"Phytochemical and Antioxidant Assessment of Cissus discolor: A Comparative Study of Leaf and Stem Extracts"

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ABSTRACT
Cissus discolor Blume, a medicinal plant from the Vitaceae family, was analyzed for its phytochemical composition and antioxidant activity using different solvents (methanol, ethyl acetate, chloroform, petroleum ether, and aqueous). Both qualitative and quantitative phytochemical analyses were conducted, revealing the presence of flavonoids, tannins, phenols, alkaloids, and other bioactive compounds. Among the extracts, the methanolic leaf extract demonstrated the highest total phenolic content (1.92 µg GAE/mg) and the strongest antioxidant activity (77.24% inhibition in the DPPH assay). The methanolic stem extract also exhibited significant phenolic content (1.54 µg GAE/mg), but overall, the leaf extracts, particularly those obtained with methanol, showed superior phytochemical richness and antioxidant potential. These findings suggest that methanol is the most effective solvent for extracting phenolic compounds, and the leaf of C. discolor holds greater potential as a source of bioactive compounds with antioxidant properties, supporting its traditional medicinal applications.

Keywords: Cissus discolor, phytochemicals, total phenolic content, antioxidant activity, DPPH assay, methanolic extract, bioactive compounds, flavonoids

INTRODUCTION

Medicinal plants have long been integral to traditional medicine systems worldwide, providing therapeutic compounds for centuries to treat various ailments and serving as a foundation for modern pharmaceuticals. Approximately 40% of global healthcare relies on traditional medicine, with 85% of these treatments derived from plants. In regions like the Kashmir Himalayas, medicinal plants are a primary healthcare resource, used extensively to treat numerous disorders based on ethnobotanical knowledge. Systems such as Ayurveda, Siddha, and Unani in India document thousands of plant species, highlighting their historical and ongoing significance in healthcare (Anmol *et al.,* 2023; Basu *et al.,* 2023). Medicinal plants possess diverse therapeutic properties, including anti-inflammatory, antimicrobial, and antioxidant effects, due to bioactive compounds like polyphenols, saponins, terpenes, and alkaloids (Šarčević-Todosijević *et al.*, 2023; Dasgupta, 2023). Despite their potential, challenges such as scientific validation, quality control, and sustainable harvesting must be addressed (Basu *et al.*, 2023). The World Health Organization (WHO) and other bodies emphasize integrating traditional knowledge with scientific research to develop new drugs for conditions like endometriosis and cancer (Rishikesan & Devi, 2023). Countries with rich traditions of medicinal plants, like India, continue to be explored for their pharmacological potential (Anmol *et al.*, 2023; Basu *et al.*, 2023).

The Vitaceae family, comprising around 910 species, includes economically significant genera such as *Vitis vinifera*, *Cissus quadrangularis*, and *Rhoicissus* (Wen *et al.*, 2018; Lu *et al.*, 2018). The family's value extends beyond grapes, with genera like *Parthenocissus* and *Cissus*, prevalent in tropical regions, contributing to its medicinal diversity (Kashikar & George, 2006; Lu *et al.*, 2012). Phytochemicals, such as flavonoids and phenolic acids, from the Vitaceae family are known for their antioxidant properties and potential to reduce cardiovascular disease risk by protecting LDL from oxidation and exhibiting anti-inflammatory effects (Quiñones *et al.*, 2012; Sabra *et al.*, 2021). The genus *Cissus*, consisting of around 800 species, is primarily found in tropical regions like India, Sri Lanka, Africa, and South Asia, and its species are recognized for their medicinal properties, including hypoglycemic, anti-inflammatory, analgesic, hepatoprotective, and antimicrobial activities (Ansarali *et al.*, 2016). Notable species include *C. quadrangularis* with analgesic and anti-inflammatory properties (Shah, 2011; Bhujade *et al.*, 2015) and *C. populnea*, which is linked to male fertility and antimicrobial effects. Other species, such as *C. cornifolia*, exhibit anticancer and anti-inflammatory properties (Mongalo *et al.*, 2023; Chipiti *et al.*, 2017), while *C. verticillata* is noted for its antidiabetic and neuroprotective effects (Kim *et al.*, 2021). The pharmacological potential of these species underlines the medicinal significance of the *Cissus* genus (Prabhavathi *et al.*, 2016; Sheikh *et al.*, 2015; Syed *et al.*, 2021).
Cissus discolor Blume, commonly known as Sangharhmai, is a vine species within the *Cissus* genus of the Vitaceae family, renowned for its striking ornamental foliage, making it popular in gardening and landscaping. This plant is valued for its

medicinal properties, particularly in treating stomach troubles and applying to itching sores. Additionally, the leaves are used for their anti-diabetic and antiseptic properties (Sawmliana, 2003). *C. discolor* has not been extensively studied for its phytochemical constituents or its antioxidant properties, which presents a significant gap in the existing literature. Given its traditional medicinal uses and potential health benefits, it is crucial to conduct comprehensive research to analyze the phytochemical profile of this species and evaluate its antioxidant activity. Our objective focuses on filling this knowledge gap by systematically investigating the phytochemical constituents and assessing the antioxidant properties of *C. discolor*, thereby contributing to a better understanding of its therapeutic potential.

MATERIALS AND METHODS

Sample Collection and Authentication

The *C. discolor* was collected during the months of May and June from the region near Hulugar Mane, Shringeri, Karnataka, India. Upon collection, the leaves and stems of the plant were carefully handled to maintain their integrity, cleaned to remove dirt or debris, and spread in a well-ventilated, shaded area for natural drying.

Extraction of plant materials

Post-drying, the samples of leaf and stem were finely ground using a blender and stored in airtight containers for future use. For the extraction process, 10 grams of shade-dried powder were subjected to solvent extraction with 100 mL of different solvents, such as water, methanol, chloroform, Petroleum ether, and ethyl acetate, utilizing a Soxhlet apparatus. The resulting extracts were concentrated by evaporating the solvents under reduced pressure using a rotary vacuum evaporator. The dried residues were subsequently stored in desiccators for later use.

Qualitative analysis of the Phytochemicals

Phytochemical analysis of various solvent extracts was performed using established methods (Harborne, 1973; Kokate, 2004). Carbohydrates were confirmed through Molisch's, Fehling's, Benedict's, iodine, and Schiff's tests, indicating the presence of reducing sugars and polysaccharides. Proteins and amino acids were detected using Ninhydrin and Biuret tests. Alkaloids were identified by Dragendorff's and Wagner's reagents, while anthraquinones were confirmed using benzene and ammonia. Flavonoids were detected through lead acetate, Shinoda, and ferric chloride tests, and phenols by ferric chloride. The foam test indicated saponins, and steroids were detected using the Liebermann-Burchard test. Tannins were identified by ferric chloride and lead acetate tests, and terpenoids using the Salkowski test. Glycosides were confirmed through Legal, Keller-Killiani, and sodium hydroxide tests. These results suggest the presence of multiple bioactive compounds, supporting the therapeutic potential of the extracts.

Quantitative analysis of the Phytochemicals

Quantitative analysis of phytochemical components was conducted using established methods (Madhu, *et al.,* 2016). Carbohydrates were estimated using the anthrone method, with a color change measured at 630 nm. Protein content was determined using the Bradford method, with absorbance read at 595 nm. Anthraquinones were quantified using acid hydrolysis and ether extraction, with the absorbance recorded at 430 nm. Total flavonoids were measured using the aluminium chloride method at 415 nm. Steroid content was estimated using a colorimetric reaction involving sulfuric acid and iron (III) chloride at 780 nm. Tannins were quantified using Folin reagent and Na_2CO_3, with absorbance read at 725 nm, and terpenoids were calculated based on weight changes after ethanol extraction. Cardiac glycosides were determined using Baljet's reagent at 495 nm, and saponins were measured using the vanillin-sulfuric acid method at 544 nm. Alkaloids were precipitated using ammonium hydroxide and weighed, while total phenolic content was estimated using Folin-Ciocalteu reagent at 765 nm, with results expressed in equivalent standards for each compound.

Antioxidant Activity

Total Phenol Content (TPC)

The total phenolic content (TPC) of leaf and stem extracts was measured by adding 200 µL of the sample to a mixture containing 80 µL distilled water, 0.5 mL Folin-Ciocalteu reagent, and 1 mL sodium carbonate solution. After a 30-minute incubation at room temperature, absorbance was recorded at 765 nm using a UV-visible spectrophotometer. A blank (without plant extract) and a gallic acid standard curve were prepared similarly. TPC was expressed as µg of gallic acid equivalents (GAE) per mL and calculated using the formula $y = 0.362x - 0.284$ (Molole *et al.,* 2022).

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

The DPPH radical scavenging activity of the extracts was evaluated following the method by Brand-Williams *et al.,* with slight modifications. A 4 mg DPPH stock solution was prepared in 100 mL methanol and stored in the refrigerator. For the assay, 20 µL of the plant extract or standard was mixed with 2 mL of the DPPH solution and incubated in the dark for 30 minutes. The absorbance was then measured at 517 nm. The percentage of radical scavenging activity was calculated using the formula:

$$\% \text{ Antioxidant Activity} = \frac{(Ac\text{-}As)}{Ac} \quad X\ 100$$

Where,
Ac= Absorbance of the control.
As = Absorbance of the sample.
Ac= Absorbance of control.

RESULTS

Plant collection and Preparation for Extraction

The *C. discolor* plant, collected from the area near Hulugar Mane, Shringeri, Karnataka, India, was authenticated by the Botanical Survey of India at the T.N.A.U. Campus, Lawley Road, Coimbatore 641 003, Tamil Nadu. A voucher specimen was deposited, and it was assigned the voucher number BSI/SRC/5/23/2022/Tech/388. The collected leaf and stem parts were dried and utilized for qualitative, quantitative, and antioxidant assays (Figure. 1A, 1B; Figure. 2A, 2B).

Qualitative analysis of the Phytochemicals

The qualitative analysis of *C. discolor* leaf and stem extracts revealed distinct phytochemical profiles depending on the solvent used. In the leaf, carbohydrates were found only in methanol, while proteins were present in ethyl acetate and chloroform extracts. Alkaloids were detected in all solvents except methanol, and flavonoids and phenols were consistently present in ethyl acetate, methanol, and chloroform extracts. Saponins and steroids were largely absent, with saponins detected only in methanol. Tannins were present in most solvents except methanol and aqueous extracts, while terpenoids and glycosides were observed only in specific extracts. In contrast, the stem extracts showed a broader phytochemical range, with alkaloids found in all solvents except the aqueous extract, and anthraquinones uniquely present in the ethyl acetate and aqueous extracts. Flavonoids were abundant in ethyl acetate, methanol, and chloroform extracts, while phenols were detected only in the methanol extract. The stem also exhibited higher levels of saponins, steroids, and terpenoids in methanol, chloroform, and petroleum ether extracts, and glycosides in chloroform, petroleum ether, and ethyl acetate extracts. Overall, the stem contained a more diverse array of secondary metabolites, especially anthraquinones, saponins, steroids, and terpenoids, compared to the leaf (Table 1).

Quantitative analysis of the Phytochemicals

The quantitative analysis of *C. discolor* extracts revealed distinct phytochemical concentrations in the leaf and stem. The leaf methanolic extract showed notable levels of carbohydrates (2.81 µg) and steroids (28.41 µg), while flavonoids were highest in the chloroform extract (2.85 µg). Tannins and terpenoids were present in moderate amounts, with glycosides detected in the petroleum ether extract (0.641 µg). In the stem, carbohydrates were detected only in the chloroform extract (1.14 µg), and anthraquinones were present in the aqueous (1.801 µg) and ethyl acetate (0.186 µg) extracts. The stem had a significantly higher concentration of steroids and terpenoids, particularly in ethyl acetate (steroids: 27.87 µg; terpenoids: 161.9 µg) and methanol (steroids: 28.02 µg). Flavonoid levels were comparable between leaf and stem, but the stem had notably higher steroid and terpenoid concentrations, highlighting its richer phytochemical profile compared to the leaf (Figure. 3).

The quantitative analysis of *C. discolor* leaf and stem extracts revealed variations in saponin and alkaloid content depending on the solvent used. In the leaf, saponins were only found in methanol (4 mg), petroleum ether (4 mg), and aqueous extracts (3 mg), while alkaloids were consistently present across all solvents, with the highest concentration in petroleum ether (9 mg). In the stem, saponins were more abundant, especially in aqueous (8 mg), methanol (7 mg), and chloroform (5 mg) extracts. Alkaloids were also more concentrated in the stem, particularly in methanol (11 mg) and petroleum ether (9 mg), with the aqueous extract having a slightly lower concentration (7 mg). Overall, the stem extracts contained higher levels of both saponins and alkaloids compared to the leaf, highlighting its greater abundance of these bioactive compounds (Figure. 4).

The phenolic content of *C. discolor* leaf and stem extracts varied significantly depending on the solvent and extract concentration. In leaf extracts, phenols were detected in ethyl acetate, methanol, and chloroform, showing a consistent increase with extract volume, with the highest levels in the chloroform extract (2.83 µg at 1000 µl). In contrast, phenols were absent in petroleum ether and aqueous extracts. The stem extracts showed phenolic content only in the methanol extract, ranging from 1.076 µg at 200 µl to 3.598 µg at 1000 µl, with no phenols detected in other solvents. These results indicate that phenol extraction is solvent-dependent, with chloroform and ethyl acetate being most effective for leaf extracts, while methanol was best for stem extracts (Figure. 5).

Antioxidant Activity

Total Phenolic Content (TPC)

The total phenolic content (TPC) of *C. discolor* extracts varied depending on the solvent used. The leaf methanol extract had the highest TPC at 1.92 µg GAE/mg, followed by ethyl acetate (1.27 µg GAE/mg) and aqueous extracts (1.08 µg GAE/mg), while chloroform and petroleum ether showed much lower levels. In the stem, the highest phenolic content was found in methanol (1.54 µg GAE/mg) and ethyl acetate (1.35 µg GAE/mg), with lower values in aqueous, chloroform, and petroleum ether extracts. Overall, methanol was the most effective solvent for extracting phenolics from the leaf, while the stem had slightly higher phenolic levels in ethyl acetate. Both leaf and stem had minimal phenolic content in non-polar solvents, indicating that polar solvents like methanol and ethyl acetate are best for phenolic extraction in *C. discolor* (Figure. 6).

DPPH Assay

The DPPH assay results for *C. discolor* showed that leaf extracts had significantly higher antioxidant activity compared to stem extracts across different solvents. The leaf methanol extract exhibited the highest antioxidant capacity (77.24%), followed by ethyl acetate (66.07%), while chloroform and petroleum ether extracts had much lower activities at 4.48% and 15.84%, respectively. The aqueous extract showed the lowest activity (2.82%). Similarly, in the stem extracts, methanol had the highest activity (51.89%), followed by ethyl acetate (35.32%), with minimal activities observed in chloroform (15.51%) and petroleum ether (5.45%). The stem's aqueous extract showed slightly better activity (10.62%) compared to the leaf's. Overall, the leaf demonstrated higher antioxidant potential, particularly in polar solvents like methanol and ethyl acetate, while non-polar solvents were less effective in both leaf and stem extracts (Figure. 7).

DISCUSSION

The qualitative analysis of *C. discolor* leaf and stem extracts revealed distinct phytochemical profiles, consistent with observations in other *Cissus* species. The presence of flavonoids and phenols in *C. discolor* supports their antioxidant role, as seen in *C. quadrangularis*, known for its high phenolic content and antioxidant activity (Kaur *et al.,* 2022). While saponins were absent in *C. discolor* leaf extracts, they were abundant in the stem, reflecting a richer phytochemical profile similar to *C. aralioides*, which is rich in phenolic acids and flavonoids (Kouassi *et al.,* 2021). This variation parallels findings in *C. hastata*, where saponins were also undetected, but alkaloids and tannins were prevalent, contributing to anti-inflammatory properties (Muhamad *et al.,* 2022). Overall, the diverse phytochemical composition of *C. discolor* aligns with trends observed across the *Cissus* genus, influencing therapeutic potential, as supported by the presence of flavonoids like quercetin and luteolin in related species (Gnanasundaram & Balakrishnan, 2018; Kaur *et al.,* 2022). The alkaloids and tannins in *C. discolor* further echo similar findings in *C. hastata* and *C. javana,* confirming their roles in anti-inflammatory and astringent activities (Muhamad *et al.,* 2022; Ningombam *et al.,* 2022).

The quantitative analysis of *C. discolor* leaf and stem extracts showed notable variations in phytochemical composition, highlighting its medicinal potential. Carbohydrates were most concentrated in the methanol extract of the leaf (2.81 µg), aligning with *C. quadrangularis*, where carbohydrates are a key component (Enechi & Odonwodo, 2004). Proteins were present in the ethyl acetate and chloroform extracts (1.512–1.603 µg), supporting findings in *C. quadrangularis* (Kaur *et al.,* 2022). Anthraquinones, detected only in the stem aqueous extract (1.801 µg), have been linked to therapeutic effects, similar to *C. populnea* (Soladoye & Chukwuma, 2012). Flavonoids were highest in the leaf's chloroform extract (2.85 µg), mirroring *C. quadrangularis* flavonoid richness (Kaur *et al.,* 2022). Tannins, found in moderate amounts, and terpenoids, abundant in the stem's ethyl acetate extract (161.9 µg), align with *C. javana* and *C. hastata* (Muhamad *et al.,* 2022). Steroids peaked in the stem's methanol extract (28.02 µg), reflecting *C. quadrangularis* content (Talreja *et al.,* 2017). The phenolic content was highest in the leaf's methanol extract, supporting literature on polar solvent effectiveness (Quilez *et al.,* 2010). Glycosides were present at lower levels, similar to *C. quadrangularis* (Kumar & Nisha, 2021). Saponins and alkaloids were more abundant in stem extracts, with alkaloids peaking in methanol (11 mg), paralleling other *Cissus* species (Kaur *et al.,* 2022). The absence of phenols in petroleum ether and aqueous extracts emphasizes solvent polarity's role in extraction efficiency (Nathar & Yatoo, 2015). Overall, the stem demonstrated a richer phytochemical profile, highlighting its importance as a bioactive compound source.

The TPC observed in *C. discolor* leaf and stem extracts aligns with findings from other *Cissus* species. *C. discolor* methanolic extracts displayed the highest phenolic concentrations, indicating methanol's efficiency for phenolic extraction, a trend also seen in *C. setosa*, where methanol yielded 78 mg GAE/g TPC (Chinnamaruthu *et al.,* 2013). In *C. hastata*, a high TPC (21.3 mg GAE/g) in the leaf extract did not correspond to high antioxidant activity, suggesting factors beyond phenolic content influence therapeutic potential (Muhamad *et al.,* 2022). The relatively lower TPC in *C. discolor* chloroform and petroleum ether extracts parallels results in *C. rotundifolia,* where solvent choice significantly impacted phenolic yield (AL-Bukhaiti *et al.,* 2019). Advanced techniques like ultrasound-assisted extraction (UAE) have improved phenolic yield and antioxidant activity in *C. woodrowii* and *C. rotundifolia*, indicating UAE could enhance TPC in *C. discolor* (Zimare, 2022; AL-Bukhaiti *et al.,* 2019). While *C. discolor* exhibits moderate phenolic content, it remains a valuable antioxidant source when extracted with polar solvents like methanol and ethyl acetate, as observed in *C. quadrangularis* (Avula *et al.,* 2021).

The DPPH assay results for *C. discolor* leaf extracts revealed strong antioxidant activity, especially in the methanol extract (77.24%), consistent with findings in *C. populnea*, where methanol extracts exhibited significant DPPH activity due to high phenolic content (Nyemb *et al.,* 2018). The ethyl acetate extract also showed notable antioxidant activity (66.07%), similar to *C. quadrangularis* ethanolic extracts (Kumar *et al.,* 2014). In contrast, the chloroform (4.48%) and petroleum ether (15.84%) extracts had lower activities, aligning with trends of non-polar solvents being less effective for antioxidant extraction (Kumar *et al.,* 2014). Stem extracts showed maximum antioxidant activity in the methanol extract (51.89%), lower than the leaves but comparable to *C. sicyoides* (Ponath *et al.,* 2022). Overall, the results highlight methanol's efficacy in extracting antioxidants from *C. discolor* and other *Cissus* species.

CONCLUSION

This study highlights the influence of solvent selection on the phytochemical and antioxidant profiles of *C. discolor* leaf and stem extracts. Both qualitative and quantitative analyses showed that polar solvents, especially methanol, were the most effective for extracting key bioactive compounds such as phenolics, flavonoids, tannins, and alkaloids. The TPC was

highest in methanolic extracts, contributing to their superior antioxidant activity, as confirmed by the DPPH assay. Leaf extracts displayed greater antioxidant potential than stem extracts, and a positive correlation between TPC and DPPH activity underscored the role of phenolics in free radical scavenging. These findings confirm the traditional medicinal uses of *C. discolor* and suggest its potential for developing nutraceutical and pharmaceutical products. Future research should focus on optimizing phenolic extraction through advanced techniques and characterizing specific compounds to further explore their therapeutic potential.

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Table 1: Qualitative Phytochemical Analysis of *C. discolor* Leaf and Stem Extracts Across Different Solvents "+" = Presence; "-" = Absence

S.No	Phytochemical tests	Solvents									
		Ethyl acetate		Methanol		Chloroform		Petroleum ether		Aqueous	
		Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
1	Carbohydrates	-	-	+	-	-	+	-	-	-	-
2	Proteins	+	-	-	-	+	-	-	-	-	-
3	Alkaloids	+	+	-	+	+	+	+	+	+	-
4	Anthraquinones	-	+	-	-	-	-	-	-	+	+
5	Flavonoids	+	+	+	+	+	+	-	-	-	-
6	Phenols	+	-	+	+	+	-	-	-	-	-
7	Saponins	-	-	+	+	-	+	-	+	+	+
8	Steroids	-	+	+	+	-	-	-	+	+	+
9	Tannins	+	+	-	+	+	+	+	+	-	+
10	Terpenoids	-	+	+	+	-	-	-	+	+	+
11	Glycosides	-	+	-	-	-	+	+	+	-	-

Figure. 1: A: C. discolor plant showing its characteristic vine structure and foliage; B: Dried and powdered leaf sample of C. discolor.

Figure. 2: A: Stem of C. discolor plant used in the study for phytochemical and antioxidant analysis; B: Dried and powdered stem sample of C. discolor prepared for extraction.

Figure. 3: Phytochemical composition of *C. discolor* leaf and stem extracts using various solvents.

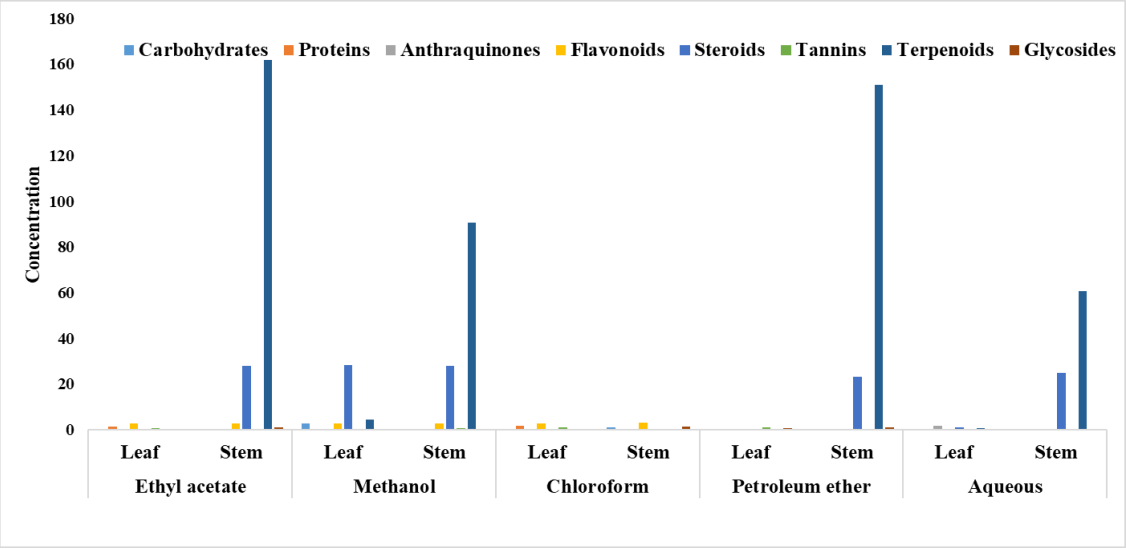

Figure. 4: Saponin and alkaloid concentrations in *C. discolor* leaf and stem extracts using different solvents.

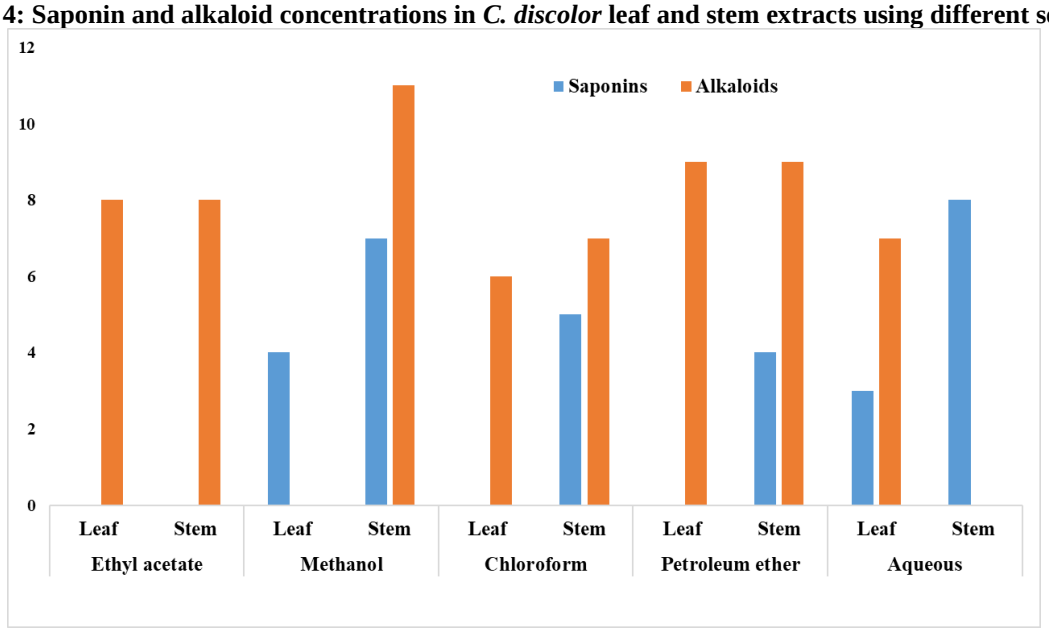

Figure. 5: Phenolic content of *C. discolor* leaf and stem extracts using different solvents (ethyl acetate, methanol, and chloroform) at various concentrations (200 µL, 400 µL, 600 µL, 800 µL, and 1000 µL).

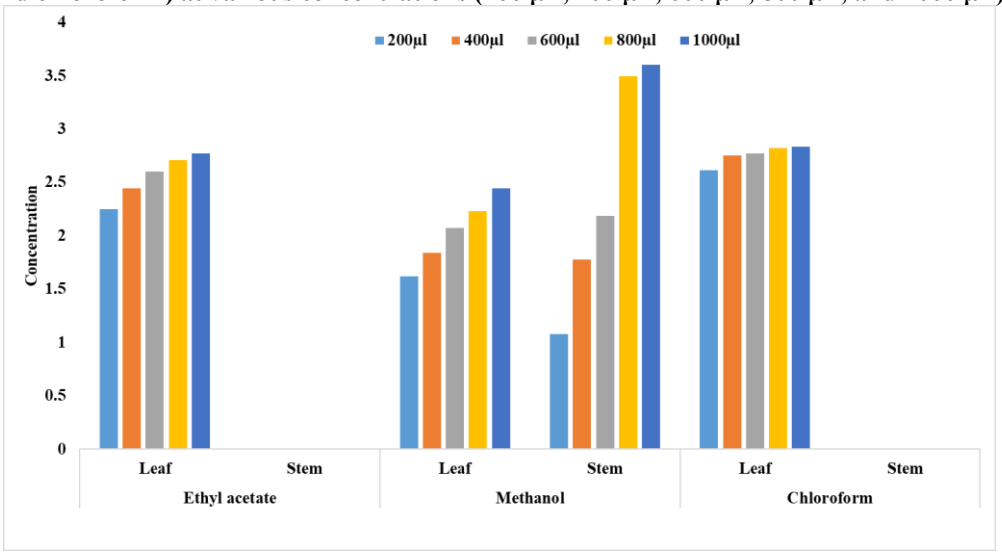

Figure. 6: Total phenolic content of *C. discolor* leaf and stem extracts across different solvents (ethyl acetate, methanol, chloroform, petroleum ether, and aqueous).

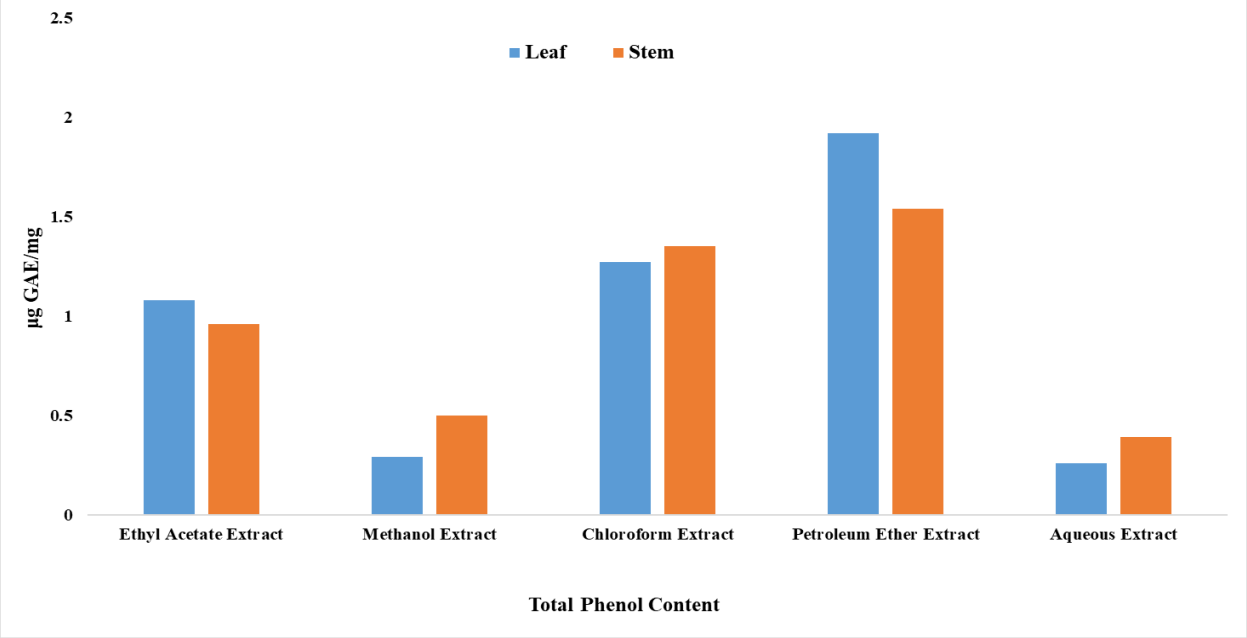

Figure. 7: DPPH radical scavenging activity of *C. discolor* leaf and stem extracts across different solvents.