Advances in Bioresearch
Adv. Biores., Special Issue (2) 2025: 41-54
©2024 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585. SPL2.25.4154

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Evaluating the Therapeutic Potential of *Cissus discolor*: Insights into Antioxidant and Antimicrobial Efficacy

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ABSTRACT
The study investigates the antioxidant potential and antimicrobial activities of Cissus discolor leaf and stem extracts. Using various solvents, the antioxidant capacity was evaluated through the reducing power assay, while the antibacterial activity was tested against four bacterial strains (Bacillus subtilis, Escherichia coli, Staphylococcus aureus, and Salmonella typhimurium) and antifungal activity against two fungal strains (Candida albicans and Aspergillus niger). The results revealed significant antioxidant activity in the methanol and aqueous extracts, while chloroform and petroleum ether extracts exhibited minimal antioxidant potential. Moderate antibacterial activity was observed against B. subtilis, with methanol and ethyl acetate extracts showing the highest efficacy, though no activity was detected against other tested bacteria. Antifungal assays indicated mild to moderate inhibition of A. niger by aqueous, chloroform, ethyl acetate, and petroleum ether extracts, whereas methanol extracts showed no effect. None of the extracts inhibited C. albicans. These findings highlight the potential of C. discolor extracts as antioxidant agents, though their antimicrobial properties may be selective, warranting further research to optimize extraction methods and identify active compounds.
Keywords: *Cissus discolor,* Antioxidant activity, Reducing power assay, Antibacterial activity, Antifungal activity.

Received 18.10.2024 Revised 28.12.2024 Accepted 22.02.2025

How to cite this article:
C. S. Kavya, Kavya B.C, Kalpashree M.M, and Krishna K. Evaluating the Therapeutic Potential of *Cissus discolor*: Insights into Antioxidant and Antimicrobial Efficacy. Adv. Biores. Special Issue [2] 2025. 41-54

INTRODUCTION

Medicinal plants and their extracts play a crucial role in modern medicine, providing a rich source of bioactive compounds that have been utilized for centuries in both traditional and pharmaceutical applications. The Vitaceae family, encompassing numerous medicinal plants, is particularly noteworthy due to its diverse phytochemical constituents and broad spectrum of biological activities. Among these phytochemicals, polyphenolic compounds such as stilbenes have garnered significant attention for their antioxidant, anticancer, and cardiovascular protective properties. Resveratrol, a well-known stilbene, has been associated with various health benefits, including the reduction of heart disease risk, as exemplified by the "French Paradox" [1]. Within this family, *Cissus setosa* has demonstrated antiulcer activity, effectively reducing gastric ulcerations in experimental models and thereby supporting its traditional use for gastrointestinal disorders [2]. Furthermore, *Cissus quadrangularis* is renowned for its anxiolytic and antiepileptic properties, attributed to its antioxidant activities and modulation of GABA neurotransmission, which contribute to its efficacy in managing epilepsy and anxiety. This species is also recognized for its content of secondary metabolites, such as alkaloids, flavonoids, and phenols, which enhance its pharmacological potential [3]. Similarly, *Cissus polyantha* has demonstrated antimicrobial activity against pathogens like *Escherichia coli* and *Staphylococcus aureus*, attributed to its phytochemical profile, which includes steroids, triterpenes, and flavonoids [4].

Cissus discolor, a less-studied species within the Vitaceae family, shares many phytochemical similarities with other members of the genus *Cissus*. While *C. discolor* is not specifically detailed in existing studies, the broader medicinal applications of the Vitaceae family provide valuable insights into its potential therapeutic uses. The genus *Cissus* is known for its diverse medicinal properties, including bone healing,

antimicrobial, anti-inflammatory, and antioxidant activities, as evidenced by the well-documented pharmacological profile of *C. quadrangularis*. This species has been used traditionally to treat conditions related to bones, muscles, and ligaments, as well as respiratory and gastrointestinal disorders [5, 6, 7]. The therapeutic potential of *C. quadrangularis* is further supported by its phytochemical composition, which includes flavonoids, triterpenoids, and alkaloids, known for their anticancer and hepatoprotective activities [5, 8, 9]. Additionally, *Cissus erosa* has demonstrated antiviral properties against Dengue and Zika viruses, attributed to its flavonoid and terpenoid content [10]. Another notable species, *Cissus assamica*, has shown significant antinociceptive and antipyretic activities, indicating its potential for managing pain and fever [11]. These medicinal properties across various ***Cissus*** species suggest that *C. discolor* may also possess similar therapeutic potential, warranting further investigation into its pharmacological profile.

The genus *Cissus* is characterized by its rich phytochemical profile, including flavonoids, phenols, alkaloids, and terpenoids, which contribute to its diverse biological activities. For instance, *Cissus incisa* leaves contain β-sitosterol, a bioactive compound identified through GC-MS analysis, known for its potential health benefits [12]. Similarly, *C. quadrangularis* is noted for its potent fracture healing properties, as well as its antimicrobial, antiulcer, antioxidative, and anticancer activities, particularly against lung cancer cell lines, where it significantly reduces cell viability in a dose-dependent manner[5, 13]. The ethanolic and methanolic extracts of *C. quadrangularis* have also demonstrated strong antioxidant properties and significant anticancer efficacy against leukemic cells, highlighting its potential as a chemopreventive agent [13]. Furthermore, *Cissus spinosa* has been studied for its antioxidant, hypoglycemic, and antilipemic actions, attributed to the presence of flavonoids and phenols, which provide protection against oxidative stress [14]. *Cissus adnata* has also shown strong antioxidant, antibacterial, anthelmintic, and antinociceptive activities, with a low toxicity profile, suggesting its potential for therapeutic applications [15]. Additionally, *Cissus trifoliata* is traditionally used in Mexican medicine for tumor management, indicating its anticancer potential, although specific phytochemicals responsible for these effects require further investigation [16].

The reducing power of plant extracts is a critical parameter in assessing their antioxidant capacity, as it reflects the ability of antioxidants to donate electrons and neutralize free radicals, thereby preventing oxidative stress and its associated damage. This property is essential because oxidative stress is linked to various chronic diseases and aging processes. Moreover, the reducing power is not only indicative of the antioxidant capacity but also provides insights into the potential health benefits of plant extracts, as it is associated with the prevention of oxidative damage in biological systems [17]. The reducing power assay is a widely used method to evaluate the antioxidant capacity of plant extracts, as it measures the ability of antioxidants to donate electrons, thereby reducing oxidized intermediates. This assay is crucial in determining the potential of plant extracts to act as antioxidants by converting ferric ions (Fe^3+) to ferrous ions (Fe^2+), which is indicative of the extract's electron-donating ability. Various studies have employed this assay to assess the antioxidant properties of different plant extracts. The research on phytochemical constituents of different plant parts further corroborated the utility of the reducing power assay, with the bark of certain plants showing the highest reducing power, thus indicating its potential as a natural antioxidant source for food and pharmaceutical applications [18]. The survey of antioxidant capacity assays also highlighted the reducing power assay as a non-radical redox potential-based method, underscoring its importance in the efficient and cost-effective evaluation of antioxidants across various samples [19]. Moreover, the study on the antioxidant activity of selected medicinal plants in the Unani system of medicine confirmed the relevance of the reducing power assay in correlating phytochemical content with antioxidant activity, particularly in plants like *Aloe barbadensis* and *Merendera persica* [20]. Lastly, the analysis of antioxidant activity in different plant species emphasized the sensitivity of the reducing power assay in discriminating antioxidant capacity, making it a preferred method for ranking species based on their antioxidant potential [21].

Screening plant extracts for antimicrobial properties is of paramount importance in the current global health landscape, primarily due to the escalating threat of antimicrobial resistance (AMR) and the limited development of new antibiotics. The traditional use of medicinal plants offers a rich repository of bioactive compounds that can be harnessed to combat resistant strains of bacteria and fungi. *Cissus* species, like many other plants, have been traditionally used in various cultures for their medicinal properties, which include antimicrobial activities. For instance, *C. cornifolia* has shown significant antimicrobial activity against a range of pathogens, including *Mycoplasma hominis* and *Candida parapsilosis*, with notable minimum inhibitory concentration (MIC) values, validating its use in traditional medicine for treating infections associated with HIV-AIDS and cancer-like infections [22]. Similarly, *C. incisa* extracts have demonstrated broad-spectrum antimicrobial activity against both Gram-positive and

Gram-negative bacteria, with the chloroform/methanol extract being particularly effective, highlighting its potential as a source of new antibacterial agents [12]. Furthermore, *Cissus welwitschii* has been found to cause significant drug accumulation in Escherichia coli, indicating its potential as a lead compound for developing antibacterial agents [23]. The antimicrobial properties of *Cissus vitiginea* have also been noted, with methanol extracts showing higher zones of inhibition against bacterial strains, suggesting the presence of bioactive compounds that could be isolated for pharmaceutical development [24]. The presence of polyphenolic compounds, such as flavonoids and tannins, in these extracts is often linked to their antimicrobial efficacy, suggesting a similar potential in *Cissus* species [25, 26, 27].

This study aims to evaluate the antioxidant potential and antimicrobial activities of *C. discolor* leaf and stem extracts using various solvents to explore their therapeutic properties. The antioxidant capacity of the extracts is assessed using the reducing power assay, while their antimicrobial efficacy is tested against selected bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium and Bacillus subtilis*) and fungal strains (*Candida albicans*, *Aspergillus niger*). The findings are compared with existing data on other *Cissus* species within the Vitaceae family to identify potential similarities or differences in their biological activities. This research seeks to identify the most effective extract and solvent combination, providing insights into the potential of *C. discolor* as a source of natural antioxidants and antimicrobial agents for pharmaceutical applications.

MATERIAL AND METHODS

Sample Collection and Authentication

The leaves and stems of *C. discolor* were gathered during May and June from the vicinity of Hulugar Mane, located in Shringeri, Karnataka, India. Special care was taken during collection to ensure the samples remained intact. The collected plant material was thoroughly cleaned to eliminate any dirt or impurities and then laid out to dry naturally in a well-ventilated, shaded environment.

Extraction of Plant Materials

After drying, the leaves and stems were ground into a fine powder using a blender and preserved in airtight containers for subsequent use. For the extraction, 10 grams of the dried powder were subjected to Soxhlet extraction using 100 mL of various solvents, including water, methanol, chloroform, petroleum ether, and ethyl acetate. The extracted solutions were then concentrated by evaporating the solvents under reduced pressure with the help of a rotary vacuum evaporator. The dried extracts were stored in desiccators to prevent moisture absorption, ensuring they remained suitable for further analysis.

Reducing power assay

The antioxidant capacity of the plant extracts was evaluated using the reducing power assay following the method described by Oyaizu. Five different concentrations of each extract, ranging from 5 mg/L to 25 mg/L, were prepared in 50% aqueous methanol. In a test tube, 2.5 mL of the plant extract, 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6), and 2.5 mL of potassium ferricyanide solution (1% w/v) were added and thoroughly mixed. The mixture was incubated in a water bath at 50°C for 20 minutes. After incubation, 2.5 mL of trichloroacetic acid (10% w/v) was added, and the solution was centrifuged at 650 rpm for 10 minutes. A 5 mL aliquot of the supernatant was collected and combined with 5 mL of distilled water and 1 mL of ferric chloride solution (0.1% w/v). The absorbance of the resulting solution was measured at 700 nm. A blank for each solvent was prepared using the same procedure, replacing the plant extract with an equal volume of the corresponding solvent. The antioxidant power of the extracts was determined based on the standard calibration curve with the equation: $y = 0.023x - 0.054$.

Antibacterial Activity Assay

The antibacterial activity of the plant extracts was evaluated in vitro against both Gram-positive and Gram-negative bacteria. The tested Gram-positive bacteria included *B. subtilis* and *S. aureus*, while the Gram-negative strains were *E. coli* and *S. typhimurium*. Prior to testing, these bacterial strains were pre-cultured in nutrient broth. The antibacterial screening was performed by determining the zone of inhibition using the disc diffusion method as described by [28].

Bacterial cultures were inoculated into conical flasks containing 100 mL of nutrient broth and incubated at 37°C for 24 hours to obtain seeded broth. The bacterial density was standardized using the McFarland method [29]. Plant extracts were dissolved in dimethyl formamide, which had been tested previously and confirmed to have no antibacterial activity. The extract solutions were prepared at a concentration of 50 mg/mL and sterilized through filtration using 0.45 µm Millipore filters. Sterile discs, each 6 mm in diameter, were impregnated with either 20 µL or 2.5 µL of the extract solution to achieve concentrations of 500 µg/disc and 250 µg/disc, respectively. These discs were then placed onto agar plates inoculated with the test bacteria. Gentamicin (10 µg/disc) and Ciprofloxacin (25 µg/disc) were used as positive controls, while the same solvents used to dissolve the extracts served as negative controls. All plates,

including those with test and control discs, were incubated at 37°C for 24 hours. After incubation, the zones of inhibition around the discs were measured to determine the antibacterial activity of the extracts.

Antifungal Activity Assay

The antifungal activity of the plant extracts was evaluated against different fungal strains, including *C. albicans* and *A. niger*. The fungal inoculum was prepared from a 5-day-old culture grown on PDA medium. To collect the inoculum, the surface of the agar plates was flooded with 8–10 mL of distilled water, and the conidia were carefully scraped using a sterile spatula. the antifungal activity was tested using the agar well diffusion method, which is commonly used to evaluate the antimicrobial potential of plant or microbial extracts. In this method, the agar surface was evenly inoculated by spreading a measured volume of the fungal inoculum across the entire plate. A well, 6–8 mm in diameter, was aseptically punched in the agar using a sterile cork borer or tip. A specified volume (20–100 µL) of the plant extract solution at the desired concentration was introduced into the well. The plates were then incubated under suitable conditions depending on the fungal strain being tested. As the antimicrobial agent diffused through the agar, it inhibited the growth of the fungal strain, and the zones of inhibition were measured to assess the antifungal activity.

RESULTS

The *C. discolor* plant, gathered from the vicinity of Hulugar Mane, Shringeri, Karnataka, India, was authenticated by the Botanical Survey of India at the T.N.A.U. Campus, Lawley Road, Coimbatore – 641003, Tamil Nadu. A voucher specimen was deposited and assigned the identification number BSI/SRC/5/23/2022/Tech/388. The harvested leaves and stems were dried and subsequently used for and antioxidant and antimicrobial assay.

Reducing Power Assay Figures

The reducing power assay results revealed differences in antioxidant activities between the leaf and stem extracts of *C. discolor* when using various solvents, highlighting variations in the extracts' efficacy. For the leaf extracts, methanol exhibited the highest antioxidative activity (15.43 %), followed closely by ethyl acetate (15.04 %) and aqueous extracts (14.40 %). In contrast, the chloroform (0.60 %) and petroleum ether (0.44 %) extracts showed minimal antioxidant activities, suggesting their ineffectiveness in extracting antioxidative compounds from the leaves. Similarly, for the stem extracts, aqueous (15.05 %) and methanol (15.04 %) showed significantly higher antioxidant activities compared to other solvents, indicating their superior extraction capacity for antioxidative compounds from the stem. However, the ethyl acetate extract demonstrated much lower antioxidant activity (2.81 %), and both chloroform (0.39 %) and petroleum ether (0.49 %) extracts had minimal activities, consistent with the trend observed in leaf extracts (Figure 1).

When comparing leaf and stem extracts, the methanol and aqueous extracts showed consistently high activities for both plant parts, suggesting these solvents are highly effective in extracting antioxidative compounds from both leaf and stem. In contrast, the ethyl acetate extract exhibited a marked variation, with high activity in the leaf but low activity in the stem, indicating that the distribution of antioxidant compounds differs between these parts. Chloroform and petroleum ether extracts consistently showed low activity in both leaf and stem, reflecting their limited effectiveness in extracting active antioxidant compounds. Overall, methanol and aqueous extracts emerged as the most effective solvents for extracting antioxidative compounds, irrespective of the plant part used.

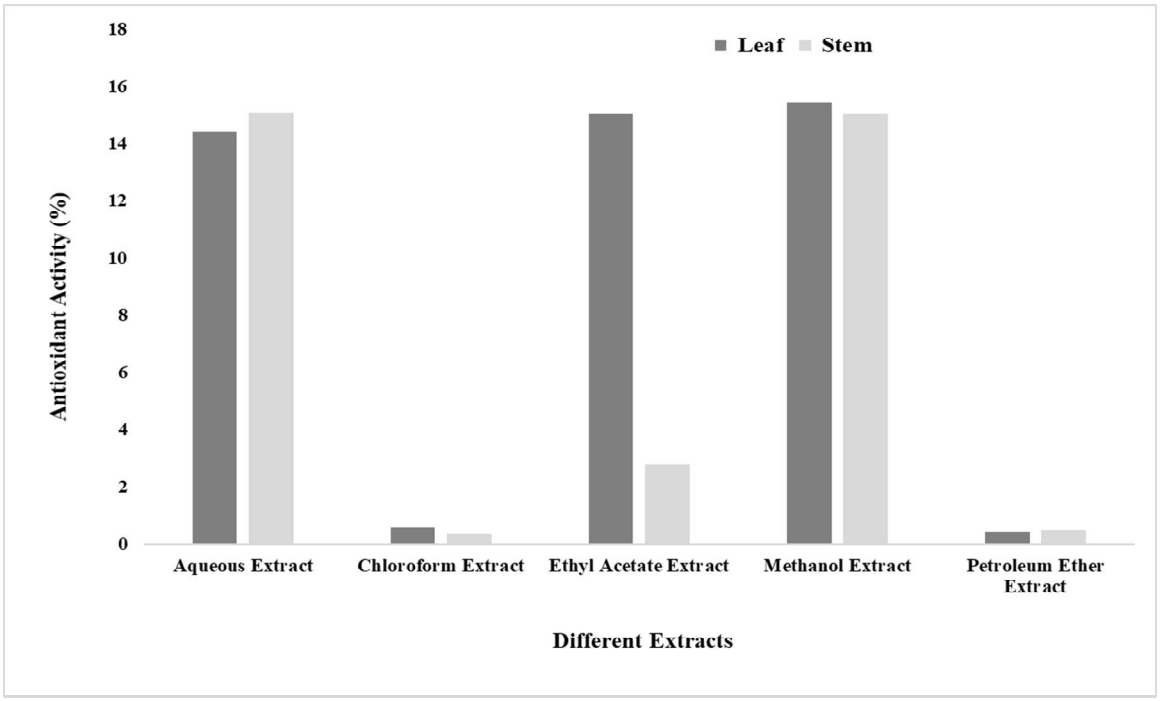

Figure 1: Reducing power assay of *C. discolor* leaf and stem extracts.

Anti-Bacterial Assay

B. subtilis

The leaf and stem extracts of *C. discolor* exhibited moderate antibacterial activity against Bacillus subtilis. The leaf extracts of ethyl acetate and petroleum ether both showed an inhibition zone of 1.0 cm (Table 1), while the methanol extract presented a slightly higher inhibition of 1.1 cm. Conversely, the aqueous and chloroform extracts were inactive against Bacillus subtilis. For the stem extracts, ethyl acetate and methanol showed an inhibition zone of 1.0 cm each, and petroleum ether demonstrated a slightly lower zone of 0.9 cm. Again, aqueous and chloroform extracts were ineffective. Despite these results, all extracts displayed lower antibacterial efficacy compared to the standard antibiotic, which exhibited an inhibition zone of 2.1 cm (Figure 2). This suggests that while the ethyl acetate and methanol extracts from both leaf and stem have some antibacterial potential against Bacillus subtilis, they are not as potent as conventional antibiotics.

E. coli

Both leaf and stem extracts showed no inhibition against Escherichia coli, indicating a lack of antibacterial activity against this Gram-negative bacterium. None of the extracts, regardless of the solvent used (aqueous, chloroform, ethyl acetate, petroleum ether, or methanol), were effective in inhibiting the growth of *E. coli* (Figure 3). This suggests that the phytochemicals present in the extracts do not possess significant antibacterial properties against this particular pathogen.

S. aureus

The leaf and stem extracts of *C. discolor* showed no antimicrobial activity against Staphylococcus aureus. Neither the leaf nor the stem extracts, regardless of solvent type, exhibited any inhibition zones, indicating that the bioactive compounds present in these extracts are not effective against this Gram-positive bacterium (Figure 4). This lack of activity suggests that these extracts lack the necessary antibacterial properties to inhibit the growth of *S. aureus*.

S. typhimurium

Similar to *S. aureus*, neither the leaf nor stem extracts of *C. discolor* demonstrated any inhibition against *S. typhimurium*. None of the extracts tested, including aqueous, chloroform, ethyl acetate, petroleum ether, and methanol, showed any antibacterial effect on this Gram-negative pathogen. This indicates that the extracts do not contain bioactive compounds capable of inhibiting the growth of *S. typhimurium*, underscoring their ineffectiveness against this bacterium (Figure 5).

Overall, the leaf and stem extracts of *C. discolor* showed selective antibacterial activity, with moderate inhibition observed only against *B. subtilis*. The ethyl acetate and methanol extracts exhibited the most consistent activity against *B. subtilis*. However, none of the extracts were effective against *E. coli*, *S. aureus*, **or** *S. typhimurium*, indicating a lack of broad-spectrum antibacterial properties.

Antifungal assay

C. albicans

The leaf and stem extracts of *C. discolor* exhibited no antifungal activity against *C. albicans*. None of the extracts, regardless of solvent type—aqueous, chloroform, ethyl acetate, petroleum ether, or methanol—showed any inhibition against this yeast strain. This indicates that the bioactive compounds present in these extracts are ineffective against *C. albicans*. The absence of inhibition zones suggests that the extracts lack the necessary antifungal properties to inhibit the growth of *C. albicans*, highlighting their limited efficacy against this fungal pathogen (Figure 6).

A. niger

The antifungal activity against *A. niger* varied among the different extracts. Leaf extracts of aqueous, chloroform, ethyl acetate, and petroleum ether solvents exhibited equal inhibition zones of 1.0 cm, indicating a consistent level of antifungal activity. In contrast, the methanol extract did not show any inhibition, suggesting it is not effective against *A. niger*. The standard antibiotic demonstrated a higher inhibition zone of 1.5 cm, indicating that it was more effective than any of the leaf extracts tested. For the stem extracts, the aqueous and chloroform extracts showed inhibition zones of 0.8 cm each, while the ethyl acetate and petroleum ether extracts showed slightly better inhibition at 0.9 cm. As with the leaf extracts, the methanol extract did not exhibit any antifungal activity against *A. niger*. The standard antibiotic displayed a larger inhibition zone of 1.7 cm, indicating superior efficacy compared to the stem extracts (Figure 7). Overall, the leaf extracts showed slightly better inhibition compared to the stem extracts, but none of the extracts were as effective as the standard antifungal agent used (Table 2).

Microbes	Zone of Inhibition (cm)											
	Aq Extract		Chl Extract		EA Extract		PE Extract		Methanol Extract	Control		Antibiotic
B. Subtilis	-	-	1	1	1.1	1.1	1.1	1.1	-	-	0	2.1
E. coli	-	-	-	-	-	-	-	-	-	-	0	0
S. aureus	-	-	-	-	-	-	-	-	-	-	0	0
S. typhimurium	-	-	-	-	-	-	-	-	-	-	0	0
C. albicans	-	-	-	-	-	-	-	-		-	0	0
A. niger	1	1	1	1	-	-	0.8	0.8	-	-	0	1.5

Table -1: Antimicrobial activity of leaf extract of *Cissus discolor*

Aq – Aqueous, Chl- Chloroform, EA –Ethyl Acetate, PE – Petroleum Ether

Microbes	Zone of inhibition (cm)											
	Aq Extract		Chl Extract		EA Extract		PE Extract		Methanol Extract		Control	Antibiotic
B. Subtilis	-	-	1	0.9	1	1.1	-	-	-	-	0	2.1
E. coli	-	-	-	-	-	-	-	-	-	-	0	0
S. aureus	-	-	-	-	-	-	-	-	-	-	0	0
S. typhimurium	-	-	-	-	-	-	-	-	-	-	0	0
C. albicans	-	-	-	-	-	-	-	-	-	-	0	0
A. niger	0.8	0.8	0.9	0.9	-	-	-	-	-	-	0	1.7

Table -2: Antimicrobial activity of stem extarcts of *Cissus discolor*

Aq – Aqueous, Chl- Chloroform, EA –Ethyl Acetate, PE – Petroleum Ether; Antibiotic : Diclofenac Sodium IP 75mg/ml

Figure 2: Antibacterial activity of different extracts against *B. subtilis*.

Figure 3: Antibacterial activity of different extracts against ***E. coli***.

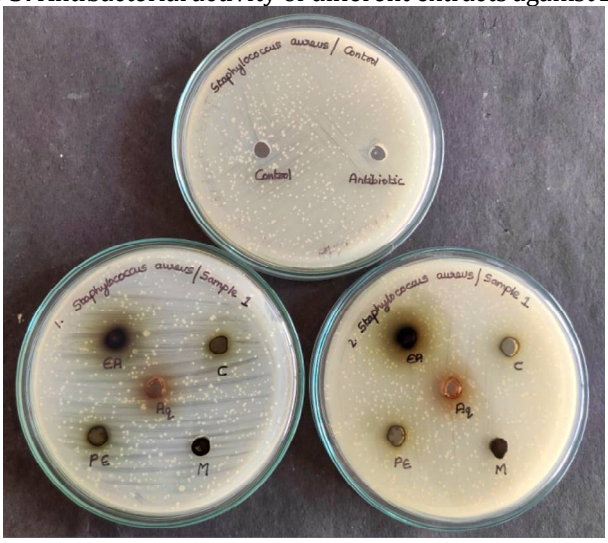

Figure 4: Antibacterial activity of different extracts against ***S. aureus***

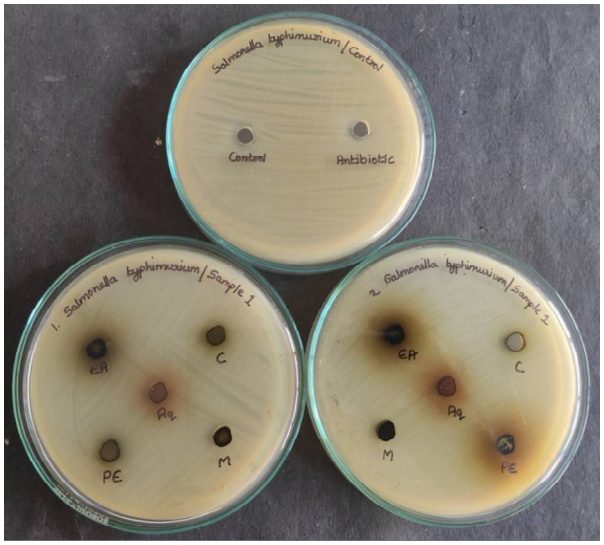

Figure-5: Antibacterial activity of different extracts against *S. typhimurium*

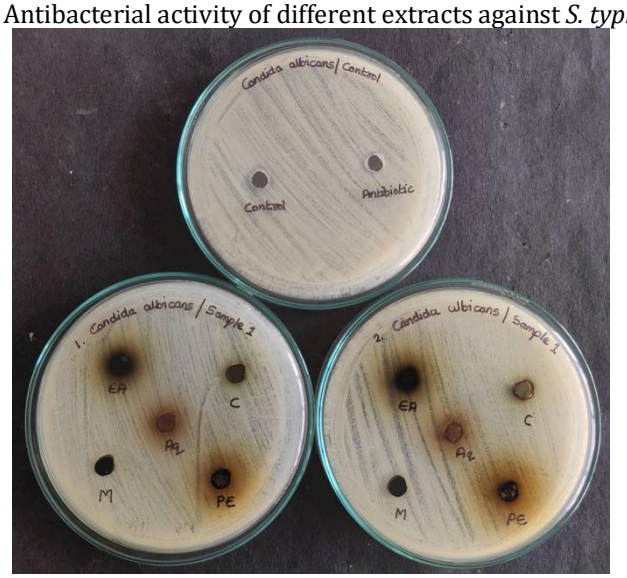

Figure 6: Antifungal activity of different extracts against *C. albicans*

Figure 7: Antifungal activity of different extracts against *A. niger*

DISCUSSION

The reducing power assay is a significant method used to evaluate the antioxidant potential of various compounds, including those found in plants from the genus ***C. discolor***. This assay measures the ability

of antioxidants to reduce ferric ions (Fe3+) to ferrous ions (Fe2+), which is indicative of their electron-donating capacity and, consequently, their potential to neutralize free radicals. The reducing power assay results of the present study revealed variations in antioxidant activities between leaf and stem extracts of the sample using different solvents, indicating differences in the efficacy of the extracts in extracting antioxidant compounds. When compared to the literature, *C. quadrangularis* and *C. setosa* have been shown to exhibit high reducing power activities in methanolic and ethanolic extracts, respectively [30, 31]. Similarly, our findings are consistent with these reports, as the methanolic extracts of both leaf and stem showed significant antioxidative activities. Additionally, in studies on *C. cornifolia*, the ethanol root extract displayed strong reducing power, attributed to its high phenolic content [32]. This correlation between high reducing power and phenolic content agrees with our study, as the solvents effective in extracting phenolic compounds (methanol and aqueous) showed the highest antioxidant activities.

The structural reactivity of phenolic compounds in different extracts may explain the differential extraction efficiency observed in our study. Phenolic compounds are typically more soluble in polar solvents, such as methanol and water, which aligns with the high reducing power activities observed in these extracts. This is supported by [33], who emphasized the influence of phenolic structure on antioxidant capacity. The low activities observed in chloroform and petroleum ether extracts could be due to their non-polar nature, which is less effective in extracting polar phenolic compounds from the plant matrix.

Thus, the findings corroborate the established literature on the antioxidant properties of *C. discolor*, highlighting the effectiveness of polar solvents like methanol and aqueous extracts in extracting antioxidant compounds. This suggests that methanolic and aqueous extracts of *C. discolor* are optimal for harnessing their antioxidant potential, as demonstrated in various studies [30, 31, 32]. Further research is needed to explore the specific phenolic constituents responsible for these activities, which could provide a deeper understanding of the mechanisms underlying the antioxidant properties of *C. discolor*.

Antibacterial Activity of *C. discolor* Extracts Against *B. subtilis*

Our findings on the antibacterial activity of *C. discolor* leaf and stem extracts against Bacillus subtilis align with previous studies in the literature. For example, the ethyl acetate and methanol extracts of *Cissus incisa* have exhibited broad-spectrum antimicrobial effects against both Gram-positive and Gram-negative bacteria [12], which is comparable to the moderate inhibition observed in our study against Bacillus subtilis. Similarly, significant antibacterial activity of methanol and ethyl acetate extracts from *C. quadrangularis* has been reported against Staphylococcus aureus and Escherichia coli [34, 35]. These findings are in line with our results, where moderate inhibition zones were recorded for methanol and ethyl acetate extracts against Bacillus subtilis, indicating that these solvents are effective in extracting bioactive antibacterial compounds from *Cissus* species.

Additionally, the lack of antibacterial activity observed in our aqueous and chloroform extracts is consistent with previous research on *Cissus welwitschii*, where methanol extracts showed stronger antibacterial effects compared to water extracts [23]. These findings suggest that methanol and ethyl acetate are more suitable solvents for isolating antibacterial constituents in *Cissus* species. The role of *B. subtilis* in plant disease management is well-documented, particularly in controlling bacterial leaf blight in rice and bacterial wilt in potatoes [36, 37], underscoring the relevance of using this bacterium in assays to evaluate the antimicrobial properties of *Cissus* extracts.

Furthermore, research exploring the molecular interactions between *C. quadrangularis* and *B. subtilis* has highlighted potential applications in drug discovery, suggesting that phytochemicals in *Cissus* species could enhance antibacterial efficacy [38]. Our findings of notable inhibition by the methanol extract further support this potential. In addition, *B. subtilis* is widely used as a biosensor to detect cell wall-targeting antibacterial compounds [39, 40], reinforcing its value as a model organism for antimicrobial screening and its suitability for evaluating the bioactivity of *C. discolor* extracts.

Antibacterial Activity of *C. discolor* Extracts Against *E. coli*

Our findings reveal that none of the *C. discolor* leaf and stem extracts demonstrated significant antibacterial activity against *E. coli*, as no inhibition zones were observed with any of the tested solvents. This lack of efficacy contrasts with several reports in the literature that highlight the potential of *Cissus* species against *E. coli*. For example, methanol extracts of *C. quadrangularis* have been reported to exhibit strong antibacterial activity comparable to standard antibiotics, with effectiveness increasing with concentration [35, 41]. Similarly, *C. incisa* has shown broad-spectrum antimicrobial effects against *E. coli*, particularly when using a combination of chloroform and methanol extracts [12]. Additionally, research on *Cissus multistriata* demonstrated that both methanol and chloroform extracts provided significant inhibition, with the chloroform extract producing a notable 25 mm inhibition zone [42].

Moreover, studies on *C. welwitschii* and *Triumfetta welwitschii* reported that methanol extracts were highly effective against *E. coli*, with the methanol extract of *T. welwitschii* achieving a MIC as low as 0.125 mg/mL and a MBC of 0.5 mg/mL. These extracts also induced substantial leakage of nucleic acids and proteins from *E. coli* cells [23]. Similarly, the ethyl acetate and methanol extracts of *C. quadrangularis* have been shown to possess considerable antibacterial potential against *E. coli*, suggesting their utility as promising antimicrobial agents [34].

In contrast, our findings are consistent with those of [34], who reported that *C. quadrangularis* extracts exhibited no inhibitory effects against *E. coli*. In our study, neither leaf nor stem extracts, irrespective of the solvent used (aqueous, chloroform, ethyl acetate, petroleum ether, or methanol), showed antibacterial activity against *E. coli*. This indicates that the bioactive compounds present in these extracts were ineffective against this Gram-negative bacterium. Similarly, found that various extracts of *C. quadrangularis*, including methanol, ethyl acetate, acetone, petroleum ether, ethanol, and water, lacked efficacy against *E. coli*. These consistent findings suggest that *E. coli* may exhibit inherent resistance to the phytoconstituents of *Cissus* species, emphasizing the need for further research to identify alternative antibacterial agents that are effective against *E. coli*.

Antibacterial Activity of *C. discolor* Extracts Against *S. aureus*

Our results differ from previous reports documenting the antibacterial potential of *Cissus* species, especially *C. quadrangularis*, against *Staphylococcus aureus*. In our study, neither the leaf nor the stem extracts of *C. discolor* demonstrated any inhibition of *S. aureus*, indicating a lack of antimicrobial activity against this Gram-positive bacterium. This finding contrasts with several studies that reported strong antibacterial activity of *C. quadrangularis* extracts against *S. aureus*. For instance, [41, 35] found that methanol extracts of *C. quadrangularis* exhibited potent antibacterial effects comparable to antibiotics like ampicillin, suggesting the presence of bioactive compounds responsible for this activity. [43] also reported that ethanolic extracts of *C. quadrangularis* showed antibacterial activity against *S. aureus*, although it was less effective than calcium hydroxide.

Other species within the *Cissus* genus, such as *C. incisa*, have also demonstrated broad-spectrum antimicrobial effects, including activity against *S. aureus*. [12] reported that a chloroform/methanol extract of *C. incisa* exhibited MIC values ranging from 125 to 500 µg/mL. Additionally, the methanol and ethyl acetate extracts of *C. quadrangularis* have consistently shown strong antibacterial effects against *S. aureus*, as documented by [34]. The antibacterial efficacy of these extracts is often attributed to phytochemicals like tannins, which have been shown to inhibit bacterial growth and enhance the effectiveness of conventional antibiotics [44, 45].

The absence of antibacterial activity in our extracts suggests that factors such as extraction methods, solvent selection, or the specific plant parts used may affect the efficacy of the extracts. In contrast, prior studies often reported higher activity with methanol and ethyl acetate extracts, indicating that the bioactive compounds responsible for antibacterial effects might not have been efficiently extracted in our study. Additionally, *S. aureus* is known for its virulence and adaptability, making it a particularly challenging target for antibacterial agents [45].

Antibacterial Activity of *C. discolor* Extracts Against *S. typhimurium*

Our results revealed that neither the leaf nor stem extracts of *C. discolor* species exhibited antimicrobial activity against *S. typhimurium*. These findings contrast with prior studies that have demonstrated the antibacterial potential of *Cissus* species and other plant-based extracts. [34] reported significant antibacterial activity of methanol and ethyl acetate extracts from *C. quadrangularis* against both Gram-positive and Gram-negative bacteria, indicating broad-spectrum antimicrobial potential. While these studies did not specifically focus on *S. typhimurium*, [12] found that *C. incisa* extracts exhibited broad-spectrum activity, with MIC values between 125 and 500 µg/mL, suggesting the possibility of effectiveness against *S. typhimurium*. Similarly, [41] reported that methanol extracts of *C. quadrangularis* were effective against multiple bacterial strains, although no specific data on *S. typhimurium* were provided.

Further studies highlight the antibacterial potential of other plant species against *S. typhimurium*. For example, *Mangkokan* leaves achieved inhibition zones of up to 11.66 mm at 100% extract concentration, while *Cassia occidentalis* exhibited antibacterial activity at concentrations as low as 40 mg/mL, with MIC and MBC values of 30 mg/mL and 60 mg/mL, respectively [46, 47]. The absence of activity in our extracts suggests that the bioactive compounds necessary to inhibit *S. typhimurium* might not have been present, or that the extraction solvents and methods used were not optimal for isolating those compounds. [48] emphasized the importance of exploring alternative antimicrobial agents in light of increasing antibiotic resistance, underscoring the need for further studies to identify effective plant-based antibacterial compounds. In summary, while our *C. discolor* extracts demonstrated selective activity against *B. subtilis*,

they were ineffective against *E. coli*, *S. aureus*, and *S. typhimurium*. These findings align with previous reports indicating variability in the antimicrobial efficacy of plant-based extracts against different bacterial strains.

Antifungal Activity of *C. discolor* Extracts Against *C. albicans*

Our study found that neither the leaf nor stem extracts of ***C. discolor*** exhibited antifungal activity against *C. albicans*, as no inhibition zones were observed for any of the tested extracts. This result contrasts with prior studies on *C. verticillata*, which reported significant antifungal potential against various *Candida* species. [49] demonstrated that hydroalcoholic extracts and fractions from *C. verticillata* leaves showed promising antifungal activity, with dichloromethane and chloroform fractions exhibiting a MIC of 125 µg/mL against *Candida krusei* and *Candida tropicalis*. These findings highlight the potential of *C. verticillata* as an antifungal agent, particularly against non-*albicans* species of *Candida*.

While our study did not observe antifungal activity in the tested ***C. discolor*** extracts, these previous studies suggest that factors such as extraction methods, solvent selection, and the specific species of *Cissus* used may play a crucial role in determining antifungal efficacy. The absence of activity in our extracts could indicate that the bioactive compounds necessary to inhibit *C. albicans* are either absent in the tested extracts or were not effectively extracted using our chosen methods. It is also possible that *C. albicans* possesses inherent resistance to certain phytochemicals found in the tested extracts, which may differ from the active compounds present in *C. verticillata*. This discrepancy underscores the importance of further research to explore different extraction techniques and to identify the specific bioactive constituents responsible for antifungal activity.

Antifungal Activity of *C. discolor* Extracts Against *A. niger*

Our findings indicated that the leaf and stem extracts of *C. discolor* displayed mild to moderate antifungal activity against *A. niger*, with aqueous, chloroform, ethyl acetate, and petroleum ether extracts producing inhibition zones ranging from 0.8 to 1.0 cm. In contrast, the methanol extract exhibited no inhibition, suggesting its ineffectiveness against this fungal strain. This lack of activity in the methanol extract diverges from previous studies that highlight the antifungal efficacy of methanolic extracts from other *Cissus* species. [50] reported that copper oxide nanoparticles (CuO NPs) synthesized using *C. quadrangularis* extracts showed an inhibition rate of up to 86% against *A. niger*, surpassing the effectiveness of the standard antifungal agent Carbendazim. The enhanced activity observed in their study may be attributed to the presence of CuO NPs, which likely augmented the antifungal properties of the plant extract.

The need to explore various extraction methods and solvents to detect antifungal compounds more effectively is also emphasized in the literature. For example, [49] found that dichloromethane and chloroform fractions of *C. verticillata* demonstrated significant MIC values against *Candida* species, a result that was not replicated in our study, where the chloroform extract showed only limited inhibition against *A. niger*. These findings suggest that further fractionation and bioactivity-guided isolation may yield more promising results.

The selective antifungal activity observed in our extracts could also be attributed to the specific resistance mechanisms employed by *A. niger*. Such mechanisms have become increasingly relevant in the context of invasive mold infections, as discussed by [51]. Molecular studies, such as those by [52], which identified antifungal compounds through molecular docking analysis, highlight the importance of understanding these resistance mechanisms. This may help explain the absence of significant antifungal activity in some of our tested extracts and guide future research efforts in identifying more effective plant-based antifungal agents.

CONCLUSION

The findings of this study demonstrate that *Cissus discolor* leaf and stem extracts possess notable antioxidant properties, particularly when extracted with methanol and water, underscoring their potential for use as natural antioxidants. However, the antibacterial activity was selective, with moderate inhibition observed only against *B. subtilis*, while the extracts were ineffective against *E. coli*, *S. aureus*, and *S. typhimurium*. Similarly, the antifungal activity was limited, with only mild to moderate inhibition of *A. niger*, and no inhibition of *C. albicans*. These results suggest that the effectiveness of *C. discolor* extracts in antimicrobial applications is dependent on the solvent used and the target microorganism. Future studies should focus on bioactivity-guided isolation of active compounds and exploration of alternative extraction techniques to enhance the antimicrobial potential of these extracts.

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