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Antibacterial activities of crude hydroethanolic extracts of Duguetia confinis(Annonaceae) bark on strains of Escherichia coli and Staphylococcus aureus

Original Research Article

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Abstract:

The emergence of bacterial resistance to antibiotics in recent years poses a significant global health threat. Pathogens like Escherichia coli and Staphylococcus aureus, particularly antibiotic-resistant strains such as extended-spectrum beta-lactamase (ESBL)-producing E. coli and methicillin-resistant S. aureus (MRSA), are increasingly difficult to treat with conventional antibiotics. This study aimed to evaluate the antibacterial activity of the hydroethanolic extract of Duguetia confinis bark against S. aureus and E. coli.

The results revealed that the D. confinis extract exhibited significant antibacterial activity, particularly against S. aureus, with a minimum inhibitory concentration (MIC) of 0.25 mg/mL. However, the extract was less effective against E. coli, with an MIC of 0.625 mg/mL. These results suggest that D. confinis could serve as a potential source of new antibacterial agents, especially against S. aureus infections.

While ofloxacin, a reference antibiotic, showed much lower MIC values, the activity of D. confinis at relatively low concentrations indicates its potential as a complementary treatment or in synergistic combinations with existing antibiotics. Further research is needed to identify the specific active compounds within the extract, evaluate their in vivo efficacy and toxicity, and explore potential synergies with other antibiotics.

Keywords: Duguetia confinis, Antibacterial activity, Minimum Inhibitory Concentration

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1. Introduction

The emergence of bacterial resistance to antibiotics in recent years poses a threat to global health (World Health Organization [WHO], 2024). The emergence of resistant strains of major pathogens such as *Escherichia coli* and *Staphylococcus aureus* is a prominent example. *E. coli*, often implicated in urinary tract and gastrointestinal infections, and *S. aureus*, known to cause skin, respiratory, and sepsis infections, have become symbols of the increasing ineffectiveness of traditional antibiotics. Resistant strains, including *extended-spectrum β-lactamase-producing E. coli (ESBL) (Matta, 2023) and methicillin-resistant* S. aureus *(MRSA), exacerbate this threat, making conventional treatments increasingly less effective (Gardam, 2000).*

Several factors are responsible for this rise in resistance. First, antibiotic resistance is a natural phenomenon (Smith, 2005). In addition, the abusive prescription of antibiotics (Augusto, 2020) and self-medication (Kannan, Carrizales, Carrizales, Alvarez, & Jouregui, 2016) have resulted in increased treatment costs. In hospitals, the emergence of antibiotic-resistant nosocomial infections results in longer hospital stays (Ben Barek & Fatheh, 2023).

Several solutions are being considered to counter this threat, such as compliance with hygiene measures in hospitals to eliminate the phenomenon of carriage (WHO, 2024), the prescription of antibiotics after an antibiogram (Augusto, 2020), and the fight against the illicit sale of medicines in developing countries (WHO, 2024). It is also essential to look for new active ingredients.

Plants have always been a source of active ingredients in the fight against diseases (Ameenah, 2006). Today, 50% of small molecules marketed for the treatment of cancer, as well as the most effective drugs for treating influenza or malaria, are still extracted or derived from plants (Faye, 2008). According to the WHO, it is estimated that 60-80% of African populations use plants as their primary source of medicine (WHO, 2013). These plants have certain advantages, including their low toxicity, low economic cost for the populations that use them, and their high potential in terms of antibacterial biomolecules. The purpose of this study was to evaluate the antibacterial activity of the *Duguetia confinis* plant on strains of *S. aureus* and *E. coli*.

2. Methods

2.1. Collection of plant material

The bark of *Duguetia confinis* was harvested in February 2024, in the district of Oveng (Southern Cameroon region) at around 8:00 a.m. Authentication was carried out at the National Herbarium of Cameroon by comparison with specimen N° 29365/HNC.

2.2. Extraction of the plant

The bark of *Duguetia confinis* was cut and dried in the shade for a week. After grinding, 162 g of powder was soaked in 2 L of 95% v/v ethanol under constant stirring for 72 h. Subsequently, the preparation was filtered through the Whatman No. 4 paper. The filtrate was evaporated at 40°C using a low-pressure rotary evaporator (Stuart RE300B) and dried to a residue of 6 g (3.65%) of crude ethanol extract of *D. confinis*.

2.3. Preparation of the stock solution and sterility test of the extract

For the tests, a stock solution of the extract was prepared. 20 mg of the extract was mixed with 10 mL of heart-brain broth (BCV), at a concentration of 2 mg/mL. A sterility test was performed to ensure that the extract used in the experiments did not contain contaminants. To do this, a sample of the stock solution was inoculated on a Mueller-Hinton solid medium and then incubated at 37 °C for 24 hours. The absence of bacterial colonies on the agar confirmed that the extract was sterile, according to established standards.

2.4. Preparation of the bacterial inoculum for the liquid test

The bacterial inoculum was prepared using colonies of bacteria less than 24 hours old, homogenized in 10 ml of BCV, and incubated for 3 hours at 37 °C to obtain a pre-culture. This pre-culture was then diluted in physiological water to create a bacterial suspension with an optical density adjusted to 0.5 according to the McFarland scale. This suspension was used as an inoculum for antimicrobial testing.

2.5. Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of hydroethanolic extracts was determined using the micro-dilution method. MIC is defined as the lowest concentration of extract capable of inhibiting any microbial growth visible to the naked eye. The antimicrobial activity of the extracts was compared to that of ofloxacin, a reference antibiotic. The tests were performed by depositing specific volumes of BCVs, the extract to be tested, and bacterial inoculum into the wells of a microplate. Growth and sterility controls were also included to ensure the validity of the results. After incubation at 37°C for 18 hours, a colored indicator, the INT, was added to visualize bacterial growth, allowing the MIC to be determined (Ginocchio, 2002).

The MICs were determined using the liquid micro-dilution method as described by NCCLS (2002), using a 0.9% NaCl solution instead of RPMI as the solvent for the dilution of inoculum solutions. In each well of a microculture plate (Nunclon, Roskilde, Denmark, 96 wells), we successively introduced:

- 100 µl of heart-brain broth throughout the titration plate;
- 100 µl of the substance to be tested, using Rank 2 micro-dilutions;
- 100 µl inoculum at 10^6 CFU/mL;
- 3 positive control wells (reference antibiotic, ofloxacin);
- 3 negative control wells (medium only).

The whole was incubated at 37 °C for 24 h on a stirrer. After 24 h of incubation, the bacterial growth was revealed using INT, the principle of which is based on the capture of protons emitted by the dehydrogenases (enzymes present on the membrane of bacteria) of living bacteria after metabolizing glucose, which reduces the INT and colors the medium pink after about 30 minutes of re-incubation.

3. Results

Analysis of the results shows that *D. confinis* extract is more active on *S. aureus* than on *E. coli*.

Paramètre d'inhibition (mg/mL)	***D. confinis***	**Ofloxacine**
HCM *E. coli*	0,625	0,0125
CMI *S. aureus*	0,25	0,002

4. Discussion

The results of this study show that the hydroethanolic extract of *Duguetia confinis* has a notable antibacterial activity, in particular against *Staphylococcus aureus* (*S. aureus*), with a minimum inhibitory concentration (MIC) of 0.25 mg/mL. However, this activity is less pronounced against *Escherichia coli* (*E. coli*), for which the MIC is 0.625 mg/mL. These values are compared to those of the reference antibiotic ofloxacin, which shows significantly lower MICs, 0.002 mg/mL for *S. aureus* and 0.0125 mg/mL for *E. coli*.

The results obtained corroborate some previous studies that have documented the antibacterial properties of plant extracts, including those of the genus *Duguetia* (Maximo de Siqueira, Correa de Oliveira, & Diamantino Boaventura, 1997). The greater sensitivity of *S. aureus* compared to *E. coli* may be due to structural differences between the cell walls of these two bacteria. *S. aureus*, being a Gram-positive bacterium, has a cell wall composed mainly of peptidoglycan, which could allow better penetration of the bioactive compounds in the extract (WHO, 2013). In contrast, *E. coli*, a Gram-negative bacterium, has an additional outer membrane that can act as a barrier limiting the entry of active substances (Carbonnelle, 1987).

These results underscore the importance of further research on medicinal plants as potential sources of new antibacterial agents. *Duguetia confinis* appears to be a promising candidate, and further studies are needed to isolate and identify the compounds responsible for the observed activity, as well as to evaluate their mechanism of action.

5. Conclusion

The study demonstrated that the hydroethanolic extract of *Duguetia confinis* has an interesting antibacterial activity, especially against *Staphylococcus aureus*. These results pave the way for further investigations to identify active molecules and assess their potential as therapeutic agents in the fight against antibiotic-resistant infections.

6. Acknowledgments

We would like to express our sincere gratitude to all those who contributed to the success of this study. Our warmest thanks go to the staff of the National Herbarium of Cameroon for their invaluable assistance in authenticating *Duguetia confinis* and to the biology laboratory team at Cosendai Adventist University for their indispensable support during experimental procedures. We greatly appreciate the advice and expertise of Prof. Assam Assam Jean-Paul, whose comments and suggestions were essential for the development of this study. Finally, we extend our thanks to our families for their encouragement and understanding throughout this research process.

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