Antimicrobial and antioxidant effect of methanolic *Crinum jagus* bulb extract in wound healing

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ABSTRACT

**Aim:** The aim of this study was to evaluate the antimicrobial and antioxidant effects of *Crinum jagus* (J. Thomps.) Dandy methanolic bulb extract in wound healing. **Materials and Methods:** Phytochemical screening revealed the presence of alkaloids, glycosides, tannins, and saponins in the extract. *In vitro* antimicrobial activity of the extract was determined by agar well diffusion method. *In vivo* antimicrobial activity of the extract was determined by microbial assay of excision wound in rats contaminated with *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Candida albicans* and treated with 300 mg/kg body weight (bw) of 10 and 5% methanolic *C. jagus* bulb extract ointment (MCJBO), respectively. Enzymatic antioxidant effect of the extract was determined *in vivo* by assaying superoxide dismutase (SOD) and catalase (CAT) activity, and malondialdehyde (MDA) level in excision wound biopsies of rats treated with 10 and 5% MCJBO, respectively, following standard methods. Non-enzymatic antioxidant effect of the extract was determined *in vitro* using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method following standard procedure. **Results:** The extract exhibited *in vitro* antimicrobial effect in a concentration-dependent manner with one hundred (100) mg/ml concentration of the extract having the highest inhibitory zone diameter for *B. subtilis* (25 mm), *S. aureus* (21 mm), and *C. albicans* (14 mm) followed by the 50, 25 and 12.5 mg/ml concentrations, respectively. *B. subtilis*, *S. aureus*, and *C. albicans* were not isolated from wounds of animals treated with both extract concentrations 10% and 5% MCJBO, respectively. Activities of the enzymatic antioxidants SOD and CAT in wound biopsies treated with 10% MCJBO were significantly (*P* < 0.05) higher when compared with those treated with 5% MCJBO. Significantly (*P* < 0.05) decreased MDA level of wound biopsies from extract-treated rats was observed. The extract exhibited non-enzymatic antioxidant (DPPH) effect in a concentration-dependent manner. **Conclusion:** This study has shown that an anti-microbial and antioxidant effects could possibly be part of mechanism by which *C. jagus* bulb extract promote wound healing process.

KEY WORDS: Antimicrobial, antioxidant, *Crinum jagus*, *in vivo*, wound healing

INTRODUCTION

Wound healing is the process of re-establishing the integrity of damaged skin [1-3]. It is an orderly intricate process initiated by a damaged tissue itself, and it involves complex mechanisms which include: Hemostasis, inflammation, proliferation, and remodeling [1,4,5]. Each of these mechanisms requires several biochemical substances to occur [4,6,8]. Thromboxane A2 and plasminogen activator inhibitor Type 1 ensures hemostasis, heme and heme proteins trigger expression of adhesion molecules, leukocytic infiltration, and release of reactive oxygen species (ROS) also called toxic free radicals or oxidants. The oxidants are detrimental to wound contaminating microorganisms and to the skin tissue itself especially when in excess [4,6,8]. Therefore, hemeoxygenase-1 elicit antioxidant effect and scavange (mop-up) the toxic free radicals, while matrix metalloproteinase ensures remodeling of the extracellular matrix [9]. The length of time it takes for wound healing to be optimum and complete is determined by factors such as availability of the needed biochemical substances, presence or absence of contaminating microorganism(s), and the toxic free radicals in the wound bed [10,11].

A myriad of wound contaminating organisms including bacteria (both Gram-positive e.g., *Staphylococcus* spp., *Streptococci*, *Bacillus* spp., etc. and Gram-negative e.g., *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus*, etc.) and fungi (both yeast e.g., *Candida albicans*, and mold e.g., *Aspergillus*) impede natural wound healing process [12-15]. They produce substances (enzymes, toxins, and free toxic radicals such as hydrogen peroxide ([H₂O₂])) that degrade biochemical substances and destroy cellular components needed for wound healing [13-15]. Majority of
wound contaminating microorganisms have developed resistance to most of the commonly used orthodox antimicrobial agents and this has garnered public attention [16-20].

Toxic free radicals (oxidants) such as superoxide (SO$_2^{-}$) and hydroxyl (OH') anions, and hydrogen peroxide (H$_2$O$_2$) causes oxidative stress [21]. These oxidants are produced by infiltrating phagocytes (during inflammation stage of healing), contaminating microbes in wound bed, and the skin itself following ultraviolet exposure [21,22]. Oxidative stress further damages wounded skin tissue by lipid peroxidation (caused by lipid peroxidase and evidenced by increased malondialdehyde [MDA] level in wound tissue), and destruction of proteins and extracellular matrix [23] - this further impedes natural wound healing process. Substances that elicit antimicrobial and antioxidant effects are often used in wound management in orthodox medicine [24,25]. These substances are used to control the growth of contaminating organisms and mop-up (scavenge) toxic free radicals to achieve optimum healing [11,16]. The radical scavenging activity of natural enzymatic antioxidants (such as superoxide dismutase [SOD], catalase [CAT], glutathione dismutase, thioredoxin reductase, etc.) produced by the skin is augmented by the orthodox medicines to promote wound healing process [21]. Reports have shown that these orthodox agents are often costly [17], and often times elicit side effects which are detrimental to the recipient [11]. Therefore, there is need for cheaper and safe alternative or complementary substances that could elicit both antimicrobial and antioxidant effects to enhance natural wound healing process.

Crinum jagus (J. Thomps.) Dandy popularly called St. Christopher or Harmattan lily, Frest crinum or Poison bulb is widely used in form of decoction by traditional practitioners in Africa, including Southeastern Nigeria, for treatment of skin wounds and several other ailments [26,27,28-32] some of which have been scientifically validated [33,34,35]. Chemical investigations revealed that it contained high amount of phenolic compounds including crinamine, lycorine, psuedolycorine, 6-hydroxycrinamine, hamayne, tetrahydro-1, 4-oxazine (morpholine), bowdensine, and demethoxy-bowdensine [36,37,38,39]. It also contained saponins, tannins, calcium oxalate, and calcium tetrata [37,38]. Phenolic compounds in plant extracts exhibited enzymatic and non-enzymatic antioxidant effect [40,41]. Plant extracts containing flavonoids, triterpernoids, and tannins exhibited antimicrobial and antioxidant effects [40,42]. Therefore, C. jagus which contains some of these bioactive substances could possibly promote wound healing process by eliciting antimicrobial and/ or antioxidant effects.

Although Adesanya et al. [37] reported anti-staphilococcal activity of C. jagus bulb, the study was not conducted in a wound healing model; and Staphylococcus is one of the numerous potential wound contaminantsthat impede wound healing process. Ode et al. [35] and Nwaechujor et al. [36] reported non-enzymatic in vitro free radical scavenging of diphenylpicrylhydrazyl (DPPH) by C. jagus bulb extract which is not related to wound healing. The antimicrobial effect of C. jagus bulb extract on common potential wound contaminating microorganisms and its enzymatic antioxidant effect in wound healing have not been evaluated. The objective of this study was to determine if C. jagus methanolic bulb extract (CJMBE) could possibly exhibit antimicrobial and/or antioxidant effect in wound healing.

**MATERIALS AND METHODS**

The experimental protocols used in this study was approved by the Ethics Committee of the University of Nigeria, Nsukka and conforms with the guide to the care and use of animals in research and teaching of University of Nigeria, Nsukka, Enugu State Nigeria.

**Animals**

A total of 97, 8-week-old male albino Wistar rats weighing between 220 and 229 g were obtained from the laboratory animal unit, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were fed on commercial growers mash (Top feeds®) and water was provided ad libitum. These rats were acclimatized for 2 weeks in the animal house at the Department of Veterinary Surgery, University of Nigeria, Nsukka.

**Plant Collection and Identification**

Fresh C. jagus bulbs were collected from Amokwe town in Udi Local Government Area Enugu State, Nigeria, in the month of May, 2014 and were identified at the International Center for Ethnomedicine and Drug Development (InterCEDD), Nsukka, by a plant taxonomist, Mr. A. Ozioko. A voucher specimen (number FRMPC/05/14) was deposited in the center’s herbarium.

**Extraction**

A kg of the C. jagus (J. Thomps.) Dandy bulbs were sliced into smaller pieces; air dried at room temperature for 2 weeks and then pulverized using the laboratory grinding machine at the Department of Crop Science, University of Nigeria, Nsukka. The pulverized bulbs were macerated in 80% methanol for 48 h with intermittent vigorous shaking at every 2 h. After 48 h, the mixture was filtered and the extract concentrated using a rotary evaporator set at 40°C. The dried CJMBE was weighed and the percentage yield calculated. The extract was then stored at 4°C in a refrigerator until needed.

**Acute Toxicity Test**

Totally, 25 adult rats were randomly divided into five groups of five animals per group. The animals were deprived water for 16 h before administration of the extract. The increasing doses of the extract 250, 500, 1000, 2000, and 5000 mg/kg body weight (bw) suspended in dimethyl sulfoxide (DMSO) was administered orally to the test groups, respectively, using a ball-tipped intubation needle fitted onto a syringe. The last group received 1 ml/kg bw of sterile distilled water and served as the control. The rats were allowed access to food and water ad libitum and were observed for 48 h for behavioral changes.
and death. The time of onset, intensity, and duration of these symptoms, if any, was recorded.

**Phytochemical Analysis for Bioactive Substances**

The extract was screened for the presence of bioactive components tannins, saponins, glycosides, flavonoids, and alkaloids following the methods of Trease and Evans [43].

**Preparation of Ointments**

The method of Okore et al. [44] was adapted in preparation of two herbal ointments containing 10 and 5% w/w of the extract in sterile soft white paraffin. Immediately after preparation, the ointments were aseptically transferred into sterile cream tubes and sealed until further needed.

**Pathogens and Preparation of Inocula**

The bacterial (Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus subtilis) and fungal (C. albicans) organisms used in this study were collected from the Department of Pharmacy, University of Nigeria, Nsukka. They were clinical wound isolates from patients in Nsukka, Nigeria, fully identified and maintained on nutrient agar slope at 4°C at the Department of Pharmaceutical Microbiology Laboratory, University of Nigeria, Nsukka. Prior to use, the bacterial organisms were sub-cultured on sterile nutrient agar plate, incubated aerobically at 37°C for 24 h, while the C. albicans was sub-cultured on sterile Sabouraud dextrose agar, incubated at 25°C for 48 h. Colonies of each organism were homogenized in sterile phosphate buffered saline (PBS) and the turbidity adjusted to correspond to 0.5 McFarland’s turbidity standard (equivalent to 1 × 10⁸ cfu/ml). The standardized broth cultures were kept at 4°C until needed.

**Preparation of Extract Concentrations**

A 100 mg/ml stock concentration was prepared by dissolving 1 g of the extract in 10 ml of DMSO. Then, 2-fold dilutions were made from the stock concentration to obtain concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml.

**Determination of In vitro Antimicrobial Effect of C. jagus Bulb Extract**

The inhibitory zone diameter (IZD) of each of the extract concentrations for each test organism was determined by agar-well diffusion method [45]. Briefly, the standardized broth cultures of the bacterial organisms were incubated at 37°C for 10 min, and then inoculated on sterile Mueller-Hinton agar plates using sterile swab sticks. Five holes of 6 mm diameter were bored into the agar plates at strategic points using sterile cork borer and labeled to correspond to the extract concentrations. Then each of the holes was filled with 50 μl of the extract concentration. The plates were allowed on the bench to ensure complete diffusion of the extract into the agar and then incubated accordingly as above. For the fungal organism, broth culture was incubated at 25°C for 10 min before inoculating sterile Sabouraud dextrose agar. Same procedure as above was undertaken and then the plates were incubated at 25°C for 48 h. After incubation, the zone of inhibition around each well was measured with a meter rule. Each test for each organism was performed in triplicate and the mean IZD calculated to the nearest whole millimeters.

**Determination of In vivo Antimicrobial Effect of C. jagus Bulb Extract**

**Creation and contamination of excision wound with test microorganisms**

Thirty six rats were anesthetized with 10 and 50 mg/kg bw of xylazine hydrochloride and ketamine hydrochloride, respectively. Their dorsum was shaved and disinfected with 70% alcohol. Then, full thickness (480 mm²) circular excision wounds were created following the method described by Morton and Malone [46]. Post-wounding, the rats were randomly assigned into 4 groups of 9 animals per group. Then, using sterile Pasteur pipettes, wound on each animal was contaminated by flooding with 1 ml of standardized broth culture of each test organism. To minimize further microbial contamination of wound, each animal was carefully placed individually in disinfected cages kept in a disinfected, clean and dust-free animal house in the Department of Veterinary Surgery, University of Nigeria, Nsukka. The wounds were not treated for 24 h post-contamination to ensure colonization and establishment of infection [11].

**Treatment of Infected Excision Wound**

Treatment of contaminated animal wound commenced 48 h post-contamination. Four treatment groups consisting of 9 animals each were treated as follows: Groups A and B were treated topically with 10% and 5% w/w methanolic CJMBE ointment (MCJBEO), respectively, Group C was treated with sterile normal saline (negative control), while Group D was treated with framycin sulfate/clotrimazole (Sofradex-f®) (positive control), respectively. Treatment of the animals continued until complete healing occurred.

**Isolation of Infective Pathogen from Contaminated Excision Wound**

At days 3, 7, and 14 post-infection (p.i.), wound swabs from 3 animals in each treatment group was taken in duplicate using sterile swab sticks. The swabs were inoculated into sterile nutrient and Sabouraud dextrose broth, incubated at 37°C for 24 h and 25°C for 48 h, for bacterial and fungal isolation, respectively. The broth cultures were observed for microbial growth (cloudiness/turbidity) and if any, a loopful of the broth culture was sub-cultured on appropriate sterile agar and incubated accordingly. Isolates of different colonial types, if any, were purified on appropriate fresh media and incubated accordingly. Morphological characteristics of pure colonies of the isolates were noted and appropriately described. Then, pure colonies of the isolates were gram stained and subjected to biochemical tests such as catalase, hemolysis and coagulase.

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for identification following standard biochemical methods. The number of animals from which each organism was isolated was appropriately recorded and the percentage calculated.

**Determination of Enzymatic Antioxidant Effect of C. jagus Bulb Extract in Wound Tissue**

A total of 36 rats were anesthetized and full thickness (480 mm²) circular excision wounds were created as described above. Four treatment groups consisting of 9 animals per group were treated as follows: Groups I and II were treated topically with 10 and 5% w/w MCJBE, respectively, while Groups III and IV were treated with sterile white soft paraffin (negative control) and framycetin sulfate/clotrimazole (positive control), respectively. Then, the wound on each of the animal was carefully bandaged using sterile gauze and adhesive tape was placed over the gauze. The animals were placed individually in a clean disinfected metal cage after grouping to avoid them biting each other’s wound. At days 3, 7, and 14 post-treatment, wound biopsy specimen was taken from 3 animals in each group and the bandages were changed. Immediately after collection of wound biopsy, specimen was placed in 10% PBS and used for biochemical assay of SOD and CAT activities, and MDA level. The SOD activity was determined following the method described by Sun et al. [47], catalase activity was determined following the procedure described by Sinha [48], while the MDA level was determined according to the method described by Draper and Hardley [49].

**Determination of In vitro Non-enzymatic Antioxidant Effect of C. jagus Bulb Extract**

Free radical scavenging activity of the extract of C. jagus was determined using DPPH assay as described by Brand-Williams et al. [50]. Briefly, 2 ml of various concentrations (10, 50, 100, 200, and 400 μg/ml) of the Crinum jagus bulb extract was added to 1 ml of DPPH (0.5 mM in 95% methanol) in a cuvette. The mixture was shaken and incubated at 30°C for 30 min in the dark. Then, the absorbance was taken at 517 nm using a spectrophotometer. Ascorbic acid at doses lower than that which have been reported to act as pro-oxidant [51,52] were used as standard compound (control) in this assay. For each extract concentration, the experiment was performed in triplicate and the mean absorbance calculated. The percentage scavenging activity was calculated as follows:

\[
\text{Scavenging effect} \% = \left( \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \right) \times 100
\]

**Statistical Analysis**

Data obtained for in vivo antimicrobial effect were expressed in percentages, while data obtained for in vivo and in vitro antioxidant effects were summarized as mean ± standard error of mean. Mean values of SOD and CAT activities and MDA levels for different groups were compared using one-way Analysis of Variance. Duncan multiple range test was used to separate variant means. \( P < 0.05 \) was considered significant.

**RESULTS**

**Extraction**

The CJMJE had an aromatic smell and was brownish in color. The percentage yield was 14.7% w/w material.

**Acute Toxicity Test**

Administration of MCJBE extract in DMSO to rats even at the highest dose of 2000 mg/kg bw did not produce any death in the treated groups. No sign of acute toxicity was also observed.

**Phytochemical Analysis**

Preliminary phytochemical analysis of CJMJE MCJBE qualitatively revealed the presence of alkaloids, tannins, saponins, and glycosides [Table 1].

**In vitro Antimicrobial Effect of Crinum jagus Bulb Extract**

The 100 mg/ml concentration of the MCJBE gave the highest IZD for B. subtilis (25 mm), S. aureus (21 mm), and C. albicans (14 mm) [Table 2]. The 50 mg/ml concentration gave IZD for B. subtilis (21 mm), S. aureus (16 mm), and C. albicans (9 mm). The 25 mg/ml concentration gave IZD for B. subtilis (15 mm) and S. aureus (10 mm) while the 12.5 mg/ml concentration gave IZD for only B. subtilis (10 mm). None of the tested concentrations of the extract inhibited the growth of P. aeruginosa.

**Table 1: Phytochemical analysis of methanolic C. jagus bulb extract**

| Phytoconstituent | Amount |
|------------------|--------|
| Alkaloids        | +++   |
| Tannins          | +++   |
| Saponins         | +     |
| Glycosides       | ++    |
| Flavonoids       | -     |

+++ : Appreciable amount, ++: Moderate amount, +: Trace amount, -: Completely absent, C. jagus: Crinum jagus

**Table 2: IZD of test concentrations of extract to each tested microorganism**

| CJMJE | IZD (mm) to each tested microorganism |
|-------|--------------------------------------|
|       | B. subtilis | S. aureus | C. albicans | P. aeruginosa |
| 100   | 25          | 21        | 14          | 0            |
| 50    | 21          | 16        | 9           | 0            |
| 25    | 15          | 11        | 0           | 0            |
| 12.5  | 10          | 0         | 0           | 0            |
| 6.5   | 0           | 0         | 0           | 0            |

MCJBE: Methanolic Crinum jagus methanolic bulb extract, B. subtilis: Bacillus subtilis, S. aureus: Staphylococcus aureus, C. albicans: Candida albicans, P. aeruginosa: Pseudomonas aeruginosa, IZD: Inhibition zone diameter
In vivo Antimicrobial Effect of *Crinum jagus* Bulb Extract in Wound

The result of frequency of reisolation of infective microorganism from wounds of animals in Groups A (treated with 10% MCJBO), B (treated with 5% MCJBO), C (treated with sterile normal saline), and D (treated with framycetin sulfate/clotrimazole) are presented in Figures 1-4, respectively. *B. subtilis* and *S. aureus* were not reisolated from wound of any animal in Groups A, B, and D at days 3, 7, and 14 post-infection. *P. aeruginosa* was reisolated from wound of all the animals in all the groups throughout the experiment. *C. albicans* was not reisolated from wound of any animal in Groups A, B, and D throughout the experiment, but was reisolated from wound of all the animals in Group C throughout the experiment.

Antioxidant Effect of *C. jagus* Bulb Extract in Wound Tissue

At day 3 post-treatment, SOD activity of Group I (treated with 300 mg/kg of 10% MCJBO) significantly ($P < 0.05$) increased when compared with Groups II (treated with...
300 mg/kg of 5% MCJBEO) and III (treated with sterile normal saline) [Table 3]. No significant (P > 0.05) difference existed in SOD activity between Groups I and IV (treated with framycetin sulfate/clotrimazole). SOD activity of Group II was significantly (P < 0.05) higher compared with Group III throughout the experiment but significantly lower compared with the Group IV. Similar trend was observed at day 7 post-treatment. At day 14 post-treatment, SOD activity of Group I was significantly (P < 0.05) higher than those of the other groups.

There were significant differences (P < 0.05) in catalase activity of wound biopsy among all the groups at day 3 post-treatment [Table 4]. There was significant (P < 0.05) increase in CAT activity of Group III when compared with the other groups. Increased significant difference (P < 0.05) existed between CAT activity of Groups I, II and III. Similar trend was observed at days 7 and 14 post-treatment.

At day 3 post-treatment, MDA level in wound biopsies of animals in Group I significantly (P < 0.05) decreased when compared with the other groups [Table 5]. No significant difference (P > 0.05) occurred in MDA levels between Groups I and IV. Similar trends were observed at days 7 and 14 post-treatment. At concentration of 10 μg/ml, the non-enzymatic free radical scavenging activity of CJMBE decreased significantly (P < 0.05) when compared with the control (ascorbic acid) [Table 6]. Similar trend was observed at concentrations of 50, 100, 200, and 400 μg/ml. Mean values of the antioxidant activity of the extract showed increasing activity with an increase in concentration.

**DISCUSSION**

In this study, the antimicrobial and antioxidant effects of CJMBE in wound healing was evaluated. Microbial wound contamination alters healing process and result in complication such as bacteremia and/or septicemia following wound invasion [53,54]. These complications are often common when resistant organisms constitute the contaminants. Ability of a substance to control the growth of a microorganism is ascertained by observing the extent to which the growth of the organism is inhibited by the substance. In the present study, *in vitro* antimicrobial study revealed that *C. jagus* bulb extract inhibited the growth of bacteria - *B. subtilis* and *S. aureus* and a yeast *C. albicans*. This indicates that the plant extract exhibited antibacterial and antifungal activities. The fact

**Bacteriological Assay of Excision Wound Post-Contamination**

Cultures of wound swabs from animals in Groups III yielded heavy growth of all the infective organisms (*S. aureus, B. subtilis, P. aeruginosa*, and *C. albicans*) throughout the study period, whereas cultures of wound swabs from animals in Group I, II, and IV yielded scanty growth of only *P. aeruginosa* at days 3, 7, and 14 post-treatment.

**Figure 4:** Frequency of reisolation of infective microorganisms from animals in Group D treated with framycetin sulfate/clotrimazole (Sofradex-f®)

**Table 3:** SOD activity in wound biopsy of animals treated with MCJBEO

| Group (treatment) | Mean±SEM SOD (μg/mg protein) in wound biopsy at days post-treatment |
|-------------------|---------------------------------------------------------------|
|                   | 3                  | 7                  | 14                 |
| I (10% MCJBEO)    | 4.22±0.09*a        | 4.57±0.02*a        | 5.24±0.06*a        |
| II (5% MCJBEO)    | 2.58±0.04*a        | 3.00±0.00*b        | 2.85±0.00*c        |
| III (sterile soft white paraffin) | 1.32±0.00*c        | 1.38±0.07*c        | 1.40±0.02*d        |
| IV (framycetin sulfate/clotrimazole) | 4.20±0.06*d        | 4.68±0.07*c        | 4.61±0.06*e        |

Different superscript*abcd* across a column indicate significant difference in means at P<0.05, MCJBEO: Methanolic *Crinum jagus* methanolic bulb extract ointment, SEM: Standard error of mean, SOD: Superoxide dismutase

**Table 4:** CAT activity in wound biopsy of animals treated with MCJBEO

| Group (treatment) | Mean±SEM CAT activity (μg/mg protein) in wound biopsy at days post-treatment |
|-------------------|--------------------------------------------------------------------------------|
|                   | 3                  | 7                  | 14                 |
| I (10% MCJBEO)    | 2.58±0.04*a        | 3.00±0.00*b        | 2.85±0.00*c        |
| II (5% MCJBEO)    | 0.15±0.03*b        | 0.11±0.00*b        | 0.10±0.00*b        |
| III (sterile soft white paraffin) | 4.62±0.52*        | 4.68±0.07*c        | 4.61±0.06*        |
| IV (framycetin sulfate/clotrimazole) | 0.62±0.00*e        | 0.64±0.07*e        | 0.64±0.06*e        |

Different superscript*abcd* across a column indicate significant difference in means at P<0.05, MCJBEO: Methanolic *Crinum jagus* bulb extract ointment, SEM: Standard error of the mean, SOD: Superoxide dismutase
that there was decreased antimicrobial activity with decrease in concentration of the extract, suggests that the antimicrobial effect of the C. jagus bulb extract is concentration-dependent. The 100 mg/ml concentration of the extract gave the highest IZD for the 3 inhibited organisms (B. subtilis [25 mm], S. aureus [21 mm], and C. albicans [14 mm]) which suggests that C. jagus jagus bulb extract exhibits the best antimicrobial effect at this (100 mg/ml) concentration. The 50 mg/ml concentration gave IZD for B. subtilis (21 mm), S. aureus (16 mm), and C. albicans (9 mm). The IZD for each organism at 50-6.25 mg/ml concentration is lower when compared with those of 100 mg/ml concentration. This result may suggest that the more the concentration of phytochemicals responsible for the antimicrobial activity, the better the effect. The fact that none of the tested concentrations of the extract inhibited the growth of P. aeruginosa indicates that C. jagus do not exhibit anti-pseudomonal effect. This suggests that the P. aeruginosa isolate used was resistant to the extract.

The antimicrobial activity of C. jagus could be attributed to its alkaloids, tannins, and saponin content as revealed by the phytochemical analysis. Studies have reported antibacterial effect of plant extract containing tannins, alkaloids, and saponins [11,17,40]. Crinamine, a phenolic alkaloid contained in C. jagus bulb have been reported to exhibit antibacterial activity [37]. The anti-candidal effect of C. jagus bulb extract observe in this study may be related to its high tannin content (as revealed by the result of the phytochemical screening). Plant extracts containing tannins have been widely reported to inhibit the growth of Candida [55,56,57]. Moreover, inhibition of growth of S. aureus by the C. jagus bulb extract in this study corroborates the report of Adesanya et al. [37]. Failure to inhibit the growth of P. aeruginosa could be attributed to its inherent resistance to most antibacterial agents [58]. It could also be that the P. aeruginosa strain is a highly-resistant isolate having been isolated from septic wound.

The result of the in vitro antimicrobial studies (highest IZD produced by 100 and 50 mg/ml) prompted us to prepare the 10 and 5% C. jagus extract ointments for the in vivo wound healing studies. Failure to re-isolate B. subtilis, S. aureus, and C. albicans from infected wound of any animal in Groups A (treated with 10% MCJBEO), B (treated with 5% MCJBEO) and D (treated with framycetin sulfate/clotrimazole) throughout the study period is attributable to the antimicrobial effect of both concentrations of the extract and of course the reference antimicrobial agent, framycetin sulfate/clotrimazole. Re-isolation of P. aeruginosa from all the animals in all the groups throughout the course of the experiment further suggests that the organism was resistant to the extract irrespective of the concentration and to the reference drug. The result of the in vivo antimicrobial studies further supports the antimicrobial effect of the extract on the tested microorganisms except P. aeruginosa.

In this study, assay of SOD and CAT activities, and MDA level was performed to determine the superoxide (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) radical scavenging activity of Crinum jagus extract, and degree of lipid peroxidation activity (cellular lipid peroxidation), respectively. Superoxide radical is considered a major biological source of ROS [59]. Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress and tissue damage [60,61]. This radical is scavenged and by SOD, an enzyme that dismutates superoxide anions (O$_2^-$) to generate hydrogen peroxide (H$_2$O$_2$) [61] which is then detoxified by catalase to water and oxygen which are non-toxic to tissues [61,62]. Therefore, the significantly increased SOD of the extract treated Groups (I and II) when compared with Group III (treated with sterile soft white paraffin), suggests that the extract increased SOD activity in the wound biopsies. However, the fact that SOD activity of Group I (treated with 10% MCJBEO) was significantly higher than the other groups, may suggest that the extract exhibited a better superoxide radical scavenging at this concentration. This suggests that C. jagus bulb extract elicited superoxide radical scavenging in a concentration-dependent manner. Interestingly, the SOD activity of wound biopsies from animals in Group I was significantly higher than all the other groups, including those treated with the reference drug, framycetin sulfate/clotrimazole, an antibiotic/antifungal combination drug. This finding suggests that at 10% concentration, superoxide radical scavenging activity of C. jagus bulb extract was better sustained than at the 5% concentration, and of course, the controls. This may explain the observed faster wound healing in animals in the group, in contrast to their counterparts in the other groups. Ability of the C. jagus extract to increase the SOD activity in the wound bed could be related to its high tannin content [63] as revealed by the result of the phytochemical screening.

### Table 5: MDA level in wound biopsy of animals treated with MCJBEO

| Group (treatment) | Mean±SEM CAT activity (μg/mg tissue protein) in wound biopsy at days post-treatment |
|-------------------|----------------------------------------------------------------------------------|
| I (10% MCJBEO)    | 0.33±0.06a                                                                        |
| II (5% MCJBEO)    | 0.54±0.04b                                                                        |
| III (sterile soft white paraffin) | 0.67±0.03c                                                                        |
| IV (framycetin sulfate/clotrimazole) | 0.41±0.00c                                                                        |

Different superscriptabc across a column indicate significant difference in means at $P<0.05$, MCJBEO: Methanolic Crinum jagus methanolic bulb extract ointment, SEM: Standard error of mean, MDA: Malondialdehyde

### Table 6: Free radical scavenging activity of MCJB

| Test substance | Percentage antioxidant activity at different test concentrations (μg/ml) |
|----------------|-------------------------------------------------------------------------|
|                | 10                        | 50                        | 100                       | 200                        | 400                        |
| MCJBE          | 6.82±0.05a                 | 45.48±0.14b               | 53.08±0.43a               | 53.34±0.69a                | 58.19±1.51a                |
| Ascorbic acid  | 73.65±0.85a                | 74.14±0.03a               | 74.63±1.16a               | 77.17±0.76a                | 79.18±0.16a                |

Different superscripta in a column indicate significant difference in mean at $P<0.05$, MCJB: Methanolic Crinum jagus methanolic bulb extract
Hydrogen peroxide, a non-reactive compound is converted to free hydroxyl radical (OH$^-$), which reacts with biomolecules to cause tissue damage and cell death [61]. It is scavenged by CAT which breaks it down into water and oxygen [62]. Significantly increased CAT activity in wound biopsies of animals in Group III (negative control) on comparison with their counterparts in the other groups may be attributed to the presence of the infective microorganisms present in the wound. Result of the post-contamination wound microbial assay revealed the presence of *S. aureus* and other infective contaminating microbes in wounds of animals in the negative control group. *S. aureus* is known to produce catalase which destroys phagocytic cells recruited to engulf them in the wound site [64]. Therefore, since the negative control group was neither treated with CJMBE0 nor the reference drug, the isolation of microbes especially *S. aureus* in the wound of animals in the group was not surprising and hence, the observed significant increase in catalase activity. Nevertheless, significantly increased CAT activity of Group I as against Groups II, and IV suggests that *C. jagus* bulb extract exhibited H$_2$O$_2$ scavenging activity in a concentration-dependent manner. This result also suggests that H$_2$O$_2$ scavenging was better sustained by 10% concentration of the extract throughout the experiment. Free superoxide radical and H$_2$O$_2$ scavenging activity of plant phenolics especially tannins have been reported [61,63]. Interestingly, the significant increase in CAT activity of Group I on comparison with Group IV (treated with framycetin sulfate/clotrimazole) suggest that the extract enhanced catalase activity more than the reference drug. This finding is supported by the fact that infective pathogens were not isolated from wounds of animals in both groups, hence there was no microbe that could have produced catalase in Group I.

The consequence of decreased SOD and CAT activities is increased cellular lipid peroxidation and delayed wound healing [65]. Lipid peroxidation is caused by the activity of lipid peroxidase which is evidenced by the presence of MDA in tissues [65,66]. MDA is toxic and causes considerable changes in the structural organization and function of cell membrane making it to be porous [66]. In the present study, a significant increase in MDA level in wound biopsies of animals in Group I compared with the other groups suggests that *C. jagus* exhibited anti-lipid peroxidation activity [65]. This finding also suggests that *C. jagus* bulb extract exhibited this activity in a concentration-dependent manner. None significant difference observed in MDA level between Groups I and IV (positive control), suggests that the extent at which the 10% MCJBE0 prevented cellular lipid peroxidation is comparable to that of the reference drug, framycetin sulfate/clotrimazole. This finding conforms to Panneerselvam and Govindasamy [65] who reported that a significant increase in SOD and CAT activities results in decreased lipid peroxidase activity indicated by decreased concentration of MDA. Therefore, the results of this study suggests that 10% MCJBE0 exhibited antioxidant activity, which resulted in low MDA level in the wound biopsies of animals in the group since increase in lipid peroxidation (MDA) level suggests increased generation of toxic free radicals [67].

In the present study, in vitro non-enzymatic free radical scavenging activity of *C. jagus* bulb extract was determined using DPPH method [41]. The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolorizes the DPPH solution. The degree of color change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test [41,68]. In the present study, the reference antioxidant (ascorbic acid) used showed significantly higher free radical scavenging activity than all the *C. jagus* bulb extract concentrations tested. However interestingly, the mean values of the antioxidant activity of the extract revealed that the extract exhibited free radical scavenging activity in a concentration-dependent manner. Optimum wound healing occurs when ROS are reduced to a level where oxidative stress is minimal [25]. Moreover, reduced ROS in wound biopsies is evidenced by increased antioxidant activities [25]. Many studies have shown that tannins contained in plant extracts exhibits antioxidant activity [41,69,70]. Therefore, the non-enzymatic antioxidant effects exhibited by the *C. jagus* bulb extract in this study could also be attributed to its high tannin content.

**CONCLUSION**

This study has shown that methanolic CJMBE exhibited antioxidant and antimicrobial effects against some common wound contaminating microorganism both in vitro and in vivo in a wound healing model. These effects could possibly be part of its mechanism in promoting wound healing, forming the basis for its use in wound management in folkloric medicine. However, further studies that would involve cell biology are recommended.

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