



PHYTOCHEMICAL SCREENING AND ANTI-OXIDANT ACTIVITY OF MEDICINAL PLANT *CORDIA DICHOTOMA*

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ABSTRACT

The medicinal plant *Cordia dichotoma* (Family: Boraginaceae) has been traditionally used for the treatment of various ailments, including digestive disorders, fever, respiratory infections, and inflammation. This study aims to evaluate the phytochemical composition and antioxidant activity of the hydroalcoholic extract of *Cordia dichotoma* leaves. Phytochemical screening revealed the presence of bioactive compounds, such as alkaloids, glycosides, flavonoids, phenols, proteins, tannins, and sterols. Thin layer chromatography (TLC) analysis confirmed the presence of flavonoids and phenolic compounds, with the R_f values corresponding to quercetin and gallic acid, respectively. The total phenol content of the extract was found to be 4.76 mg/100mg, and the total flavonoid content was 1.41 mg/100mg. The antioxidant activity was assessed using the DPPH radical scavenging assay, where the hydroalcoholic extract exhibited significant antioxidant activity, with 84.54% scavenging effect at a concentration of 100 µg/ml. The IC₅₀ value for the hydroalcoholic extract was 61.68 µg/mL, indicating moderate antioxidant potential compared to the standard ascorbic acid (IC₅₀ = 34.30 µg/mL). These findings suggest that *Cordia dichotoma* leaves are a promising source of natural antioxidants and bioactive compounds with potential therapeutic applications in managing oxidative stress-related diseases.

Keywords: *Cordia dichotoma*, Phytochemical screening, Antioxidant activity, DPPH assay, Flavonoids, Phenols, Thin layer chromatography (TLC), Hydroalcoholic extract, Oxidative stress, Natural antioxidants.

INTRODUCTION

Cordia dichotoma, a member of the *Boraginaceae* family, is a widely recognized medicinal plant found in tropical and subtropical regions. It has been used for centuries in traditional medicine systems for the treatment of various ailments such as digestive disorders, fever, respiratory infections, and inflammatory conditions. The plant is known for its diverse pharmacological properties, including antimicrobial, anti-inflammatory, antidiabetic, and antioxidant

activities. These therapeutic effects are attributed to the bioactive compounds present in its leaves, fruits, seeds, and bark (Ali *et al.*, 2013; Gupta *et al.*, 2016).

Phytochemical screening plays a crucial role in identifying the presence of various bioactive compounds such as alkaloids, flavonoids, terpenoids, saponins, tannins, and phenolic compounds. These compounds are responsible for the plant's medicinal properties and are the primary focus of research into its pharmacological activities

(Pandey *et al.*, 2019). The rich phytochemical profile of *Cordia dichotoma* indicates its potential as a source of natural antioxidants, which are compounds capable of neutralizing free radicals and reducing oxidative stress in the body (Muthuraman *et al.*, 2014).

Oxidative stress is a key contributor to the pathogenesis of various chronic diseases, including cardiovascular diseases, cancer, diabetes, and neurodegenerative disorders. Antioxidants play a vital role in preventing or mitigating oxidative damage by scavenging free radicals and enhancing the body's defense mechanisms. As such, *Cordia dichotoma* has gained significant attention for its potential antioxidant properties, which could be harnessed for the development of therapeutic agents aimed at combating oxidative stress-related diseases (Pandey *et al.*, 2019; Priyanka *et al.*, 2020).

Recent studies have highlighted the importance of *Cordia dichotoma* in mitigating oxidative stress through various mechanisms, including scavenging free radicals, chelating metal ions, and enhancing endogenous antioxidant defense systems. The plant's antioxidant activity is attributed to the presence of phenolic compounds, flavonoids, and other secondary metabolites that have been shown to exhibit potent free radical-scavenging abilities (Gupta *et al.*, 2016; Priyanka *et al.*, 2020).

This research aims to explore the phytochemical composition of *Cordia dichotoma* through qualitative and quantitative analysis, followed by an evaluation of its antioxidant activity. The study will provide insight into the plant's potential as a natural antioxidant and its

therapeutic applicability in preventing or managing oxidative stress-induced diseases.

MATERIALS AND METHODS

The leaves of *Cordia dichotoma* were collected from District Bhopal, Madhya Pradesh on the basis of geographical availability. Samples were crashed and transferred into glass container/ extraction bottle and preserved until extraction procedure was performed in the laboratory.

Extraction of *Cordia dichotoma* by microwave assisted extraction technique

The leaves of *C. dichotoma* (30g) was extracted by microwave assisted extraction technique at room temperature in 250 mL of hydroalcoholic solvent (methanol: aqueous; 75:25v/v). The plant extract was filtered through a whattman filter paper and from filtrate the excess of solvent (aqueous) was removed using a rotary evaporator. The extracts were preserved in airtight containers and stored at 4°C till further use. Percent yield of crude extract was calculated (Sharma *et al.*, 2020).

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \times 100$$

Phytochemical screening

Medicinal plants are traditional pharmaceutical commodities and many of the current medicinal drugs are derived indirectly from plants. Phytochemical materials consist of two main bioactive components (Chlorophyll, vitamins, amino acids, sugar etc.) and secondary bioactive components; (Alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical analyses were performed

according to the normal protocols for extract. Phytochemical examinations were carried out for all the extracts as per the standard methods (Mane *et al.*, 2020).

TLC Profile

For TLC analysis aluminium plate precoated with Silica gel as the stationary phase, 7 X 6 cm was cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1- μ l of sample by using capillary at distance of 1 cm at 5 track, by the mobile phase used a mixture of Toluene: Ethyl acetate: formic acid (different solvent system).

In solvent system I- Toluene: Ethyl acetate: formic acid (5:4:1) for flavonoids (quercetin) and In solvent system II- Toluene: Ethyl acetate: formic acid (7:5:1) for Phenol (Gallic acid) used. The movement of the analyte was expressed by its retention factor (Rf), values were calculated for different samples (Mane *et al.*, 2020).

$$Rf = \frac{\text{Distance Traveled by solute}}{\text{Distance Traveled by the solvent}}$$

Estimation of total phenolic and flavonoid content

Total flavonoids content estimation (TFC)

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25 μ g/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% AlCl₃ methanolic solution was added to 3 ml of extract or standard and allowed to stand

for 15 min at room temperature; absorbance was measured at 420 nm (Balmik *et al.*, 2019).

Total phenolic content estimation (TPC)

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50 μ g/ml was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol. 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer (Balmik *et al.*, 2019).

In-vitro antioxidant activity by DPPH free radical scavenging assay

Antioxidant activity was performed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. Sample (10 mg) was dissolved in methanol (10 mL) to obtain a stock concentration of 1000 μ g/mL. The stock solution was further diluted to final concentrations of 10, 20, 40, 60, 80 and 100 μ g/mL in methanol. The DPPH solution was freshly prepared in MeOH (6mg in 100ml methanol). Then, 1.5 mL of DPPH solution was added to 1.5 mL of sample solution of different concentrations.

An equal volume of DPPH and methanol except sample was used as control. Methanol was used as blank in this method. The mixture was allowed to react at room temperature in the dark. After 15 minutes, the absorbance of the reaction mixture was recorded at 517 nm.

Then, Final decrease in absorbance was noted of DPPH with the sample at different concentration. The percentage inhibition (%) was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

The concentration of plant extract necessary to scavenge 50% of radicals (IC₅₀) was calculated by plotting inhibition percentages against concentrations of sample (Dwivedi *et al.*, 2019).

RESULTS AND DISCUSSION

In this study, *Cordia dichotoma* was evaluated for its phytochemical composition, antioxidant activity, and potential as a natural source of bioactive compounds. The results from the various tests conducted have provided significant insights into the plant's medicinal potential.

Table 1 shows the extractive yield obtained from the hydroalcoholic extraction of *Cordia dichotoma*. The yield of 4.26% suggests that the plant contains a substantial amount of extractable bioactive compounds, which could contribute to its therapeutic properties. This yield is consistent with other studies on plants with similar chemical composition, where higher yields often correlate with higher concentrations of bioactive phytochemicals.

Preliminary phytochemical screening (Table 2) revealed the presence of various bioactive compounds in the hydroalcoholic extract of *Cordia dichotoma* leaves. Alkaloids, glycosides, phenols, flavonoids, proteins, tannins, and sterols were all found to be present, which aligns with the plant's traditionally reported uses in treating inflammatory and infectious diseases (Gupta *et al.*, 2016). The presence of alkaloids,

flavonoids, and phenolic compounds is particularly notable, as these groups of compounds are known for their antioxidant, anti-inflammatory, and antimicrobial activities (Muthuraman *et al.*, 2014). On the other hand, saponins and diterpenes were absent, which might suggest that these compounds are not the primary contributors to the plant's therapeutic effects in the context of this study.

Thin Layer Chromatography (TLC) analysis (Table 3 and Table 4) further supports the presence of flavonoids and phenols as secondary metabolites in *Cordia dichotoma*. The R_f values of the hydroalcoholic extract of *Cordia dichotoma* matched closely with those of standard quercetin (flavonoid) and gallic acid (phenolic compound), confirming their presence in the extract. The multiple spots observed in the TLC analysis of the hydroalcoholic extract (Table 4) indicate a complex mixture of compounds, which likely contribute to the plant's diverse biological activities.

The total phenol content in the hydroalcoholic extract of *Cordia dichotoma* was found to be 4.76 mg/100mg, and the flavonoid content was 1.41 mg/100mg (Table 5). These values suggest that *Cordia dichotoma* is a moderate source of phenolic compounds and flavonoids, which are well-known for their antioxidant properties. Phenolic compounds, particularly flavonoids, have been shown to exhibit strong free radical scavenging abilities, contributing to the plant's therapeutic potential in managing oxidative stress-related diseases.

The DPPH radical scavenging assay (Table 6) demonstrated the ability of the hydroalcoholic extract to neutralize free radicals. The

antioxidant activity increased with higher concentrations of the extract, with 84.54% scavenging activity at 100 µg/ml, indicating the extract's potent antioxidant capacity. The IC₅₀ value of the hydroalcoholic extract was found to be 61.68 µg/mL, which is relatively higher compared to ascorbic acid (IC₅₀ = 34.30 µg/mL) (Table 7). Although the hydroalcoholic extract showed good antioxidant activity, it was less potent than

ascorbic acid, a standard antioxidant. This difference in potency suggests that while *Cordia dichotoma* may not be as effective as ascorbic acid in neutralizing free radicals, it still possesses significant antioxidant potential, possibly due to the presence of phenolic compounds and flavonoids, which are known for their radical scavenging properties.

Table 1: Extractive values obtained from *Cordia dichotoma*

S. No	Solvent	Time of extraction (Hour)	Weight of sample (gm)	Weight of extract (gm)	% Yield
1	Hydroalcoholic	24	30	1.28	4.26

Table 2: Preliminary phytochemical screening of *Cordia dichotoma* leaves extract

S. No.	Phytoconstituents	Test Name	Hydroalcoholic extract
1.	Alkaloids	A) Wagner's Test	+
		B) Hager's Test	+
2.	Glycosides	Leagel's test	+
3.	Saponins	Foam test	-
4.	Diterpenes	Copper acetate test	-
5.	Phenols	A) Ferric chloride test	+
		B) Folin-Ciocalteu	+
6.	Carbohydrates	A) Fehling test	-
		B) Benedicts test	-
7.	Flavonoids	A) Lead acetate	+
		B) Alkaline reagent test	-
8.	Proteins	Xanthoproteic test	+
9.	Tannin	Gelatin test	+
10.	Sterol	Salkowski test	+

(+) Positive, (-) Negative

Table 3: Result of flavonoid and phenol with Rf Value for TLC Analysis

S. No.	Secondary metabolites	Standard	Solvent system	Solvent Ratio	Spot	Rf Value (cm)
1	Flavonoid	Quercetin	Toluene: Ethyl acetate: formic acid	5:4:1 (in ml)	1	0.6
2	Phenol	Gallic acid	Toluene: Ethyl acetate: formic acid	7:5:1(in ml)	1	0.44

Table 4: Result of hydroalcoholic extract of *Cordia dichotoma* with Rf Value for TLC Analysis

S. No.	Plant extract (C. dichotoma)	Solvent system	Solvent Ratio	Number of spots	Rf Value (cm)
1	Hydroalcoholic	Toluene: Ethyl acetate: formic acid	5:4:1 (in ml)	4	0.20
					0.56
					0.70
					0.80
2	Hydroalcoholic	Toluene: Ethyl acetate: formic acid	7:5:1(in ml)	8	0.40
					0.52
					0.54
					0.60
					0.70
					0.74
					0.80
					0.90

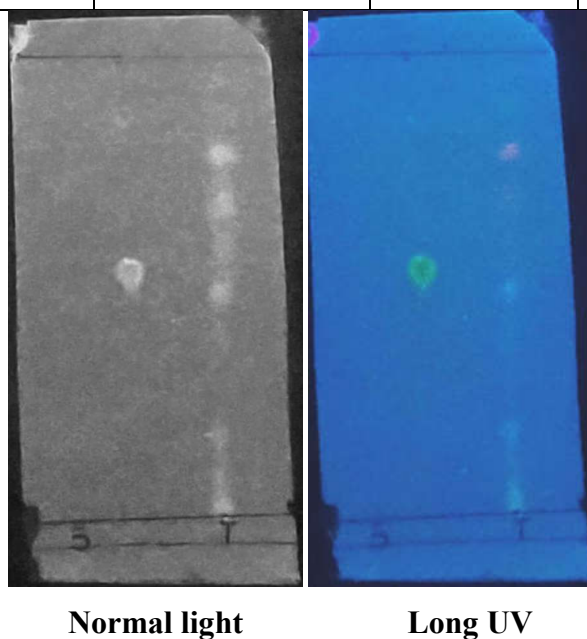


Figure 1: TLC plate viewed under UV light (Quercetin)

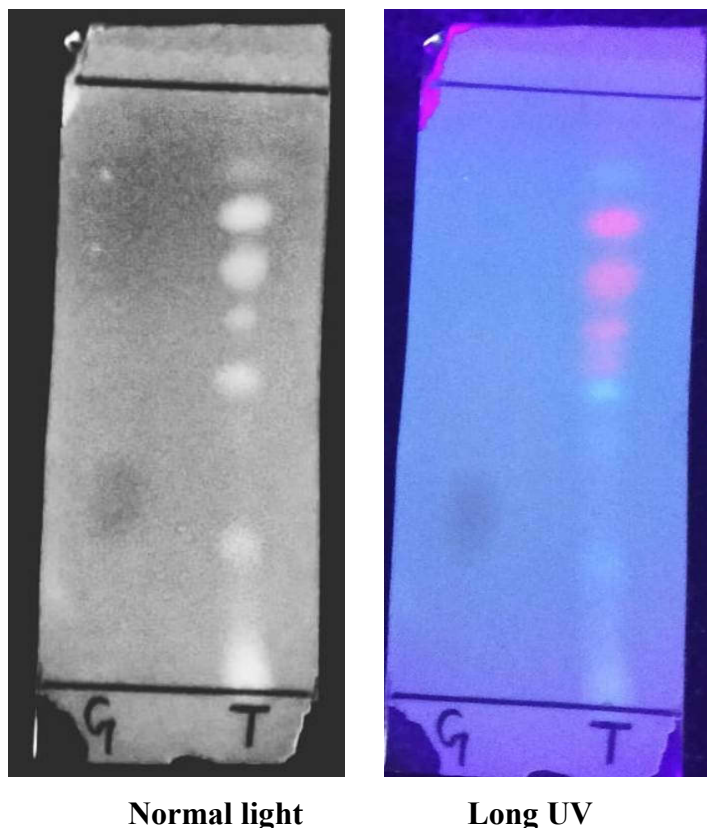


Figure 2: TLC plate viewed under UV light (Gallic acid)

Table 5: Phenolic and flavonoid content (%) in the leaves of *C. dichotoma* L

Hydroalcoholic extract	Total phenol content mg/100mg	Total flavonoids content mg/100mg
<i>C. dichotoma</i>	4.76	1.41

Table 6: Percentage (%) DPPH radical scavenging activity of *C. dichotoma* L

Plant Extract (<i>C. dichotoma</i>)			
S. No.	Concentration (µg/ml)	Absorbance at 517 nm	DPPH radical scavenging activity (%)
1	10	0.512	4.66
2	20	0.459	14.53
3	40	0.317	40.97
4	60	0.293	45.44
5	80	0.213	60.34
6	100	0.083	84.54

Control absorbance = 0.537

Table 7: IC₅₀ value for DPPH assay of Ascorbic acid and hydroalcoholic extract of *C. dichotoma*

S. No	Standard / Plant Extract	IC ₅₀ (µg/mL)
1.	Ascorbic acid	34.30
2.	Hydroalcoholic extract	61.68

CONCLUSION

The findings of this study demonstrate that *Cordia dichotoma* contains a variety of bioactive compounds, including alkaloids, glycosides, phenols, flavonoids, tannins, and sterols, which likely contribute to its therapeutic effects. The hydroalcoholic extract exhibited promising antioxidant activity, indicating its potential for use in managing oxidative stress-related diseases. Although the extract showed a lower IC₅₀ value compared to ascorbic acid, its antioxidant potential, along with its phytochemical composition, makes it a valuable plant for further exploration in the development of natural antioxidant agents. Future studies could focus on isolating specific bioactive compounds and evaluating their individual contributions to the plant's overall antioxidant and therapeutic properties.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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