



ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF ROOT EXTRACT OF CADABA FRUTICOSA (L.) DRUCE.

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Communicated : 26.05.2024

Revision : 30.05.2024 & 29.06.2024
Accepted : 09.08.2024

Published : 30.09.2024

ABSTRACT:

This research was conducted with the aim of examining the antibacterial and antifungal properties of Roots of *Cadaba fruticosa* (L.) Druce. Additionally, it aimed to assess the zone of inhibition of extracts on specific bacterial and fungal strains. In this study, the microbial efficacy of Dimethyl Sulfoxide extracts of Root of *Cadaba fruticosa* (L.) Druce was assessed for possible antimicrobial properties against clinically relevant bacterial and fungal strains. The antimicrobial efficacy was evaluated in the extracts using the Agar disc diffusion technique (Kirby-Bauer method). The antibacterial and antifungal effects of extracts (62.5, 125, 250, 500, 1000 µg/ml) of *Cadaba fruticosa* (L.) Druce were assessed against two Gram-positive—*Bacillus subtilis*, *Staphylococcus aureus*; two Gram-negative—*Escherichia coli*, *Pseudomonas aeruginosa* human pathogenic bacteria; and two fungal strains—*Candida tropicalis* and *Trichoderma asperellum*. Zones of inhibition of extracts were compared with that of standards such as Ciprofloxacin for antibacterial effectiveness and Amphotericin B for antifungal effectiveness. The findings indicated that the extract exhibits weak to moderate antibacterial and No antifungal activity against the tested organisms.

Keywords:- Dimethyl Sulfoxide, *Trichoderma asperellum*, Ciprofloxacin, *Pseudomonas aeruginosa*, *Cadaba fruticosa*.

INTRODUCTION :

The increasing prevalence of multidrug-resistant microbial pathogens has necessitated the search for novel antimicrobial agents from natural sources. The rising occurrence of multidrug-resistant microbial pathogens has made it essential to seek new antimicrobial agents from natural sources. For centuries, medicinal plants have served as a fundamental aspect of traditional medicine, providing a vast array of bioactive compounds with therapeutic possibilities. *Cadaba fruticosa*, a perennial shrub that is part of the Capparaaceae family, is found extensively in tropical and subtropical areas, including regions of Africa, Asia, and the Middle East. Historically, *Cadaba fruticosa* has been utilized to address various health

issues, such as skin infections, wounds, gastrointestinal problems, and inflammatory disorders (Kumar et al., 2012; Rani et al., 2018). Although the leaves and bark of this plant have been investigated for their medicinal attributes, the antimicrobial capabilities of its roots have yet to be thoroughly examined.

This study aims to evaluate the antibacterial and antifungal activities of *Cadaba fruticosa* Root extract. The findings could provide scientific validation for the traditional use of this plant and contribute to the development of novel antimicrobial agents to combat drug-resistant pathogens.

MATERIAL AND METHODS

Plant Material Collection and Preparation

Fresh roots of *Cadaba fruticosa* were collected from Ner taluka of Yavatmal District, M.S (India) during the month of October. The plant material was identified and authenticated by a botanist at Arts, Commerce and Science College, Maregaon, and a voucher specimen was deposited in the College herbarium. The plant parts, such as Leaves, Bark, Root, and Fruits, were washed thoroughly with distilled water to remove dirt and impurities, air-dried at room temperature for 7–10 days, and ground into a fine powder using a mechanical grinder.

Preparation of Plant Extract

The Soxhlet extraction method was employed to prepare the extract. Approximately 20 g of powdered root was placed in a thimble and extracted using 200 mL of Methanol solvent. The extraction process was carried out for 6–8 hours at a temperature of 40 °C. The solvent was then evaporated under reduced pressure using a rotary evaporator to obtain a concentrated crude extract. The extract was stored in an airtight container at 4°C until further use. The solvent was subsequently evaporated under reduced pressure with a rotary evaporator to yield a concentrated crude extract. The extract was kept in an airtight container at 4°C until it was needed for further use. The methanolic extract was then evaporated in pre-weighted Petri plates and then dissolved in DMSO Solvent. The concentration was made as mg/ml. The DMSO extract was used further for assessing antibacterial and Antifungal activity.

Microorganisms

The antimicrobial efficacy of the extract was assessed against a variety of both gram-positive and gram-negative bacterial cultures that include (*Bacillus subtilis*, MTCC-1133), (*Escherichia coli*, MTCC-452), (*Pseudomonas aeruginosa*, MTCC-3541), (*Staphylococcus aureus*, MTCC96), and fungal cultures (*C.*

tropicalis- MTCC1000), (*Trichoderma asperellum*, MTCC 4347) which were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC) in Chandigarh.

Antibacterial assay (Hudzicki, J.,2009).

The Zone Inhibition Method (Kirby-Bauer method) was utilized for the assay. In summary, Mueller-Hinton agar was inoculated with the corresponding microbial cultures. The MHA plates were inoculated by spreading 100 µl of bacterial culture, (Inoculum was prepared by adjusting to a 0.5 McFarland Unit - approximately cell density (1.5 X 10⁸ CFU/mL from Mueller-Hinton Broth), followed by placing the discs (Whatman No 1 filter paper discs (5mm) containing various concentrations of extracts (0 to 100 mg/ml). One disc on each plate was loaded with the solvent DMSO alone, which acted as the vehicle control. All the Discs were punched into the agar, and 100 µL of the extract (at varying concentrations, e.g., 62.5, 125, 250, 500, 1000, upto **2500** mg/mL) was loaded on each disc. The plates were incubated at 37°C for 24 hours. The zones of inhibition were measured in millimetres and compared with standard antibiotics e.g., ciprofloxacin for bacteria. The clear zones created around the disc were measured and recorded.

Antifungal activity

The antifungal activity was assessed using the Zone of Inhibition Method (Kirby-Bauer method) (<https://asm.org/getattachment>). The SDA plates were inoculated by spreading 100 µl of the fungal culture, *C. tropicalis* and *Trichoderma spp.*, (the inoculum was prepared by adjusting to 0.5 McFarland Unit - approximately cell density (1.5 X 10⁸ CFU/mL from Sabouraud dextrose broth), followed by placing the discs containing various concentrations (0 to 100 mg/ml). One disc in each plate was loaded with DMSO solvent alone, which acted as a vehicle control, and an Amphotericin B disc was used as a positive control. All discs were inserted into

the agar, and 100 µL of the extract (at different concentrations, such as 62.5, 125, 250, 500, 1000, up to 2500 µg/disc) was placed onto each disc. Each extract was examined in triplicate. The plates were incubated at 28°C for 48 hours. The clear zones formed around the discs were measured and documented.

Statistical Analysis

All experiments were conducted in triplicate, and the data were represented as mean ± standard deviation.

RESULTS AND DISCUSSION:

The results of the study demonstrated significant but less antibacterial and antifungal activities of *Cadaba fruticosa* root extract. The extract exhibited dose-dependent inhibition against all tested microbial strains. The positive controls (PC) exhibited significant inhibition activity against all the bacteria even at very low concentrations. The highest zone of inhibition was observed in *E. coli* (**23.33 ± 0.58 mm**) and *B. subtilis* (**24 ± 0 mm**), indicating their susceptibility to the standard antimicrobial agents used (Table 1, figure 1, 3). The extract showed moderate antibacterial activity against *B. subtilis*. The lowest concentration (62.5 µg/disc) produced a small Zone of Inhibition (6.67 ± 0.58 mm), which increased slightly with concentration. The maximum inhibition (8.33 ± 0.58 mm) was observed at 1000 µg/disc and above. Compared to the PC (24 mm), the inhibition is significantly lower, indicating only weak to moderate antibacterial activity. In *Staphylococcus aureus* (Gram-positive) bacteria the extract had no activity at lower concentrations (62.5–500 µg/disc). The Zone of Inhibition of 8 mm was observed at 500 µg/disc and above. Compared to the PC (22.33 ± 0.5 mm), the extract shown a much weaker effect, suggesting low efficacy against *S. aureus*. Also the *Escherichia coli* (Gram-negative) the extract showed no activity up to 156.25 µg/disc. From 312.5 µg/disc onwards, weak inhibition (6–8.33

mm) was observed. The maximum inhibition (8.33 mm at 1250 µg/disc and 2500 µg/disc) is still much lower than the PC (23.33 mm), indicating low antibacterial potential. Although *P. aeruginosa* exhibited some resistance, with no inhibition at 62.5–156.25 µg/disc. From 312.5 µg/disc onwards, weak inhibition (7.67–9.67 mm) was observed. The highest inhibition (9.67 ± 0.58 mm at 2500 µg/disc) remains significantly lower than the PC (16 mm). This suggests the extract has only mild activity against *P. aeruginosa* (Table 1, figure 1, 3). In this investigation, the extract did not show any antifungal activity against *Candida tropicalis* and *Trichoderma* species. However the positive control has shown potent antifungal (12mm) activity against tested fungal strain (Table 2, Figure 2,3).

Although previous research on the antimicrobial potential of *Cadaba fruticosa* has shown potent antimicrobial activity of leaf extract. For instance, Kumar et al. (2012) reported significant antibacterial activity of the plant's leaf extract against Gram-positive and Gram-negative bacteria. Similarly, Al-Snafi (2016) highlighted the therapeutic potential of *Cadaba fruticosa* in traditional medicine.

Juliet et al., 2018 also found the potent antibacterial and Antifungal activity of Ethanolic and Methanolic extracts of Wild and Tissue-cultured propagated leaf parts of *Cadaba fruticosa*.

Similar findings were reported by Lavinya, et al 2014 on the Methanolic and aqueous leaf extract of *Cadaba fruticosa*. However, this study is among the first to focus on the root extract, highlighting it as a potential source of antimicrobial agents but may be in synergistic effect with other part of the plant body of *Cadaba fruticosa*.

The results also highlight the importance of exploring traditional medicinal plants to combat antimicrobial resistance. Further studies are

needed to isolate and characterize the active compounds responsible for the observed activities and to evaluate their mechanisms of action.

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Table 1-Antimicrobial activity of *Cadaba fruticosa* Root Extract.

| <i>Bacillus subtilis</i> | | <i>S. aureus</i> | | <i>E.coli</i> | | <i>P. aeruginosa</i> | |
|--------------------------|--------------------------|------------------|--------------------------|------------------|--------------------------|----------------------|--------------------------|
| Amount (µg/disc) | Zone of Inhibition in mm | Amount (µg/disc) | Zone of Inhibition in mm | Amount (µg/disc) | Zone of Inhibition in mm | Amount (µg/disc) | Zone of Inhibition in mm |
| PC | | PC | | PC | | PC | |
| (3 µg) | 24 ± 0 | (10µg) | 22.33±0.5 | (10 µg) | 23.33 ±0.58 | (3µg) | 16 ± 0 |
| 62.5 | 6.67 ± 0.58 | 62.5 | 0 | 156.25 | 0 | 156.25 | 6.67 ± 0.58 |
| 125 | 7 ± 0 | 125 | 0 | 312.5 | 6 ± 0 | 312.5 | 7.67 ± 0.58 |
| 250 | 7.33 ± 0.58 | 250 | 0 | 625 | 8 ± 1 | 625 | 8 ± 0 |
| 500 | 8 ± 0 | 500 | 8±0 | 1250 | 8.33 ± 0.58 | 1250 | 8 ± 0 |
| 1000 | 8.33 ± 0.58 | 1000 | 8±0 | 2500 | 8.33 ± 0.58 | 2500 | 9.67 ± 0.58 |

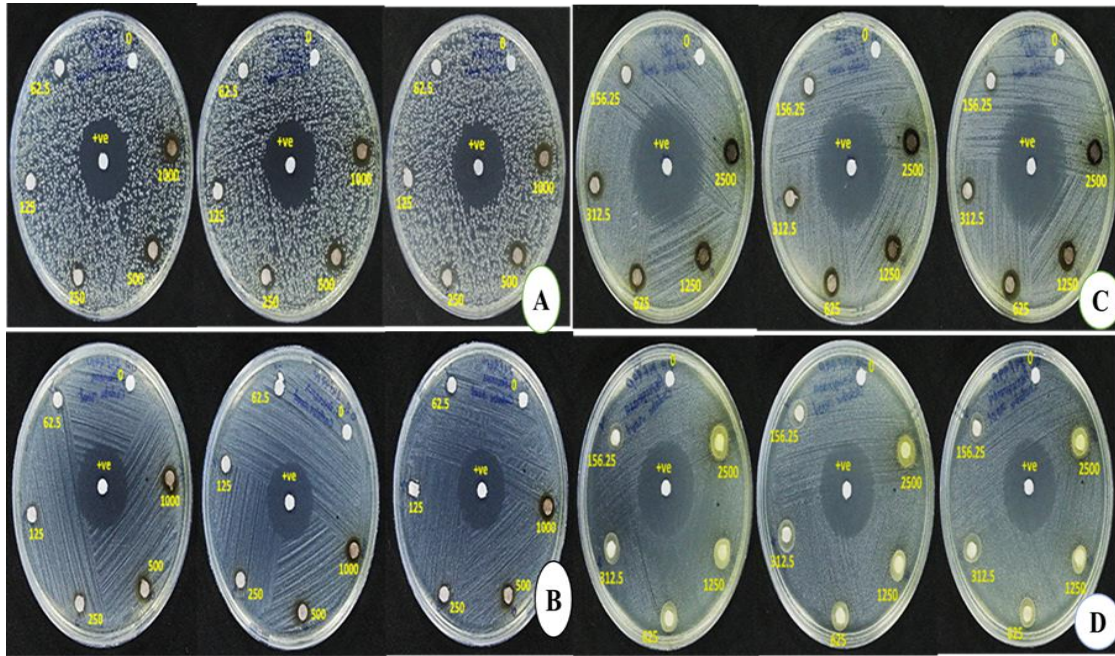


Figure 1-Antimicrobial activity of *Cadaba fruticosa* Root extract .Bacteria A – *Bacillus subtilis*, B- *Staphylococcus aureus* , C- *Escherichia coli* , D- *Pseudomonas aeruginosa*

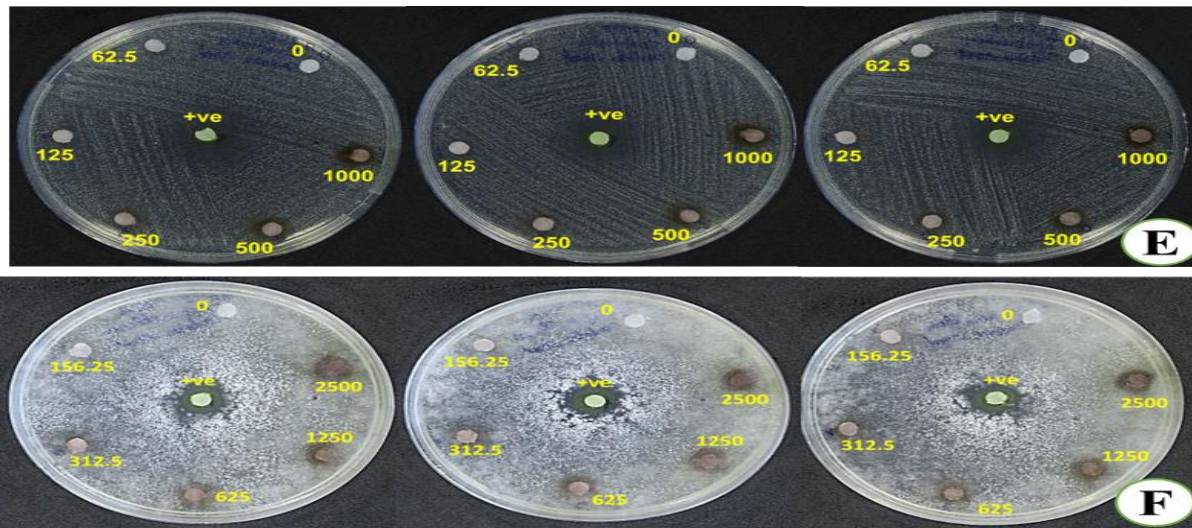


Figure 2. Antimicrobial activity of *Cadaba fruticosa* Root extract of Fungi E –*Candida tropicalis* , F- *Trichoderma asperellum* .

| Table 2 - Antifungal activity of <i>Cadaba fruticosa</i> (L.) Druce. Root extract | | | |
|---|--------------------------|-------------------------------|--------------------------|
| <i>Candida tropicalis</i> | | <i>Trichoderma asperellum</i> | |
| Amount(μ g/disc) | Zone of Inhibition in mm | Amount(μ g/disc) | Zone of Inhibition in mm |
| PC (100 μ g) | 12 | PC (120 μ g) | 12 |
| 62.5 | 0 | 156.25 | 0 |
| 125 | 0 | 312.5 | 0 |
| 250 | 0 | 625 | 0 |
| 500 | 0 | 1250 | 0 |
| 1000 | 0 | 2500 | 0 |

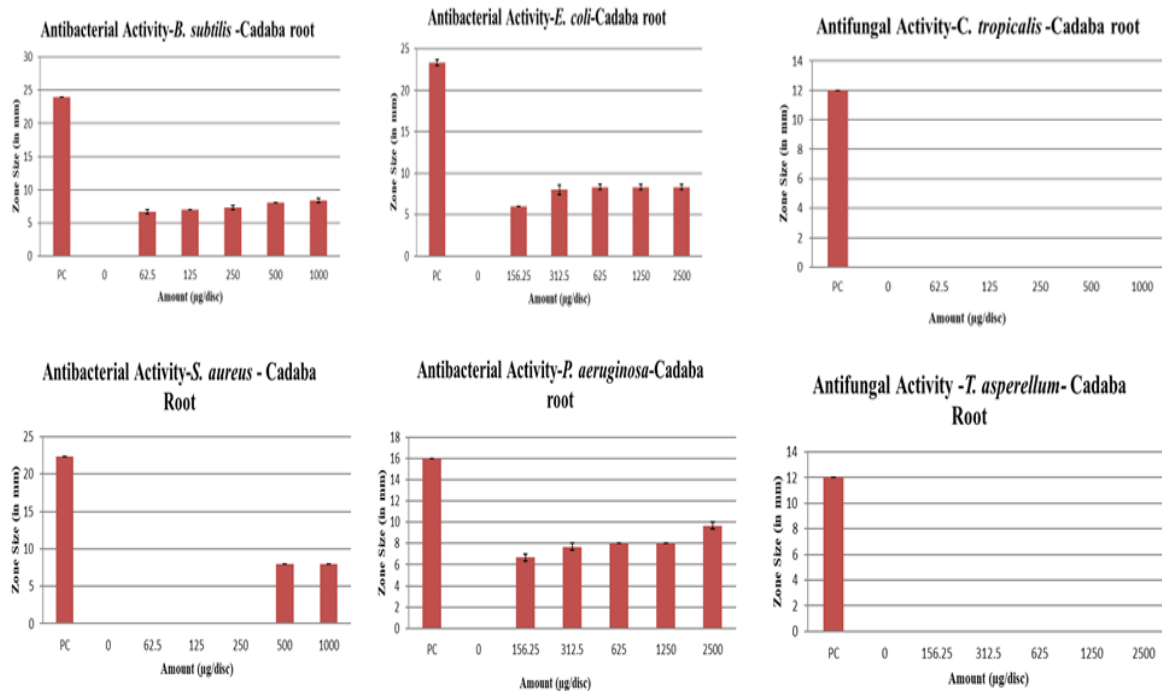


Figure 3. Bar Graph showing Anti-bacterial and Anti-fungal Activity Of *Cadaba fruticosa* (L.) Druce.