PHYTOCHEMICAL COMPOSITION AND ANTIMICROBIAL POTENTIAL OF *Combretum hispidum* LEAF EXTRACT ON CLINICAL BACTERIAL ISOLATES

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ABSTRACT

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| **Aim:** 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. This study aimed to evaluate the phytochemical composition, characterize the bioactive constituents, and assess the in vitro antimicrobial activity of *C. hispidum* leaf extract.**Methodology:** Fresh leaves of *C. hispidum* were collected, authenticated, shade-dried, and extracted using ethanol via Soxhlet extraction. Phytochemical constituents were determined through standard qualitative and quantitative techniques. Antimicrobial activity was evaluated against clinical and standard strains of *Enterobacter spp.*, *Klebsiella spp.*, *Salmonella typhi*, *Citrobacter spp.*, and *Shigella spp.* using the disc diffusion method.**Results:** 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. The highest zone of inhibition was observed against *Enterobacter spp.* (7.58 ± 0.50 mm at 50% concentration) and *Salmonella typhi* (7.33 ± 0.45 mm at 50%).**Conclusion:** The study confirms that *Combretum hispidum* leaf extract contains diverse bioactive phytochemicals with measurable antimicrobial properties, supporting its ethnomedicinal relevance. |

*Keywords: Combretum hispidum*, phytochemicals, antimicrobial, medicinal plants, in vitro

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 the population in developing countries relies 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 *Thalictum 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 20th 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 14 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 the cold maceration method. Fresh leaves of *C. hispidum* were dried under shade at room temperature on a laboratory bench for 14 days and ground into fine powder using a manual blender. A total of 300 g of the powdered sample was macerated in 1.5 L of 96% ethanol in a sealed glass container and left undisturbed for 48 hours at room temperature. The mixture was filtered using muslin cloth followed by Whatman No. 1 filter paper. The filtrate was evaporated to dryness at 40 °C using a hot air oven to yield a brown pasty extract.

The total weight of the dried extract obtained was 9.25 g. Extraction efficiency was calculated using the formula:

% Yield = (Weight of extract / Weight of plant material) × 100 = (9.25 g / 300 g) × 100 = 3.08%

**Figure 1: Step by step schematic diagram for extraction of *C. hispidum* leaf**



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

Qualitative and quantitative phytochemical studies of *C. hispidum* leaf extract was 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 Test Organisms**

The Microorganisms used were the strains of *Escherichia coli* (ATCC 25922 gram negative), *Staphylococcus aureus* (ATCC 25923 gram positive), *Enterococcus 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 used to assess the antimicrobial activity of the plant extract (Yao et al., 2021). Paper discs (6 mm diameter) were prepared by perforating Whatman No. 1 filter paper and sterilized in a hot air oven at 140 °C for 1 hour. Each disc was impregnated with 20 µL of the plant extract at concentrations of 1 mg/mL, 10 mg/mL, and 100 mg/mL, then dried in an incubator at 50 °C**.** Discs impregnated with DMSO alone served as negative controls. All treatments were replicated twice.

The dried discs were placed on MHA plates previously inoculated with the test organisms. Plates were labeled and incubated at 37 °C for 24 hours. After incubation, the zones of inhibition were measured in millimeters using a metric ruler. No microbial growth was observed in the negative control plates, confirming aseptic conditions and the effectiveness of sterilization procedures.

The Kirby-Bauer disc diffusion technique was used to evaluate the antimicrobial activity of the plant extract (Yao et al., 2021). Paper discs measuring 6 mm in diameter were obtained by perforating Whatman No. 1 filter paper and were sterilized in a hot air oven at 140 °C for 1 hour. Each disc was impregnated with 20 µL of the plant extract at concentrations of 1 mg/mL, 10 mg/mL, and 100 mg/mL, and then dried in an incubator at 50 °C. Discs impregnated with DMSO alone served as negative controls. All treatments were replicated twice.

The dried discs were placed on Mueller-Hinton agar (MHA) plates previously inoculated with standardized suspensions of the test organisms. The plates were appropriately labeled and incubated at 37 °C for 24 hours. After incubation, the diameter of the zones of inhibition was measured in millimeters using a metric ruler, following the procedures of Strika *et al*. (2017) and Ikpeama *et a*l. (2014). No microbial growth was observed in the negative control plates, confirming aseptic conditions and the effectiveness of sterilization procedures.

**2.4.5 Determination of minimum inhibitory concentration (MIC)**

For each test organism, 126 sterile test tubes containing 1 mL of Mueller-Hinton broth were arranged in 7 rows of 18 tubes. One milliliter of the plant extract (100 mg/mL) was added in triplicate to the test tubes in the first row. Serial two-fold dilutions were performed from the first to the seventh row by transferring 1 mL of broth-extract mixture to the next row and discarding 1 mL from the last test tube. The final concentrations of extract across rows were: 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 mg/mL.

Two negative controls were included: one set of test tubes containing 1 mL of Mueller-Hinton broth without extract (organism control), and another set containing 1 mL of broth with 1 mL of DMSO (DMSO control). All tubes were inoculated with 50 µL of standardized bacterial suspension, except the sterile control (containing only broth). All tubes were incubated at 37 °C for 18 hours. The lowest concentration (highest dilution) of extract preventing microbial growth is considered minimal inhibitory concentration (MIC) (Fatope et al., 1993).

**2.4.6 Minimum Bactericidal Concentration (MBC)**

Minimum bactericidal concentration was carried out by inoculating sample from the MIC tubes showing no bacterial growth on nutrient agar plates and was incubated at 370C for 24hrs. The plates were then observed for the presence or absence of microbial growth. The least concentration of extract showing no bacterial growth was considered the MBC (Fatope et al., 1993).

**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 sample means 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. These include 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. 2).

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, 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. 2: 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 Zone Diameters (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). While this study identified 23 bioactive compounds via GC-MS—including oleic acid, 2,4-di-tert-butylphenol, and palmitic acid, the specific compounds responsible for antimicrobial activity were not isolated. However, existing evidence suggests that oleic acid disrupts bacterial cell membranes, increasing permeability and leakage in Gram-positive bacteria (Yoon *et al*., 2018). Likewise, phenolic compounds like 2,4-di-tert-butylphenol exert antimicrobial effects by generating oxidative stress and damaging microbial cell structures (Zhao *et al*., 2020; Ayswarya *et al*., 2022). These compound-specific actions likely underpin the inhibitory effects observed in *C. hispidum* and warrant further targeted studies.

**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 |
| *Klebsiella 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 |
| *Citrobacter* | 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.

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