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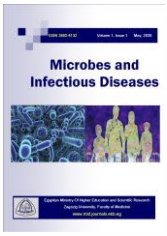

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Original article

Antibiogram and antimicrobial potency of *Chromolaena odorata* leaf crude extracts on post-operative wound bacterial flora from hospital setting

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ABSTRACT

Background: This study is aimed at identifying the bioactive components in *Chromolaena odorata* active against multiple antibiotics resistant bacteria (MARB) isolated from post-operative wound infections. **Methods:** A total of 69 septic wound samples were collected from patients (male and female) with clinically suspected post-operative wound infections. All methods were conducted using standard microbiological techniques. **Results:** *Staphylococcus epidermidis* was susceptible to gentamycin (10µg) at 24.50±0.50 mm, streptomycin (30µg) at 20.00±0.00 mm, ciprofloxacin (10µg) at 28.00±0.00 mm and amoxicillin (30µg) and are resistant to all other antibiotics tested. The 300 mg/ml concentration of the methanol crude extract had the highest zone of inhibition (ZOI) at 22.55±0.55 mm on *Staphylococcus aureus* and 22.30±0.30 mm ZOI was recorded on *Enterococcus faecalis* while the least ZOI at 6.45±0.25 mm on *Streptococcus pyogenes.* 12.5 mg/ml was the least inhibitory concentration on *Klebsiella pneumoniae* while 50 mg/ml was recorded as the highest minimum bactericidal concentration (MBC) on *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* at 50 mg/ml. **Conclusion:** The identification and characterization of bioactive compounds from *Chromolaena odorata* leaves could help develop more effective antimicrobial treatments for the management of post-operative wound infections. Further research is needed to isolate and characterize the active bioactive chemical compounds in *Chromolaena odorata* responsible for the antimicrobial activity and to evaluate their safety and efficacy in clinical settings for the treatment of post-operative wound infections.

Introduction

Post-operative wound infections also known as surgical site infections (SSI) occur within few days to few months after a surgical operation has been carried out and are associated with the contamination of the wound by bacteria which could be during the surgery or in the wards during treatment and care [1]. Post-operative wound infections can sometimes be superficial infections involving only the skin or more serious and can involve tissues under the skin, organs, or implanted material as reported by **Anderson et al.** [2]. Post-operative infection remains the most common post-operative complications and one of the most frequently encountered nosocomial infections worldwide [3, 4]. The most common pathogens of

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hard to heal post-operative wound infections are *Enterococcus* species *(*spp.), *Escherichia coli*, and *Staphylococcus aureus (S. aureus)* [5]. Other frequently identified microorganisms in infected post-operative wounds include *Citrobacter*, coagulase-negative *Staphylococci, Klebsiella* spp., *Proteus* spp. *and Pseudomonas aeruginosa (P. aeruginosa)* [5]. Their role as infectious agents is due to the increasing number of highly virulent organisms that can survive in hospital conditions.

The widespread use of broad-spectrum antibiotics has likely led to the development of antibiotic-resistant and multi-drug-resistant strains, such as methicillin-resistant *S. aureus* (MRSA) and *P. aeruginosa* [6, 7]. Antibiotic prophylaxis significantly reduces the risk of post-operative infections; however, there is still disagreement about the duration of the antibiotic therapy and the choice of antibiotics [8]. Beta-lactam antibiotics, quinolones, and lincosamide antibiotics are used in order to treat bacterial infections of post-operative wounds [5]. The fresh leaves of *Chromolaena odorata* or its decoction has been used in traditional medicine to treat human burns, soft issue wounds, ulcerated wounds, burn wounds, postnatal wounds and also for treatment of leech bites, indigestion and skin infection [9].

Post-operative wound infections are important cause of morbidity and mortality in patients undergoing surgery [10]. Treatment of post-operative wound infections with antibiotics is becoming a challenge for surgeons as multiple antibiotics resistance is on the increase [11]. Hence, this study will reveal the bioactive compounds present in *Chromolaena odorata* (*C. odorata*) plant extract and evaluate the antibacterial activity of the plant on MDR bacteria to at least one agent in three or more antimicrobial categories from post-operative wound samples. The specific goals of the study are to isolate and identify pathogenic bacteria from post-operative wound; investigate the MDR bacteria resistant to at least one agent in three or more antimicrobial categories present by subjecting the isolates to antibiotics susceptibility assay; evaluate the phytochemical constituents of the methanol and petroleum ether extracts of *C. odorata* leaf; assess the leaf extracts of *C. odorata* for antibacterial activity on the selected multiple antibiotics resistant bacteria and identify the bioactive compounds of the methanol and petroleum ether extracts of *C. odorata* using Gas Chromatography-Mass Spectrophotometry (GC-MS).

Materials and methods

Ethical consideration

Ethical consideration for the collection of postoperative wound swab samples was collected from Ondo State Health Research Ethics Committee, Ministry of Health, Akure and from the Ethics and Research Committee of Federal Medical Centre, Owo. Informed consent was obtained from patients prior to specimen collection using a standardized and well-structured consent form, with the confidentiality of patients' data strictly adhered to.

Study area

This research was conducted at Federal Medical Centre, Owo, Ondo State, Nigeria.

Swab sample and study population

The study was conducted from February 2022 – June 2022. Post-operative wound swabs were collected from in-patients suspected of surgical site infections.

Inclusion and exclusion criteria

The inclusion criteria were having surgical wound with pus discharge, purulent discharge, signs of sepsis (warmth, erythema, in duration and pain) and with physician diagnosis suspected of surgical site infection; surgical site infection means having the above signs and symptoms assessed by physicians based on CDC criteria (Surgical Site Infection (cdc.gov) from male and female surgical ward according to the standard guidelines to prevent surgical site infections as stated by **Anderson et al.** [2]. The exclusion criteria entailed the omission of patients with pre-existing surgical wounds currently on antibiotic therapy.

Sample collection and handling

Following guidelines of clinical and laboratory standard institute [12] from surgical patients developing post-operative wound infection, swab samples were collected from the depth of the wound using a sterile swab stick under an aseptic condition. The samples were placed in ice bag and immediately transported to microbiology laboratory of Federal University of Technology, Akure and analyzed within 1 hour of collection.

Isolation of bacteria

The plate streaking technique was used for isolation of bacteria. Swab sticks were used to streak the

samples on the already solidified sterile blood agar, Eosin methylene blue (EMB) agar (Oxoid, United Kingdom, UK), MacConkey agar (Oxoid, United Kingdom, UK), and mannitol salt agar (MSA) (Oxoid, United Kingdom, UK), and incubated at 37ºC for 24hours. Pure cultures of isolate were obtained by sub-culturing unto freshly prepared nutrient agar plates. The pure cultures were kept in a sterile nutrient slant and stored for further identification tests [13].

Cultural and morphological characteristics of bacteria isolates

The isolated bacteria were identified using their cultural and morphological characteristics on media. This was followed by microscopic examination of the bacteria isolates. The cultural characteristics included shape, elevation, surface, edge, colour, opacity and consistency. Physiological and biochemical tests were carried out to confirm their identification [13]. Biochemical characterization was done according to the methods of **Bayode et al.** [13].

Standardization of bacterial isolates from wound swab samples

A loopful of the bacteria culture was aseptically inoculated into freshly prepared sterile nutrient broth and incubated for 24 hours. 0.2ml from the 24 hours broth culture of the test organism was dispensed into 20ml sterile nutrient broth and incubated for another 4 hours to standardize the culture to 0.5 McFarland standards (106 Cfu/ml) before use as conducted by **Bayode et al.** [13].

Antibiotic susceptibility testing of bacterial isolates from wound swab samples

The Antibiotic susceptibility test was carried out using the Kirby-Bauer disc diffusion method as described by **Prabhu et al.** [14] and the Clinical Laboratory Standard Institute [12]. The standardized bacterial isolates were aseptically introduced on the surface of sterile Mueller Hinton agar (MHA) plates with the aid of a sterile swab stick using spread method. Commercially available antibiotics disc inclusive of pefloxacin (10µg) (c), gentamycin (10 µg) (Oxoid, United Kingdom, UK), ampiclox (30µg), Oxoid, United Kingdom, UK; zinnacef (20 µg) Oxoid, Oxoid, United Kingdom, UK United Kingdom, UK, amoxicillin (30µg), Oxoid, United Kingdom, UK ceftriaxone (25 µg), ciprofloxacin (10µg), Oxoid, United Kingdom, UK. streptomycin (30µg), Oxoid, United Kingdom, UK septrin (30µg), Oxoid, United Kingdom, UK and erythromycin (10µg) were used to determine the sensitivity of Gram-positive bacteria while disc inclusive of septrin (30 µg), Oxoid, United Kingdom, UK chloramphenicol (30 µg), Oxoid, United Kingdom, UK sparfloxacin (10µg), Oxoid, United Kingdom, UK ciprofloxacin (30 µg), Oxoid, United Kingdom, UK amoxicillin (30 µg), augmentin (10 µg), gentamycin (30 µg), pefloxacin (30 µg), ofloxacin (10 µg), and streptomycin (30 µg) were used to determine the sensitivity of Gram-negative bacteria were aseptically placed on the surface of the solidified agar with the aid of sterile forceps and allowed to diffuse for 5-10 minutes. The seeded plates were incubated for 18 hours at 37ºC. After incubation, diameters of zones of inhibition were measured to the nearest millimeter (mm) using transparent meter rule. The bacterial isolates that were sensitive to the antibiotics produced clear zones of inhibition. The diameter zone of inhibition was recorded and the results were interpreted using standard interpretative charts as recommended by the Clinical Laboratory Science Institute (CLSI), [15]. Seeded plates without antibiotics disks served as the control. The antibiotics sensitivity profile was carried out in triplicates. Multiple antibiotic-resistant were indicated by the organism that shows resistance to a minimum of 5 different antibiotics.

Determination of multiple antibiotics resistance (MAR) index of bacterial isolates

The Multiple Antibiotic Resistance Index was calculated as the ratio of the number of antibiotics to which an isolate is resistant to the total number of antibiotics which the isolates were exposed to (a/b), where "a" represents the number of antibiotics to which the isolates were resistant to and "b" represents the total number of antibiotics to which the isolates were exposed to [13].

Collection and preparation of plant materials

Collection of plant materials

Fresh plants of *Chromolaena odorata* (L.) were obtained from a farmland in Okitipupa, Ondo State and identified by a botanist in Ekiti State University. The leaves were washed and air-dried at room temperature until they were fully dried, after which they were grinded to fine powder, packed in clean polythene bag and stored.

Preparation of plant materials

Solvent extracts were obtained using methanol and petroleum ether. 100g of powdered plant leaves was soaked in 1000mL of each solvent for 72 hours and stirred frequently [15]. The solution was sieved

using muslin cloth, filtered through Whatmann No. 1 filter paper, concentrated to dryness using rotary evaporator (RE-52A, Union Laboratories, England) under reduced pressure at 37ºC and stored in air-tight containers at 4ºC [16].

The percentage yield of crude extract powder was calculated by the following equation:

Percentage Yield (%) =Extract yield ×100

Dried plant yield 1

The extracts were concentrated to dryness using the drying oven under reduced pressure at 37 ℃. The extracts were preserved in sterile air-tight containers at 4 ℃ for further use.

Phytochemical Screening of *Chromolaena odorata* Leaf extracts

Qualitative and quantitative screening of *Chromolaena odorata* crude leaf extracts was carried out as described by **Alabi et al.** [17]. Tests for terpenoids, steroids, flavonoids, alkaloids, saponins, tannins, phlobatannins, phenols, and cardiac glycosides were the qualitative tests conducted. Quantitative tests for tannin, flavonoid, saponin, alkaloids, steroids, cardiac glycosides, and terpenoids on the crude *C. odorata* leaf extracts.

Reconstitution of plant extracts

The dried plant extracts were reconstituted with 30% Dimethyl sulphoxide (DMSO) to give different concentrations prior to antibacterial susceptibility testing.

Antibacterial activities of *Chromolaena odorata* Leaf extracts

Antibacterial activities of *C. odorata* leaf extracts (methanol and petroleum ether) against isolates that showed multiple antibiotic resistance was determined using agar well diffusion method as described by **Akinyemi et al.** [18]. Each standardized test organisms were streaked on the surface of sterile Mueller-Hinton agar (MHA) plates and allowed to stand for 15 minutes. A sterile 6mm cork-borer was used to bore wells on the solidified MHA plates and 0.1ml of the extracts was introduced into each well. Ciprofloxacin (50µg) was used as positive control and DMSO as negative control and the plates were incubated at 37ºC for 18 hours. The extracts were tested for antibacterial activity. Clear diameter around the wells were indicative of inhibition and were measured in millimeter (mm) using a transparent meter rule. A consistent value of the inhibition zone that is ≥ 10mm was selected to study the minimum inhibitory concentration (MIC) and minimum bactericidal concentration.

Determination of minimum inhibitory concentration (MIC) of *C. odorata* leaf extracts against bacterial isolates from wound swab samples

The minimum inhibitory concentration of the extracts from *C. odorata* was determined using the broth dilution method. 1ml of the reconstituted extracts at a concentration of 200 mg/ml was diluted serially to give different concentrations of 100mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml in test tubes. 1ml of an 18-hour culture of the standardized inoculum was added to each of the test tubes and mixed thoroughly. The tubes were then incubated at 37 ℃ for 18 hours alongside the control test tubes. The test tubes containing growth medium and *C. odorata* extract without inoculum was used as negative control while the test tube containing the growth medium and inoculum without *C. odorata* leaf extract was used as positive control as demonstrated by **Akinyemi et al.** [18].

MIC was determined as the lowest concentration (mg/ml) of the extracts with no visible growth when compared with the positive and negative control tubes. The MBC was determined by sub-culturing the test tubes with no growth on sterile Mueller Hinton agar (MHA) plates and further incubated at 37 ℃ for 24 hours. The lowest concentration (mg/ml) with no growth after sub-culturing on MHA was regarded as the MBC **Akinyemi et al.** [18].

Partial purification of *Chromolaena odorata* leaf extracts

Purification of *C. odorata* leaf extracts Using column chromatography

Plant extracts were purified using column chromatography. A column was rinsed with water and allowed to dry completely. A glass wool was fixed to the bottom of the column, then the column was packed with silica gel and sufficient amount of petroleum ether was run through the packed column in order to make the silica gel completely settle. The sample was prepared by mixing 3g of the extract with silica gel and properly stirred till it formed 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 (in that order). Fractions were collected in clean containers. The collected fractions were analyzed by thin layer chromatography (TLC) to determine if the fractions

contain more than one component or if the fractions can be combined. Fractions containing similar Rf values were combined [19].

Qualitative analysis of fraction using thin layer chromatography

Thirty (30)g of silica gel G (with CaSO4 as the binder) was placed in a beaker shaken vigorously with 65 ml of distilled water for 1 minute till it was made slurry, the slurry paste was transferred to the applicator and spread uniformly on the surface of the 20 × 20 cm TLC plate. The coated plates were allowed to dry for 10 minutes in dust-free conditions. The gel was activated prior to use for 5 minutes at 110oC in a hot oven. The gel was divided into a number of lanes by drawing lines with a lead. A micro syringe was used to spot the extracts in various lanes carefully on the line 2.5 cm away from each other. The appropriate mobile phase was poured into a chromatographic tank. The plate was inserted with the solvent system below the level of the spots and left for about 50 minutes [20]. The plate was removed from the tank and the solvent front was marked. The plate was then air-dried and visualized. The retention factor (Rf) value was obtained for each of the extracts using the formula below:

Retention factor (Rf) = (Distance moved by solute)/(Distance moved by solvent)

Structural elucidation of *Chromolaena odorata* crude petroleum ether and methanol Leaf Extracts Using Gas Chromatography Mass Spectrophotometry (GC-MS)

The structural elucidation of methanol and petroleum ether extracts of *C. odorata* leaf was done to obtain the bioactive constituents of *C. odorata* leaf extract using Gas-Chromatography/Mass-Spectrophotometry (GC-MS) analysis as described by **Bodunrinde et al.** [20]. The analysis was carried out by injecting samples (2 mL) into a Varian 3800/4000 gas chromatograph /mass spectrophotometer (GC/MS) system fitted with a 30-m DB-5 capillary column (30 m length × 0.25 mm diameter × 0.25 µm thickness). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Nitrogen gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/ min, and an injection volume of 2 µl was employed (a split ratio of 10:1). The injector temperature was maintained at 250 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C/min to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scanning interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GCMS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total area. The mass detector used in this analysis was Turbo-Mass Gold-Perkin–Elmer, and the software adopted to handle mass spectra and chromatograms was Agilent ChemStation ver-5.2. Identification of phyto-components Interpretation on mass-spectrum GC-MS was conducted using the database of the National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight, and structure of the components of the tested materials were ascertained.

Statistical analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 26. The one-way analysis of variance test was used to determine significant differences in the means of the dependent variable across the groups of independent variables at $p<0.05$, the significant differences were separated using Turkey's Honesty Significant Test.

Results

Rate of occurrence of bacterial isolates in the study area

The rate of occurrence of bacteria isolates from wound swab samples of patients attending Federal Medical Centre, Owo, Ondo State is presented in Figure 1. The 62 culture that showed growth yielded 78 isolates. Gram negative organisms were more prevalent than Gram positive organisms accounting for 40 (51.28%) and 38 (48.72%) of the isolates respectively. The following bacteria were isolated from post-operative wound swabs comprising *Staphylococcus aureus* 21 (26.92%), *Pseudomonas aeruginosa* 19 (24.36%), *Staphylococcus epidermidis* 10 (12.82%), *Escherichia coli* 8 (10.26%), *Klebsiella pneumoniae* 7 (8.97%), *Proteus mirabilis* 6 (7.69%), *Streptococcus pyogenes* 4 (5.13%), and *Enterococcus faecalis* 3 (3.85%). The biochemical characteristics of bacterial isolates are shown in **table (1).**

Antibiotics sensitivity patterns of bacterial isolates

Table 2 and 3 showed the antibiotics sensitivity pattern of selected Gram-positive and Gram-negative bacterial isolates to conventional antibiotics. All the Gram-positive bacteria tested were resistant to pefloxacin (10µg), ampliclox (30µg), erythromycin (10µg), zinnclof (20µg), and rocephin (25µg). *Staphylococcus aureus* and *Streptococcus* pyogenes were resistant to all the conventional antibiotics used in this study. *Staphylococcus epidermidis* was susceptible to gentamycin (10µg) at 24.50±0.50 mm, streptomycin (30µg) at 20.00±0.00 mm, ciprofloxacin (10µg) at 28.00±0.00 mm and amoxicillin (30µg) and are resistant to all other antibiotics tested. *Enterococcus faecalis* was susceptible to gentamycin (10µg) at 18.50±0.50 mm, septrin (30µg) at 22.00±0.00 mm, amoxicillin (30µg) at 24.50±0.50 mm and resistant to all other antibiotics test. All the Gram-negative bacteria tested were resistant to the antibiotics used for this study. **Table 4** showed the multiple antibiotic resistance index of the resistant bacterial isolates where the % MARI ranged between 60 – 100 %.

Percentage extraction yield and phytochemical constituent of *Chromolaena odorata* leaf crude extract

Table 5 showed the percentage yield observed in crude *C. odorata* leaf extracts. Methanol extract has the highest yield of 18.90% while petroleum ether crude had 13.5 % yield. **Table 6** showed the presence of phytochemical compounds comprising tannins, alkaloids, steroids and terpenoid present in both methanol and petroleum ether crude extracts. Anthraquinone and phlobotannin were absent in both crude extracts. **Figure 2** showed the qualitative and quantitative phytochemical constituents of different crude extracts of *C. odorata* leaf crude extract. Petroleum ether crude extract of *C. odorata* has the highest phytochemical constituent (4%), while methanol extract had 3.8 %. Flavonoid was the more predominant phytochemical (checa3.0%) in methanol crude extract compared to petroleum ether (2.9 %).

Chromatographic purification of *C. odorata* leaf crude extracts

Table 7 and 8 showed the different fractions from the column chromatography of extracts of *C. odorata* leaf and their corresponding retention factor from the thin layer chromatogram. **Table 7** showed fractions from the column chromatography of methanol extract of *C. odorata* leaf and their corresponding retention factor from thin layer chromatography. Four fractions were separated, and using ethyl acetate as mobile phase solvent, the retention factor ranged between 0.3548 – 0.7903. **Table 8** showed fractions from column chromatography of extract of *C. odorata* leaf and their corresponding retention factor from thin layer chromatography. Four fractions were separated, and using chloroform as mobile phase solvent, the retention factor ranged between 0.4833 – 0.7667.

Antibacterial activity of *Chromolaena odorata* crude extracts on bacterial isolates from post-operative wound swabs

The 300 mg/ml concentration of the methanol crude extract had the highest zone diameter (ZOI) at 22.55±0.55 mm on *Staphylococcus aureus* and 22.30±0.30 mm zone of inhibition ZOI was recorded on *Enterococcus faecalis* while the least ZOI at 6.45±0.25 mm on *Streptococcus pyogenes.* The 200 mg/ml concentration also had a high ZOI of 22.40±0.40 mm on Staphylococcus epidermidis, the 300 mg/ml concentration of crude methanol also had ZOI of 19.35±0.35 mm and 18.45±0.45 mm on *Pseudomonas aeruginosa* and *Escherichia coli* respectively as illustrated in **table (9).** The 300 mg/ml concentration of petroleum ether had the highest ZOI of 26.20±0.20 mm on *S. pyogenes* while 100 mg/ml concentration had the least ZOI at 6.40±0.10 mm on *Proteus mirabilis.* A zone of inhibition of 19.80±0.30 mm was also recorded at the same concentration on *S. epidermidis.* The 200 mg/ml concentration also had a ZOI of 17.05±0.05 mm and 19.20±0.20 mm respectively on *S. epidermidis* and *S. pyogenes* as shown in **table (10).**

The minimum inhibitory and bactericidal concentration of *C. odorata* crude extracts on bacterial isolates from post-operative wound swabs

Twelve and half (12.5) mg/ml was the least inhibitory concentration on Klebsiella pneumoniae while 50 mg/ml was recorded as the highest minimum inhibitory concentration (MIC) on *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* at 50 mg/ml. *Enterococcus faecalis, Streptococcus pyogenes, Staphylococcus epidermidis* and *Escherichia coli* all had a minimal killing concentration (minimum bactericidal concentration) of 100 mg/ml a displayed in **table (11).** *Pseudomonas aeruginosa* and *Staphylococcus*

epidermidis both had the highest MIC of 50 mg/ml while all the bacterial isolates from the post-operative wound swabs had a MBC of 100 mg/ml.

The presence of bioactive chemical compounds in the crude methanol leaf extract of *Chromolaena odorata*

Various fatty acids and their esters such as hexadecanoic acid, octadecanoic acid, and their methyl esters, Octadecatrienoic acid (Z, Z, Z), a form of linolenic acid, 1,2-Benzenedicarboxylic acid, diheptyl ester, a phthalate ester were present as bioactive chemical compounds in the methanol crude leaf extract of *C. odorata* as illustrated in **table (12).** 1, 2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester and a benzene-dicarboxylic acid derivative were present in the petroleum ether *C. odorata* crude extract as shown in **table (13).**

Table 1. Biochemical characteristics of bacteria isolates from post-operative wound swabs.

Gram reaction	Shape	Catalase	Coagulase	Citrate	Oxidase	Urease	H_2S	Gas	Lactose	Glucose	Sucrose	Indole	Motility	MR	VP	Fructose	Maltose	Mannitol	Galactose	Suspected Organisms
+	Cocci	+	+	+	-	+	-	-	+	+	+	-	-	+	+	+	+	+	+	*Staphylococcus aureus*
+	Cocci	-		+	-	-	-	-	+	+	+	-	-	+	+	+	+	+	+	*Enterococcus faecalis*
-	Rod	+	-	+	+	-	-	+	-	-	-	-	+	-	-	-	-	+	-	*Pseudomonas aeruginosa*
-	Rod	+	-	-	-	-	-	+	+	+	-	+	+	+	-	-	-	+	+	*Escherichia coli*
+	Cocci	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	+	*Streptococcus pyogenes*
-	Rod	+	+	+	-	+	-	+	+	+	+	-	-	-	+	+	+	+	+	*Klebsiella pneumoniae*
-	Rod	+	-	+	-	-	+	+	-	+	-	-	+	+	NA	NA	-	-	NA	*Proteus mirabillis*
+	Cocci	+	-	+	-	+	+	+	+	+	+	-	-	-	+	+	+	-	+	*Staphylococcus epidermidis*

Keys: + = present; - = absent, MR= Methyl Red test, VP = Voges- Proskauer test, H2S: Hydrogen sulphide gas production test; NA- Not applicable

Table 2. Antibiotic sensitivity patterns of Gram-positive bacteria isolated from post-operative wound swabs

Organisms	**Antibiotic zone of inhibition (diameter in mm)**									
	CN S = ≥15 I =13-14 R = ≤12	**PEF** S = ≥16 I =13-15 R = ≤12	**S** S = ≥15 I =12-14 R = ≤11	**SXT** S = ≥16 I =11-15 R = ≤10	**CPX** S = ≥21 I =16-20 R = ≤15	**AM** S = ≥17 I =14-16 R = ≤13	**APX** S = ≥17 I =14-16 R = ≤13	**E** S = ≥23 I =14-22 R = ≤13	**Z** S = ≥23 I =15-22 R = ≤14	**R** NA
S. aureus	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	8.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S. epidermidis	24.50±0.50^{e}	0.00±0.00^{a}	20.00±0.00^{c}	10.00±0.00^{b}	28.00±0.00^{f}	21.50±0.50^{d}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S. pyogenes	0.00±0.00^{a}	6.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}	12.00±0.00^{d}	0.00±0.00^{a}	10.50±0.50^{d}	8.00±0.00^{c}	0.00±0.00^{a}	0.00±0.00^{a}
E. faecalis	18.50±0.50^{c}	0.00±0.00^{a}	10.50±0.50^{b}	22.00±0.00^{d}	0.00±0.00^{a}	24.50±0.50^{e}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Keys: CN- Gentamycin 10 µg, S- Streptomycin 30 µg, PEF- Pefloxacin 10 µg, SXT-Septrin 30µg, CPX- Ciprofloxacin 10 µg, AM- Amoxicillin 30 µg, APX-Ampiclox 30µg, E- Erythromycin 10 µg, Z-Zinnclof 20 µg, R-Rocephin 25 µg.

Data are represented as mean± SE (standard error). Values with the same superscript letter(s) along the same rows are significantly different ($p<0.05$) according to Tukey's Honestly Significant Difference. Each value is a mean of three (3) replicate

Table 3. Antibiotic sensitivity patterns of Gram-negative bacteria isolated from post-operative wound swabs

Organisms	Antibiotic zone of inhibition (diameter in mm)									
	CN S = ≥15 I =13-14 R = ≤12	**PEF** S = ≥16 I =13-15 R = ≤12	**OFL** **S = ≥16** **I =13-15** R = ≤12	**S** **S = ≥15** **I =12-14** R = ≤11	**SXT** **S = ≥16** **I =11-15** R = ≤10	**CH** **S = ≥18** **I =13-17** R = ≤12	**SP** **S = ≥19** **I =16-18** R = ≤15	**CPX** **S = ≥21** **I =16-20** R = ≤15	**AM** **S = ≥17** **I =14-16** R = ≤13	**AU** **S = ≥1** **I =14-1** R = ≤13
E. coli	0.00±0.00[a]	6.00±0.00[b]	10.00±0.00[c]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	7.00±0.00[b]	13.00±0.00[d]	0.00±0.00[a]	6.00±0.00[b]
P. mirabilis	12.00±0.00[d]	0.00±0.00[a]	0.00±0.00[a]	8.00±0.00[b]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	10.00±0.00[c]	0.00±0.00[a]	0.00±0.00[a]
K. pneumonia	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	10.50±0.50[c]	0.00±0.00[a]	8.00±0.00[b]	0.00±0.00[a]
P. aeruginosa	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	12.00±0.00[c]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	7.00±0.00[b]	0.00±0.00[a]

Keys: CN- Gentamycin 10 µg, S- Streptomycin 30 µg, PEF- Pefloxacin 10 µg, OFL- Tarivid 10 µg, SXT-Septrin 30µg, CH- Chloramphenicol 30µg, SP- Sparfloxacin 10 µg, CPX- Ciprofloxacin 10 µg, AM- Amoxicillin 30 µg, AU- Augmentin 30 µg. Data are represented as mean± SE (standard error). Values with the same superscript letter(s) along the same rows are significantly different ($p<0.05$) according to Tukey's Honestly Significant Difference. Each value is a mean of three (3) replicates

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Table 4. Multiple antibiotic resistance index of the resistant bacteria isolates.

	A	**B**	**MARI (%)**
P. aeruginosa	10	10	100
S. aureus	10	10	100
S. epidermidis	6	10	60
E. coli	10	10	100
K. pneumoniae	10	10	100
P. mirabilis	10	10	100
E. faecalis	7	10	70
S. pyogenes	10	10	100

Keys: A: Number of antibiotics indicating resistance; **B:** Total number of antibiotics tested; **MARI:** Multiple antibiotic resistance index = A/B

Table 5. Phytochemical (qualitative) constituents of *C. odorata* leaf extracts.

Constituents	**Petroleum ether extract**	**Methanol extract**
Tannins	+	+
Alkaloids	+	+
Terpenoids	+	+
Steroids	+	+
Saponins	-	+
Flavonoids	+	+
Cardiac glycosides	-	+
Anthraquinones	-	-
Phlobatannins	-	-

Key: + = Present; - = Absent

Table 6. Colour and Percentage Yield of the Extracts obtained from *C. odorata* crude extracts.

Solvent	**Dried plant yield (g)**	**Extract yield (g)**	**Percentage (%)**	**Colour**
Methanol	100	18.9	18.9	Dark green
Petroleum ether	100	13.5	13.5	Dark green

Table 7. Retention factor for methanol extract of *C. odorata* whole leaf.

Fraction	**Sample font**	**Solvent font**	**R_f value**	**Solvent for mobile phase**
A	2.2	6.2	0.3548	Ethyl acetate
B	3.0	6.2	0.4839	Ethyl acetate
C	3.9	6.2	0.9290	Ethyl acetate
D	4.9	6.2	0.7903	Ethyl acetate

Table 8. Retention factor for the petroleum ether extract of *C. odorata* leaf.

Fraction	Sample font	Solvent	R_f value	Solvent for mobile phase
A	2.9	6.0	0.4833	Chloroform
B	3.2	6.0	0.5353	Chloroform
C	3.7	6.0	0.6167	Chloroform
D	4.6	6.0	0.7667	Chloroform

Table 9. Antibacterial activity of methanol crude extract of *C. odorata* leaf on bacterial isolates from post-operative wound swabs.

Isolates	Concentration (mg/ml)			Positive control	Negative control
	100 mg/ml	200 mg/ml	300 mg/ml		
S. aureus	9.70 ± 0.10^{b}	15.10 ± 0.10^{c}	19.35 ± 0.35^{d}	22.30 ± 0.30^{e}	0.00 ± 0.00^{a}
S. epidermidis	19.20 ± 0.20^{b}	22.40 ± 0.40^{c}	22.55 ± 0.55^{d}	27.70 ± 0.75^{d}	0.00 ± 0.00^{a}
S. pyogenes	6.45 ± 0.25^{a}	11.20 ± 0.80^{b}	15.40 ± 0.40^{d}	24.20 ± 0.20^{e}	0.00 ± 0.00^{a}
E. faecalis	15.50 ± 0.50^{b}	17.25 ± 0.25^{bc}	22.30 ± 0.30^{d}	19.00 ± 1.00^{c}	0.00 ± 0.00^{a}
P. aeruginosa	9.75 ± 0.25^{ab}	12.30 ± 0.30^{ab}	19.35 ± 0.35^{ab}	28.75 ± 8.75^{b}	0.00 ± 0.00^{a}
K. pneumoniae	8.75 ± 0.25^{b}	11.50 ± 0.50^{c}	12.55 ± 0.05^{c}	16.40 ± 0.40^{d}	0.00 ± 0.00^{a}
E. coli	10.30 ± 0.30^{b}	14.15 ± 0.15^{c}	18.45 ± 0.45^{d}	20.15 ± 0.15^{e}	0.00 ± 0.00^{a}
P. mirabilis	8.75 ± 0.25^{b}	10.85 ± 0.15^{c}	16.20 ± 0.20^{d}	22.70 ± 0.30^{e}	0.00 ± 0.00^{a}

Data are represented as mean± SE (standard error). Values with the same superscript letter(s) along the same rows are not significantly different ($p<0.05$) according to Tukey's Honestly Significant Difference. Each value is a mean of three (3) replicates

Table 10. Antibacterial activity of petroleum ether extract of *C. odorata* leaf on bacterial isolates from post-operative wound swabs.

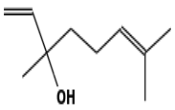
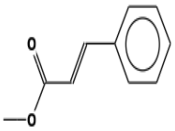
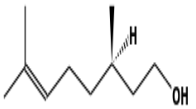
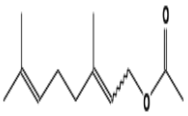
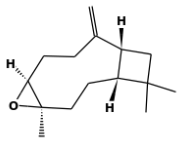
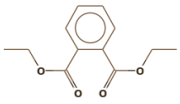
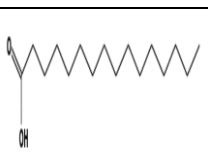
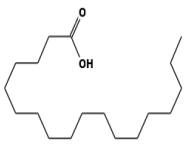
Isolates	Concentration (mg/ml)			Positive control	Negative control
	100 mg/ml	200 mg/ml	300 mg/ml		
S. aureus	8.10 ± 0.10^{b}	12.50 ± 0.50^{c}	16.70 ± 0.30^{d}	23.80 ± 0.80^{e}	0.00 ± 0.00^{a}
S. epidermidis	13.15 ± 0.85^{b}	17.05 ± 0.05^{c}	19.80 ± 0.30^{d}	22.50 ± 0.50^{e}	0.00 ± 0.00^{a}
S. pyogenes	13.30 ± 0.20^{b}	19.20 ± 0.20^{c}	26.20 ± 0.20^{d}	28.20 ± 0.20^{e}	0.00 ± 0.00^{a}
E. faecalis	10.50 ± 0.50^{b}	14.25 ± 0.25^{c}	16.05 ± 0.05^{d}	21.15 ± 0.15^{e}	0.00 ± 0.00^{a}
P. aeruginosa	7.90 ± 0.10^{b}	10.30 ± 0.30^{b}	17.50 ± 0.50^{c}	32.40 ± 2.40^{d}	0.00 ± 0.00^{a}
K. pneumoniae	10.50 ± 0.50^{b}	12.20 ± 0.20^{c}	15.10 ± 0.10^{d}	20.30 ± 0.30^{e}	0.00 ± 0.00^{a}
E. coli	9.85 ± 0.15^{b}	13.40 ± 0.40^{c}	16.80 ± 0.20^{d}	21.60 ± 0.20^{e}	0.00 ± 0.00^{a}
P. mirabilis	6.40 ± 0.10^{b}	10.50 ± 0.50^{c}	14.40 ± 0.40^{d}	19.25 ± 0.25^{e}	0.00 ± 0.00^{a}

Values with the same superscript letter(s) along the same rows are not significantly different ($p<0.05$) according to Tukey's Honestly Significant Difference

Table 11. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *C. odorata* extracts.

Bacterial isolates	**Methanol**		**Petroleum ether**	
	MIC (mg/ml)	**MBC (mg/ml)**	**MIC (mg/ml)**	**MBC (mg/ml)**
S. aureus	25	100	50	100
S. epidermidis	50	50	50	100
S. pyogenes	25	100	25	100
E. faecalis	25	100	50	100
P. aeruginosa	50	100	50	100
K. pneumoniae	12.5	50	25	100
E. coli	25	100	25	100
P. mirabilis	25	50	50	100

Table 12: Presence of bioactive chemical compounds in the methanol and petroleum ether crude leaf extracts of *Chromolaena odorata*.

Peak #	RT	Compound Detected	Mol. Formula	MW	Peak Area%	Comp %w	m/z	Structures
1	4.49	Benzene, 1,4-dichloro-	$C_6H_4Cl_2$	147	0.94	1.05	50, 111, 147	
2	15.00	Linalool	$C_{10}H_{18}O$	154	0.95	1.08	43, 71, 154	
3	16.41	2-Propenoic acid, 3-phenyl-, methyl ester, €-	$C_{10}H_{10}O_2$	162	3.79	3.40	51, 103, 162	
4	18.00	6-Octen-1-ol, 3,7-dimethyl-	$C_{10}H_{20}O$	156	2.84	3.00	41, 69, 156	
5	20.00	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	$C_{12}H_{20}O_2$	196	9.46	5.92	43, 69, 196	
6	21.50	Caryophyllene oxide	$C_{15}H_{24}O$	220	2.57	2.90	41, 69, 154	
7	23.51	Diethyl Phthalate	$C_{12}H_{14}O_4$	222	15.43	15.82	50, 149, 222	
8	27.00	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.30	1.16	43, 74, 270	
9	29.52	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	17.26	18.93	43, 73, 256	
10	30.50	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.82	1.94	43, 73, 284	

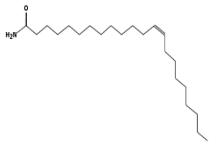
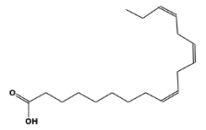
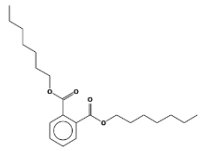
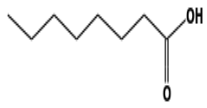
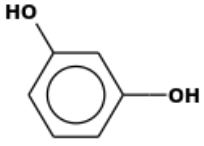
11	32.00	Isopropyl stearate	$C_{21}H_{42}O_2$	326	2.82	3.10	43, 102, 326	
12	35.00	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	1.89	1.73	41, 56, 296	
13	38.00	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	337	7.67	7.62	41, 72, 337	
14	40.14	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	21.58	22.92	41, 79, 278	
15	42.00	1,2-Benzenedicarboxylic acid, diheptyl ester	$C_{22}H_{34}O_4$	362	5.98	5.46	41, 149, 362	
16	43.60	Squalene	$C_{30}H_{50}$	410	3.67	3.95	41, 69, 410	

Table 13. Presence of bioactive chemical compounds in the methanol and petroleum ether crude leaf extracts of *Chromolaena odorata*.

Peak #	**RT**	**Compound Detected**	**Mol. Formula**	**MW**	**Peak Area %**	**Comp %w**	**m/z**	**Structures**
1	4.49	Hexanal	$C_6H_{12}O$	100	0.93	1.09	41, 56, 100	
2	12.81	Octanoic acid	$C_8H_{16}O_2$	144	4.25	3.92	43, 60, 144S	
3	15.93	Resorcinol	$C_6H_6O_2$	110	6.60	5.09	53, 82, 110	

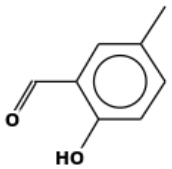
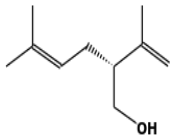
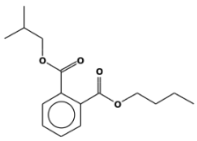
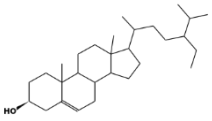
4	19.70	2-Hydroxy-5-methylbenzaldehyde	$C_8H_8O_2$	136	16.82	8.11	77, 107, 136	
5	20.21	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, (R)-	$C_{10}H_{18}O$	154	6.49	4.87	41, 69, 154	
6	26.52	2-Pentadecanol	$C_{15}H_{32}O$	228	1.01	1.42	43, 45, 228	
7	32.00	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$	322	4.85	5.15	43, 73, 322	
8	32.68	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	$C_{25}H_{42}$	278	17.56	17.94	41, 149, 278	
9	35.00	Isopropyl linoleate	$C_{21}H_{38}O_2$	322	2.73	3.40	43, 67, 322	
10	35.75	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308	2.13	2.35	41, 70, 308	
11	37.91	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	19.42	24.61	43, 73, 256	
12	43.37	β-Sitosterol	$C_{29}H_{50}O$	414	6.38	7.06	43, 107, 414	

Figure 1. Percentage occurrence of bacteria isolates from post-operative wound swabs

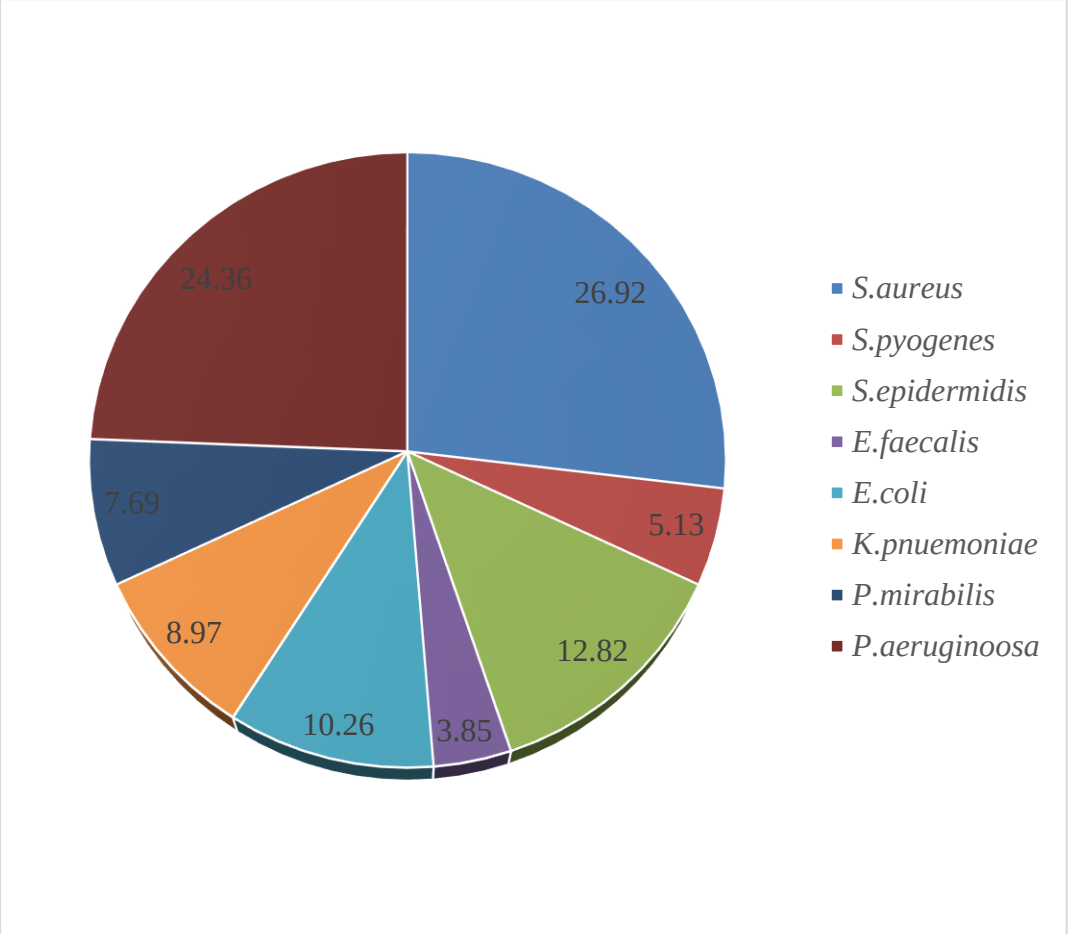
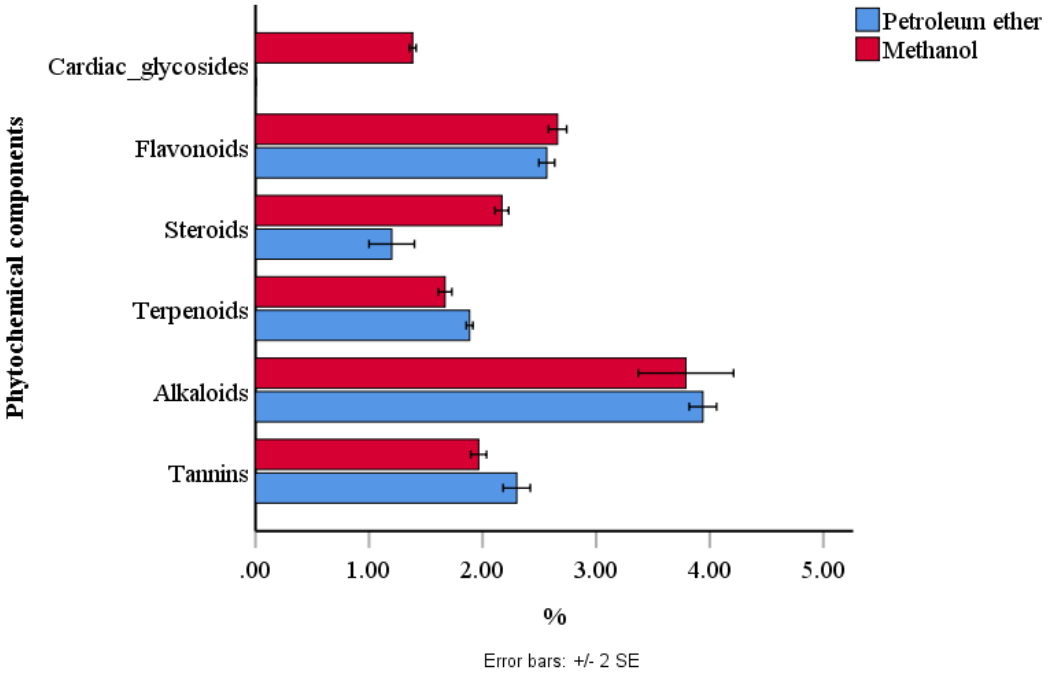


Figure 2. Phytochemical (quantitative) constituents of *C. odorata* leaf crude extracts



Discussion

This study was conducted between February 2022 and June 2022 and was aimed to investigate the antimicrobial potency of methanol and petroleum ether crude *C. odorata* leaf extracts on bacteria isolated from post-operative wound swabs. The findings revealed a higher prevalence of wound infections in males aged 30-39 years and a majority of the wound swabs showing bacterial growth. The study identified various bacterial isolates, with *S. aureus* (26.92%) and *P. aeruginosa* (24.36%) being the most common. The observed antibiotic resistance in *Staphylococcus aureus* and *Streptococcus pyogenes* is a significant concern, as these bacteria can cause severe infections as reported by **Jubeh et al.** [21]. The high multiple antibiotic resistance index (MARI) observed in this study ranging between 60-100% further emphasizes the need for alternative antimicrobial agents in clinical settings for the management of post-operative wounds as replicated by **Loh et al.** [22]. One study conducted in Abuja, Nigeria, provides baseline data on the occurrence of SSIs at a hospital as reported by **Olowo-Okere et al.** [23].

The antibiotic resistance observed in this study, particularly in *S. aureus* and *S. pyogenes*, is consistent with the growing concern of antibiotic resistance worldwide. A study by **Vivas et al.** [24] highlights the increasing prevalence of antibiotic-resistant bacteria and the urgent need for alternative antimicrobial agents. The study found that the majority of the study population was male (53.62%), with the highest incidence rate of wound infection observed in males aged 30-39 years (32.43%). This could be attributed to various factors such as occupation, lifestyle, and exposure to environmental contaminants as similarly observed by **Akhlagh et al.** [25]. The fact that 89.86% of the wound swabs showed bacterial growth highlights the prevalence of post-operative wound infections and the need for effective antimicrobial treatments.

The presence of phytochemical compounds such as tannins, alkaloids, steroids, and terpenoids in the crude extracts of *C. odorata* suggests potential antimicrobial properties as previously stated by **Vijayaraghavan et al.** [26] who performed a multi-spectrum study on the phytochemical screening, free radical scavenging and antimicrobial potential of *Chromolaena odorata* leaf extracts against pathogenic bacterium in wound infections. The identification of bioactive compounds such as benzene, 1,4-dichloro, linalool, 2-Propenoic acid, 3-phenyl-, methyl ester, and hexanal in *C. odorata* crude leaf extracts further supports the potential antimicrobial properties of *C. odorata* [27]. These compounds have been reported to exhibit antimicrobial activity in previous studies, the study findings suggest that the crude extracts of *C. odorata* leaves possess antimicrobial properties against various bacterial strains isolated from post-operative wound swabs. The presence of phytochemical compounds and bioactive compounds in the extracts supports their potential use as alternative antimicrobial agents. The presence of tannins, alkaloids, steroids, and terpenoids in both methanol and petroleum ether crude extracts of *C. odorata* suggests that these compounds may contribute to the observed antimicrobial activity on post-operative wound infection [28].

The study found that the methanol crude extract of *C. odorata* exhibited the highest zone of inhibition (ZOI) against *S. aureus* and *Enterococcus faecalis*, while the petroleum ether extract showed the highest ZOI against *S. pyogenes*. These findings support the potential use of *C. odorata* extracts as alternative antimicrobial agents, as suggested by other studies [26]; [21]. The minimum inhibitory concentrations (MICs) of the methanol crude extract varied among the bacterial isolates, with the lowest MIC observed for *Klebsiella pneumoniae* and the highest MIC for *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. This indicates that the antimicrobial activity of the extract may be more effective against certain bacterial strains. The study found that the methanol crude extract exhibited the highest ZOI against S. *aureus* and *E. faecalis*, while the petroleum ether extract showed the highest ZOI against *S. pyogenes.* This indicates that the antimicrobial activity of the extracts may vary depending on the bacterial strain and the type of extract used. The MICs of the methanol crude extract varied among the bacterial isolates, suggesting that the antimicrobial activity of the extract may be more effective against certain bacterial strains. Understanding the MICs for different bacterial strains can help guide the development of targeted antimicrobial treatments using *C. odorata* extracts as demonstrated by **Omokhua** [29].

A study by **Danish et al.** [30] reported the antimicrobial activity of Aloe vera gel extracts against various bacterial strains infected post-operative wound, including *S. aureus, Escherichia coli*, and *P. aeruginosa* in clinical settings. The

study found that the Aloe vera extracts exhibited significant inhibitory effects on the growth of these bacteria, suggesting its potential use as an alternative antimicrobial agent. A study by **Paw et al.** [31] investigated the antimicrobial activity of Curcuma longa rhizome extracts against various bacterial and fungal strains in North-East India. A study by **Kwiatkowski et al.** [32] evaluated the antimicrobial activity of Thymus vulgaris essential oil against various bacterial strains infected post-operative wound, including *S. aureus*, *Escherichia coli*, and *P. aeruginosa* from clinical settings [33]. The study found that the essential oil exhibited significant antimicrobial activity, with the highest inhibitory effects observed against *S. aureus*. A study by **Archana et al.** [34] investigated the antimicrobial activity of Azadirachta indica leaf extracts against various bacterial strains from infected post-operative wounds, including *S. aureus*, *Escherichia coli*, and *P. aeruginosa* from hospital environment.

Chromolaena odorata leaves have been reported to contain various bioactive compounds that contribute to its antimicrobial potency, particularly against bacterial infections associated with post-operative wound swabs as similarly reported by **Oladejo et al.** [35] who observed potent antibacterial activity of Jatropha tanjorensis leaf extracts against bacteria associated with wound infections from clinical settings in Ado-Ekiti. 1, 4-Dichlorobenzene is an aromatic compound with potential antimicrobial properties. It has been reported to exhibit bacteriostatic and bactericidal effects against various Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus* and *Escherichia coli* as observed by **Bai et al.** [36]. **Omeke et al.** [37] also reported *Pseudomonas aeruginosa* isolates from wound infections were susceptible to the *C. odorata* methanol crude extract at a high concentration of 400 mg/ml.

Hexanal is an aldehyde compound with potential antimicrobial properties. It has been reported to exhibit bacteriostatic and bactericidal effects against various Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus* and *Escherichia coli* from infected post-operative wounds as demonstrated by **Salehi et al.** [38]. Its antimicrobial activity is attributed to its ability to disrupt bacterial cell membranes, leading to cell lysis and death.

Conclusion and recommendation

The identification and characterization of bioactive compounds from *Chromolaena odorata* leaves could help develop more effective antimicrobial treatments for the management of post-operative wound infections. Further research is needed to isolate and characterize the active bioactive chemical compounds in *Chromolaena odorata* responsible for the antimicrobial activity and to evaluate their safety and efficacy in clinical settings for the treatment of post-operative wound infections.

Authors' contributions

AKB designed the study. AKB developed the methodology, acquired and analyzed the data and wrote the first draft of the manuscript. BJA supervised the study. BJA reviewed and revised the manuscript. All authors read and approved the final draft of the manuscript.

Ethical approval

Ethical approval was procured and approved by the Ondo State Ministry of Health, Akure, Nigeria. The ethics statement document carries a Health Research Ethics Committee assigned number of NHREC/18/08/2016 and a protocol number of OSHREC/20/8/2021/365.

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Conflict of interest

Not declared.

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