PHYTOCHEMICAL SCREENING AND *IN VITRO* BIOACTIVITY INVESTIGATION OF *CISSUS DISCOLOR* BLUME (TABINDING MYA NAN) STEM

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Abstract

The stem of *Cissus discolor* Blume (Tabinding Mya Nan) was investigated for its phytochemical components and their bioactivities, including antioxidant, antimicrobial, and antiarthritic properties. A quantitative analysis was conducted to determine the phenolic and flavonoid content, which are known for their diverse bioactive potential. Antioxidant activity was evaluated using ethanol and aqueous extracts, demonstrating significant activity. Antimicrobial activity was assessed against six microorganism strains using the agar well diffusion method, revealing promising results. Antiarthritic activity was examined *in vitro* using the protein denaturation method, where the crude extracts exhibited activity comparable to standard drugs, with relatively high efficacy. The findings suggest that the antioxidant, antimicrobial, and antiarthritic properties of *C. discolor* extracts highlight their potential for developing enhanced traditional medicines.

Keywords: *Cissus discolor* Blume, elemental contents, phytochemical constituents, antioxidant, antimicrobial and antiarthritic activities

Introduction

Cissus discolor Blume (Tabinding Mya Nan), a member of the Vitaceae family, is native to tropical and subtropical regions of Asia. Figure 1 represents images of plant and dried powder of the stem of *C. discolor*. The plant is rich in bioactive components, including alkaloids, flavonoids, glycosides, cinnamic acids, gallic acid, quercetin, and sitosterol. *C. discolor* has been traditionally used in folk medicine to treat ailments such as cough, cholera, and stomach disorders. Its pharmacological activities include anti-inflammatory, antimicrobial, wound-healing, pain-relieving, analgesic, antibiotic, antidiabetic, antioxidant, anticancer, and antiviral properties. Notably, the combination of honey and the root of *C. discolor* has been reported to aid in treating gastric cancer (Khin *et al.*, 2017).

Recent studies have demonstrated that *C. discolor* possesses anti-inflammatory, antifungal, and antimycobacterial properties. Despite these findings, synthetic agents used for disease treatment are often associated with adverse effects and limited therapeutic efficacy. Although the biological activities of *C. discolor* have been studied extensively over the years, the total phenolic and flavonoid contents—key compounds known to reduce oxidative stress and relieve joint pain—have yet to be quantified. Therefore, this study aimed to quantitatively determine the total phenolic and flavonoid contents, and the antioxidant, antimicrobial, and antiarthritic activities of the stem solvent extracts of *C. discolor*, to provide further evidence supporting its potent antioxidant and antiarthritic properties.

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(a) **(b)**

Figure 1. Images of (a) plant and (b) dried powder of *Cissus discolor* Blume (Tabinding Mya Nan) stem

materials and methods

Sample Collection and Preparation

The stem of *C. discolor* was collected from Myo Ma Market in Taunggyi in December 2023. Distilled water was used to clean the stems, removing dust and impurities thoroughly. The cleaned samples were then shade-dried to preserve bioactive components before being ground into a fine powder using a grinding machine. To prevent moisture absorption and contamination, the powdered samples were stored in air-tight containers. These dried and powdered samples were subsequently used for chemical and biological analyses.

Determination of Moisture and Ash Contents

The 2 g sample was used for the determination of moisture and ash contents.

Moisture content determination: The sample was placed in a pre-weighed porcelain crucible and kept in an oven at 105 °C for 2 h. After removal from the oven, it was cooled in a desiccator and then weighed. The process of heating, cooling, and weighing was repeated until a constant weight was achieved. The loss in weight corresponds to the moisture content, and the percentage of moisture was then calculated.

Ash content determination: The sample was placed in a pre-dried, cooled, and weighed porcelain crucible. It was gently heated over a burner until thoroughly charred, then transferred to a muffle furnace at 550 °C until the residue was free of carbon. The crucible and residue were cooled in a desiccator and weighed. This process was repeated until a constant weight was achieved. The ash content was then calculated.

Elemental Analysis of *C. discolor* by Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometer

The relative abundance of elements in the stem of *C. discolor* was determined using an Energy Dispersive X-ray Fluorescence (EDXRF) spectrometer (Shimadzu EDX-700) at the Department of Chemistry, Monywa University.

Preliminary Phytochemical Constituents Tests

Preliminary phytochemical tests were conducted on the sample following standard methods to classify the types of organic constituents present. These included alkaloids, α-amino acids, cyanogenic glycosides, flavonoids, phenolic compounds, saponins, steroids, tannins, and terpenoids. The tests were performed using established protocols as described by M-Tin Wa (1972) and Harborne (1984).

Quantitative Determination of Total Phenolic and Flavonoid Contents

The antioxidative factors, total phenolic content, and total flavonoid content, of watery and ethanol extracts were measured spectrophotometrically according to the Folin-Ciocalteu method (Saxena *et al.,* 2013) and the aluminium chloride colorimetric assay (Zhishen *et al.*, 1999). The absorbance of the reaction mixture was read at λ_{max} 765 nm to determine phenolic content and 415 nm for flavonoid content using a spectrophotometer (Jenway, 6300 Spectrophotometer). The concentrations in terms of gallic acid equivalent of each of the extracts were calculated by using the linear regression equation from the standard curve of gallic acid. Total phenolic contents in the watery and ethanol extracts were expressed as mg of gallic acid equivalent per 1 g extracts. Similarly, the concentration of quercetin equivalent in the extracts was calculated using the linear regression equation from the standard calibration curve of quercetin. The total flavonoid contents of the watery and ethanol extracts were expressed as mg quercetin equivalent per 1 g sample extract.

Investigation of Antioxidant Activity

DPPH radical scavenging activity of ethanol and watery extracts was spectroscopically determined using a UV-visible spectrophotometer (Marinova and Batchvarov, 2011). The control solution was prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of ethanol. The sample solution was also prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of test sample solution. These prepared solutions were kept in brown bottles. Then the solutions were incubated at room temperature and shaken on the shaker for 30 min. After 30 min, the absorbance of different concentrations of tested samples was measured at 517 nm using Jenway, 6300 Spectrophotometer. Absorbance measurements were done three times for each solution and the mean value so obtained was used to calculate the percentage of radical scavenging activity. The butylated hydroxytoluene (BHT) was used as a standard for this activity.

Screening of Antimicrobial Activity

The antimicrobial activity of petroleum ether, ethyl acetate, ethanol, and watery extracts of the sample was determined against six strains of microorganisms such as *B. pumilus, B. subtilis, C. albicans, E. coli, P. aeruginosa,* and *S. typhi* by employing agar well diffusion method (Finegold and Martin, 1982). The tests were conducted at the Department of Chemistry, Madalar University.

In Vitro Determination of Antiarthritic Activity

The *in vitro* antiarthritic activity was studied using the Bovine Serum protein denaturation method and the Egg Albumin denaturation method at a concentration of 125 to 500 μg mL^{-1}. In the Bovine Serum protein denaturation method, the reaction mixture (5 mL) consisted of 0.2 mL of BSA, 2.8 mL of phosphate-buffered saline (pH 6.4), and test solutions of different concentrations (500, 250, and 125 μg/mL) were mixed to form a reaction mixture of 5 mL. Double distilled water of the same volume served as control. The samples were incubated at 37±2 ºC in an incubator for 15 min followed by heating at 70 ºC for 5 min. UV-vis spectrophotometer (Jenway, 6300 Spectrophotometer) was used to measure the absorbance at 660 nm. Similarly, in the egg albumin denaturation method, egg albumin was used instead of BSA.

Results and Discussion

Moisture and Ash Contents of *C. discolor* (Tabinding Mya Nan) Stem

The analysis revealed that the dried stem powder contained **2 % moisture** and **8 % ash.** The low moisture content suggests that the stem can serve as a stable and suitable ingredient for use in conventional medicine, supporting its potential application in treating

various diseases. Moreover, the ash content indicates a high mineral presence, highlighting the stem as a valuable source of essential minerals.

Semiquantitative Elemental Analysis of *C. discolor* (Tabinding Mya Nan) Stem

The semi-quantitative elemental analysis of *C. discolor* stem was conducted using the **EDXRF (Energy Dispersive X-Ray Fluorescence) technique**, which identified the presence of **K, Ca, S, Fe, Cu, Zn, Mn, Rb, and Sr** in the sample. The EDXRF spectra data, illustrating the relative abundance of these elements in the dry powdered stem sample, are presented in **Figure 2** and summarized in **Table 1.** The results are further depicted as bar graphs in **Figure 3.**

The analysis revealed that **calcium (1.247 %)** was the most abundant element in the sample, followed by significant amounts of **potassium (0.519 %)** and **sulphur (0.109 %)**. Calcium and potassium are essential for several physiological functions, including **valence regulation, electrolyte balance, muscle contraction, and blood pressure regulation**. Potassium also plays a critical role in promoting the health of **teeth and bones**. Sulphur contributes to **food metabolism** and supports various bodily functions, including maintaining healthy **skin**. Furthermore, trace elements such as **manganese (Mn)**, **iron (Fe)**, **copper (Cu)**, and **zinc (Zn)** were also detected, highlighting the nutritional value of the sample. Based on these findings, *C. discolor* stem may be considered a beneficial resource for promoting health.

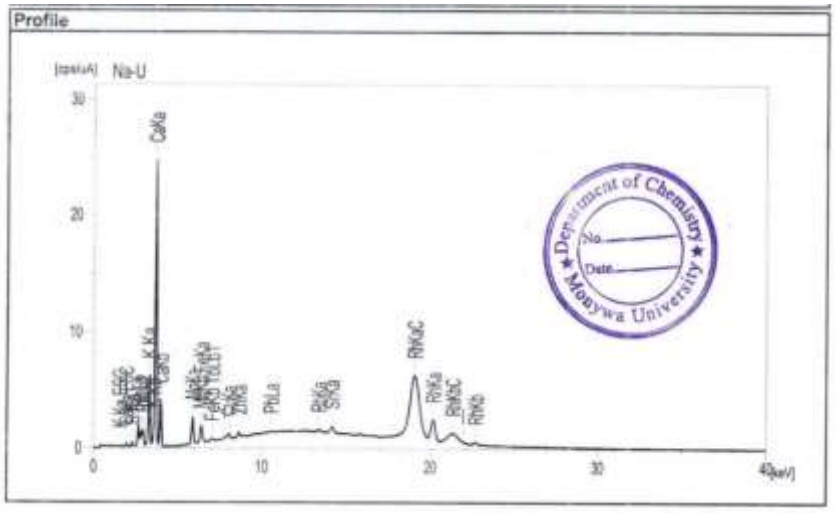

Figure 2. EDXRF spectrum of *C. discolor* (Tabinding Mya Nan) stem

Table 1. Relative Abundance of Some Elements in *C. discolor* (Tabinding Mya Nan) Stem

Elements	Relative abundance (% w/w)
Ca	1.249
K	0.519
S	0.109
Mn	0.022
Fe	0.009
Cu	0.002
Zn	0.001
Sr	0.001
Rb	0.001
C, H	98.086

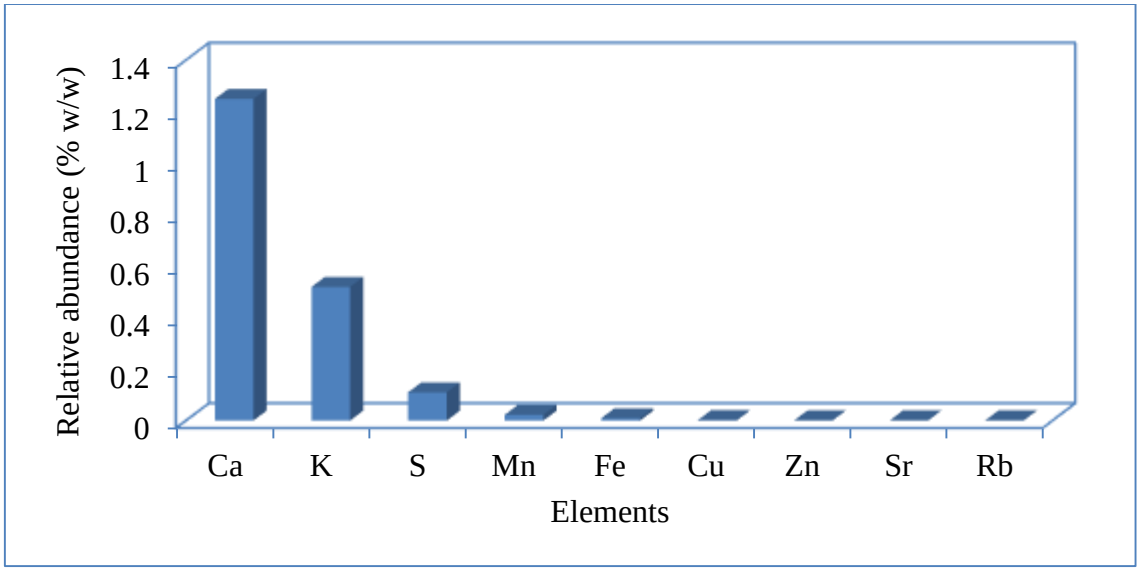

Figure 3. A bar graph of the relative abundance of elements in *C. discolor* (TabindingMya Nan) stem

Phytochemical Constituents of *C. discolor* (Tabinding Mya Nan) Stem

When humans consume phytochemicals, these compounds can exhibit significant biological activity. Phytochemicals are primarily obtained from various plant-based foods, including fruits, vegetables, whole grains, and seeds. This literature survey suggests a more systematic approach is needed to conduct preliminary phytochemical studies on the *C. discolor* stem.

Various phytochemical tests were conducted to determine the presence or absence of alkaloids, α-amino acids, cyanogenic glycosides, flavonoids, and phenolic compounds. The results indicate that all bioactive compounds, except cyanogenic glycosides, are present.

This study provides valuable insights into the presence of diverse phytochemicals, which may contribute to the tested sample's potential antioxidant, antimicrobial, and antiarthritic properties.

Total Phenolic Content of Watery and Ethanol Extracts of *C. discolor* (Tabinding Mya Nan) Stem

The Folin-Ciocalteu (F-C) assay is one of the most widely used methods for phenolic analysis. The principle of the F-C assay is based on the reduction of the Folin-Ciocalteu reagent in the presence of phenolic compounds, resulting in the formation of molybdenum-tungsten blue. This blue complex is measured spectrophotometrically at 765 nm, with the intensity of the colour increasing linearly with the concentration of phenolic compounds in the reaction medium. A higher concentration of phenolic compounds in the extracts leads to a darker colour and higher absorbance.

Gallic acid (3,4,5-trihydroxybenzoic acid) was used to construct a standard calibration curve. The total phenolic content, expressed as micrograms of gallic acid equivalent per milligram (µg GAE/mg) of crude extract, was calculated from this standard curve (Saxena *et al.*, 2013). The total phenolic content of ethanol extract (84.5 ± 0.04 µg GAE/mg extract ± SD) was observed to be nearly twice as high as that of the watery extract (43.5 ± 0.01 µg GAE/mg extract ± SD). The data supporting these findings is presented in Table 2.

Total Flavonoid Content of Crude Extracts of *C. discolor* (Tabinding Mya Nan) Stem

To confirm the presence of active compounds in *C. discolor* (particularly flavonoids), a content analysis was conducted using a colourimetric assay specifically designed to target flavonoids conveniently and appropriately (Cook, 1996). The aluminum chloride colourimetric assay, as reported by Woisky and Salatino (Rice-Evans, 1995), is effective in detecting flavonoids in the flavone and flavanol groups, such as quercetin. In this assay, aluminum chloride reacts with quercetin to form a stable, colour-signature complex.

The reaction involves the C-4 keto group and either the C-3 or C-5 hydroxyl group of the flavonoid compounds to produce an acid-stable complex. Additionally, reactions within the ortho-dihydroxyl groups in flavonoids' A- and B-ring can also form several acid-labile complexes (Agati, 2012). These complexes, formed by flavonols (including kaempferol, rutin, quercetin, and quercitrin), exhibit maximum absorbance at 415 nm (Cook, 1996). By employing the aluminum chloride colourimetric assay, the total flavonoid content can be accurately measured.

A standard calibration curve of quercetin was constructed using varying concentrations of quercetin. From this experimental study, the total flavonoid content of the watery and ethanol extracts of the *C. discolor* stem is presented in Table 2. The results indicate that the ethanol extract (104.03 mg QE/g) contained a higher flavonoid content compared to the watery extract (55.64 mg QE/g).

Table 2. Total Phenolic Content and Total Flavonoid Content in Watery and Ethanol Extracts of *C. discolor* (Tabinding Mya Nan) Stem

Extracts	TPC (µg GAE/mg extract ± SD)	TFC (mg of QE/ g of extract ± SD)
Watery	43.5 ± 0.01	55.64 ± 0.002
Ethanol	84.5 ± 0.04	104.03 ± 0.001

Radical Scavenging Activity of Crude Extracts of *C. discolor* (Tabinding Mya Nan) Stem by DPPH Radical Scavenging Assay

According to the description by Marinova and Batchvarov (2011), the antioxidant activity of watery and ethanol extracts of the *C. discolor* stem was evaluated using a DPPH radical scavenging assay. The assay is based on the violet-coloured ethanolic DPPH solution, which has a maximum absorption wavelength of 517 nm. Neutralisation of the DPPH free radical results in a colour change from violet to pale yellow under daylight. Test substances with anti-radical properties cause decolourisation of the original colour. As the concentration of the test substance increases, absorption values decrease, indicating stronger antioxidant activity.

In this study, butylated hydroxytoluene (BHT) was used as a standard. Experimental data revealed that the *C. discolor* stem exhibits high antioxidant activity. The IC_{50} values of the stem extracts and the standard BHT, along with their percent radical scavenging activity, are presented in Figure 4. Additional details are provided in Table 3.

The antioxidant potency of the ethanol extract (IC_{50} = 13.60 µg/mL) was found to be higher than that of the watery extract (20.49 µg/mL); however, both are lower than the standard BHT (IC_{50} = 3.43 µg/mL).

Evidence suggests a positive association between the scavenging ability against DPPH radicals and the presence of phenolic and flavonoid compounds. This finding indicates that these

compounds contribute significantly to the antioxidant capacity of both ethanol and watery extracts of the *C. discolor* stem.

Table 3. Radical Scavenging Activity of Watery and Ethanol Extracts of *C. discolor* Stem and Standard BHT

Extracts	% RSA ± SD of different concentrations (µg/mL)				IC_{50} (µg/mL)
	3.125	6.25	12.5	25	
Watery	44.44 ± 0.175	46.67 ± 0.175	48.37 ± 0.175	50.92 ± 0.175	20.49
Ethanol	46.75 ± 0.232	48.37 ± 0.355	49.53 ± 0.235	54.85 ± 0.23	13.60
BHT (Std.)	49.11 ± 0.009	58.31 ± 0.008	68.69 ± 0.001	82.05 ± 0.001	3.43

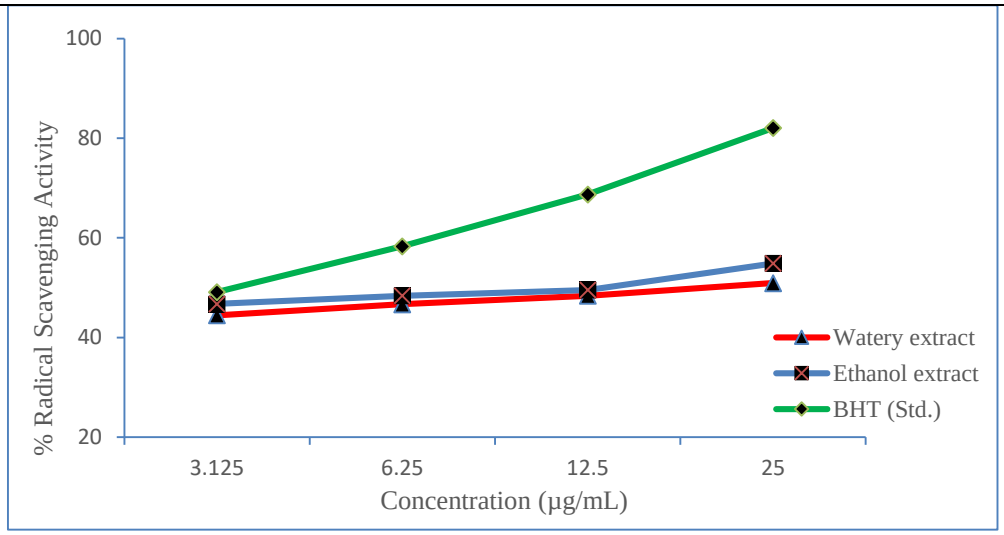

Figure 4. Percent radical scavenging activity vs concentration (µg/mL) of watery and ethanol extracts of stem of *C. discolor* and standard BHT

Screening of Antimicrobial Activity of Crude Extracts of *C. discolor* Stem by Agar Well Diffusion Method

In this study, the antimicrobial activity of four crude extracts (H_2O, EtOH, EtOAc, and PE) obtained from the stem of *C. discolor* was evaluated against six different microbial strains: *B. subtilis*, *B. pumilus*, *C. albicans*, *E. coli*, *P. aeruginosa*, and *S. typhi*. The agar well diffusion method was used to assess antimicrobial activity, with the measurable zone of inhibition (including the 8 mm well diameter) indicating the degree of effectiveness. A larger zone diameter corresponds to greater antimicrobial activity.

Photographic records of the results are presented in Figure 5, while the inhibition zone diameters are summarized in Table 4. The results revealed that the ethyl acetate (EtOAc) and ethanol (EtOH) extracts exhibited mild antimicrobial activity against all tested microorganisms, whereas the petroleum ether (PE) and watery (H_2O) extracts showed no activity.

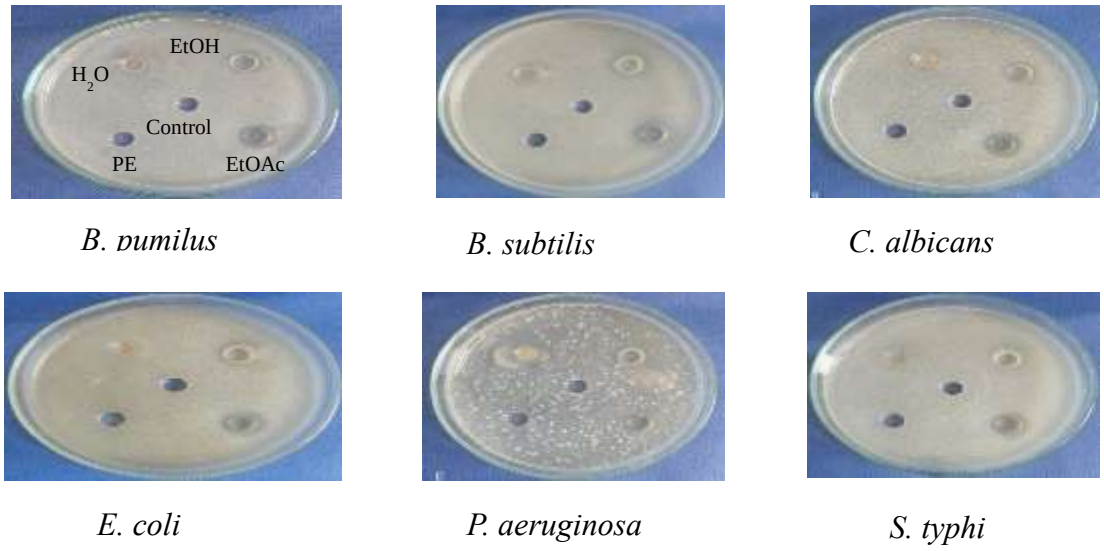

Figure 5. Photo records showing inhibition zone due to the effect of different crude extracts *C. discolor* stem on six different microorganisms

Table 4. Inhibition Zone Diameters of Various Crude Extracts of *C. discolor* Stem on Six different Microorganisms

Microorganisms	Inhibition zone diameter of different extracts (mm)			
	PE	**EtOAc**	**EtOH**	**H_2O**
B. pumilus	-	12.03	12.89	-
B. subtilis	-	13.10	12.00	-
C. albicans	-	11.75	11.00	-
E. coli	-	12.50	11.30	-
P. aeruginosa	-	10.33	11.21	-
S. typhi	-	12.00	12.50	-

Agar well (8 mm); 9 mm ~ 14 mm = mild activity; 15 mm ~ 20 mm = medium activity;
21 mm and above = high activity; (-) = no activity

Antiarthritic Activity of Crude Extracts of *C. discolor*

In the anti-arthritis assay using the protein denaturation method, both extracts demonstrated significant results, as detailed in Table 5. A decrease in the absorbance of test samples compared to the control indicates protein stabilization. The ethanol extract exhibited a higher inhibition percentage than the aqueous extract. The IC_{50} value for the inhibition of bovine serum albumin (BSA) protein denaturation was 744.05 µg/mL for the ethanol extract and 856.16 µg/mL for the watery extract. Similarly, in the egg albumin protein denaturation method, the IC_{50} value for the ethanol extract was 415.28 µg/mL, compared to 775.90 µg/mL for the watery extract. These results are illustrated in Figure 6. As a result, the ethanol extract was found to exhibit a higher inhibition of protein denaturation compared to the watery extract. However, both extracts were less potent than the standard drug Allopurinol (IC_{50} = 204.75 µg/mL in BSA assay and 335.57 µg/mL in egg albumin assay)

Protein denaturation is a primary cause of arthritic diseases (Hossain *et al.*, 2015). Based on this study, it can be concluded that both extracts possess anti-arthritic properties and can prevent the efflux of intracellular components. These properties are likely due to the production of various phytoconstituents within the plant. Phenolics and flavonoids are well-documented for their biological activities, including antioxidant, anticarcinogenic, anti-inflammatory, cardiovascular protection, and cell proliferation effects (Rice-Evans et al., 1996). According to Singh and Sharma (2016), anti-arthritic activity can be attributed to phenols, tannins, and flavonoids. Additionally, many flavonoids and related polyphenols contribute to the antioxidant and anti-inflammatory activities of numerous plants (Govindappa *et al.*, 2011). Therefore, the presence of these bioactive compounds in the extracts suggests that the samples possess membrane-stabilizing properties.

Table 5. Antiarthritic Activity of Crude Extracts of *C. discolor* Stem on Protein Denaturation Method by Using Bovine Serum Albumin and Egg Albumin

Extracts	Conc. (µg/mL)	% Inhibition		IC_{50} (µg/mL)	
		BSA	EA	BSA	EA
Watery	125	15.78	19.31	856.16	775.90
	250	21.05	30.68		
	500	36.84	43.18		
	1000	52.63	55.36		
Ethanol	125	14.85	19.64	744.05	415.28
	250	25.74	41.07		
	500	47.77	53.57		
		57.89			
Allopurinol	125	47.36	30.36	204.75	335.57
	250	52.63	51.79		
	500	54.89	64.29		

Conc.= Concentration; BSA = Bovine Serum Albumin; EA = Egg Albumin

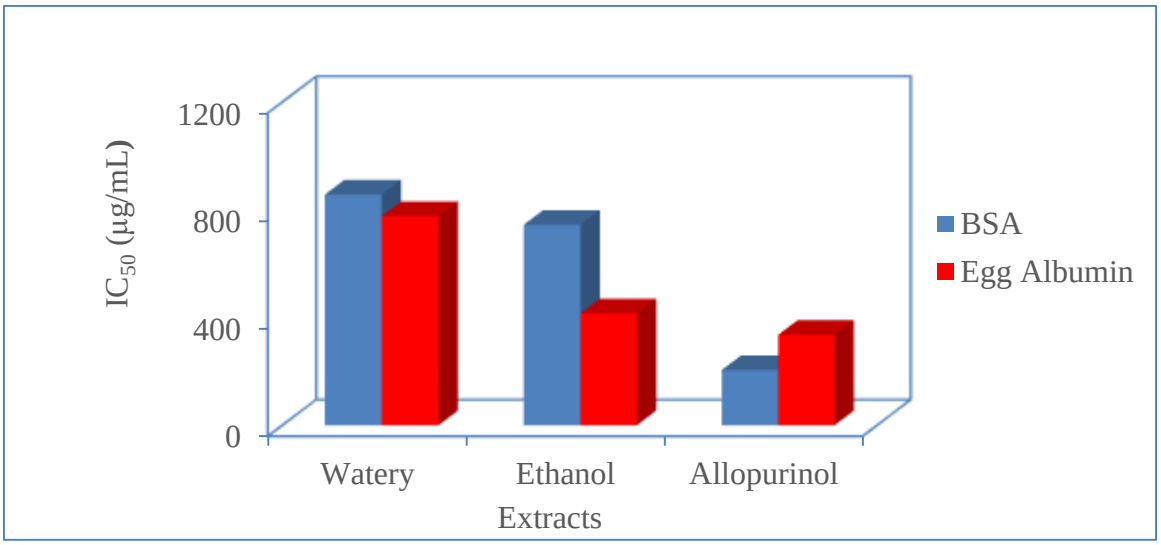

Figure 6. A bar graph of IC_{50} values of watery, ethanol, and Allopurinol in bovine serum albumin and egg albumin protein denaturation

Conclusion

The study analyzed the composition and bioactivities of the *C. discolor* (Tabinding Mya Nan) stem, including its moisture, ash, elemental, and phytochemical contents, as well as antioxidant, antimicrobial, and antiarthritic properties. High ash and low moisture contents highlight its suitability for medicinal use, with significant levels of calcium, potassium, and sulfur supporting micronutrient-related treatments.

Phytochemical screening revealed all tested constituents except cyanogenic glycosides. The ethanol extract, rich in phenolic and flavonoid compounds, exhibited strong antioxidant activity but limited antimicrobial efficacy. Both ethanol and aqueous extracts demonstrated antiarthritic activity, suggesting their potential as natural therapeutic agents.

Overall, the stem of *C. discolor* demonstrates potential as a natural alternative medicine, exhibiting antioxidant, antimicrobial, and antiarthritic properties.

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