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Olufemi Lionel Okunye
Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Olabisi Onabanjo University, Ogun State, Nigeria

Kotun Bunmi Comfort
Department of Biological Sciences and Biotechnology, College of Pure and Applied Sciences, Caleb University, Imota Lagos State, Nigeria

Oyinloye Oladapo Elijah
Department of Pharmacology and Toxicology, Faculty of Pharmacy, Olabisi Onabanjo University, Ogun State, Nigeria

Olufunke Caroline Babalola
Department of Pharmaceutics and Pharmaceutical Technology Faculty of Pharmacy, Olabisi Onabanjo University, Ogun State, Nigeria

Brenda Iloka Chijioke
Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Olabisi Onabanjo University, Ogun State, Nigeria

Oluwaseun Eunice Adewole
Department of Microbiology, Faculty of Science, University of Ilesa, Osun state, Nigeria

Ayedun Joshua Seun
Department of Biological Sciences and Biotechnology, College of Pure and Applied Sciences, Caleb University, Imota Lagos State, Nigeria

Ajayi Peter Omotayo
Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Olabisi Onabanjo University, Ogun State, Nigeria

Ade-Adekunle Olusegun Ayo
Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Olabisi Onabanjo University, Ogun State, Nigeria

Coker Morenike Eunice
Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria

Correspondence:
Olufemi Lionel Okunye
Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Olabisi Onabanjo University, Ogun State, Nigeria
Email: okunyelionel@oouagoiwoye.edu.ng

Comparative antimicrobial activity of *Moringa oleifera*, *Nigella sativa* seed extracts, and gentamicin against *Staphylococcus aureus* from burn wounds

Olufemi Lionel Okunye, Kotun Bunmi Comfort, Oyinloye Oladapo Elijah, Olufunke Caroline Babalola, Brenda Iloka Chijioke, Oluwaseun Eunice Adewole, Ayedun Joshua Seun, Ajayi Peter Omotayo, Ade-Adekunle Olusegun Ayo, Coker Morenike Eunice

ABSTRACT

Background: In folklore, *Nigella sativa* and *Moringa oleifera* seeds have long been associated with multifaceted antimicrobial properties, although without extensive empirical scientific validation. Natural products of plant origin have been recognized as potential sources of antimicrobial agents. The antibacterial activity of *Moringa oleifera* and *Nigella sativa* against clinical isolates of *Staphylococcus aureus* has been previously reported in contexts other than burn infections. **Objective:** This study aimed to compare the antimicrobial effects of *Nigella sativa* seed and *Moringa oleifera* seed extracts with gentamicin against *Staphylococcus aureus* isolates from burn wound infections. The investigation was inspired by ethnobotanical surveys, ethnopharmacological records, and traditional medicinal claims regarding the therapeutic potential of these plants. **Materials and Methods:** *Staphylococcus aureus* isolates from burn wound infections were exposed to varying concentrations of *Moringa oleifera* and *Nigella sativa* seed extracts (5 µg/mL, 2.5 µg/mL, and 1.25 µg/mL), prepared via cold maceration. Gentamicin at 5 µg/mL served as the standard antibiotic control. All preparations were incubated at 37°C for 24 hours, and antimicrobial activity was assessed by measuring the zone of growth inhibition. **Results:** Both aqueous and methanol extracts of *Moringa oleifera* and *Nigella sativa* seeds demonstrated anti-staphylococcal activity at concentrations ranging from 5 µg/mL to 1.25 µg/mL, particularly at dilution factors of 1:2 to 1:4. However, antimicrobial activity diminished at higher dilutions (1:16 and 1:32). Notably, *Nigella sativa* seed extract exhibited more pronounced anti-staphylococcal effects than *Moringa oleifera*. Gentamicin, a broad-spectrum aminoglycoside antibiotic, displayed 100% anti-staphylococcal activity at all tested dilutions. **Conclusion:** Methanol extracts of *Moringa oleifera* and *Nigella sativa* seeds exhibit promising anti-staphylococcal activity and may hold potential for clinical applications as alternative therapies. Gentamicin remains a highly effective standard treatment for *Staphylococcus aureus* infections, including those associated with burn wounds.

Keywords: Plant extract, *Moringa oleifera*, *Nigella sativa*, Gentamicin, *Staphylococcus aureus*, Burn wound infections.

INTRODUCTION

In folklore, *Moringa oleifera* seeds and *Nigella sativa* seeds have been associated with multifunctional properties but without much elaborate empirical scientific backups. *Moringa oleifera* is a world-renowned plant herb for its extra ordinary nutritional and medicinal properties. It has natural antihelminthic, antibiotic, detoxifier properties and it is an outstanding immune builder. Numerous antibacterial compounds have been isolated from *Moringa oleifera,* including; glucosinolates, rhamanose, ptrygospermin and isothiocyanates. Specifically, these compounds include 4-(4-O-acetyl-a-L rhamnopyranosyloxy) benzyl isothiocyanate, niazinicin, pterygospermin and benzyl glucosinolate. *Moringa oleifera* is endowed with very many great medicinal compounds. *Moringa oleifera* is rich in various phytochemicals, including flavonoids, terpenoids, alkaloids, saponins, steroids, phenolic acids, tannins, anthocyanins, aproanthocyaidins and various other compounds which contributes to its antioxidants and antimicrobial properties [1].

Nigella sativa is an annual flowering plant in the family Ranunucleaceae native to western Asia. It is naturalized in Europe and Africa. *Nigella sativa* also known as black seed in Nigeria contains some other compounds in trace amounts. Many active compounds have been isolated, identified and reported so far in different varieties of black seeds. The most important active compounds are thymoquinone, thymohydroquinone, dithymoquinone, p-cymene, carvacrol,4-terpineol, t-anethol, sesquiterpene longifolene and α-pinene and thymol. *Nigella sativa* contains two different types of alkaloids; *i.e.*

isoquinoline alkaloids *e.g.* nigellicimine and nigellicimine-N-oxide, and pyrazol alkaloids or indazole ring bearing alkaloids which include nigellidine and nigellicine [2].

The most effective extracts were the crude alkaloid, and thymoquinone bioactive compounds which can cause oxidative stress-induced cell apopstosis, enhance membrane permeability and exert potent antimicrobial effects. These diverse pharmacological properties make *Nigella sativa* a promising herb for the development of new drugs and therapy for a wide range of diseases. *Nigella sativa* ethanolic extract was reported to have antibacterial activity against clinical isolates of methicillin resistant *Staphylococcus aureus* [3].

Burns, a coagulative necrosis of the skin and mucous membrane with or without the underlying structures, are damage to the skin caused by a variety of non-mechanical sources including chemicals, electricity, heat, sunlight and nuclear radiation. The severity of burn wound is determined by the degree of tissue damage and size of the area affected. Approximately 500,000 burns are treated each year by hospitals worldwide, 40,000 and required prolonged hospitalizations [4]. Burn wound can affect the entire skin as well as tissues that lie beneath it but burns to the face, hands or feet are considered severe because of the resulting complication with airway management, dexterity and movement associated with these organs [5]. The skin damage and scar if unkempt can be a good source of microbial metabolites which may cause a further decomposition and deterioration at such loci. The symptoms of burns will depend on the depth of injury and causes of the burn. Some of the symptoms of burns wound include; changes in the colour of the skin which may be red, pink, white or brownish appearance blister on the skin, pain in the burn area, respiratory or airways symptoms. Organisms originate from the patient's own skin, gut and respiratory flora, as well as from contact with contaminated health care environments and workers [6].

Staphylococcus aureus is a microbial pathogen and has been isolated from numerous burns and wounds, and including surfaces in medical facilities. *Staphylococcus aureus* is difficult to treat because of its armaments of virulence determinants and toxin production potentials. These determinants include both structural components and secreted cellular products. The ability to persist, avoid phagocytosis, elaborate biofilms and survive within the epithelial cell coupled with its antimicrobial resistance profiles made this bug, a potential threat [7].

Staphylococcus aureus and other opportunistic pathogens had been reported to have been isolated from burns and wound samples; normal commensals of the body may also take advantage of the damaged cell associated with weakened immune to cause more deterioration to the burnt surface [8].

This study investigated the antimicrobial activity of Moringa *oleifera* and Nigella *sativa* methanol seeds extract and also gentamicin - a conventional broad-spectrum antibiotic, on isolates of *Staphylococcus aureus* from burns infection.

MATERIALS AND METHODS

Experimental Design

This was a laboratory experimental study to investigate the antibacterial activity of methanol seed extract of *Moringa oleifera* and *Nigella sativa* and also gentamicin a standard antibiotic on *Staphylococcus aureus* obtained from cases of burns infections. The test pathogens were obtained from local primary health care center in Ibadan and the plant extract assayed for their anti-staphylococcal activity using agar cup diffusion method. The research was carried out in the Department of Pharmaceutical microbiology of the Olabisi Onabanjo University.

Collection of samples

Swabs from the burn infections were collected from the routine laboratory benches of a local community Primary Health Care centers Ibadan. The samples were culture on mannitol salt agar, incubated aerobically at 37ºC for 48 h and thereafter Gram stained with other conventional biochemical tests that include; catalase, coagulase, DNase and purity test for further confirmation. The isolates were stored as slant in the refrigerator at 4ºC for further use.

Collection of plant materials

Moringa oleifera seeds were collected from the forest of Olonde-Ogunmakin axis of Ogun state while *Nigella sativa* seed were collected from the forest along Ilesa-Ife axis of Osun state. The seeds were air dried at room temperature for 21 days and thereafter blended aseptically to obtain a powdered form for the extraction work.

Authentications of plant materials

The whole plants; *Moringa oleifera* and *Nigella sativa* was authenticated with a voucher numbers PCGH132 and PCGH 136 respectively at the herbarium unit of the Department of Pharmacognosy of the Olabisi Onabanjo University where the vouchers were archived.

Procurement of Gentamicin

Gentamicin injection packaged sterile and sealed (80 mg/2 mL vials ampoule) from Shanxi zhongbao shuguang with batch number 250222 was procured from a Crown pharmacy chemist store in Challenge, Ibadan. It was used at 5 µg /mL in aqueous dilution, as practiced by the hospital in the use of antibiotics.

Extraction (cold extraction)

A quantity of 1000 grams of each of the powdered seeds was soaked separately in 2.5 L of sterile distilled water in sterile conical flasks and left for 3 days with intermittent shaking. The same procedure was repeated using 80% v/v of distilled methanol and water. The mixtures were then filtered and concentrated using rotary evaporator. The extracts were kept in a sterile container and stored at 4ºC in a refrigerator [9].

Antimicrobial assay of the extracts (Agar cup diffusion method)

An overnight nutrient broth culture of the isolates of *Staphylococcus aureus*, equivalent to 10^6cfu/mL, was diluted in 1:100 dilutions and seeded into 20 mL of molten Mueller Hinton Agar. Wells were dug with flamed-sterilized cork borer of 6 mm internal diameter. *Moringa oleifera* and *Nigella sativa* seeds powder from the stock concentration of each dilution (5 µg/mL, 2. 5 µg/mL and 1.25 µg/mL) as well as 5 µg/ml of gentamicin were filled into the wells. The cultured plates were allowed to remain on the bench for a pre-diffusion period of 1 hour 30 minutes, followed by incubation in an upright position at 37ºC for 24 h. The isolates were then checked for the zone of growth inhibition as an indication of susceptibility [10].

Ethical Clearance

Ethical approval with reference number NHREC/28/11/2025 was obtained from the Health Research Committee office of the Hospital Management Board of the University before embarking on this study.

RESULT AND DISCUSSION

The anti-staphylococcal activities of the fractional dilutions of *Moringa oleifera* seeds extract, *Nigella sativa* seeds extract, and gentamicin powder were quantitatively assessed in this study.

Selection of *Moringa oleifera* and *Nigella sativa* plants for this study was based on ethno-botanical survey which has been considered by

many researchers as a better approach to drug discovery and bioactive chemical constituents in medicinal plants [11].

Figure 1 exhibited varied zones of growth inhibitions which as an index of measuring antimicrobial activity of plant extracts which in similarity with related study by Mohamed et.al., (2019) on of antibiotic susceptibility test results [11]. The antimicrobial activity of *Moringa oleifera, Nigella sativa,* and gentamicin evaluated elicited varied activity. At concentration of 5 µg/mL, the undiluted stock of *Moringa oleifera* methanol seed extract elicited a pronounced antimicrobial activity on the five isolates of *Staphylococcus aureus* exposed and the 1:2, 1:4 fractional dilutions but a decline in antimicrobial activity was observed at 1:8, 1:16, and 1:32 dilutions as indicated by the zones of growth inhibition recorded as shown in Table 1. Similar trend of antimicrobial activity was observed on *Nigella sativa* methanol seeds extracts as shown in Table 2, - an expression of more antimicrobial activity at a lower dilution factor, which could be due to reduction in concentration of the extract along with halving dilution factors, which agrees with the study of Aaser et.al., (2024) on *Moringa oleifera*: Recent Insights for Its Biochemical and Medicinal Applications [12].

The activity of *Moringa oleifera* methanol seed extract and *Nigella sativa* methanol seed extract at a concentration 2.5 µg/ml as shown in Table 4 and 5 from the zones of growth inhibition exhibited indicated an antimicrobial effectiveness but decline along the line of halving concentration and dilution factors, which corroborates the similar findings of Altoparlak, et.al., 2004 on the time-related changes of antimicrobial resistance patterns and predominant bacterial profiles of burn wounds and body flora of burned patients [13].

At concentration 1.25 µg/mL, *Moringa oleifera* both methanol and aqueous extract elicited no antimicrobial activity at 1:8, 1:16, 1:32 as exhibited in Table 5 while at the same dilution factors, Nigella sativae methanol and aqueous extract exhibited little antimicrobial activity in comparison with *Moringa oleifera* seed extract. Having compared *Moringa oleifera, Nigella sativa* activities on the isolates of *Staphylococcus aureus from* burns infections in this study. It was observed that undiluted *Moringa oleifera* seed extracts, at both lower and higher dilution factors in relation to concentrations, showed remarkable but lesser anti-staphylococcal activity than the extract of *Nigella sativa* at the same concentration as exhibited by their zones of growth inhibition as showed in Table 1-6. The variation observed from Table 1 to 6 on the antimicrobial activity of extracts of *Moringa oleifera* seeds extracts and Nigella *sativa* seed extract respectively, could also be attributed to variation in concentrations, phytochemical composition of the seed extract, inherent genetic composition or strain variation of the isolates of *Staphylococcus aureus* exposed [14].

The zones of growth inhibition recorded for both *Moringa oleifera* and *Nigella sativa* seed extract could be attributed to the biological metabolites of *Moringa oleifera* and *Nigella sativa*. Both aqueous and methanol crude extracts *Moringa oleifera* seeds elicited elaborate remarkable zones of inhibition in comparison with *Nigella sativa* seeds. But the methanol extracts from both plants elicited more antimicrobial activity than water extract. This could be as a result of a selective reaction of the composition of the extract to chemicals of different polarities, since methanol higher polarity make it an excellent extractant which corroborates the study of Nana Ama Amissah (2017) on Epidemiology of *Staphylococcus aureus* in a burn unit of a tertiary care center in Ghana [15].

Gentamicin, a broad-spectrum standard antibiotic used in this study was effective on the five of *Staphylococcus aureus* as showed in Table 7, at every various dilution. The zone of growth inhibition observed in 5 fractional dilutions prepared established the efficacy of gentamicin, a broad-spectrum antibiotic on *Staphylococcus aureus* which agreed with the study of Adeleke et.al., (2005) on comparative antibacterial activity of honey and gentamicin against *Escherichia coli* and *Pseudomonas aeruginosa* [16]. The clinical relevance of the studied plant in comparison with gentamicin, a standard antibiotic, are the antimicrobial activity of the plants explored on the isolates of *Staphylococcus aureus* which can be of therapeutic value in the management of staphylococcal mediated burn infections.

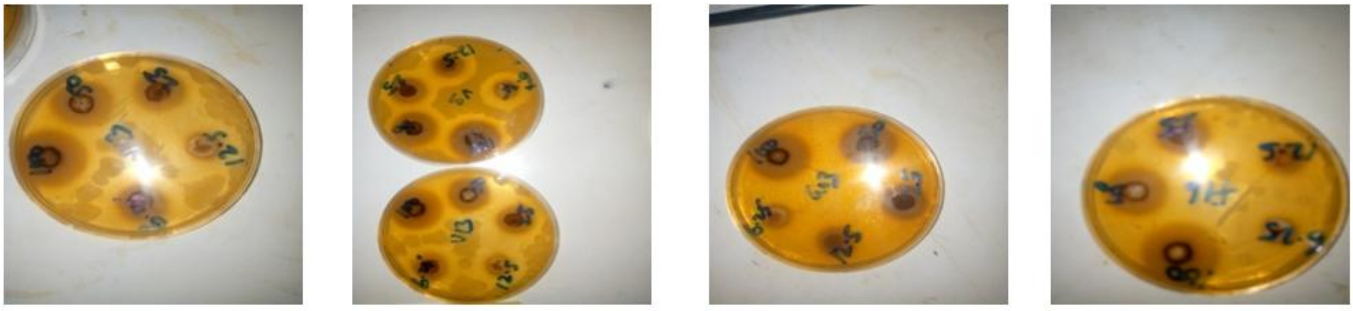

Figure 1: Plates showing zones of growth inhibition A and B (*Nigella sativa* extract) C and D (*Moringa oleifera* extract)

Table 1: Zones of growth inhibition of *Moringa oleifera* seeds (5 µg/mL) against *Staphylococcus aureus* isolates

	Zone of growth inhibition in (mm)												**Negative control**	
Isolates	0		1:2		1:4		1:8		1:16		1:32		Water	50% methanol
	AQ	ME	AQ	ME	AQ	ME	AQ	ME	AQ	ME	AQ	ME		
S. aureus 1	16	21	9	14	8	12	8	10	7	10	8	12	0	0
S. aureus 2	14	25	12	22	10	15	10	18	8	12	8	10	0	0
S. aureus 3	10	20	12	17	9	11	9	13	9	12	8	10	0	0
S. aureus 4	20	24	14	21	11	16	10	13	8	10	8	12	0	0
S. aureus 5	21	28	11	18	11	17	10	14	9	12	10	14	0	0

AQ: Aqueous extract ME: Methanol extract Cork borer size: 6mm

Table 2: Zones of growth inhibition of *Nigella sativa* seeds (5 µg/mL) against *Staphylococcus aureus* isolates

	Zone of growth inhibition in (mm)												Negative control	
Isolates	0		1:2		1:4		1:8		1:16		1:32		Water	50% methanol
	AQ	ME	AQ	ME	AQ	ME	AQ	ME	AQ	ME	AQ	ME		
S. aureus 1	19	26	12	16	10	14	9	12	9	10	8	12	0	0
S. aureus 2	21	32	14	16	10	15	10	14	8	11	-	14	0	0
S. aureus 3	23	30	12	15	9	12	9	11	9	9	8	11	0	0
S. aureus 4	20	24	14	21	11	16	10	13	8	10	7	13	0	0
S. aureus 5	21	28	11	18	11	17	10	14	9	12	-	14	0	0

AQ: Aqueous extract ME: Methanol extract Cork borer size: 6mm

Table 3: Zones of growth inhibition of *Moringa oleifera* seeds (2.5 µg/mL) against *Staphylococcus aureus* isolates

	Zone of growth inhibition in (mm)												Negative control	
Isolates	0		1:2		1:4		1:8		1:16		1:32		Water	50% methanol
	AQ	ME	AQ	ME	AQ	ME	AQ	ME	AQ	ME	AQ	ME		
S. aureus 1	12	18	8	14	8	14	8	12	7	11	8	12	0	0
S. aureus 2	14	24	12	20	10	14	10	16	8	11	8	9	0	0
S. aureus 3	13	21	12	15	9	12	9	12	9	12	8	10	0	0
S. aureus 4	18	22	13	19	10	17	10	14	8	12	-	-	0	0
S. aureus 5	16	20	10	20	12	15	10	16	9	14	-	-	0	0

AQ: Aqueous extract ME: Methanol extract Cork borer size: 6mm

Table 4: Zones of growth inhibition of *Nigella sativa* seeds (2.5 µg/mL). against *Staphylococcus aureus* isolates

	Zone of growth inhibition in (mm)												Negative control	
Isolates	0		1:2		1:4		1:8		1:16		1:32		Water	50% methanol
	AQ	ME	AQ	ME	AQ	ME	AQ	ME	AQ	ME	AQ	ME		
S. aureus 1	12	18	8	14	8	14	8	12	7	11	8	12	0	0
S. aureus 2	14	24	12	20	10	14	10	16	8	11	8	9	0	0
S. aureus 3	13	21	12	15	9	12	9	12	9	12	8	10	0	0
S. aureus 4	18	22	13	19	10	17	10	14	8	12	10	14	0	0
S. aureus 5	16	20	10	20	12	15	10	16	9	14	10	14	0	0

AQ: Aqueous extract ME: Methanol extract Cork borer size: 6mm

Table 5: Zones of growth inhibition of *Moringa oleifera* seeds (1.25 µg/mL) against *Staphylococcus aureus* isolates

	Zone of growth inhibition in (mm)												Negative control	
Isolates	0		1:2		1:4		1:8		1:16		1:32		Water	50% methanol
	AQ	ME	AQ	ME	AQ	ME	AQ	ME	AQ	ME	AQ	ME		
S. aureus 1	8	10	8	12	8	8	-	-	-	-	-	-	0	0
S. aureus 2	10	14	8	10	8	9	-	-	-	-	-	-	0	0
S. aureus 3	8	10	8	9	-	8	-	-	-	-	-	-	0	0
S. aureus 4	14	18	10	12	-	-	-	-	-	-	-	-	0	0
S. aureus 5	10	15	9	10	8	10	-	-	-	-	-	-	0	0

AQ: Aqueous extract ME: Methanol extract Cork borer size: 6mm

Table 6: of growth inhibition of *Nigella sativa* seeds (1.25 µg/mL) against *Staphylococcus aureus* isolates

	Zone of growth inhibition in (mm)												Negative control	
Isolates	0		1:2		1:4		1:8		1:16		1:32		Water	50% methanol
	AQ	ME	AQ	ME	AQ	ME	AQ	ME	AQ	ME	AQ	ME		
S. aureus 1	10	16	8	14	8	12	8	10	7	11	8	9	0	0
S. aureus 2	12	20	11	18	10	13	10	14	8	10	7	8	0	0
S. aureus 3	11	19	12	13	9	11	9	12	8	10	7	8	0	0
S. aureus 4	16	21	13	19	10	16	10	14	8	10	8	9	0	0
S. aureus 5	14	20	10	18	12	15	9	16	8	10	8	12	0	0

AQ: Aqueous extract ME: Methanol extract Cork borer size: 6mm

Table 7: Zones of growth inhibition of Gentamicin (5 µg/mL) against *Staphylococcus aureus* isolates

	Zone of growth inhibition in (mm)					
Isolates	0	1:2	1:4	1:8	1:16	1:32
S. aureus 1	35	33	30	27	25	24
S. aureus 2	35	30	28	25	24	21
S. aureus 3	30	30	27	23	21	20
S. aureus 4	30	25	22	17	15	12
S. aureus 5	35	32	30	27	25	22

Cork borer size: 6mm

CONCLUSION

Nigella sativa and *Moringa oleifera* seed extracts separately elicited antimicrobial activities against the isolates of *Staphylococcus aureus* in this study. It is therefore recommended that *Nigella sativa* and *Moringa oleifera* seeds extracts when further purified could be used for the therapeutic management of *Staphylococcus aureus* from burns infections. Further studies may be necessary in fas approaching future to unravel the minimum inhibitory concentration, minimum bactericidal concentration, *in vivo* study using animal model, and other antimicrobial parameters

Study limitation

Most of the time, in-vitro results do not translate *in vivo* activity. Also, it is impossible to quantify the amount of the antimicrobial substance from the plant that diffuse to agar medium. The results obtained from this study elicited antimicrobial effect on isolates of *Staphylococcus aureus* obtained from burns infection at varied concentration.

Conflict of interest

The authors declared no conflict of interest.

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ORCID ID

Olufemi Lionel Okunye: https://orcid.org/0000-0001-6444-563X

Oyinloye Oladapo Elijah: https://orcid.org/0000-0003-2094-1855

Olufunke Caroline Babalola: https://orcid.org/0000-0002-1742-8762

Brenda Iloka Chijioke: https://orcid.org/0009-0000-6514-4265

Oluwaseun Eunice Adewole: https://orcid.org/0000-0001-7712-5037

Ayedun Joshua Seun: https://orcid.org/0009-0006-1062-5540

Ajayi Peter Omotayo: https://orcid.org/0009-0002-1448-1434

Ade-Adekunle Olusegun Ayo: https://orcid.org/0000-0002-8268-7824

Coker Morenike Eunice: https://orcid.org/0000-0002-9063-5688

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