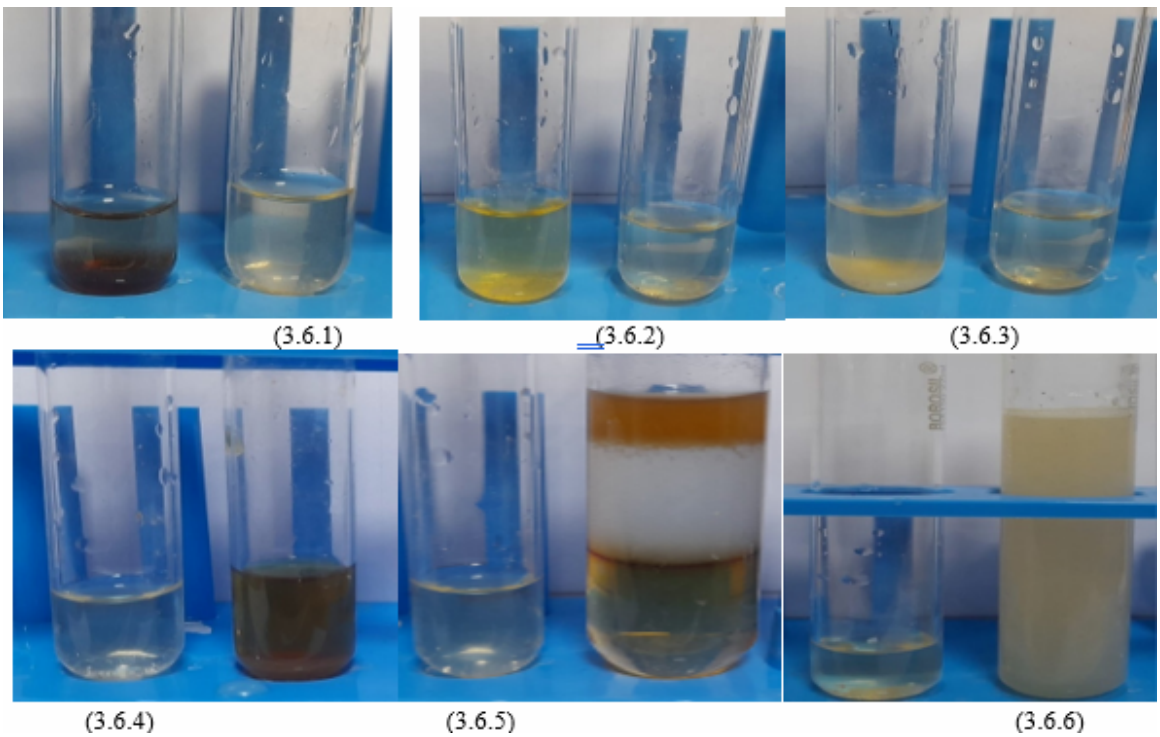


Fig 10: - Phytochemical analysis of aqueous extract of *Cissus quadrangularis* L. (3.6.1) Wagner's reagent test. (3.6.2) Alkaline reagent test. (3.6.3) Lead acetate test. (3.6.4) Ferric chloride test. (3.6.5) Salkowaski test. (3.6.6) Saponins forth test.

Comparative analysis of phytochemicals in MACDHAq extracts of stems of the plant *Cissus quadrangularis* L.

Table 3: - Comparative analysis of phytochemicals in MACDHAq extract of the plant *Cissus quadrangularis* L.

Different phytochemicals	The stem extract of different solvents of the plant *Cissus quadrangularis* L.						
	Name	Methanol	Acetone	Chloroform	Dichloro-methane	Hexane	Aqueous
	Alkaloids	+	+	+	+	+	+
	Flavonoids	+	-	+	+	+	+
	Phenols	+	-	+	+	+	+
	Steroids	+	+	-	+	+	+
	Saponins	+	+	-	-	+	+
	Tannins	-	-	-	-	-	+

The results of phytochemical screening offer an empirical foundation for the use of medicinal plants in traditional therapies. The phytochemical constituents are key contributors to the pharmacological effects of these plants. The percentage yields of plant extracts were 4, 6.5, 6, 4.5, 6 and 5.5 of Acetone, Aqueous, Chloroform, Dichloromethane, Hexane and Methanol respectively (Table - 2).

Presence of different phytochemicals are analyzed as follow: Radish brown precipitate indicate the presence of alkaloids by Wagner's reagent test. Yellow colour indicate the presence of flavonoid by alkaline reagent test. Formation of precipitate indicate the presence of tannin and phenolic compounds by lead acetate test. Presence of dark bluish black color indicate the tannin by ferric chloride test. Formation of two layers, the upper layer turn red and H_2SO_4 layer showed yellow with green fluorescence indicates presence of steroids by Salkowaski test. And presence of forth indicates saponins by saponins forth test.

The phytochemical screening of methanolic extract showed the presence of alkaloids, flavonoids, phenols, steroids and saponins but absence of tannins (Fig-5), the similar work reported by Murthy et al, 2003 [19], Kannaki and Venket, 2019 [20] & Mummed et al, 2018 [21]. Acetone extract showed the presence of alkaloids, steroids and saponins but absence of flavonoid, Phenols & tannins (Fig-6), the result supported by the work of Kuppuramalingam, A. P., et al, 2018 [22]. Chloroform extract showed the presence of alkaloids, flavonoids & phenols but absence of steroids, saponins & tannins (Fig-7), this result is supported by the work of Kannaki and Venket, 2019 [20]. Dichloromethane extract showed the presence of alkaloids, flavonoids, phenols & steroids but absence of saponins & tannins (Fig-8), the result is supported by the work of Anitha & Suji, 2012 [23].Hexane extract showed the presence of alkaloids, flavonoids, phenols, steroids & saponins but absence of tannins (Fig-9). Presence of steroids in hexane extract also supported by the work of Murthy et al, 2003 [19]. Aqueous extract showed the presence of all the phytochemical examined (Fig-10), this result also supported by the work of Kalpana et al, 2017 [24], Anitha & Suji, 2012 [23,] and Kuppuramalingam, A. P et al, 2018 [22].

IV. Conclusion

The result of preliminary phytochemical profiling of stem extracts in different solvent of the plant *Cissus quadrangularis* L. showed that alkaloids were present in all solvent extracts, tannins were present in only aqueous extract, phenols and flavonoids were present in all extract except acetone, steroids were present in all extracts except chloroform and saponins were present in all extract except dichloromethane and chloroform.

On an average, we can conclude that, the stems of the plant *Cissus quadrangularis* L. contain all the tested phytochemicals (i.e. alkaloids, flavonoids, phenols, tannins, steroids and tannins). The result also correlates with previous research work that have been reported the existence of compounds alkaloids, flavonoids, phenols, tannins, steroids and tannin as describe in result and discussion section.

Acknowledgement

We wish to thank HOD, Botany and Principal T.P.S. College, PPU and HOD, University department of Botany, PPU for their support and encouragement.

Conflict of interest

We declared that there is no conflict of interest and the work that have been done by me was authentical and original.

Abbreviation

MACDHAq – Methanol acetone chloroform dichloromethane hexane aqueous.

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