

## Research Article

# Antimicrobial and Phytochemical Screening of Combretum Leaf Against Two Pathogens

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## Article Info

**Keywords:** Ethanol, Aqueous, Methanol, Combretum, Muller Hinton Agar and Phytochemical Analysis.

Received: 29.04.2026;

Accepted: 13.06.2026;

Published: 20.06.2026

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## Abstract

The *combretum* leaf extracts were investigated for phytochemical screening and antimicrobial activity on *Staphylococcus aureus* and *Eseherichia coli* by agar well diffusion method using Augmentin as standard antibiotics. The phytochemical screening revealed the presence of saponins, flavanoids, alkaloids, terpenoids, tannins whereas glycosides and steroids are absent in ethanol, methanol and aqueous extracts respectively. The ethanol, methanol and aqueous extracts were used in varying concentrations. The zone of inhibition was observed in both strains and the three extracts were found to show a very high antimicrobial activity but the ethanol extracts possessed more activity than the methanol and aqueous extracts, thus they were found to have Inhibitory action. The minimum inhibitory concentration (MIC) was found to range from 0.45 to 2.5mg/ml respectively. *combretum* leaf extract is thus recommended for the treatment of human infections caused by these organisms.

## 1. Introduction

Herbal medicine is a primary form of medicine that uses plant parts to cure human sickness which is as old as the ailment itself [1]. Medicinal plants have always been regarded as a source of healthy living for people, and their therapeutical capabilities are highly effective in the treatment of a variety of disorders, with the benefits of these medicinal plants being natural [2]. For hundreds of years, medicinal plants have been employed as antibacterial, antifungal, and antiviral agents in various parts of the world [3].

Plants are thought to play an important role in maintaining healthy health [4]. Plants and plant-based products are the foundations of many modern medications, according to Kamba and Hassan [5]. Despite the availability of improved conventional medications, there has been an increase in interest in complementary and alternative medicines, particularly among people who are concerned about the toxicity and safety of current treatments [6]. Plants have a huge potential for developing novel medications that will benefit humanity. Because traditional medicine is readily available and economically feasible in African countries, around 80% of the population relies on it for treatment [7]. The World Health Organization (WHO) estimates that almost 70 percent of the world population relies on traditional medicine, mainly medicinal plants, for their basic health care requirements [8]. Many research groups have screened plant extracts for secondary metabolites with relevant biological activity in order to find new lead molecules [9]. Many phytochemicals, such as alkaloids, glycosides, tannins, and saponins, have been linked to antibacterial and biological activity in plants [10].

*Combretum* is a big genus with over 250 species that can be found in both temperate and tropical climates [11]. Scrotal elephantiasis, diarrhea, ring worms, syphilis, typhoid fever, eye sores, and ear aches are all treated using *Combretum's* roots, stem, bark, and leaves [12]. *Combretum* stem bark and leaves are also used as antipyretics and in the treatment of stomach ache, gonorrhea, and typhoid fever, while

Daniella oliveri's stem bark is used to cure fever, boils, and back discomfort [13]. Scrotal elephantiasis, ring worms, typhoid fever, impotence, eye sore and ear ache, stomach pains, snake bite, leprosy, dysentery, diarrhea, general body swellings, arthritis and other inflammatory conditions, and abortion are all treated with *Combretum sokodense* roots, stem, bark, and leaf [14]. Some *Combretum* species are traditionally used for the treatment of mumps, syphilis, pneumonia, and other diseases [15, 16]. Nose bleeds, sore throats, fattening babies, gall stones, hook worm, urinary tract infection, scorpion sting, infertility in women, heart disease, dysmenorrhoea, and overall debility, according to traditionalists, might be treated with *Combretum* species [1].

## 2. Methods

*Combretum* leaf was collected from All saints Anglican church Eke Agbani Enugu. It was authenticated by Prof Ngwu of the Department of Agronomy/Crop Science ESUT, Enugu state.

### 2.1. Sample preparation

The fresh leaf of *Combretum* plant was conveyed to the laboratory using a black nylon bag. The fresh leaf was washed using running tap water, air dried, and was grinded into fine powdery form using mill grinder and stored in an Air tight plastic container until ready for used.

### 2.2. Preparation o plant extract

The *Combretum* leaf was air dried for three (3) weeks and grinded into fine powder. Approximately 50 g of the sample was weighed using weighing balance into a beaker and 300 mL of the solvent (aqueous, methanol and ethanol) was added into the clean beaker. The mixture was heated for 60 mins at a temperature of 60°C. The extract was filter using sterile filter paper (whatman number 1) into a clean conical flask and stored in refrigerator at 4°C.

### 2.3. Preparation of microorganisms

The test organisms used were all green leaf clinical origin and were isolated from *Combretum* plant. One Gram-positive bacterium (staphylococcus aureus) and one Gram-negative bacterium were used to test the antibacterial properties of the extracts. They were kept as stock cultures at 4°C in the Department of Applied Microbiology and Brewing, Faculty of Applied Natural Science, ESUT, on a nutrient agar slant.

### 2.4. Antimicrobial assay

The agar well diffusion method was used to test the antibacterial activity of water, ethanol, and methanol extracts of *Combretum* leaf. 1.20 g of each extract was dissolved in DMSO and then various quantities of the extracts (0.1 mL, 0.2 mL, and 0.3 mL) were obtained to test antibacterial activity as described by Maragathavalli et al [17]. In duplicates, standard inoculums of  $1.5 \times 10^8$  cells were distributed on the surface of sterile Muller Hinton agar plates that matched 0.5 McFarland standards. On the Muller Hinton agar plates, a sterile 6 mm cork borer was used to cut a hole in which 0.1 mL of each of the plant extracts was introduced. The plates were incubated for 72 h at 37 °C. Measurements of inhibition zones in millimeters were used to detect antibacterial activity. Standardized discs were also employed to test bactericidal activity of synthetic antibiotics, and zones of inhibition were determined.

### 2.5. Determination of the minimum inhibitory concentration (MIC)

This was determined using Maragathavalli et al. broth dilution method [17]. The lowest concentration of a substance required to impede the development of microorganisms was established as the minimum inhibitory concentration [18]. The extracts were made at various strengths (0.1 mL, 0.2 mL, and 0.3 mL). 0.1 mL of standardized test organisms were put into tubes holding various amounts of extracts, with solvents and test organisms without extract serving as controls. These were incubated for 24 h at 37°C. After culture, the tube with the lowest concentration of extract without growth was chosen as the minimal inhibitory concentration.

### 2.6. Phytochemical analysis

The ethanol, n-hexane and Aqueous extract were subjected to phytochemical screening to detect the presence of flavonoids, glycosides, saponins, steroids, tannins, alkaloids and terpenoids as described by Yahaya [19].

#### Test for Flavonoids

2 mL each of the extracts was treated with ethyl acetate solution and heated for 5 mins. The mixture was allowed to cooled, filtered. The filtrate was stirred constantly with 4% aluminium chloride solution and left for 10 mins. The formation of yellow coloration indicated the presence of flavonoid.

#### Test for Saponins

1 mL (milliliter) of extract was warmed with 1.5 mL of distilled water and boiled for 3 mins, the mixture was filtered while hot and the filtrate was stirred constantly with a drop of olive oil. The formation of emulsion reviews the presence of saponins.

### Test for Tannins

2 mL of the extracts was warmed with 30% ethanol for 8 mins. The mixture was allowed to cooled and filtered. Then the filtrates were treated with few drops of lead acetate solution. The formation of gelatinous precipitate indicates the presence of tannins.

### Test for Alkaloids

3 drops of dragendoffs reagent was added to 2.5 mL of the extract with 3 mL dilute HCl in water bath for 5 mins. The mixture was cooled and filtered. The formation of a red precipitate indicates the presence of alkaloids.

### Test for Terpenoid

5 mL (milliliter) of the extract was treated with 1 mL of acetic anhydride and 1 mL of Chlorofoam. Then concentrated sulphuric acid solution was added slowly and red violet colour was observed for terpenoid.

## 3. Results

It was observed that the ethanol extract of the leaf showed the highest level of inhibition on *Escherichia coli* when compared with *S.aureus* in Table 1. In Table 2 the methanol extract of the leaf showed the highest level of inhibition on *S.aureus* when compared with *E.coli* and in Table 3, the aqueous extract has appreciable effect on the tested organisms for *E.coli*. The MIC in Table 4 was found to range from 0.45 mg/mL to 2.5 mg/mL for all the extracts which shows that evaluation of MIC is sufficient for measuring bacterial activity. The MIC result showed that ethanol extract was more effective against the tested bacterial strains than other extracts.

**Table 1:** Antimicrobial activity of ethanolic extract of *Combretum* leaf

Test organism	Concentration of extract (mg/ml)			Standard Augmentin (10µg)
	0.1	0.2	0.3	
<i>E. Coli</i>	14	13	12	18
<i>S.aureus</i>	13	12	10	17

**Table 2:** Antimicrobial activity of Methanolic extract of *Combretum* leaf

Test organism	Concentration of extract (mg/ml)			Standard Augmentin (10µg)
	0.1	0.2	0.3	
<i>E. Coli</i>	11	10	9	18
<i>S.aureus</i>	12	11	10	17

**Table 3:** Antimicrobial activity of aqueous extract of *Combretum* leaf

Test organism	Concentration of extract (mg/ml)			Standard Augmentin (10µg)
	0.1	0.2	0.3	
<i>E. Coli</i>	10	9	8	18
<i>S.aureus</i>	9	8	7	17

**Table 4:** Minimum inhibitory concentration (mg/ml) of *Combretum* leaf extract of ethanol, Methanol and aqueous on test organisms

Test organism	Ethanol	Methanol	Aqueous	Standard
<i>E.coli</i>	2.5	0.45	0.45	5.60
<i>S.aureus</i>	2.5	0.45	0.45	5.66

## 4. Discussion

Many studies have revealed that plant parts extraction using ethanol were more inhibitory than methanol and aqueous extract as a result of ethanol being a good extractant [20]. Raja *et al* [21] who reported that leaf extract exhibited strong antimicrobial activity against the organisms tested at all concentrations.

The phytochemical screening of *Combretum* leaf revealed the presence of saponins, flavanoids, alkaloids, terpenoids, and tannins. However saponins and terpenoids were absent only in methanol extracts while glycosides and steroids are absent in ethanol, methanol and aqueous extracts respectively in Table 5. The presence of these active ingredients in plants has been linked to many of the pharmacological and biological activities of plants [22]. Tannin-rich plants, for example, have been used to treat intestinal disorders like diarrhoea and dysentery [23]. Furthermore, tannins have been found to have astringent properties that aid in the healing of wounds and inflamed mucous membranes. Flavonoids are potent antioxidants that have a wide range of health benefits and apart from antioxidant activity; these molecules have antiviral, anticancer, anti-inflammatory, and anti-allergic properties [24]. Saponins have also been shown in studies to have health benefits such as cholesterol reduction, antitumor and anti-mutagenic activities, which can reduce the risk of human cancer, immunity

booster, bone loss reduction, and so on [25]. Glycosides are also used as cardiac drugs, laxatives, anti-irritants, analgesics, they prevent renal infections, and they have anti-rheumatic, anti-inflammatory, expectorant, and antispasmodic properties [26]. Alkaloids have been shown to inhibit bacterial growth and lower blood pressure [27].

**Table 5:** Phytochemical result of the *Combretum* leaf extracts

S/N	Phytonutrients	Ethanol	Methanol	Aqueous
1	Flavonoids	++	+	++
2	Glycosides	-	-	-
3	Saponins	+	-	+
4	Steroids	-	-	-
5	Alkaloids	+++	++	++
6	Terpenoids	++	-	+
7	Tannins	++	+	++

## 5. Conclusion

The presence of some phytochemicals in *Combretum* leaves demonstrated antibacterial potentials that are as effective as standard antimicrobial drugs against gram positive and gram negative bacteria extract. The extract performed better than the vast majority of bacteria tested. The observed activity suggests that the leaves, like other plant parts, have therapeutic properties. As a result, *Combretum* leaves should be used as a source of antibacterial agents.

## Article Information

**Acknowledgments:** My profound gratitude goes to Dr. Ike, Christian and Prof. Okoro, U.C. I also appreciate all those that have contributed to the success of this work.

**Author Contributions:** I.O.C. - Conceptualization, Methodology, Writing – review & editing, Supervision; O.C.C. - Methodology, Writing – original draft, Writing – review & editing; O.N.J. - Conceptualization, Formal analysis.

**Funding / Financial Support:** The authors received no financial support for the research, authorship, and/or publication of this article.

**Conflict of Interest (Competing Interests):** Authors have declare that no competing interests exist.

**Ethical Approval:** Not Applicable.

**Informed Consent:** Not Applicable.

**Data Availability Statement:** Not Applicable.

**Clinical Trial Registration:** Not Applicable.

**Disclaimer (Artificial Intelligence):** The author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.), and text-to-image generators have been used during writing or editing of manuscripts.

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