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Antimicrobial Screening and Phytochemical Analysis of *Elaeis guineensis* (Ewe Igi Ope) against *Salmonella* Strains

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OEA and SIA designed the study and wrote the protocol. Authors OEA and SIA wrote the first draft of the manuscript. Author FNO managed the literature searches and author AGO performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The study aimed at determining the *in vitro* anti-salmonella effect of *Elaeis guineensis* on *Salmonella* species isolated from clinically suspected typhoid fever patients in FUTA, Nigeria.
Study Design: The study evaluated the prospective use of *Elaeis guineensis* as an alternative to conventional drugs in the treatment of salmonellosis and gastroenteritis.
Place and Duration of Study: Federal University of Technology, Akure (FUTA) Health Centre was used for the study which Investigation was done during June to September, 2015.
Methodology: Two hundred (200) blood samples were collected from clinically suspected typhoid fever patients attending FUTA Health Centre in Akure, Ondo State, Nigeria. *Salmonella* Typhi and *Salmonella* Typhimurium were isolated from the blood samples using selective media. The identities of the isolates were verified using conventional microbiological techniques. *Elaeis guineensis* leaves were collected from Federal University of Technology Akure (FUTA) Teaching and Research Farm, and the plant's identity was authenticated using standard manuals at Crop Science and Pest

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Department, FUTA. The antibacterial effect of methanolic, acetone and hexane extracts of the plant leaves on *Salmonella* Typhi and *Salmonella* Typhimurium were evaluated. Quantitative and qualitative phytochemical screening were performed on the leaf extracts. Antibiotics susceptibility profile of the isolates was also assessed using commercially available antibiotics.

Results: The highest zone of inhibition (21.67±1.20 mm) was observed with the methanolic extract at concentration of 100 mg/mL for *Salmonella* Typhimurium. No zone of inhibition was observed for hexane at the concentration of 6.25 and 12.5 (mg/mL). Highest zone of inhibition (17.00±0.58 mm) was observed with the methanolic extract at concentration of 100 mg/ml for *Salmonella* Typhi and the least (0.33±0.33 mm) was observed with the hexane extract at a concentration of 25 mg/mL. Phytochemical analysis of the extracts revealed the presence of many secondary metabolites including alkaloids, flavonoids, tannins and saponins. Flavonoids was highest (118.03±0.29) in methanol extract and least (8.00±0.00) in hexane extract. Same trend was observed for other phytochemicals present. As for the conventional antibiotics, *Salmonella* Typhi had the highest zone of inhibition (23.00 mm) with ciprofloxacin and the least (1.33 mm) with augmentin, *Salmonella* Typhimurium recorded the highest zone of inhibition (23 mm) with ciprofloxacin and pefloxacin, while the lowest (4 mm) was recorded with tetracycline.

Conclusion: These findings showed that *Elaeis guineensis* is a potential source of reliable phytotherapy in combating salmonellosis and gastroenteritis.

Keywords: Elaeis guineensis; antimicrobial; typhoid fever; phytochemicals; Salmonella Typhimurium; Salmonella Typhi.

1. INTRODUCTION

For several decades, nature has constituted a repository of medicinal plants; and a huge number of modern drugs have been isolated from various natural sources including plants [1]. The traditional system of medicines which evolved over 60,000 years ago incorporates usage of plants and herbs for therapeutic purposes. The use of herbal medicine has enjoyed wide acceptability, being very encouraging and having a strong potential to be widely used in traditional health care around the globe [2]. Due to emerging drug resistant bacterial strains, many bacterial diseases do not respond to prescribed antibiotic treatments options and thus infectious diseases caused by various bacterial pathogens are flaring up and becoming uncontrollable [3].

The emergence of drug resistance as well as modern developments in therapeutic field have revived the use of traditional medicines and the plant-based remedies as potential source of therapeutic aids in health systems all over the world for both humans and animals [4,5]. Resistance of microbes to commonly used antibiotics has enhanced morbidity and mortality, and has triggered the search for new drugs [6]. These approaches include a combination of immunological, biotechnological and molecular methods [7,8]. Out of all these alternative and novel therapies, herbal therapy is gaining much momentum and particularly attaining wide popularity and speeding up with a good pace [9,10]. They possess multi-dimensional health benefits and show high utility in alternative and complementary medicinal systems as effective prophylactic and therapeutic regimens.

The African oil palm (*Elaeis guineensis*-Ewe Igi Ope) is one of the plants that are central to the lives of traditional societies in West Africa. It is a monocotyledon perennial tree crop of the order Spodiciflorae and family Palmae, grouped under the Cocoineae tribe [11]. All parts of this plant are useful. The fruit mesocarp oil and palm kernel oil are administered as a poison antidote, and are also used externally with several other herbs as a lotion to treat skin diseases. Palm kernel oil is applied to convulsing children to regulate their body temperature. Oil palm is a folk remedy for cancer, headaches, and rheumatism. [11].

Salmonella is a primary cause of food poisoning worldwide. The Center for Disease Control and Prevention [CDC] reports that approximately 1.4 million cases of salmonellosis were annually reported in the United States [12]. The World Health Organisation (WHO) estimated an annual infection rate of 21.6 million and approximate death rate of 600, 000 with the highest percentage in Africa and Asia. In developing countries, typhoid is more severe due to poor hygiene, indiscriminate use of antibiotics, and a rapid rise in multidrug resistance. Resistance to the first line drugs, chloramphenicol,

ciprofloxacin, and amoxicillin, in the course of salmonellosis management has been reported [13].

The present study was thus designed to evaluate the antibacterial efficacy of *E. guineensis* leaf, extract on multidrug resistant *Salmonella* Typhi and *S*. Typhimurium of blood origin, as well as with a view to proffering better treatment alternatives for infections caused by them.

2. MATERIALS AND METHODS

2.1 Ethical Clearance

Informed consent was sought in clinically suspected cases and approval for the study (reference number AD.4293/48) was obtained from the Ethics Committee of the Ondo State Ministry of Health. Confidentiality was maintained in accordance with standards of medical practice.

2.2 Collection of Blood Samples

Blood samples used were collected from Federal University of Technology, Akure Health Centre. The blood samples were kept in Ethylene Diamine Tetra Acetic (EDTA) bottles and transferred to the laboratory for analysis.

2.2.1 Bacteria isolation

The *S.* Typhi and *S*. Typhimurium isolates tested were isolated from Blood samples of presumptive typhoid fever patients attending the Health Center. The Blood samples collected from the patients were inoculated onto Brain Heart Infusion Broth in the ratio of 2:20 that is 2 mL of blood into 20 mL of Brain Heart Infusion Broth, and incubated at 37℃ for 48 hours. After incubation, a loopful of the culture was streaked on *Salmonella- Shigella* agar (SSA) and incubated at 37℃ for 24 hours.

2.2.2 Sub-culturing of isolates

A colony of the grown organism was sub-cultured on a fresh *Salmonella- Shigella* agar plate and incubated for 24 hours at 37℃. This was done to get a pure and distinct colony strain on plate.

2.2.3 Identification of the bacterial isolates

The bacterial isolates were identified using their colony morphological characteristics. The appearance of each colony on the agar media and characteristics such as shape, edge, colour, elevation and texture were observed as described by Olutiola et al. [14]. The isolates were thereafter subjected to relevant biochemical tests and identified using the taxonomic scheme of Bergey's Manual of Determinative Bacteriology.

2.3 Collection and Authentication of Plant Materials

The plant leaf used (*Elaeis guineensis*) was collected from the Federal University of Technology Akure Obakekere farm, Ondo State. The plant was identified by a taxonomist in Crop Science and Pest Department (CSP) of the Federal University of Technology, Akure (FUTA) and reference was made to the handbook of West African weeds. Collected plant material was air-dried under shade at room temperature, ground with an electric grinder into fine powder and stored in airtight container.

2.3.1 Preparation of plant extracts

Three solvents: hexane, acetone, and methanol were used to soak 50 g of leaf powder in a separate container and left for 72 hours. The extracts were filtered off using muslin cloth and filter paper and thereafter concentrated to dryness in rotary pressure evaporator and preserved for antimicrobial study on the isolates. The 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.50 mg/mL concentrations of the extract were prepared by dissolving 500 mg, 250 mg, 125 mg and 62.5 mg of the leaf extract respectively into different bottles containing 500 mL of 20% tween each.

2.3.2 Determination of the antibacterial assay of the plant leaf extract

The antibacterial activity of the plant leaf extract was determined using the agar well diffusion method described by Irobi et al. [15]. Muller Hinton agar was prepared according to the manufacturer's instruction and allowed to cool to about 40-50℃. The freshly prepared and cooled media was poured into Petri dishes and allowed to solidify at room temperature. About 0.2 mL of the standardized test inoculum was evenly spread on the surface of the solidified media using a sterile swab stick. Five equidistant wells of 5 mm in diameter each were made on the seeded agar plate using a sterile cork borer; a 5mL of tween 20 was used in re-constituting the extracts after which the extract with concentrations ranging from 6.25-100 mg/ml

were introduced into the bored holes. The plates were incubated at 37℃ for 24 hrs. The resulting zones of inhibition were measured using a ruler calibrated in millimetres. The average of the three readings was taken to be zone of inhibition around the bacteria isolate at that particular concentration.

2.4 Determination of the Antibiotics Sensitivity Profile of the Bacterial Isolates

The antibiotics sensitivity test was carried out by using commercial gram negative antibiotics disc containing augmentin 30 µg, ceftriazone 30 µg, nitrofurantoin 200 µg, gentamicin 10 µg, cotrimoxazole 25 µg, ofloxacin 5 µg, amoxicillin 25 µg, ciprofloxacin 10 µg, tetracycline 30 µg and pefloxacin 5 µg. Muller Hinton agar was prepared accordingly and allowed to solidify. Test organisms were streaked on the agar, left for some minutes for adherence of the organism to the surface of the agar, and antibiotics disc was placed firmly with a sterile forceps on the solidified agar. The plates were thereafter incubated at 37℃ for 24 hours.

2.5 Phytochemical Screening of *Elaeis guineensis* Extract

The quantitative and qualitative phytochemical screening of the plant extract was done according to the methods of Ayoola et al. [16-20].

2.6 Statistical Analysis

All experiments were carried out in triplicates. Data obtained were analysed using one way Analysis of Variance (ANOVA) and treatment means were compared using New Duncan's Multiple Range Test. Differences were considered significant at $P<0.05$.

3. RESULTS

3.1 Antibacterial Activity of the Plant Extract on the Test Bacteria

Out of two hundred (200) blood samples, ten (10) were positive for the presence of Salmonella with three S. Typhi and seven *S.* Typhimurium isolates. The morphological characterization of the test bacteria showed that the isolates were *Salmonella* Typhi and *Salmonella* Typhimurium while the cultural morphological and microscopic tests attested that they were in pure forms. The antibacterial assay of the crude extract of *Elaeis guineensis* leaf revealed that the test bacteria were susceptible to the extracts of different solvents at different concentrations. With methanol extracts of the leaf (Table 1), highest activity (17.00±058 mm) was recorded against *S.* Typhi at 100 mg/mL while lowest inhibition was recorded against the bacterium at 6.25 mg/mL. Acetone extracts showed mild activity (1.33±0.33 mm) against *S.* Typhi, however, it demonstrated good activity at higher concentrations from 25 mg/ml upward with zones of inhibition ranging from 4.00±0.00 mm to 10.00±0.58 mm. With hexane extract, the test was not sensitive at lower concentrations of 6.25 mg/mL and 12.5 mg/mL whereas very weak activity was observed at higher concentrations (25 mg/mL upward) with inhibition ranging from 0.33±0.33 mm to 1.33±0.33 mm.

Table 2 shows the antibacterial activity of the crude extracts of the leaf of *Elaeis guineensis* on *S.* Typhimurium. Highest zone of inhibition (21.67±1.20 mm) against the test isolate was observed at 100 mg/mL while lowest inhibition (8.33±0.33 mm) was recorded at 6.25 mg/mL. Acetone extract demonstrated mild activity against *S.* Typhimurium with zone of inhibition ranging from 1.67±0.33 mm to 6.33±0.33 mm corresponding with 12.5 mg/mL and 100 mg/mL. With hexane extract, at concentration up to 25 mg/ml the bacterium (*S.* Typhimurium) was not susceptible. Even at higher concentration (50 and 100 mg/mL) very weak activity was still observed (0.67±0.33 mm and 1.33±0.33 mm respectively) against the isolate.

3.2 Phytochemical Screening Results

The results of the qualitative analysis of the leaf extracts of *Elaeis guineensis* showed the presence of alkaloid, saponin, flavonoid, glycoside and terpenoid (Table 3). The quantitative phytochemicals assessments (Table 4) revealed that 4.44±0.08 mg/g, 5.15±0.08 mg/g and 7.97±0.04 mg/mL contents of alkaloids were present in n-hexane, methanolic and acetone extracts of *E. guineensis* respectively. For the glycoside concentrations, 8.00±0.00 mg/g was found in hexane extract, 11.81±0.55 mg/g and 13.05±0.19 mg/g in acetone and methanolic extracts respectively. Tannin contents were observed to be 85.37±0.14 mg/g, 23.38±0.54 mg/g and 68.42±0.05 mg/g correspondingly in methanol, n-hexane and acetone extracts. Highest flavonoids content was obtained in

methanolic extract (118.03±0.29 mg/mL) while the least was recorded in n-hexane (5.24±0.12 mg/g). A 23.00±0.58 mm and 17.33±0.67 mm zone of inhibition was recorded against the isolates respectively.

The results of the qualitative analysis of the leaf extracts of *Elaeis guineensis* (Table 3) showed the presence of alkaloid, saponin, flavonoid, glycoside and terpenoid (Table 3). The quantitative phytochemicals assessments (Table 4) revealed that 4.44±0.08 mg/g, 5.15±0.08 mg/g and 7.97±0.04 mg/mL contents of alkaloids were present in n-hexane, methanolic and acetone extracts of *E. guineensis* respectively. For the glycoside concentrations, 8.00±0.00 mg/g was found in n-hexane extract, 11.81±0.55 mg/g and 13.05±0.19 mg/g in acetone and methanolic extracts respectively. Tannin contents were observed to be 85.37±0.14 mg/g, 23.38±0.54 mg/g and 68.42±0.05 mg/g correspondingly in with methanol, n-hexane and acetone extracts. Highest flavonoids content was obtained in methanolic extract (118.03±0.29 mg/mL) while the least was recorded in hexane (5.24±0.12 mg/g).

3.3 Antibiotics Susceptibility Profile of the Isolates

The antibiotics susceptibility profile of the two test bacteria are shown in Fig. 1. Both *S.* Typhi and *S.* Typhimurium were susceptible to ciprofloxacin with 23.00±0.58 mm and 17.33±0.67 mm zones of inhibition recorded against the isolates respectively. Pefloxacin and ofloxacin inhibited the growth of *S.* Typhimurium with 23.00±0.58 mm and 22.00±0.58 mm inhibition zones, while *S.* Typhi was resistant to the same antibiotics with inhibition zones of 4.67±0.33 mm and 14.00±0.58 mm respectively. Both isolates were however resistant to tetracycline, ceftriaxone, nitrofuranton and cotrimoxazole. The zones of inhibition were interpreted using [21].

Table 1. Antibacterial activity of *Elaeis guineensis* leaf extract on *S.* Typhi

Concentration (mg/mL)	Methanol	Acetone	Hexane
6.25	5.67^{a}±0.33	1.33^{a}±0.33	0.00^{a}±0.00
12.5	8.34^{b}±0.33	4.00^{b}±0.00	0.00^{a}±0.00
25	12.00^{c}±0.58	5.33^{c}±0.33	0.33^{a}±0.33
50	14.33^{d}±0.33	7.33^{d}±0.33	0.67^{ab}±0.33
100	17.00^{e}±0.58	10.00^{e}±0.58	1.33^{b}±0.33

Values with the same alphabet along the column are not significantly (P<0.05) different

Table 2. Antibacterial activity of *Elaeis guineensis* leaf extract on *S.* Typhimurium

Concentration (mg/mL)	Zone of inhibition (mm)		
	Methanol	Acetone	Hexane
6.25	8.33^{a}±0.33	0.00^{a}±0.00	0.00^{a}±0.00
12.5	11.33^{b}±0.67	1.67^{b}±0.33	0.00^{a}±0.00
25	14.67^{c}±0.67	2.67^{c}±0.33	0.00^{a}±0.00
50	18.67^{d}±0.67	4.33^{d}±0.33	0.67^{ab}±0.33
100	21.67^{e}±1.20	6.33^{e}±0.33	1.33^{b}±0.33

Values with the same alphabet along the column are not significantly (P<0.05) different

Table3. Qualitative phytochemical of analysis of *Elaeis guineensis* leaf extract

Phytochemicals	Methanol	Acetone	N-Hexane
Saponin	+	+	+
Tannin	+	+	+
Flavonoid	+	+	+
Steroid	-	-	-
Terpenoid	+	+	+
Alkaloid	+	+	+
Glycoside	+	+	+

Antibiotics

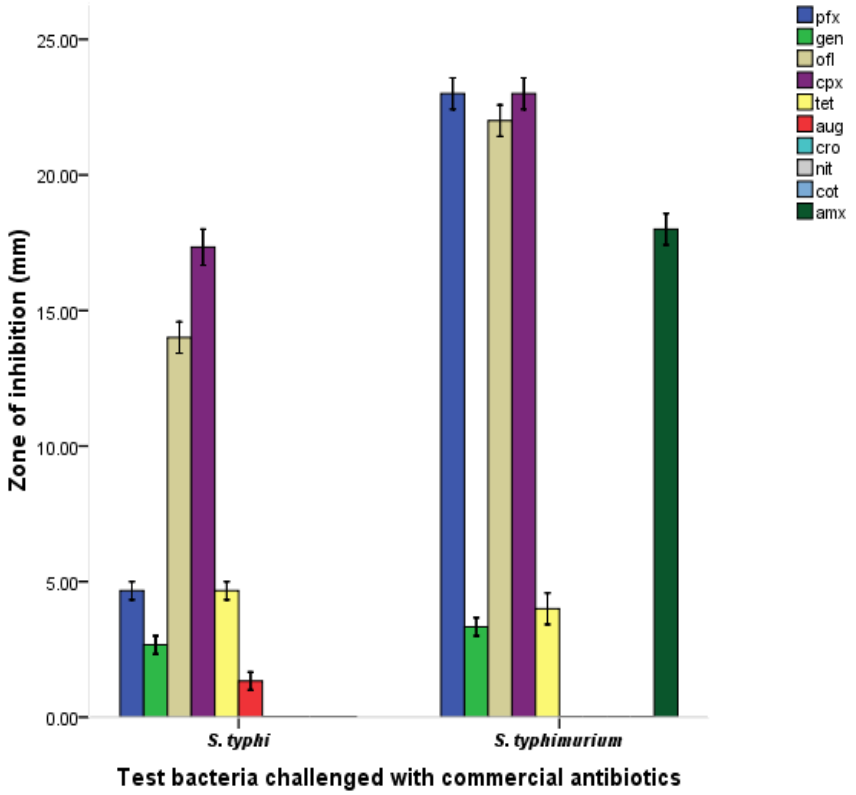

Fig. 1. Antibiogram of *S.* Typhi and *S.* Typhimurium to commercial antibiotics

Table 4. Quantitative Phytochemical Analysis of *Elaeis guineensis*

Phytochemicals	Methanol (mg/g)	Hexane (mg/g)	Acetone (mg/g)
Glycoside	13.05^{b}±0.19	8.00^{b}±0.00	11.81^{b}±0.55
Terpenoid	41.82^{c}±0.18	14.87^{c}±0.13	34.76^{c}±0.49
Tannin	85.37^{d}±0.14	23.38^{d}±0.54	68.42^{e}±0.05
Saponin	108.24^{e}±0.00	57.17^{f}±0.35	58.35^{d}±0.11
Flavonoid	118.03^{f}±0.29	25.24^{e}±0.12	70.97^{f}±0.49
Alkaloid	5.15^{a}±0.08	4.44^{a}±0.08	7.97^{a}±0.04

4. DISCUSSION

Plants contain various biologically active compounds which have proven potential for development as medical agent. *Elaeis guineensis* is one of the herbs that finds application in many aspects of human life especially with respect to its health benefits. In this study, methanolic leaves extracts of *E. guineensis* showed comparatively higher antibacterial activity against *Salmonella* Typhi and *S.* Typhimurium than the acetone and hexane extracts. These results agree with those of [22] that evaluated the antibacterial activity of *E. guineensis* on some selected bacterial pathogens of clinical source. Tannins and flavonoids have been implicated as the main phenolic compound in most plants, and serve as supplements in combating diseases of bacterial origin [23]. Reports by [24] revealed that ethanolic extract of *E. guineensis* leaf was potent against some human pathogenic bacteria of which prominent among which are *S.* Typhi and *S.* Typhimurium. Phytochemical screening showed the presence of saponin, terpenoid, flavonoid and tannins in the *E. guineensis* methanolic leaves extracts. Previous report by [25] had shown that the methanolic leaf extract of

oil palm comprised tannins, alkaloids, reducing sugars, steroids, saponins, terpenoids and flavonoids in bountiful amounts. Tannins and flavonoid are the main phenolic compounds in most plants and can serve as supplements in fighting against various diseases [26]. The quantification of the amounts of the phytochemicals present in oil palm leaves confirmed the presence of flavonoid and tannin as the main phytochemical constituents in them. Based on the study, the high content of phenolic, tannin and flavonoid compounds may have contributed to its antibacterial activity. Indeed, many antimicrobial activities in plants have been reported to be a result of the bioactive compounds present in plants, such as alkaloids, saponins, tannins, anthraquinones, steroids, flavonoids, terpenoids and so on [27,28]. Saponins and tannins which are known antimicrobial agents have been reported to prevent the development of microorganisms by precipitating microbial protein and thus making nutritional proteins unavailable for them [29]. The quantitative phytochemical tests showed that the highest tannin and saponin contents were observed in methanolic extract. This is in consonance with the work of [30] who equally observed high contents of these phytochemicals. Moreover, flavonoids are well documented to have important effects on various biological systems. Flavonoids have been referred to as "nature's biological response modifiers" because of strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses, and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anticancer activities [31]. Many plants containing flavonoids and alkaloids besides antimicrobial property; have anti-inflammatory, antispasmodic, diuretic and analgesic properties [28].

The *E. guineensis* leaf extract was also found to be rich in tannin. Smirnoff, [32] documented that tannins are used for treating intestinal disorders such as diarrheoa and dysentery. Antimicrobial agents have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them [32]. Ogunleye [33] opined that medicinal herbs owe their medicinal values to the phytochemicals present in them.

The results of the antibiotic sensitivity pattern showed that both *S.* Typhi and *S.* Typhimurium were multidrug resistant. The most effective of all antibiotics against the isolates was ciprofloxacin. Ogunleye, [33] in his research observed the same effect when he challenged ciprofloxacin with bacterial pathogens isolated from well water, and he attributed the potency of antibiotic to its broad spectrum activity against both Gram-positive and Gram-negative bacteria. Ciprofloxacin belongs to the group of floroquinolone, a broad spectrum antibiotic. Resistance to antibiotics is mainly driven by the selective pressure imposed by their inappropriate use especially in developing countries where people do not have minimal awareness of resistance to antibiotics and infections. Often times, people want systematic relief to which the health professionals respond by prescribing antibiotics for quick recovery. Infections with drug resistant microorganisms are associated with severity of the patient's illness, increased patient contact with health care personnel and length of stay in the hospital. [34,35]

Chandy et al. [36] reported that the occurrence of antibiotic resistance in middle income countries like Nigeria may be due to poor access to doctors thus encouraging the unacceptable practice of buying antibiotics without due prescription.

5. CONCLUSION

The findings of this study revealed that methanolic extract of *Elaeis guineensis* demonstrated good antibacterial property against the multidrug resistant *Salmonella* Typhi and *Salmonella* Typhimurium. The multidrug *salmonella* strains which showed considerable susceptibility to the extract were obtained from blood samples of selected typhoid fever patients in contrast to previous findings where isolates were obtained from other clinical sources. The rich phytochemicals present in *Elaeis guineensis* leaves are responsible for its antibacterial activities and may serve as an affordable novel source for the treatment of infections of bacterial origin. Toxicological and pharmacological studies will be of immense usefulness in confirming the effectiveness of *E. guineensis* leaves as less toxic and cost effective alternative in combating human infections.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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