PHYTOCHEMICAL COMPOSITION AND ANTIMICROBIAL POTENTIAL OF *Combretum hispidum* LEAF EXTRACT

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ABSTRACT

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| **Aims:** Antimicrobial resistance is a pressing global health threat that has intensified the search for novel therapeutic agents, particularly from natural sources. *Combretum hispidum*, a medicinal shrub widely used in West African ethnomedicine, has been traditionally employed in treating infections, fever, wounds, and gastrointestinal ailments.**Methodology:** This study evaluated the phytochemical composition and antimicrobial potential of *C. hispidum* leaf extract using experimental rat models and in vitro assays. Fresh leaves of *C. hispidum* were collected, authenticated, shade-dried, and extracted using ethanol via Soxhlet apparatus. Phytochemical constituents were determined through standard qualitative and quantitative techniques, including gas chromatography–mass spectrometry (GC-MS). Antimicrobial activity was assessed against clinical and standard strains of *Enterobacter spp., Klebsiella spp., Salmonella typhi, Cytrobacter* spp., and *Shigella* spp. using the disc diffusion method and determination of minimum inhibitory concentration (MIC).**Results:** Quantitative phytochemical analysis revealed alkaloids (37.14 ± 0.39 mg/100 g) as the most abundant compound, followed by flavonoids (19.73 ± 0.61 mg/100 g), phenols (14.36 ± 0.33 mg/100 g), and saponins (12.71 ± 0.16 mg/100 g), while glycosides (4.90 ± 0.09 mg/100 g) were least abundant. GC-MS analysis identified 23 bioactive compounds, with oleic acid being most prominent. Antimicrobial assays showed dose-dependent inhibition, with the highest zone of inhibition observed against *Enterobacter* spp. (7.58 ± 0.50 mm at 50% concentration) and *Salmonella typhi* (7.33 ± 0.45 mm at 50%), while *Shigella* spp. showed moderate sensitivity (5.83 ± 0.98 mm at 100%).**Conclusion:** The study confirms that *Combretum hispidum* leaf extract contains a diverse range of bioactive phytochemicals with measurable antimicrobial activity, supporting its ethnomedicinal use. The results suggest its potential as a source of plant-derived antimicrobial agents. Further studies should explore the isolation and characterization of the active compounds, evaluate their mechanisms of action, and assess in vivo efficacy and safety to facilitate the development of standardized phytomedicines from *C. hispidum.* |

*Keywords: Combretum hispidum, phytochemicals, antimicrobial activity, GC-MS analysis*

1. INTRODUCTION

Medicinal plants remain a cornerstone of healthcare systems globally, especially in low-resource settings where access to modern medicine is limited. The World Health Organization (WHO) estimates that approximately 80% of populations in developing countries rely on traditional medicine for primary healthcare, with plant-based remedies forming the core of these practices (WHO, 2018; Akinyemi *et al*., 2018). This dependence arises from factors like affordability, cultural relevance, and perceived reduced side effects compared to synthetic pharmaceuticals (Monib, 2024). In resource-constrained regions, traditional medicinal plants provide critical therapeutic options due to economic barriers and inadequate healthcare infrastructure (Karunamoorthi *et al*., 2013; Akinyemi *et al*., 2018). Indigenous communities have preserved extensive ethnobotanical knowledge, utilizing over 50,000 identified plant species to treat diverse ailments through empirically validated practices (Monib, 2024; IUCN, 2023). These plants are repositories of bioactive phytochemicals such as alkaloids, flavonoids, and terpenoids which exhibit antimicrobial, anti-inflammatory, and antioxidative properties (Gu *et al*., 2014). Modern drug discovery increasingly leverages these compounds, isolating them for development into novel pharmaceuticals or using them as templates for synthetic analogs (Gu *et al*., 2014; Karunamoorthi *et al*., 2013).

One of the most pressing global health challenges today is antimicrobial resistance (AMR). AMR has escalated into a severe crisis, with resistant pathogens causing an estimated 1.27 million deaths in 2019 and contributing to nearly 5 million fatalities overall (Murray *et al*., 2022). The World Health Organization categorizes AMR as one of the greatest threats to health, food security, and development, as misuse and overuse of antibiotics especially in low- and middle-income countries continue to drive resistance. Predictions estimate that, by 2050, AMR could claim up to 10 million lives annually and incur economic losses up to USD 100 trillion, intensifying the urgency for discovering new antimicrobial agents (Chanel and Doherty, 2020).

This dire therapeutic landscape underscores an urgent need for novel antimicrobial compounds, particularly from natural sources. Plants produce complex phytochemicals such as flavonoids, alkaloids, tannins, and saponins that have evolved as defense mechanisms against microbial pathogens (Robino, 2024; Ushie *et al*., 2019). These compounds exhibit diverse mechanisms of action, including disruption of bacterial cell membranes, inhibition of energy metabolism, and interference with nucleic acid synthesis, making them promising candidates against multidrug-resistant strains (Sweet *et al*., 2023; Robino, 2024; Ushie *et al*., 2019). For instance, phytochemical screening of plants like *Terminalia rhynchocarpum* has revealed extracts with minimum inhibitory concentrations (MIC) as low as 0.48 μg/mL against *S. aureus* and *E. coli*, surpassing the efficacy of conventional antibiotics like gentamicin (Dubale *et al*., 2023). Similarly, thymol—a monoterpene phenol demonstrates broad-spectrum activity, reducing viable populations of *Salmonella enterica* by 66.8% and *Listeria monocytogenes* by 70.2% at 0.5 mg/mL concentrations (Sweet *et al*., 2023). These phytochemicals offer a chemically diverse scaffold for developing new antimicrobials, with synergistic interactions enhancing their potency against resistant pathogens (Nascimento *et al*., 2000).

Within this context, *Combretum hispidum* emerges as a promising candidate for scientific exploration. It is a shrub species within the Combretaceae family, a taxon comprising approximately 600 species of trees, shrubs, and lianas predominantly distributed across tropical regions (Ogbole *et al*., 2016; de Morais Lima *et al*., 2012). Ethnomedical practices in West Africa historically utilize this plant for treating infections, fever, wound management, and diarrheal diseases, aligning with broader regional applications of *Combretum* species as antimicrobial agents (Silén *et al*., 2023; Ejidike *et al*., 2023). Prior phytochemical analyses of congeners like *Combretum micranthum* reveal 155 bioactive compounds—including flavonoids, phenolic acids, and alkaloids that demonstrate broad-spectrum antibacterial and antiviral activities (Tine *et al*., 2024; Taura *et al*., 2009). Similarly, *Combretum molle* bark extracts exhibit significant efficacy against Gram-positive and Gram-negative pathogens, with acetone extracts showing minimum inhibitory concentrations as low as 1.25 mg/mL against *Streptococcus pyogenes* and *Escherichia coli* (Ally, 2021; Parusnath *et al*., 2023). These findings not only affirm the antimicrobial potential of *Combretum* species but also support the hypothesis that *C. hispidum* may harbor similar or even novel bioactive agents.

Despite this promise, *Combretum hispidum* remains largely uncharacterized in terms of phytochemistry and in vivo pharmacological validation. This study is justified because, despite ethnopharmacological evidence, *C. hispidum* has yet to undergo comprehensive phytochemical profiling or in vivo pharmacological validation to substantiate its traditional antimicrobial uses.

This study therefore aims to evaluate the phytochemical composition and antimicrobial potential of *Combretum hispidum* leaf extract thereby contributing to the search for novel plant-based therapeutic agents.

2. material and methods

**2.1 Collection and identification of the plant**

Fresh leaves of *C. hispidum* were plucked from a farm settlement in Ahaba Oloko in Ikwuano Local Government Area, Umuahia Abia State, Nigeria and were authenticated at the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike in the month of June, 2022 by Dr. Emmanuel Udoka. The leaves were washed with distilled water and spread on a clean mat in a well-ventilated room with regular turning to enhance even drying and to avoid decaying. Drying of the leaves took place over a period of 21 days. Plant material was validated on <http://www.worldfloraonline.or/taxon/wfo-0001328093>, accessed on: August, 7th 2024. Dried sample of the material was assigned voucher number MOUAU/ZEB/HERB/22/002 and preserved in the herbarium of the Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike.

**2.2 Preparation of plant extracts**

The leaf extract was prepared using a soxhlet extractor. Briefly, fresh leaves were dried under shade for 14 days, after which they were pulverised to fine powder using a manual blender. Eighty (80) grams of the powdered sample was introduced into the extraction chamber of the soxhlet extractor and extraction was done using ethanol as solvent. Temperature was maintained at 70oC through-out the extraction period of 48 hours. At the end of the period, the collected extract in ethanol was dried in a laboratory oven at 40oC to obtain a brown pasty extract which weighed 8.89 g representing an extract yield of 11.11%.

**2.3 Phytochemical analysis of *C. hispidum* leaf extract**

Qualitative and quantitative phytochemical studies of *C. hispidum* leaf extract were carried out using the methods of Harborne (1998) as reported by Deka and Kalita, (2012), while gas chromatography mass spectrometry (GCMS) analysis of the extract was carried out as was reported by Ukpai *et al*., (2023).

**2.4 Determination of antimicrobial activity of *C. hispidum***

**2.4.1 Microorganisms**

The Microorganisms used were the strains of *Esherichia coli* (ATCC 25922 gram negative), *Staphylococcus aureus* (ATCC 25923 gram positive), *Enterococus fecalis* (ATCC 7080 gram positive), *Pseudomonas aeruginosa* (ATCC 27853 gram negative) and *Salmonella typhi* (clinical isolate). The organisms were obtained from the Center for Molecular Biosciences and Biotechnology, Michael Okpara University of Agriculture, Umudike.

**2.4.2 Reactivation of stock cultures of test organisms**

The test organisms were reactivated from nutrient agar slants onto freshly prepared nutrient agar plates. A cell suspension of each micro­organism was prepared by transferring 3-5 colonies from the nutrient agar plates to a sterile bottle containing physiological saline. The turbidity of the suspension was adjusted to 0.5 McFarland turbidity standards with sterile physiological saline.

**2.4.3 Inoculation of test organisms**

Plates of Mueller-Hinton agar (MHA) were prepared following the manufacturer's directives. The MHA plates were allowed to set and using a sterile swab stick, the cell suspension of each test organisms was aseptically spread on the agar surface.

**2.4.4 Testing for anti-microbial activity**

The Kirby-Bauer disc diffusion technique was employed for anti-microbial testing (Yao *et al*., 2021). This is a method used to determine the sensitivity of microorganisms to specific antimicrobial drugs. Greater drug efficacy yields larger microbe-free zones surrounding drug-containing disks after overnight growth on solid media. The method involves the placing of antimicrobial impregnated paper discs onto the surface of agar which has previously been seeded with the bacteria to be tested. The antimicrobial agent subsequently diffuses into the agar where it may inhibit bacterial growth in a zone surrounding the disc.

Paper disc measuring 6mm in size, obtained by perforating filter paper (Whatmann No l), were sterilised in hot air oven set at 140°C for 1 hour. Thereafter, each paper disc was impregnated with 20µ of the plant extracts and fractions solutions at the various concentrations of I mg/ml, l0 mg/ml and l00 mg/ml. The discs were left in an incubator set at 50°C to dry. Gentamicin discs and discs impregnated with DMSO were also set up as controls. Each treatment was replicated twice.

The discs containing the extracts and the control were placed on the MHA plates where the respective test organisms have been inoculated and the plates appropriately labelled.

The plates were incubated at 37°C for 24 hours the diameter of any clear zone obtained around the discs were measured with a metre rule. This was according to the method of Strika *et al*., 2017 and Ikpeama *et al*., 2014.

**2.4.5 Determination of minimum inhibitory concentration**

Minimum inhibitory concentration (MIC) is the lowest concentration of an anti­microbial drug that prevents visible bacteria growth of a microorganism after overnight incubation in a media. For each test micro-organism to be studied 126 test tubes with each containing 1 ml of sterile Mueller Hinton broth were set up 18 test tubes in a row of 7 rows; One milliliter of the plant extract and of the fraction (100 mg/ml) was then respectively added in triplicate to the test tubes in the 1st row. These were serially diluted row by row by transferring 1ml from each test tubes in the first row to each test tubes of the 2nd row serially and from each test tubes in the second row 1 ml was taken and serially transferred to the test tubes in the 3rd row down to the last test tube of the 7th row from where 1 ml was taken and discarded. The 1st row down to the 7th row contained concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.13 mg/ml, 1.51 mg/ml and 0.76 respectively. Similarly, the same procedure was carried out for gentamicin (positive control) during which 1 ml of 0.005 mg/ml of dissolved gentamicin was taken in triplicate into test tubes labelled antibiotic control containing 1 ml of Mueller Hinton broth, 1 ml was taken out and discarded. Two sets of negative control were prepared; the first set of three test tubes containing 1 ml of Mueller Hinton broth and no plant extract was labelled as organism control and another three sets containing 1 ml of Mueller Hinton broth and 1 ml of DMSO labelled DMSO control. Each of the test tubes was inoculated with 50 µ of the test bacterial suspension except the sterile control (containing only Mueller Hinton broth). All test tubes were incubated at 37°C for 18 hours. The MIC was defined as the lowest concentration that prevented visible growth of the organisms in the test tubes.

The MIC values were confirmed by streaking the incubated culture tubes on freshly prepared nutrient agar plates. Scanty growth on the plate around the point of streak suggested MIC while complete absence of growth was indicative of MBC (Minimum Bactericidal Concentration).

**2.5 Statistical analysis**

Results were presented as mean values ± standard deviations (mean ± SD). The replicates in each treatment were subjected to one-way analysis of variance (ANOVA) and the difference between the samples mean were tested by LSD *post-hoc* test using Statistical Package for Social Sciences (SPSS) software version 25. P-values ≤ 0.05 were considered statistically significant.).

3. results and discussion

**3.1 Phytochemical composition of *C. hispidum***

The most abundant phytochemical found in leaf extract of *C. hispidum* was alkaloids (37.14±0.39 mg/100 g) followed by flavonoids (19.73±0.61 mg/100 g), phenols (14.36±0.33 mg/100 g) and saponins (12.71±0.16 mg/100 g) while the least in abundance was glycosides (4.90±0.09 mg/100 g). Others were tannins (7.86±0.46 mg/100 g), steroids (6.82±0.14 mg/100 g) and terpenoids (5.29±0.09 mg/100 g) (Table 1). The chromatogram of the mass spectra result ofthe extract presented in Fig. 1 showed the presence of 23 bioactive compounds: 2,4-Di-tert-butylphenol, 13-Octdecenal, 17-pentatriacontene, 1-Decanol, 1-Docosene, 1-Ethanone, 1-Nonadecene, 3-Eicosene, 9,12-Octadecadienal, 9-Octadecenal, 9-Octadecenoic acid, Benzene, Carbonic acid, Cyclohexadecane, Dibutyl phthalate, Erucic, Heptane, Hexadecane, Hydroxylamine, Oleic acid, Palmitic acid, Tetracosane and Undecane with oleic acid as the most occurring member (Fig. 1).

The phytochemical composition of *Combretum hispidum* leaf extract in this study aligns with findings from other studies on *C. hispidum* and related species, which consistently report the presence of these key secondary metabolites known for their antimicrobial and pharmacological properties. For instance, Ikpeazu *et al*. (2020) identified nineteen bioactive compounds in *C. hispidum* leaves via GC-MS, including phenolic compounds and fatty acids such as oleic acid, which was also the most prevalent compound in the chromatogram of the present study. The presence of oleic acid and other fatty acids corroborates their role in antimicrobial activity, as these compounds can disrupt microbial membranes and inhibit pathogen growth. Comparatively, GC-MS analysis of *C. hispidum* roots by other researchers also revealed diverse bioactive compounds, including benzoic acid derivatives and triazine compounds, highlighting the chemical complexity within different plant parts and supporting the ethnomedicinal use of the species (Ikpeazu *et al*., 2020). This chemical diversity is consistent with the genus *Combretum*, where species such as *Combretum molle* have been reported to contain over 200 isolated compounds, including alkaloids, flavonoids, tannins, and terpenoids, all contributing to their antimicrobial and antioxidant activities (Parusnath *et al*., 2023; Mathipa *et al*., 2022). The quantitative abundance of alkaloids and flavonoids in *C. hispidum* leaves parallels findings in other *Combretum* species, where these phytochemicals are principal contributors to antimicrobial efficacy. The detection of tannins, steroids, and terpenoids in moderate quantities also aligns with previous phytochemical screenings of *Combretum* species, which emphasize their synergistic roles in antimicrobial and anti-inflammatory effects. For example, tannins have been shown to precipitate microbial proteins and inhibit enzymes, while terpenoids disrupt microbial membranes, collectively enhancing antimicrobial potency (Mathipa *et al*., 2022). The relatively lower concentration of glycosides in *C. hispidum* is consistent with reports from other *Combretum* species, where glycosides are often present but not dominant (Mathipa *et al*., 2022).

**Table 1: Phytochemical content of *C. hispidum* ethanol leaf extract**

|  |  |  |
| --- | --- | --- |
| Phytochemicals | Qualitative test inferences | Quantitative availability (mg/100 g) |
| Saponins  | ++ | 12.71±0.16 |
| Tannins  | + | 7.86±0.46 |
| Phenolic  | ++ | 14.36±0.33 |
| Flavonoids  | +++ | 19.73±0.61 |
| Steroids  | + | 6.82±0.14 |
| Terpenoids  | + | 5.29±0.09 |
| Glycosides  |  + | 4.90±0.09 |
| Alkaloids  | +++ | 37.14±0.39 |

*\*Keys: + = present in low amount, ++ = present in moderate amount, +++ = present in high amount. Values of quantitative test results are as means ± standard deviation (n = 3).*

**Fig. 1: Chromatogram showing peaks of compounds present in *C. hispidum* ethanol leaf extract**

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**Table 2: Bioactive compounds identified in *C. hispidum* leaf extract by GCMS**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **PEAK** | **Retention****Time** | **COMPOUND NAME** | **STRUCTURE** | **% COMPOSITION** |
| 1. | 5.238  | 9,12 Octadecadienal  |  | 9.10  |
| 2.  | 6.884  | Hexadecane  |  | 1.23  |
| 3.  | 7.426  | Benzene  |  | 0.45  |
| 4.  | 8.522  | Carbonic acid  |  | 1.45  |
| 5.  | 8.878  | 17 pentatriacontene  |  | 3.58  |
| 6. | 8.985  | Undecane  |  | 3.35  |
| 7.  | 9.138  | Tetracosane  |  | 1.18  |
| 8.  | 9.430  | Carbonic acid  |  | 9.06  |
| 9.  | 9.558  | 17-Pentatriacontene  |  | 1.37  |
| 10.  | 9.621  | Heptane  |  | 3.22  |
| 11.  | 9.815  | Carbonic acid  |  | 8.64  |
| 12.  | 10.159  | Undecane  |  | 8.30  |
| 13.  | 10.579  | Hydroxylamine  |  | 2.09  |
| 14.  | 12.804  | Hexadecane  |  | 1.71  |
| 15.  | 15.708  | Hexadecane  |  | 1.72  |
| 16.  | 18.284  | Cyclohexadecane  |  | 1.78  |
| 17.  | 18.478  | Palmitic acid  |  | 1.94  |
| 18.  | 21.107  | 1-Decanol  |  | 0.93  |
| 19.  | 22.130  | 2,4-Di-tert-butylphenol  |  | 9.67  |
| 20.  | 23.442  | Erucic  |  | 2.24  |
| 21.  | 28.091  | 1-Nonadecene  |  | 2.97  |
| 22.  | 30.529  | Dibutyl phthalate  |  | 1.60  |
| 23.  | 30.704  | 3-Eicosene  |  | 2.54  |
| 24.  | 30.886  | 1-Ethanone  |  | 0.64  |
| 25.  | 32.036  | Oleic acid  |  | 0.57  |
| 26.  | 32.181  | 1-Docosene  |  | 1.27  |
| 27.  | 32.591  | 9-Octadecenal  |  | 2.05  |
| 28.  | 32.934  | 9-Octadecenal  |  | 3.41  |
| 29.  | 33.106  | Oleic acid  |  | 2.36  |
| 30.  | 33.225  | Oleic acid  |  | 0.64  |
| 31.  | 33.375  | Oleic acid  |  | 2.11  |
| 32.  | 33.679  | 9-Octadecenoic acid  |  | 1.61  |
| 33.  | 33.875  | 9-Octadecenoic acid  |  | 0.97  |
| 34.  | 34.000  | Oleic acid  |  | 0.95  |
| 35.  | 34.116  | Oleic acid  |  | 1.42  |
| 36. | 34.544  | Oleic acid  |  | 1.45  |
| 37.  | 37.065  | 13-Octdecenal  |  | -0.25  |

**3.2 Antimicrobial activity of *C. hispidum* leaf extract against some common pathogenic bacteria**

Results of antimicrobial activity of *C. hispidum* leaf extract presented in Table 3 showed that the extract significantly inhibited microbial growth in *in vitro* media with inhibition zones ranging from 2.77 – 7.58 mm. The maximum zone of inhibition diameter (7.58±0.50 mm) produced by the extract was against *Enterobacter spp* at 50% extract concentration, followed by an inhibition zone diameter of 7.33±0.45 mm against *Salmonella typhi* also at 50% extract concentration. The minimum zone of inhibition diameter of 5.83±0.98 mm was also observed against *Shigella spp* following 100% extract application.

The antimicrobial assessment of *Combretum hispidum* leaf extract exhibited dose-dependent efficacy against Gram-negative pathogens, with inhibition zones (IZDs) increasing from 6.25% to 50% concentrations before plateauing or declining—a trend that resonates with observations in other *Combretum* species. Notably, *Enterobacter* spp. showed IZDs rising from 3.21 mm to 7.58 mm, while *Klebsiella* spp. reached up to 7.05 mm. This mirrors the dose-responsive behavior reported for *C. molle* acetone extracts, where IZDs ranged from approximately 11–18 mm across various bacteria and correlated with increasing concentrations (Asres *et al*., 2006)). Furthermore, *Salmonella typhi* and *Shigella* spp. achieved IZDs above 7 mm at higher extract dosages, comparable to the inhibition of *Escherichia coli* and *Shigella* spp. by *C. molle* stem bark, which were as effective as ciprofloxacin in certain assays (Asres *et al*., 2006). The antibacterial action against both *S. typhi* and *Shigella* spp. in *C. hispidum* is consistent with prior studies noting broad-spectrum activity of *Combretum* species, especially against gastrointestinal pathogens.

The moderate but clear inhibitory activity at 12.5% and 25% concentrations supports findings in *Combretum pincianum*, where methanol extracts yielded IZDs between 10–20 mm at 25 mg/mL, along with MICs aligned with conventional antibiotics (Silén *et al*., 2023). Although *C. hispidum* produced smaller zones (~4–6 mm at mid concentrations), these results remain significant given the lower doses applied. The bactericidal capacity suggested by ZID amplitude parallels the mechanisms attributed to *C. molle* extracts, where hydrolysable tannins were identified as key antimicrobials (Ikpeazu *et al*., 2020; Asres *et al*., 2006). The rich phytochemical content in *C. hispidum* including alkaloids, flavonoids, phenols, and saponins likely contributes to its antimicrobial efficacy, supporting established roles of these compounds in membrane disruption, enzyme inhibition, and oxidative damage to pathogens. The slight decrease in IZD at 100% concentration (notably with *Enterobacter* and *S. typhi*) may reflect a plateau effect often observed in plant extract studies, possibly due to compound precipitation or microbial adaptation at higher extract loads—phenomena previously documented in dose-response analyses of crude botanical extracts (Mgonja and Ally, 2021).

**Table 3: Diameter of zones of inhibition of the extract against some bacteria isolates**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  Test isolates | IZD in mm at 6.25% | IZD in mm at 12.5 % | IZD in mm at 25% | IZD in mm at 50 % | IZD in mm at 100% |
| Control | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a |
| *Enterobacter spp* | 3.21±0.26b | 4.33±0.36c | 7.24±0.62b | 7.58±0.50c | 6.60±0.61b |
| *Klebsiala spp* | 3.08±0.21b | 4.12±0.36c | 4.51±0.39c | 6.16±0.42b | 7.05±0.59b |
| *Salmonella typhi* | 4.39±0.22c | 3.51±0.37c | 6.03±0.72b | 7.33±0.45d | 2.77±0.32c |
| *Cytrobacter* | 3.66±0.37b | 4.32±0.41b | 4.39±0.48c | 5.19±0.19e | 3.03±0.55c |
| *Shigella spp* | 3.21±0.23b | 5.58±0.34d | 3.72±0.43d | 4.98±0.33e | 5.83±0.98b |

*Values are presented as mean ± standard deviation (n = 3) and values with different superscripts are significantly (P<.05) different from any paired mean within each column. Means on the same row with different number superscripts are significantly different (P<.05) from any paired mean across the row.*

4. Conclusion

The findings of this study confirm that *Combretum hispidum* leaf extract contains diverse bioactive phytochemicals, with alkaloids, flavonoids, phenols, and saponins being the most abundant. These compounds likely contribute to its observed antimicrobial effects. The extract demonstrated dose-dependent inhibitory activity against multiple Gram-negative pathogens, reinforcing its ethnomedicinal use in treating infections. While its antimicrobial potency was moderate, the results support *C. hispidum* as a promising source of plant-based antimicrobial agents and provide a foundation for further pharmacological and toxicological investigations.

References

1. World Health Organization. (2018). *Traditional and complementary medicine in primary health care* (WHO/HIS/SDS/2018.37). <https://iris.who.int/bitstream/handle/10665/326299/WHO-HIS-SDS-2018.37-eng.pdf?sequence=1>
2. Akinyemi, O., Oyewole, S. O., and Jimoh, K. A. (2018). Medicinal plants and sustainable human health: a review. *Horticulture International Journal*, 2(4), 194–195.
3. Monib, P. N. A. W. (2024). The role of plants in traditional and modern medicine. *Journal of Pharmacognosy and Phytochemistry*, 13(2), 643–647.
4. Karunamoorthi, K., Jegajeevanram, K., Vijayalakshmi, J., and Mengistie, E. (2013). Traditional medicinal plants: a source of phytotherapeutic modality in resource-constrained health care settings. *Journal of Evidence-Based Complementary and Alternative Medicine*, 18(1), 67–74.
5. IUCN. (2023). *Cultural practice: Collection of medicinal plants* [PDF]. <https://iucn.org/sites/default/files/2023-05/practice_c.medicinal-plants_final.pdf>
6. Gu, R., Wang, Y., Long, B., Kennelly, E., Wu, S., Liu, B., ... and Long, C. (2014). Prospecting for bioactive constituents from traditional medicinal plants through ethnobotanical approaches. *Biological and Pharmaceutical Bulletin*, 37(6), 903–915.
7. Murray, C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Aguilar, G. R., Gray, A., ... and Tasak, N. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*, 399(10325), 629–655.
8. Chanel, S., and Doherty, B. (2020, September 10). 'Superbugs' a far greater risk than Covid in Pacific, scientist warns. *The Guardian*. [https://www.theguardian.com](https://www.theguardian.com/)
9. Robino, I. (2024). Exploring natural sources for new antibiotics. *Journal of Antimicrobial Agents*, 10(1), 366.
10. Ushie, O. A., Neji, P. A., Longbap, B. D., Shibdawa, M. A., and Apeh, O. B. (2019). Comparative estimation of alkaloids, flavonoids and saponin in selected medicinal plants in Taraba State. *Dutse Journal of Pure and Applied Sciences (DUJOPAS)*, 5(1b), 123–131.
11. Sweet, R., Booth, C., Gotts, K., Grove, S. F., Kroon, P. A., and Webber, M. (2023). Comparison of antibacterial activity of phytochemicals against common foodborne pathogens and potential for selection of resistance. *Microorganisms*, 11(10), 2495.
12. Nascimento, G. G., Locatelli, J., Freitas, P. C., and Silva, G. L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, 31, 247–256.
13. Ogbole, O. O., Ayeni, F. A., and Ajaiyeoba, E. O. (2016). In-vitro antibacterial screening of methanol extracts of three Combretum species against seven strains of methicillin resistant *Staphylococcus aureus* (MRSA). *Nigerian Journal of Pharmaceutical Research*, 12(2), 149–154.
14. de Morais Lima, G. R., De Sales, I. R. P., Caldas Filho, M. R. D., De Jesus, N. Z. T., de Sousa Falcão, H., Barbosa-Filho, J. M., ... and Batista, L. M. (2012). Bioactivities of the genus *Combretum* (Combretaceae): a review. *Molecules*, 17(8), 9142–9206.
15. Silén, H., Salih, E. Y., Mgbeahuruike, E. E., and Fyhrqvist, P. (2023). Ethnopharmacology, antimicrobial potency, and phytochemistry of African *Combretum* and *Pteleopsis* species (Combretaceae): a review. *Antibiotics*, 12(2), 264.
16. Ejidike, I. P., Mtunzi, F. M., Ledwaba, I., Phele, M. J., Ejidike, O. M., Ogunleye, O., ... and Eze, M. O. (2023). *Combretum* species around Africa as alternative medicine: Ethnopharmacological and ethnobotanical importance. *Journal of Applied Pharmaceutical Science*, 13(8), 012–029.
17. Tine, Y., Sene, M., Gaye, C., Diallo, A., Ndiaye, B., Ndoye, I., and Wele, A. (2024). *Combretum micranthum* G. Don (Combretaceae): A review on traditional uses, phytochemistry, pharmacology and toxicology. *Chemistry and Biodiversity*, 21(5), e202301606.
18. Taura, D., Arzai, A., and Oyeyi, T. (2009). Evaluation of antimicrobial activities of *Combretum micranthum* L. *Bayero Journal of Pure and Applied Sciences*, 2(1), 183–185.
19. Ally, M. H. S. (2021). Antimicrobial properties of velvet bush willow (*Combretum molle*) crude bark extracts on selected bacteria species. *International Journal of Medicinal Plants and Natural Products (IJMPNP)*, 7(1), 28–34.
20. Parusnath, M., Naidoo, Y., Singh, M., Rihan, H., and Dewir, Y. H. (2023). Phytochemical composition of *Combretum molle* (R. Br. ex G. Don.) Engl. and Diels leaf and stem extracts. *Plants*, 12(8), 1702.
21. Ikpeazu, O. V., Otuokere, I. E., and Igwe, K. K. (2020). GC–MS Analysis of Bioactive Compounds Present in Ethanol Extract of *Combretum hispidum* (Laws)(Combretaceae) leaves. *International Journal of Trend in Scientific Research and Development*, 4(5), 307–313.
22. Ikpeazu, O. V., Otuokere, I. E., and Igwe, K. K. (2020). Gas Chromatography–Mass Spectrometric Analysis of Bioactive Compounds Present in Ethanol Extract of *Combretum hispidum* (Laws)(Combretaceae) Root. *Communication In Physical Sciences*, 5(3), 325–337.
23. Mathipa, M. M., Mphosi, M. S., and Masoko, P. (2022). Phytochemical profile, antioxidant potential, proximate and trace elements composition of leaves, stems and ashes from 12 *Combretum* spp. used as food additives. *International Journal of Plant Biology*, 13(4), 561–578.
24. Harborne, A. J. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis*. Springer Science and Business Media.
25. Deka, A., Kalita, J. C., Singh, Y. R., and Deka, M. (2012). Determination of estrogenicity of Asoca plant (*Saraca asoca* Linn.) in adult female ovariectomized mice. *International Journal of Recent Scientific Research*, 6, 7661–7664.
26. Ikpeama, A., Onwuka, G. I., and Nwankwo, C. (2014). Nutritional composition of turmeric (*Curcuma longa*) and its antimicrobial properties. *International Journal of Scientific and Engineering Research*, 5(10), 1085–1089.
27. Mgonja, F. R., and Ally, M. H. S. (2021). Assessment of antimicrobial activity of velvet bush willow (*Combretum molle*) crude bark extracts on selected bacteria species. *[Unspecified Journal – Possibly same as ref 19]*.
28. Dubale, S., Kebebe, D., Zeynudin, A., Abdissa, N., and Suleman, S. (2023). Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. *Journal of Experimental Pharmacology*, 51–62.
29. Asres, K., Mazumder, A., and Bucar, F. (2006). Antibacterial and antifungal activities of extracts of *Combretum molle*. *Ethiopian Medical Journal*, 44(3), 269.