

ANTIBACTERIAL ACTIVITY OF *HYPTIS PECTINATA* AND *OCIMUM GRATISSIMUM* LEAVES EXTRACTS AGAINST *SALMONELLA TYPHI* AND *STAPHYLOCOCCUS AUREUS*

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Abstract

The research explored the antibacterial efficacy of leaf extracts from *Hyptis pectinata* and *Ocimum gratissimum* on *Salmonella typhi* and *Staphylococcus aureus*. Leaves were sourced from Modibbo Adama University (MAU), Yola, and stool samples were obtained at the University clinic from individuals experiencing diarrhea. Additionally, 20 nasal samples were collected from students residing in the university girls' Hostel. The investigation encompassed the preparation of culture media for stool and nasal mucosa samples, identification of *Staphylococcus aureus* colonies through diverse tests, and extraction of crude extracts via cold maceration and ethanol. The Minimum Inhibitory Concentration (MIC) of both *Hyptis pectinata* and *Ocimum gratissimum* extracts were carried out and were determined using their level of turbidity. Likewise, the Minimum Bactericidal Concentration (MBC) of both plant extracts was carried out and was also determined using their growth response. Phytochemical analysis was performed to detect saponins, alkaloid, flavonoid, phenols, tannins, and glycosides. The results underwent scrutiny using various biochemical tests.

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The study confirmed that ethanolic extracts of *Ocimum gratissimum* and *Hyptis pectinata* effectively inhibit *S. typhi* and *S. aureus*, with MICs of 100 mg/ml and 200 mg/ml, respectively. Phytochemical analyses confirmed the presence of antibacterial compounds like alkaloids, flavonoids, saponins, and tannins, which are likely to contribute to their inhibitory effects. These findings validate the traditional use of these plants in treating bacterial infections and position them as promising candidates for the development of alternative antimicrobial therapies.

Keywords: *Antibacterial agents, Hyptis pectinata leaf extracts, Ocimum gratissimum, Salmonella typhi, Staphylococcus aureus*

Introduction

Enteric infections, stemming from microorganisms like bacteria, viruses, and parasites, often result from the consumption of contaminated food or water and can be transmitted from person to person. The reported cases of enteric and food-borne illnesses to public health are believed to be only a fraction of the actual occurrences (Abebe *et al.*, 2020). Infections associated with *S. typhi*, and *S. aureus* poses significant global public health challenges. Globally, non-typhoidal *Salmonella* leads to over one billion cases of diarrhea annually, resulting in three million deaths. In developing countries, Staphylococcal infections cause around 200 million cases and over 650,000 deaths yearly, primarily affecting children and young adults (Stanaway *et al.*, 2019).

S. typhi, an obligate anaerobe with genotypic and phenotypic similarities to the *Salmonella* genus, differs in reactions to various biochemical tests (Ashurst *et al.*, 2022). It is a leading cause of foodborne illness globally, causing conditions such as salmonellosis, typhoid fever, and septicemia, manifesting as fever, diarrhea, abdominal cramps, nausea, vomiting, chills, and prostration. Salmonellosis is generally self-limiting, but severe cases can be fatal, especially for vulnerable populations (Gordon *et al.*, 2020; Mkangara, 2023). *S. aureus* is a common cause of a spectrum of skin and soft tissue infections. It is frequently isolated in surgical site infections, cutaneous abscesses, and purulent cellulitis. Hospital staff, due to their ability to carry the pathogen, play a crucial role in its transmission to patients (Masiuk *et al.*, 2021). *Ocimum gratissimum*, a native African medicinal plant, is used for culinary and medicinal purposes, with various local names such as scent leaf, “efinrin,” “nchanwu,” and “daidoya.” It is traditionally employed in treating respiratory tract infections, diarrhea, headache, ophthalmic issues, skin diseases, pneumonia, cough, fever, and conjunctivitis (Imosemi, 2020). Cultivated mainly in Nigeria, it contains phytochemicals like tannins, saponins, alkaloids, glycosides, phenols, and flavonoids (Ibeabuchi *et al.*, 2023).

S. typhi and *S. aureus* are both pathogens that can be linked to foodborne and enteric infections, though they affect different body systems and cause distinct symptoms. *S. typhi* is primarily associated with typhoid fever, a systemic infection resulting from contaminated food or water (Brockett *et al.*, 2020). It causes severe gastrointestinal symptoms and can lead to complications if untreated, making it a major foodborne pathogen globally (Khan and Shamim, 2022).

On the other hand, *S. aureus*, although often associated with skin infections, can cause food poisoning when food is contaminated by staphylococcal enterotoxins. These toxins, which are resistant to heat, can survive cooking processes, leading to foodborne illnesses when ingested. Symptoms of *S. aureus* food poisoning are generally short-lived but include nausea, vomiting, and abdominal cramping, highlighting its role as a foodborne pathogen (Andrade *et al.*, 2023).

Both pathogens underscore the importance of food safety, as they can be transmitted through improper handling or contamination of food, emphasizing the critical need for measures to prevent the spread of both enteric and foodborne diseases.

Hyptis pectinata, another Lamiaceae family plant, is found in tropical regions of North and South America and West Africa, including Nigeria. Known as bush mints, it has aromatic characteristics and has demonstrated anti-inflammatory, antioxidant, antinociceptive, and antimicrobial effects in studies (Pavel *et al.*, 2016; Mnisi *et al.*, 2022). With the increasing antibiotic resistance in *S. typhi* and *S. aureus*, finding effective and natural treatments using readily available herbal and medicinal plant extracts with minimal side effects is crucial (Arvanitakis *et al.*, 2019). This study aims to assess the antibacterial activity of *Hyptis pectinata* and *Ocimum gratissimum* leaf extracts against specific strains of *S. typhi* and *S. aureus*.

Materials and Methods

Sample Collection and Identification

Plant Material

Leaves of *Hyptis pectinata* and *Ocimum gratissimum* were collected from the botanical garden at Modibbo Adama University (MAU), Yola. The leaves were identified in the Plant Science Department of MAU. They were then washed with fresh water, rinsed, and air-dried in a shaded area until they reached a consistent weight. The dried leaves were ground into a fine powder using a sterile mortar and pestle.

Biological Samples

Stool samples were collected in sterile containers from patients with diarrhea at the MAU clinic. Nasal swabs were obtained from 20 students residing in the university girls' hostel, using sterile swabs, and transported to the microbiology laboratory for analysis.

Preparation and Culturing of Samples

Stool Sample Culture

The media was prepared according to the manufacturer's instructions. Using the streak plate method, the stool sample was inoculated onto prepared Salmonella-Shigella agar (SSA) plates. The incubation of the plates was done for 24 h at 37°C. Colonies that appeared large, colorless, or translucent with black centers on SSA were suspected of being *S. typhi*. The colonies were identified using appropriate biochemical methods for identification.

Nasal Mucosa Sample Culture

Nutrient agar was prepared following the manufacturer's guidelines. The nasal samples were inoculated on the prepared media plate using a streak plate method. Incubation of the plates was done for 24 h at 37°C. Subsequent stage involved testing colonies suspected to contain *S. aureus* using appropriate biochemical methods for identification.

Identification of Isolates

Isolates were identified using the gram staining technique, as well as the following biochemical test, coagulase, catalase, mannitol fermentation, DNase, triple sugar iron agar, indole, methyl red, urease, and citrate utilization test.

Extraction of Crude Extracts

Ethanol (99%) was utilized as the extraction solvent, following the cold maceration method as described by Nerlekar *et al.* (2024). Plant samples were initially washed with distilled water to eliminate surface contaminants, then air-dried in

a shaded, ventilated environment to prevent direct sunlight exposure and potential degradation of bioactive compounds. Once a constant weight was achieved, indicating complete moisture loss, the dried leaves were ground into a fine powder for extraction.

For the extraction process, 50 g of powdered leaf material was placed in a clean container, to which 250 ml of ethanol was added. The mixture was stirred thoroughly and sealed with aluminum foil to prevent solvent evaporation and protect from light exposure. The preparation was maintained at ambient temperature with periodic agitation over a 72-hour period. Post-maceration, the mixture was filtered first through muslin cloth and subsequently with Whatman no. 1 filter paper. The resulting filtrate was concentrated to dryness in a water bath to remove any residual solvent. The final crude extract was then stored in airtight containers for further analysis. The extraction yield was calculated using the formula below:

$$\text{Percentage (\%)} \text{yield} = \frac{\text{Weight of extract}}{\text{Weight of dry leaf materials}} \times 100 \quad (1)$$

Phytochemical Analysis

Test for Saponin

About 0.5 g of the powder (crude extract) was weighed and poured into a test tube containing 5 ml of distilled water and vigorously shaken for a few minutes, foam production that persisted for more than 10 min confirmed the presence of Saponin (Shaikh and Patil, 2020).

Test for Flavonoid

To test for the presence of flavonoids, 5 ml of the crude plant extract was measured, ensuring the extract was thoroughly filtered to remove any solid particles. Following this, 2-3 drops of sodium hydroxide (NaOH) solution were added to the extract in a test tube, which was then shaken vigorously to ensure thorough mixing. The development of an intense yellow color indicated the presence of flavonoids. For confirmation, 2-3 drops of diluted hydrochloric acid (HCl) were subsequently added to the mixture. A shift from yellow to colorless upon this addition confirmed the presence of flavonoids in the sample as described by Alamgir and Alamgir (2018).

Test for Phenols

A powdered sample (50 mg) was dissolved in 5 mL of distilled water. Two or three drops of neutral ferric chloride were then added into the test tube, and the presence of phenolic was confirmed by the dark-green coloration, as stated by Omar and Mohammed, (2023).

Test for Tannin

A few drops of ethanol ferric chloride solution were added to 0.2 g of the extract; the presence of Tannins is indicated by a blue-black to blue-green color, as described by Bankole *et al.* (2016).

Test for Glycosides

A mixture was created by combining 2 mL of glacial acetic acid, which included 2 drops of 2% FeCl₃, with the extract. This combined solution was then transferred into a separate test tube containing 2 mL of concentrated sulfuric acid. The presence of glycosides was confirmed by the formation of a brown ring at the interphase (Shaikh and Patil, 2020).

Testing for Antibacterial Susceptibility and Standardizing Inoculum for Sensitivity Analysis

To assess the antibacterial activity of the test extract, Mueller-Hinton Agar plates were inoculated with standardized microbial suspensions using the spread plate technique, distributing 1 mL of each suspension evenly across the agar surface. After a 5-10 minute drying period, a 6 mm well was created in each plate with a sterilized cork borer, and the well base was sealed with molten Mueller-Hinton Agar to prevent lateral diffusion of the test extract. Triplicate wells were prepared with 20 mg and 30 mg of the extract dissolved in 1 mL, yielding final concentrations of 20 mg/mL and 30 mg/mL, respectively. The plates were left at room temperature for 15 min to allow the extract to diffuse into the agar and were then incubated at 37°C for 24 h. Post-incubation, zones of inhibition were observed around the wells, indicating antibacterial activity, and their diameters were measured to the nearest millimeter. Ciprofloxacin (10 µg/disc) and metronidazole (30 µg/disc) served as positive controls, with distilled water as the negative control.

The sensitivity test was performed using the well diffusion method, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS). Three to five colonies of similar morphology from each agar plate were transferred to a test tube containing 5 mL of nutrient broth for sub-culturing. The turbidity of every microbial suspension was modified to attain an optical density comparable to the 0.5 McFarland turbidity standards, leading to a suspension that contained roughly 0.5×10^6 CFU/ml (Ewansiha, 2020).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Assay

The minimum inhibitory concentration (MIC) of the crude extracts was determined using the micro-tube dilution method. Serial dilutions of

the crude extracts were prepared to achieve final concentrations of 200, 100, 50, 25, 12.5, and 6.25 mg/mL. Initially, 200 mg of the crude extract was accurately weighed using an analytical balance and dissolved in 1 mL of dimethyl sulfoxide (DMSO) to create a stock solution with a concentration of 200 mg/mL. The solution was mixed thoroughly to ensure complete dissolution.

Subsequent concentrations were prepared through stepwise dilution of the stock solution with DMSO. For the 100 mg/mL concentration, 500 µL of the 200 mg/mL stock solution was mixed with 500 µL of DMSO. To prepare the 50 mg/mL concentration, 500 µL of the 100 mg/mL solution was diluted with an equal volume of DMSO. Similarly, 25 mg/mL was prepared by mixing 500 µL of the 50 mg/mL solution with 500 µL of DMSO. The 12.5 mg/mL concentration was obtained by diluting 500 µL of the 25 mg/mL solution with 500 µL of DMSO, and finally, 6.25 mg/mL was prepared by mixing 500 µL of the 12.5 mg/mL solution with 500 µL of DMSO. The DMSO concentration remained consistent throughout the dilution series.

The sterile nutrient broth was used as the negative control, with no inoculation, and dispensed into separate test tubes. The MIC was identified as the lowest concentration of the extract that exhibited no visible turbidity post-incubation when compared with the control.

For the determination of the minimum bactericidal concentration (MBC), aliquots from tubes that showed no visible turbidity, starting from the MIC concentration, were sub-cultured onto freshly prepared Mueller-Hinton agar plates. The plates were incubated at 37°C for 18-24 h, and the MBC was determined as the lowest concentration at which no bacterial growth was observed.

Correlation Analysis for Bacterial Type

To examine the relationship between extract concentration and antibacterial efficacy across bacterial types, a Pearson correlation analysis was conducted. This analysis measured the correlation between the concentration of *Hyptis pectinata* and *Ocimum gratissimum* extracts and their antibacterial effects on *S. aureus* and *S. typhi*. Correlation coefficients were calculated to evaluate the strength of the relationship between extract concentration and antibacterial activity, offering insight into the effectiveness of each plant extract at varying concentrations against these bacterial types.

Statistical Analysis

Statistical methods such as Pearson correlation were used to assess the correlation between extract

concentration and antibacterial efficacy for each bacterium.

Results and Discussion

Results

The ethanol extraction of *Ocimum gratissimum* and *Hyptis pectinata* leaves resulted in yields of 80% and 90%, respectively, as shown in Table 1. These high yields reflect the effectiveness of ethanol in extracting bioactive compounds from the plants.

Phytochemical tests confirmed the presence of alkaloids, flavonoids, saponins, and tannins in both extracts. Notably, steroids were detected only in *Hyptis pectinata*, while anthraquinones were absent in both plants (Table 2).

Initial culturing and biochemical tests identified *S. typhi* and *S. aureus* as the primary

organisms for testing, with colony characteristics detailed in Tables 3 and 4.

Using the well diffusion method, the antibacterial activity of the extracts was observed at 20 mg/mL and 30 mg/mL concentrations. Higher concentrations yielded larger inhibition zones, with *Ocimum gratissimum* and *Hyptis pectinata* showing notable antibacterial effects against both bacteria (Figure 1).

The minimum inhibitory concentrations (MIC) required to inhibit bacterial growth were determined to be 100 mg/mL for *Ocimum gratissimum* against both *S. aureus* and *S. typhi*, while *Hyptis pectinata* exhibited an MIC of 200 mg/mL for the same bacteria. These findings, summarized in Table 5, indicate that *Ocimum gratissimum* demonstrates slightly higher antimicrobial potency compared to *Hyptis pectinata*. For the minimum bactericidal concentration (MBC), complete bacterial eradication

Table 1. Percentage yield of the plant extracts

S/N	Solvent	Plant sample	Actual weight of the Powder (g)	Experimental Yield (g)	Percentage of Yield (%)
1	Ethanol	<i>Ocimum gratissimum</i>	7	10	0.7
1	Ethanol	<i>Hyptis pectinata</i>	7	9	0.8

Table 2. Phytochemical constituents of *Ocimum gratissimum* and *Hyptis pectinata*

S/N	Phytochemical constituent	<i>Ocimum gratissimum</i>	<i>Hyptis pectinata</i>
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Tannin	+	+
4	Saponins	+	+
5	Steroids	-	+
6	Anthraquinones	-	-

Table 3. Morphological and biochemical characteristics of isolated organism

Parameters	A
Gram reaction	-ve
Cellular morphology	Rod-like shape, Non-capsulated, Non-spurring
Coagulase test	+
Catalase test	+
Mannitol fermentation test	+
DNase Test	+

Most likely organism

A = *S. aureus*

Table 4. Morphological and biochemical characteristics of isolated organism

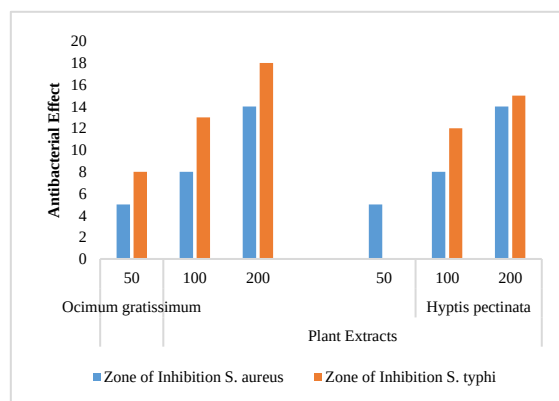
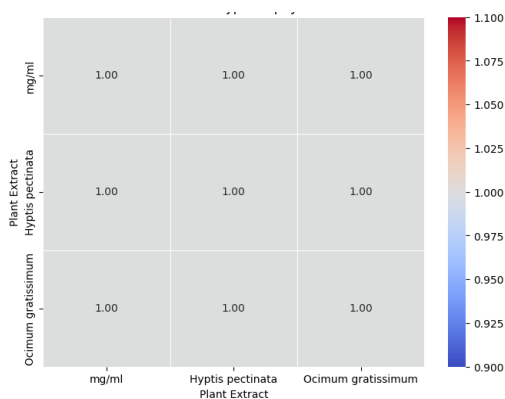
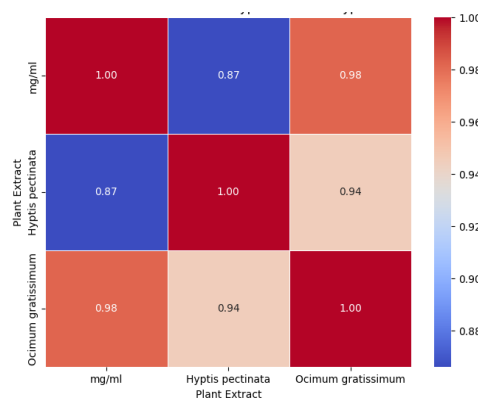
Parameters	B
Gram reaction	+ve
Cellular morphology	Round, yellow colonies with yellow coloration not sticky
Triple Sugar Iron (TSI) Agar test	H ₂ S production and Gas production
Indole test	-
Methyl red test	+
Urease test	-
Citrate utilization test	+

Most likely organism

B = *S. typhi*

Table 5. Result of Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC)

Plant Extract (mg/ml)	MIC				MBC			
	<i>Hyptis pectinata</i>		<i>Ocimum gratissimum</i>		<i>Hyptis pectinata</i>		<i>Ocimum gratissimum</i>	
	<i>S. aureus</i>	<i>S. typhi</i>	<i>S. aureus</i>	<i>S. typhi</i>	<i>S. aureus</i>	<i>S. typhi</i>	<i>S. aureus</i>	<i>S. typhi</i>
200	-	-	-	-	-	+	-	+
100	-	+	-	+	+	+	+	+
50	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	+
12.5	+	+	+	+	+	+	+	+
6.25	+	+	+	+	+	+	+	+

**Figure 1. Antibacterial Effects of *Hyptis pectinata* and *Ocimum gratissimum* Extracts on *S. aureus* and *S. typhi* at Various Concentrations****Figure 2. Correlation for *S. aureus*****Figure 3. Correlation for *S. typhi***

was achieved at 200 mg/mL for *S. aureus* with both extracts, whereas *S. typhi* required higher concentrations for bactericidal efficacy (Table 4).

Pearson correlation analysis showed a strong positive correlation between the concentration of each extract and their antibacterial efficacy. Specifically, *Ocimum gratissimum* exhibited a correlation coefficient of 0.98, while *Hyptis pectinata* demonstrated a correlation of 0.94, indicating that higher concentrations consistently increased antibacterial activity against both organisms (see Figures 2 and 3).

Discussion

The present study confirmed the antibacterial activity of *Ocimum gratissimum* and *Hyptis pectinata* extracts against *S. aureus* and *S. typhi*, corroborating their ethnopharmacological relevance. Previous studies, such as those by Omale *et al.* (2020) and Ajao *et al.* (2023), observed that these plant extracts, rich in phytochemicals like alkaloids, flavonoids, tannins, and saponins, demonstrate potent antibacterial properties. These bioactive compounds interact with bacterial cell walls and membranes, causing increased permeability and cell

lysis, as suggested by Thawabteh *et al.* (2019). Alkaloids, for example, disrupt nucleic acid synthesis by inhibiting enzymes like dihydrofolate reductase and topoisomerase, which are crucial for bacterial replication and survival.

In terms of the comparative efficacy of *Ocimum gratissimum*, recent studies have shown that flavonoids and phenolic compounds in this plant play significant roles in antibacterial mechanisms by disrupting cell membrane integrity and inducing oxidative stress in bacterial cells (Kasta, 2020). This aligns with the observed MIC and MBC values in our study, where *Ocimum gratissimum* demonstrated notable efficacy against both gram-positive and gram-negative bacteria. Studies by Masiuk *et al.* (2021) also highlight that gram-positive bacterium, such as *S. aureus*, are more susceptible to phenolic-rich extracts due to the simpler structure of their peptidoglycan layer, which allows greater penetration of bioactive compounds.

Our findings on the MIC values further corroborate previous research that found ethanolic extracts more effective than aqueous counterparts. Kasta (2020) demonstrated that ethanol, as a solvent, extracts a broader spectrum of phytochemicals, enhancing the overall antimicrobial efficacy. This could explain the greater inhibition zones observed for both *S. aureus* and *S. typhi*, with our MIC results indicating that concentrations as low as 100 mg/ml for *S. aureus* were effective. This supports findings by Saharkhiz *et al.* (2022), who reported that high ethanol concentrations yield extracts with stronger antibacterial action due to the high solubility of active compounds like flavonoids and alkaloids in ethanol, which facilitates better penetration into microbial cells.

Furthermore, studies by Ewansiha (2020) and Stanaway *et al.* (2019) provide a broader perspective on the clinical relevance of these findings, especially given the increasing antibiotic resistance of pathogens like *S. aureus* and *S. typhi*. The dose-dependent response observed in this study, with increasing concentrations of extracts correlating with enhanced antibacterial efficacy, underscores the potential of these plant extracts as alternative antimicrobial agents. Ewansiha's study highlighted similar results in a clinical context, suggesting that consistent dosing of plant-based treatments could mitigate the onset of resistance by targeting multiple bacterial mechanisms simultaneously, unlike single-target synthetic antibiotics.

The perfect positive correlations observed between extract concentration and antibacterial efficacy, especially against *S. aureus*, reflect findings by Thawabteh *et al.* (2019), who discussed the synergistic effects of multiple bioactive compounds within a single extract. This synergy is

thought to enhance antimicrobial action by concurrently disrupting different cellular processes, such as membrane stability, metabolic enzyme function, and genetic material synthesis.

In addition, the higher MIC values required to inhibit *S. typhi* compared to *S. aureus* are consistent with findings by Khan and Shamim (2022), who reported that gram-negative bacteria generally display higher resistance due to their outer membrane, which limits the diffusion of antimicrobial compounds. The findings here reinforce the potential of *Ocimum gratissimum* and *Hyptis pectinata* as natural antimicrobial candidates, especially in regions where antibiotic resistance in gram-positive bacteria presents significant public health concerns.

These results align with the growing consensus in recent literature that plant extracts with high levels of phenolic compounds can serve as effective alternatives to synthetic antimicrobials, particularly for drug-resistant infections (Andrade *et al.*, 2023). By establishing a clear link between phytochemical presence and antimicrobial activity, this study supports further exploration into optimizing extraction methods and standardizing concentrations for clinical applications.

Conclusions

In conclusion, this study demonstrated that ethanolic extracts of *Ocimum gratissimum* and *Hyptis pectinata* exhibit significant antibacterial activity against *S. typhi* and *S. aureus*. The presence of phytochemicals such as alkaloids, flavonoids, saponins, and tannins in these plants supports their traditional use in managing bacterial infections. These findings suggest the potential of these plant extracts as alternative antimicrobial agents and highlight their relevance in developing cost-effective therapeutic options. Further research is recommended to explore other bioactive components in these plants and assess their efficacy against a broader range of pathogens.

Recommendations

From the research, additional studies to explore other potential biological activities of the extracts should be conducted. Moreover, screening other morphological parts of the plants is advisable for a more comprehensive understanding of their medicinal properties. Given the easy availability of the plant and the simplicity of extracting its compounds through uncomplicated processes such as maceration or infusion, it could serve as

a cost-effective and viable alternative to conventional drugs.

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Ethical Approval Statement

The Health Research Ethics Committee of Modibbo Adama University, Yola, granted ethical approval (Ref. No. MAUTH/HREC/23/359) on March 29, 2023, for the study titled “Antibacterial Activity of *Hyptis pectinata* and *Ocimum gratissimum* Leaves Extracts Against *Salmonella typhi* and *Staphylococcus aureus*”. Approval was conditioned on obtaining informed consent, maintaining participant confidentiality, handling samples responsibly, adhering to ethical standards, and submitting the final project report.

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