



## Pharmacological Research

Antimicrobial and antifungal activities of *Cordia dichotoma* (Forster F.) bark extractsPankaj B. Nariya<sup>1</sup>, Nayan R. Bhalodia<sup>1</sup>, V. J. Shukla<sup>2</sup>, R. N. Acharya<sup>3</sup>

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## Abstract

*Cordia dichotoma* Forst.f. bark, identified as botanical source of *Shlesmataka* in Ayurvedic pharmacopoeias. Present study was carried out with an objective to investigate the antibacterial and antifungal potentials of *Cordia dichotoma* bark. Antibacterial activity of methanol and butanol extracts of the bark was carried out against two gram negative bacteria (*Escherichia coli*, and *Pseudomonas aeruginosa*) and two Gram positive bacteria (*St. pyogenes* and *Staphylococcus aureus*). The antifungal activity of the extracts was carried out against three common pathogenic fungi (*Aspergillus niger*, *A.clavatus*, and *Candida albicans*). Zone of inhibition of extracts was compared with that of different standards like Ampicilline, Ciprofloxacin, Norfloxacin and Chloramphenicol for antibacterial activity and Nystain and Greseofulvin for antifungal activity. The extracts showed remarkable inhibition of zone of bacterial growth and fungal growth and the results obtained were comparable with that of standards drugs against the organisms tested. The activity of extracts increased linearly with increase in concentration of extract (mg/ml). The results showed the antibacterial and antifungal activity against the organisms tested.

**Key words:** Antibacterial, antifungal, *Cordia dichotoma*, gram positive, gram negative, *in vitro*

## Introduction

Antibiotics are one of the most important weapons in fighting bacterial infections and have greatly benefited the health related quality of human life since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less effective against certain illnesses not only because many of them produce toxic reactions, but also due to emergence of drug resistant bacteria. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. Infectious diseases are the second leading cause of death world-wide.<sup>[1]</sup> In industrialized nations, despite the progress made in the understanding of microbiology and their control, incidents of epidemics due to drug resistant microorganisms and the emergence of hitherto unknown disease-causing microbes, pose enormous public health concerns.<sup>[2]</sup> The emergence of multidrug-resistant bacteria has created a situation in which there are few or no treatment options for infections with certain microorganisms.<sup>[3]</sup> Along with bacterial infections, the

fungal infections also are a significant cause of morbidity and mortality despite advances in medicine and the emergence of new antifungal agents.<sup>[4]</sup>

Although the need for new antimicrobials is increasing, development of such agents faces significant obstacles.<sup>[5]</sup> A number of factors make antimicrobial agents less economically attractive targets for development than other drug classes.<sup>[6]</sup> Pharmaceutical research and development costs which are estimated to be \$400–\$800 million per approved agent,<sup>[7]</sup> pose a considerable barrier to new drug development in general. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides, and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine.<sup>[8,9]</sup> The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the plant.<sup>[10,11]</sup> Historically, plants have provided a good source of anti-infective agents; emetine, quinine, and berberine remain highly effective instruments in the fight against microbial infections. Phytomedicines have shown great promise in the treatment of intractable infectious diseases including opportunistic HIV infections. Plants containing protoberberines and related alkaloids, picralima-type indole alkaloids, and garciniabiflavonones used in traditional African system of medicine, have been found to be active against a wide variety

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of micro-organisms.<sup>[12]</sup> Many plants have been reported to have antifungal activity.<sup>[13,14]</sup>

*Cordia dichotoma* Linn. (Boraginaceae) is tree of tropical and subtropical regions, commonly known as Lasura in Hindi and *Shlesmataka* in Sanskrit. It is a medium sized tree with short crooked trunk, leaves simple, entire and slightly dentate, elliptical-lanceolate to broad ovate with round and cordate base, flower white, fruit drupe, yellowish brown, pink or nearly black when ripe with viscid sweetish transparent pulp surrounding a central stony part.<sup>[15]</sup> It grows in sub-Himalayan tract and outer ranges, ascending up to about 1500 m elevation.<sup>[16]</sup> It is used as immunomodulator, antidiabetic, anthelmintic, diuretic and hepatoprotective in folklore medicine. Seeds have disclosed the presence of  $\alpha$ -Amyrin, betulin, octacosanol, lupeol-3-rhamnoside,  $\beta$ -sitosterol,  $\beta$ -sitosterol-3-glucoside, hentricontanol, hentricontanol, taxifolin-3,5-dirhmnoside, and hesperitin-7-rhamnoside.<sup>[17]</sup> Preliminary phytochemical analysis of *C. dichotoma* bark indicated the presence of relatively high levels of alkaloids, flavonoides, steroids, and terpenoids. Hence, the present investigation was undertaken to determine the antioxidant potential of *C. dichotoma* bark.

## Materials and Methods

### Plant materials

*Cordia dichotoma* bark was collected from its natural habitat, Jamnagar, Gujarat, India, in the month of April-May 2009. The plant was authenticated by the Pharmacognosy Laboratory, I.P.G.T. and R.A. Jamnagar, Gujarat, India.

### Preparation of extracts

The bark was shade dried and crushed to make coarse powder. The powder (303 g) was successively extracted by soxhlet extraction with solvents of increasing polarity beginning with 2 L petroleum ether (60–80°C) and then extracted with 3 L of methanol (95%v/v) by continuous extraction method for 48 h. In this methanolic extract, solvent was distilled off and the extract was concentrated and dried under reduced pressure, which yielded a brownish green mass. The extract was preserved at 2–4°C and butanol (BuOH) extracts were obtained after successive partition from methanol (MeOH) extracts. MeOH and BuOH extracts were used in this study.

### Preliminary phytochemical screening of extract

The methanolic extract was analysed to detect for the presence of different chemical groups as per the methods described in Ayurvedic Pharmacopoeia of India. Preliminary phytochemical screening shows the presences of relatively high levels of alkaloids, coumarines, flavonoides, steroids, terpenoids, tannin, etc.

### Microorganism

The microorganisms employed in the current study were procured from Microcare Laboratory, Surat (Gujarat); standard cultures of different species of two gram positive and two gram negative bacteria including pathogenic and nonpathogenic and three pathogenic fungi strains were used.

### Media

Nutrient broth, nutrient agar, malt extract broth, and Sabouraud dextrose agar, from Himedia Laboratories, Mumbai (India), were used in this study.

## Antimicrobial and antifungal agents

Ampicilline, Ciprofloxacin, Norfloxacin, Chloramphenicol for antibacterial activity and Nystain and Greseofulvin for antifungal activity.

## Antibacterial and antifungal activity

The antibacterial activity was evaluated on four common pathogenic bacteria viz. *Escherichia coli* MTCC 96, *Pseudomonas aeruginosa* MTCC 424, *Staphylococcus aureus* MTCC 96, and *S. pyogenes* MTCC 442. The antifungal activity of the extracts was evaluated on three common pathogenic fungi viz. *Aspergillus niger* MTCC 282, *Candida albicans* MTCC 227, and *A. clavatus* MTCC 1323.

For evaluation of antibacterial and antifungal activities of alcohol and butanol extract of bark, agar diffusion assay method was used.<sup>[18]</sup> For investigation of antibacterial activity, Sterile Muller Hinton agar media (Hi-media) was prepared in petridishes. The bacteria ( $1 \times 10^8$  bacteria/ml) was inoculated separately in the media. In each petridish, four wells (diameter 6 mm) were prepared under aseptic conditions. In these, various concentrations of the extracts were prepared (i.e., 5  $\mu$ g/ml, 25  $\mu$ g/ml, 50  $\mu$ g/ml, 100  $\mu$ g/ml, and 250  $\mu$ g/ml) with DMSO. Same procedure applies in standard drug. All the dishes were incubated at 35°C for 24 hrs.

For investigation of antifungal activity, sterile potato dextrose agar media (Hi-media) were prepared in petri dishes. The fungal spores ( $1 \times 10^6$  spores/ml) were inoculated separately in the media. In each petri dish, four wells (diameter, 6 mm) were prepared under aseptic conditions. In these various concentrations, the extracts were prepared (i.e., 5  $\mu$ g/ml, 25  $\mu$ g/ml, 50  $\mu$ g/ml, 100  $\mu$ g/ml, and 250  $\mu$ g/ml) with DMSO. Same procedures apply for standard drug. DMSO is used as a blank. All the dishes were incubated at 35°C for seven days. At the end of the incubation period, the media were observed for zone of inhibition. The zones of inhibition were measured in millimeter using Vernier Calipers.

## Results

The results of investigation of antibacterial and antifungal activities of bark were studied in different concentrations (5  $\mu$ g/ml, 25  $\mu$ g/ml, 50  $\mu$ g/ml, 100  $\mu$ g/ml, and 250  $\mu$ g/ml) against four pathogenic bacterial strains two gram positive- *S. pyogenes* MTCC 442 [Figure 1] and *S. aureus* MTCC 96 [Figure 2]; two gram negative- *E. coli* MTCC 443 [Figure 3], *P. aeruginosa* MTCC [Figure 4] and three fungal strains- *A. niger* MTCC 282 [Figure 5], *A. clavatus* MTCC 1323 [Figure 6], *C. albicans* MTCC 227 [Figure 7].

The results of the antibacterial activities are presented in Tables 1–6. The antibacterial and antifungal activity of the extract increased linearly with increase in concentration of extract (mg/ml). The results revealed that *E. coli* and *P. aeruginosa* were more sensitive as compared to *S. aureus* and *S. pyogenes*. The growth inhibition zone measured ranged from 10–20 mm for all the sensitive bacteria, and ranged from 12–21 mm for fungal strains. The antibacterial and antifungal activity of the extract increased linearly with increase in concentration of extract (mg/ml) as compared with standard

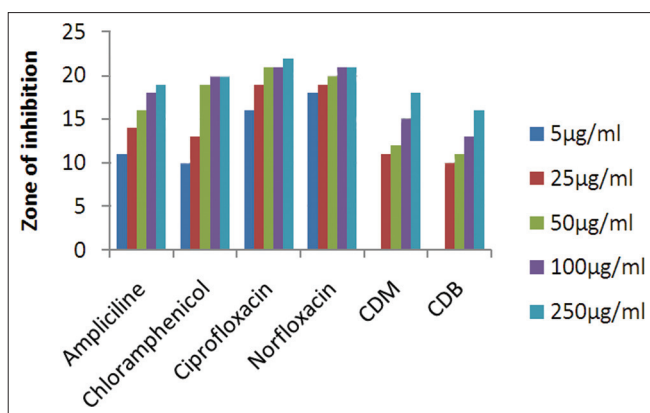


Figure 1: Antibacterial activity against *S. pyogenes* (MTCC 442)

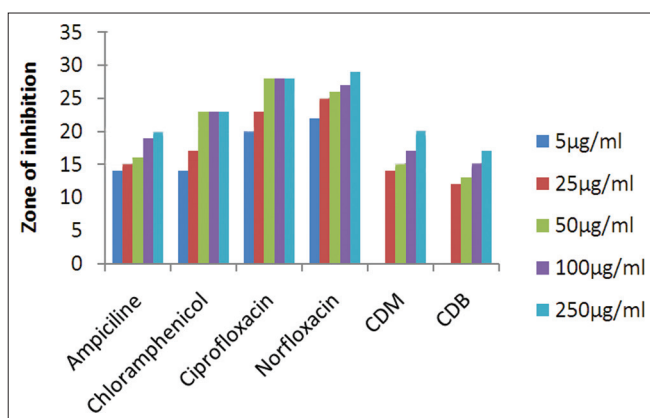


Figure 3: Antibacterial activity against *E. coli* (MTCC 443)

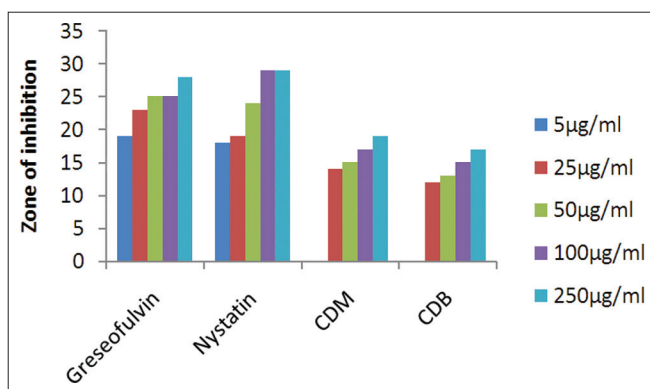


Figure 5: Antifungal activity against *A. niger* (MTCC 282)

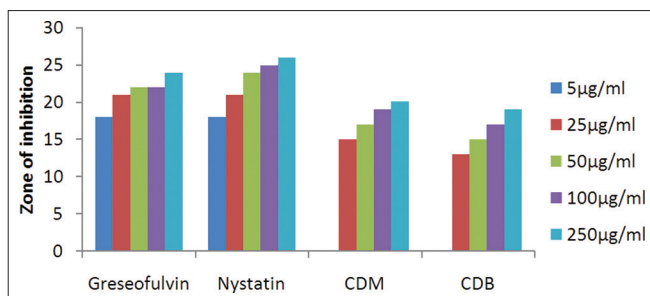


Figure 7: Antifungal activity against *C. albicans* (MTCC 227)

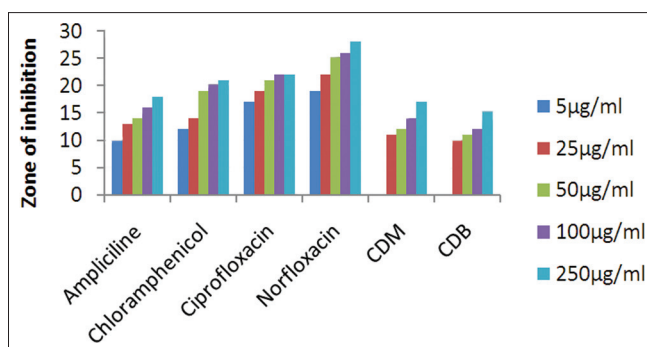


Figure 2: Antibacterial activity against *S. aureus* (MTCC 96)

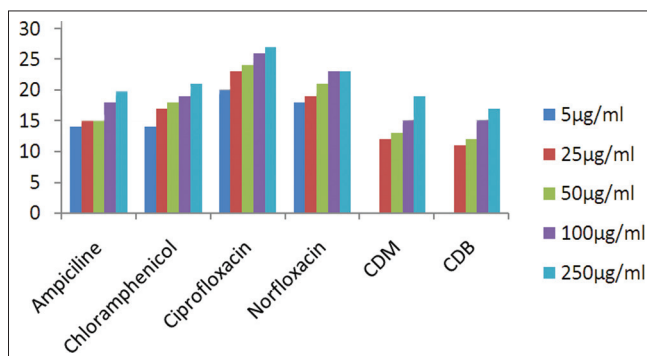


Figure 4: Antibacterial activity against *P. aeruginosa* (MTCC 424)

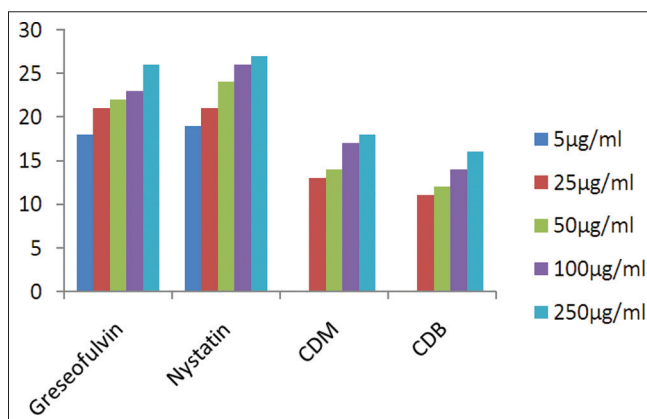


Figure 6: Antifungal activity against *A. clavatus* (MTCC 1323)

drugs. For fungal activity, *C. albicans* shows good result as compared to *A. niger* and *A. clavatus*.

The results show that *Cordia dichotoma* bark extracts were found to be more effective against all the microbes tested.

## Discussion

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies. In the present work, methanolic and butanol extracts obtained from bark shown remarkable activity against most of the tested bacterial and fungal strains. The results were compared with standard drugs.

**Table 1: Antibacterial activities of MeOH and BuOH extracts of bark of *CDB* against gram positive organism**

Antibacterial activity [zone of inhibition]											
Sr. no	Code no	<i>S. aureus</i> MTCC 96					<i>S. pyogenes</i> MTCC 442				
Diameter of zone of inhibition in mm											
		5	25	50	100	250	5	25	50	100	250
1	CDM @	11	12	14	17	-	11	12	15	18	
1	CDB @	10	11	12	15	-	10	11	13	16	

@ - No zone of inhibition, CDM - *Cordiadihotoma* MeOH extract, CDB - *Cordiadihotoma* BuOH extracts

**Table 2: Antibacterial activities of MeOH and BuOH extracts of bark of *CDB* against gram negative organism**

Antibacterial activity [zone of inhibition]											
Sr. no	Code no	<i>E. coli</i> MTCC 443					<i>P. aeruginosa</i> MTCC 424				
Diameter of zone of inhibition in mm											
		5	25	50	100	250	5	25	50	100	250
1	CDM @	14	15	17	20	@	12	13	15	19	
1	CDB @	12	13	15	17	@	11	12	15	17	

@ - No zone of inhibition, CDM - *Cordiadihotoma* MeOH extract, CDB - *Cordiadihotoma* BuOH extracts

**Table 3: Antibacterial activities of MeOH and BuOH extracts of bark of *CDB* against fungal organism**

Antifungal activity [zone of inhibition]																
Sr. no.	Code no.	<i>A. niger</i> MTCC 282					<i>A. clavatus</i> MTCC 1323					<i>C. albicans</i> MTCC 227				
Diameter of zone of inhibition in mm																
		5	25	50	100	250	5	25	50	100	250	5	25	50	100	250
1	CDM @	14	15	17	19	@	13	14	17	18	@	15	17	19	20	
1	CDB @	12	13	15	17	@	11	12	14	16	@	13	15	17	19	

@ - No zone of inhibition, CDM - *Cordiadihotoma* MeOH extract, CDB - *Cordiadihotoma* BuOH extracts

**Table 4: Antibacterial activities of standard drugs against gram positive organism**

Antibacterial activity [zone of inhibition]										
Standard drugs	<i>S. aureus</i> MTCC 96					<i>S. pyogenes</i> MTCC 442				
Diameter of zone of inhibition in mm										
	5	25	50	100	250	5	25	50	100	250
Ampicilline	10	13	14	16	18	11	14	16	18	19
Chloramphenicol	12	14	19	20	21	10	13	19	20	20
Ciprofloxacin	17	19	21	22	22	16	19	21	21	22
Norfloxacin	19	22	25	26	28	18	19	20	21	21

**Table 5: Antibacterial activities of standard drugs against gram negative test organism**

Antibacterial activity [zone of inhibition]										
Standard drugs	<i>E. coli</i> MTCC 443					<i>P. aeruginosa</i> MTCC 1688				
Diameter of zone of inhibition in mm										
	5	25	50	100	250	5	25	50	100	250
Ampicilline	14	15	16	19	20	14	15	15	18	20
Chloramphenicol	14	17	23	23	23	14	17	18	19	21
Ciprofloxacin	20	23	28	28	28	20	23	24	26	27
Norfloxacin	22	25	26	27	29	18	19	21	23	23

**Table 6: Antifungal activities of standard drugs against fungal strains**

Antibacterial activity [zone of inhibition]																
Standard drugs	<i>A. niger</i> MTCC 282					<i>A. clavatus</i> MTCC1323					<i>C. albicans</i> MTCC 227					
Diameter of zone of inhibition in mm																
	5	25	50	100	250	5	25	50	100	250	5	25	50	100	250	
Greseofulvin	19	23	25	25	28	18	21	22	23	26	18	21	22	22	24	
Nystain	18	19	24	29	29	19	21	24	26	27	18	21	24	25	26	

## Conclusion

In the current investigation, the methanolic and butanol extracts of *C. dichotoma* bark was found to be active on bacteria and fungi's in comparison to standard drug. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds.

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## हिन्दी सारांश

# कॉर्डिया डायकोटोमा (फॉर्स्टर एफ.) काण्डत्वक सत्व की सूक्ष्मजीवविरोधी तथा कवकविरोधी क्रिया – एक प्रायोगिक परीक्षण

पंकज बी. नारीया, नयन आर. भालोडिया, वी. जे. शुक्ला, आर. एन. आचार्य

आयुर्वेदीय भेषजसंहिता द्वारा कॉर्डिया डायकोटोमा को श्लेष्मातक का वानस्पतिक स्रोत निर्धारित किया गया है। इसके काण्डत्वक के सूक्ष्मजीवविरोधी तथा कवकविरोधी क्रियाशक्ति की जाँच करना, प्रस्तुत अध्ययन का उद्देश्य है। इस काण्डत्वक के मिथेनॉल तथा ब्यूटेनॉल में कर्षित सत्वों की सूक्ष्मजीवविरोधी क्रियाशक्ति, एश्वेरिशिया कोलाइ एवं सुडोमोनास एरुजिनोजा इन दो ग्राम निगेटीव तथा स्ट्रेप्टोकोकस पायोजेन्स एवं स्टेफेलोकोकस ऑरियस इन दो ग्राम पॉजिटीव बैक्टेरिया पर परखी गयी। तथा कवकविरोधी क्रियाशक्ति एस्पेरजिलस नायगर, ए. क्लेवेटस एवं केण्डिडा अल्बिकन्स नामक तीन कवकों पर परखी गयी। इन सत्वों द्वारा उत्पादित प्रतिबन्धित क्षेत्रोंकी तुलना सूक्ष्मजीवविरोधी मानक यथा एम्पिसिलिन, सिप्रोफ्लोक्सेसिन, नोरफ्लॉक्सेसिन एवं क्लोरेम्फिनेकॉल तथा कवकरोधी मानक निस्टेन तथा ग्रेसोफ्लुविन इन मानकों के साथ की गयी। परीक्ष्य सत्वों की क्रिया द्वारा प्रयोग में लिये गये सूक्ष्मजीव तथा कवकों के वृद्धिक्षेत्र में लक्षणीयप्रतिबन्ध पाया गया। यह प्रतिबन्ध, मानक औषधियों द्वारा उत्पादित प्रतिबन्ध से तुलना करने योग्य था। इन सत्वों की क्रियाशक्ति, सत्वों की संहति के साथ सीधे एकमार्गी अनुपात में बढ़ती गयी। प्राप्त परिणाम परीक्षित जीवों पर सूक्ष्मजीवविरोधी तथा कवकविरोधी क्रिया प्रदर्शित करते हैं।