## Original Article

# A Comparative Study of the Antibacterial Effect of Three Ethnomedical Plants (*Ocimum gratissimum*, *Vernonia amygdalina* and *Cymbopogon citratus*) on Certain Clinical Isolates

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### **Abstract**

**Background and Aim:** Ethnomedicinal plants are used by indigenous populations all over the world as remedies for various maladies. The present study aimed at evaluating the antibacterial susceptibility of the leaves of *Cymbopogon citrates*, *Vernonia amygdalina* and *Ocimum gratissimum* against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus*.

Materials and Methods: Active agents in the leaves were extracted with methanol using the Soxhlet extraction technique. The extracts were then tested for antibacterial activity using the agar well diffusion method. MIC was determined by the tube dilution technique.

**Results:** The results revealed that the methanolic extract of *C. citrates* had the best mean zones of inhibition against *P. aeruginosa, S. aureus, E. coli* and methicillin-resistant *S. aureus*. The mean zones of the inhibition of *C. citrates* against *P. aeruginosa, S. aureus, E. coli* and methicillin-resistant *S. aureus* were  $11.5 \pm 1.5$  mm (31.25 mg/ml),  $11.5 \pm 0.5$  mm (125 mg/ml),  $12.0 \pm 1.0$  (125 mg/ml) and  $12.0 \pm 1.0$  (500 mg/ml) respectively. The activities of the extracts in relation to the activity of gentamycin (positive control) and DMSO (negative control) were also determined. The methanolic extract of *C. citrates* had the highest activity (38 %) against *Pseudomonas aeruginosa* at 31.25 mg/ml and *S. aureus* (54%) at 250 mg/ml and (52%) against MRSA at 500 mg/ml. *V. amygdalin* showed the highest activity (35%) *against E. coli* at 125 mg/ml. The therapeutic efficacy was also determined using the breakpoint of  $10 \mu g$  gentamycin (the positive control).

**Conclusion:** The findings of this study suggest that *C. citrates*, *V. amygdalina* and *O. gratissimum* could be explored by pharmaceutical companies as raw materials for the synthesis of new antibiotics for the treatment of bacterial infections, particularly as a cocktail.

**Keywords:** Ethnomedical plants, Therapeutic efficacy, *Ocimum gratissimum*, *Vernonia amygdalina* and *Cymbopogon citratus* 

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### Introduction

Ethnomedicinal plants are used by indigenous populations all over the world as remedies for various maladies. A report by the World Health Organization (WHO) revealed that surprisingly large number of the world's population, i.e. 80%, are estimated to resort to traditional medicine for their primary healthcare needs (1). Several factors, including unaffordability, potency or efficacy, ignorance, and unavailability of modern healthcare facilities, contribute to the aforementioned high dependence on these plants. In developed countries, 25% of the medical drugs are obtained from plants and their derivatives (2; 3). Likewise, the implication of this phenomenon is the necessity of conducting research on plants. Moreover, the use of traditional medical information is of considerable interest nowadays. Antimicrobial properties of plants are rooted, in part, in the compounds synthesized in the secondary metabolism of plants that might act individually, additively, or in synergy. The combined actions of these materials are likely to affect the activity of the main medicinal components by accelerating or reducing the pace of its assimilation in the body. The secondary metabolites from plants' origin contribute to the increased stability of the active compounds (phytochemicals), reduce the rate of unfavorable harmful side effects, and induce additive, potentiating, or antagonistic effects (4). The three ethnomedical plants investigated in this study included Ocimum gratissimum, Vernonia amygdalina and Cymbopogon citratus.

Ocimum gratissimum is categorized as a plant belonging to the group of herbs known as spices. This plant is an erect small plumb with many barnacles that typically are not more than 1 m high (5). It belongs to the family of Labiatae, genus Ocimum and species gratissimum (6). In traditional medicine, leaves have been utilized as tonic and anti-diarrhea agents. They have also been used for the treatment of conjunctivitis via instilling directly into the eyes. When mixed with alcohol, the leaf oil is applied as a lotion to treat skin infections, and is taken internally for bronchitis. In Nigeria, it is called Efinrin in Yoruba, Diadoyal in Hausa and Nchuanwu

in Igbo (7). Some of the components of *O. gratissimum* are alkaloids, saponins, tannins, phlobatannins, anthraquinones, steroids, terpenoids, flavonoids, and cardiac glycosides (8).

Ocimum gratissimum is extensively used in the traditional medicine. Certain Nigerian tribes use the extract of its leaves in the treatment of diarrhea, epilepsy, and high fever. Furthermore, the cold leave infusions are used in order to relieve stomach upset and hemorrhoids. They are also used in the management of the baby's cord, and also to keep the wound surfaces sterile. Moreover, they are utilized in the treatment of fungal infections, cold and catarrh (10; 11; 9). O. gratissimum is used by the Ibos of the South Eastern Nigeria. This plant is widely used in traditional medicine to cure various maladies, including upper respiratory tract infection, diarrhea, headache, disease of the eyes, skin diseases, pneumonia, cough, fever and conjunctivitis (12). The infusion of O. gratissimum leaves is used as pulmonary antisepticum, antitussivum antispasmodic agents (13). Various formulations of the leaf essential oil of O. gratissimum (Ocimum Oil) have been incorporated in a wide range of bases as topical antiseptics, and also in the treatment of minor wounds, boils and pimples (14).

As a member of the Asteraceae family, Vernonia amygdalina is a small ever green shrub that grows in tropical Africa. This shrub, whose height ranges from 1-3 m, has petiole leaf of about 6 mm in diameter and elliptical in shape (15). This plant is predominantly found in West Africa where its leaves, called bitter leaf, are the part with the highest use. The odorous leaves of this plant are dark green in color, and have a bitter flavor when chewed. However, they are delicious when cooked due to its pleasant nostalgic bitterness when it interacts with proteinous ingredients such as fresh or dry fish in the soup. This plant has several local names in Nigeria, including Kiriologbo (in Ijaw); Onugbu (in Igbo), Ewuro (in Yoruba) and Shiwaka (in Hausa). The bitterness is likely to be caused by certain determinants such as the existence of alkaloids, saponins, tannins and glycosides that are found in bitter leaves (16).

This plant is claimed to exhibit anti-helminthic and anti-malarial properties as well as anti-tumorigenic

properties (21; 22). Locally it is used in the treatment of stomachache. The expressed extract is used in treating skin infections such as ringworm, itching, rashes and eczema. The leaf extracts also exhibited antimicrobial and anti-tumorigenic properties. Flavonoids are also found in bitter leaves and three flavones-luteolin, luteolin 7-0-beta-glucuronoside and luteolin 7-0-beta-glucoside have been identified. Cymbopogon citratus, which is widely referred to as lemon grass or oil grass, is considered as a member of the Poaceae family. It is a tropical plant from South Asia that was introduced into Nigeria. The leaves of this plant are used as stimulant, sudorific, antiperiodic, and anti-catarrhal in the traditional medicine of India, whereas its essential oil is utilized as a carminative, depressant, analgesic, antipyretic, antibacterial, and antifungal agent (27; 18; 19; 20). Laboratory studies have exhibited several properties for this plant, including cytoprotective, antioxidant, and anti-inflammatory properties in vitro. Moreover, antifungal properties have been found for this plant, even though Cymbopogon martini was found to be more efficient in that research. A small, randomized, controlled trial utilized an infusion made from C. citratus as a low-cost remedial procedure for the treatment of oral thrush in HIV/AIDS patients.

A low-dose research did not find any effect of Cymbopogon citratus essential oils on the human body (33). Nevertheless, subsequent studies have indicated that the plant's essential oil could enhance gamma-Aminobutyric acid, or γ-aminobutyric acid (GABA-ergic) neuro transmission at sufficient doses with an anxiolytic threshold dose of 10 mg/kg. Even though the pharmacological activity has been confirmed, an average adult male would need 600-800 mg of the pure essential oil for experiencing the reduction of anxiety in a clinically significant level. The present study is a comparative examination of these three ethnomedicinal plants. The aim of this research was to evaluate the antimicrobial activities of the plants, their minimum inhibitory concentrations and also their antimicrobial efficacy on a spectrum of selected clinical isolates.

## **Materials and Methods**

#### The Collection of Plant Materials

Fresh leaves of C. citratus, V. amygdalina and O.

gratissimum were collected from the vicinity of Okada in Ovia North East Local Government area of Edo State, Nigeria, during May and were taken to the Department of Biological Sciences, Igbinedion University, for proper identification. The fresh leaves were washed with distilled water, and then were crushed and subsequently extracted.

#### **Extraction of Active Compounds**

The leaves of the three plant species were separately extracted with methanol. The extraction was performed with the Soxhlet apparatus. Fifty grams of the crushed leaves was extracted with 200 ml of methanol. The leaves were packed in the Soxhlet apparatus and were sandwiched between cotton wool. A water condenser was fixed to the top of the Soxhlet apparatus, and the apparatus was fitted into the neck of a flask containing 200 ml of the methanol that was continuously heated on a magnetic stirring heater. The vapor from methanol reached the Soxhlet apparatus through the side tubes, and was condensed as it was passing into the condenser. Subsequently, the condensed solvent was dropped on the sandwiched leaves, and then the required active agents were extracted. The extract was then filtered through the cotton wool in the Soxhlet apparatus into the siphon and ultimately into the flask bearing the solvent. This operation was pursued for a duration of 6 hours to obtain a significant extraction of the extractable substances. After the accomplishment of the extraction process, the extract was transferred into a clean preweighed beaker and was evaporated until a light brown oily liquid was obtained. The weight of the concentrated extract was subsequently derived, and the percentage yield was calculated by dividing the final weight of the concentrated extract by the initial weight of leaves multiplied by 100 as expressed below:

% Yield = 
$$\frac{\text{Weight of the concentrated extract}}{\text{Initial weight of leaves prior to extraction}} \times 100$$
 (1)

#### **Collection of Test Organisms**

Clinical isolates of *P. aeruginosa*, *E. coli*, *S. aureus* and methicillin-resistant *S. aureus* (MRSA) were obtained from the Igbinedion University Teaching Hospital, Nigeria.

### **Confirmation of Test Organisms**

The identity of the clinical isolates was confirmed with phenotypic tests that had been previously described by Barrow and Feltham (34).

#### **Standardization of Test Organisms**

The microorganisms were inoculated into sterile nutrient broth and incubated at 37°C for 18 to 24 hours. After the incubation, turbidity of the broth was adjusted to match 0.5 McFarland standard (equivalent to approximately  $1 \times 10^8$  cfu/ml) (23; 24).

### **Bacterial Susceptibility Tests:**

#### **Antibacterial Activity Assay**

The agar well diffusion technique was employed by Perez and Bazevque (25). Sterile Mueller Hinton agar plates were prepared, and the fresh standardized broth culture of each test organism was inoculated. Subsequently, a sterile cork borer of 5 mm diameter was used to punch 5 wells on each of the plates. One hundred micro-liters (100 µl) of each of the varying concentrations (15.6, 31.3, 62.5, 125, 250 and 500 mg/ml) of the test extracts was dropped into four of the wells, while the remaining two wells were filled with 100 µl of gentamycin (4 mg/ml) and 100 µl of sterile DMSO (10 mg/ml). The plates were then left for one hour to allow the contents in the well to diffuse into the agar, followed by incubation at 37 °C for 24 hours. The diameter zones of inhibition were then measured in millimeters (mm). The antibacterial activity (26; 31) was calculated by applying the expression below to measure the percentage activities of the extracts:

% Activity =  $\frac{\text{inhibition zone of the extract-inhibition zone of the negative control}}{\text{inhibition zone of the positive control}} > 100$  (2)

### **Determination of the Minimum Inhibitory** Concentration (MIC)

MIC was determined by the tube dilution technique according to Baron and Fingold (28). One ml of each of the varying concentrations of the test extracts was added into 9 ml of sterile nutrient broth in test tubes and inoculated with standard size of the bacterial suspension (1×10<sup>8</sup> cfu/ml). The tubes were then incubated at 37°C for 24 hours, and MIC was subsequently taken as the least concentration which showed no turbidity (visible growth of the test organisms) (29).

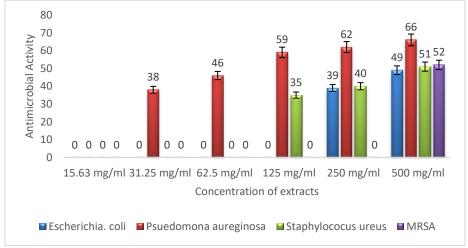
### **Determination of the Therapeutic Efficacy**

The clinical interpretation of the inhibition zones exhibited by the test extracts of C. citrates, V. amygdalina and O. gratissimum was implemented according to the guidelines prescribed by the Clinical and Laboratory Standards Institute (30). The zones of inhibition obtained from the test extracts were compared with breakpoints (interpretive criteria) stipulated for gentamycin (the positive control in this study) by the Clinical and Laboratory Standards Institute to determine the extent of susceptibility of each test extract against the respective bacteria employed in this study. Based on this comparison, the therapeutic efficacy of the test extracts reported as subsequently either sensitive susceptible, intermediately susceptible, or resistant.

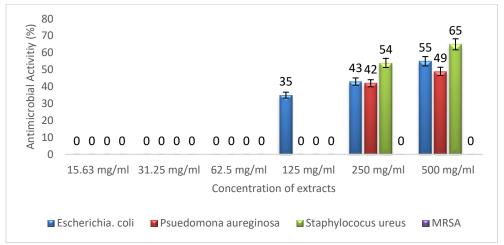
### **Results and Discussion**

#### **Percentage Yield of the Extract**

The percentage yields of the methanolic extracts of *C. citrates*, *V. amygdalina* and *O. gratissimum* were



**Figure 1.** The Antimicrobial Activity of the Methanolic Extracts of *C. citratus* (A), on *E. coli*, *P. aeruginosa*, S. *aureus* and Methicillin-Resistant *S. aureus*.



**Figure 2.** The Antimicrobial Activity of the Methanolic Extracts of *Vernonia. amygdalina* on *E. coli*, *P. aeruginosa*, *S. aureus* and Methicillin-Resistant *S. aureus*.

15.6%, 16.4% and 16.8% respectively.

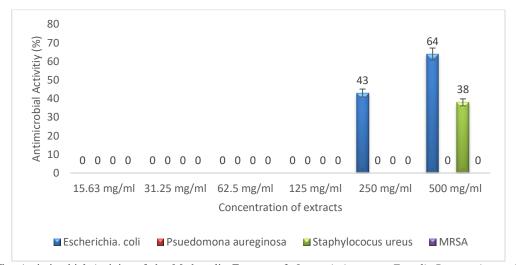
#### **Confirmation of Clinical Isolates**

The results of the phenotypic tests obtained for the test organisms (*S. aureus*, methicillin-resistant *S. aureus*, *P. aeruginosa* and *E. coli*) were significantly consistent with expected standard results for these organisms (34).

#### **Antibacterial Susceptibility**

Table 1 represents the mean zones of inhibition derived from the antimicrobial susceptibility assays. Comparatively, the methanolic extract of *C. citrates* had the only inhibition zone against *P. aeruginosa*. It

had the largest zone of inhibition compared to the other plants at a much lower concentration. There was no inhibition zone from the other extracts against any of the test organisms at that concentration or lower. *C. citrates* also had the best zone of inhibition against *s. aureus* and also exhibited inhibition zones against all the test organisms at and to MRSA. *V. amygdalina* exhibited the highest zone of inhibition against *E. coli* but had no inhibition against MRSA. *O. gratissimum* showed the poorest inhibition of the three plant extracts against *S. aureus*. All the plant extracts showed inhibitions against *E. coli* and *S. aureus*.



**Figure 3.** The Antimicrobial Activity of the Methanolic Extracts of *O. gratissimum* on *E. coli*, *P. aeruginosa*, *S. aureus* and Methicillin-Resistant *S. aureus*.

**Table 1:** The Antibacterial Susceptibility of the Methanolic Extracts of *C. citrates*, *V. amygdalina* and *O. gratissimum* on *E. coli*, *P. aeruginosa*, *S. aureus* and Methicillin-Resistant *S. aureus*.

Plants	Organisms		Me	Positive Control	Negative Control				
1 141145	O' gamsms			Gentamyci n	DMSO				
		15.63	31.25	62.50	125	250	500	4 mg/ml	10 mg/ml
	E. coli	0.0 ± 0.0	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$0.0 \pm 0.0$	13.0 ± 1.0	16.5 ± 1.5	$33.5 \pm 1.5$	$0.0 \pm 0.0$
C. citrates	P. aeruginosa	0.0 ± 0.0	11.5 ± 1.5	14.0 ± 1.0	18.0 ± 0.0	19.0 ± 1.0	20.0 ± 0.0	$30.5 \pm 1.5$	$0.0 \pm 0.0$
	S. aureus	0.0 ± 0.0	$0.0 \pm 0.0$	$0.0 \pm 0.0$	11.5 ± 0.5	13.0 ± 0.0	15.5 ± 0.5	$32.5 \pm 1.5$	$0.0 \pm 0.0$
	MR S. aureus	0.0 ± 0.0	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$0.0 \pm 0.0$	12.0 ± 1.0	$23.0 \pm 1.0$	$0.0 \pm 0.0$
	E. coli	0.0 ± 0.0	$0.0 \pm 0.0$	$0.0 \pm 0.0$	12.0 ± 1.0	15.0 ± 2.0	19.0 ± 2.0	$34.5 \pm 1.5$	$0.0 \pm 0.0$
V.	P. aeruginosa	0.0 ± 0.0	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$0.0 \pm 0.0$	12.0 ± 1.0	14.0 ± 1.0	$28.5 \pm 1.5$	$0.0 \pm 0.0$
amygda lina	S. aureus	0.0 ± 0.0	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$0.0 \pm 0.0$	18.5 ± 1.5	22.0 ± 1.0	$34.0 \pm 1.0$	$0.0 \pm 0.0$
•	MR S. aureus	0.0 ± 0.0	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$21.5 \pm 0.5$	$0.0 \pm 0.0$
	E. coli	0.0 ± 0.0	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$0.0 \pm 0.0$	13.0 ± 1.0	19.5 ± 1.5	$30.5 \pm 0.5$	$0.0 \pm 0.0$
0.	P. aeruginosa	0.0 ± 0.0	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$25.0 \pm 1.0$	$0.0 \pm 0.0$
gratissi mum	S. aureus	0.0 ± 0.0	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$0.0 \pm 0.0$	12.0 ± 1.0	$31.5 \pm 1.5$	$0.0 \pm 0.0$
	MR S. aureus	0.0 ± 0.0	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$22.5 \pm 0.5$	$0.0 \pm 0.0$

MR: Methicillin-resistant

Figure 1-3 indicate the antibacterial activity of the extracts against the different test-organisms in relation to the activity of gentamycin (positive control) and DMSO (negative control). The methanolic extract of *C. citrates* had the highest activity (38 %) against *Pseudomonas aeruginosa* at 31.25 mg/ml and *S. aureus* (54 %) at 250 mg/ml. *V. amygdalin* showed the highest activity (35%) *against E. coli* at 125 mg/ml. It also had activity (52%) against MRSA at 500 mg/ml.

The minimum inhibitory concentrations of the methanolic extracts of *C. citrates*, *V. amygdalina* and *O. gratissimum* against *E. coli*, *P. aeruginosa*, *S. aureus* and methicillin-resistant *S. aureus* have been presented in Table 2. The minimum inhibitory concentrations of *C. citrates* were 250 mg/ml, 7.8 mg/ml, 62.5 mg/ml and 500 mg/ml for *E. coli*, *P. aeruginosa*, *S. aureus* and methicillin-resistant *S. aureus* respectively. *V. amygdalina* extract had the minimum inhibitory concentrations of 125 mg/ml,

250 mg/ml and 125 mg/ml for *E. coli*, *P. aeruginosa*, and *S. aureus* respectively; while the minimum inhibitory concentrations of *O. gratissimum* extract for *E. coli* and *S. aureus* were reported to be 125 mg/ml and 500 mg/ml respectively.

#### Therapeutic Efficacy

Therapeutic efficacy (Table 3) in this context is the extent to which a given concentration of the plant extracts effectively inhibits bacterial infections by significantly inhibiting the proliferation of causative clinically irrelevant pathogens levels. Staphylococcus aureus and Escherichia coli were susceptibility at a minimum concentration of 500 mg/ml, and Pseudomonas aeruginosa was at a minimum concentration of 125 mg/ml. to C. citrates extract. Staphylococcus aureus and Escherichia coli were susceptible to V. amygdalina at a minimum concentration of 250 mg/ml. Escherichia coli was the only isolate that was susceptible to O. gratissimum at a minimum concentration of 500 mg/ml. Methicillin-

Dlama	0	Concentrations of the Extracts (mg/ml)												
Plants	Organisms	3.9	7.8	15.6	31.3	62.5	125	250	500					
	E. coli	+	+	+	+	+	+	-	-					
C. citrates	P. aeruginosa	+	-	-	-	-	-	-	-					
C. curates	S. aureus	+	+	+	+	-	-	-	-					
	MR S. aureus	+	+	+	+	+	+	+	-					
	E. coli	+	+	+	+	+	-	-	-					
V amus dalina	P. aeruginosa	+	+	+	+	+	+	-	-					
V. amygdalina	S. aureus	+	+	+	+	+	-	-	-					
	MR S. aureus	+	+	+	+	+	+	+	+					
	E. coli	+	+	+	+	+	-	-	-					
O anatiasimum	P. aeruginosa	+	+	+	+	+	+	+	+					
O. gratissimum	S. aureus	+	+	+	+	+	+	+	-					
	MR S. aureus	+	+	+	+	+	+	+	+					

**Table 2:** The Antibacterial Susceptibility of the Methanolic Extracts of *C. citrates*, *V. amygdalina* and *O. gratissimum* on *E. coli*, *P. aeruginosa*, *S. aureus* and Methicillin-Resistant *S. aureus*.

resistant *Staphylococcus aureus* was resistant to all the plant extracts.

The resistance of microorganisms to most of the antibiotics has led to more adverse clinical conditions, including morbidity and mortality induced by treatment failure. Moreover, it has resulted in the rise of health care costs. Even though a number of antibiotics are still efficient (32), the rising capability of microbes to develop resistance to several drugs has motivated researchers to conduct new studies dealing with new, safe and efficient bioactive therapeutic agents of herbal origin (32).

The antibacterial susceptibility assays (Tables 1 and 2; Figures 1, 2, and 3) performed in this study revealed that C. citrates, V. amygdalina and O. gratissimum could exhibit different forms of susceptibility to the bacterial organisms as corroborated by the antibacterial activities of these extracts (Figure 1). C. citrates had the highest zone of inhibition and activity against P. aeruginosa and methicillin-resistant S. aureus. Only C. citrate exhibited activities with MIC values less than 8 mg/ml against P. aeruginosa. When extracts exhibit activities with MIC values below 8 mg/ml, their antimicrobial activities are confirmed (41). V. amygdalina had the highest inhibition zone of 12.0  $\pm$ 1.0 mm against E. coli at a concentration of 125 mg/ml. O. gratissimum was the poorest of the three plant extracts. The methanolic extracts of these plants exhibited higher degrees of activity at higher concentrations than at lower concentrations indicating that the inhibition of bacterial growth might be dose dependent. The findings of the present research were consistent with previous studies regarding the spectrum of activity of these plant extracts. Nevertheless, our findings largely disagreed with the concentrations at which they were previously reported to have exhibited these activities (35; 37; 36; 40; 39). The antibacterial activities that were observed could be due to a combination of bioactive phytochemicals (alkaloids, cardiac glycosides, steroids, tannins, flavonoids and saponins) in these extracts (39).

These findings suggest that susceptibility differences might not be induced by cell wall structural distinctions between the categories of bacteria that were examined. Since the inhibition zones might not be commensurate to the efficacy of the extracts due to the variable diffusivity of the extracts in the agar medium, the minimum inhibitory concentration was computed (Table 2). The scientific evaluation and dissemination of the local ethnomedical preparations and prescriptions of plant origins is highly recommended. The therapeutic efficacy of plant extracts was also determined (Table 3).

Furthermore, further studies are required to be conducted concerning the botanical preparation of the traditional sources of medicinal plants in various fields, including pharmacology, phytochemistry, ethnobotany and other biological activities associated with drug recovery. Hence, traditional herbs might prove to be new antimicrobial sources with stable, biologically active ingredients that could make a scientific basis for the use of plants in modern

 $<sup>- \</sup> represents \ no \ growth \ of \ test \ organism; \ + represents \ growth \ of \ test \ organism; \ MR: \ methicillin-resistant.$ 

medicine.

## **Conclusion**

This research suggests the exploration of *C. citrates*, *V. amygdalina* and *O. gratissimum* as sources of natural products for future use in the management of bacterial infections but not against resistant strains, except at high doses that must have been pharmaceutically determined. Even though a cocktail of the plants was not explored in this study as

Table 3: The therapeutic Efficacy Exhibited by the Methanolic Extracts of the Leaves of C. citrates, V. amygdalina, and O. gratissimum against Certain Bacteria.

	C	Staphylococcus aureus				Pseudomonas aeruginosa						Escherichia coli					Methicillin-Resistant Staphylococcus aureus				
Plant Extracts	(mg/m l)	Break-Point for 10 µg Gentamycin (mm)		M (mm )	Т	Break-Point for 10 µg Gentamycin (mm)		M (mm )	Т	Break-Point for 10 µg Gentamycin (mm)		M (mm )	T	Break-Point for 10 μg Gentamycin (mm)			M (mm)	T			
		S	I	R			S	I	R			S	I	R			S	I	R		
C. citrates	500	≥ 15	13 – 14	≤ 12	15.5	S	≥ 15	13 – 14	≤ 12	20.0	S	≥ 15	13 – 14	≤ 12	16.5	S	≥ 15	13 - 14	≤ 12	12.0	R
	250	≥ 15	13 - 14	≤ 12	13.0	I	≥ 15	13 – 14	≤ 12	19.0	S	≥ 15	13 – 14	≤ 12	13.0	I	≥ 15	13 – 14	≤ 12	0.0	R
	125	≥ 15	13 – 14	≤ 12	11.5	R	≥ 15	13 – 14	≤ 12	18.0	S	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R
	62.5	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	14.0	I	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R
	31.25	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	11.5	R	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R
	15.63	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R
V. amygdalin a	500	≥ 15	13 – 14	≤ 12	22.0	S	≥ 15	13 – 14	≤ 12	14.0	I	≥ 15	13 – 14	≤ 12	19.0	S	≥ 15	13 – 14	≤ 12	0.0	R
	250	≥ 15	13 – 14	≤ 12	18.5	S	≥ 15	13 – 14	≤ 12	12.0	R	≥ 15	13 – 14	≤ 12	15.0	S	≥ 15	13 – 14	≤ 12	0.0	R
	125	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	12.0	R	≥ 15	13 – 14	≤ 12	0.0	R
	62.5	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R
	31.25	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R
	15.63	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R
O. gratissimu m	500	≥ 15	13 – 14	≤ 12	12.0	R	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	19.5	S	≥ 15	13 – 14	≤ 12	0.0	R
	250	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	13.0	I	≥ 15	13 – 14	≤ 12	0.0	R
	125	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R
	62.5	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R
	31.25	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R
	15.63	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R	≥ 15	13 – 14	≤ 12	0.0	R

S: Sensitive/Susceptible; I: Intermediate susceptibility; R: Resistant; C: Concentration of extract; M: Mean zones of inhibition exhibited by plant extracts; T: Therapeutic efficacy of plant extracts at different concentrations

commonly carried out traditionally, there might be a better activity if that process is undertaken. The findings could also be of commercial interest to both pharmaceutical companies and research institutes.

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### **Conflict of Interest**

The authors declare that they have no conflict of interest.

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