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In vitro evaluation of antioxidant and antimicrobial properties of *Cordia mocleodii* leaf extract

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ABSTRACT

Cordia macleodii is known as a medicinal plant with hepatoprotective and wound-healing properties. *Cordia macleodii* contains relatively high levels of flavonoids, alkaloids, steroids, and terpenoids. This study aimed to investigate the *in vitro* antibacterial and antioxidant properties of *Cordia macleodii* methanol leaf extract. The antioxidant activity of the methanol extract of *Cordia macleodii* leaves was determined using the DPPH scavenging test, total phenol content (TPC), and total flavonoid content (TFC). The antibacterial activity was evaluated using the disc diffusion method. The extract exhibited significant dose-dependent antioxidant activity comparable to ascorbic acid. The total flavonoid content was 611.9 mg/g, and the total phenol content was 164.4 mg/g. The DPPH free radical scavenging assay indicated an ascorbic acid production of 523.21 µg/ml, while routine tests showed 18.35 µg/ml. Additionally, the leaf extracts demonstrated strong antibacterial activity against various bacteria, even exceeding the effectiveness of Ciprofloxacin. Further research is necessary to develop targeted therapies, potentially opening new avenues for harnessing the medicinal properties of *Cordia macleodii*. Clinical studies are also needed to investigate its potential as an alternative medicine.

INTRODUCTION

Despite the rise of modern medicine, medicinal plants remain crucial in healthcare, serving as complementary therapies or potential alternatives to certain treatments [1, 2]. This stems from the vast diversity of plant life, characterized by a rich tapestry of structural and biological variations [3, 4]. Within plants lie unique biochemical compounds called phytochemicals, many of which possess potent inhibitory effects against pathogenic microorganisms [5]. Moreover, several chronic illnesses, including diabetes, arthritis, atherosclerosis, cancer, and Alzheimer's, are linked to free radicals, highly reactive chemicals in the body [6]. Evidence suggests that apoptosis and necrosis, specific cell death mechanisms, can be triggered and amplified by these free radicals and reactive nitrogen species [7]. This opens doors for targeted antioxidant interventions, such as mitochondria-targeted ubiquinone, which has shown promise in alleviating liver damage from alcohol consumption [8]. However, despite the potential of herbal medicine, its therapeutic capabilities remain largely unexplored, hindering its wider adoption within mainstream healthcare [9, 10]. Therefore, a pressing need exists to systematically evaluate the efficacy of these botanical-based remedies.

Cordia macleodii, an endangered medicinal plant belonging to the Boraginaceae family (known locally as Dahipalas/Dahiman), is indigenous to the moist-dry deciduous forests of Central India. This plant exhibits a wide array of pharmaceutical potentials, including hepatoprotective, anti-inflammatory, antioxidant, antibacterial, antifungal, wound healing, and anti-venom activities [11, 12]. Preliminary phytochemical analysis of plant

leaves using various solvents revealed the presence of tannins, phenols, flavonoids, saponins, alkaloids, and glycosides [13, 14]. The growing interest in natural remedies within traditional medicine systems further underscores the significance of *Cordia macleodii* and its promising therapeutic properties [15].

Screening of natural antioxidant and antibacterial properties in *Cordia macleodii* is essential for several compelling reasons. First, *Cordia macleodii* has a long history of use in traditional folk medicine. Studies have demonstrated the plant's safety and effectiveness as an alternative medicinal agent, which could legitimize and broaden its application in contemporary medicine [16]. Antioxidants play a crucial role in combating oxidative stress, which is associated with various chronic illnesses, including cancer, heart disease, and neurological conditions. By identifying and evaluating the antioxidant capabilities of *Cordia macleodii*, researchers can assess its potential in managing or preventing these illnesses [17].

Moreover, the increasing prevalence of antibiotic-resistant microorganisms necessitates the discovery of new antimicrobial agents. Screening of *Cordia macleodii* for antibacterial properties may reveal compounds effective against resistant strains, contributing to the development of new antibiotics [18]. The findings from antioxidant and antibacterial screenings can also enhance the creation of nutraceutical products. These food-based products can be marketed as natural health supplements, appealing to consumers interested in holistic health solutions that go beyond basic nutrition [19]. Screening *Cordia macleodii* for antioxidant and antibacterial properties has great significance in therapeutic potential, combating illnesses, addressing antibiotic resistance, developing dietary supplements, and supporting conservation efforts. The aim of this study was to screen natural antioxidant and antimicrobial properties of *Cordia mocleodii* leaf extract. We evaluated the bioactive compound, and antimicrobial performance using disk diffusion assay. Initially, we have confirmed the presence of several secondary metabolites and the antibacterial potential of *Cordia mocleodii.*

MATERIALS AND METHODS

Plant collection and extract preparation

Cordia macleodii leaves were gathered from Savar, Dhaka, Bangladesh, and meticulously cleaned to remove extraneous plant material. After sun-drying for a week, the leaves were pulverized into a coarse powder and securely stored in airtight containers under controlled conditions. Subsequently, 180 g of the powder was soaked in 1000 ml of 90% methanol for ten days in a sealed container, with regular agitation. The resulting solution was filtered through cotton cloth and Whatman filter paper, and the filtrate was then evaporated to obtain the crude extract. This carefully collected and processed extract formed the basis for subsequent analyses, and this experiment was done three times to get the result perfectly.

Chemicals used during the experiment

This research utilized methanol, carbon tetrachloride, dichloromethane, petroleum ether, sodium carbonate, Folin-Ciocalteu reagent, quercetin, and gallic acid, all obtained from Merck Co. in Germany. Additionally, ascorbic acid was sourced from the same supplier. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich (United States), while standard ciprofloxacin discs were acquired from a local pharmacy. Furthermore, the streptokinase drug was supplied by Incepta Pharmaceuticals Ltd., Bangladesh.

Test microorganisms

The research utilized ten bacterial strains, comprising five Gram-positive (*Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Sarcina lutea*, and *Staphylococcus aureus*) and five Gram-negative (*Escherichia coli*, *Salmonella paratyphi*, *Salmonella typhi*, *Shigella boydii*, and *Vibrio mimicus*) strains. These were obtained from the Institute of Nutrition and Food Science at the University of Dhaka in Bangladesh for the antimicrobial assay evaluation.

Phytochemical screening

Standard chemical assays facilitated the identification of various phytochemical groups in the samples based on their characteristic color changes. Specifically, lead acetate, alkaline reagent, ferric chloride, and ammonia were utilized to identify flavonoids and phenolics [20]. Dragendroff's and Mayer's tests confirmed the presence of alkaloids, employing lead acetate, potassium hydroxide, ferric chloride, and potassium dichromate. Additionally, tannins were detected using lead acetate, potassium hydroxide, ferric chloride, and potassium dichromate. Furthermore, glycosides were identified through the Legal, Keller-Kiliani, and Borntrager tests, while saponins were detected using the froth and Salkowski tests. Finally, the Molisch test was employed to quantify the carbohydrate content.

Measurement of total flavonoids content

The *Cordia macleodii* extract was examined for its total flavonoid content (TFC) employing the aluminum chloride colorimetric method [21]. A solution comprising aluminum chloride, potassium acetate, and the extract was prepared and diluted with distilled water. The absorbance of this solution was measured at a specific wavelength and compared to a blank solution lacking the extract. By employing a standard solution of quercetin, the quantity of flavonoids in the extract was determined and expressed in terms of quercetin equivalents.

Determination of total phenolic content

The quantification of total phenolic compounds (TPC) in *Cordia macleodii* extracts was measured utilizing the Folin-Ciocalteu method [22] with gallic acid serving as the standard (0-100 μg/ml). In brief, 5 ml of methanol containing either 10 mg crude extract or a 2 mg/ml extract aliquot was combined with 2.5 ml of Folin-Ciocalteu reagent and 2.0 ml of 7.5% (w/v) Na2CO3 solution, then incubated at room temperature for 20 minutes. To determine the total phenolic content in the samples, absorbance at 760 nm was measured using a UV-vis spectrophotometer from Shimadzu, Japan. Researchers constructed a calibration curve based on absorbance readings of known gallic acid solutions, enabling them to express the results as milligrams of gallic acid equivalents (GAE) per gram of extract.

DPPH test for free radical scavenging activity

We utilized the Brand-Williams et al., technique to assess the plant fractions' ability to neutralize the specific free radical DPPH [23] with ascorbic acid serving as the standard reference compound. Stock solutions of ascorbic acid and each fraction (1000 μg/ml) were prepared in methanol and serially diluted to obtain concentrations ranging from 0.977 to 500 μg/ml. A DPPH solution (5 mg/250 ml methanol) was prepared, and 3.0 ml aliquots

were combined with 2.0 ml aliquots of each concentration of standard or fraction solution. The extent of the reaction in each mixture was evaluated by measuring its absorbance at 517 nm following a 30-minute incubation at room temperature in the absence of light, with methanol serving as a control. The percentage inhibition of DPPH radical scavenging was determined using the formula:

% inhibition = [1 - (A sample/A blank)] × 100

Where, A sample represents the light absorption of a solution containing the standard or fraction, and A blank represents the background absorption solely from the reagents used in the measurement. The 50% inhibitory concentration (IC50) for DPPH radical scavenging was then determined from a percentage inhibition vs. concentration curve.

Measurement of antimicrobial activity

The effectiveness of the extract against various bacteria, including both Gram-positive and Gram-negative types, was assessed using a disc diffusion method as described by Bauer et al., [24]. These bacteria included *Bacillus cereus*, *B. megaterium*, *B. subtilis*, *Sarcina lutea*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella paratyphi*, *S. typhi*, *Shigella boydii*, and *Vibrio mimicus*. Bacterial strains were obtained from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Sterilized 6 mm filter paper discs were impregnated with 400 µg of extract dissolved in 10% v/v DMSO. These discs, along with blank discs as negative controls and ciprofloxacin discs (5 µg/disc) as positive controls, were placed on nutrient agar plates pre-inoculated with the respective test microorganisms. Following a 24-hour incubation at 4°C to enable diffusion of the test substance, bacterial growth was assessed via a subsequent 24-hour incubation at 37°C. The antimicrobial activity of the extract was determined by measuring the diameter of inhibition zones surrounding the sample discs.

Statistical analysis

The statistical analysis of the experiments' data is presented as mean ± SEM. One-way analysis of variance (ANOVA) with Dunnett's multiple comparisons test was used to assess differences between groups. A p-value less than 0.05 was considered statistically significant and the statistical software R was used for all analyses.

RESULTS

Presence of phytochemicals in *Cordia macleodii* extract

Phytochemical screening unveiled the presence of several pharmacologically active constituents in the leaf extracts, including phenolics, saponins, flavonoids, tannins, glycosides, alkaloids, and carbohydrates (Table 1).

Effect of *Cordia macleodii* extract on the free radical scavenging activity

DPPH is commonly employed as a reagent to assess the antioxidant capability of substances in absorbing free radicals [25]. This free radical can be stabilized by the introduction of a donor radical, such as an electron or a hydrogen radical [26]. The *Cordia macleodii* extract exhibited robust antioxidant activity, demonstrated by its increasing efficacy in neutralizing DPPH radicals with rising concentration (Figure 1). This effect displayed a strong positive correlation (r ~ 0.7863) with concentration, indicating a nearly

perfect linear relationship. The IC50 value of *Cordia macleodii* extract was 523.21 µg/ml compared to the ascorbic acid 18.35 µg/ml of leaf extract (Table 2).

Table 1. Presence/absence of key phytochemical constituents in *Cordia mocleodii* leaves.

Sl. No.	Phytochemicals	*Cordia mocleodii* leaves
1.	Phenolics	+
2.	Saponins	+
3.	Flavonoids	+
4.	Tannins	+
5.	Reducing sugar	-
6.	Glycosides	+
7.	Alkaloids	+
8.	Phytosterols	-
9.	Carbohydrates	+

N.B: (+) = Presence (-) = Absence

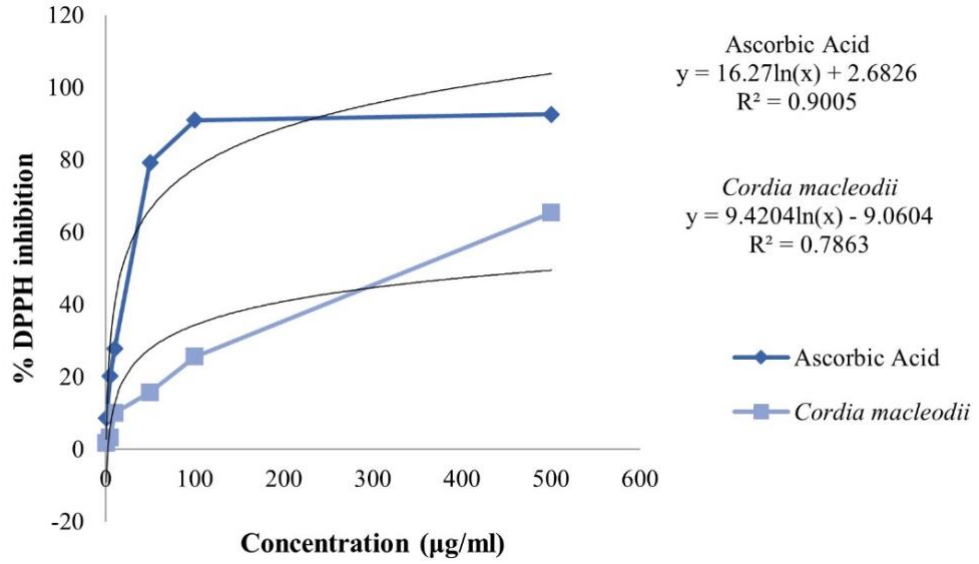

Figure 1. Standard carve of ascorbic acid and *Cordia macleodii* leaves extract. The figure shows the DPPH activity of *Cordia macleodii* extract with a standard antioxidant ascorbic acid.

Table 2. Comparative analysis of DPPH inhibition by ascorbic acid and *Cordia macleodii*.

Concentration (µg/ml)	Ascorbic acid		*Cordia macleodii*	
	Absorbance(nm)	% of DPPH inhibition	Absorbance(nm)	% of DPPH inhibition
1	1.528	8.61	1.040	1.60
5	1.333	20.27	1.024	3.12
10	1.208	27.75	0.952	9.93
50	0.347	79.25	0.891	15.71
100	0.151	90.97	0.787	25.54
500	0.124	92.58	0.366	65.37

Effect of *Cordia macleodii* extract on bacterial growth

The antimicrobial activity of *Cordia macleodii* leaf extract was evaluated using agar disk diffusion assay. The bacterial strains showed various potential against the pathogenic bacterial strains including gram-positive (*B. megaterium*, *B. subtilis*, *S. aureus*, *B. cereus*, *S. lutea*) and five gram-negative (*S. typhi*, *E. coli*, *V. mimicus*, *S. paratyphi*, *S. boydii*) strains (Figure 2). Notably, the methanol extract exhibited potent antibacterial activity against *S. typhi*, *E. coli*, and *S. boydii* as evidenced by significantly larger inhibition zones compared to other tested microbes. Additionally, *B. megaterium* bacteria showed a higher zone of inhibition compared to other gram-positive bacteria.

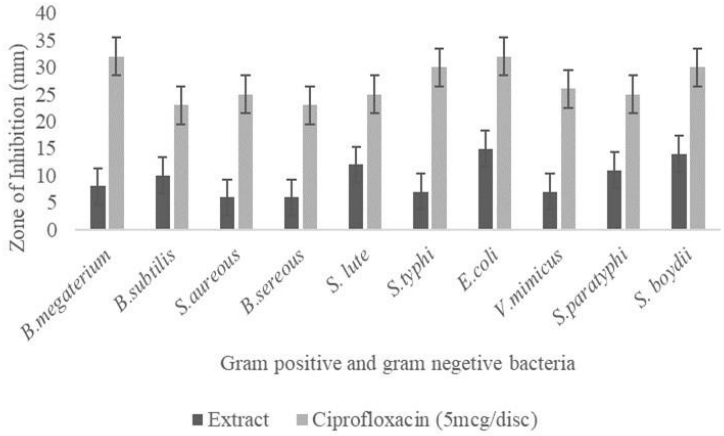

Figure 2. The diameter of the zone of inhibition where the *Cordia macleodii* extract showed less antibacterial activity compared to the ciprofloxacin.

DISCUSSION

The vast array of bioactive compounds found in natural products has sparked significant interest due to their proven efficacy in treating various ailments. Plants, in particular, are a treasure trove of novel chemicals with diverse therapeutic applications. For example, tannins exhibit well-documented anti-inflammatory and anticancer properties [27, 28]. Additionally, the complex interplay of secondary metabolites in medicinal plants can lead to multiple mechanisms of antimicrobial action, potentially hindering the development of resistance [29]. This study investigated the antibacterial and antioxidant potential of extracts derived from *Cordia macleodii* leaves. The findings demonstrate that the extracts possess broad-spectrum antibacterial activity against various microorganisms, potentially exceeding the efficacy of conventional antibiotics. This aligns with the growing interest in natural products as a source of novel antimicrobials due to their complex composition, which can hinder resistance development [30]. The observed activity might be attributed to the identified secondary metabolites, including alkaloids, terpenoids, saponins, phenols, tannins, and steroids, all of which have documented antimicrobial properties [28]. Interestingly, this finding differs slightly from previous studies on *Cordia macleodii*, which reported the presence of flavonoids alongside the detected metabolites [31].

The DPPH free radical scavenging assay revealed potential antioxidant activity in the methanol extract. This aligns with the established role of phenolic compounds, such as tannins, in scavenging free radicals and mitigating oxidative stress, a key player in various diseases [32, 33]. While previous research suggests a positive correlation between *Cordia macleodii* extract concentration and its antioxidant capacity [34], this study focused

on a single concentration. Future studies could explore a dose-dependent response to further elucidate the antioxidant potential. The identified phytochemicals, including glycosides, alkaloids, flavonoids, and saponins, are known contributors to various biological activities in plants [35-37]. Notably, these compounds have been linked to potential thrombolytic effects, warranting further investigation into this specific activity of *Cordia macleodii* extracts. Isolating and evaluating the individual roles of these compounds would provide a deeper understanding of their contribution to the observed functionalities.

CONCLUSION

This study revealed promising antibacterial, and antioxidant properties within extracts from *Cordia macleodii* leaves. Additionally, the extracts demonstrated promising antibacterial action against a variety of microorganisms, occasionally outperforming traditional medications. These results motivate further investigation to identify the specific bioactive substances responsible for these effects, and in vivo studies and exploration of the underlying mechanisms are crucial next steps. This study highlights the potential of *Cordia macleodii* extracts as a source of natural antimicrobials and antioxidants. Further research is recommended to isolate and characterize the active compounds, explore their mechanisms of action, and investigate their potential for therapeutic applications.

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AUTHOR CONTRIBUTIONS

Research design and initial draft preparation: SRA, MRHS, SAE, OI; data collection and experimental assistance: SRA, MRHS, EH; data analysis: SRA, MNU, OI; manuscript review and editing: LJ. The final version of the manuscript was approved by all authors. All authors provided consent to publish this work.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors related to this work.

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