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Antimicrobial Sensitivity on the Seed Extracts of *Combretum Micranthum* (kinkheliba) on Some Bacteria of Public Health Importance

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ABSTRACT

This study explores the antimicrobial effects of seed extracts from *Combretum micranthum* against two bacterial pathogens: *Staphylococcus aureus* and *Escherichia coli*. The extracts were prepared using methanol and water, and their antibacterial properties were assessed in vitro using the agar diffusion method. A phytochemical analysis revealed the presence of resins, alkaloids, saponins, and tannins in the extracts. The methanolic extract demonstrated notable antibacterial activity, inhibiting *S. aureus* with a 17 mm zone of inhibition and *E. coli* with a 20 mm zone of inhibition. In contrast, the aqueous extract only exhibited antibacterial effects against *S. aureus* (8 mm inhibition zone) and had no impact on *E. coli*. These results indicate that *Combretum micranthum* seeds may serve as a potential source of antibacterial agents for treating bacterial infections. Further research is needed to identify and isolate the specific active compounds responsible for these antimicrobial properties.

Keywords: *Combretum micranthum*, Antimicrobial activity, Phytochemical analysis, *E.coli*.

1. Introduction

In recent years, ethnobotanical studies have gained significant global attention due to the increasing reliance on traditional medicine, especially plant-based remedies. The World Health Organization (WHO) reports that 80% of the population in developing countries primarily depends on traditional medicine, often involving medicinal plants. In Cameroon, while traditional medicine remains a cornerstone of healthcare, its integration into the formal health system is hindered by organizational challenges (Nkongmeneck et al., 2007; Snigdha et al., 2024).For centuries, people have utilized herbal remedies for various ailments, and contemporary advancements in phytochemical analysis have facilitated the identification and characterization of bioactive compounds with potential therapeutic applications (Adejuwon et al., 2011; Adekunle et al., 2009). The screening of higher plants for medicinal purposes represents a significant endeavor in the ongoing quest for novel, safer, and more efficacious drugs to combat pathogenic bacteria. *Combretum micranthum*, a member of the Combretaceae family, is a plant species widely employed in traditional medicine to treat a diverse range of ailments, including gastrointestinal disorders, fever, and colic. Medicinal plants offer a compelling alternative to synthetic drugs, often mitigating adverse side effects while demonstrating efficacy in treating infectious diseases. Numerous studies have identified bioactive compounds within plants that exhibit potent antimicrobial activity. Some traditional remedies have even yielded compounds effective against antibiotic-resistant bacterial strains. This study aims to investigate the antimicrobial properties of C. micranthum seed extract against a panel of clinically relevant bacteria. Infectious diseases are the most dreaded human ailments globally, resulting in approximately 50,000 fatalities daily (Akeem et al., 2012; Osonwa et al., 2012; Tanwieh et al., 2024; Taura et al., 2009). The incidence of antibiotic-resistant bacteria is increasing (Akeem et al., 2012; Osonwa et al., 2012). The emergence of resistance to novel antibiotic compounds by bacteria responsible for the majority of infectious illnesses has exacerbated the problem. Over the past three decades, pharmaceutical companies have developed new antibiotics (3rd Generation). Nonetheless, these antibiotics remain ineffective in inhibiting the proliferation of several bacteria, particularly those with the potential to acquire resistance. Consequently, the researcher intends to investigate the antimicrobial sensitivity of C. micranthum seed extract against several microorganisms of public health significance. In this study, we screened *Combretum micranthum* for antibacterial medicine and determinate the phytochemical content.

2. Experimental part

2. 1. Materials

The seed of *Combretum micranthum* was harvested in a food market in dry form and ground to a powder in the Bamenda North West region in April 2019. After weighing the powder form of the seed, the extraction was done shaking intermittently for 24h. The extraction was filtered with Whatman No.1 filter paper, put into Petri dishes, and allowed to evaporate to dryness in the incubator at 50 °C.

2. 2. Test Organisms

2. 2. 1. Isolation and purification of *Staphylococcus. aureus*

A pus specimen was first collected from an infected wound, inoculated on nutrient agar, and incubated for 18 hours. After gram staining and the colonies' growth, a distinct colony of gram-positive cocci was picked and subcultured on nutrient agar and incubated aerobically at 37 °C for 24 hours for a purity test. Catalase and coagulase tests were then performed to confirm that the cocci were *Staphylococcus aureus*.

2. 2. 2. Isolation and purification of *Escherichia coli*

A specimen of slaughtered pigs (faeces) was collected in Bamenda II, inoculated on MacConkey agar, and incubated for 24 hours. After gram staining, the colonies' growth (pink in colour) and a distinct colony of the gram-negative rod were picked and subcultured on MAC and incubated aerobically at 37 °C for 24 hours for the purity test. The presence of pink colonies when subcultured and the lactose-fermenting colonies was observed to confirm that it is Escherichia coli.

2. 2. 3. Inoculum standardization

Standard bacterial cultures (*S. aureus* and *E. coli*) were prepared by sub-culturing a loopful of each bacteria into 10ml sterile peptone water and incubating overnight at 37 ^{0}C.

2. 3. Geographical Distribution of *Combretum micranthum*

Combretum micranthum belongs to the *Combretaceae* family, which consists of 18 genera. It is a highly regarded medicinal plant in Africa, with roots, barks, seeds, fruits, and leaves being used. It grows with annual rainfall between 300 and 1500 mm and at altitudes from sea level to 1000 mm. *Combretum* is a very large genus, comprising about 250 species and distributed worldwide in the tropics and subtropics. About 140 species occur in tropical Africa, and 20 are endemic to Madagascar (Schmelzer & Gurib-Fakim, 2008). In Africa, it is widespread in the savanna zone of West Africa (Burkhill, 1985). The flowers are usually bisexual and are borne as auxiliary clusters on scaly stalks. The fruits are small and scaly, while the leaves are dorsoventrally or rarely centric with short stalk. The leaves turn green to orange over time and are useful in herbal medicine (Watson & Dallwitz, 1991). The plant is very drought- and fire-resistant; it's a browse plant for cattle and is of some importance for forage during the dry season in northern Nigeria.

- **Botanical classification**
- **Kingdom**: Plantae
- **Order**: Myrtales
- **Family**: Combretaceae
- **Genus**: Combretum
- **Species**: *Combretum micranthum*

Figure 1. picture of *Combretum micranthum* fruit that contain s

Figure 2. Picture of *Combretum micranthum* tree

2. 4. Extraction

2. 4. 1. Aqueous Extraction

100g of the powdered seed was weighed and put into a flask/bottle, and 800 ml of distilled water was added, mixed, and shaken intermittently for 24 hours. The materials were then filtered using Whartman No1 filter paper, and the filtrates were distributed into three separate clean, sterile dishes and were evaporated to dryness in the incubator at 50°C.

2. 4. 2. Alcoholic Extraction

100 g of the powdered seed was weighed and deposited in a clean and sterile bottle/flask, and 800 mL of methanol was added. The bottle was shaken intermittently for 24 hours. The materials were subsequently filtered with Whartman No1 filter paper, and the filtrates were allocated into three distinct clean, sterile dishes and evaporated to dryness in an incubator at 50°C.

2. 5. Antimicrobial Sensitivity Assay

The antimicrobial assay was conducted utilizing the agar well diffusion technique. (Habamu et al., 2010; Perez, 1990). Wells measuring 4mm in diameter were created in MHA plates using sterilized radio antennas. The examined organisms in sterile peptone water were introduced onto the media in each section of the three wells. Two wells were filled with 0.1 ml of the extract. 1 mg/ml of Doxycycline was added to the third well and functioned as a control. The plates were pre-incubated for 15 minutes to facilitate extract diffusion prior to overnight incubation at 37°C. The diameter of the clean zone was quantified in ml utilizing a ruler.

2. 6. Phytochemical Screening

The seed powder of *Combretum micranthum* (Kinkeliba) served as the sample for qualitative phytochemical screening of resins, alkaloids, saponins, tannins, glycosides, and flavonoids, adhering to the standard protocols outlined by (Evans, 1997) and (Fon et al., 2009).

- **Detection of Resins**

1 g of seed powder was incorporated into 4 ml of boiling ethanol. The solution was passed through Whatman No1 filter paper, and the filtrate was diluted with 4 ml of 1% aqueous HCl, after which the degree of coloring was measured. The emergence of a dense resinous precipitate signified the existence of resins (Evans, 2009).

- **Detection of Alkaloids**

1g of seed powder was stirred with 10 ml of 2N HCl in a steam bath. This was filtered, and 1 ml of the filtrate was tested with a few drops of Wagner's reagent. The formation of a precipitate after 15-20 min indicated the presence of alkaloids.

- **Detection of Saponins**

1g of seed powder was stirred with 10 ml of distilled water in a test tube. After heating and filtration, agitation must be applied; the appearance of a persistent foam indicates the presence of saponins.

- **Detection of Tannins**

1 g of seed powder was stirred with 10 ml of boiling distilled water. The filtrate was subjected to filtration, followed by the addition of a few drops of 6% Ferric Chloride. The appearance of deep green coloration indicated the presence of tannins. The subsequent portion of the filtrate was treated with several ml of iodine solution. The emergence of a subtle bluish hue validated the existence of tannins (Evans, 2009).

- **Detection of Glycosides**

1 g of seed powder was stirred with 10 ml of boiling distilled water. The filtrate was subjected to hydrolysis with 2 ml using a few drops of strong HCl, followed by the addition of a few drops of ammonium solution to achieve alkalinity. Five drops of this solution were incorporated into 2 ml of Fehling qualitative reagents and subsequently heated to boiling. The emergence of a reddish-brown precipitate signifies the existence of glycosides (Evans, 2009).

- **Detection of Flavonoids**

1 g of the seed powder was dissolved in 2 ml of weak sodium hydroxide. A little quantity of concentrated H_2SO_4 was subsequently introduced. The loss of color signified the presence of flavonoids (Evans, 2009).

3. Results

Table 1. Results of phytochemical analysis of *Combretum micranthum***.**

Phytochemicals	Abundance
Resins	+
Alkaloids	++
Saponins	+++
Tannins	++
Glycoside	_
Flavonoids	_

-: absent, +: present but trace, ++: present but less abundant, +++: present abundant

Table 1 shows the types of phytochemicals present in *Combretum micranthum* seeds. The highest being saponins and the least glycosides and flavonoids.

Table 2. Sensitivity testing to determine the zone of inhibition in mm

	Staphylococcus aureus	Escherichia coli
Control (doxycycline)	Sensitive (Ø=20mm)	Sensitive (Ø=20mm)
Methanolic extraction	Sensitive (Ø=17mm)	Sensitive (Ø=20mm)
Aqueous extraction	Sensitive (Ø=8mm)	Resistant

Ø=diameter of inhibition of growth

Table 2 shows that *E. coli* was resistant to aqueous extraction and sensitive to methanolic extraction, while *S. aureus* was sensitive to all extracts.

Figure 3. Mueller Hinton agar showing clear zone of inhibition of growth by the extracts. (*E.coli)*

Figure 4. Mueller Hinton agar shows clear zone of inhibition of growth by the extracts.(*S.aureus*)

4. Discussion

The aqueous and methanol extracts of *Combretum micranthum* seed inhibited *Staphylococcus aureus and Escherichia coli.* These results are similar to those obtained on the stem bark (Akeem et al., 2012), leaves (Osonwa et al., 2012), and roots (Taura et al., 2009). The phytochemical analysis indicated the existence of bioactive substances with in vitro antibacterial properties. The results indicated the existence of bioactive substances such as resins, saponins, tannins, and alkaloids. Nonetheless, it is shown that the plant composition is contingent upon the geographical origin. (Rao & Rout, 2003). Tannins were present but in fewer amounts, approximately similar to works done on the leaves (Karou et al., 2005) and stem bark (Akeem et al., 2012). Herbs containing tannins have been reported to aid in treating inflamed or ulcerated tissues, as well as intestinal disorders like diarrhea and dysentery (Parekh & Chanda, 2007). Tannins are also known to exhibit bacteriostatic or bactericidal effects against Staphylococcus aureus (Chung et al., 1998). This study found a high presence of alkaloids, which contrasts with previous research on the leaves (Osonwa et al., 2012) and stem bark (Akeem et al., 2012), where this compound was absent. Alkaloids have been associated with antimicrobial, antidiarrheal, and anthelmintic properties (Tiwari et al., 2011). Additionally, saponins were detected in high amounts, while resin was present only in trace quantities.

5. Conclusion

This investigation has enabled us to ascertain the antibacterial susceptibility of C. micranthum seed extract against some bacterial pathogens (*S. aureus and E. coli*).The methanol extracts eradicated the bacteria entirely, although the aqueous extract had a moderate antimicrobial activity.

The findings from these experiments indicate that the seed extracts of Combretum micranthum demonstrate antimicrobial sensitivity against the selected isolates, with the exception of *E. coli*, which exhibited resistance to aqueous extraction.Phytochemical substances found in plants that possess antibacterial effects include resins, saponins, tannins, and alkaloids.

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Data Availability

No data was used for the research described in the article.

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