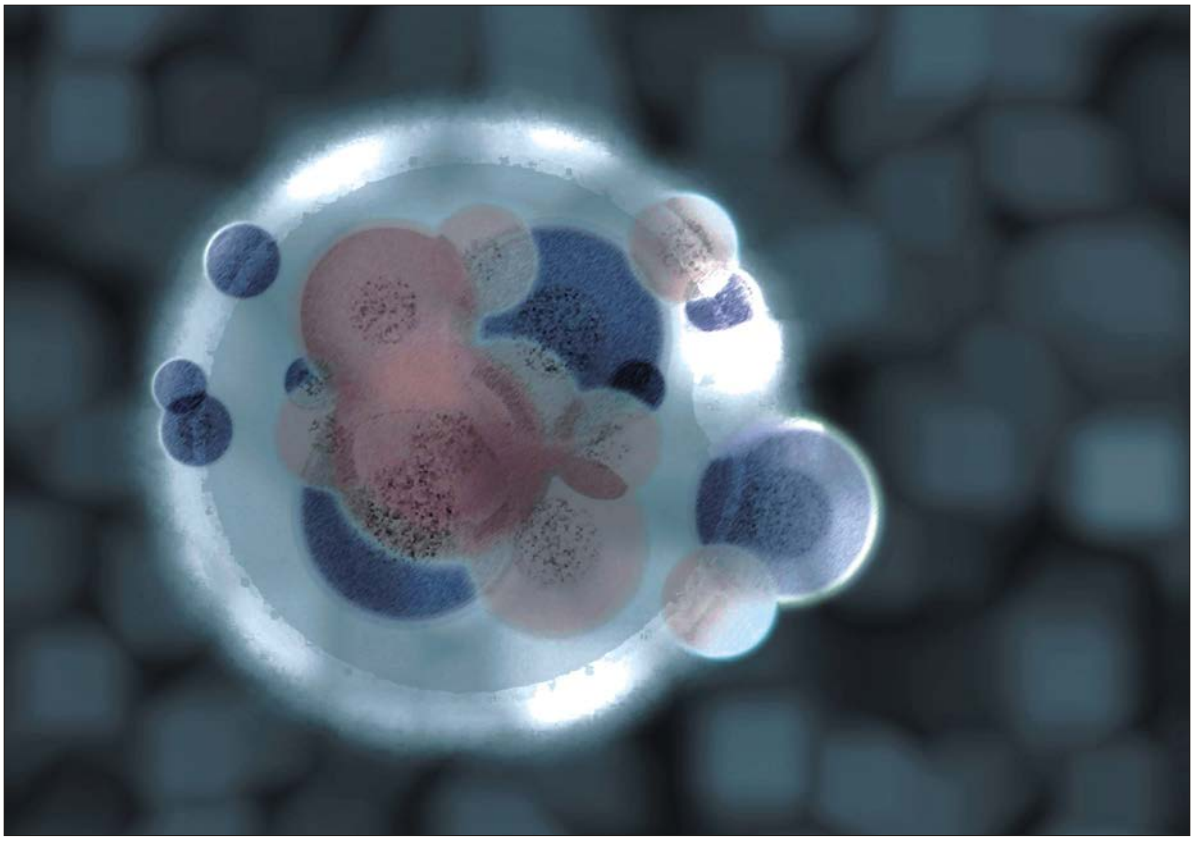

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2025.1.12

Research Article
Assessment of Wound Healing Potentials of Methanol Extract of *Anthocleista vogelii* Leaves on Female Wistar Rats

Onyinye Gift Acha, Joyce Oronne Akaninwor, Charity Uchechi Ogunka-Nnoka and Precious Philip Ogamba

Department of Biochemistry, Faculty of Science, University of Port Harcourt, Choba, Rivers State, Nigeria

Abstract

Background and Objective: Conventional drugs used in wound treatment are not only expensive but also have challenges of allergies and drug resistance. This necessitates the need to seek alternatives, especially in the area of medicinal plants which is still not fully explored. In this article wound healing potentials of methanol extract of *Anthocleista vogelii* leaves were studied. **Materials and Methods:** The 25 female Wistar rat (144-176 g) were divided into 5 groups of 5 rats each. Group 1 (normal control), group 2 and 3 were topically administered 0.1 g of Vaseline and 0.1 g Gentamicin, while groups 4 and 5 were administered 0.1 g Vaseline along with 0.1g extract (*A. vogelii* 1) and 0.2 g extract (*A. vogelii* 2), respectively for 14 days after inducing excision wound (2 cm) on the dorsal skin. An assay for biochemical and wound healing parameters was carried out using standard methods. **Results:** Wound healing studies revealed a significant increase in oestrogen level in group 4 and a significant decrease in iron level in group 5 when compared with group 1, 2 and 3. Hematological results revealed an increase in PCV, Hb, RBC, Platelets and RBC, WBC, neutrophil, eosinophil and monocyte on administration of *Anthocleista vogelii* leaves. **Conclusion:** The study thus revealed that *Anthocleista vogelii* leaves possess wound healing potential.

Key words: *Anthocleista vogelii* leaves, methanol extract, wound healing, oestrogen, iron, haematology

Citation: Acha, O.G., J.O. Akaninwor, C.U. Ogunka-Nnoka and P.P. Ogamba, 2025. Assessment of wound healing potentials of methanol extract of *Anthocleista vogelii* leaves on female Wistar rats. J. Biol. Sci., 25: 1-12.

Corresponding Author: Onyinye Gift Acha, Department of Biochemistry, Faculty of Science, University of Port Harcourt, Choba, Rivers State, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Wounds can be said to be injuries that affect living tissue that lead to the interruption of its normal physical and chemical structure and function[1]. Wounds arise as a result of physical, chemical, thermal or even microbial or immunological injury to the tissue[2]. Wound healing is a complex biological process that aims to restore the structure and function of damaged tissue[3]. It occurs in four stages: hemostasis, inflammation, proliferation and tissue remodeling[4]. During hemostasis, blood vessels constrict and platelets form clots by attaching to exposed collagen and extracellular matrix, triggering the coagulation cascade to prevent blood loss[5]. Platelets release growth factors like Transforming Growth Factor-Beta (TGF-β) and Fibroblast Growth Factor (FGF), which along with cytokines, help recruit monocytes and neutrophils to the wound site, starting the inflammatory phase[6]. Neutrophils reach the wound at inflammatory phase, eliminate invading microorganisms through phagocytosis and release reactive oxygen species and proteolytic enzymes[7]. In addition to penetration, neutrophils also discharge IL-8 that helps in chemotaxis thus attracting monocytes and other cells to the site of the wound[5]. After cleansing of the wound, which is the last stage of the Inflammation phase, the proliferation phase begins. During this phase, granulation tissue forms, angiogenesis occurs and the wound undergoes contraction and epithelialization[8]. Fibroblasts, keratinocytes and endothelial cells are actively involved in this process. Granulation involves the deposition of a newly produced extracellular matrix composed of collagen, fibrin and fibronectin, which replaces the damaged tissue[9]. Fibroblasts and myofibroblasts help contract the wound by pulling its edges together, while keratinocytes move granulation tissue from the wound's edges to the center, aiding in wound closure and protecting the underlying tissue[6]. The final stage of wound healing is tissue remodeling, which happens after the wound closes. This phase focuses on maximizing the flexibility of the newly formed tissue, though the wound never regains the original tissue's full tensile strength. Remodeling involves collagen fibers forming cross-linkages, reducing scar thickness and decreasing vascularity as the scar matures[10]. Cells involved in wound repair are removed through apoptosis since they are no longer needed[11].

The quest for effective wound healing agents has led to the exploration of numerous medicinal plants, known for their bioactive compounds that can enhance the healing process. Medicinal plants, particularly those used in traditional medicine, have been sources of novel therapeutic agents. Plant extracts promote wound healing through various mechanisms. They can enhance collagen synthesis, stimulate the proliferation of fibroblasts and keratinocytes and modulate the inflammatory response. Methanol extracts, in particular, are effective in extracting a broad range of polar and non-polar compounds, making them suitable for isolating bioactive compounds from plants[12]. A few notable African medicinal plants known for their wound-healing properties include Aloe vera, African marigold (*Tagetes erecta*), *Bulbine frutescens*, *Sutherlandia frutescens* and many others[13-16].

Anthocleista vogelii which belongs to the family Gentianaceae and is commonly found in West Africa is widely used in African traditional medicine. The plant is traditionally used to treat various ailments such as malaria, diabetes, gastrointestinal disorders and skin diseases, including wounds. The use of *Anthocleista vogelii* in wound healing in traditional medicine suggests that it may contain active compounds that can promote tissue repair and regeneration. The plant contains a variety of bioactive compounds including alkaloids, saponins, flavonoids, tannins and phenolic compounds, which are known for their antimicrobial, anti-inflammatory and antioxidant properties[17]. These compounds are critical in the wound healing process as they help in reducing inflammation, preventing infections and promoting tissue regeneration. Oladimeji *et al.*[18] evaluated the antimicrobial activity of the methanol extract of *Anthocleista vogelii* and found it effective against several wound-infecting pathogens, including *Staphylococcus aureus* and *Escherichia coli*.

Research on the wound healing potential of *Anthocleista vogelii* is still emerging. However, some studies have demonstrated the wound healing potential of the *Anthocleista* genus. For instance, a study by Ugwu *et al.*[19] showed that the methanol extract of *Anthocleista vogelii* significantly accelerated wound contraction and closure in animal models. Hence, this study investigated the wound healing potentials of methanol extract of *Anthocleista vogelii* leaves on female Wistar rats.

MATERIALS AND METHODS

Study area: The research was carried out from December, 2021 to March, 2022. Wistar rats were purchased from the Department of Pharmacology and Toxicology Animal House, University of Port Harcourt, Nigeria. All chemicals used in the study were of analytical grade and obtained from local suppliers and distributors.

Table 1: Experimental animal grouping based on treatments

Group	Treatment
Control	Untreated rats (natural healing)
Vaseline	Rats treated with 0.1 g Vaseline (vehicle)
Gentamicin	Rats treated with Gentamicin
A. vogelii 1	Rats treated with 0.1 g extract+0.1 g Vaseline
A. vogelii 2	Rats treated with 0.2 g extract+0.1 g Vaseline

Plant sample collection and identification: Freshly harvested leaves of *Anthocleista vogelii* were obtained from Abuja Campus University of Port-Harcourt. Samples were identified by Dr. Chimezie Ekeke of the Department of Plant Science and Biotechnology, University of Port-Harcourt Herbarium and authenticated with voucher number UPH/P/340.

Preparation of extract: The leaves collected from the plant were washed carefully and air-dried for 28 days. The dried leaves were ground into powder form using a grinding mill. About 1042 g of the powdered leaves were dissolved in 3000 mL of 70% methanol for 72 hrs, it was then filtered through Whatman No.1 filter paper and the clear filtrate obtained was further separated using a Rotary Evaporator (05-51, 0200 rpm China) at 45°C and later transferred to evaporating dish placed in a water bath (HH-WA20 Finotech instruments). The final extract obtained was weighed, stored, labelled in screw-capped bottle and preserved at 25°C for further studies.

Experimental animals: The 25 Wistar albino rats weighing between 144 to 176 g were purchased from the Department of Pharmacology and Toxicology Animal House. Rats were grouped into five study groups of 5 rats each (Table 1) and acclimatized for 7 days at room temperature (36°C) and relative humidity. Rats were fed with rat chow and water *ad libitum*.

Experimental design: An excision wound (2 cm) was induced on the dorsal skin of 25 Wistar rats, according to the method reported by Zaki *et al.*[20] with little modification. The dorsal region was fully shaved, cleaned and disinfected with methylated spirit. Rats were held in their normal standing posture and an excision wound was made to the point of relaxed subcutaneous tissue using a surgical blade and forceps. The wound site was cleaned with methylated spirit before topical application was done once in 24 hrs and the wound site was measured using a ruler. The study was carried out for 14 days. The rats were sacrificed and blood was collected for wound healing study parameters (total protein level, iron level, estrogen level and haematology) intervals on days 0, 7 and 14.

Determination of iron in plasma (nitro-PAPs method-monoreagent)[21]

Procedure: The 3 cuvettes labelled Al (sample), A2 (standard) and A3 (blank) were assembled. A1: 1000 µL of reagent and 50 µL of sample were pipetted, A2: 1000 µL of reagent and 30 µL of Nitro PAPs stabilizer (standard) and to A3: 1000 µL of reagent and 50 µL distilled water. They are then mixed and incubated at 25°C for 10 min. Absorbance of sample A (sample) and standard A (standard) against the blank A (RBL) was read in a spectrophotometer (Genesys 10-S) USA) at 578 nm.

Calculations:

$$C = 30 \times \frac{SA\ (s)}{SA\,(STD)}\ (\mu mol/)$$

Determination of oestrogen (estradiol AccuBind™ microplate ELISA test system)[22]

Procedure: The microplate's wells of control, serum reference and sample to be analysed were formatted. The 25 µL were pipetted into the labelled well for control, serum reference and sample. About 50 µL Estradiol Botin Reagent was added to every well and the microplate was swirled gently to mix, covered and incubated. Another 50 µL Estradiol Enzyme Reagent was added directly to every well and the plate was swirled gently to mix, covered and incubated. The content was eliminated by decanting or aspirating. For decantation, the plate was blotted dry with absorbent paper. Then 350 µL wash buffer was added, decanted and aspirated. This step was done twice for three washes. Into every well, 100 µL of substrate solution was added and incubated. The 50 µL stop solution was then added and mixed. The absorbance for every well was recorded at 450 nm.

Haematological parameters (microhaematocrit method)[23]

Packed cell volume procedure: The microhaematocrit tube was filled to three-quarters with well-mixed EDTA-treated blood. The open end of the tube was sealed with a sealant and placed in a microhaematocrit rotor, then centrifuged at 1200 rpm for 5 min. The packed cell volume (PCV) was subsequently measured using a microhaematocrit reader (ZCP-EZRD-HEM7).

Hemoglobin[23]: Hemoglobin levels were measured using an automated cell counter from a tube containing well-mixed blood treated with EDTA as an anticoagulant, filled to a specified level. In this process, all forms of hemoglobin were

converted into cyanomethemoglobin, a colored protein and quantified using a colorimeter (Hach DR100 Colorimeter P/N 41100-58)

Red blood cell count[24]

Procedure: Formal citrate (diluting fluid) was measured (4.0 mL) and dispensed into a test tube. The 0.2 mL of properly mixed EDTA blood was measured into the mixture and mixed. The counting chamber was then assembled and filled with well-mixed samples and kept for 3 min for proper sedimentation. The cells were examined using a 10X objective lens. The small squares having the red cells were counted and the total red cells per litre were read.

Platelet count[24]

Procedure: In a test tube, 0.38 mL of diluting fluid and 0.02 mL of properly mixed EDTA blood were added and mixed. The assembled and filled counting chamber was kept for 20 min, placed on a dampened tissue in a Petri dish and overlaid with the lid. The counting chamber was examined using a 40X objective lens. The small square having the platelet was counted and the total platelet per litre of blood was reported.

White blood cell count[24]

Procedure: In a test tube, 0.38 mL diluting fluid, 0.20 mL of well-mixed EDTA blood were added and then mixed thoroughly and a counting chamber then assembled and remixed. One chamber grid was carefully filled and kept to stand for 2 min for proper sedimentation. Cells were examined using 10X objective lens. The large squares were counted and a number of white cells for every liter of blood was reported.

Calculation:

$$\text{WBC count (per liter)} = \frac{N \times DF \times 10}{A \times D}$$

Where:

N = Number of cells counted
DF = Dilution factor
A = Area counted
D = Depth of the chamber, 0.1

Percentage rate of wound healing: The wound area was photographed on day 0 as 2 cm and subsequently on days 7 and 14. Rate of wound healing was calculated with the formula modification of Zaki *et al.*[20]:

$$\text{Rate of wound healing (\%)} = \frac{\text{Initial wound area-Wound area nth day (0, 7, 14)}}{\text{Initial wound area}} \times 100$$

Statistical analysis: All data were analysed statistically. The values were reported as Means±SD while one way analysis of variance was employed to detect differences in tested groups using the Statistical Package for Social Sciences (SPSS) version 23. The results were represented as significant at $p<0.05$. Tukey's *post hoc* test was conducted to indicate where results differ statistically.

Ethical consideration: Ethical approval was obtained for use of laboratory animal in this study from the University of Port Harcourt Ethical Committee with the code: UPH/CERAMAD/REC/MM77/063.

RESULTS

Iron (µmol/L) level: The result of the Iron (Fe) level of rats treated with methanol extract of *A. vogelii* leaf for 14 days was represented in Table 2. The Iron level of the Control group indicated a significant decrease from the baseline to day 14. Comparison of the Control group with others revealed a significant difference ($p<0.05$) in Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 on day 7 and a significant difference in all other groups on day 14. When Gentamicin group was compared with Control, Vaseline, *A. vogelii* 1 and *A. vogelii* 2 group, a significant difference ($p<0.05$) was observed in all other groups on day 7 and day 14.

Oestrogen (pg/mL) level: The oestrogen level of the control group as shown in Table 3 revealed a significant decrease ($p<0.05$) from day 0 to 7 and a significant increase ($p<0.05$) on day 14. The Vaseline and *A. vogelii* 2 group estrogen level significantly increased ($p<0.05$) from day 0 to 7 and significantly decreased ($p<0.05$) on day 14, while the Gentamicin and *A. vogelii* 1 group estrogen level significantly increased from day 0 to 14. Comparing the control group to the other group revealed a significant difference ($p<0.05$) in all other groups on day 7 and in Vaseline, *A. vogelii* 1 and *A. vogelii* 2 group on day 14. Comparison of Control, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group with Gentamicin group revealed statistical difference ($p<0.05$) in Control, Vaseline, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and in Vaseline, *A. vogelii* 1 and *A. vogelii* 2 group on day 14.

Table 2: Effect of methanol extract of *A. vogelii* leaf on iron level of wound treated female Wistar rats

Group	Baseline	Day 7	Day 14
Control	50.00±4.31	35.33±6.43[b]	38.00±9.54[b]
Vaseline	50.00±4.31	35.67±3.51[b]	39.67 ±1.53[a,b]
Gentamicin	50.00±4.31	29.67±4.51[a]	31.67±3.06[a]
A. vogelii 1	50.00±4.31	23.33±0.58[a,b]	31.67±1.53[a,b]
A. vogelii 2	50.00±4.31	38.33±4.04[a,b]	26.67±3.51[a,b]

Values are Mean±SD of triplicate determination. Values with superscript "a" are statistically different (p<0.05) when Control group is compared with Vaseline, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14, superscript "b" is statistically different (p<0.05), when Gentamicin group is compared with Control, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14. Values without any superscript show that there was no statistical difference when Control and Gentamicin were compared with other groups

Table 3: Effect of methanol extract of *A. vogelii* leaf on oestrogen level of wound treated female Wistar rats

Group	Baseline	Day 7	Day 14
Control	70.80±2.77	58.33±0.58[b]	77.67±0.58
Vaseline	70.80±2.77	80.00±2.00[a,b]	64.00±2.65[a,b]
Gentamicin	70.80±2.77	72.00±1.00[a]	76.00±2.65
A. vogelii 1	70.80±2.77	86.67±1.53[a,b]	101.00±4.00[a,b]
A. vogelii 2	70.80±2.77	92.67±2.52[a,b]	64.33±3.51[a,b]

Values are Mean±SD of triplicate determination. Values with superscript "a" are statistically different (p<0.05) when Control group is compared with Vaseline, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14, superscript "b" is statistically different (p<0.05), when Gentamicin group is compared with Control, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14. Values without any superscript show that there was no statistical difference when Control and Gentamicin were compared with other groups

Haematological profile: The result of the present study gives reference data on the effect of topical application of methanol extract of *A. vogelii* leaf on some hematological parameters.

Packed CVell Volume (PCV): The result of the packed cell volume in the Control group as shown in Fig. 1 revealed an increase from baseline to day 7 and an increase from day 7 to 14. Control, Vaseline and Gentamicin group showed a significant increase (p<0.05) from day 7 to 14, while *A. vogelii* 1 and *A. vogelii* 2 group had a similar result of a significant increase from baseline to 7 and a decrease on day 14. Comparison of Control group to other group revealed a significant difference (p<0.05) in Vaseline, Gentamicin, *A. vogelii* 2 group on day 7 and Vaseline, Gentamicin, *A. vogelii* 1 group on day 14. For Gentamicin group with Control, Vaseline, *A. vogelii* 1 and *A. vogelii* 2 group, significant difference (p<0.05) occurred in *A. vogelii* 1 on day 7 and *A. vogelii* 1 and *A. vogelii* 2 group on day 14 (Fig. 1).

Hemoglobin (Hb): The hemoglobin result from the effect of methanol extract of *A. vogelii* leaf on wound-treated female Wistar rats revealed that the hemoglobin level in the Control, Vaseline and Gentamicin group has significantly increased (p<0.05) from baseline to day 14, while *A. vogelii* 1 and *A. vogelii* 2 group significantly increased (p<0.05) from baseline to day 7 and decreased on day 14. Comparison of the Control group with other groups revealed a significant difference (p<0.05) in Gentamicin and *A. vogelii* 2 group on day 7 and with Vaseline and Gentamicin group on day 14. Comparison of Gentamicin with Control, Vaseline, *A. vogelii* 1 and *A. vogelii* 2 group revealed a statistical difference (p<0.05) in Control and *A. vogelii* 1 on day 7 and Control, *A. vogelii* 1 and *A. vogelii* 2 group on day 14 (Fig. 2).

Red blood cell (RBC) count: The red blood cell result of the present study was presented in Fig. 3. The RBC count of the Control, Vaseline and *A. vogelii* 1 group significantly increased from baseline to day 14, while the RBC count of Getamicin and *A. vogelii* 2 group significantly increased from baseline to day 7 and significantly decreased (p<0.05) on day 14. When the Control group was compared with the other groups, they had no significant difference (p<0.05) on day 7 and 14. Comparison of the Vaseline group with Control, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 revealed a significant difference (p<0.05) in *A. vogelii* 1 in day 7 and no significant difference (p<0.05) on day 14 in other groups. Comparison of the Gentamicin group with Control, Vaseline, *A. vogelii* 1 and *A. vogelii* 2 revealed no significant difference (p<0.05) in all groups on day 7 and14 (Fig. 3).

Platelet: There was a significant increase (p<0.05) in the Control and Gentamicin group from baseline to day 14, while the Vaseline and *A. vogelii* 2 group showed a significant increase (p<0.05) from baseline to day 7 and a significant decrease on day 14. The *A. vogelii* 1 group showed a significant decrease (p<0.05) from baseline to 14. Comparison of the Control group with other group revealed a significant difference (p<0.05) with Vaseline, Gentamicin and *A. vogelii* 2 group on day 7 and with *A. vogelii* 1 and *A. vogelii* 2 group on day 14. Comparison of the Gentamicin group with the Control, Vaseline, *A. vogelii* 1 and *A. vogelii* 2 group revealed a significant difference (p<0.05) with Control, *A. vogelii* 1 and *A. vogelii* 2 group on day 7, Vaseline, *A. vogelii* 1 and *A. vogelii* 2 group on day 14 (Fig. 4).

White blood cell (WBC): Control and *A. vogelii* 1 group significantly decreased (p<0.05) from baseline on day 7 and increased significantly (p<0.05) on day 14. Gentamicin, Vaseline and *A. vogelii* 2 group revealed a significant increase

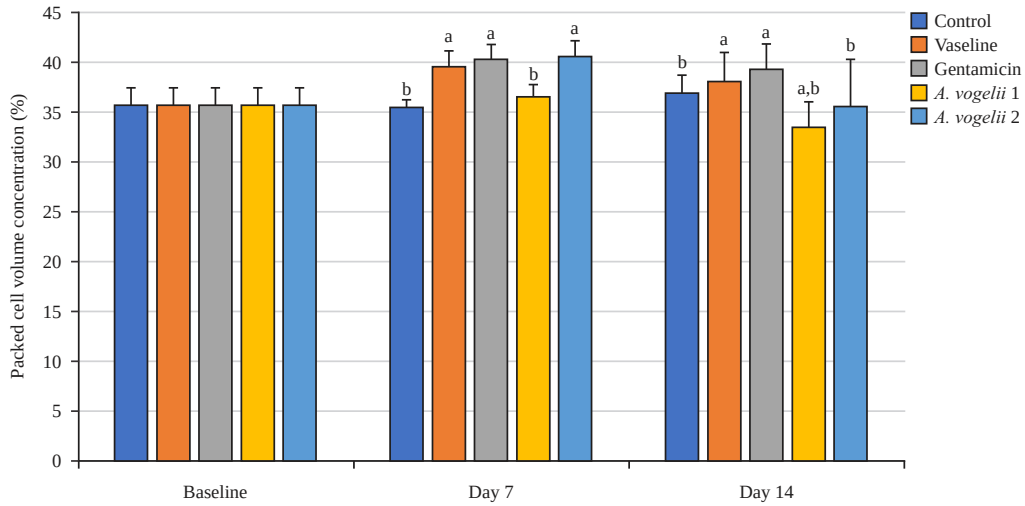

Fig. 1: Effect of methanol extract of *A. vogelii* leaf on Packed Cell Volume, PCV (%) level of wound treated female Wistar rats

Values are Mean±SD of triplicate determination. Values with superscript "a" are statistically different ($p<0.05$) when the Control group is compared with Vaseline, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14, superscript "b" is statistically different ($p<0.05$) when Gentamicin group is compared with Control, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14. Values without any superscript show no statistical difference when Control and Gentamicin were compared with other groups

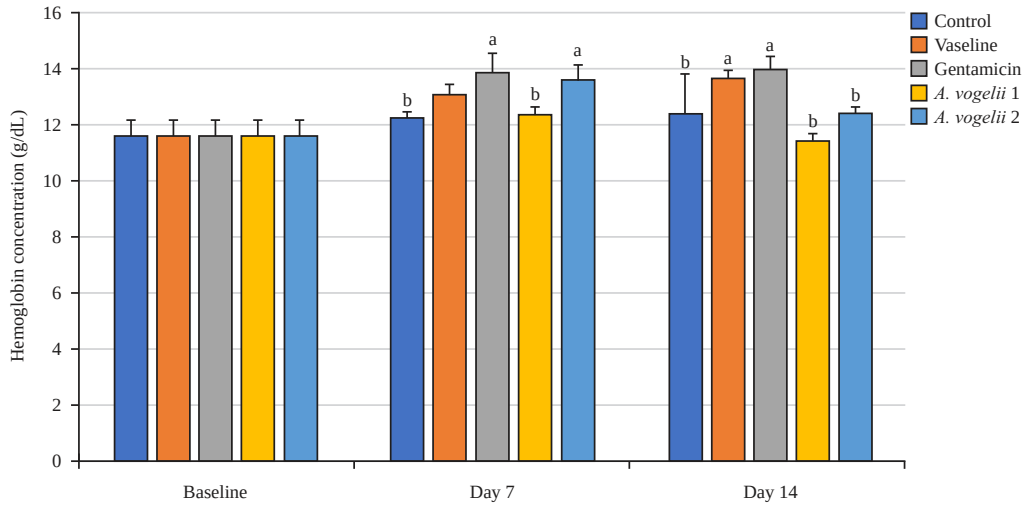

Fig. 2: Effect of methanol extract of *A. vogelii* leaf on hemoglobin concentration (g/dL) of wound treated female Wistar rats

Values are Mean±SD of triplicate determination. Values with superscript "a" are statistically different ($p<0.05$) when Control group is compared with Vaseline, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14, superscript "b" is statistically different ($p<0.05$) when Gentamicin group is compared with Control, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14. Values without any superscript show that there was no statistical difference when Control and Gentamicin were compared with other groups

in the level of WBC from baseline to day 14. Comparison of Control with other groups revealed a significant difference ($p<0.05$) with Vaseline, Gentamicin and *A. vogelii* 2 on day 7 and no significant difference ($p<0.05$) in all groups on day 14. Comparison of Gentamicin group with other groups revealed a significant difference ($p<0.05$) with Control and *A. vogelii* 1 on day 7 and no significant difference ($p<0.05$) in all groups on day 14 (Fig. 5).

Neutrophil: The level of neutrophil from the present study showed that the level of neutrophil in the Control group, Gentamicin group and *A. vogelii* 2 group showed a significant increase ($p<0.05$) from baseline to day 14. Result of the neutrophil level in the Vaseline and *A. vogelii* 1 group result showed a significant increase ($p<0.05$) from baseline to day 7 and a significant decrease ($p<0.05$) on day 14. Comparison of the Control group with the other groups

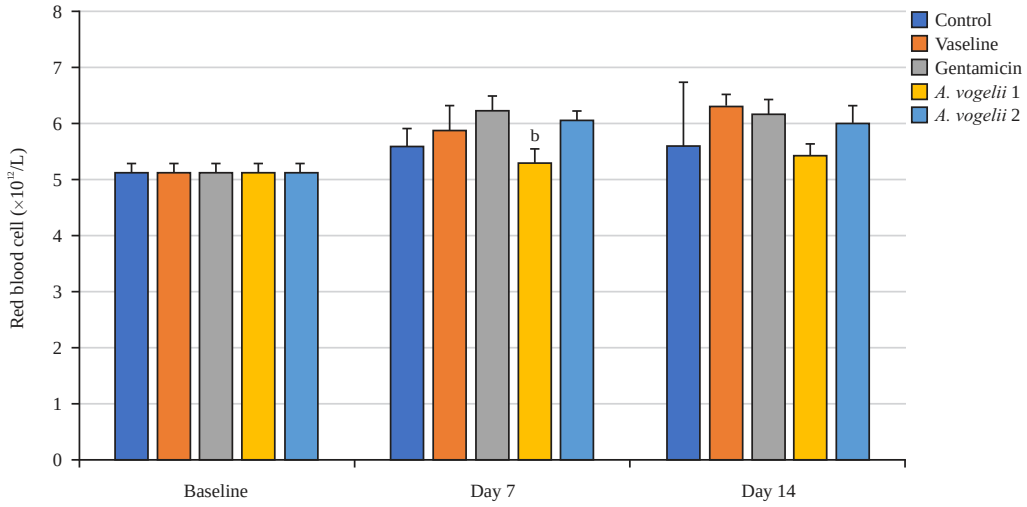

Fig. 3: Effect of methanol extract of *A. vogelii* leaf on red blood cell count (×10^{12}/L) of wound treated female Wistar rats

Values are Mean±SD of triplicate determination. Values with "b" is statistically different ($p<0.05$) when Gentamicin group is compared with Control, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14. Values without any superscript show that there was no statistical difference when Control and Gentamicin were compared with other groups

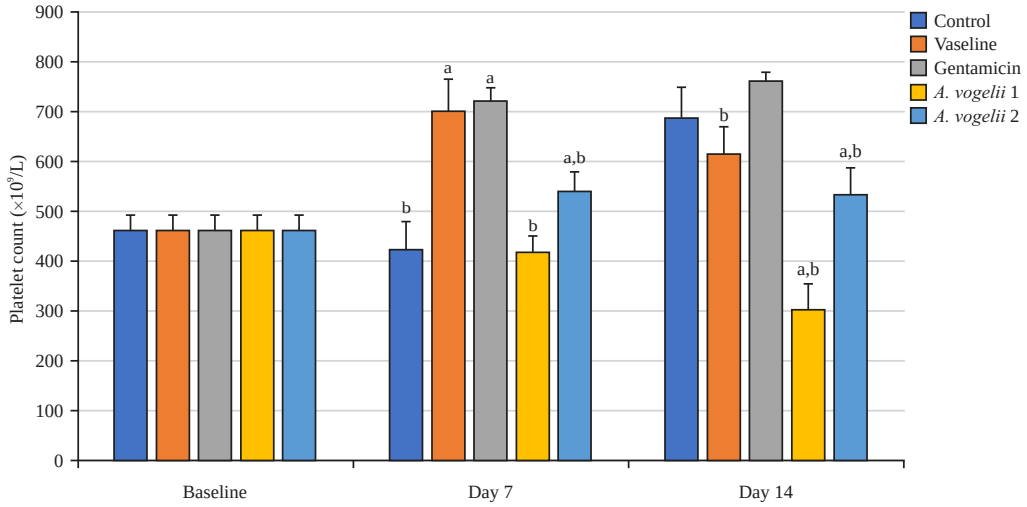

Fig. 4: Effect of methanol extract of *A. vogelii* leaf on platelet count (× 10^{9}/L) of wound treated female Wistar rats

Values are Mean±SD of triplicate determination. Values with superscript "a" are statistically different ($p<0.05$) when Control group is compared with Vaseline, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14, superscript "b" is statistically different ($p<0.05$) when Gentamicin group is compared with Control, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14. Values without any superscript show that there was no statistical difference when Control and Gentamicin were compared with other groups

revealed a significant difference between Gentamicin group on day 7 and with Vaseline and *A. vogelii* 1 group on day 14. When Gentamicin group was compared with Control, Vaseline, *A. vogelii* 1 and *A. vogelii* 2 a significant difference ($p<0.05$) was observed on Control and *A. vogelii* 2 on day 7 and Vaseline and *A. vogelii* 1 group on day 14 (Fig. 6).

Lymphocyte: The lymphocyte result revealed that the Control, Gentamicin and *A. vogelii* 2 group decreased from baseline to day 14, while the lymphocyte level in the Vaseline and *A. vogelii* 1 group revealed a significant decrease ($p<0.05$) from baseline to day 7 and a significant increase ($p<0.05$) on day 14. Comparing the Control group with other groups revealed a significant difference ($p<0.05$) with the Vaseline, Gentamicin group on day 7 and no significance difference ($p<0.05$) with other groups on day 14. Comparison of the Gentamicin group with the Control group, Vaseline group, *A. vogelii* 1 and *A. vogelii* 2 group

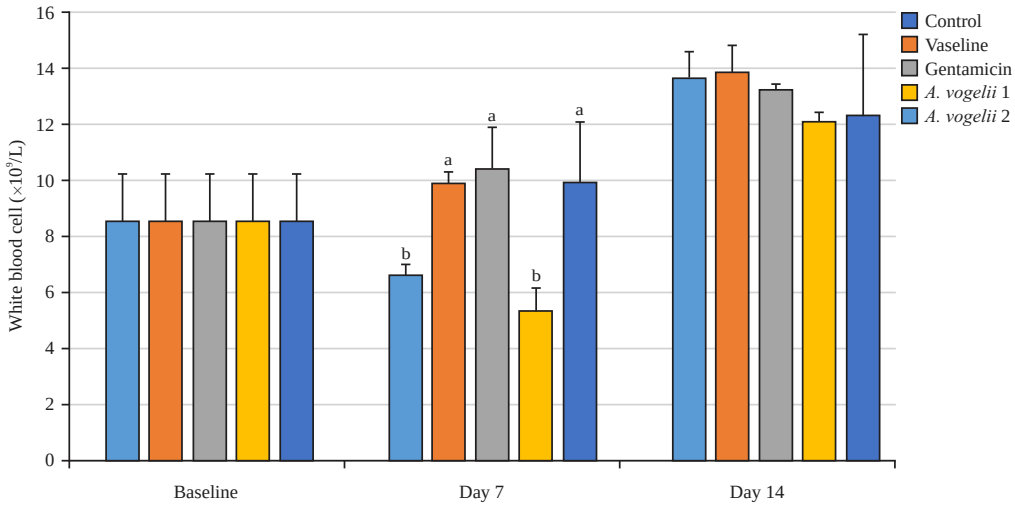

Fig. 5: Effect of methanol extract of *A. vogelii* leaf on white blood cell (× 10^9/L) of wound treated Wistar female rats

Values are Mean±SD of triplicate determination. Values with superscript "a" are statistically different (p<0.05) when Control group is compared with Vaseline, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14, superscript "b" is statistically different (p<0.05) when Gentamicin group is compared with Control, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14. Values without any superscript show that there was no statistical difference when Control and Gentamicin were compared with other groups

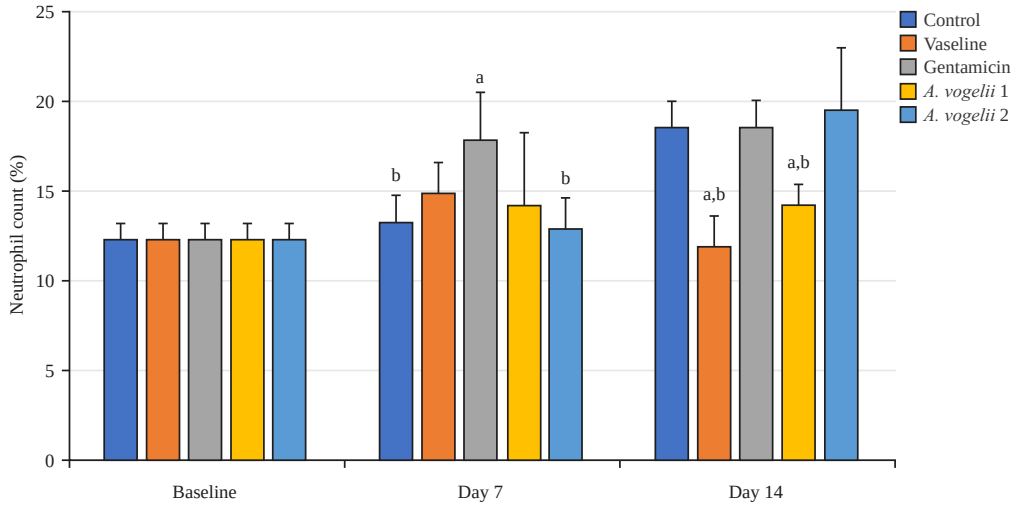

Fig. 6: Effect of methanol extract of *A. vogelii* leaf on neutrophil count (%) of wound treated female Wistar rats

Values are Mean±SD of triplicate determination. Values with superscript "a" are statistically different (p<0.05) when Control group is compared with Vaseline, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14, superscript "b" is statistically different (p<0.05) when Gentamicin group is compared with Control, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14. Values without any superscript show that there was no statistical difference when Control and Gentamicin were compared with other groups

showed a significant difference (p<0.05) with Control, *A. vogelii* 1 and *A. vogelii* 2 group on day 7, Vaseline and *A. vogelii* 1 group on day 14 (Fig. 7).

Eosinophil: The result of the eosinophil level revealed that the Control, Vaseline and Gentamicin, *A. vogelii* 1 group significantly increased (p<0.05) from baseline to 7 and decreased from day 7 to day 14, while the *A. vogelii* 2 group showed a significant decrease (p<0.05) from baseline to day 7 and a significant increase (p<0.05) from 7 to 14. Comparison of the Control group with other groups revealed a significant difference (p<0.05) with Vaseline and Gentamicin on day 7 and Gentamicin and *A. vogelii* 2 group on day 14. There was significant difference (p<0.05) observed in Control, *A. vogelii* 1 and *A. vogelii* 2 when Gentamicin group was compared with Control, Vaseline,

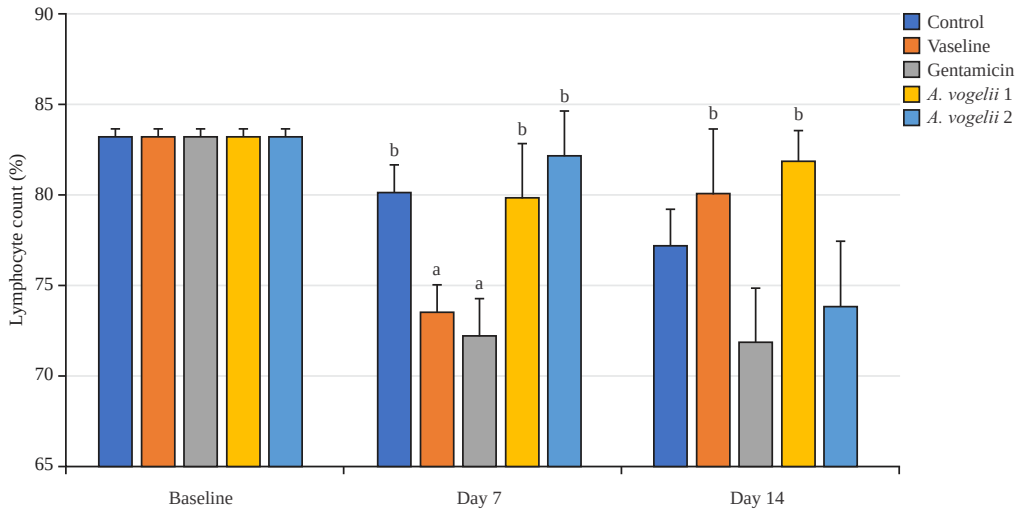

Fig. 7: Effect of methanol extract of *A. vogelii* leaf on lymphocyte count (%) of wound treated female Wistar rats

Values are Mean±SD of triplicate determination. Values with superscript "a" are statistically different (p<0.05) when Control group is compared with Vaseline, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14, superscript "b" is statistically different (p<0.05) when Gentamicin group is compared with Control, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14. Values without any superscript show that there was no statistical difference when Control and Gentamicin were compared with other groups

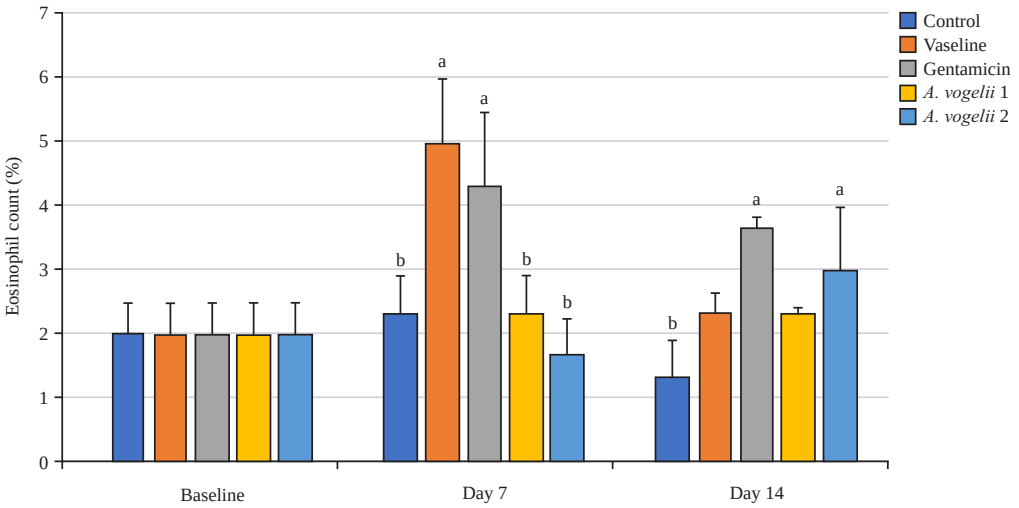

Fig. 8: Effect of methanol extract of *A. vogelii* leaf on eosinophil count (%) of wound treated female Wistar rats

Values are Mean±SD of triplicate determination. Values with superscript "a" are statistically different (p<0.05) when the Control group is compared with Vaseline, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14, superscript "b" is statistically different (p<0.05) when Gentamicin group is compared with Control, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14. Values without any superscript show that there was no statistical difference when Control and Gentamicin were compared with other groups

A. vogelii 1 and *A. vogelii* 2 group on day 7 and a significant difference (p<0.05) was observed on Control group on day 14 (Fig. 8).

Monocyte: The monocyte result revealed that the Control group, Gentamicin group and *A. vogelii* 1 group significantly increased (p<0.05) from baseline to 14. Monocyte level in the Vaseline group significantly increased (p<0.05) from baseline to day 7 and decreased on day 14. The *A. vogelii* 2 group showed a significant decrease (p<0.05) from baseline to day 7 and a significant increase on day 14. Comparison of the Control group with the other groups revealed a significant difference (p<0.05) in Vaseline, Gentamicin and *A. vogelii* 1 group on days 7 and 14. A significant difference (p<0.05) was observed in Control, *A. vogelii* 1 and *A. vogelii* 2 on day 7 and Vaseline, *A. vogelii* 1 and *A. vogelii* 2 on day 14 when the Gentamicin group was compared with Control, Vaseline, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14 (Fig. 9).

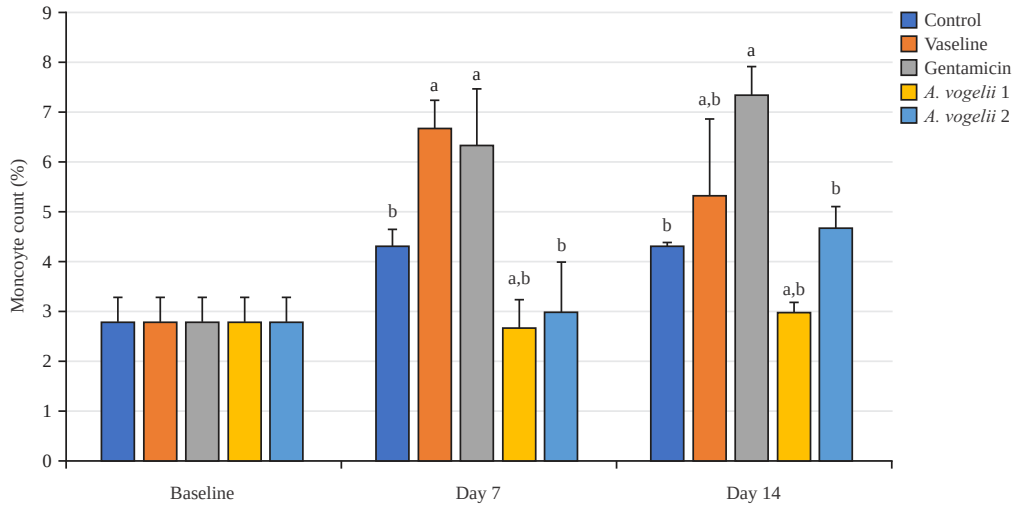

Fig. 9: Effect of methanol extract of *A. vogelii* leaf on monocyte count (%) of wound treated female Wistar rats

Values are Mean±SD of triplicate determination. Values with superscript "a" are statistically different ($p<0.05$) when Control group is compared with Vaseline, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14, superscript "b" is statistically different ($p<0.05$) when Gentamicin group is compared with Control, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14. Values without any superscript show that there was no statistical difference when Control and Gentamicin were compared with other groups

Table 4: Effect of methanol extract of *A. vogelii* leaf on percentage rate of wound healing of wound treated female Wistar rats

Group	Baseline	Day 7	Day 14
Control	0.00	55.00±9.13	87.50±3.54
Vaseline	0.00	60.00±7.07	85.00 ±0.00
Gentamicin	0.00	51.25±2.50	92.50±3.54
A. vogelii 1	0.00	53.75±12.50	72.50±3.54
A. vogelii 2	0.00	47.50±8.75	67.50±3.54

Values are Mean±SD of triplicate determination. Values without any superscript shows that there was no statistical difference when Control and Gentamicin was compared with other groups

Percentage rate of wound healing: The percentage rate of wound healing in rats treated with methanol extract from *A. vogelii* leaves is presented in Table 4. The result revealed a significant increase in the percentage rate of wound healing in all groups with the Gentamicin group showing the highest percentage rate of wound healing at 92.50% on day 14. There was no statistical difference ($p<0.05$) in the rate of wound healing across the group during the period of research. Also, no statistical difference was observed at $p<0.05$ when the control group was compared with Vaseline, Gentamicin, *A. vogelii* 1 and *A. vogelii* 2 group on day 0 and 14, when Gentamicin group was compared with Control, Vaseline, *A. vogelii* 1 and *A. vogelii* 2 group on day 7 and 14.

DISCUSSION

The study revealed the wound healing potential of methanol extract of *Anthocleista vogelii* leaves on female Wistar rats. The significant reduction in iron levels in the Gentamicin and *A. vogelii* groups might indicate that these treatments enhance wound healing by altering iron metabolism, which is crucial for various biological processes including cell proliferation and repair[25]. The greater reduction in *A. vogelii* 1 suggests a strong effect, potentially due to its bioactive compounds affecting iron homeostasis.

Oestrogen is known to influence wound repair by promoting cell proliferation and collagen synthesis[26]. Oestrogen levels increase gradually during adolescence until maturity is reached. Oestrogen levels during the reproductive years fluctuate according to the menstrual cycle. The *A. vogelii* 1 and *A. vogelii* 2 significantly increase estrogen levels by day 7, with the effect continuing through day 14. This suggests that these extracts may have estrogenic activity, which could influence various physiological processes including wound healing[27]. Estrogen is known to influence wound repair by promoting cell proliferation and collagen synthesis[28].

The haematological result shows that there was a significant increase in the level of PCV, Hb, RBC, Platelets, RBC, WBC, neutrophil, eosinophil and monocyte on administration of *A. vogelii* leaves. This may significantly contribute to the wound healing process. Hematological parameters, such as Red Blood Cell (RBC) count, hemoglobin concentration and White Blood Cell (WBC) count, play vital roles in wound healing by supporting oxygen delivery, immune response and inflammation management. The RBCs and hemoglobin are essential for transporting oxygen from the lungs to tissues throughout the body, including wound

sites. Oxygen is crucial for cellular metabolism and energy production, which are necessary for effective wound healing[8]. Oxygen is also vital for collagen synthesis, a critical component of wound repair and for the bactericidal activity of neutrophils. An increase in RBC count and hemoglobin levels ensures that the wound receives adequate oxygen, promoting faster wound healing. In the context of wounds, higher oxygen availability can enhance fibroblast function, keratinocyte migration and epithelialization[29]. The WBCs, especially neutrophils and macrophages, are crucial for the inflammatory phase of wound healing. They help clear pathogens and debris from the wound site and release cytokines and growth factors that promote tissue repair and regeneration[30]. An increase in WBC count suggests a robust immune response, which is vital for preventing infection and promoting clean wound healing. A well-regulated increase in WBCs also ensures an effective transition from the inflammatory phase to the proliferative phase, characterized by new tissue formation. This balance is critical because prolonged inflammation can impede healing, whereas a timely resolution can accelerate tissue repair[31]. Platelets are another critical hematological component involved in wound healing. Platelets contribute to clot formation and secrete various growth factors like Platelet-Derived Growth Factor (PDGF) and Transforming Growth Factor-beta (TGF-β), which are crucial for wound healing[32].

The wound healing size indicated a gradual reduction for every group. Wound closure is due to contraction which takes place in the proliferative phase. Wound contraction is caused by fibroblast and myofibroblasts by pulling the wound edges together. Keratinocytes move the granulation tissue from the wound edges to the center to close the wound and also protect the tissue beneath[6]. The percentage rate of wound healing suggests that Gentamicin is the most effective treatment, followed by *A. vogelii* 1. The lower healing rates in *A. vogelii* 2 compared to other groups suggest that while *A. vogelii* 2 has some healing potential, but it is less effective than the other treatments. The differences in effectiveness could be due to variations in the concentration or composition of the extracts used.

CONCLUSION

The methanol extract of *Anthocleista vogelii* leaves in this study has shown potential in promoting wound healing in female Wistar rats. The extract demonstrated significant wound closure and reduction in wound area, indicating its ability to facilitate the healing process. This can be attributed to the presence of bioactive compounds such as flavonoids, alkaloids and phenolic compounds, which are known to possess antioxidant and ant-inflammatory properties. The result of the study therefore revealed that the methanol extract of *Anthocleista vogelii* possesses enough wound healing potential and can be considered in wound treatment. Further studies are needed to elucidate the underlying mechanisms and optimal dosage for achieving optimal wound healing effects.

SIGNIFICANCE STATEMENT

The purpose of the research was to assess the wound healing potentials of methanol extract of *Anthocleista vogelii* leaves on female Wistar rats. The results revealed a significant increase in hematological parameters (PCV, Hb, RBC, Platelets, RBC, WBC, neutrophil, eosinophil and monocyte) on the administration of *A. vogelii*. This suggests that methanol extract of *Anthocleista vogelii* leaves can be used as an alternative to conventional drugs used in wound treatment as these drugs are not only expensive but also have problems such as allergies and drug resistance.

REFERENCES

1. Velnar, T., T. Bailey and V. Smrkolj, 2009. The wound healing process: An overview of the cellular and molecular mechanisms. J. Int. Med. Res., 37: 1528-1542.
2. Abubacker, M.N. and P.K. Devi, 2016. Evaluation of wound healing activity of ethanolic extract of *Lepidagathis cristata*. Willd. (Acanthaceae) in experimental animal models. Eur. J. Pharm. Med. Res., 3: 410-416.
3. Desmet, C.M., V. Préat and B. Gallez, 2018. Nanomedicines and gene therapy for the delivery of growth factors to improve perfusion and oxygenation in wound healing. Adv. Drug Delivery Rev., 129: 262-284.
4. Guo, S. and L.A. DiPietro, 2010. Factors affecting wound healing. J. Dent. Res., 89: 219-229.
5. Qing, C., 2017. The molecular biology in wound healing & non-healing wound. Chin. J. Traumatol., 20: 189-193.
6. Shedoeva, A., D. Leavesley, Z. Upton and C. Fan, 2019. Wound healing and the use of medicinal plants. Evidence-Based Complementary Altern. Med., Vol. 2019. 10.1155/2019/2684108.
7. Kobayashi, S.D., N. Malachowa and F.R. DeLeo, 2018. Neutrophils and bacterial immune evasion. J. Innate Immun., 10: 432-441.
8. de Oliveira Gonzalez, A.C., T.F. Costa, Z. de Araújo Andrade and A.R.A.P. Medrado, 2016. Wound healing-A literature review. Anais Brasileiros Dermatologia, 91: 614-620.

9. Ovais, M., I. Ahmad, A.T. Khalil, S. Mukherjee and R. Javed *et al*., 2018. Wound healing applications of biogenic colloidal silver and gold nanoparticles: Recent trends and future prospects. Appl. Microbiol. Biotechnol., 102: 4305-4318.
10. Young, A. and C.E. McNaught, 2011. The physiology of wound healing. Surgery, 29: 475-479.
11. Akasaka, Y., I. Ono, T. Kamiya, Y. Ishikawa and T. Kinoshita *et al*., 2010. The mechanisms underlying fibroblast apoptosis regulated by growth factors during wound healing. J. Pathol., 221: 285-299.
12. Rajkumar, S.R.J., M.S.A.M. Nadar and P.M. Selvakumar, 2018. Plant-derived compounds for wound healing- A review. Org. Med. Chem. Int. J., Vol. 5. 10.19080/OMCIJ.2018.05.555653.
13. Agyare, C., Y.D. Boakye, E.O. Bekoe, A. Hensel, S.O. Dapaah and T. Appiah, 2016. Review: African medicinal plants with wound healing properties. J. Ethnopharmacol., 177: 85-100.
14. Pather, N., A.M. Viljoen and B. Kramer, 2011. A biochemical comparison of the *in vivo* effects of *Bulbine frutescens* and *Bulbine natalensis* on cutaneous wound healing. J. Ethnopharmacol., 133: 364-370.
15. Dube, P., S. Meyer, A. Madiehe and M. Meyer, 2020. Antibacterial activity of biogenic silver and gold nanoparticles synthesized from *Salvia africana-lutea* and *Sutherlandia frutescens*. Nanotechnology, Vol. 31. 10.1088/1361-6528/abb6a8.
16. Mabona, U. and S.F. van Vuuren, 2013. Southern African medicinal plants used to treat skin diseases. S. Afr. J. Bot., 87: 175-193.
17. Ikpe, E.E., U.D. Archibong, C.U. Michael and E.G. Ukpong, 2020. Chemical composition of *Anthocleista vogelii* and anti-oxidant effect of its methanolic and acetone extracts. Chem. Sci. Int. J., 29: 7-18.
18. Oladimeji, S.O., J.O. Igbalaye and C.L. Coleshowers, 2014. Immunological biomarkers determined in female rats administered with pro-fertility extract of *Anthocleista vogelii*. J. Nat. Sci. Res., 4: 113-122.
19. Ugwu, C.N., E.N. Ezeibe, C.S. Nwagwu, C.C. Eze and S.A. Evurani *et al*., 2019. Preparation and evaluation of burns wound healing ointment base of leaves and stem bark of *Anthocleista djalonensis* (L) extract using animal model. Int. J. Pharm. Educ. Res., 1: 29-36.
20. Zaki, M.Z.M., A. Hamid and M. Abdul Latif, 2011. Effects of *Alocasia* sp. stem juice on open wound healing in rats. Adv. Environ. Biol., 5: 3734-3742.
21. Ramsay, W.N.M., 1953. The determination of iron in blood plasma or serum. Biochem. J., 53: 227-231.
22. Zhao, M.P., Y.Z. Li, Z.Q. Guo, X.X. Zhang and W.B. Chang, 2002. A new competitive Enzyme-Linked Immunosorbent Assay (ELISA) for determination of estrogenic bisphenols. Talanta, 57: 1205-1210.
23. Jones, R.F., 1961. Determination of packed cell volume by centrifugation. J. Clin. Pathol., 14: 198-199.
24. Tana, S., T.R. Saraswati and E.Y.W. Yuniwarti, 2018. Hematology and blood chemistry status of most frequently consumed ruminants in community. Biosaintifika: J. Biol. Biol. Educ., 10: 341-347.
25. Recalcati, S., E. Gammella, P. Buratti, A. Doni, A. Anselmo, M. Locati and G. Cairo, 2019. Macrophage ferroportin is essential for stromal cell proliferation in wound healing. Haematologica, 104: 47-58.
26. Shi, S., L. Wang, C. Song, L. Yao and J. Xiao, 2023. Recent progresses of collagen dressings for chronic skin wound healing. Collagen Leather, Vol. 5. 10.1186/s42825- 023-00136-4.
27. Plackett, T.P., M.S. Gregory and E.J. Kovacs, 2015. Effects of high estrogen levels on monocyte chemoattractant protein-1 and wound healing. Adv. Wound Care, 4: 92-99.
28. Prabhu, V., B.S.S. Rao, A.C.K. Rao, K. Prasad and K.K. Mahato, 2022. Photobiomodulation invigorating collagen deposition, proliferating cell nuclear antigen and Ki67 expression during dermal wound repair in mice. Lasers Med. Sci., 37: 171-180.
29. Sen, C.K., 2009. Wound healing essentials: Let there be oxygen. Wound Repair Regener., 17: 1-18.
30. Mescher, A.L., 2017. Macrophages and fibroblasts during inflammation and tissue repair in models of organ regeneration. Regeneration, 4: 39-53.
31. Broughton II, G., J.E. Janis and C.E. Attinger, 2006. Wound healing: An overview. Plast. Reconstructive Surg., 117: 1e-S-32e-S.
32. Kaltalioglu, K. and S. Coskun-Cevher, 2015. A bioactive molecule in a complex wound healing process: Platelet-derived growth factor. Int. J. Dermatol., 54: 972-977.