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Original article

Evaluation of phytochemical constituents and antibacterial efficacy of *Chromolaena odorata* (Linnaeus) leaf extracts on bacteria associated with patient's wounds

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ABSTRACT

Background: This research was designed to show evaluation of antibacterial activities of *Chromolaena odorata* (*C. odorata*) Linn extracts on bacteria associated with wound infections. **Methods:** A total of 127 wound exudate samples were collected from the patients with chronic wound infections attending the Federal Medical Centre, Owo, First Mercy Hospital and University of Medical Teaching Hospital, Akure, Ondo State, Nigeria, between November 2021 and January 2022. Questionnaires were served to each patient in order to obtain information about the wound. Morphological and biochemical characterization of bacteria were determined using standard microbiological methods. The *C. odorata* extract was subjected to Soxhlet and maceration extraction methods. The solvents used were cold water, hot water, N-hexane and ethanol. Phytochemical evaluation was carried out on the extracts. The antibiotic susceptibility patterns of the isolates were determined by testing against antibiotics and extracts. Qualitative phytochemical analysis showed the presence of alkaloid, flavonoid, glycoside, phenol, phlobatannin, saponin, steroid, tannin, and terpenoid in the extracts of *C. odorata*. **Results:** A total of 13 bioactive compounds were observed in the ethanol extract of *C. odorata*. The highest leakages of Na+ and K+ were observed in *Proteus mirabilis* at 12.9 mol/kg and 30.0 mol/kg. There was higher leakages of Na+ in all the test isolates than K+. **Conclusions:** The antibacterial efficacy of leaf extracts of *C. odorata* has been evidently proven against bacterial isolates associated with wound infections, suggestive of good alternative treatment materials.

Introduction

Wounds are a common occurrence in our daily lives, ranging from minor cuts and bruises to more severe injuries [1]. They can be caused by accidents, surgeries, or even diseases [2-4]. Understanding wounds, their types, healing process, and management is crucial for individuals, healthcare professionals, and caregivers.

Wound infections refer to infections that occur in a wound or surgical incision [5]. When a wound is not properly cleaned, protected, or cared for, bacteria or other microorganisms can enter the wound, leading to an infection [6]. Wound infections can be caused by various types of bacteria, including *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Escherichia coli*, among others [7]. It is however important to note

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that not all wounds will become infected, and the severity of an infection can vary. If wound infection is suspected, appropriate medical attention should be sought [8].

Wound infections are often characterized by the invasion of bacteria into open or damaged skin, resulting in inflammation and impaired healing [9]. Bacterial infections can significantly delay the recovery process, increase morbidity, and, in severe cases, lead to life-threatening complications [10]. Understanding the bacteria associated with wound infections, their characteristics, and their antibiotic resistance patterns is crucial for effective diagnosis, treatment, and prevention strategies. Some of the bacteria associated with wound infections are *Pseudomonas aeruginosa, Streptococcus pyogenes, Staphylococcus aureus*, *Escherichia coli* and *Enterococcus* species [7].

Large numbers of antibiotics have been usually combined for the treatment of wound infection. Narrow and broad spectrums antibiotics are available for the treatment. However, improper and irrational use of antibiotics as well as genetic and non-genetic drug resistant mechanisms of bacteria may result into antibiotic resistance [11]. The development of antibiotic-resistant bacteria has greatly limited the effectiveness of conventional antimicrobial therapy [12]. This is why alternative therapeutic measures have been sought in the past years.

Many species of plants and herbs with wound healing activities have been identified in Africa and developing countries as a result of ethno-botanical research advancement. The use of medicinal plants in wound management and care includes debridement, disinfection and provision of suitable environment for natural healing process [13]. Studies have shown that active ingredients from medicinal plants are less toxic, with low or no side effects compared with orthodox therapeutic agents; hence, the increased and renewed interest in the use and application of medicinal plants in the wound healing process both in diabetic and non-diabetic conditions [13]. In 2015, **Rajasree et al.** [14] established the fact that *C. odorata* had bioactive therapeutic substances that produced effects on wound healing. Also, the constituents of the plant extracts modulate one or more of the overlapping wound healing stages. Traditional use of *C. odorata* leaves involves pasting the ground leaves topically.

In this study, we evaluated the phytochemical constituents of leaf extracts of *C. odorata* as well as their antibacterial activities against bacteria isolated from wound exudates.

Materials and methods

Sample size determination

The WHO (2009) formula with ± 10 % precision level for large sample was applied since the population was homogenous.

The formula; n = N/ 1 + N (e) 2

Where; n = the sample size; N = size of the population; e = level of significance (or limit of tolerable error).

Collection of clinical samples and isolation of bacteria

A total of 127 clinical samples were obtained by means of rotation of sterile swabs (transport swab) from wounds between November, 2021 and April, 2022 from different hospitals which include Federal Medical Centre, Owo (FMC), University of Medical Sciences Teaching Hospital (UNIMEDTH) and First Mercy Hospital, Akure within the duration from 8 am to 10 am daily and transported in an ice pack to the Research laboratory FUTA for further analysis within 1 hour of collection for microbiological analysis.

Identification of bacterial isolates

Biochemical tests were carried out for the identification of bacterial species. The following were the tests carried out; Gram staining, catalase, citrate, urease, oxidase, indole, voges-proskauer, and sugar fermentation tests [15].

Standardization of bacterial isolates from wound swab samples

Standardization of the culture to 0.5 McFarland's standard (108 ¬CFU/ml) was done as described by **Isunu et al.** [16].

Collection and preparation of leaf samples of *C. odorata*

Fresh leaf samples of *C. odorata* were collected from Ojajere Quarters at Ilara Mokin, Ifedore Local Goverment Area of Ondo State between the hours of 6 - 8 am at a prevailing temperature of about 30±2 ℃. All the collections were done in the month of October 2021. The plants were identified and authenticated by Prof. M. K. Oladunmoye (Pharmaceutical microbiologist) at the Department of Medical Microbiology, Federal University of Technology, Akure (FUTA), Ondo State.

The leaves of *C. odorata* were allowed to dry at 28 ℃ for 4 weeks and then pulverized to a fine powder with the aid of a Binatone blender (Model BLG-621). Four solvents were used for the preparation of the extracts, namely cold distilled water, hot distilled water, ethanol 60% concentration and n-hexane. The extracts were prepared according to the method described by **Isunu et al.** [16].

Percentage recovery of the leaf extracts of *C. odorata*

The percentage yield of the extract of *C. odorata* were calculated thus [17]:

$$\text{Percentage recovery of extract (\%)} = \frac{\text{Weight of extract recovered after extraction}}{\text{Initial weight of plant before extraction}} \times \frac{100}{1}$$

Evaluation of the phytochemical constituents of leaf extracts of *C. odorata*

The plant extracts of ethanol, n-hexane, cold and hot aqueous were qualitatively screened as described by **Akinmoladun et al.** [18]. Plant extracts were screened for the presence of cardiac glycoside, terpenoids, steroids, flavonoids, alkaloids, saponin, tannin, phlobatannin and phenol. The number of phytochemical constituents in the extracts were also carried out using methods described by AOAC [19].

Antibacterial activity of leaf extracts of *C. odorata*

The assay for the antibacterial activity of *C. odorata* extracts was carried out as described by Isunu et al. [16] with modifications. The reconstitution of the extracts was done in accordance to the various concentration intended for use in this study. Positive control was maintained with 2mg/ml of Ciprotab ®. The plates were then incubated for 18 hours at 37 ℃ and the diameter of zones of inhibition were measured in mm.

Investigation of amount of sodium ion leakages

Sodium ion concentration was estimated by colorimetric method based on the modified Maruna and Trinder method. Sodium was precipitated together by magnessium uranyl acetate as uranyl magnesium sodium acetate salt. Excess uranyl salts reacts with potassium ferrocynide to produce a brownish colour. The intensity of the colour is inversely proportional to the sodium concentration in the specimen and is measured photometrically at 530 nm (500 nm – 540 nm).

Statistical analysis

Data obtained in this study were subjected to one-way analysis of variance (ANOVA), and differences between means were compared by Duncan's New Multiple Range Test at 95% confidence interval using Statistical Package for Social Sciences (SPSS) version 20.

Results

Distribution of frequency of pathogenic bacterial from wound source in relation to age and sex **(Figure 1).**

A total of 127 wound samples were collected from the patients chronic wound infections. The highest frequency was seen among the male patients between the age of 21 and 30 years while the lowest frequency was obtained among patients between ages of 1 and 10 years.

Identification of bacterial isolates from wound exudates

The biochemical characteristics of the bacterial isolates from wound samples are shown in **table (1).** Both Gram positive and Gram negative bacteria were identified as the bacteria involved in wound infections in this study. The bacteria were identified as *Staphylococcus aureus* (*S. aureus)* (N = 55), *Pseudomonas aeruginosa* (*P. aeruginosa*) (N = 42), *Proteus mirabilis (P. mirabilis)* (N = 38), *Klebsiella pneumoniae (K. pneumoniae*) (N = 24) and *Escherichia coli (E. coli*) (N = 9).

Percentage occurrence of bacteria isolated from wound swabs

The percentage occurrence of the bacteria isolated from wound samples of patients are; *S. aureus* (33%), *P. aeruginosa* (25%), *P. mirabilis* (23%), *K. pneumoniae* (14%), and *E. coli* (5%). Gram positive bacteria especially *Staphylococcus* species has the highest percentage occurrence of the bacteria involved in wound infections **(Figure 2).**

Percentage yield of the leaf extracts of *C. odorata*

The percentage yield of the extracts varied from solvent to solvent. The recovery rate for Ethanol, hot water, cold water and N-hexane are 58.57 %, 51.73 %, 46.85 % and 14.59 % respectively **(Figure 3).**

Phytochemical constituents of the leaf extracts of *C. odorata*

Ethanol leaf extract of *C. odorata* was found to have the highest quantity of cardiac glycoside (882.32±20.58 mg/g) while N-hexane leaf extract of *C. odorata* (0.00±0.00mg/g) have the least quantity

of cardiac glycoside, tannin and terpenoids respectively. Hot water and cold water extracts of *C. odorata* have the highest (479.31±2.51) and least (88.57±4.29) quantity of flavonoid respectively. Phenol had the highest quantity in hot water extract of *C. odorata* (479.31±2.51 mg/g). Alkaloid had the highest quantity (16.81±0.03 mg/g) in N-hexane leaf extract of *C. odorata*. The qualitative and quantitative phytochemical constituents are shown in **tables (2, 3).**

Antibacterial activities of leaf extracts of *C. odorata*

The antibacterial activities of leaf extracts of *C. odorata* at 100 mg/mL showed that the purified ethanol extract produced the widest zone of inhibition (17.67±1.76 mm) against *K. pneumoniae*, while crude N

-hexane extract produced no zone of inhibition against *S. aureus* and *P. mirabilis* **(Table 4).**

Leakage of sodium (Na+) and potassium ions (K+) from bacterial isolates by the ethanol leaf extract of *C. odorata*

The leakage of sodium (Na+) and potassium ions (K+) from bacterial isolates by the ethanol leaf extract of *C. odorata* is presented in **figure (4).** The highest leakages of Na+ and K+ were observed in *P. mirabilis* with values of 12.9 mol/kg and 30.0 mol/kg respectively. There was higher leakage of K+ in all the test isolates than Na+.

Table 1. Biochemical characteristics of bacteria isolated from wound samples.

S/N	Gram Reaction	Catalase	Coagulase	Citrate	Oxidase	Motility	Glucose	Fructose	Lactose	Sucrose	Maltose	Organisms
1	-ve rod	-	-	-	-	NM	AG	AG	AG	AG	AG	*P. aeruginosa*
2	+ve cocci clusters	+	+	+	-	NM	AG	AG	AG	AG	AG	*S. aureus*
3	-ve rod	+	-	+	-	NM	AG	AG	AG	AG	AG	*K. pneumoniae*
4	-ve rod	+	-	-	-	M	AG	AG	AG	AG	AG	*E. coli*
5	-ve rod	+ve	-ve	+	-	M	AG	AG	AG	AG	AG	*P. mirabilis*

Key: NM: Non motile, M- Motile; AG-Acid and Gas production, A- Acid production only, +: present, -: absent, +ve: Gram positive, -ve: Gram negative

Table 2. Qualitative phytochemical constituents of the leaf Extracts of *C. odorata*

Phytochemicals	**Cold water**	**Hot water**	**Ethanol**	**N-hexane**
Tannin	+	+	+	-
Saponin	+	+	+	-
Steriod	+	+	+	-
Terpenoid	+	+	-	-
Phenol	+	+	+	+
Glycoside	+	+	+	+
Alkaloid	-	-	-	-
Phlobatannin	-	-	-	-
Flavonoid	+	+	+	+

Keys: Present +; Absent –

Table 3. Quantitative phytochemical constituents of the leaf extracts of *C. odorata*

Phytochemicals	**Cold water**	**Hot water**	**Ethanol**	**N-hexane**
Tannin	32.14 ± 1.99^{b}	47.82 ± 0.43^{b}	57.94 ± 3.86^{a}	0.00 ± 0.00^{a}
Terpenoid	2.16 ± 0.29^{a}	$9.90\pm.54^{a}$	13.67 ± 0.71^{a}	0.00 ± 0.00^{a}
Phenol	$146.14\pm.19^{e}$	$365.34\pm.61^{e}$	283.43 ± 57.58^{c}	128.89 ± 6.33^{b}
Glycoside	194.37 ± 6.75^{f}	289.13 ± 13.19^{d}	882.32 ± 20.58^{d}	359.15 ± 16.78^{d}
Alkaloids	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	16.81 ± 0.03^{a}
Flavonoid	88.57 ± 4.29^{d}	479.31 ± 2.51^{f}	163.12 ± 2.51^{b}	247.61 ± 11.71^{c}
Saponin	70.84 ± 2.59^{c}	74.14 ± 20.09^{c}	1129.72 ± 17.50^{e}	0.00 ± 0.00^{a}
Steriod	$1.52\pm.06^{a}$	2.01 ± 0.10^{a}	3.77 ± 0.07^{a}	0.00 ± 0.00^{a}

Values are presented as Mean±SE of duplicates, values in the same column carrying same superscript are not different significantly ($p<0.05$) according to new Duncan's Multiple Range test.

Table 4: Comparative susceptibility pattern of crude and purified leaf extract of *C. odorata* at 100 mg/ml on bacterial isolates from clinical samples

Isolate	PEC	CEC	PNH	CNH	CCW	PCW	PHW	CHW
S. aureus	16.33±0.88^{a}	11.67±0.33^{a}	10.67±0.33^{ab}	0.00±0.00^{a}	10.67±0.33^{a}	11.67±0.33^{a}	13.33±0.33^{a}	11.00±0.58^{ab}
P. aeruginosa	16.00±1.15^{a}	12.00±0.58^{a}	11.33±0.33^{b}	10.67±0.00^{b}	10.67±0.67^{a}	12.00±0.58^{a}	14.00±0.58^{a}	10.67±0.58^{a}
K. pneumoniae	17.67±1.76^{a}	13.67±0.61^{a}	13.00±0.00^{c}	10.67±0.67^{b}	10.33±0.33^{a}	12.33±0.33^{a}	15.33±0.67^{a}	12.67±0.33^{ab}
E. coli	16.33±.88^{a}	12.33±0.88^{a}	12.67±0.58^{c}	11.00±0.58^{b}	11.00±0.58^{a}	13.33±0.88^{a}	15.33±0.88^{a}	13.00±1.00^{b}
P. mirabilis	16.33±.88^{a}	13.67±0.88^{a}	10.00±0.00^{a}	0.00±0.00^{a}	12.00±0.58^{a}	13.33±0.67^{a}	14.67±.88^{a}	12.00±0.58^{ab}

Values are presented as Mean±SE of duplicates, values in the same column carrying same superscript are not different significantly ($p<0.05$) according to new Duncan's Multiple Range test.
Keys: PEC- Purified Ethanol Chromolaena; CEC- Crude Ethanol Chromolaena; PNH- Purified N-hexane; CNH- Crude N-hexane; CCW- Crude Chromolaena Cold water; PCW- Purified Chromolaena Cold water; PHW- Purified Hot Water ; CHW- Crude Hot Water

Figure 1. Distribution of frequency of pathogenic bacteria from wound source in relation to age and sex.

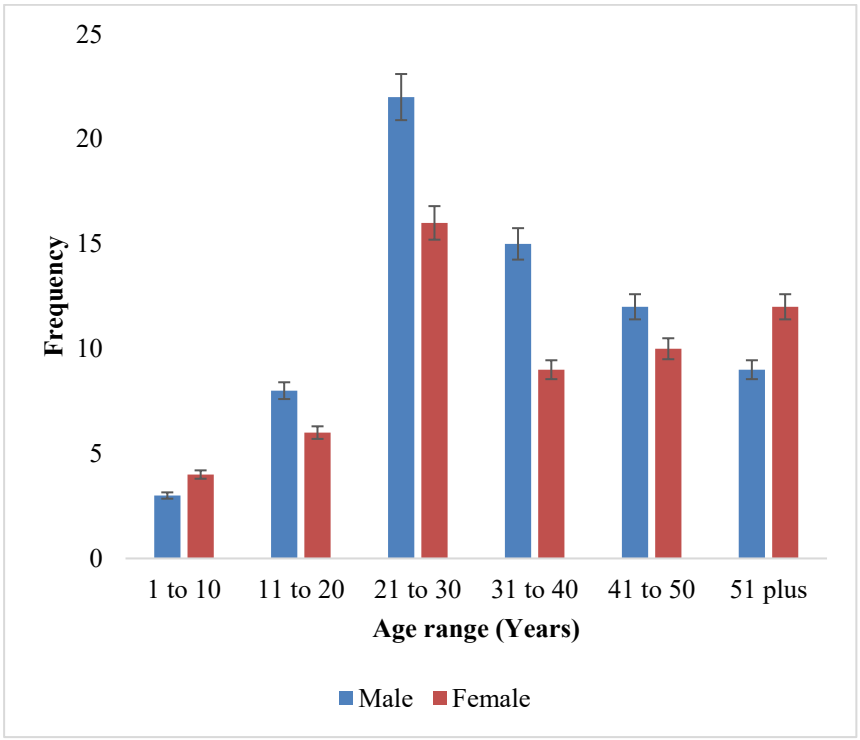


Figure 2. Percentage occurrence of bacteria isolated from wound swabs.

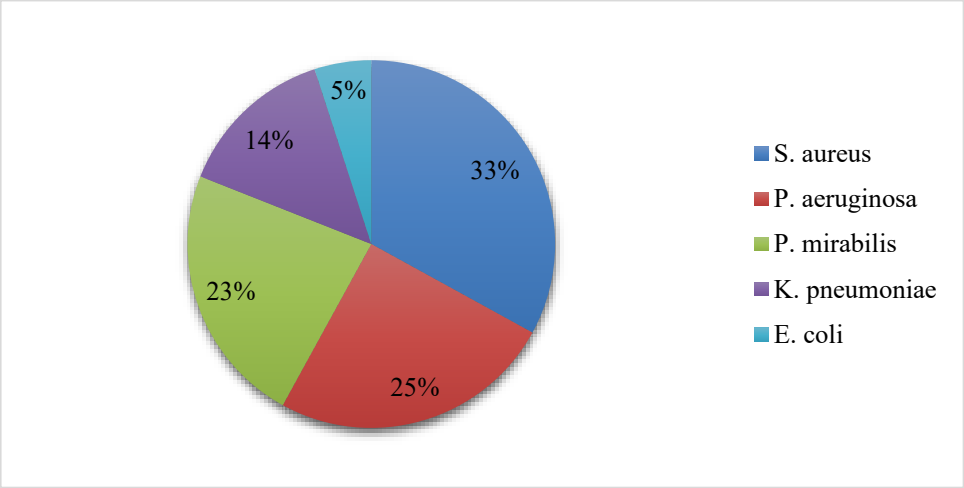


Figure 3. Percentage yield (%) of extracts of *C. odorata*

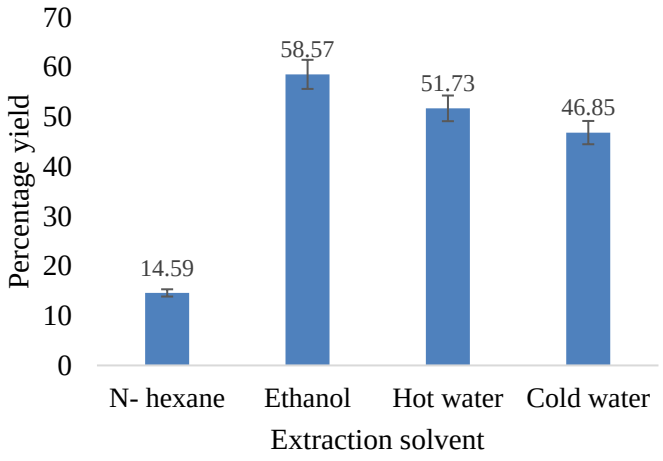
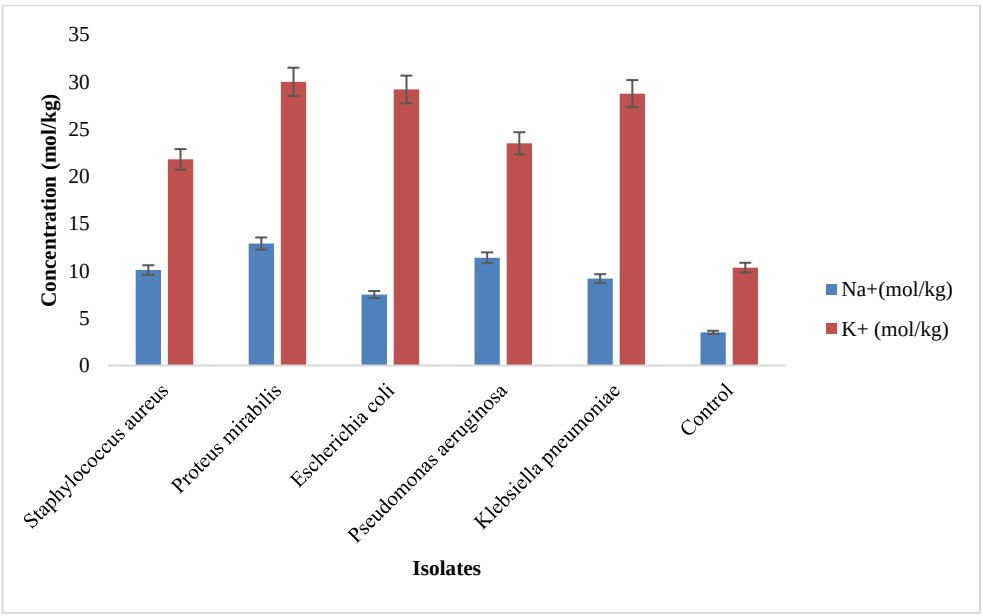


Figure 4. Leakage of potassium ions (K+) and sodium ion (Na+) from bacterial isolates by the ethanol leaf extract of *C. odorata*



Discussion

In this study, it was observed that the rate of wound pathogenic organisms was most pronounced among male patients that were within the third decade of life and it was distributed across the ages and sex. This is similar to the findings of **Seleh et al.** [20] and **Tom et al.** [21], who asserted that the predominance among patients in this category is most likely due to the fact that male exposure to a possible wound and/or trauma is greater as they represent majority of the workforce responsible for hard/risky labour.

Gram positive bacteria especially *S. aureus* was the most predominant bacteria observed and has percentage occurrence of 33 % among the isolated bacteria involved in wound infections while *E. coli* was the least with percentage occurrence of 5%. Similar study was conducted from different parts of Nigeria [22].

The ethanol leaf extract of *C. odorata* has the highest percentage recovery compared to the aqueous and N-hexane extraction solvent. The N-hexane extraction solvent has poor yield of recovery of the extracts. The differences in polarity of the solvents could affect the solubility of grinded plant, percentage recovery and odour of the extracts [23].

Tannins, saponins, terpenoids, phenols, flavonoid, steroid and alkaloids were present in all the extracts of *C. odorata*. This is supported by **Vijayaraghavan et al.** [24] who purported that the medicinal values of plants lie in their phytochemical constituents.

The findings revealed that the ethanol extracts of *C. odorata* could be attributed to the nature of active compounds and the stronger extract ion capacity of ethanol could have produced greater number of active constituents responsible for the antibacterial activity.

The leaf extracts exhibited antibacterial activities on the test bacterial isolates, showing varying zones of inhibitions. Similar findings have been reported in other studies [25,26]. The ethanol and aqueous leaf extracts of *C. odorata* showed antibacterial effect on both Gram positive and Gram negative bacteria tested suggesting that its bioactive components possess broad spectrum antibacterial activity.

The highest leakages of Na+ and K+ were observed in *P. mirabilis* at 12.9 mol/kg and 30.0 mol/kg. There was higher leakage of Na+ in all the test isolates than K+. It is well known that the cytoplasm of living cells, generally, contains more potassium ions than sodium ions [27].

Conclusion

This study has shown that *C. odorata* leaf extracts could be effectively used in wound treatment as it inhibited the in vitro growth of bacteria isolated from infected wounds. The leaf extracts of *C. odorata* if properly harnessed could be a source of active antibacterial agents for the development of drugs against the pathogenic bacteria responsible for wound infections.

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Author's contributions

Author Oladunmoye, K. M., designed the study. Author Lambe, E. F., developed the methodology, acquired the data, analysed and interpreted the data. Author Lambe, E. F., wrote the first draft of the manuscript. Both authors read and approved the final draft of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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