

Evaluation of Phytochemical Constituents and Antibacterial Activity of *Chromolaena Odorata* L. Leaf Extract against Selected Multidrug Resistant Bacteria Isolated From Wounds

ABSTRACT

Aim: To study the antibacterial effect of *Chromolaena odorata* extracts on multidrug resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* from wounds.

Place of study: University of Medical Sciences Teaching Hospital, Akure, Ondo State Nigeria, between January and June 2019.

Methodology: A total of 87 wound swabs were collected from patients of University of Medical Sciences Teaching Hospital, Akure, Air-dried and powdered *C. odorata* leaves were extracted using hot water, ethanol and methanol as extraction solvents and concentrated using a rotary evaporator. The concentrated *C. odorata* extracts were purified using chromatographic techniques. Qualitative and quantitative phytochemical screening of *C. odorata* extracts were done by standard methods. Antibiotics susceptibility pattern of bacterial isolates to a panel of ten (10) conventional antibiotics was determined by disc diffusion method.

Results: *C. odorata* methanolic extract had the highest extract yield (26.2%). From the multidrug resistance analysis, 66.7% of bacterial isolates tested had multidrug resistance index (MDRI) of 100%. *Pseudomonas aeruginosa* was susceptible to 100 mg/ml of *C. odorata* ethanolic extract but resistant (0.00 ± 0.00) to 100 mg/ml of hot aqueous *C. odorata* extract.

Conclusion: This study reveals the inhibitory activities of *C. odorata* extracts on multidrug resistant bacteria isolated from wounds and an indication of their potential in the treatment of wound infection.

Keywords: Susceptible, Inhibitory activity, Leaf extracts, Phytochemicals, Wounds.

1. INTRODUCTION

A wound is a disruption in the structure of the skin leading to the exposure of the subcutaneous tissue [1] and providing a condition suitable for microbial colonization and proliferation. The proliferation of microorganisms either from the body (endogenous) or from the environment (exogenous) in a wound causes wound infection [2]. The commonest pyogenic bacteria often associated with infected wounds are *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pneumococcus* species and coliform bacilli, such as *Escherichia coli*, *Proteus* species, *Pseudomonas aeruginosa*, other enteric bacilli and some fungi [3]. In chronic infection that are slow to heal and in pus showing no other microbes, there is a high possibility of infection with *Mycobacterium tuberculosis* and other *Mycobacteria* [2].

Originally, an antibiotic refers to an organic compound produced by a microorganism but has the potential to destroy another microorganism although presently the term also include those produced synthetically [4]. Antibiotics resistance has occurred when a microorganism is not affected by the antibiotics used against it [5]. This results majorly from the indiscriminate use of antibiotics and is a primary threat to the success of modern health care [6] and has led to the studying of medicinal plants as a conceivable means of alternative therapy [7]. For many centuries, medicinal plants have been used as medicines in the treatment of many infections and diseases either as whole plants or plant extracts [8] because of bioactive compounds present in them which offers protection against microorganisms and insects. Medicinal plants are broadly used to cure various infections and also used as a precursor for the synthesis of natural drugs. Bioactive compounds responsible for

therapeutic effects of plants are normally stored in plant cells as secondary metabolites and may vary in concentration depending on plant part, season, climate and growth phase [9].

Chromolaena odorata L. belongs to the family Asteraceae and is widespread over many parts of the world including Nigeria. The weed's common names include Siam weed, common floss flower and christmas bush [10]. *C. odorata* is commonly referred to as 'Obu inenawa' by the igbos (South-eastern Nigeria), 'ewe awolowo' by the yorubas (South-western Nigeria) and 'bienqua' among the Ijaws in the Niger Delta region of Nigeria [11]. It has been reported for its anti-inflammatory, analgesic, anthelmintic, antidiarrhoeal, astringent, antispasmodic, antihypertensive, diuretic tonic, antipyretic, heart tonic, antigonorrhoeal, antioxidants and wound healing activities [12, 13]. A decoction of the leaf is used as a cough remedy. It can also be mixed with lemon grass and guava leaves for the treatment of malaria. Extract of *C. odorata* has been reported to be effective in the treatment of sore throat and pile. Fresh juice squeezed out from its leaves can also be used to stop bleeding [11]. The decoction of the leaves and stems of *C. odorata* has been reported to be effective against the treatment of skin disease [10]. Ethanol and methanol extracts of *C. odorata* has been reported for antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Propionibacterium acnes* associated with skin infection [13]. Naidoo *et al.* [14] also reports the antimicrobial potential of *C. odorata* extracts on *Bacillus cereus*, *S. aureus*, *S. epidermidis*, *Proteus vulgaris*, *Aspergillus flavus*, *A. glaucus*, *Candida albicans* and *Trichophyton rubrum*.

The wound healing effect of *C. odorata* have been credited to saponins, tannins, essential oils and phenolic compounds present in the plant [15]. Phytochemical screening of *C. odorata* has revealed the presence of phenol, saponin, terpenoids, glycosides, steroids, tannins and flavonoids [16-18]. Flavonoids are a major group of plant phenolic compounds which act as antioxidants forming a complex with extracellular soluble proteins while terpenoids are naturally occurring organic chemicals which damages the cell membrane [18]. The problem of bacterial resistance to conventional antibiotics is growing so the outlook on its reliance in the future is still uncertain. This study investigated on antibacterial potentials of different extracts of *C. odorata* on multidrug resistant bacteria associated with wound infection.

2. MATERIALS AND METHODS

2.1 Collection of Clinical Samples and Isolation of Bacteria

Ethical approval for the collection of wound swab samples was collected from Ondo State Health Research Ethics Committee and each patient filled a consent form. Wound swabs were collected from patients in medical and surgical wards of University of Medical Sciences Teaching Hospital, Akure, Ondo State. A swab sample was aseptically collected from patients. Swabs were collected and immediately transported to the Department of Microbiology laboratory, Federal University of Technology, Akure, Nigeria. For the isolation of bacteria, wound swabs were evenly spread on prepared sterile nutrient agar plates and incubated at 37 °C for 24 hours [19]. After incubation, distinct colonies were picked from the primary isolation plates and subcultured on sterile nutrient agar plates and incubated at 37 °C for 24 hours. Pure colonies were inoculated on fresh nutrient agar slants, incubated at 37 °C for 24 hours and preserved at 4 °C for further studies. The bacterial isolates were maintained in the form of culture as collected from the Department of Microbiology, Federal University of Technology, Akure.

2.2 Presumptive of Identification of Selected Bacterial Isolates

The selected bacteria were identified using colonial morphology and biochemical characteristics. Colonial characteristics used for presumptive identification of bacterial isolates include colony opacity, colour, elevation, surface, edge and shape. Biochemical characteristics include Gram's reaction, catalase, citrate, urease, oxidase and sugar fermentation tests [20].

2.3 Collection of Typed Bacterial Isolates

Three pure typed cultures of bacterial species were collected from Federal Institute of Industrial Research, Oshodi (FIIRO), Nigeria and ascertained in Department of Microbiology, Federal University of Technology, Akure. A loopful of the bacteria was streaked on the surface of freshly prepared sterile nutrient agar plates and incubated at 37 °C for 24 hours. Colonial characteristics used for the confirmation include colony opacity, colour, elevation, surface, edge, shape and biochemical characteristics such as Gram's reaction, catalase, citrate, urease, oxidase and sugar fermentation tests [18].

2.4 Preparation of *C. odorata* Leaf Extracts

C. odorata leaves were collected from Ogbomoso, Oyo State, Nigeria in November 2018 and identified in the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure. The leaves were washed with water and air-dried at room temperature for 28 days, grinded and stored in sterile paper bags. For the extraction, organic solvent extracts were prepared by

soaking 100 g of the powdered *C. odorata* leaves in 1000 ml each of methanol and ethanol for 72 hours and stirred frequently [14]. The aqueous extract was obtained by pouring 1000 ml of hot (100 °C) distilled water on 100 g of the pulverised plant leaves and soaked in it for 24 hours and stirred frequently [21]. The solutions were sieved using muslin cloth, filtered through Whatman No. 1 filter paper, concentrated using rotary evaporator (SCI100-S) and stored at 4 °C for further studies [13]. The percentage yield of crude extract was calculated as [11]:

$$\text{Percentage Yield (\%)} = \frac{\text{Extract Yield}}{\text{Dried plant yield}} \times 100$$

2.5 Phytochemical Screening of Extracts

Phytochemical screening was carried out on the hot aqueous, methanolic and ethanolic extracts for the qualitative determination of phytochemical constituents as described by Akinmoladun *et al.* [22]. Plant extracts were screened for the presence of terpenoids, steroids, flavonoids, alkaloids, saponin, tannin, phlobatannin and phenol. Quantitative analyses of phytoconstituents in the extracts were also performed using methods described by Mir *et al.* [23].

2.6 Purification of Plant Extracts using Column Chromatography

A glass wool was fixed to the bottom of a clean column, the column was packed with silica gel and petroleum ether was passed through it for proper settling of the gel. Three grams of the extract was properly mixed with silica gel till it forms a free flowing powder, and loaded to the top of the prepared column. The packed column was eluted by gradient method using petroleum ether, chloroform, ethyl acetate and methanol as solvents [24].

2.7 Standardization of Bacterial Inoculum

A total of ten (10) *S. aureus*, twenty-seven (27) *P. aeruginosa* and thirteen (13) *K. pneumoniae* cultures were selected for this study. The bacterial inoculum was standardized to 0.5 McFarland standard using the method of Akinyemi and Ogundare [25]. A loopful of pre-cultured test bacteria was inoculated into 5 ml sterile nutrient broth and incubated for 24 hours at 37 °C. The broth was compared with the 0.5 McFarland standard for turbidity match.

2.8 Antibiotics Sensitivity Pattern of Bacterial Isolates

Susceptibility of isolated bacteria to conventional antibiotics was determined using the Kirby-Bauer (disc diffusion) method [25]. Exactly 0.1 ml of the standardized inoculum was introduced to prepared sterile Mueller-Hinton (HiMedia) Agar plates using three-way swab method. Commercially available antibiotics disc inclusive pefloxacin (10 µg), gentamicin (10 µg), ampiclox (30 µg), zinnacef (20 µg), amoxicillin (30 µg), rocephin (25 µg), ciprofloxacin (10 µg), streptomycin (30 µg), septrin (30 µg) and erythromycin (10 µg) was used to determine the sensitivity of Gram positive bacteria. Disc inclusive septrin (30 µg), chloramphenicol (30 µg), sparfloxacin (10 µg), ciprofloxacin (30 µg), amoxicillin (30 µg), augmentin (10 µg), gentamicin (30 µg), pefloxacin (30 µg), ofloxacin (10 µg) and streptomycin (30 µg) was used for the determination of sensitivity of Gram negative bacteria to antibiotics. These antibiotics discs were aseptically placed on the agar plates using sterile forceps. The Mueller-Hinton plates were incubated at 37 °C for 18 hours. After incubation, diameters of zones of inhibition were measured to the nearest millimetre (mm) using a transparent meter rule.

2.9 Resistance Profile of Bacterial Isolates

Following the method of Oladoja and Onifade [26], the resistance of bacteria to conventional antibiotics was determined following the Clinical and Laboratory Standards Institute (CLSI) recommendation for each drug. The multidrug resistance index (MDRI) was calculated using the formula:

$$\text{MDRI (\%)} = \frac{(\text{Number of antibiotics indicating resistance})}{(\text{Total number of antibiotics used})} \times 100$$

2.10 Antimicrobial activity of *C. odorata* Extracts

One gram of *C. odorata* methanolic, ethanolic and hot aqueous extracts were reconstituted in 30% Dimethyl sulphoxide (DMSO) to obtain extract concentration of 100 mg/ml. The inhibitory activity of the extract on the bacterial isolates was determined using agar well diffusion method. A volume of 0.1 ml of the standardized inoculum of the test bacteria was transferred to the surface of sterile Mueller Hinton agar plates and streaked by three way method using a sterile swab stick to achieve a confluent growth. A 6mm cork-borer was used to bore wells on the solidified agar plates and 100 µg (0.1 ml) of purified extracts was introduced into each well. These plates were incubated at 37°C for 24 h and the zones of inhibition were measured in millimetres [7, 25]

2.11 Statistical Analysis

Data were analysed using one-way analysis of variance and presented as mean \pm standard error. Statistical significance was determined at values $P \leq 0.05$ and the means were separated using Duncan's New Multiple Range Test by SPSS 20.0.

3. RESULTS AND DISCUSSION

The bacterial isolates tested against conventional antibiotics and *C. odorata* extract in this study are common bacteria associated with wound infection and has been reported as the most prevalent pathogens [27]. In this study, varied percentage yield of *C. odorata* extract was observed with methanol having the highest percentage yield as shown in Figure 1. This is in accordance with the findings of Ngo *et al.* [28] who reported highest yield of *Salacia chinensis* methanol extract compared to other solvents tested. The difference recorded on the percentage yield may be due to the difference in plant used or as a result of the varied polarity of the tested solvents; methanol and ethanol solvents are less polar compared to water. It could also be due to the disparity of the quality and quantity of their phytoconstituents.

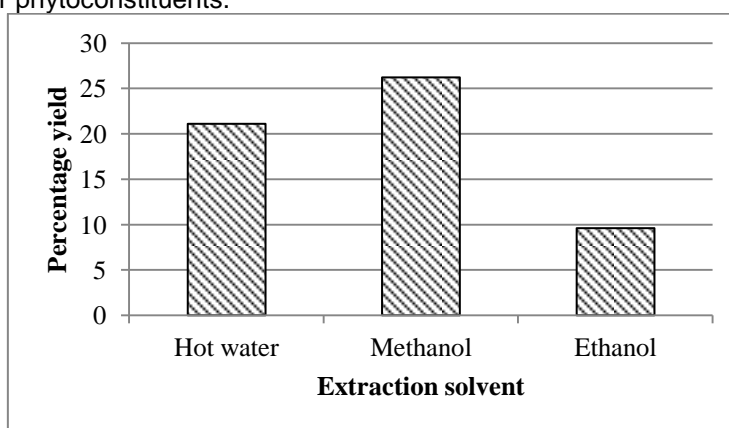


Figure 1: Percentage yield (%) of extracts of *Chromolaena odorata*

Table 1 shows the quantitative phytochemical components of *C. odorata* extracts. The qualitative phytochemical analyses of *C. odorata* extracts reveal the presence of phenol, saponin, tannin, glycosides, steroids, terpenoids and flavonoids in methanolic and ethanolic extracts. The phytochemical analyses reveal the presence of phenol, saponin, tannin, glycosides, terpenoids and flavonoids in hot water extract. The presence of these phytochemicals in *C. odorata* was also been reported by other researchers [29, 30]. These phytoconstituents have been reported for antibacterial activities such as formation of complex with extracellular soluble proteins and bacterial cell wall, disruption of cell membrane, precipitation of microbial protein and inactivation of microbial adhesion, enzymes, and cell envelope transport proteins [16].

Table 1: Quantitative phytochemical constituents of *C. odorata* extracts

Phytochemical	Extracts		
	Hot water	Ethanol	Methanol
Saponin	130.73 \pm 0.18 ^c	34.00 \pm 0.18 ^a	110.73 \pm 0.18 ^b
Flavonoid	0.82 \pm 0.02 ^a	0.79 \pm 0.02 ^a	1.09 \pm 0.02 ^b
Terpenoid	29.55 \pm 0.03 ^b	36.17 \pm 0.03 ^c	0.00 \pm 0.00 ^a
Steroid	0.00 \pm 0.00 ^a	2.35 \pm 0.03 ^b	2.29 \pm 0.02 ^b
Tannin	0.55 \pm 0.01 ^a	1.90 \pm 0.01 ^c	1.50 \pm 0.01 ^b
Glycosides	9.03 \pm 0.34 ^b	0.77 \pm 0.32 ^a	0.74 \pm 0.32 ^a
Phenol	13.41 \pm 0.06 ^a	13.88 \pm 0.06 ^a	17.51 \pm 0.06 ^b

Results are presented as mean \pm SE. Values carrying the same alphabet in the same row are not significantly different ($p \leq 0.05$)

As presented in figure 2, the ten (10) *S. aureus* isolates tested were resistant to erythromycin (10 μ g) while only 50% were resistance to ciprofloxacin (10 μ g) and amoxicillin (30 μ g). As presented in figure 3, all twenty seven (27) *P. aeruginosa* isolates were resistant to augmentin (10 μ g). As shown in figure 4, the highest

antibiotics resistance of *K. pneumoniae* was to augmentin (10 µg) (76.9%) while the least was to ciprofloxacin (30 µg) (30.8%). Resistance in bacteria can be either vertical by mutating existing genes or horizontal by acquiring new genes from other strains or species [31].

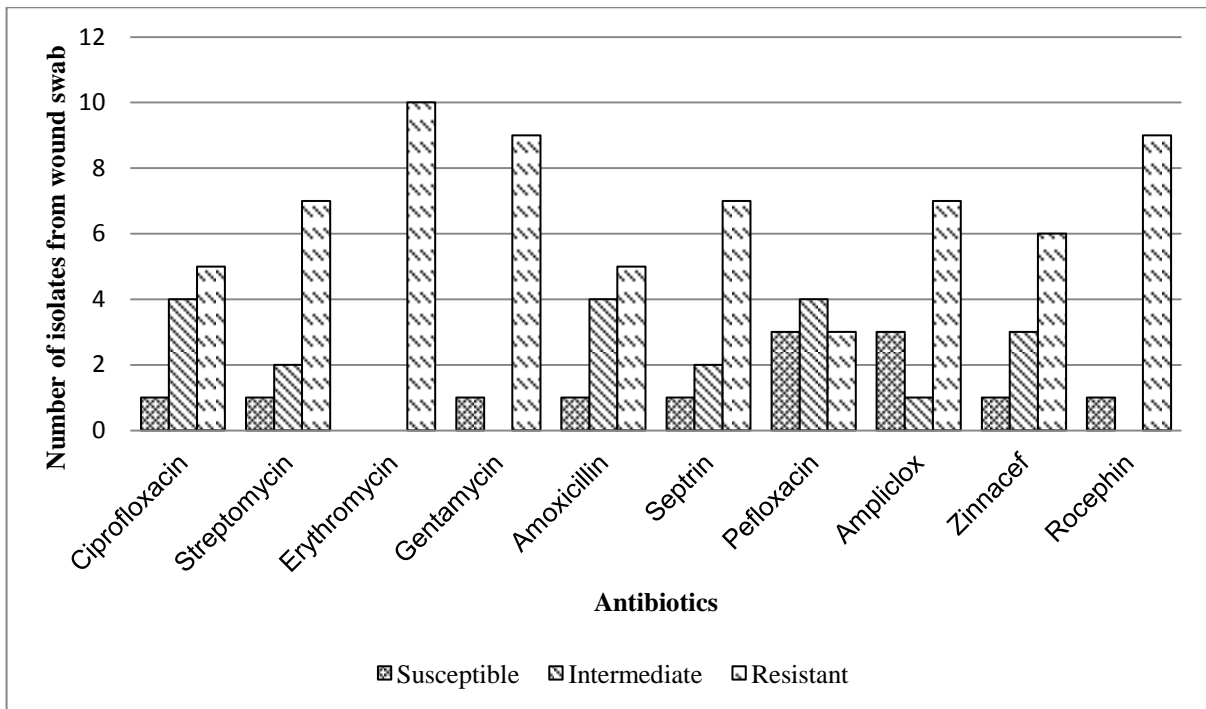


Figure 2: Antibiotics sensitivity pattern of strains of *Staphylococcus aureus* isolated from wound swab samples
Total number of isolates =10

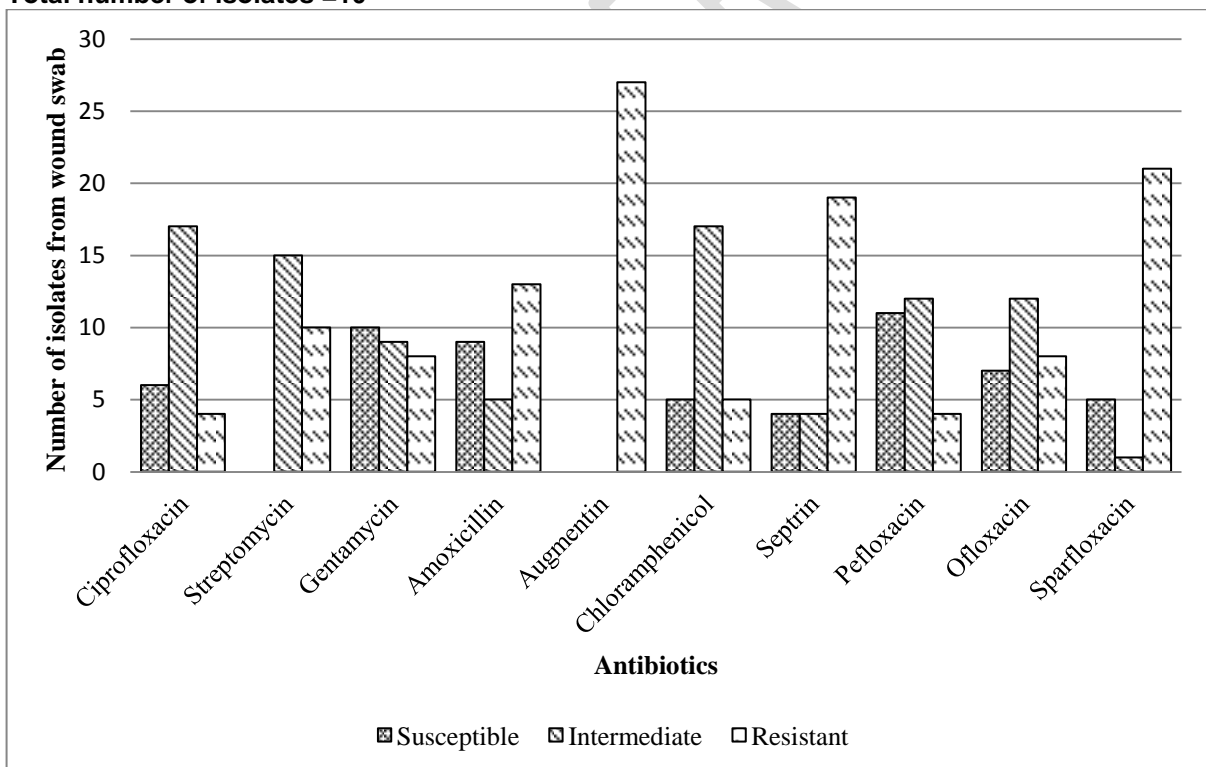


Figure 3: Antibiotics sensitivity pattern of strains of *Pseudomonas aeruginosa* isolated from wound swab samples
Total number of isolates =27

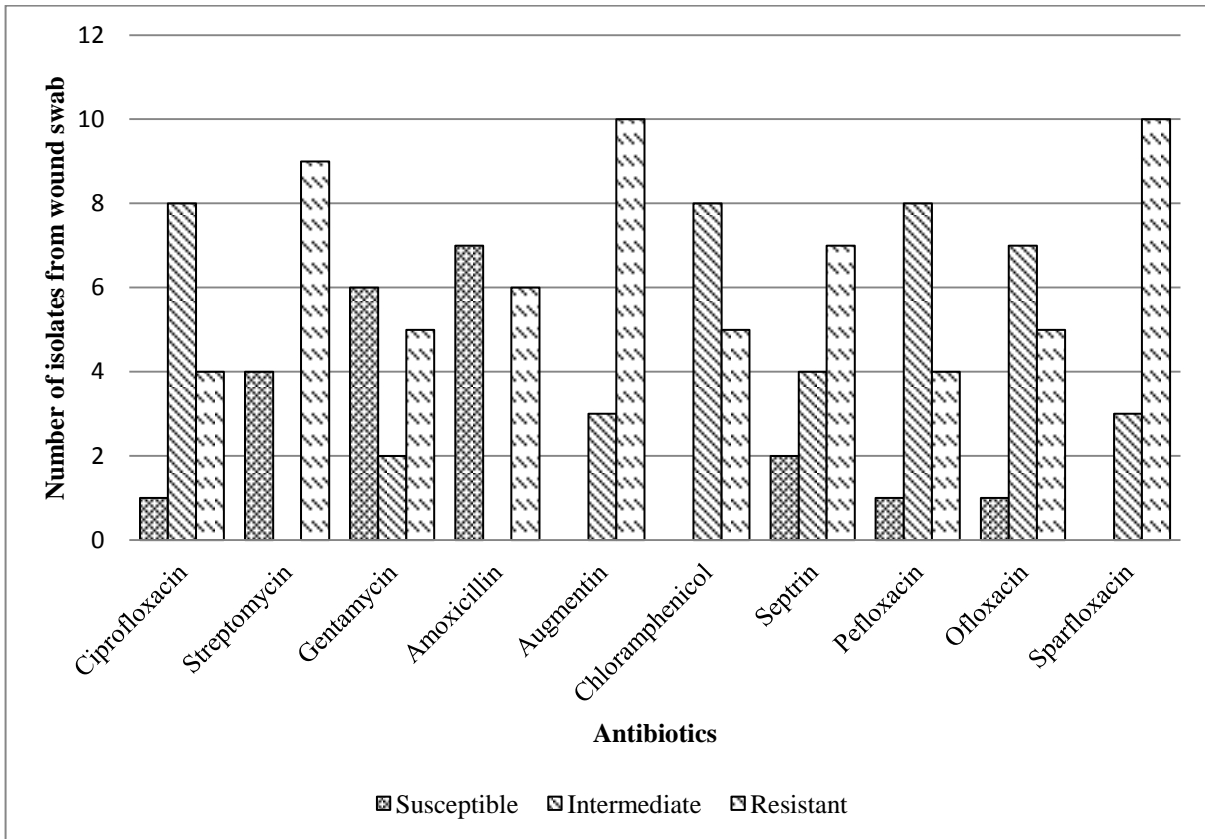


Figure 4: Antibiotics sensitivity pattern of strains of *Klebsiella pneumoniae* isolated from wound swab samples
Total number of isolates =13

UNDER PEER REVIEW

The present high rate of antimicrobial resistance has been attributed to indiscriminate use of antibiotics and infections with drug-resistant bacteria remain an important problem in clinical practice. Ciprofloxacin has the least resistance across bacterial isolates tested compared to other antibiotics used in this study. Its effectiveness on bacteria isolated from wounds was also reported by Aynalem et al. [32] and Akinkunmi et al. [26].

Zones of inhibition of purified *C. odorata* extracts on typed and clinical bacterial isolates are presented in Table 2. No zone of inhibition was observed with hot aqueous extract. This finding corresponds with the result of Naidoo et al. [12] and Mbajiuka et al. [10] who reported no zones of inhibition against bacteria by *C. odorata* aqueous extract. The efficiency of extraction procedure depends on the accessibility of the constituent to the solvent [3]. Methanolic and ethanolic extracts of *C. odorata* showed antibacterial effect on both Gram positive and Gram negative bacteria tested which may suggest that its bioactive component possess broad spectrum antibacterial activity [33]. At 100 mg/ml *C. odorata* methanolic and ethanolic extracts exhibited antimicrobial potential with better activity observed with typed bacterial isolates than with the clinical. This result corresponds with the finding of Srisuda et al. [11] which stated the best extraction solvent of *C. odorata* leaves for antibacterial activity to be methanol and ethanol. This observed antibacterial activity of organic solvent extracts can be due to the accessibility of solvent to more bioactive component of the plant leaf which could have acted singly or in combination. Medicinal plants have been known to possess antimicrobial properties against multidrug resistant bacteria and thus offers a prospective solution to the problem of reduced efficacy of antibiotics [34] and confirms the traditional use of *C.odorata* in the treatment of wounds [35]. Although water extract exhibited no inhibitory activity, methanol and ethanol extracts of *C. odorata* showed inhibitory activity against bacteria tested. Hence, these extracts can be considered as potential antibacterial and wound healing agent.

Table 2: Antibacterial activity of *C. odorata* extracts on bacteria isolated from wounds
Zones of inhibition (mm)

Isolates	Hot water	Methanol	Ethanol
<i>Staphylococcus aureus</i>	0.00±0.00 ^a	9.33±0.33 ^b	10.00±0.58 ^b
<i>Pseudomonas aeruginosa</i>	0.00±0.00 ^a	16.00±0.58 ^c	9.67±1.21 ^b
<i>Klebsiella pneumoniae</i>	0.00±0.00 ^a	9.67±0.33 ^b	12.33±0.33 ^c
<i>S. aureus</i> NTCC 6571	0.00±0.00 ^a	12.67±0.58 ^b	12.00±0.58 ^b
<i>P. aeruginosa</i> ATCC 10145	0.00±0.00 ^a	15.00±0.58 ^c	12.33±0.33 ^b
<i>K. pneumoniae</i> ATCC 13883	0.00±0.00 ^a	14.67±0.33 ^c	13.00±0.58 ^b

Results are presented as mean±SE. Values carrying the same alphabet in the same row are not significantly different (P≤0.05)

4. CONCLUSION

This study revealed the multidrug resistant bacteria associated with wounds. The study also reveals the presence of phenol, saponin, tannin, glycoside, steroid, terpenoid, and flavonoid in methanolic and ethanolic extracts of *C. odorata*. The antibacterial activity exhibited by methanolic and ethanolic extracts of *C. odorata* against multidrug resistant bacteria from wounds suggests their usage as an antibacterial agent in alternative to conventional antibiotics. The study confirmed the susceptibility of multidrug resistant bacteria to *C. odorata* methanolic and ethanolic extracts. Against the daily increasing threat of antimicrobial resistance, *C. odorata* can serve as a possible source of alternative medicine and can also be used to discover bioactive components which will serve as leads for the manufacture of new drugs rather than removal of the weed by destruction. Further studies are recommended on biosafety assay of the extracts.

Ethical Approval

Ethical approval for the collection of wound swab samples from patients of University of Medical Sciences Teaching Hospital, Akure, Nigeria was obtained from Ondo State Health Research Ethics Committee, Ministry of Health, Ondo State, Nigeria.

Consent :

Health Research Ethics Committee and each patient filled a consent form.

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