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## Research Paper

# Antibacterial activity of various crude extracts of *Ficus callosa* against Multi-Drug-Resistant (MDR) pathogens

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## ABSTRACT

A major worldwide health concern that contributes to a high annual death rate is multidrug-resistant (MDR) microorganisms. Thus, new antibacterial substances derived from biological sources must be developed. This study looked at the antibacterial properties of several *Ficus callosa* crude extracts against multidrug-resistant bacteria. The disc-diffusion method was used to determine the antibiograms of five isolated bacteria: *Burkholderia cepacia* (ATCC 25416), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Citrobacter freundii* (ATCC 8090), *Enterobacter cloacae* (ATCC 13047), and *Enterobacter faecalis* (ATCC 29212). Additionally, the antibacterial efficacy of plant extracts was observed. Most antibiotics at the time did not work on the isolated bacterium. Three sections of the *F. callosa* plant and five extracts were utilised. A maximal zone of inhibition of 19 to 20 mm was observed on bacterial lawns induced by *Citrobacter freundii* ethyl acetate extract of fruit and bark. There was no zone of inhibition seen in the petroleum ether extract of any of the bacteria or components. Its efficacy was demonstrated by the chloroform extract, which had the lowest Minimum Inhibitory Concentration (MIC) of 10 mm against MDR *Enterobacter cloacae* and *Burkholderia cepacia*. Nevertheless, it also had the greatest total MIC value.

## INTRODUCTION

One of the most urgent worldwide public health concerns of the twenty-first century is antimicrobial resistance (AMR) [1,2]. One of the

main causes of morbidity and death around the globe is bacterial pathogen-induced infections [3]. In cases like cancer treatment, organ transplantation, managing preterm infants, or major operations, antibiotic treatment is

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unquestionably one of the most important tactics used in contemporary medicine to fight infections or microorganisms [4]. Resistance in infections is caused by the overuse, abuse, and underuse of antibiotics [5]. Additionally, the rise, dissemination, and survival of multidrug-resistant (MDR) bacteria, sometimes known as "superbugs," have made antibiotic resistance a major danger to the health of people, animals, and the environment [6]. Even though there are 4,000,000 plant species on the globe, 21,000 of them have been certified by the WHO as therapeutic plants. Because they are less expensive, have fewer side effects, and are multi-component/multipotent than synthetic medications, a lot of studies are being done on plant-derived therapeutic agents. The extract's antibacterial activity against MDR Gram-negative bacterial isolates was assessed *in vitro* using the agar well diffusion technique. When compared to the Streptomycin standard, the fresh inoculum of MDR bacterial isolates was evenly distributed throughout the surface of sterile MHA plates [7]. Whatman filter paper discs with a 6 mm diameter were used to create Sevan wells in every plate. Using a micropipette, 30µl of extract (100µg/ml) from the chosen plants was then added to each disc [8]. After 15 minutes of extract diffusion over the plates, they were incubated at 37°C for the whole night and checked for the zone of inhibition.

## MATERIALS AND METHODS

### Collection of samples:

The most important wild medicinal plants are found in the Coonoor shola woods in the Western Ghats. These plants have been firmly recognised by the Southern Regional Centre of the Botanical Survey of India in Coimbatore, Tamil Nadu, and have been given the certification number BSI/SRC/5/23/2023-24/Tech-437. We harvested *F. callosa*'s leaves, fruit, and bark [9]. Rinsed well

under running water, then dried in the shade and ground into a fine powder.

### Extraction procedure for dry leaf, fruit, and bark samples:

In the Soxhlet extraction procedure, around 70 g of powdered leaf, fruit, and bark was extracted with 1 L of several solvents, including petroleum ether, chloroform, ethyl acetate, ethanol, and aqueous solutions, to make the extractions. The extractions were dried before being examined further [10]. In a rotary evaporator (Heidolph, Germany), the extracts were combined, the solvent was vacuum-evaporated at 40°C, and the dried extracts were kept at 4°C for the antibacterial test.

### Collection of bacterial strains

The clinical isolates of the following bacterial strains were acquired from the Department of Microbiology at Orbito Asia Diagnostics in Coimbatore, Tamil Nadu, India: *Citrobacter freundii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Escherichia coli*, and *Burkholderia cepacian*. A nutrient agar slant was used to keep these cultures at 4°C [11]. The antibacterial activity *in vitro* was assessed using Muller-Hinton Agar (MHA), which was acquired from Hi Media in Mumbai.

### Disc diffusion method

The disc diffusion technique, modified [12], was used to assess the antibacterial activity of *F. callosa* extracts. Twenty millilitres of sterile MHA were added to petri dishes. The plates were then employed in the susceptibility test after being given time to harden. To inoculate MHA plates, a swab was streaked with bacterial suspensions containing 108 colony-forming units (CFU) per millilitre over the agar surface. After that, the plates were left to dry for ten minutes. Sterile discs were loaded with various extracts impregnated with

30 µl of concentration (300 µg/disc) of crude



extracts after the extracts had been dissolved in 10% DMSO under aseptic conditions <sup>[13-15]</sup>. To make sure the extract-containing discs made contact with the infected agar surface, they were carefully pushed onto the medium surface using sterile forceps. A positive control of streptomycin (30 µg/disc) and a blind control of 10% DMSO were employed in each test <sup>[16]</sup>. Following inoculation, the plates were incubated for eight hours or overnight at 37°C. The millimetres of the inhibitory zone were measured.

## RESULT AND DISCUSSION

The antibacterial activity of several extracts against different bacterial species is shown in Table 1 and Plate 1. *Citrobacter freundii*, *Escherichia coli*, *Burkholderia cepacia*, *Enterobacter cloacae*, and *Klebsiella pneumoniae* were among the bacterial species examined <sup>[17]</sup>. Petroleum ether, chloroform, ethyl acetate, ethanol, and aqueous solutions were the extracts that were utilised. controls both the positive (Streptomycin) and negative (DMSO) controls. The table's numbers, which indicate the extent of bacterial growth suppression, most likely correspond to inhibition zone diameters (in millimetres). The antibacterial activity of ethanol, aqueous extracts, chloroform, and ethyl acetate varies, with ethyl acetate consistently displaying bigger inhibition zones. Ethyl acetate has the biggest inhibition zone (19 mm), indicating that *Klebsiella pneumoniae* is most impacted. The antibacterial activity of aqueous extracts is the lowest. No microorganisms are inhibited by petroleum ether. The strongest inhibitory zones are seen in the positive control, streptomycin, confirming the anticipated antibacterial action. The most effective extracts against these bacterial strains seem to be ethanol and ethyl acetate, indicating that the active ingredients in these solvents have substantial antibacterial properties.

Effectiveness might be confirmed with the use of additional research, such as Minimum Inhibitory Concentration (MIC) testing. In Plate 2 and Table 2, the bactericidal activity of several fruit extracts from *F. callosa* is demonstrated. One significant discovery about *Citrobacter freundii* is that the ethyl acetate extract generated an inhibitory zone of 20 mm, which is rather similar to the 22 mm seen with streptomycin. This suggests the presence of strong antibacterial chemicals in the ethyl acetate fraction <sup>[18]</sup>. *Enterobacter cloacae*, *Burkholderia cepacia*, *Escherichia coli*, and *Klebsiella pneumoniae*. According to Kavitha and Satish <sup>[19]</sup>, the extracts' inhibitory zones for these strains differ. The inhibition zones of the chloroform extract, for example, range from 10 to 11 mm, indicating mild action. For the majority of species, however, the ethyl acetate and ethanol extracts typically exhibit marginally greater inhibitory zones, albeit somewhat lower than those of streptomycin, the common antibiotic. The bactericidal properties of several bark extracts from *F. callosa* are displayed in Table 3 and Plate 3. With the strongest antibacterial action, the ethyl acetate extract is the one with the highest concentration of bioactive chemicals. The ethanol and chloroform extracts likewise exhibit encouraging outcomes; however, the petroleum ether extract does not work. All of the active extracts are efficient against *Klebsiella pneumoniae* and *Citrobacter freundii*, although they are not all equally potent against other bacteria.

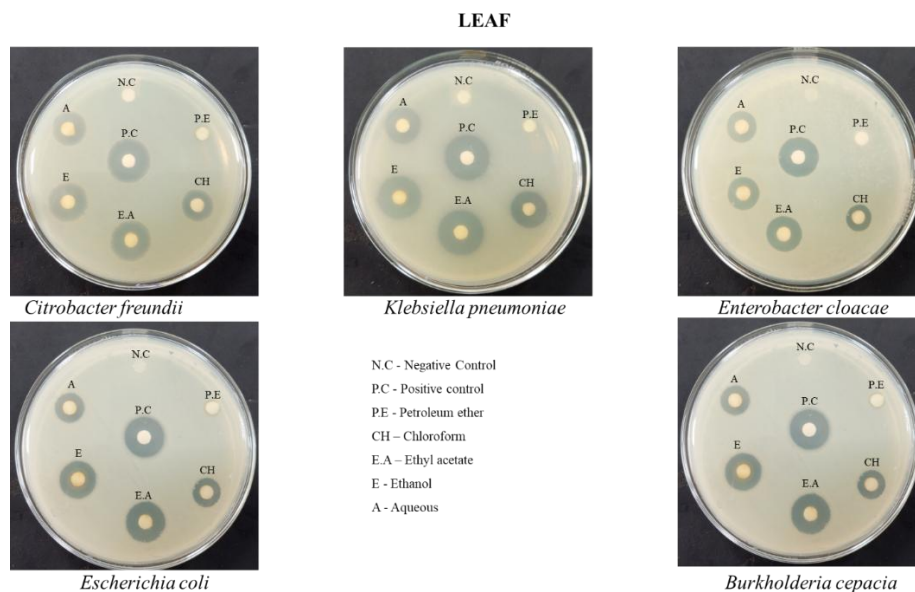
## CONCLUSION

The antimicrobial analysis of *F. callosa* extracts demonstrates the robust antibacterial properties of ethyl acetate, especially against *Citrobacter freundii* and *Klebsiella pneumoniae*, with inhibition zones comparable to those of streptomycin. According to the results, further study is necessary to determine the therapeutic



uses of the active ingredients in ethyl acetate. Future research, such as testing using Minimum Inhibitory Concentration (MIC), may shed light on how well these extracts work to prevent bacterial infections.

**PLATE 1.** Antibacterial activity by different extractives of the leaves of *F. callosa* through the disc diffusion method

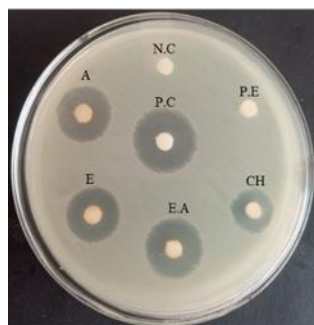
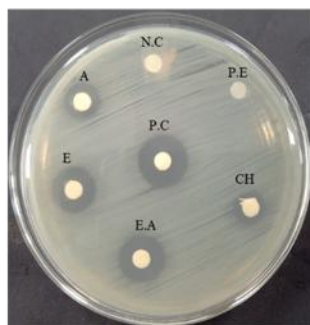
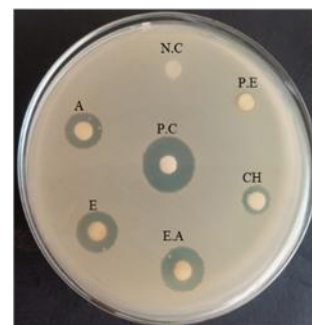
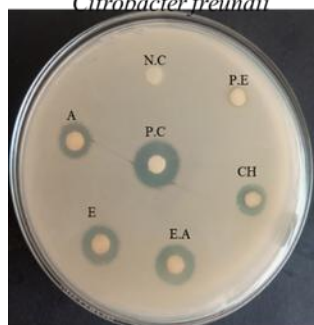


**Table 1.** Antibacterial activity by different extractives of the leaves of *F. callosa* through the disc diffusion method

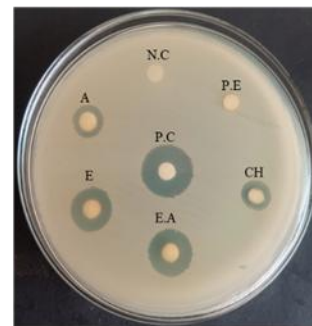
| Extracts                     | Zone of inhibition mm |            |               |         |         |                         |                                 |
|------------------------------|-----------------------|------------|---------------|---------|---------|-------------------------|---------------------------------|
|                              | Petroleum ether       | Chloroform | Ethyl acetate | Ethanol | Aqueous | Negative control (DMSO) | Positive control (Streptomycin) |
| <i>Citrobacter freundii</i>  | -                     | 13         | 16            | 15      | 14      | -                       | 17                              |
| <i>Klebsiella pneumoniae</i> | -                     | 15         | 19            | 17      | 14      | -                       | 20                              |
| <i>Enterobacter cloacae</i>  | -                     | 11         | 15            | 13      | 12      | -                       | 18                              |
| <i>Escherichia coli</i>      | -                     | 12         | 16            | 15      | 12      | -                       | 17                              |
| <i>Burkholderia cepacia</i>  | -                     | 12         | 17            | 16      | 12      | -                       | 17                              |

**PLATE 2.** Antibacterial activity by different extractives of the fruit of *F. callosa* through the disc diffusion method

## FRUIT

*Citrobacter freundii**Klebsiella pneumoniae**Enterobacter cloacae**Escherichia coli*

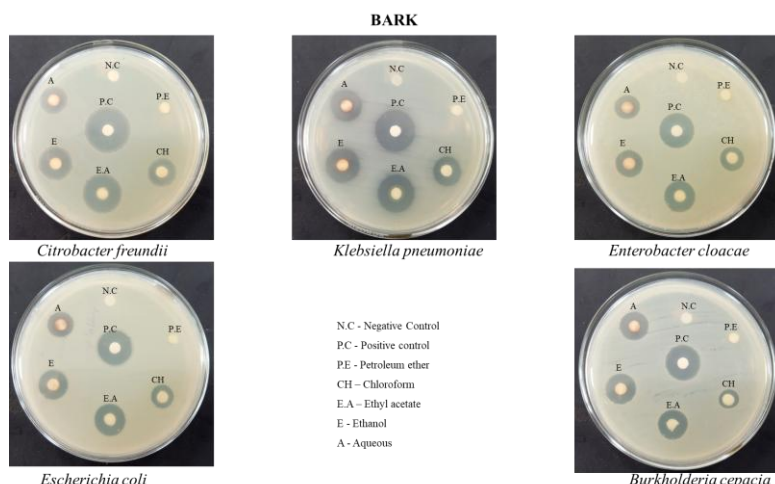
N.C - Negative Control  
 P.C - Positive control  
 P.E - Petroleum ether  
 CH - Chloroform  
 E.A - Ethyl acetate  
 E - Ethanol  
 A - Aqueous

*Burkholderia cepacia*

**Table 2. Antibacterial activity by different extracts of the fruit of *F. callosa* through the disc diffusion method**

|                              | Zone of inhibition mm |            |               |         |         |                         |                                 |
|------------------------------|-----------------------|------------|---------------|---------|---------|-------------------------|---------------------------------|
|                              | Petroleum ether       | Chloroform | Ethyl acetate | Ethanol | Aqueous | Negative control (DMSO) | Positive control (Streptomycin) |
| <i>Citrobacter freundii</i>  | -                     | 14         | 20            | 18      | 18      | -                       | 22                              |
| <i>Klebsiella pneumoniae</i> | -                     | 11         | 15            | 15      | 13      | -                       | 18                              |
| <i>Enterobacter cloacae</i>  | -                     | 10         | 15            | 14      | 13      | -                       | 18                              |
| <i>Escherichia coli</i>      | -                     | 11         | 14            | 13      | 11      | -                       | 16                              |
| <i>Burkholderia cepacia</i>  | -                     | 10         | 15            | 14      | 10      | -                       | 18                              |

**PLATE 3.** Antibacterial activity by different extractives of the bark of *F. callosa* through the disc diffusion method



**Table 3.** Antibacterial activity by different extractives of the bark of *F. callosa* through the disc diffusion method

| Extracts                     | Zone of inhibition mm |            |               |         |         |                         |                                 |
|------------------------------|-----------------------|------------|---------------|---------|---------|-------------------------|---------------------------------|
|                              | Petroleum ether       | Chloroform | Ethyl acetate | Ethanol | Aqueous | Negative control (DMSO) | Positive control (Streptomycin) |
| <i>Citrobacter freundii</i>  | -                     | 15         | 20            | 18      | 15      | -                       | 23                              |
| <i>Klebsiella pneumoniae</i> | -                     | 15         | 19            | 17      | 16      | -                       | 21                              |
| <i>Enterobacter cloacae</i>  | -                     | 12         | 17            | 14      | 13      | -                       | 19                              |
| <i>Escherichia coli</i>      | -                     | 12         | 17            | 15      | 13      | -                       | 18                              |
| <i>Burkholderia cepacia</i>  | -                     | 10         | 16            | 16      | 15      | -                       | 19                              |

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