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# Chemical composition and healing potential of essential oil of *Dennettia tripetala* on methicillin-resistant *Staphylococcus aureus*: infected wound model

Solayide A. Adesida<sup>1\*</sup>, Michael I. Iyebeye<sup>1</sup>, Oluwagbemiga O. Aina<sup>2</sup>, Rebecca F. Peters<sup>3</sup>, Charles C. Ezeaku<sup>4</sup> and Elizabeth A. Amosun<sup>5</sup>

## Abstract

**Background:** *Dennettia tripetala* (Pepperfruit) is an important medicinal plant in some West African communities. This study was designed to examine the antibacterial properties and wound-healing abilities of *D. tripetala* essential oil on mice with methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Gas chromatography-mass spectrometry (GC-MS) was used to analyse the essential oil (EO) extracted by hydro-distillation from the dried fruits of *D. tripetala*. MRSA was identified using 30 µg cefoxitin disk, CHROMagar, and Polymerase Chain Reaction. Congo red agar and tube technique were used to assess the production of biofilms. The antimicrobial susceptibility for both antibiotics and essential oil was determined by Kirby Bauer and broth dilution methods. Eleven male mice were used in the *in vivo* study, and each animal had wound infection on the dorsal inter-scapular skin region created with a 6 mm biopsy punch and 50 µl (adjusted to 0.5 McFarland standard) of MRSA. The size of the wound and its histological characteristics were used to estimate healing rate.

**Results:** The GC-MS investigation of the essential oil revealed six compounds, with benzene (2-nitroethyl) being the most prominent. Out of the eighteen (18) isolates examined, 12 MRSA strains were identified using the three methods for methicillin resistance determination, with about 80% of them being classified as biofilm producers. More than 60% of the MRSA isolates were resistant to erythromycin, fusidic acid, gentamicin and trimethoprim/sulfamethoxazole. The essential oil had greater antibacterial activity than the reference antibiotic, vancomycin. The essential oil had a minimum inhibitory concentration of 80 l/ml and a minimum bactericidal concentration of 160 l/ml (v/v). Haematoxylin and eosin staining revealed that the skin tissue that had been exposed to *D. tripetala* essential oil had a thicker epithelial layer, numerous fibroblasts, a build-up of collagen, and many blood cells.

**Conclusions:** The results showed that *D. tripetala* essential oil has powerful anti-staphylococcal properties as well as the capacity to expedite wound healing. This suggests that *D. tripetala* essential oil could be a successful candidate for developing a topical agent for wound management.

**Keywords:** Antibacterial activity, *Dennettia tripetala*, Essential oil, Methicillin-resistant *Staphylococcus aureus*, Wound healing

\*Correspondence: sadesidashittu@gmail.com

<sup>1</sup> Department of Microbiology, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria

Full list of author information is available at the end of the article

## 1 Background

Medicinal plants have long been used to manage a variety of diseases in both humans and animals, due to their distinct antibacterial, anti-inflammatory and antioxidant properties since ancient times [1, 2]. This practice is assuming global importance owing to the rising resistance of microbes to antibiotics which has become the hallmark of infectious diseases. Most medicinal plants contain biologically active volatile organic components such as phenylpropenes, aromatic hydrocarbons, aliphatic and cyclic terpenoid chemicals, demonstrating their long-standing traditional uses [3]. Essential Oils (EOs) produced from most plants, including those from *Dennettia tripetala* (Annonaceae), have been examined for their antifungal, insecticidal, antioxidant, antinociceptive, antihyperlipidemic, and anti-inflammatory effects [4–6].

*Dennettia tripetala*, commonly referred to as Pepperfruit, is frequently used as condiments in West African countries [1] and widely grown in the South-East and Western regions of Nigeria. Previous studies have shown that the essential oil of its fruit and seeds contains significant bioactive components that could serve as interesting targets for new antimicrobials, antioxidants, and preservatives [5, 7, 9], in addition to its local usage for cough, sore throat, fever, and nausea [8]. It has been noted that some of its bioactive components, including 2-phenyl nitroethane, linalool, and (6E)-nerolidol [10], have hydrophobic qualities that could interface with various sites of microbial cells and activities of membrane-associated enzymes [11]. These observations reinforce the importance of ethnopharmacological studies on *D. tripetala* and encourage the development of antimicrobials against resistant pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA).

Methicillin-resistant *S. aureus* is one of the most prominent and recalcitrant pathogens impacting wound healing process. The World Health Organization (WHO) has designated the organism as one of the twelve priority pathogens posing threats to human health. Infections due to MRSA have become significant issue since the organism can develop resistance to routinely used antibiotics through the possession of *mecA* gene, thereby limiting therapeutic options to a few expensive drugs. Furthermore, the capacity of some strains to produce biofilm tends to make treatment more difficult, resulting in a longer hospital stay and higher medical costs. Its involvement in wound infections is becoming a major medical issue around the world [12].

A wound is described as any interruption in the continuity of the skin, mucous membranes, or internal tissues. [13]. Skin wounds are typically classified into acute and chronic wounds, where acute wounds are

painful and can heal over time according to the regular wound healing process [14]. Chronic wounds are tissue injuries that do not heal in a timely and orderly manner due to bacterial infection. MRSA-infected wounds are likely to affect the structure of the skin and may result in sepsis, scarring, gangrene, lengthy hospitalisation, and increased expenses [15]. Although, there have been a few studies on the antibacterial and antioxidant properties of *D. tripetala* essential oil, there has been limited research on the anti-MRSA effect and the anticipated benefits of its fruit oil in wound healing. To this end, we evaluated the antimicrobial activity of the *D. tripetala* EO against MRSA and its inherent capacity to expedite wound healing in mice with MRSA-infected wounds.

## 2 Methods

### 2.1 Staphylococcal isolates and study design

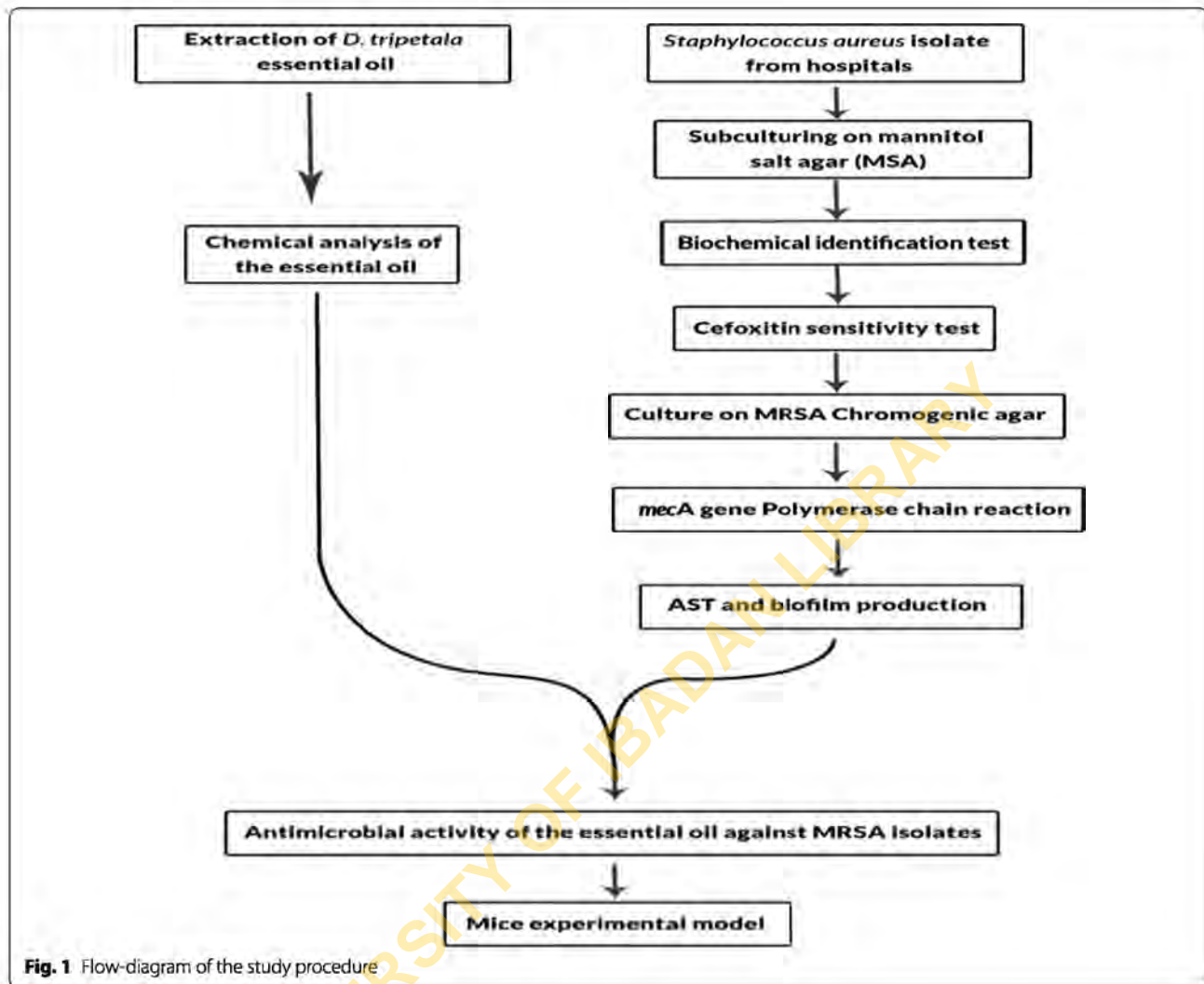
*Staphylococcus aureus* isolates (n=18) from wounds were obtained from three medical centres in Lagos (Lagos University Teaching Hospital, Lagos State University Teaching Hospital, and National Orthopaedic Hospital, Lagos) between April and October, 2019. The isolates were subjected to biochemical characterization based on standard conventional procedures [16]. Ethical approval for animal experimentation was obtained from the Health Research Committee, College of Medicine, University of Lagos, Nigeria, prior to the start of the investigation, and the guidelines for animal studies were strictly followed (Ethical approval reference number: CMUL/HREC/000608/201919). The workflow of the entire methodology is depicted schematically in Fig. 1.

### 2.2 Processing and extraction of essential oil from *Dennettia tripetala*

Dried fruits of *D. tripetala* were purchased from a fruit and spice market in Onitsha, South-East Nigeria, and authenticated at the University of Lagos, Botany Department, Faculty of Science. Five hundred grams of dried fruits were hydro-distilled for 5 h using a Clevenger-type apparatus. The essential oil was collected using n-hexane, concentrated with a rotary evaporator, and stored in sealed vials at 4 °C pending analysis.

### 2.3 Characterisation of essential oils by gas chromatography-mass spectrometry (GC-MS)

Essential oil analysis was carried out with a GC-MS system (7890A Agilent Technologies Inc., Santa Rosa, CA, USA). The GC-MS system's conditions and parameters included those defined by Wang et al. [17].



By comparing their mass spectra to those in the NIST14.L MS library (National Institute of Standards and Technology, Gaithersburg, MD, USA) [18], the separated constituents were identified.

#### 2.4 Phenotypic identification of methicillin-resistant *Staphylococcus aureus*

The methicillin susceptibility of all isolates was determined by using 30 µg cefoxitin disks on Mueller–Hinton agar (MHA) plates incubated at 35 °C for 24 h. The zone of inhibition was interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations: (susceptible ≥ 22 mm, resistant < 22 mm) [19]. All isolates were subcultured on CHROMagar medium (CHROMagar, France). Based on the manufacturer's recommendations, isolates that had characteristic mauve colour within 24 h were MRSA.

#### 2.5 *mecA* PCR

Staphylococcal DNA was extracted from overnight pure culture by boiling method [20]. The PCR was carried out in 20 µl of a reaction mixture using the protocol described by Murakami et al. [21]. The amplification product was run on 1.5% agarose gel. Ethidium bromide staining was used to visualise DNA bands after electrophoresis and a 100 bp DNA ladder was used as molecular weight standard.

#### 2.6 Biofilm detection

The potential of the isolates to produce biofilm was evaluated qualitatively by Congo red and tube techniques as described previously [22].

## 2.7 Antibiotic susceptibility testing

### 2.7.1 Disk diffusion assay

Antibiotic susceptibility pattern was determined on Mueller–Hinton agar (MHA) (Oxoid, England) and interpreted based on EUCAST [19] recommendations. The antimicrobial agents tested were: Erythromycin (15 µg), gentamicin (10 µg), trimethoprim/sulfamethoxazole (25 µg), fusidic acid and piperacillin-tazobactam (100 µg/10 µg) (Oxoid, England). *S. aureus* ATCC 25923 was used for quality control.

### 2.7.2 Determination of minimum inhibitory concentration (MIC) for vancomycin

The MIC of vancomycin for the MRSA isolates was determined by the broth microdilution method [23]. MIC ≤ 2 µg/ml was considered as sensitive, 4–8 µg/ml as vancomycin-intermediate *S. aureus*, and ≥ 16 µg/ml as vancomycin-resistant *S. aureus*.

## 2.8 Antimicrobial activity of *Dennettia tripetala* essential oil

### 2.8.1 Disk diffusion method

The bactericidal activity of essential oils was first tested through the disk diffusion method [24]. Sterile filter paper disks (Whatman no 1, England, 6 mm diameter) were permeated with 50 µl of the oil and allowed to stand at room temperature for 20 min. Coconut oil was used as the diluent. An inoculum was aseptically prepared by suspending two colonies from an overnight culture plate into 2mls of distilled water. The impregnated disks were carefully placed on a uniform lawn made from bacterial culture grown overnight (adjusted to 0.5 McFarland standard) on MHA plates. The plates were left at room temperature for 30 min to allow for oil diffusion before being incubated at 37 °C for 24 h. A filter paper disk impregnated with coconut oil was used as negative control while vancomycin (30 µg) was used as the positive control. To prevent evaporation of the essential oil, all plates were sealed with sterile aluminium foil and incubated at 37 °C for 24 h. The zones of inhibition were measured in millimetres.

### 2.8.2 Evaluation of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Following the methods outlined by Okoh et al. [5], the MIC of the essential oil was determined via the broth microdilution method. The essential oil stock was dissolved in polyethylene glycol (5%). Aliquots of 1600 µl, 800 µl, 400 µl, 200 µl, and 100 µl were added into test tubes containing 360 µl, 1160 µl, 1560 µl, 1760 µl, and 1860 µl of Mueller–Hinton broth. The mixture was vortexed and 40 µl of bacterial suspension (adjusted to 0.5

McFarland) was subsequently added to bring the final volume to 2 ml and concentration of 160 µl/ml, 80 µl/ml, 40 µl/ml, 20 µl/ml, and 10 µl/ml (V/V), respectively. The MIC was defined as the lowest concentration that inhibited bacterial growth after being cultured at 37 °C for 24 h. The lowest concentration of the EO that kills 100% of the initial bacterial population and showed no colonies on the MHA plates at 37 °C for 24 h was recorded as the MBC. Five percent polyethylene glycol was used as negative control while vancomycin (16 µg/ml) prepared as described previously [23] was used as a positive control.

## 2.9 Mice experimental model

### 2.9.1 Experimental mice

This experiment involved eleven (11) male albino mice weighing 26–32 g obtained from the Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria. The mice were caged for ten to fourteen days for acclimatisation. They were kept in different iron cages with access to air, humidity, and a day and night cycle at an ambient temperature of 23 ± 3 °C. They were all fed sterile rodent laboratory diets and water ad libitum. The iron cage was swabbed every day with 70% ethanol to reduce microbial contamination. All animal handling protocols were carried out in strict accordance with international guidelines for the use of experimental animals [25].

### 2.9.2 Wound initiation and dressing

The mice were divided into 3 groups. Group 1 comprised of 3 mice while group 2 and 3 comprised of 4 mice each. Diluted essential oil (Coconut Oil + Essential Oil; 2:1) was topically applied to the 1st Mouse in group 1, the second Mouse received Coconut Oil (the carrier oil used for diluting the essential oil), while the last Mouse served as the negative control. In group 2 and 3, the first mouse received diluted essential oil, the second mouse received honey, the third mouse received mupirocin (positive control) while the last mouse served as the negative control. After anaesthesia, a circular wound was created by excising the skin on the dorsal inter-scapular shaved region of each animal using a 6 mm biopsy punch. A suspension of MRSA prepared with 50 µl phosphate buffered saline and diluted to 0.5 McFarland was applied to the wound. The wounds were left open for 24 h before the oil was applied topically once a day until the wound healed completely. Digital photographs were taken for each wound every day. The percentage of wound contraction was determined by calculating the wound area using ImageJ software. Based on the initial and final wound areas, the percentage of wound closure was estimated as follows:

$$\text{Wound closure Percentage} = \frac{\text{Wound area on Day 0} - \text{Wound area on Day X}}{\text{Wound area on Day 0}}$$

where X = day post-injury.

### 2.9.3 Bacteriological examination of the wound area

On day 3, 5, and 7, the total bacterial count was determined by collecting sections from the wound sites with sterile swab sticks and homogenising them in test tubes containing 2 ml of tryptone soy broth. The homogenised sample was serially diluted in 9 ml sterile distilled water before being cultured on MHA and MRSA ChromAgar. The plates were incubated aerobically for 24 h at 37 °C. After incubation, all colonies were counted and the results were expressed as Colony Forming Unit (CFU)/ml.

### 2.10 Histopathological analysis

Mouse was euthanised by cervical dislocation ten days after the wound was created, and samples from the wound area, including epidermis, dermis, and subcutaneous area, as well as 1 to 2 mm around the normal skin, were removed. Cut portions of observable lesions were fixed in 10% neutral-buffered formalin before placing in well-labelled warm molten paraffin blocks embedding tissue cassette [26] after which they were processed using a 24 h automatic tissue processor (Microm STP 125 Thermo-fisher—USA). The tissue sections were stained with Haematoxylin and Eosin. It was mounted using Dibutylphthalate Polystyrene Xylene and the slides were reviewed microscopically with 10X and 40X magnification.

### 2.11 Statistical analysis

Statistical differences were evaluated through a one-way analysis of variance (ANOVA) along with the Dunnett and Tukey test (Minitab 22.3 Version, LLC). Differences were considered significant at the  $p < 0.05$  level.

## 3 Results

### 3.1 Chemical Component of *Dennettia tripetala* essential oil

The GC–MS analyses of the essential oil of *D. tripetala* fruits identified six compounds (Table 1). The most abundant chemical was benzene (2-nitroethyl), which made up 51.74% of the total constituents. Linalool, the second most prominent component, had a molecular weight of 154 g/mol and retention time of 3.793 min. Caryophyllene oxide was identified at a retention time of 10.629 min. Caryophyllene oxide and 1, 6, 10-Dodecatrien-3-ol, 3, 7, 11-trimethyl- (Nerolidol) which are sesquiterpenoids accounted for 22.9% of the overall oil content. The mass spectrum, structural formula, and molecular weight of the various compounds identified are shown in Fig. 2.

### 3.2 Biofilm production and antibiotic susceptibility of MRSA isolates

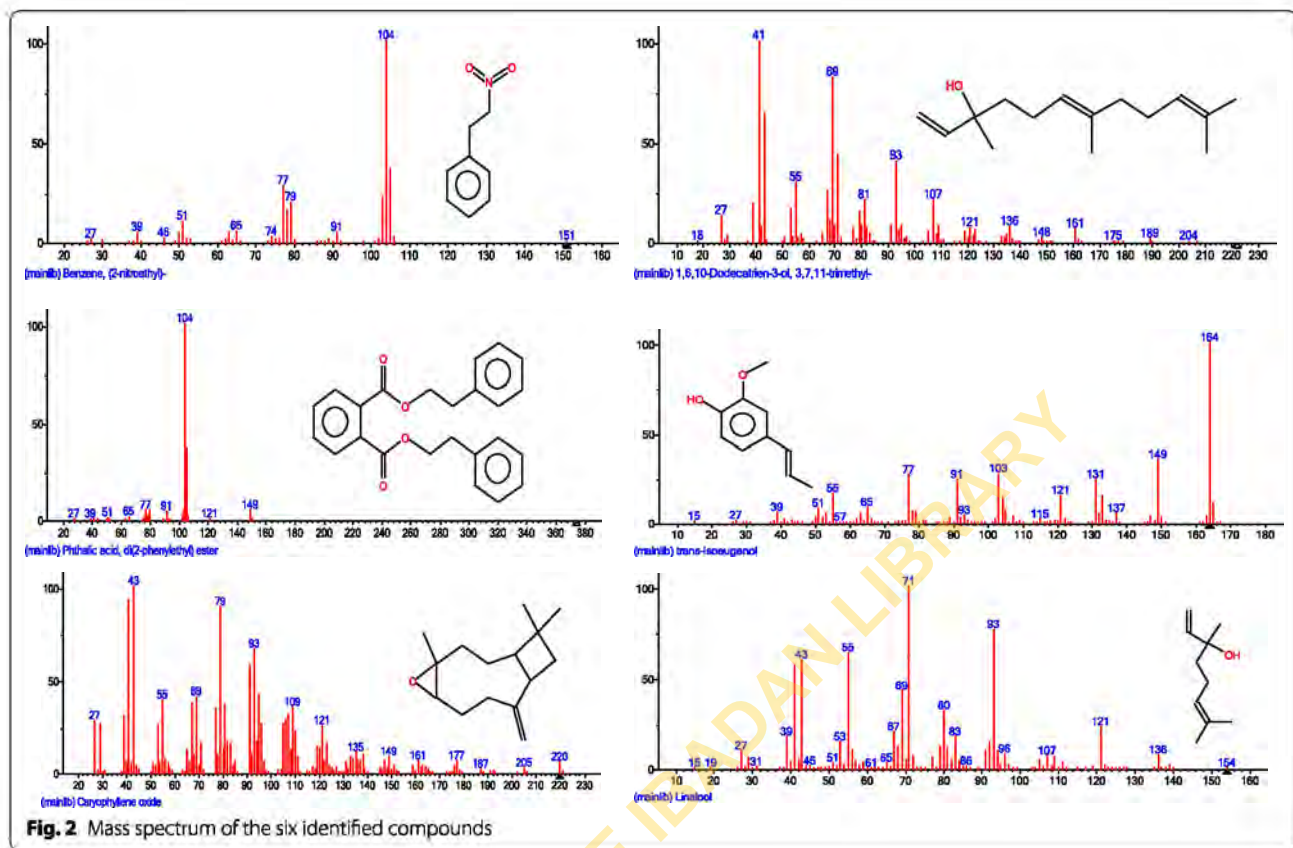
Out of the 18 *S. aureus* isolates analysed, 12 (66.7%) were identified as MRSA. Ten of the 12 MRSA strains were able to produce a varying degree of biofilm by both the tube method and Congo red method. Each antibiotic tested in this study represents a class and the results indicated the multidrug resistance feature of the MRSA isolates. Nine strains were resistant to trimethoprim/sulfamethoxazole, a folate pathway inhibitor, 7 were resistant to gentamicin, an aminoglycoside and 6 strains exhibited resistance to erythromycin, a macrolide. Only one isolate had piperacillin/tazobactam resistance, while fusidic acid resistance was 100%.

### 3.3 Antimicrobial activities of *Dennettia tripetala* essential oil and vancomycin

Undiluted *Dennettia tripetala* essential oil (EO) inhibited all the tested MRSA strains. The minimum inhibitory concentration of vancomycin to three strains of MRSA

**Table 1** Constituents in the essential oil of *Dennettia tripetala*

Retention time (Min.)	Molecular weight (g/mol)	Percentage Composition	Name of Identified compound
3.793	154	24.24	Linalool
6.589	151	51.74	Benzene, (2-nitroethyl)
7.208	374	0.36	Phthalic acid, di(2-phenylethyl) ester
10.033	164	0.75	Trans-Isoeugenol
10.424	222	21.51	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl- (Nerolidol)
10.629	220	1.9	Caryophyllene oxide



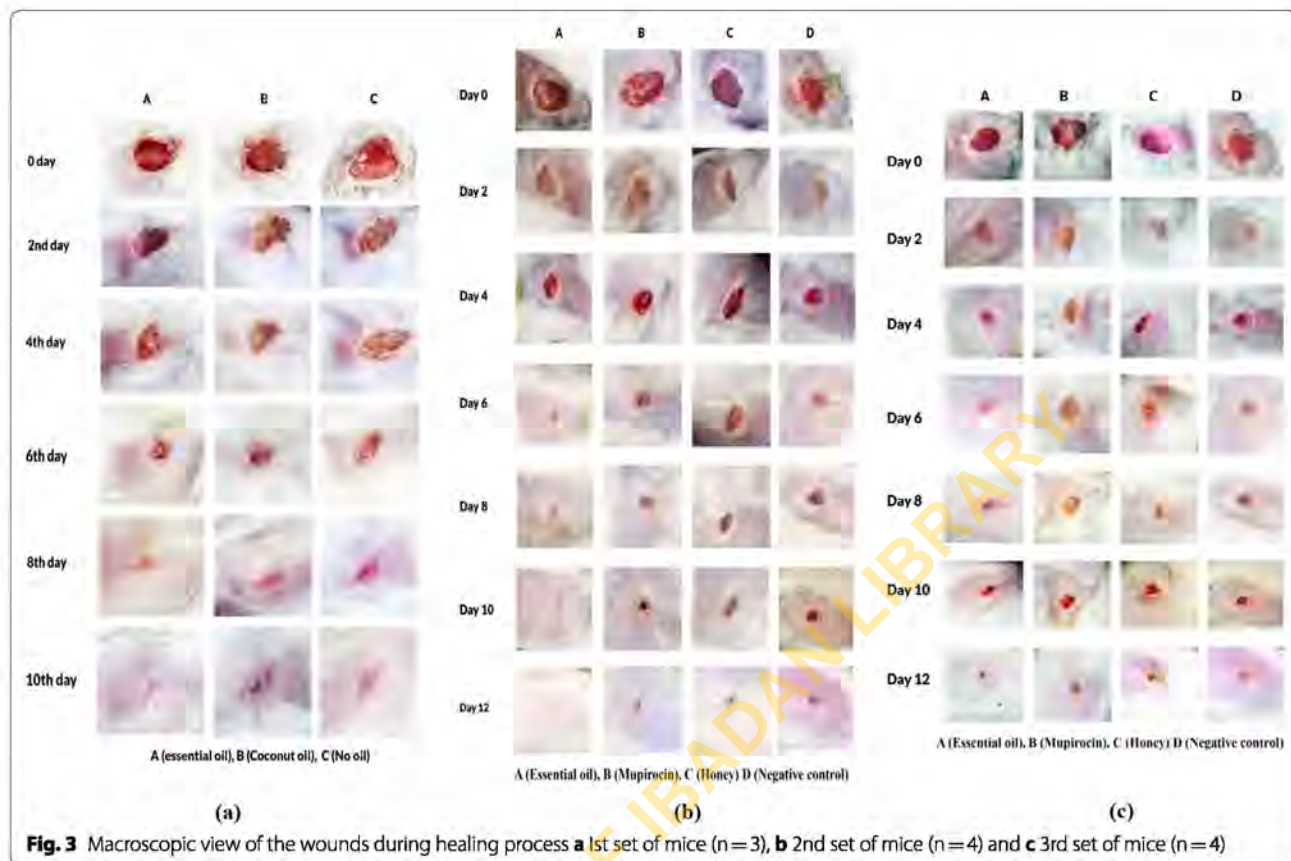
**Table 2** Susceptibility of MRSA to various concentrations of *Dennettia tripetala* Essential Oil

Isolate Codes	Disk Diffusion: zone of inhibition (mm)		MIC						MBC	
	<i>D. tripetala</i> EO	NC	PC (30 µg)	160 µl/ml	80 µl/ml	40 µl/ml	20 µl/ml	10 µl/ml	NC	PC (16 µg/l)
WS101/19 N	40	0	0	—	+	+	+	+	+	—
W101	41	0	23	—	+	+	+	+	+	—
W164	40	0	21	—	+	+	+	+	+	—
W81	28	0	23	—	+	+	+	+	+	—
W001	34	0	0	—	+	+	+	+	+	—
W163	34	0	23	—	+	+	+	+	+	160 µl/ml
WS101/19	40	0	0	—	+	+	+	+	+	+
WS88	44	0	14	—	+	+	+	+	+	—
1158	30	0	20	—	+	+	+	+	+	—
1100	9	0	0	—	+	+	+	+	+	+
W107	40	0	22	—	—	+	+	+	+	—
W113	50	0	23	—	—	+	+	+	+	80 µl/ml

NC Negative control (undiluted coconut oil), — (No visible growth), + (visible growth), NC Negative control (5% Polyethylene glycol), PC Positive control (vancomycin, 16 µg/ml)

was  $\geq 16 \mu\text{g/ml}$ , thereby indicating resistance. The Minimum Inhibitory Concentration (MIC) of the EO ranged from 80 to 160  $\mu\text{l/ml}$  with only two isolates inhibited

at 80  $\mu\text{l/ml}$  (Table 2). The minimum bactericidal concentrations were the same as the minimum inhibitory concentrations.



### 3.4 Mice experimental model

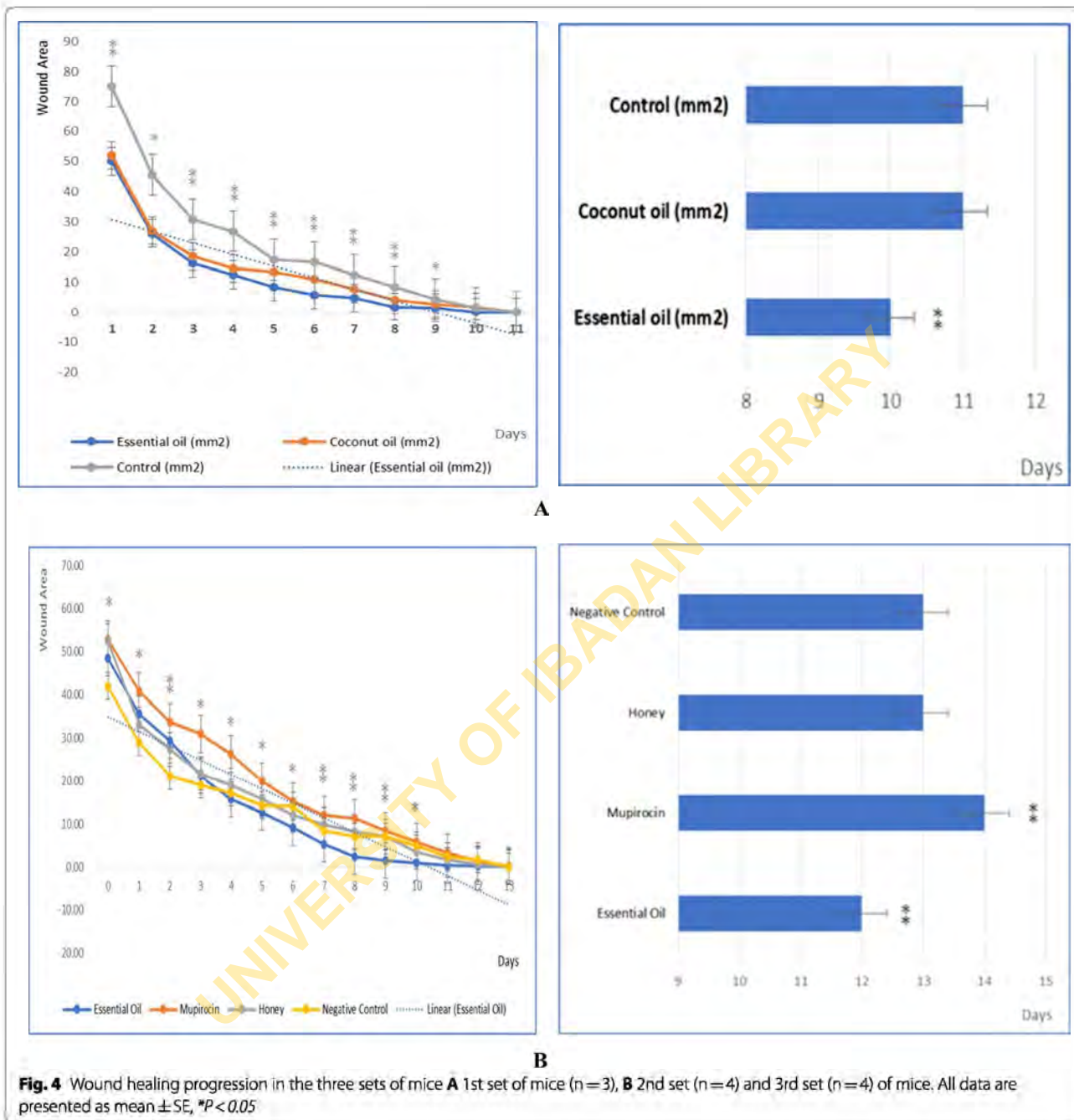
The macroscopic view of the wound during the healing period for the three sets of mice is shown in Fig. 3. Wounds treated with *D. tripetala* EO had a faster rate of healing and required fewer days to recover completely than wounds treated with the control (Figs. 3, 4). The topical application of EO accelerated wound closure between the 5th and 10th day following incision (Figs. 3, 4). The total bacterial and MRSA count significantly decreased with the application of the essential oil compared to mupirocin, honey, coconut oil, and the negative control (Fig. 5). Histopathological evaluation of the tissues of the essential oil-treated group demonstrated well formed granulation. Tissue sections from the negative control and coconut oil-treated animals, on the other hand, showed less/weaker granulation (Fig. 6).

## 4 Discussion

In this investigation, six compounds were identified in the essential oil of dried seeds of *D. tripetala*. In the literature, the chemical profiles of *D. tripetala* EO have been shown to differ substantially. Okoh et al. [5] detected thirty-three and twenty-seven compounds in riped and

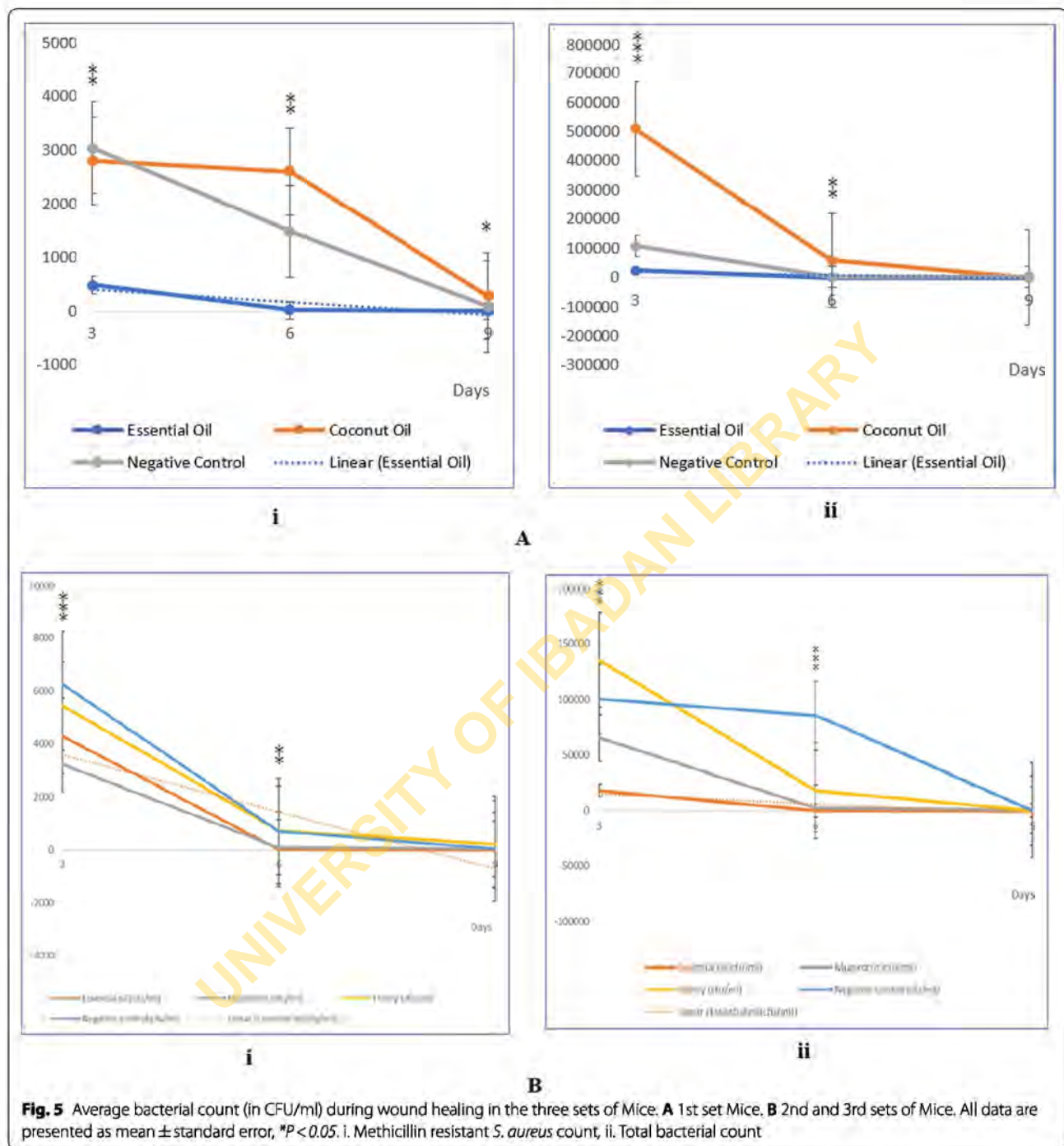
un-ripped fruits of *D. tripetala* EO, respectively. Oyemitan et al. [10] detected nineteen (19) compounds from the fresh fruits and eight (8) from the dried seeds. The discrepancies in the components of *D. tripetala* EO may be due to differences in geographical locations, storage conditions, drying processes, humidity, and extraction methods of the EO. We found that benzene (2-nitroethyl) was the most abundant mono-substituted aromatic compound, accounting for 51.74% of the total composition of the essential oil. This is in line with previous findings in which the absolute composition of benzene (2-nitroethyl) was 53.70% [27]. Benzene (2-nitroethyl), also known as phenyl-nitroethane, is a colourless oil that is slightly heavier than water and recognised to play a key role in therapeutic potentials of essential oils. Linalool constitutes 24.24% of the essential oil and was the second most abundant. The only monoterpenoid found in this investigation were linalool and trans-isoeugenol. This is consistent with the findings of Oyemitan et al. [10].

Findings from the present study suggested that MRSA was likely to be widespread in the hospitals surveyed. Sixty-seven percent of the 18 *S. aureus* isolates were identified as MRSA. This is consistent with the findings of Ariom and colleagues [28]. While some researchers



[29] documented a lower frequency (8.7%), Garoy and colleagues [30] observed high incidence (72%; 59/82) of MRSA emancipating from wounds. However, our observation confirms that the rate of MRSA varies from settings to settings and may be influenced by a number of variables, including the use of antibiotics in the study location. All MRSA strains in the current study, with the exception of two, demonstrated the ability to produce biofilm. The presence of biofilm-forming bacteria has

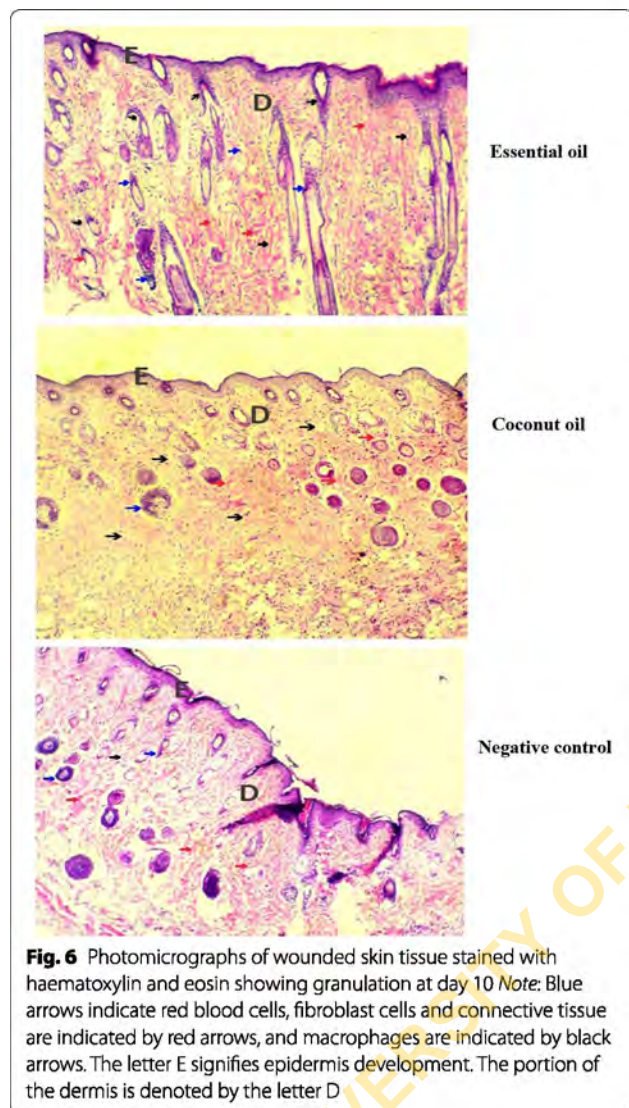
become a serious problem in the treatment of wound infections. This trait is thought to confer virulence and persistence. Several writers alleged that biofilms may grow on any wound especially when planktonic bacteria are not eliminated by the host immune system or antimicrobial treatment [31]. We considered that the biofilm-forming characteristics of the MRSA isolates recognised in this study increased their pathogenicity, which was



exacerbated by their high level resistance to the six antibiotics tested.

Additionally, the minimum inhibitory concentration of vancomycin for three strains of the MRSA was MIC > 16 µg/ml, suggesting reduced susceptibility to this antibiotic. Vancomycin is a well-known front-line drug of choice for treating MRSA infections. The emergence of

vancomycin-resistant *S. aureus* plays a significant role in the treatment failure of staphylococcal infections which is becoming a significant issue in our settings. Similarly, this investigation found that the MRSA isolates were entirely resistant to fusidic acid, as an earlier research in Nigeria had shown [32]. Thus, it appears that a substantial proportion of *S. aureus* in our setting is resistant to



**Fig. 6** Photomicrographs of wounded skin tissue stained with haematoxylin and eosin showing granulation at day 10 Note: Blue arrows indicate red blood cells, fibroblast cells and connective tissue are indicated by red arrows, and macrophages are indicated by black arrows. The letter E signifies epidermis development. The portion of the dermis is denoted by the letter D

fusidic acid. The occurrence of fusidic acid resistance in clinical isolates of *S. aureus* has been found to be rising globally [33]. Future study on the genotypes of fusidic resistant *S. aureus* as well as long-term surveillance studies, particularly in these hospitals will be of interest.

Nonetheless, our findings highlight the potential threat posed by MRSA-infected wounds if such isolates become extensively disseminated.

In this study, the essential oils extracted from the dried fruit of *D. tripetala* displayed a strong inhibitory effect against the tested MRSA strains. This is consistent with the findings of Oyemitan et al. [10] who observed high antimicrobial activities of the EO from the dried seed of *D. tripetala* against certain bacterial strains including *S. aureus* (NCTC 6571). Although, we did not study each individual component of the essential oil, some

investigators have deduced that the antimicrobial activity of the *D. tripetala* essential oil may depend on one or two of the major constituents that make up the oil or trace components in the crude essential oils which can also exhibit synergistic effects [34, 35]. However, we observed that the antibacterial activity of the essential oil was concentration-dependent, and the rate of inhibition varied among the MRSA strains.

In this study, it was interestingly discovered that topical application of *D. tripetala* essential oil significantly decreased the total bacterial count compared to control groups (one-way ANOVA, Turkey, and Dunnett test;  $p < 0.05$ ). Wound healing is a highly organised process involving inflammatory reactions, fibroplasia/proliferation and remodeling or maturation [36]. The process may be complicated by the presence of resistant bacteria with virulent traits like MRSA. The enhanced healing rate might be attributed, on the basis of the current data, to a reduced inflammatory phase and faster proliferation and maturation stages. Between the 5<sup>th</sup> and 10<sup>th</sup> days after the initial development of the wound, it was noticed that wound closure advanced more quickly when the EO was applied topically. This suggests that the wound healing properties of this essential oil peaked at the proliferation (2<sup>nd</sup> phase) and the remodeling phases (3<sup>rd</sup> phase). Inflammation could destroy the extracellular matrix, cell senescence, and delays in wound healing. The anti-inflammatory activity of *D. tripetala*, as discovered by Oyemitan et al. [4], possibly account for the decrease in local oedema, and the prevention of tissue function loss, hence a faster rate of wound healing.

The histopathological analyses revealed that, in contrast, to the skin tissue treated with coconut oil and the negative control, the skin tissue treated with essential oil had a thicker epithelial layer, a lot of fibroblasts, collagen deposition, and many blood cells (one-way ANOVA, Turkey, and Dunnett test;  $p < 0.05$ ). The enhanced re-epithelisation of the impaired tissue, which is caused by the remodelling of the cell adhesion molecules at the wound edge keratinocytes, may be responsible for the considerable rate of wound closure observed from the 5<sup>th</sup> to the 10<sup>th</sup> days of the healing process. These cells can migrate to assist in improving the lesion due to the lack of adhesion with other keratinocytes and basement membrane proteins [37]. In the injured tissue treated with *D. tripetala* EO, macrophages and fibroblasts were seen in larger numbers. In essence, as compared to controls, the application of the EO considerably reduced the overall bacterial and MRSA count.

Observably, the antimicrobial activity of *D. tripetala* EO must have played a vital role in the wound healing process by drastically reducing the microbial load which

would have delayed the progression. These assertions have been explored by various authors regarding essential oils from some plant species such as *Cinnamomum zeylanicum* [38]. One of the components discovered, isoeugenol, has been shown to possess antimicrobial properties [39]. The primary component of the essential oil under study, benzene (2-nitroethyl), may also have reacted with the bacterial cells through a number of different mechanisms, such as altering the fatty acid composition of the cell membrane, inhibiting enzymes and proteins, causing ion and metabolite leakage, or altering the proton motive force [40]. We therefore opined that *D. tripetala* essential oil could be used therapeutically to promote wound healing and treat wound infections. As a result, we believed that *D. tripetala* essential oils may be utilised medicinally to foster wound healing and treat wound infections. Nevertheless, more research is needed to determine its safety.

## 5 Conclusions

This study revealed promising antimicrobial activities of *D. tripetala* essential oil against all the tested MRSA isolates with zones of inhibition ranging from 9 to 50 mm and the MICs and MBCs within the range of 80 µl/ml and 160 µl/ml (v/v). This information would be useful in the design and formulation of *D. tripetala* essential oils as a bioactive agent. This study also suggests that the *D. tripetala* EO can enhance the healing of MRSA-infected wound by inhibiting the growth of the bacteria and increasing the granulation of tissues. Due to its potent antibacterial properties, *D. tripetala* essential oil may therefore be a viable choice for the production of topical creams for wound healing.

### Abbreviations

PCR: Polymerase chain reaction; EO: Essential oil; MRSA: Methicillin-resistant *Staphylococcus aureus*; GC-MS: Gas chromatography-mass spectrometry; MIC: Minimum inhibitory concentration; MHA: Mueller–Hinton agar; MBC: Minimum bactericidal concentration; CFU: Colony forming unit (CFU).

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### Author contributions

All the authors listed in this article participated and contributed to the research. SAA and MIJ conceptualized and designed the study, OOA and MIJ performed the lab work, SAA, MIJ and EAA prepared the manuscript. RFP and CCE carried out the initial bacteriological analyses of the isolates. The final manuscript was read and approved by all authors.

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### Availability of data and materials

All data generated or analysed during this study are contained within this published article.

## Declarations

### Ethics approval and consent to participate

No human was used in this research. All animal research procedures were followed as specified by the Health Research Committee, College of Medicine, University of Lagos, Nigeria (Reference number: CMUL/HREC/000608/201919).

### Consent for publication

Not applicable.

### Competing interests

There are no competing interests declared by the authors.

### Author details

<sup>1</sup>Department of Microbiology, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria. <sup>2</sup>Biochemistry and Nutrition Department, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria. <sup>3</sup>Department of Medical Microbiology and Parasitology, Lagos University Teaching Hospital, Idi-Araba, Lagos, Nigeria. <sup>4</sup>Pathology Department, National Orthopaedic Hospital, Yaba, Lagos, Nigeria. <sup>5</sup>Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.

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