Effects of Methanol and N-hexane Extracts of *Raphia vinifera* Fruit and *Elaeis guineensis* Seeds Against *Staphylococcus aureus* and *Escherichia coli*

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Abstract: - The aim of this study was to assess the impact of methanol and n-hexane extracts derived from the mesocarp of *Raphia vinifera* fruit and *Elaeis guineensis* seeds on the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria. The antibacterial properties of methanol and n-hexane extracts of *Raphia vinifera* fruit mesocarp and palm kernel seeds against *Staphylococcus aureus* and *Escherichia coli* were evaluated using the agar-well diffusion method. The average diameter of the areas where growth was inhibited by the n-hexane extract of palm kernel seed and *Raphia vinifera* fruit mesocarp was 15 mm and 9 mm, respectively, for *S. aureus*. The diameter of the inhibition zone for *Staphylococcus aureus* was 11 mm for the *Elaeis guineensis* seeds extract and 8 mm for the *Raphia vinifera* fruit mesocarp extract for S. Aureus. The n-hexane extract of *Elaeis guineensis* seeds and *Raphia vinifera* fruit mesocarp showed inhibition zones with mean diameters of 15 mm and 12 mm, respectively, for *Escherichia coli*. Similarly, the methanol extract of *Elaeis guineensis* seeds and *Raphia vinifera* fruit mesocarp exhibited inhibition zones with mean diameters of 5 mm and 9 mm, respectively, for *Escherichia coli*. Among the solvents utilised for extraction in this work, n-hexane demonstrates the highest antibacterial efficacy compared to methanol extracts against all test species, including *S. aureus* and *E. coli*. Thus, both *Elaeis guineensis* seeds and *Raphia vinifera* fruit mesocarp have the potential to serve as alternate antibacterial agents and significant reservoirs of medicinal compounds for treating diverse illnesses.

Key-Words: - *Staphylococcus aureus*, *Escherichia coli*, *Raphia vinifera*, *Elaeis guineensis* seeds, Methanol, N-hexane

Received: June 29, 2022. Revised: September 11, 2023. Accepted: October 16, 2023. Published: November 14, 2023.

1 Introduction

Microbial resistance to antibiotics has been observed mostly in certain developing nations. Enhancing our understanding of the medicinal properties of plants could provide valuable scientific and medical knowledge for building a strategic and successful approach to combating drug-resistant bacteria. Plants are a highly abundant source of medications used in traditional medicine, modern medicine, nutraceuticals, food supplements, folk medicine, pharmaceutical intermediates, and chemical compounds for synthetic drugs [1].

The utilization of plants and botanical substances for therapeutic purposes can be traced back to the inception of human civilization. Medicinal plants possess significant economic significance globally. We have been endowed with a bountiful botanical abundance by nature, consisting of a wide array of plant species that thrive in various regions of our country [2].

The therapeutic properties of certain plants are attributed to the presence of certain bioactive compounds found in different sections of these plants and their extracts. The active compounds referred to as phytochemicals have the ability to have unique physiological effects on target organisms [3, 4]. Plants synthesize a wide array of phytochemical compounds, which are secondary metabolites [5]. These secondary metabolites have distinct mechanisms of action and are utilized either directly or indirectly in the pharmaceutical business [6].

The advent of antibiotics led to a substantial decrease in the incidence of illness and death caused by infections. However, the improper use and mishandling of these antibiotics by patients has given rise to a more serious issue: the growth of drug-resistant organisms. Currently, this issue is causing significant apprehension in the field of medical practice and has introduced a fresh perspective to the challenge of treating infections. This issue is further exacerbated in underdeveloped nations where the regulation of antibiotic sales is inadequate [7].

Regrettably, the exponential growth of the human population has rendered contemporary health facilities incapable of adequately meeting global health needs. Consequently, there is an increased reliance on the use of natural herbal health therapies. The current challenges related to the use of antibiotics include the rise in the number of pathogenic bacteria strains that are resistant to multiple drugs, such as methicillin-resistant *Staphylococcus aureus, Helicobacter pylori*, and MDR *Klebsiela pneumoniae*. This has led to a renewed interest in plants that have antimicrobial properties [8, 9].

The presence of antimicrobial resistance in pathogens might lead to higher expenses and more problems with procedures and treatments [10]. As stated by Sani et al. [11], the development of resistance in microorganisms to antibiotics that were previously effective can be attributed to the acquisition of resistant genes from other species or strains within the same culture. According to Emine et al. [12], overcrowding and subpar infection control procedures in hospitals facilitate the spread of this resistance. Aisha et al. [13] found that there is an increasing prevalence of bacterial isolates in Nigeria that exhibit widespread resistance to β-lactam antibiotics and flouroquinolones.

The evolution of antibiotic-resistant bacteria is often attributed to poor antimicrobial treatment, which refers to the ineffective treatment of infection. Contributing factors to insufficient antimicrobial treatment in hospital patients with infections include pre-hospital antibiotic usage, prolonged antibiotic exposure, the exorbitant cost of highly effective antibiotics, improper utilization of broad-spectrum antibiotics, and the use of invasive medical devices [12].

Various plants have been utilized for medicinal purposes in the management and treatment of multiple illnesses. *Raphia vinifera* is an exemplar of such botanical specimens. *Raphia vinifera*, commonly known as bamboo palm, is a plant with medicinal and nutritional properties. It is found in large quantities in swamps and other moist areas along the creeks of the Niger Delta and other deltaic states in Nigeria. It is also distributed from Benin to the Democratic Republic of the Congo [14]. The palm tree is a solitary, hapazanthic plant with a thick, unbranched stem that reaches a height of 5 meters. It belongs to the family "Arecaceae" [15].

Evidence has shown that many components of the *Raphia vinifera* plant possess therapeutic qualities. Specifically, the root of the plant is prepared and used as a remedy for toothache. The fibers extracted from the leaf sheath possess properties that can alleviate digestive issues, while the fermented fluid derived from the inflorescence acts as a natural laxative. The fruit pulp is used as a remedy for dysentery, while an infusion of the fruit effectively stops hemorrhaging. In West Cameroon, the apical bud of *R. vinifera* is brewed to create a medicinal liquid for treating gonorrhoea and other illnesses of the genital and urinary systems. Additionally, the leaf of this plant is employed to counteract the effects of poison and combat numerous sexually transmitted diseases [16]. The oily mesocarp of the fruit is consumed and can be subjected to fermentation to produce a potent beverage for ceremonial purposes or to enhance sexual prowess in accordance with traditional medicine [16].

The African oil palm, scientifically known as *Elaeis guineensis* Jacquin, yields two distinct types of oil: palm oil and palm kernel oil [17]. The pericarp is composed of three distinct layers: the exocarp, which is the outermost skin; the mesocarp, which is the outer pulp that contains palm oil; and the endocarp, which is a hard shell that encloses the kernel or endosperm. The kernel, or endosperm, contains oil, known as kernel oil. [18]. This study sought to assess the impacts of methanol and n-hexane extracts derived from the mesocarp of *Raphia vinifera* fruit and *Elaeis guineensis* seeds on *Staphylococcus aureus* and *Escherichia coli.*

2 Materials and Methods

Collection of *Raphia vinifera* fruit mesocarp and *Elaeis guineensis* seed Samples

The fruit mesocarp of *Raphia vinifera* and the seeds of the *Elaeis guineensis* seeds were acquired from Ekowe market in Southern Ijaw Local Government Area, Bayelsa State. The specimens were desiccated for a duration of three days under the sun and then pulverized using a grinder machine. The resulting powder was then stored in an airtight container for laboratory analysis.

Methods

Plant Sample Extraction

The solvents used for extraction were n-hexane and methanol. A beaker containing approximately 10 grams of the *Raphia vinifera* fruit mesocarp and *Elaeis guineensis* seed samples was filled with 25 milliliters of n-hexane and thoroughly mixed using vortexing. The mixture was thereafter subjected to centrifugation at a speed of 3000 revolutions per minute for a duration of 10 minutes. The liquid portion, known as the supernatant, was obtained and moved to a sealed test tube using the process of filtration. The supernatant obtained was dried using a moderate nitrogen stream and then reconstituted in 10 ml of dimethyl sulfoxide. The mixture was well mixed by vortexing. The identical method was replicated with the methanol solvent [19].

Preparation of Dried Filter Paper Discs

Discs were prepared using Whatman filter paper no. 102. A perforator was used to create a hole with a diameter of approximately 5 mm. These were inserted in a petri plate after sterilization in an autoclave.

Raphia vinifera fruit mesocarp and *Elaeis guineensis* seeds Extract Disc Placement

A plant disc with a concentration of 3 ml (3 µl), along with Raphia vinifera fruit mesocarp and palm kernel seeds, was prepared using filter paper. The prepared disc was then carefully placed on the plates using sterile forceps. A single aseptic antibiotic disc was carefully positioned onto the agar plate surface using a forceps. The forceps were sterilized by submerging them in alcohol before being placed on another antibiotic disc. The disc was thereafter delicately compressed using forceps to establish full contact with the agar surface and positioned at a distance from the plate's edge for convenient measurement. After positioning all the discs, the plates were turned upside down and placed in a 37°C incubator for a duration of 24 hours.

Culture Media Preparation

The media utilized were produced in accordance with the instructions provided by the manufacturer. 3.4 grams of nutritional agar were weighed to produce a 500-gram pack for three plates. A volume of one milliliter of a water sample was applied to the solid nutrient agar surface and then evenly distributed around the medium using a glass spreader. Following the spread plate procedure, the culture was subsequently incubated in an inverted orientation in the incubator for 24 hours at a temperature of 37 ^{o}C.

Eosin Methylene Blue Agar (EMB): A 500-gram pack of EMB was prepared by weighing 4.3 g for 3 plates. A water sample was introduced or inoculated onto the medium, and the plates were incubated in an inverted position at 37 ^{o}C for 48 hours. The isolates were then subcultured in buffered peptone water and incubated at 37 ^{o}C for 24 hours to ensure that they were in their exponential growth phase. The isolated specimen obtained from the wide was introduced onto a solid nutritional agar medium and evenly distributed using the spread plate technique. The antimicrobial susceptibility test was conducted by placing the disc on the plate. The plates were subjected to a 24-hour incubation period at a temperature of 37 ^{o}C in order to observe the response of the plants to the antibiotic disc [20].

Gram Staining Method

Approximately 5 drops of crystal violet dye were applied to the fixed culture and allowed to sit for 60 seconds. The stain was decanted, and the surplus stain was delicately washed away using a steady flow of water. Approximately 5 drops of the iodine solution were applied to the smear, ensuring that the fixed culture was fully covered. The solution was left undisturbed for 30 seconds. The iodine solution was decanted, and the slides were rinsed with flowing water, removing any excess water by shaking them off the surface. A small amount of decolorizer was added to facilitate the downward flow of the solution on the slide. After a duration of 5 seconds, the substance was washed away using water, and the process was halted after the solvent ceased to exhibit any coloration while passing over the slide. The specimen was stained with 5 drops of safranin solution and left for 20 seconds. The safranin solution was rinsed with water and then blotted with bibulous paper to eliminate any residual water. Subsequently, the slide was scrutinized using a microscope [21].

3 Results

Antibacterial Susceptibility

The susceptibility of the extracts from *Raphia vinifera* fruit mesocarp and palm kernel seeds, which have different levels of antibacterial activity against Gram-positive species (*Staphylococcus aureus*) and Gram-negative species (*Escherichia coli*), was tested using methanol and n-hexane extracts. The results are presented in Tables 1 and 2 below. Table 3 displays the biochemical traits of the test organisms, specifically *S. aureus* and *E. coli*.

Table 1: Antibacterial activity of *Raphia vinifera* fruit mesocarp and *Elaeis guineensis* seeds in against *Staphylococcus aureus*

S/N	Plant Samples	Extraction solvent	MIC	Susceptibility in zone of	Interpretation

				inhibition	
1	*Elaeis guineensis* seeds	n-hexane	0.5ml	15mm	Sensitive
2	*Elaeis guineensis* seeds	Methanol	0.5ml	11mm	Sensitive
3	*Raphia vinifera* fruit	n- hexane	0.5ml	9mm	Sensitive
4	*Raphia vinifera* fruit	Methanol	0.5ml	8mm	Sensitive

Data are given as mean of the diameter of zone of inhibition (mm) of triplicate determination

Table 2: Antibacterial activity of *Raphia vinifera* fruit mesocarp and *Elaeis guineensis* seeds in against *Escherichia coli*

S/N	**Plant Samples**	**Extraction solvent**	**MIC**	**Susceptibility in zone of inhibition**	**Interpretation**
1	*Elaeis guineensis* seeds	n-hexane	0.5ml	15mm	Sensitive
2	*Elaeis guineensis* seeds	Methanol	0.5ml	5mm	Sensitive
3	*Raphia vinifera* fruit	n- hexane	0.5ml	12mm	Sensitive
4	*Raphia vinifera* fruit	Methanol	0.5ml	9mm	Sensitive

Data are given as mean of the diameter of zone of inhibition (mm) of triplicate determination

Table 3: Biochemical Characteristics of *Staphylococcus aureus* and *Escherichia coli*

Microorganism	*Escherichia coli*	*Staphylococcus aureus*
Cell morphology	Rod	Coccus
Colony shape	Spindle	Circular
Gram staining reaction	Positive	Negative
Biochemical test		
Oxidase	Negative	Negative
Catalase	Positive	Positive
Methyl red	Positive	Positive
V.P	Negative	Positive
Indole	Positive	Negative
Citrate	Negative	Positive
Urease	Negative	Positive

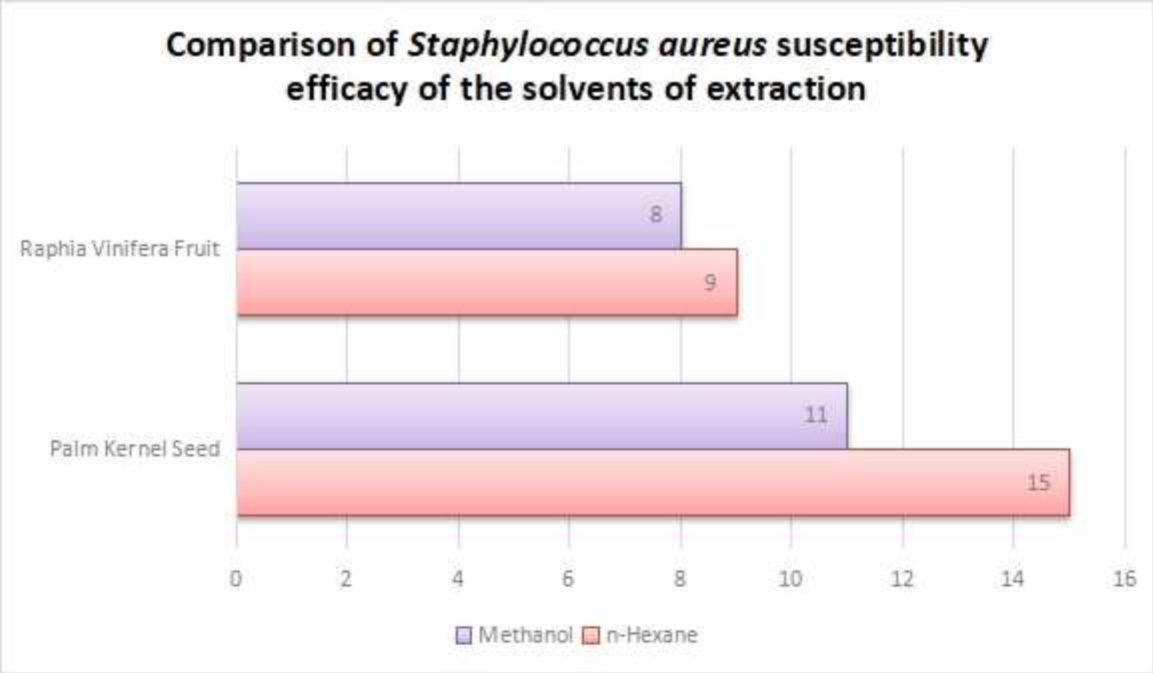

Figure 4.1: Comparison of *Staphylococcus aureus* susceptibility efficacy of the solvents of extraction

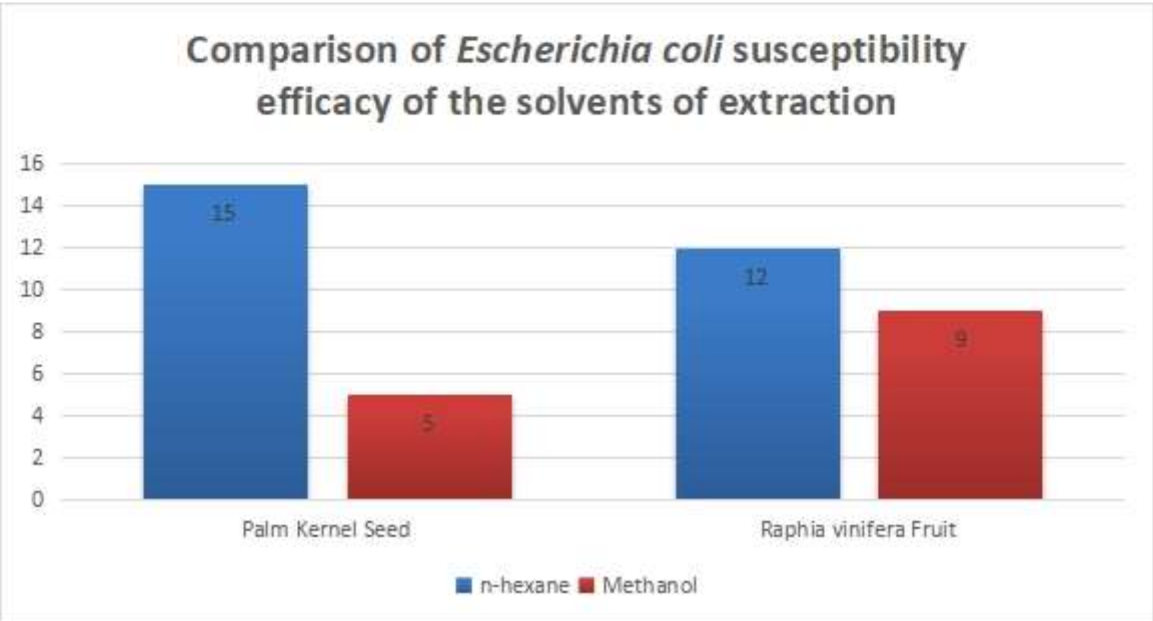

Figure 2: Comparison of *Escherichia coli* susceptibility efficacy of the solvents of extraction

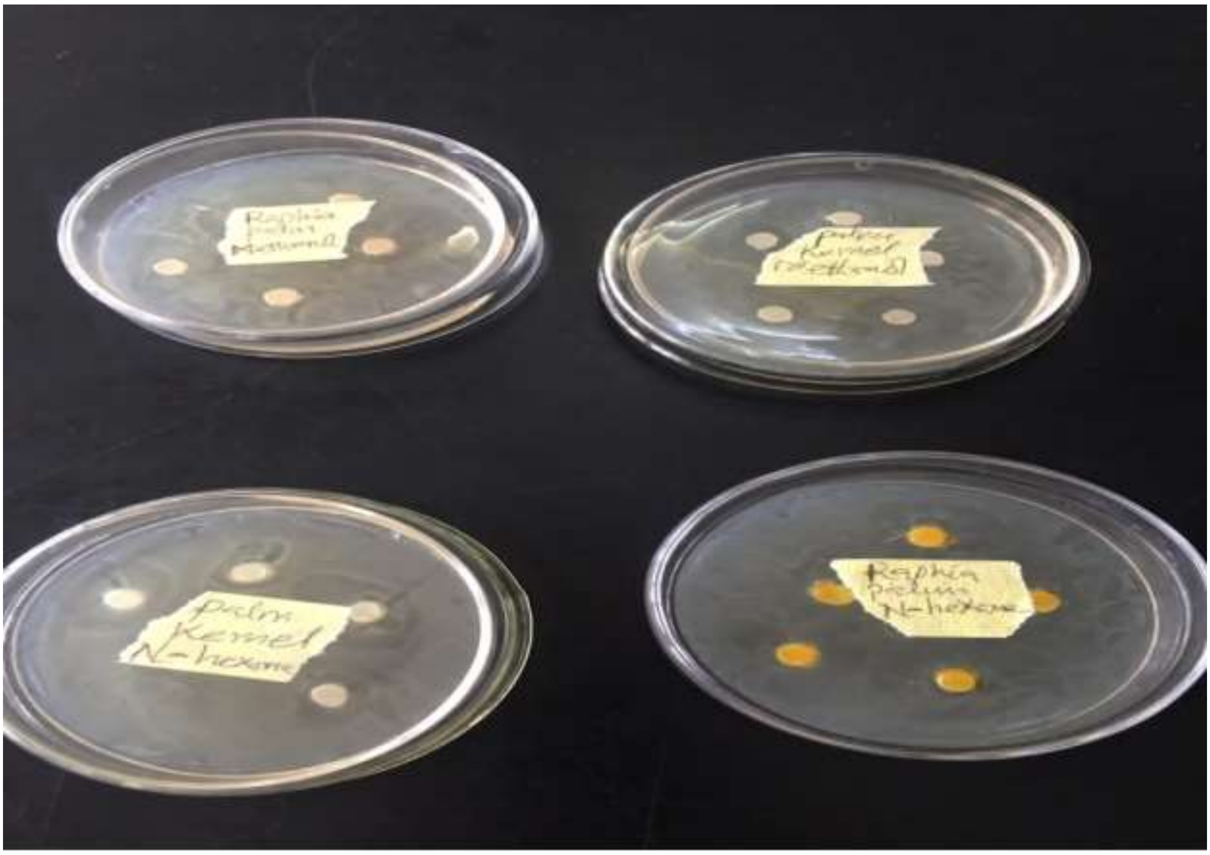

Plate 1: Antibacterial activities of methanol and n-hexane extracts of *Stapholococcus aureus* and *Escherichia coli*

4 Discussion

Recently, there has been a growing quest for novel antimicrobial agents in response to their limited efficacy, adverse effects, and potential medication interactions with patients. Additionally, the antibacterial and antifungal agents used appear to be resistant to infections, as the lesion keeps recurring. Consequently, this finding has spurred the quest for novel antimicrobial agents derived from natural substances. The susceptibility of extracts from *Raphia vinifera* fruit mesocarp and palm kernel seeds, with different levels of antibacterial activity against *Staphylococcus aureus* (a Gram-positive species) and *Escherichia coli* (a Gram-negative species), was tested using methanol and n-hexane extracts. The results are presented in Tables 1 and 2.

The average diameter of the zones of inhibition caused by the n-hexane extract of palm kernel seed and *Raphia vinifera* fruit mesocarp was 15 mm and 9 mm, respectively, for *S. aureus*. The diameter of inhibition zones for *S. aureus* was 11 mm and 8 mm for the methanol extract of *Elaeis guineensis* seed and *Raphia vinifera* fruit mesocarp, respectively. The n-hexane extract of palm kernel seed and *Raphia vinifera* fruit mesocarp exhibited inhibition zones with mean diameters of 15 mm and 12 mm, respectively, against *Escherichia coli*. Similarly, the methanol extract of *Elaeis guineensis* seeds and *Raphia vinifera* fruit mesocarp showed inhibition zones with mean diameters of 5 mm and 9 mm, respectively, against *Escherichia coli*. This finding is consistent with studies by Ekwenye and Ijeomah [17] and Ugbogu and Akukwe [22].

Among the solvents utilized for extraction in this study, n-hexane exhibited the highest antibacterial activity compared to methanol extracts against all test organisms (*S. aureus and E. coli*), as depicted in Figures 1 and 2. This finding contradicts the earlier findings of Aarti and Astha [23], Ogidi et al. [24, 25], Madhavi et al. [26], and Ogidi et al. [27], who discovered that the methanol extract exhibited greater sensitivity against bacteria and fungi compared to other extracts.

5 Conclusion

The antibacterial findings from this investigation indicate that the n-hexane and methanol extracts of the palm kernel seed and *Raphia vinifera* fruit mesocarp have the potential to be utilized in the creation of more effective and powerful antibacterial agents. Nevertheless, the n-hexane extract derived from both palm kernel seed and *Raphia vinifera* fruit mesocarp exhibited greater efficacy in terms of broad-spectrum antibacterial activity against S. aureus and E. coli. This finding provides further support for the potential therapeutic application of palm kernel seed and *Raphia vinifera* fruit mesocarp in traditional medicine.

Additional, for further study, *in vivo* investigations are necessary to determine the impact of palm kernel seed and *Raphia vinifera* fruit mesocarp extracts on skin and soft tissue infections, namely abscesses, furuncles, and cellulitis. This study would have a significant influence on the prospective treatment of these diseases and other associated conditions. Conservation of the species *Raphia vinifera* and *Elaeis guineensis* trees is necessary. The majority of the colossal foliage species have nearly disappeared from numerous environments where they were once abundant. Encouraging the consumption of *Elaeis guineensis* seeds and *Raphia vinifera* fruit mesocarp is recommended due to their potent antibacterial properties.

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Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

Odangowei Inetiminebi Ogidi: was responsible for the conceptualization, validation, writing - original draft and actively participated in all the publication stages of this manuscript.

Henshaw Ememobong Carbom: was responsible for laboratory work, reviewing, and editing the manuscript and actively participated in all the publication stages of this manuscript.

Akpofiniere Monica Tawariowei: was responsible for reviewing and editing the manuscript and actively participated in all the publication stages of this manuscript.

Sources of Funding for Research Presented in a Scientific Article or Scientific Article Itself
No funding was received for conducting this study.

Conflict of Interest
The authors have no conflicts of interest to declare that are relevant to the content of this article.