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Anti-oxidant and Wound Healing Potentials of *Gnetum africanum* Welw and *Ficus vogelii* Miq Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. Author MEC conceptualized and designed the study. Authors MEC and OAA collected and assembled data. Authors MEC, OAA and BOE analysed and interpreted the data. Author OAA wrote the first draft. Authors MEC and BOE critically reviewed the draft. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Gnetum africanum* and *Ficus vogelii* are vegetables consumed in some parts of Africa. They are used in ethno medicine for treatment of different diseases, and are particularly known to hasten wound healing. The study aimed at investigating the wound healing properties of

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the plants in a rat model.
Materials and Methods: The powdered leaves of the two plants were successively extracted with hexane, ethyl acetate and methanol using a Soxhlet apparatus. The crude methanol extract was screened for secondary metabolites and anti-oxidant properties. The wound healing activity was evaluated using excision wound model. Thirty healthy female Wistar albino rats (150-200 kg) were used for the experiment and randomized into 5% extract + ointment, 3.5% extract + ointment, simple ointment, and gentamicin treatment groups. The ointments were administered topically daily, and wound contraction was measured every alternate day. The percentage wound closure rate and histopathology of healed wound area were determined. The antioxidant activity of the plants was determined using the DPPH scavenging activity and ferric ion reducing antioxidant power assay (FRAP).
Results: The methanol extracts of both plants showed the presence tannins, alkaloids, flavonoids, saponins, and steroids in varying amounts. The antioxidant assays revealed that the extracts of both plants had good anti-oxidant properties. Extracts of *Gnetum africanum* at 3.5% w/w and *Ficus vogelii* 5% w/w exhibited potent healing activity, eliciting 100% wound closure by day 7.
Conclusion: The study revealed that *Gnetum africanum* and *Ficus vogelii* have wound healing properties which scientifically justifies its use for treatment of wounds traditionally and could be developed into useful drugs for wound treatment and management.

Keywords: Gnetum africanum; Ficus vogelii; wound; ointment; ethnomedicine.

1. INTRODUCTION

A wound is a disorder that disrupts cellular and anatomical tissue; it is usually caused by physical, chemical, thermal, microbial, or immunological damage to the tissues. A wound might be acute or chronic; acute wounds generally include abrasions, minor cuts, bites, punctures, surgical incisions, and burns (heat, chemical, cold, or friction); they usually are properly managed and heal in a short period. Chronic wounds, on the other hand, are wounds that refuse to heal within three months; that is the functional and anatomical integrity has not been restored within this time. Chronic wounds are highly associated with underlying diseases such as diabetes and some other vascular conditions and are commonly found on the lower legs. It has been estimated that about 6 million people suffer from chronic wounds [1,2,3].

Wound infection is a common problem especially in developing countries like the Sub-Saharan Africa. Wounds provide an environment suitable for colonization and proliferation of microbes, this is because they expose the subcutaneous tissues which are usually moist, warm and nutritionally rich, any wound is at a high risk of getting infected because the skin's microbiome includes potentially pathogenic and opportunistic pathogens that can infect compromised skin [4]. Infected wounds are often difficult to manage and often fail to heal, leading to prolonged patient suffering from pain and trauma, and high cost of treatment of the infected wound [5,6].
Wound healing is an intricate and dynamic process that entails the repair and regeneration of a tissue that has suffered past injury [1]. The wound healing process has four interconnected and overlapping phases: hemostasis, inflammation, proliferation and tissue remodeling or resolution [7,8].

Medicinal plants have been used globally to cure a wide range of ailments. According to the World Health Organization, almost 80% of people in developing countries use plants as their primary source of medication. Medicinal herbs have been found to possess high contents of alkaloids, flavonoids, tannins, triterpenes, saponins, naphthoquinone, and other phytochemicals. As a result, they have been used for a long time to enhance the healing process of wounds by improving the quality and rate of healing of cutaneous wounds [9]. Plants with wound healing capabilities are expected to have the ability to promote the growth of fibroblasts, stimulate the proliferation and differentiation of keratinocytes, enhance the production of collagen and exhibit antioxidant, antimicrobial and anti-inflammatory characteristics. In most cases, for a medicinal plant or natural product to be considered an effective wound healing agent, it must possess two or more of the above properties [10]. *Ficus vogelii* Miq. is a tree that thrives in the tropical and sub-tropical regions worldwide. The West African rubber tree is widespread in countries such as Nigeria, Mali, Ghana and Senegal [11]

and is thus commonly referred to as the 'West African rubber tree' [12]. Traditional practitioners have purportedly employed the leaves of *Ficus vogelii* to cure diabetic diseases, anemia, dysentery and diarrhoea [13].

Gnetum africanum Welw is a shade loving dioecious perennial plant that can grow up to about 10m long [14]. It belongs to the section of Gnetophytes known as Gnetum and the sub-section of microgenomes (The Gymnosperm database). The leaves and seeds of the plants have been used to treat enlarged spleen, pain relief during child birth, as antidote for poisonous snake bites [15] and excessive urination in diabetic patients [16]. The leaves have been previously reported to have anti-carcinogenic, anti- oxidant and anti- inflammatory properties [17].

The aim of this study was to investigate the wound healing properties of *Gnetum africanum* and *Ficus vogelii* to provide insight on the scientific basis of their use in ethnomedicine.

2. MATERIALS AND METHODS

2.1 Plant Collection and Preparation

The leaves of *Gnetum africanum* and *Ficus vogelii* were collected from the Botanical Garden, University of Ibadan, Ibadan, Nigeria and verified at the Department of Botany, University of Ibadan, with voucher numbers UIH-22557 for *Gnetum africanum* and UIH-22856 for *Ficus vogelii*. The leaves were air dried under the shade at ambient temperatures, pulverized and weighed.

2.2 Plant Extraction

The powdered leaves of *Gnetum africanum* (1500g) and *Ficus vogelii* (2088g) were successively extracted with hexane, ethyl acetate and methanol using a Soxhlet apparatus. The crude extracts were concentrated to dryness using a rotary vacuum evaporator, weighed and stored in a refridgerator at 4ºC for further analysis.

2.3 Phytochemical Screening

The crude extracts obtained from both plants were analyzed for the presence of secondary metabolites using conventional techniques [18].

2.4 *In vitro* Antimicrobial Screening of Plant Extracts

The assessment of the plant extracts' antibacterial activity was carried out using the agar-well diffusion method. A 0.5 McFarland standard equivalent suspension of each isolate was made in 0.85 % saline and 0.1 mL of resulting isolate suspension was used to inoculate the plates of Mueller Hinton Agar. Wells of equal distances were bored with the aid of a standard sterile 8mm cork borer and 100 µL of different concentrations of extracts and control were placed into the corresponding wells. Gentamicin (10 µg) was used as the standard drug control. To facilitate the diffusion of the extract, the plates were left at room temperature for about one hour. Incubation of the plates was carried out at 37 °C for 24 h. All tests were done in duplicates and the average values recorded for the results. The extracts with the most promising activity was used for further downstream studies.

2.5 *In vivo* Wound Healing Potentials of Most Active Extracts

2.5.1 Animals

The animal research utilized experimental and handling procedures that were compliant with regulations set forth by national and institutional bodies. Every experiment has undergone thorough examination and received approval from the relevant ethics committee.

Thirty (30) Wistar female albino rats in good condition, weighing between 150 and 200 kg were divided into six (6) groups of five animals each. Each group was individually confined in plastic cages. Animals were fed with standard pellet diet and water *ad libitum*. The experiments commenced after a 10-day acclimatization period in the laboratory environment.

2.5.2 Excision wound model

The experiments were carried out using the method described by Murthy et al. [19] with some modifications. Full thickness excision wound of about 600 mm^2 was made on the rats. The wound was then inoculated with 0.1 mL suspension of *Staphylococcus aureus* (ATCC 25923), adjusted to 0.5 McFarland standard. Infected wounds were left for 48 hours before administration of extract preparations. The methanol extract ointments of *Gnetum africanum* and *Ficus vogelii* at 3.5% and 5%. One group of rats each received the preparation of *Gnetum africanum* at 3.5% and 5%, for a total of two groups while another two groups received similar

concentration of ointment from *Ficus vogelii*. One control group received the simple ointment base used in preparation of extract-ointment while another group received gentamicin ointment. All ointments were applied topically to the lesion sites till the wounds were fully healed. The wound healing potential was determined by the rate of wound contraction and wound closure time. Wound area was measured using a translucent ruler on the day it was formed, and subsequently every alternate day until the wound had fully healed. The degree of wound healing was calculated. Wound contraction was calculated as percentage reduction in wound area using the formula;

> % of wound closure = (Wound area on day 0 - Wound area on day N)/ (Wound area on day 0) × 100

N: number of days.

2.5.3 Histopathology

Biopsy from wound site was collected after 7 days of treatment, fixed in 10% formalin and sectioned for histology examination [20].

Sampled rats in each group were euthanized at the end of the experiment.

2.6 Antioxidant activity of *Gnetum africanum* and *Ficus vogelii*

Two methods were used to determine the anti-oxidant properties of the methanolic extracts of the plants

2.6.1 DPPH scavenging activity

The DPPH scavenging activity was carried out according to the method described by Manzocco et al. [21] with slight modifications. The sample extract (1 mL) was diluted with 1mL of DPPH solution (0.3mM). After 30 min, the absorbance was measured at 517 nm. The percentage of the DPPH radical scavenging was calculated using the equation below:

> % DPPH scavenging = (% inhibition of DPPH radical Abr – Aar)/ Abr) × 100

Where Abr is the absorbance control and Aar is the absorbance of the sample after the reaction.

2.6.2 Ferric ion reducing antioxidant power assay (FRAP)

Ferric ions reducing power was measured according to the method of Benzie [22] with a slight modification. Methanolic extracts of both plants in different concentrations ranging from 100ug/ml to 500ug/ml were mixed with 2.5ml of 20 mM phosphate buffer and 2.5 ml 1%, w/v potassium ferricyanide, and then the mixture was incubated at 50 °C for 30 min. Afterwards, 2.5 ml of 10%, w/v trichloroacetic acid and 0.5 ml 0.1%, w/v ferric chloride were added to the mixture, which was kept aside for 10 min. Finally, the absorbance was measured at 700 nm. Ascorbic acid was used as positive reference standard. Each assay was performed in triplicate.

3. RESULTS

3.1 Phytochemical Screening

Quantitative and qualitative phytochemical screening of methanolic extract of *Gnetum africanum and Ficus vogelii* revealed the presence of saponins, alkaloids, flavonoids, tanins, coumarins, steroids, terpenoids, anthraquinones and phenols as reported previously by Coker et al. [23,24].

3.2 *In vivo* wound healing potentials

The methanol extracts of both plants were tested against excision wounds made on rats. For *Ficus vogelii,* the extracts had a better activity at a concentration of 5% w/w showing approximately 100% wound closure at day 7 while for *Gnetum africanum*; the extracts had a better activity at a concentration of 3.5% w/w showing approximately 100% wound closure at day 7.

The wound biopsy results revealed that the groups treated with 5% *Ficus vogelii* and 3.5% *Gnetum africanum* had rapid healing and the wounds were in the final stages of wound healing by the 7th day with no lesion which further confirmed the results obtained from observing percentage contractions.

Ficus vogelii showed better wound healing properties at a concentration of 5% w/w than at 3.5% w/w while *Gnetum afr*icanum showed better wound healing properties at a concentration of 3.5% w/w than at 5% w/w (Fig. 1). The maturation phase of wound healing of rats treated with plant extracts are shown in Fig. 2 and 3.

3.3 Antimicrobial activity of extracts

The antimicrobial assay carried out on the methanol extracts also revealed that both plants had *in vitro* anti-bacterial activity against clinical

isolates from wound infections. Table 1 shows the average diameter of zones of inhibition against various clinical isolates.

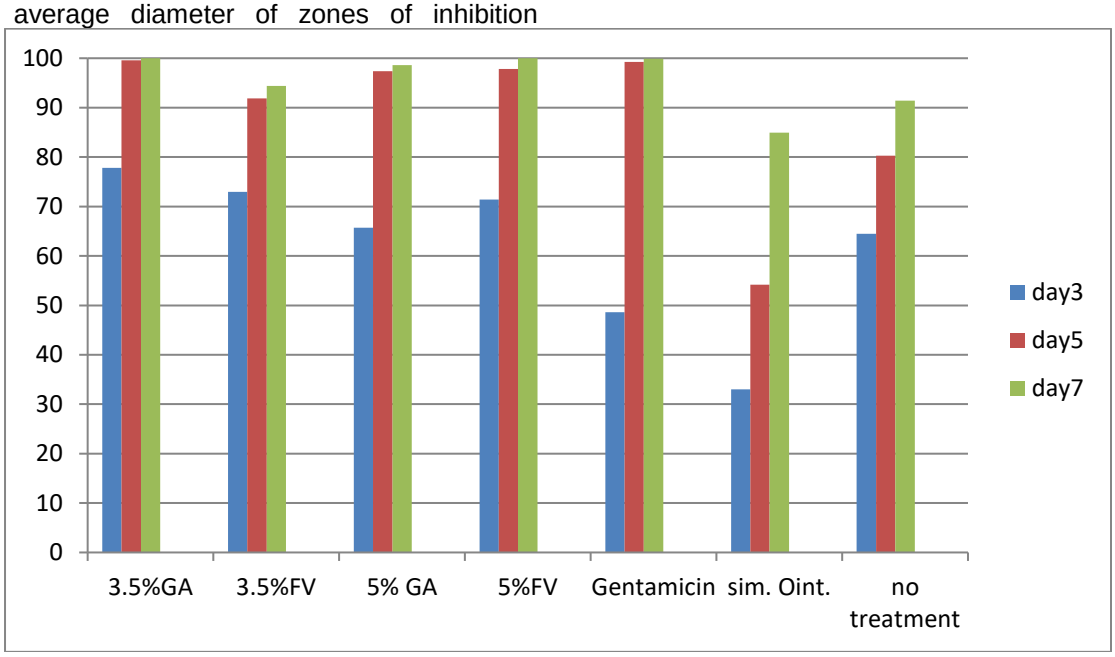


Fig. 1. Percentage wound contraction of 3.5%, 5% *Gnetum africanum, Ficus vogelii,* gentamicin, simple ointment and no treatment groups on day 3, 5 and 7

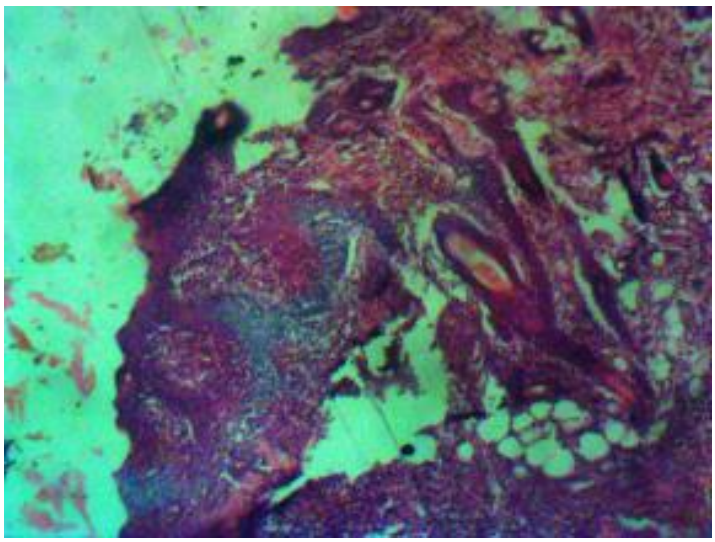
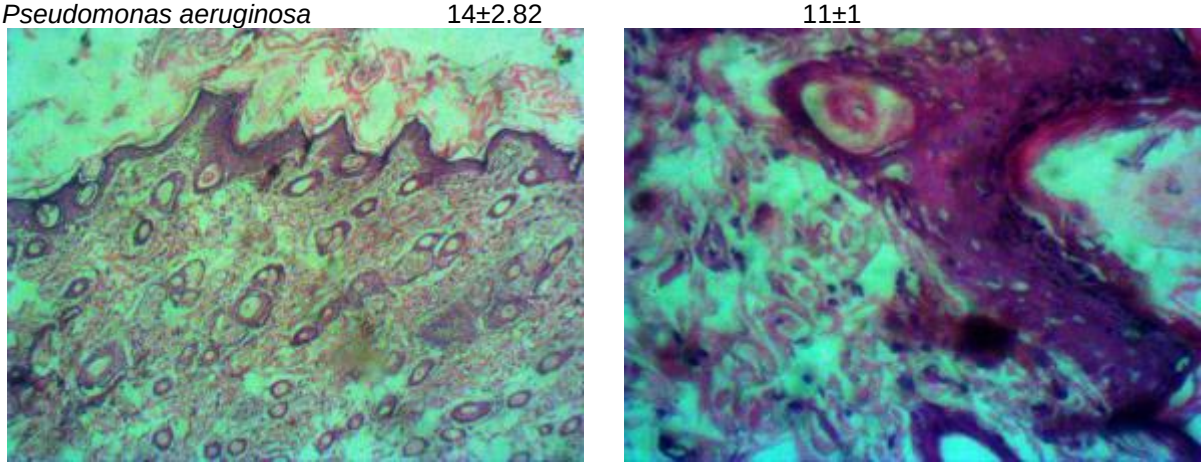


Fig. 2. Skin biopsy of rat treated with 3.5% *Gnetum africanum* after treatment showing maturation phase of wound healing. There are few acute inflammatory cells and proliferation of connective tissues HE x100

Table 1. Average diameter of zone of inhibition of *Gnetum africanum* and *Ficus vogelii* at 100mg/ml

Clinical Isolates	zone of inhibition (mm)	
	Gnetum africanum	***Ficus vogelii***
Escherichia coli	16.33±2.87	11±1.2
Staphylococcus aureus	16.8±2.77	13.8±1.76
Klebsiella pneumoniae	18±6.22	13.7±2.4

Proteus mirabilis	17.5±1.87	12.6±0.96
Pseudomonas aeruginosa	14±2.82	11±1



A **B**

Fig. 3. Skin biopsy of rat treated with 5% *Ficus vogelii* after treatment showing proliferative phase of wound healing. There is keratosis, acanthosis and acute inflammatory cells in the epidermis. A-HE x100, B- HE x 400

3.4 Anti-oxidant Property of Extracts

The assays consistently revealed that the methanol extracts of both plants had good anti-oxidant property**.** The percentage inhibition increased as concentration increased for the DPPH assay. The percentage inhibition of the extracts at concentrations of 200-500µg/ml was higher than 50%. The ferric reducing power tests also showed that the methanol extracts of the plants had good anti-oxidant properties when comparing the absorbance of the extract to that of the standard antioxidant drug (ascorbic acid). The results generally revealed that both *Gnetum africanum and Ficus vogelii* had good anti-oxidant properties, although *Gnetum africanum* had higher anti-oxidant properties than *Ficus vogelii* (Figs. 4&5).

4. DISCUSSION

The methanol extracts of both *Gnetum africanum and Ficus vogelii* contained a variety of phytochemicals which may be responsible for their antibacterial, anti-oxidant and wound healing potentials. Both plants contained tannins, alkaloids, steroids, anthraquinones, saponins and flavonoids which have also been reported in

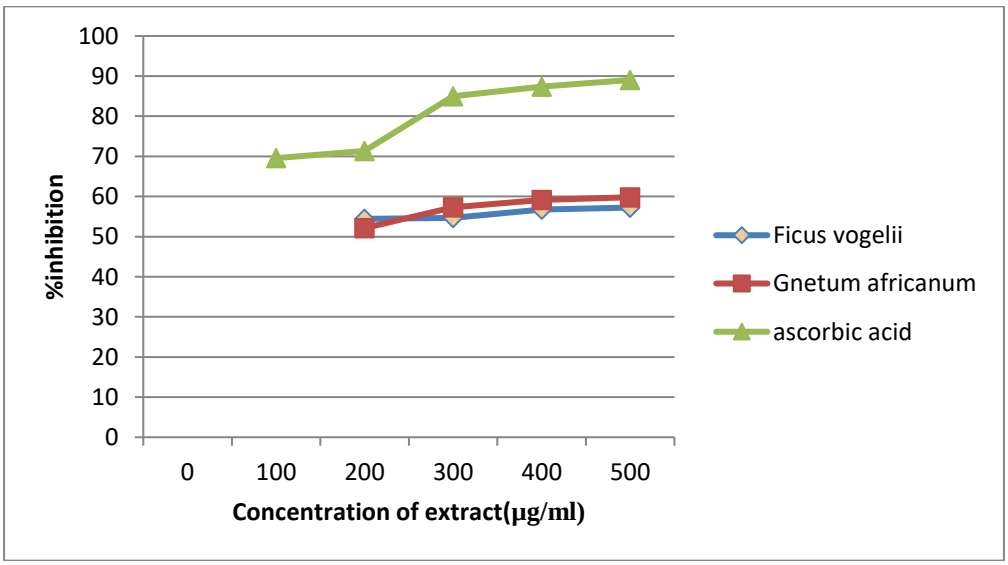


Fig. 4. DPPH radical scavenging showing percentage inhibition and absorbance at different concentrations (µg/ml) of methanol extract of *Ficus vogelii* and *Gnetum africanum*

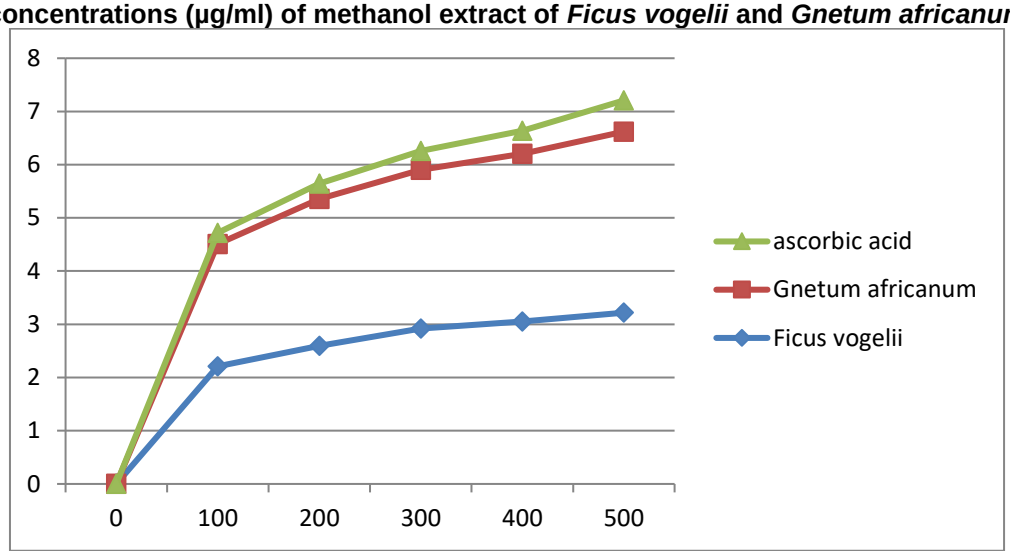


Fig. 5. Graph of Absorbance (nm) of FRAP against concentration of methanol extract of *Ficus vogelii* and *Gnetum africanum*

similar studies [23,24,25]. Eneh et al. [25] reported the presence of cardiac glycosides which were absent in the methanol extract of *Gnetum africanum* in this study. This difference may be as a result of slight difference in the choice of solvents used for extraction and the method of extraction used. These phytochemicals have been said to have astringent and excellent antioxidant properties that can hasten wound healing and treat inflamed mucous membranes 26].

The *in vivo* studies showed that methanol extract ointment from both plants had good wound healing properties. The infected excision wounds inflicted in the rat models healed rapidly with the application of the plants' extract ointment. The *Gnetum africanum* extract ointment showed a slightly better activity at 3.5%w/w than at 5%w/w while the *Ficus vogelii* extract ointment showed a better activity at 5%w/w than at 3.5%w/w (Fig. 1). The rate of healing, however, at both concentrations for both plants was faster than in the positive control (Gentamicin) group, although by day 7 the percentage wound closure rate was similar to that of the Gentamicin group. The negative control groups also experienced wound contraction only at much slower rate. The wound biopsy results revealed that the groups treated with 5% *Ficus vogelii* and 3.5% *Gnetum africanum* had rapid healing and the wounds were in the final stages of healing by the 7th day with no lesions which further confirms the results obtained from observing percentage contractions. The wound healing potential of *Gnetum africanum* reported in this study agrees with the claims stated by Ali Assanta & Robert [17] where he reported that the plant has been used ethnomedicinally for treatment of wounds due to its anti-inflammatory and anti-oxidant properties. The antioxidant and wound healing properties of the *Ficus vogelii* extracts in this study agrees with work done by Yadav et al. [27].

The antioxidant studies revealed that both *Gnetum africanum* and *Ficus vogelii* have good antioxidant properties when compared to the standard antioxidant, Vitamin C. [28] Ali Assanta & Robert [17] stated that the plant *Gnetum africanum* has antioxidant properties. This was also reported by Eleazu and Eleazu [29], and this study further confirmed their claims. Yadav et al. [30] also mentioned in their work that members of the *Ficus* genus were generally known to have antioxidant properties; the results of the result of this study further confirmed that claim as *Ficus vogelli* demonstrated good antioxidant properties in the two assays highlighted above. The ferric-reducing tests showed that *Gnetum africanum* had excellent antioxidant properties similar to vitamin C, used as the standard positive control. The presence of antioxidant in plants helps to confer protection against diseases that are associated with free radicals [31]. The excellent antioxidant property of both plants is proposed to be involved in their wound-healing potential and

can be exploited for drug discovery for wounds and other infectious conditions.

The results of the antimicrobial susceptibility tests revealed that the methanol extracts of *Gnetum africanum and Ficus vogelii* had considerable anti-bacterial activities against the clinical isolates from wounds, as previously reported by Coker et al. [23,24]. This is consistent with the observations of Ayuk et al. [26] which reported that extracts of *Gnetum africanum* had activity against organisms such as *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa.* The result is also consistent with what was reported by Eneh et al. [25] on the antimicrobial activity of aqueous and methanol extract against various bacterial isolates. The report by Eneh et al. [25] showed that the extracts were not active against *Escherichia coli* isolates but this study showed that the methanol extracts of *Gnetum africanum* possessed activity against all the clinical isolates of *Escherichia coli* isolates tested. The differences may either be due to the strains of isolates tested or the manner in which the plants extracts were processed. Different strains of bacteria can exhibit high variation in antimicrobial susceptibility depending on genetics and gene expression. Also, plant processing, time of plant collection, location and age of plants, amongst other factors, can influence phytochemical composition and by extension, antimicrobial activity.

5. CONCLUSION

This study showed that the leaves *Gnetum africanum* and *Ficus vogelii* contain bioactive compounds that have excellent wound healing potentials. The wound healing potential of the extracts is comparable to that of gentamicin ointment and the plants are thus a potential source for potent antimicrobials. The extracts contained secondary metabolites like alkaloids, flavonoids, tannins, terpenoids, steroids, and anthraquinones. These metabolites have been reported to have pharmacological uses and could be responsible for the antibacterial, wound healing and antioxidant properties exhibited by the plant extracts in this study.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was obtained from the Animal Care Use and Research Committee (UI-ACUREC/052-0521/26) of the University of Ibadan, Nigeria.

DATA AVAILABILITY STATEMENT

All raw data from this study were generated by the authors and are available upon request from the corresponding author. The analyzed form of all data are however present within the article.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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