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IN-VIVO AND *IN-VITRO* EFFICACY OF *Ocimum gratissimum* AND *Vernonia amygdalina* ON ALBINO RATS AND SOME CLINICAL ISOLATES

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ABSTRACT

Phytochemical activity, in-vivo and in-vitro efficacy of *Vernonia amygdalina* and *Ocimum gratissimum* on albino rats and on some clinical isolates were investigated. Fifty samples comprising of twenty five (25) urine samples and twenty five (25) stool samples were collected from male and female patients at the State Hospital Ijebu Ode, Ogun State. The samples were analysed using standard microbiological method. The *Vernonia amygdalina* and *Ocimum gratissimum* were collected from Ago-Iwoye and its environs in Ogun State. The leaves extract was subjected to preliminary phytochemical screening and antibacterial tests. These investigations revealed the presence of some constituents like; alkaloids, flavonoids, saponins and tannins. The antibacterial activity of the plant extract was assayed using the agar plate disc and well diffusion techniques. The isolated microorganisms were: *Escherichia coli, Klebsiella spp, Pseudomonas aeruginosa, Salmonella typhi*, and *Staphylococcus aureus.* The plant extracts showed antibacterial activities against the isolates at 6.25mg/ml with zone of inhibition of 1 mm (*Klebsiella sp*), *Pseudomonas aeruginosa* (2mm), *Escherichia coli* (4 mm) and *Staphylococcus aureus* with 5 mm while the extract was resistance on *Salmonella sp* at 6.25mg/ml. Both male and female (20) albino rats (*Rattus norvegicus)* were used in this experiment. The highest zone of inhibition was at 100mg/ml with *Staphylococcus aureus* showing the highest zone of inhibition with 36 mm, followed by *E. coli* (34 mm), *Pseudomonas aeruginosa* and *Klebsiella sp* (28 mm) and *Salmonella sp* (25 mm).

Keywords: Albino rats, Bacterial isolates, Phytochemical, Plant extracts, Sensitivity

INTRODUCTION

Around the world, traditional and folk medicines are crucial to health care services. Approximately 75% of people on the planet get their medical care from plants and their extracts (Sen and Chakraborty, 2017). Many people in our country rely heavily on herbal cures, especially those who live in rural areas. The majority of these herbal medicines have proven effective throughout time, especially when it comes to treating allergy, metabolic, and cardiovascular conditions (Shyam *et al.,* 2024).

In addition to having a high therapeutic potential, medicinal higher plants have been widely exploited as a source of many active ingredients for the treatment of human ailments (Afolabi *et al* 2020). Since antibiotic resistance has become a global concern, the first goal to assess the significance of these plants is the in vitro antibacterial or antifungal assay (Dagne *et al*., 2025). Due to their accessibility, affordability, demonstrated specificity, biodegradability, low toxicity, and minimal residual toxicity in the ecosystem, plant extracts and phyto-products are becoming more popular (Agu *et al*., 2024). Numerous studies have been conducted to demonstrate the antifungal and antibacterial qualities of several plant species (Dagne *et al*., 2025).

The plant *Vernonia amydalina* is commonly fully-fledged in Brazil, Nigeria, Tanzania, South Uganda, Kenya, Ethiopia, and Yemen (Bhattacharjee *et al*., 2013). Because of its bitter taste and flavor, *Vernonia amygdalina* is known as bitter leaf. It can be employed as an active anticancer, antibacterial, anti-malarial, and anti-parasitic agent (Danladi *et al*., 2018; and Dagne *et al*., 2025). Pharmacologically beneficial complex active components are found in this plant according to Habtamu and Melaku, 2018. In ethnomedicine, fever, hiccups, renal issues, and stomach discomfort are all treated using the roots and leaves according to Degu *et al*., 2024. Many West African nations, including Cameroon, Ghana, and Nigeria, employ the stem and root stripped of the bark as chew sticks (Burkill, 1985; and Adebayo *et al*., 2019). Additionally, *V. amygdalina* has been used historically in blood dressing and has been shown to significantly lower blood glucose levels at the post-prandal time point (Uchenna *et al*., 2008). According to Fasola *et al*. (2010), *V. amygdalina* exhibits hypoglycemic action. Following treatment with varying doses of the aqueous leaf extracts, they saw a dose-dependent decrease in the fasting blood sugar level in alloxan-induced diabetic rats. Additionally, *V. amygdalina* leaf extracts were shown by Yedjou *et al*. (2008) to be a DNA-damaging anticancer agent in the treatment of breast cancer.

Ocimum gratissimum is called 'efinrin' by the Yoruba's of the southwestern part of Nigeria, 'nchanwu' by the Igbo's and 'Dai'doya' by the Hausa's and generally known as scent leaf (Effraim *et al.,* 2003; Carr and Vissers, 2013). It is an herbaceous plant which belongs to the family "Labiatae". It is reported that it contains terpenoids, eugenol, thymol, saponins and alkaloids as stated by Imohiosen *et al*. (2021). *Ocimum gratissimum* is germicidal and has found widely use in toothpastes and mouth wash as well as some topical ointments (Holets *et al.*, 2003). It is used as an excellent gargle for some throats and tonsillitis. It is also used as an expectorant and a cough suppressant. The plant extract is used against gastrointestinal helminths of animal and man (Fakae, 2000). *Ocimum gratissimum* is also a suitable option for unsettled stomachs due to its carminative qualities. It is used to treat hemorrhoids and as an emetic. In vitro antifungal efficacy against some dermatophytes has already been documented for

Ocimum gratissimum. *Ocimum gratissimum's* antifungal properties were demonstrated by Lexa *et al*. (2006). At a dosage of 62.5µg/ml, the extract's chloroformic fraction inhibited 23 isolates (92%) of *Cryptococcus neoformans*.

Recent research on *Ocimum gratissimum* has demonstrated that the plant extract can be used as a source of treatment for those with AIDS, HIV, and acquired immune deficiency virus (Elujoba, 2000). It was reported by Charalampos *et al*. (2013); and Airaodion, *et al*. (2019), that *Ocimum gratissimum* has antioxidant properties which says that natural antioxidants exert a key role in maintenance of health, prevention of the degenerative and chronic diseases such as cardiac and cerebral ischema, atherosclerosis, rheumatic disorder, DNA damage and ageing, carcinogenesis, neurodegenerative disorders and diabetic.

Pharmaceutical Activities of the Plants

Anti-diabetic: Diabetes mellitus is the most prevalent endocrine condition that affects glucose homeostasis, leading to serious diabetes consequences such as retinopathy, angiopathy, nephropathy, and neurological problems because of disruptions in glucose utilization. *V. amygdalina* and *Ocimum gratissimum* are widely known for treating diabetes, according to the Ayurvedic medical system and Amsalu (2020).

Wound healing: In Ayurvedic medicine, *V. amygdalina* and *Ocimum gratissimum* is used as a wound healing. Cheklie (2020) examined the rate of wound healing enclosure and the histology of healed wounds in rats using an aqueous extract of the whole plant of *V. amygdalina* and *Ocimum gratissimum*. The findings clearly show the positive and noteworthy effects of accelerating the rate of wound healing enclosure in the experimentally induced wounds in rats.

Antidiarrheal: According to Kindie, (2023) assessed ethanol extracts of *V. amygdalina* and *Ocimum gratissimum* and found that they significantly inhibited PGE2-induced enter pooling and castor oil-induced diarrhea in rats. In rats given charcoal meal tests, these extracts also significantly decreased gastrointestinal motility. The outcomes demonstrate that all of these plant components are effective anti-diarrheal agents.

In this work, the therapeutic qualities of the plants *Ocimum gratissimum* and *Vernonia amygdalina* are investigated by a test for their antibacterial effects on specific human pathogenic bacteria belonging to the family *Enterobacteriaceae*, including *Salmonella typhi* and *Escherichia coli.* The purpose of this study is to evaluate *V. amygdalina* leaf extracts' antimicrobial efficacy against certain urinary tract infections in comparison to ciprofloxacin treatments.

MATERIALS AND METHODS

Plant Collection

The plant *Ocimum gratissimum* and *Vernonia amygdalina* leaf samples were collected from Ago Iwoye and the surrounding area in Ogun State, Nigeria. At Olabisi Onabanjo University's Department of Plant Science in Ago Iwoye, the gathered plant materials were identified. The fresh plant samples were allowed to air dry at ambient temperature before being grounded into a powdered form in the lab using a Sonik blender (Japan).

Animal Collection

At first, ethical clearance was attained from Health Research Ethics Committee of Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State, Nigeria with Ref. No. NHRC/23/05/2017, before the commencement of the research.

Twenty (20) male and female albino rats (*Rattus norvegicus*) were used for this experiment. They were purchased from the University of Ibadan animal house and breed in the university (Olabisi Onabanjo University) animal house. All well fed with standard commercial chicken grower's mash feed (Animal care feeds, Nigeria) and clean running tap water, the cages and environment were adequately cleaned on a daily bases, the rats were acclimatized to the new environment for 2 weeks before the commencement of inoculation and the treatment. The rats were tested before inducing and after inducing them with the clinical organisms.

Collection of Clinical Samples and Culturing of Microorganisms

Urine and stool samples were taken from the State Hospital in Ijebu Ode, Ogun State, Nigeria. The hospital's symptomatic patients provided the samples. This study was conducted at Olabisi Onabanjo University's Department of Microbiology laboratory in Ago-Iwoye, Ogun State. Twenty-five urine and twenty-five fecal samples, totaling fifty samples, were collected in sterile Universal bottles, transferred to the departmental laboratory, and cultured right away.

A loopful of the urine and stool samples was added to nutrient agar, MacConkay, and *Salmonella Shigella* agar that had already been prepared. The mixture was then incubated at 37 ^{0}C for a whole day. The plates were analyzed (morphological and microscopic investigation) following an overnight incubation.

Test Organisms

Clinical isolates of *Salmonella typhi* and *Escherichia coli* from urine and feces samples served as the test organisms for this investigation. For confirmation, the organisms were cultural, morphological, and biochemical characterization utilizing procedures outlined in Cheesebrought, 2022. For later usage, pure cultures of the verified isolates were preserved on slants in suitable media and refrigerated at 40 ^{0}C.

Gram Staining

Using sterile techniques, a smear was prepared from 24 hours old pure culture by adding a drop of normal saline in the middle of clean grease-free glass slide. The inoculating wire-loop was sterilized by flaming and then cooled by passing it through the flame. The colony from the culture plate was picked with the loop and emulsified. The smear was allowed to air dry and then the reverse side of the slide was passed through the flame quickly to fix the bacteria so as to avoid washing off of the bacteria cells during staining. A drop of Lugol's iodine, which acts as a mordant, was applied to the smear for 60 seconds after it had been soaked with crystal violet stain, rinsed under a slow running tap, and drained. After draining the Lugol's iodine, it was carefully cleaned under the faucet. After that, the slides were decolored for five seconds using 95% ethanol (care was taken to prevent over-decolorization). The slides were counterstained with Safranin for 60 seconds after being washed under the tap for five seconds. The stain was drained and cleaned right away. After adding oil immersion, the slides were allowed to air dry before being examined under a microscope at x100 magnification (Manasa and Thomas, 2022).

Biochemical Characterization

After subculturing each isolate, the colonies were streaked on a freshly made agar plate. For biochemical testing, colonies were selected from the recently generated isolates. According to Yadav *et al.* (2021), biochemical tests used to identify organisms include:

Catalase test: The purpose of the catalase test was to determine whether the microbe could use citrate as its only carbon source. A colony was selected from the pure culture using a sterile straight wire loop. The microorganism-containing straight wire loop was then used to pierce the citrate slant's butt, tighten the slant's cover, and incubate it for 24 to 48 hours at 37 ^{0}C. The color shift was noticed and noted.

Indole test: The indole test is a qualitative method that uses tryptophan reduction to assess a bacterium's capacity to make indole. The test organisms were inoculated in a bijou bottle with three milliliters of peptone water and nutritional broth, and they were incubated at 35–37 degrees Celsius for a maximum of twenty-four hours. Following incubation, a few drops of Kovac's reagent (0.5 ml) were added, gently shaken, and left to stand. Indole production in the tubes was detected by the pinkish to red ring-like color at the top layer, and the outcomes were noted.

Oxidase test: The oxidase test was used to identify organisms that use oxygen as an electron acceptor when reduced cytochrome C is oxidized to produce water and oxidized cytochrome C. A piece of No. 1 Whatman filter paper was placed in a petri dish with a glass slide, and a few colonies of the test organisms were added to the filter paper along with a few drops of fresh 1% aqueous solution of tetramethyl-paraphenylenediamine hydrogen chloride (oxidase reagent). A purple hue within ten seconds is indicative of a delayed positive result.

Coagulase test: This was used to identify *Staphylococcus aureus* producing the coagulase enzyme which causes plasma to clot by converting fibrinogen to fibrin. The slide method was used; a drop of sterile distilled water was placed on each of a sterile slide. Then a colony of the organism was emulsified on each spot to make two thick suspensions. A loopful of plasma was added to one of the suspensions and mixed gently. The slide was examined for clumping or clotting of the organisms within 10 seconds. Plasma was not added to the second suspension which serves as control.

Citrate test: Catalase is found in most aerobic microorganisms, this test was used to determine the ability of enzyme to produce catalase, which breaks down Hydrogen peroxide to give water and oxygen bubbles. A drop of 3% Hydrogen peroxide solution was dropped at the center of a clean-grease free slide. A colony of the isolate organism was placed on the hydrogen peroxide, after 5 seconds gas bubbles were observed which indicated the presence of catalase. This was done to differentiate between Staphylococci and Streptococci. Effervescence of gas indicated the presence of gram positive organism where H_2O_2 was broken into O_2 and H_2O; $2H_2O_2$======$2H_2O + O_2$.

Plant Extraction

In this investigation, the maceration method of extraction was used in accordance with Afolabi *et al*. (2020). In a different beaker, 100 grams (100g) of powdered *Ocimum gratissimum* and *Vernonia amygdalina* leaves were weighed and soaked in 700 milliliters of 70% ethanol and distilled water for 48 hours. The extracts were then filtered using filter paper (Whatman No. 1) and placed in a sterile conical flask. The ethonolic extract was then evaporated to dryness over a subwater bath at 70 0C, while the aqueous extract was evaporated at 80 0C. The dried extracts were weighed and kept in a refrigerator at 40°C in tight, sterile bottles with labels until needed.

Extraction efficiency % = (Final dry weight of extract/ Initial weight of dried plant material) × 100 as described by Shilpakar *et al.,* (2011).

Phytochemical Screening

Phytochemical screening was carried out on the plant samples to test for the active ingredients present in them. Plant leaves were analysed according to qualitative method of identification by Afolabi *et al*., (2020), and the test include;

Saponin

Frothing method was used to detect the presence of saponins. One gram of powdered sample were transferred into a test tube containing 10 mL of distilled water and then boiled for five minutes and then filtered. The filtrate was mixed vigorously and observed. The presence of froths indicated the presence of Saponins. The mixture was shaken vigorously and kept for 3min after which was observed. A honey comb like froth was formed and it showed the presence of saponins.

Taninns

For tannin, the Braemer's test was employed**.** One gram of the powdered sample were decocted with 10 mL of distilled water by boiling for 10 minutes and filtered while hot and allowed to cool. 0.1% Ferric chloride reagent was added to the filtrate and observed. A blue-black, green or blue green precipitate indicated presence of tannins.

Phlobatanins

One gram of powdered sample were transferred into a test tube containing 10 mL of distilled water and then boiled for five minutes and filtered. To the filtrate 5 mL of 1% HCL and boiled for 5 mins. The presence of precipitate is a positive result.

Cardiac Glycoside

One (1 ml) of glacial acetic acid and 5 % ferric chloride was added. To 2 ml of plant extract followed by the addition of 3 drops of concentrated sulphuric acid.

Anthraquinones

For anthraquinone glycosides, the borntrager's test method was employed: One gram of powdered sample were heated with 2 mL of 10% HCl for five minutes and filtered while still hot, then allowed to cool. The filtrate were partitioned with equal volume (aliquot) of chloroform and mixed gently. The presence of delicate rose-pink layer on the test solution indicated the presence of anthraquinones glycosides.

Flavonoids

One gram of powdered sample were added to 10mL of ethanol and 3 drops of $FeCl_3$ solution was added, dark green colour observed indicated the presence of flavonoid. One gram of powdered sample were added to distilled water and heated for 3 mins. It was allowed to cool and 2 mL conc. H_2SO_4 was also added, a yellow coloration disappear which shows the presence of flavonoid.

Steroids

Two ml (2 mLs) of acetic anhydride were added to 0.5 g of ethanol extract of each sample with 2 mLs H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Deoxy Sugar

Zero point five grams (0.5g) of the filtered plant extract were dissolved in 2 mls of glacial acetic acid containing one drop of ferric chloride. It was then underplay with 1ml of concentrated sulphuric acid. Violet ring was observed after few minutes, which indicates positive test.

Alkaloids

For alkaloids, one gram of the dried powdered sample were heated with 10 mL of 10% HCl on water bath for five minutes. The extract were then filtered and allowed to cool. The pH were then adjusted to about 6-7 by adding 10% ammonia and using litmus paper. The presence of turbidity or precipitation indicated presence of alkaloid.

Bacterial Sensitivity Testing Using Antibiotic Disc

Inocula measured up to 1ml from the peptone water in bijou bottle were introduced on to the surface of sterile Mueller Hinton base agar plates. It was evenly distributed by rocking the plates and allowed to dry but covered to prevent atmospheric contamination. A sterile antibacterial agent (antibiotic disc) confirmed to be sensitive to the organisms was carefully placed with a forceps on to the centre of the labelled plates of each bacterial species. The plate were incubated at 37 ^{0}C and examined growth after 24hours, zones of inhibition were measured in (mm) using transparent meter rule. Antibiotics' disc was used as control (Sossou *et al.,* 2024).

Procedure for Sensitivity Test of Plant Extracts

Tube dilution method was used for the antibacterial sensitivity testing according to Agu *et al.,* (2023). 10 tests tubes were arranged in a rack. 5ml of the sterilized broth were poured into these test-tubes, the first tube serves as control containing only the broth and inoculum. 5ml of the extract concentration were poured into the second and third tubes with the second being 100% concentration. From the third test tube, 5ml of the mixture of broth and extract stock solution were pipetted in serial dilution order resulting in different concentrations of 50 %, 25 % respectively. 1ml of the harvested organism were then poured into the test tubes and incubated overnight at a temperature of 25 ^{0}C.

The antimicrobial sensitivity of the extract was determined by the Minimum inhibitory concentration (MIC) using agar well diffusion method. The minimum inhibitory concentration is the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism after overnight incubation. The plant extracts were concentrated into, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml.

Procedure

i. The test organisms were inoculated on each plates of Muller Hinton Agar randomly
ii. An ager well diffusion was used with a standard cork borer of 6 mm in diameter; this cork borer was used to bore holes into the plates.
iii. 0.2 ml of the test solution of plants extracts from each concentration were added into the wells.
iv. The plates were incubated at 37 ^{0}C for 24 hours

After 24hours, the zones of the inhibitions were measured and documented.

Experimental Design

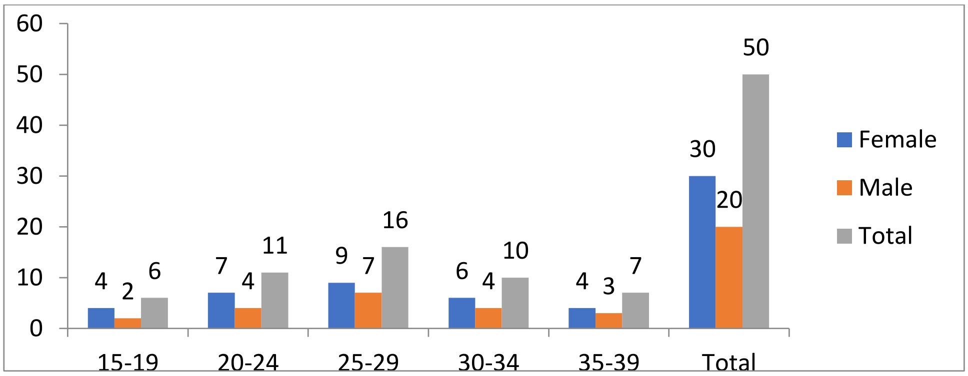
The albino rats were randomly assigned into 4 groups 'A-D' of 5 rats each. The four groups of Albino rats consisting of 5 rats per group were studied to assess the *in vivo* antimicrobial effect of the aqueous and ethanolic plants (*Ocimum gratissimum)* extract against diarrhoeagenic bacteria. Group A rats were introduced with organisms and treated with Aqueous *Ocimum gratissimum* plant extract while Group B rats were induced with the organisms only and serve as negative control, group C rats were induced with organism and treated with ethanolic *Ocimum gratissimum* plant extract, and group D received nothing. Rats in all groups were observed hourly for 3 to 4 days (incubation period) for the presence or absence of watery stool.

The rats which were randomly assigned into 4 groups A-D of 5 rats each and treated orally as follows; Group A rats were given 1ml of 18 hours broth culture of the test organisms to induce diarrhoea and later treated with 2mls of 100mg/kg of Aqueous *O. gratissimum* plant extract, group B rats were induced with organisms only, Group C were induced with organism and later treated with 2mls of 100mg/kg of ethanolic *O. gratissimum* plant extract respectively, group D rats received nothing. The treatment was repeated for each of the 2 test organisms. The treated rats were then placed in separate cages over clean sack and observed for the presence of diarrhoea everyone to seven days for the onset of diarrhoea, number of diarrhoea episodes and mortality rate (numbers of rats that died during observation period in relation to total number of rats used in each group). Incidence of loose stool that was >2 was considered proof of diarrhoea, while presence of loose stool that was = or <2 was recorded as a protection from diarrhoea.

RESULTS AND DISCUSSION

Results

This finding according to the objectives are represented below with chats and tables describing each results. Thirty (30) of the specimens were obtained from female patients while 20 were from the male patients, making a total of 50 samples. The age distribution of the patients in relation to sex from whom symptomatic specimens were obtained as represented in chat 1 below indicate that within the age range of 25- 29 years, female patient has the highest number of patients with 9 while male has 7 patients under the same age range as described in table 1 below which depict the cellular morphology of the clinical samples where urine samples appeared whitish yellow, some light pink, small and irregular colony margin while the stool samples gives blue green coloration, blackish coloration with smooth colony appearance.



Chat 1: Sample Collection in Relation to Age and Sex

Table 1: The Morphological Characteristics of the Isolates from the Clinical Sample

Sample	Cellular Appearance
Urine	Whitish yellow, small and irregular colony margin
Urine	Light pink colony
Urine	Rose pink with entire margin colony
Stool	Blackish colouration with smooth colony
Stool	Blue green colouration with smooth colony

Phytochemical Analysis

The active ingredients present in the *Ocimum gratissimum* and *Vernonia amygdalina* leaf extracts as shown in Table 2 below: to aqueous extract, *O. gratissimum* showed high concentration to saponins, and deoxy-sugar was found totally absent while alkaloid was found in a high concentration to *V. amygdalina* and phlobatanins was completely absent as well. To ethanolic extract of *Ocimum gratissimum* and *Vernonia amygdalina,* tannins was present in high concentration in the two, while Cardiac glycoside were entirely absent in the two plant extracts.

Table 2: Phytochemical Analysis of Aqueous and Ethanolic Extracts of *Ocimum gratissimum* And *Vernonia amygdalina*

Phytochemical Constituents	Aqueous Extract		Ethanolic Extract	
	O. gratissimum	***V. amygdalina***	***O. gratissimum***	***V. amygdalina***
Saponins	+++	+	-	+
Tannins	+	++	+++	+++
Phlobatanins	+	-	++	++
Cardiac glycoside	++	+	-	-
Anthraquinones	+	+	+	+
Flavonoids	+	++	+	++
Terpenes	+	+	+	+
Deoxy-sugar	-	+	+	-
Alkaloids	++	+++	++	-

Keys:

- = Not detected
+ = Present in small concentration
++ = Present in moderately high concentration
+++ = Present in high concentrations

Biochemical Analysis

The biochemical test carried out on the isolates reveal the chemical composition of each isolate, based on these analyses, the isolates were characterized and identified and represented in the table 3 below.

Table 3: Gram Reaction and Biochemical Characterization of the Isolated Microorganisms

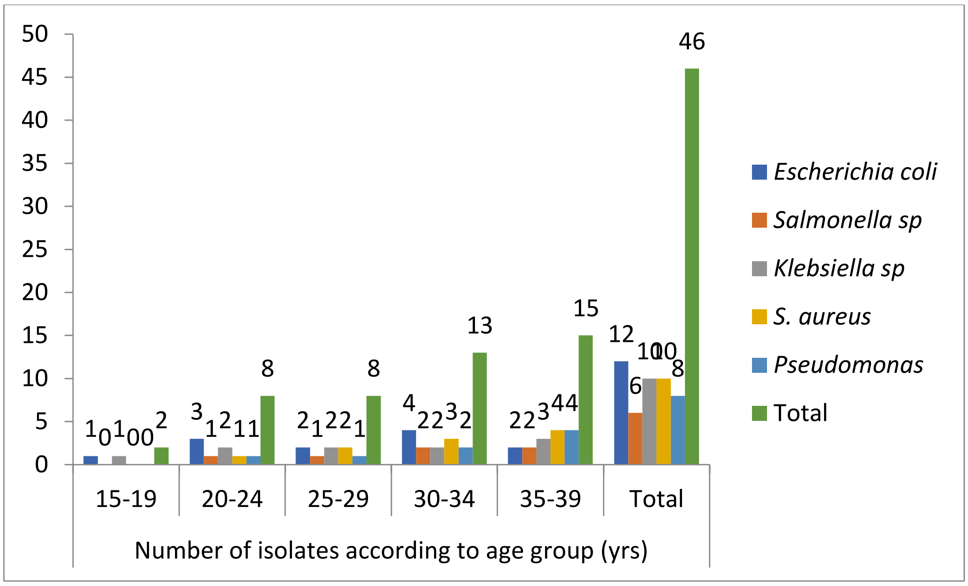
Clinical sample	Media Used	Gram reaction	Catalase	Coagulase	Citrate	Indole	Oxidase	Probable Microorganism
Urine	MacConkey agar	GNB	+	-	+	-	-	*Klebsiella sp*
Urine	MacConkey agar	GNB	+	-	-	+	-	*Escherichia coli*
Urine	Nutrient Agar	GPC in clusters	+	+	-	-	-	*Staphylococcus aureus*
Urine	Nutrient Agar	GN cocobacilli	+	-	+	-	+	*Pseudomonas aeruginosa*
Stool	SS Agar	GNB motile	+	-	+	-	-	*Salmonella sp*

Key:

(-): Negative
(+): Positive
GNB: Gram negative Bacilli
GPC: Gram positive Cocci
SSA: Salmonella Shigella Agar

Isotates According to Age Range

From the 50 samples collected 46 isolates were isolated. Twelve samples yielded *Escherichia coli;* six samples as *Salmonella*, ten samples as *klebsiella,* ten samples at *S. aureus,* while *pseudomonas* were isolated from eight samples as represented in the chat below. *Pseudomonas aeruginosa* and *Staphylococcus aureus* showed increased number to 35 to 39 age range while *Escherichia coli* to 30 to 34 age range with four isolates respectively. The isolates; *Salmonella sp, Pseudomonas aeruginosa* and *Staphylococcus* were totally absent from age 15 to 19 age range.



Chat 2: Incidence of the Organisms According to Age

Antibiotic Sensitivity Testing
The zone of inhibition of each isolates varied to different antibiotics disc. To cefotaxime, *E. coli* has 25 mm clear zones of inhibition which is the highest inhibition exhibited among all the isolates while *S. aureus* has no zones of inhibition at to the same antibiotics. Salmonella has the highest inhibition zones to ceftazidime with 24 mm; *P. aeruginosa* and *Salmonella* has the same zones of inhibition to ceftriaxone with 22 mm. However, *S. aureus* was the only isolate that showed clear zones of inhibition to amoxicillin with 20 mm while *E. coli*, *P. aeruginosa*, *Klebsiella sp*. and *Salmonella. Sp*. was not determined. These illustrations are described in the table 4.

Table 4: Zones of Inhibition of Antibiotics against the Isolates

Antibiotics	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella sp.*	*Salmonella sp.*
	Zones of inhibition (mm)				
Cefotaxime	-	25 mm	20 mm	-	20 mm
Ceftazidime	ND	-	22 mm	20 mm	24 mm
Ceftriaxone	ND	20 mm	22 mm	17 mm	22 mm
Amoxicillin	21 mm	ND	ND	ND	ND

Key:
\- (Resistant)
ND: (Not determined)

Antimicrobial Assay of Ethanolic Extracts against the Bacterial Isolates
The ethanolic extracts of the *Ocimum gratissimum* plant showed antimicrobial activities against the isolates at 6.25mg/ml with zones of inhibition of 1mm (*Klebsiella sp)*, *Pseudomonas aeruginosa* (2 mm), *Escherichia coli* (4 mm) and *Staphylococcus aureus* with 5 mm while the extract was not effective on *Salmonella sp* at 6.25mg/ml. The highest zone of inhibition was at 100mg/ml with *Staphylococcus aureus* showing the highest zone of 36 mm, then *E. coli* (34 mm), *Pseudomonas aeruginosa* and *Klebsiella sp* (28 mm) and *Salmonella sp* (25 mm).
While ethanoliac extract of *V. amygdalina* showed lower zones of inhibition to that of *O. gratissimum* at 12.5mg/ml with *S. aureus* (10 mm), *E. coli* and *Salmonella sp* (8 mm), while other microorganisms were not susceptible at the concentration of 12.5mg/ml; highest zones were shown at concentration of 100mg/ml with *Straphylococcus aureus, E. coli, Salmonella sp., Pseudomonas aeruginosa, Klebsiella sp.* (28 mm, 24 mm, 23 mm, 22 mm and 21 mm) respectively as shown in table 5.

Table 5: Zones of Inhibition of Ethanolic Extracts of *O. Gratissimum* and *V. amygdalina* at Different Concentrations

S/No	Microorganisms	EOE 100mg/ml	EVE	EOE 50mg/ml	EVE	EOE 25mg/ml	EVE	EOE 12.5mg/ml	EVE	EOE 6.25mg/ml	EVE
1	*Staphylococcus aureus*	36	28	28	22	18	17	10	10	5	-
2	*Escherichia coli*	34	24	22	19	20	14	12	8	4	-
3	*Pseudomonas aeruginosa*	28	22	20	16	15	10	7	-	2	-
4	*Klebsiella sp.*	28	21	19	19	14	10	5	-	1	-
5	*Salmonella sp.*	25	23	18	18	12	11	5	5	-	-

* All zones in mm (millimetre)

Key:
EOE (Ethanolic *O. gratissimum Extract*)
EVE (Ethanolic *V. amygdalina Extract*)

Antimicrobial Assay of Aqueous Extracts Against the Bacteral Isolates
The aqueous extract was also effective against the isolates at 12.5mg/ml for the *O. gratissum* with lowest zone of 8 mm and highest zone of 34 mm at 100mg/ml; while that of aqueous *V. amygdalina* extract is 25mg/ml except for *Staphylococcus aureus* which was susceptible to *V. amygdalina* at 12.5mg/ml (6 mm) as shown in table 6.

Table 6: Zones of Inhibition of Aqueous Extracts of *O. Gratissimum* and *V. amygdalina* at Different Concentrations

S/No	Microorganisms	AOE 100mg/ml	AVE	AOE 50mg/ml	AVE	AOE 25mg/ml	AVE	AOE 12.5mg/ml	AVE	AOE 6.25mg/ml	AVE
1	*Staphylococcus aureus*	34	25	25	20	18	14	10	6	-	-
2	*Escherichia coli*	30	22	23	18	15	10	10	-	-	-
3	*Pseudomonas aeruginosa*	26	20	22	15	12	8	8	-	-	-
4	*Klebsiella sp.*	28	21	22	15	10	7	8	-	-	-
5	*Salmonella sp.*	27	22	23	18	10	9	8	-	-	-

* All zones in mm (millimetre)

Key:
AOE (Aqueous *O. gratissimum Extract*)
AVE (Aqueous *V. amygdalina Extract*)

Discussion
Plants like *O. gratissimum* and *V. amygdalina* are widely used in Nigeria for the treatment of various diseases such as diarrhoea, skin infections, stomach disorders, wound healing, sterility and diabetics mellitus.
The active phytochemical analysis carried out on *Ocimum gratissimum* and *Vernonia amygdalina* leaf extracts indicate that saponins, Cardiac glycoside, Tannins, Flavonoids and Alkaloids are abundantly present in aqueous extracts of both plants while Tannins, Phlobatanins, Flavonoids, and Alkaloids are also present in high concentration. This work is in line with Airaodion *et al.* (2019) which indicate that saponins, alkaloids, Tannins, Phlobatannin, Flavonoid, and Phenols are present in the leave extracts of *O. gratissimum* and *Telfairia. occidentalis*.
In this present study, the clinical samples are urine and stool in which *Klebsiella sp., Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated from urine sample while *Salmonella sp.* from stool sample. The ethanolic leaf extracts of the plant showed antimicrobial activities against the isolates at 6.25 mg/ml with zone of inhibition of 1 mm (*Klebsiella sp)*, *Pseudomonas aeruginosa* (2 mm), *Escherichia coli* (4 mm) and *Staphylococcus aureus* with 5 mm while the extract was not effective on *Salmonella sp.* at 6.25mg/ml. The highest zone of inhibition was at 100 mg/ml with *Staphylococcus aureus* showing the highest zone of 36 mm, then *E. coli* (34 mm), *Pseudomonas aeruginosa* and *Klebsiella sp.* (28 mm) and *Salmonella sp*, (25 mm). Zone of inhibition varied to the tested bacteria pathogen and plant fraction extract. The ethanolic *V. amygdalina* extract showed lower zones of inhibition to the ethanolic *O. gratissimum* extracts at 12.5mg/ml with *S. aureus* (10 mm), *E. coli* and *Salmonella sp* (8 mm), while other microorganisms were not susceptible at the concentration of 12.5mg/ml, While highest zones were shown at concentration of 100mg/ml with *Staphylococcus aureus, E. coli, Salmonella sp., Pseudomonas aeruginosa, Klebsiella sp.* (28 mm, 24 mm, 23 mm, 22 mm and 21 mm) respectively. This research corresponded to the work done by Raji *et al*. (2025) that the antibacterial efficacy of *V. amygdalina* was found to be solvent-dependent, with ethanolic extracts exhibiting greater antibacterial activity than aqueous extracts.
Aqueous extract was also effective against the isolates at 12.5mg/ml for the *O. gratissimum* with lowest zone of 8 mm and highest zone of 34 at 100 mg/ml; while that of aqueous *V. amygdalina* extract is 25mg/ml except for Staphylococcus aureus which was susceptible to *V. amygdalina* at 12.5mg/ml (6 mm) in accordance to the research of Raji, *et al*. (2025) that aqueous extract showed narrow antibacterial activity, predominantly against *E. coli*, which displayed complete resistance at lower concentrations. On the other hand, at higher concentrations, zones of inhibition were observed ranging from 2.0 mm at 25 mg/mL to 10 mm at 200 mg/mL against *S. aureus*, and from 5.0 mm at 100 mg/mL to 8.0 mm at 200 mg/mL against *E. coli* isolate. Also, These results are in agreement with those reported by Evbuomwan *et al.* (2018) and David and Musyoki (2024), strengthening the reduced effectiveness of aqueous extracts in contrast to ethanolic extracts. The deprived performance of aqueous extracts in antibacterial assays may be allied to the low solubility of certain phytochemicals in water as a solvent.
The antibacterial activities of ethanolic extracts of *O. gratissimum* and *V. amygdalina* in the treatment of ailments have been previously reported. The antibacterial activity of *O. gratissimum* and *V. amygdalina* extracts could be due to the presence of all the bioactive compounds, such as flavonoids, alkaloids, tannins, saponins and setroids. This statement was also confirmed by Raji, *et al*., 2025.
The phytochemical test of the crude methanolic extract of *O. gratissimum* and *V. amygdalina* revealed the presence of alkaloids, carbohydrates, flavonoids, saponins and tannins. This relates to the research of Usunomena and Ngozi (2016), who also revealed the presence of flavonoids, alkaloids, saponins, tannins, triterpenoids, steroids, and cardiac glycosides, while Steroid and monosaccharides were found to be absent. The antibacterial activity exhibited could be due to the presence of phytochemicals (flavonoids, saponins, terpendois, phenol and tannins) and the occurrence of phenolic compounds in the extract.
This study indicates some levels of antibiotic resistance among all the bacterial isolates. The antimicrobial test carried out on the isolates using the following antibiotics: Cefotaxime, Ceftazidime, Ceftriaxone and Amoxycillin.
The result of the susceptibility test performed on the bacterial isolates showed that the gram negative bacteria (*E.coli, P. aeruginosa, Klebsiella sp* and *Salmonella sp*) are sensitive to Ceftrixome antibiotics. The gram positive organism *Staphylococcus aureus* is only sensitive to tetracycline but show no inhibition zone to the rest of the antibiotics.

CONCLUSION

In this present study, the plant extract exhibited a strong antibacterial activity against the human pathogenic organisms investigated. The study therefore, supports the therapeutic uses of the plant in traditional medicine and suggests the need to isolate, identify and characterize the active principles responsible for its activity. The antimicrobial activities of *Ocimum gratissimum* and *Vernonia amygdalina* on *Escherichia coli, Staphylococcus aureus, P. aeruginosa, K. pneumonia* and *salmonella sp* have been experimented. The result revealed that the organisms were active against *Ocimum gratissimum* extract even at 6.25mg/ml concentration, but the organism may develop resistance while *Vernonia amygdalina*, the organisms were not as active except for *salmonella sp* which showed considerable sensitivity. It is highly recommended that people infected with bacterial infections (*Salmonella sp.*) can be treated with antibiotics. Moreover, people in the rural area with less chance of getting the drug can use the plant for the treatment of bacterial infection; the chance of curing whole infection is low.

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