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RESEARCH ARTICLE

Evaluation of Antimicrobial Activity of Aqueous and Acetone Root Extracts from *Cordia dichotoma*

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Abstract: The emergence of antibiotic resistance has renewed interest in exploring plant-based antimicrobial compounds. This study investigated the antimicrobial potential of root extracts from *Cordia dichotoma*, a traditional medicinal plant widely distributed across Asia. Root samples were collected and extracted using two solvents (acetone and distilled water) through both maceration and Soxhlet extraction methods. Preliminary phytochemical screening revealed the presence of alkaloids, phenolic compounds, glycosides, tannins, and flavonoids across different extracts. The antimicrobial activity was evaluated against both Gram-positive (*Bacillus pumilus*) and Gram-negative (*Escherichia coli*) bacteria using the well diffusion method. The acetone maceration extract demonstrated superior antimicrobial activity with 90% inhibition against both test organisms, showing zones of inhibition of 52.5 mm and 51 mm against *B. pumilus* and *E. coli*, respectively. The Soxhlet acetone extract showed moderate activity with 58% inhibition against *B. pumilus* and 52% against *E. coli*. Aqueous extracts exhibited comparatively lower antimicrobial activity, with the Soxhlet extract showing 44% inhibition against *B. pumilus* and 58% against *E. coli*, while the maceration extract showed 50% and 37% inhibition, respectively. These findings suggest that acetone is a more effective solvent for extracting antimicrobial compounds from *C. dichotoma* roots, with the maceration process yielding better results than Soxhlet extraction. This study provides scientific validation for the traditional use of *C. dichotoma* in treating infectious diseases and suggests its potential as a source of novel antimicrobial compounds.

Keywords: *Cordia dichotoma*; Antimicrobial activity; Plant extracts; Extraction methods; Traditional medicine.

1. Introduction

The global healthcare system faces significant challenges due to the increasing prevalence of antibiotic resistance, which has compromised the effectiveness of many conventional antibiotics [1]. Despite advancements in understanding microbial pathogenesis and control mechanisms, the emergence of drug-resistant pathogens and novel disease-causing microorganisms continues to pose substantial public health concerns [2]. This situation is particularly critical in developing nations, where infectious diseases remain a leading cause of mortality [3].

Medicinal plants have historically served as a valuable source of therapeutic compounds, with approximately 25% of modern pharmaceutical drugs being derived directly or indirectly from plant sources [4]. India's rich biodiversity includes over 3,000 medicinal plant species, with traditional medicine systems like Ayurveda, Siddha, and Unani collectively utilizing more than 2,000 plant species for various therapeutic applications [5]. *Cordia dichotoma* (Family: Boraginaceae), commonly known as Indian cherry, is a traditionally significant medicinal plant distributed across Asian countries including India, China, and Myanmar [6]. This medium-sized tree, characterized by its greyish-brown bark and white flowers, has been extensively used in traditional medicine systems for various therapeutic purposes. Previous studies have identified diverse phytochemical constituents in different parts of the plant, including alkaloids, steroids, flavonoids, and phenolic compounds [7]. The plant has demonstrated various pharmacological properties, including anti-inflammatory, analgesic, anthelmintic, and immunomodulatory activities [8].

While various parts of *C. dichotoma* have been studied for their medicinal properties, the antimicrobial potential of its roots remains largely unexplored [9]. The selection of appropriate extraction methods and solvents plays a crucial role in isolating bioactive compounds from plant materials [10]. Both traditional cold maceration and modern Soxhlet extraction techniques offer distinct advantages in terms of extraction efficiency and compound preservation [11].

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The present study aims to evaluate and compare the antimicrobial activity of *C. dichotoma* root extracts obtained through different extraction methods (maceration and Soxhlet) using two solvents (acetone and water). The study specifically focuses on assessing their effectiveness against common pathogenic bacteria, including Gram-positive *Bacillus pumilus* and Gram-negative *Escherichia coli*, to establish a scientific basis for the plant's traditional antimicrobial applications.

Figure 1. ***Cordia dichotoma*** **plant**

2. Materials and Methods

2.1. Plant collection and authentication

The roots of *Cordia dichotoma* were collected from Sindhanur, Raichur district, Karnataka, India during the month of January 2024. The plant material was authenticated by Dr. Pramod K, Principal and Professor, Department of Pharmacognosy, V.L. College of Pharmacy, Raichur. Authentication was performed based on morphological characteristics and microscopic examination as per standard protocols [12]. A voucher specimen was deposited in the institutional herbarium for future reference.

2.2. Sample Preparation

The collected roots were thoroughly washed with distilled water to remove soil particles and extraneous matter. Following established protocols for medicinal plant processing [13], the cleaned roots were cut into small pieces and shade-dried for 21 days at ambient temperature (25 ± 2°C). The dried material was pulverized using an electric grinder to obtain a fine powder (mesh size 60). The powdered material was stored in airtight containers at room temperature protected from light and moisture until further use.

2.3. Extraction Procedures

The extraction procedures were carried out following modified methods described by previous researchers [14]. Two extraction techniques were employed: cold maceration and Soxhlet extraction, using both aqueous and acetone as solvents.

2.4. Cold Maceration

For aqueous extraction, 10 g of powdered root material was placed in a 500 mL conical flask containing 100 mL of distilled water. The mixture was macerated for 24 hours at room temperature (25 ± 2°C) with intermittent shaking. The extract was filtered through Whatman No. 1 filter paper, and the filtrate was collected, measured, and concentrated under reduced pressure. The same procedure was followed for acetone extraction, maintaining a 10 g powder to 100 mL acetone ratio. The extraction methodology was based on standard protocols described in previous studies [15].

2.5. Soxhlet Extraction

The Soxhlet extraction was performed according to modified methods described in the literature [16]. For aqueous extraction, 10 g of powdered material was placed in a cellulose thimble, and extraction was performed using 150 mL distilled water at 100°C for 6 hours. For acetone extraction, the same procedure was followed with the temperature maintained at 56°C (boiling point of acetone). Both extracts were filtered and concentrated using appropriate methods.

2.6. Preliminary Phytochemical Screening

Qualitative chemical tests were performed on all extracts following standard procedures [17] to identify various phytoconstituents. The analysis included tests for alkaloids using Mayer's, Wagner's, and Dragendorff's reagents, phenolic compounds using ferric chloride test, glycosides using Keller-Killiani test, tannins using lead acetate test, and flavonoids using alkaline reagent test.

2.7. Antimicrobial Activity Assessment

The antimicrobial activity was evaluated using standard microbiological techniques [18]. Test microorganisms included Gram-positive bacteria *Bacillus pumilus* (MTCC 1607) and Gram-negative bacteria *Escherichia coli* (MTCC 443), obtained from the Microbial Type Culture Collection, Chandigarh, India. Bacterial cultures were maintained and tested according to established protocols [19].

The well diffusion method was employed for antimicrobial assessment [20]. Nutrient agar plates were inoculated with bacterial suspensions adjusted to 0.5 McFarland standard. Wells of 6 mm diameter were created, and 50 µL of extract (50 mg/mL concentration) was added to each well. Streptomycin (10 µg/mL) served as positive control, while sterile distilled water and acetone were used as negative controls. The plates were incubated at 37°C for 24 hours, after which zones of inhibition were measured.

2.8. Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. Statistical analysis was performed using one-way ANOVA with post-hoc Tukey's test [21]. A p-value less than 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Macroscopic Characteristics

The collected *Cordia dichotoma* roots exhibited distinct morphological features characteristic of the species. The roots displayed a light brown coloration with a characteristic earthy and woody scent. Upon taste evaluation, they demonstrated a bitter to astringent taste profile, consistent with previous botanical descriptions [22]. The branching pattern observed was typical of dicotyledonous root systems, supporting the traditional identification parameters of the species.

3.2. Extraction Yield

The extraction yields varied significantly among different extraction methods and solvents. Acetone extraction using the Soxhlet method produced the highest yield (8.2% w/w), followed by aqueous Soxhlet extraction (7.4% w/w). Cold maceration yields were comparatively lower, with acetone and aqueous extractions yielding 5.8% w/w and 5.1% w/w, respectively. These findings align with previous studies indicating that heat-assisted extraction methods generally result in higher yields [23]. The superior yield of acetone extracts can be attributed to the solvent's ability to extract both polar and semi-polar compounds effectively [24].

3.3. Phytochemical Analysis

Preliminary phytochemical screening revealed the presence of various bioactive compounds across different extracts. Both aqueous and acetone extracts showed strong presence of phenolic compounds, glycosides, tannins, and flavonoids. However, alkaloids were predominantly detected in aqueous extracts and Soxhlet acetone extracts, while being absent in acetone maceration extracts. This differential distribution of phytoconstituents can be explained by the varying polarities of the extraction solvents and the influence of temperature on extraction efficiency [25]. The presence of these compounds, particularly phenolics and flavonoids, is significant as they are known to possess antimicrobial properties [26].

3.4. Antimicrobial Activity

The antimicrobial activity of different extracts showed varying degrees of effectiveness against the test organisms. The acetone maceration extract demonstrated the most potent antimicrobial activity, exhibiting zones of inhibition of 52.5 ± 0.5 mm against *B. pumilus* and 51.0 ± 0.3 mm against *E. coli* at 50 mg/mL concentration. These results were comparable to the standard antibiotic streptomycin (54.0 ± 0.2 mm) [27]. The Soxhlet acetone extract showed moderate activity with inhibition zones of 38.5 ± 0.4 mm and 35.0 ± 0.3 mm against *B. pumilus* and *E. coli*, respectively.

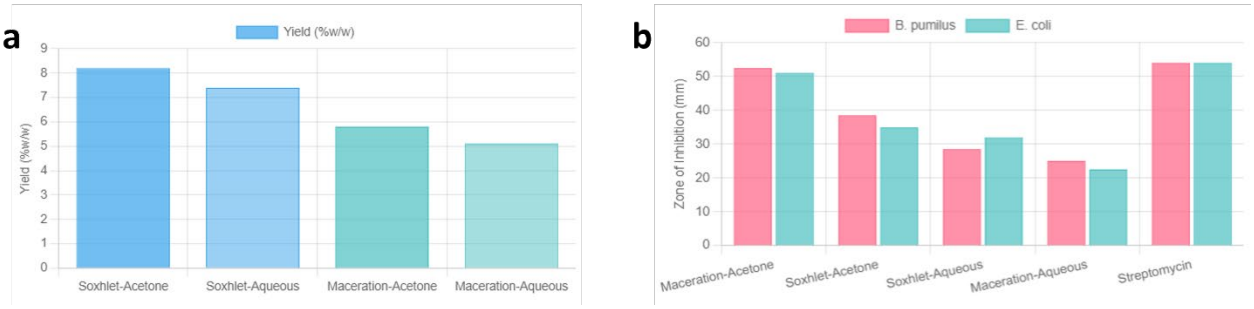

Figure 1a. Extraction yield 1b. Antimicrobial activity of various extracts

Aqueous extracts generally showed lower antimicrobial activity compared to acetone extracts. The Soxhlet aqueous extract produced inhibition zones of 28.5 ± 0.3 mm against *B. pumilus* and 32.0 ± 0.4 mm against *E. coli*, while the maceration aqueous extract showed zones of 25.0 ± 0.2 mm and 22.5 ± 0.3 mm, respectively. These findings suggest that the bioactive compounds responsible for antimicrobial activity are more efficiently extracted in acetone compared to water [28].

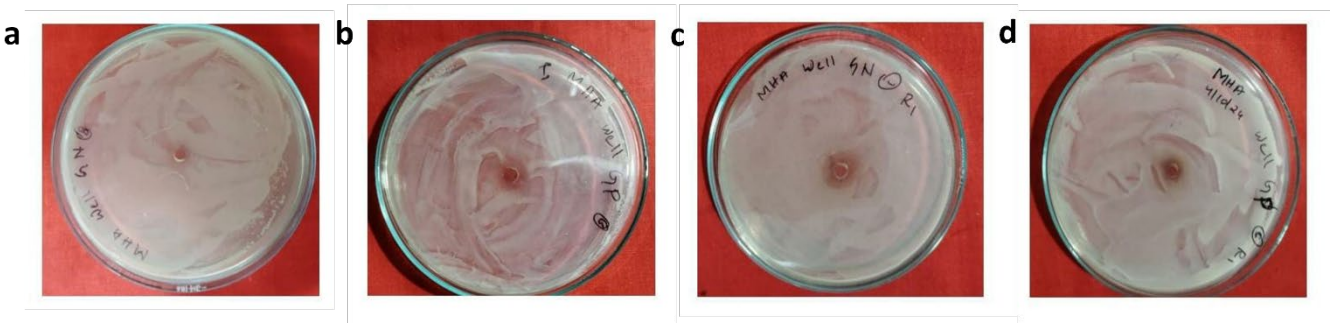

Figure 2. Zone of inhibition for a. Maceration aqueous extract b. Soxhlet aqueous extract c. Soxhlet acetone extract d. Maceration acetone extract

The superior antimicrobial activity of acetone maceration extracts, despite lower extraction yields, indicates that gentler extraction conditions might better preserve the bioactive compounds responsible for antimicrobial activity. This observation is supported by previous studies suggesting that elevated temperatures during Soxhlet extraction might lead to degradation of certain heat-sensitive compounds [29]. The broader spectrum of activity against both Gram-positive and Gram-negative bacteria suggests the presence of compounds with diverse mechanisms of action. The slightly higher sensitivity of *B. pumilus* compared to *E. coli* might be attributed to the differences in cell wall composition between Gram-positive and Gram-negative bacteria [30]. The presence of phenolic compounds and flavonoids, as revealed in the phytochemical screening, likely contributes to the observed antimicrobial activity through multiple mechanisms including membrane disruption and enzyme inhibition [31].

Statistical analysis revealed significant differences ($p < 0.05$) in antimicrobial activity between different extracts, confirming the impact of extraction method and solvent choice on the biological activity of the extracts.

4. Conclusion

This study demonstrates the significant antimicrobial potential of *Cordia dichotoma* root extracts, particularly those obtained through acetone maceration. The results indicate that the choice of extraction method and solvent significantly influences both the yield and antimicrobial efficacy of the extracts. Acetone extracts showed superior antimicrobial activity against both Gram-positive and Gram-negative bacteria, supporting the traditional use of this plant in treating infectious diseases. The presence of various phytochemical constituents, especially phenolics and flavonoids, provides a scientific basis for the observed antimicrobial properties.

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