Research Article

Antimicrobial activity of extracts of *Murraya paniculata* on bacteria isolated from patients with oral cavity infection

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Abstract

Oral diseases like dental caries and periodontal diseases have become important health challenges worldwide. This study was carried out on the effects of leaves and bark extracts of *Murraya paniculata* on identified bacterial strains from the oral cavity of patients with oral infection. The microorganisms isolated were *Chryseobacterium meningosepticum*, *Ochrobactrum anthropi*, *Moellerella wisconsensis*, *Eikenella corrodens* and *Chromobacterium violaceum* and the antibacterial activities of methanolic, hot water and cold water extracts of the *Murraya paniculata* leaves and bark on these microorganisms were observed. The methanolic extract of the leaves had zones of inhibition within the range of 17-32 mm, while the hot water and cold water extracts had none. The methanolic, hot water and cold water extracts of the plant bark had no inhibitory effect on the isolates. The extracts of both the leaves and bark of the plant were compared with the antibacterial effects of commercially available toothpastes (CUP, CUH, DHO, DHM, CFC, CFT and NCH), which showed that some of the toothpastes (CUP and CFC) had inhibitory effect on the isolates within the range of 14-21mm while others (CUH, DHM, DHO, CFT and NCH) had none. The antibiotic susceptibility test carried out showed that the isolates were susceptible to some antibiotics; ofloxacin, gentamycin and levofloxacin and resistant to others like cefuroxime, ampiclox, cefotaxime, imipenem/cilastatin, nalidixic acid, amoxicillin/clavulanate. The gas chromatography-mass spectrophotometry (GC-MS) analysis of the *Murraya paniculata* leaves extract revealed seventeen active compounds, while the *Murraya paniculata* bark extract, showed twenty-two compounds. This study revealed that the methanolic extract of the plant leaves was potent against the tested isolates. These antimicrobial activities may be attributed to the presence of active compounds in the extracts, which could be incorporated into conventional toothpastes.

1. Introduction

Oral microbiology is the study of microorganisms that inhabit the oral cavity and their interactions with the host [1]. The oral cavity is a suitable habitat for microorganisms; it provides a source of water and nutrients, as well as a moderate temperature. Oral health issues persist as a significant global concern, with dental caries and periodontal diseases, ranking among the foremost oral health challenges on a worldwide scale [2-3]. Toothaches are a widespread issue that frequently affects people all around the world. The World Health Organization (WHO) has identified the alleviation of toothaches as a top priority in their global agenda for promoting oral health [4]. More than 750 species of bacteria inhabit the mouth, and some of these are causes of oral diseases, including toothache [5]. According to Hollist

[6], traditional health practices can effectively address oral and dental issues using plant-based remedies which include toothache/decay, gingivitis, ulcerative gingivitis, angular stomatitis, mouth ulcers, swollen tonsil, oral thrush, tonsillitis, and black tongue. Resident microbes of the mouth adhere to the teeth and the gums to resist mechanical flushing from the mouth to the stomach where acid sensitive microbes are destroyed by hydrochloric acid [7–8]. Anaerobic bacteria in the oral cavity include: *Actinomyces*, *Arachnia*, *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Fusobacterium*, *Lactobacillus*, *Leptotrichia*, *Peptococcus*, *Selenomonas* etc. [9]. Genera of fungi include: *Candida, Cladosporium, Aspergillus, Fusarium, Glomus, Alternaria, Penicillium* and *Cryptococcus* [10]. Existence of medicinal herbs has helped with oral health globally. These herbs have anti-inflammatory, anti-bacterial, anti-viral, anti-fungal activity on oral microorganisms [11]. These herbs include *Aloe vera*, ginger, rosemary, clove, garlic, peppermint, fenugreek etc. *Aloe vera* gel has been found more effective in controlling bacteria that cause cavities than other commercially available toothpaste. Cloves are also effective at fighting cavity and are often added to oral products such as toothpaste and mouthwash [11–12]. *Murraya paniculata* is a potential plant for the treatment of toothache and merits scientific attention towards the discovery of possibly novel drugs [13]. It is used especially for its essential oil, and also for its medicinal values. It is extensively cultivated as a garden and hedge plant and has a mildly bitter-minty flavour, providing a warming effect with analgesic properties promote blood circulation and can relieve contusions [14]. Research has identified that the oral cavity is the 'home' of various microorganisms, which include bacteria, fungi, viruses and protozoa. After the human gut, the oral cavity hosts the second-largest and most diverse microbiota, comprising more than 700 bacterial species. The mouth, with its diverse niches, is an exceptionally complex habitat where microbes inhabit the hard surfaces of the teeth and the soft tissues of the oral mucosa, tongue, soft palate and hard palate. The oral microbiome consists of millions of bacteria; dominant among them are *Bacteroides*, Actinobacteria, etc. *Candida* naturally inhabits the mouth, but on occasion, it can experience overgrowth leading to symptoms [15]. The objectives of the study are to isolate and identify bacterial strains present in the oral cavity; extract the active components from the leaves and bark of *Murraya paniculata*; determine the effects of the leaf and bark extracts on the bacterial strains isolated from the mouth; compare antibacterial effect of *Murraya paniculata* extracts with commercially available toothpastes and conventional antibiotics and ascertain the chemical constituents of the leaves and bark of *Murraya paniculata*.

2. Materials and methods

2.1 Isolation of microorganisms

Microorganisms were isolated from the mouth of patients with oral diseases and infections from the Department of Dental Services, State General Hospital, Ota, Ogun State, Nigeria. The patients used sterilized water to gargle their mouths and the water sample was collected into a sterile universal bottle for microbiological analysis in the laboratory.

The water sample collected was streaked directly onto the sterile Nutrient Agar plate and the plates were incubated at 37 °C for 24 hours. After incubation, the microorganisms were then re-cultivated on a sterile Nutrient Agar plate for pure cultures to be obtained. An Analytical Profile Index (API) kit (API 20E) was used to identify the microorganisms.

2.2 Collection of plant materials

The leaves and bark of *Murraya paniculata* were individually gathered separately into a polythene bag from the field located in Bells University of Technology, Ota, Ogun State, Nigeria. The fresh leaves and bark of the plant were rinsed thoroughly under a running tap and sterile water, the leaves and bark were spread on disposable papers and allowed to dry at room temperature in the laboratory for about 4 weeks causing a reduction in their moisture content.

2.3. Extraction of the active components of *Murraya paniculata* leaves and barks

After drying the leaves and bark of *Murraya paniculata* for about 4 weeks, the dried leaves and bark were ground in an electric blender to powder. Glass containers used for the extraction of leaves and bark were sterilized at 170 °C for 1 hour using the hot air oven. The powdered leaves and bark were dissolved separately using hot water, cold water, and methanol as solvents. The solutions were prepared in a ratio of 1:10, using 50 g of powdered bark and leaves and 500

mL of the solvents in separate sterilized containers. The containers were sealed using foil paper and covered for the nutrients of the leaves and bark to dissolve into the solvents in a dry, cool place and the Whatman No. 1 filter paper was used to filter the solution after 5 days. The filtrate was kept in a hot water bath at 50 °C to evaporate and a thick concentration was obtained. The extracts were then stored in the refrigerator.

2.4. Antimicrobial assay of extracts

The following concentrations of the leaves and bark in cold water, hot water and methanolic extracts were measured; 5, 15, 25, 50, 75 and 100 mg/mL and dissolved in 1 mL of dimethyl sulfoxide (DMSO) each. The preparation of Mueller-Hinton agar plates was followed by the manufacturer instruction. Wells were drilled in the agar plates using a sterilized cork borer (6.0 mm in diameter). After boring of the hole, swab stick was used to streak the bacterial suspension on the Mueller-Hinton agar plates. A 0.l mL of the solution (the leaves and bark extracts and 1 mL of dimethyl sulfoxide (DMSO) solution were used to fill the Mueller-Hinton agar plates containing 6 holes for the concentration. The Mueller-Hinton agar plates were then incubated at 37 °C for 24 hours and the zones of inhibition were measured.

2.5 Preparation of bacterial suspension

Distinct colonies on the pure culture of the isolates grown on the nutrient agar plate were picked and mixed with 9 mL sterile distilled water in McCartney bottles and the turbidity of the suspension was compared with 0.5 McFarland standard equivalents to 1×10^8 CFU/mL. The turbidity of the suspension must be the same as the McFarland standard [16].

2.6 Preparation of Mcfarland standard

One mililitre of concentrated sulphuric acid was added to 99 mL of distilled water to prepare a 1%v/v solution of sulphuric acid, the solution was mixed properly. Then 0.5g of dihydrate barium chloride ($BaCl_2.2H_2O$) was added into 50 mL of distilled water to prepare 1%v/v of barium chloride. Thereafter, 0.6 mL of the barium chloride solution was added to 99.4 mL of the sulphuric acid solution, and then mixed thoroughly. 9 mL of the turbid solution was transferred into a McCartney bottle of the same type as used in preparing the test inoculum [17].

2.7 Antibiotic susceptibility test

The antibiotic test was carried out on each isolate. Multiple discs having twelve different antibiotics; cefuroxime, ampiclox, cefotaxime, imipenem/ cilastatin, ofloxacin, gentamicin, nalidixic acid, levofloxacin, ceftriaxone sulbactam, amoxicillin/ clavulanate, cefixime and nitrofurantoin were aseptically placed with the aid of sterile forceps over the surface of Mueller-Hinton Agar which had been previously inoculated with the test isolates with prescribed turbidity (compared to that of McFarland standard).

2.8. Preparation of commercial toothpaste extracts

Different toothpastes (CUP, CUH, DHO, DHM, NCH, CFT and CFC) were used to compare the effects of the leaves and bark extract of *Murraya paniculata* on the test isolates. Both herbal (CUH, DHO, DHM and NCH) and ordinary toothpaste (CUP, CFT and CFC) were used in this comparison. The following concentrations of the toothpaste were measured: 5, 15, 25, 50, 75 and 100 mg/mL and dissolved in 1 mL of dimethyl sulfoxide (DMSO) solution each. Mueller-Hinton agar plates were prepared following the manufacturer's instructions. Wells were bored in the agar plates using a sterile cork borer (6.0 mm in diameter), after boring the hole, swab stick was used to streak the bacterial suspension on the Mueller-Hinton agar plates. 0.l mL of the solution (the toothpaste extracts and 1 mL of dimethyl sulfoxide (DMSO) solution were used to fill the Mueller-Hinton agar plates containing 6 holes for the concentration. The Mueller-Hinton agar plates were then incubated at 37 °C for 24 hours and the zones of inhibition were measured.

2.9. Gas chromatography-mass spectrometry (GC-MS) analysis

To identify the chemical composition of the methanolic extracts of *Murraya paniculata* leaf and bark, the analysis was performed using Shiamadzu GC-MS-QP2010 Plus (Japan). The separation was carried out using a Restek RTX-5MS fused silica capillary column (5 % diphenyl and 95 % dimethylpolysiloxane) of 30 m x 0.25 mm internal diameter (di) and 0.25 mm in film thickness.

The column oven temperature was programmed at 60°C and was held for 1.0 minute, raised to 180 °C for 3 minutes and finally to 280 °C held for 5 minutes.

Table 1. Antibacterial activity of methanolic extracts of *Murraya paniculata* leaves on microorganisms isolated from the oral cavity

S/N	Isolates	Concentration of extract (mg/mL)					
		5	15	25	50	75	100
		Zone of inhibition (mm)					
1	*Chryseobactrium meningosepticum*	18	22	27	27	28	29
2	*Ochrobactrum anthropi*	R	R	R	R	R	R
3	*Moellerella wisconsensis*	R	R	R	24	25	32
4	*Eikenella corrodens*	R	R	R	17	20	21
5	*Chromobacterium violaceum*	R	R	R	R	R	R

R: Resistance

The injection temperature was 250 °C, the rate flow of the helium gas was 1.80 mL/min and the film thickness was 0.25μm. Compounds were identified at the Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria.

3. Results and discussion

The Analytical Profile Index (API) results indicated that *Chryseobacterium meningosepticum*, *Ochrobactrum anthropi*, *Moellerella wisconsensis*, *Eikenella corrodens* and *Chromobacterium violaceum* were the microorganisms isolated from the oral cavity. The isolates were identified to be Gram-negative bacteria. *Chryseobacterium meningosepticum* now known as *Elizabethkingia meningosepticum*, was once classified as *Flavobacterium* species and it is widely distributed in nature [18]. Environmental studies have revealed that *Chryseobacterium meningosepticum* can also be found in chlorine treated municipal water supplies and it is a potential reservoir for hospital infection [19]. *Ochrobactrum anthropi,* formerly classified as *Achromobacter* species, has been known to be distributed widely in soil, environmental and water sources. They are related to the genus *Brucella,* is an opportunistic rare pathogen in the human body [20]. *Moellerella wisconsensis,* a member of the *Enterobacteriaceae* has been isolated widely in nature but only from human clinical samples [21–22]. This microorganism has been isolated from the oral cavity of a wild raccoon [23]. *Eikenella corrodens* known to cause severe invasive diseases in humans, is present as endogenous microbiota in the mouth and upper respiratory tract [24–25]. *Chromobacterium violaceum* is a free-living microorganism whose infection usually occurs through abrasions in the skin or by ingestion

of contaminated soil and water and it was reported to be found in the oral cavity of humans [26-29].

Table 1 indicates the antibacterial activity of the methanolic extract of *Murraya paniculata* leaves on the isolates, and the results showed that *Chryseobacterium meningosepticum* was susceptible to all concentrations of the methanolic extract of plant leaves, while *Ochrobactrum anthropi* and *Chromobacterium violaceum* were resistant. *Moellerella wisconsensis* and *Eikenella corrodens* were resistant to low concentrations (5-25 mg/mL and susceptible at higher concentrations of 50-100 mg/mL. The highest inhibitory effect of the methanolic extract, within the range of 17-32 mm, may be due to the better solubility of the active compounds in the organic solvent [30]. Its greater efficacy as against the cold water and hot water extracts with no inhibitory effect on the bacterial isolates may also be due to the fact that different solvents have different polarities, hence different degrees of solubility for the various phyto-constituents [31]. The methanolic, hot water and cold water extracts of the *Murraya paniculata* bark had no inhibitory effects on the isolates. The great difference between the inhibitory effect of the leaves and the bark extracts may be due to the different chemical constituents present in these parts of the plant. Although, the leaves had fewer chemical components compared to bark. However, leaves had a wider range of inhibitory effects compared to bark with no inhibitory effect. The Gram-negative bacteria with the highest zone of inhibition was *Moellerella wisconsensis* with a maximum of 32 mm. *Ochrobactrum anthropi* and *Chromobacterium violaceum* were resistant to the leaf extracts (Table 1). Njimoh *et al.* [32] reported that methanolic extract of *Aframomum danielli* and *Albizia lebbeck* exhibited microbial activity against

Table 2. Antibacterial activity of CUP toothpaste extract on microorganisms isolated from the oral cavity

S/N	Isolates	Concentration of extract (mg/mL)					
		5	15	25	50	75	100
		Zone of inhibition (mm)					
1	*Chryseobacterium meningosepticum*	R	R	R	14	20	21
2	*Ochrobactrum anthropi*	R	R	R	R	R	R
3	*Moellerella wisconsensis*	R	R	R	R	R	R
4	*Eikenella corrodens*	R	R	R	R	R	R
5	*Chromobacterium violaceum*	R	R	R	R	R	R

R: Resistance, CUP: Toothpaste brand

Table 3. Antibacterial activity of CFC toothpaste extract on microorganisms isolated from the oral cavity

S/N	Isolates	Concentration of Extract (mg/mL)					
		5	15	25	50	75	100
		Zone of inhibition (mm)					
1	*Chryseobacterium meningosepticum*	R	R	R	R	R	20
2	*Ochrobactrum anthropi*	R	R	R	R	R	R
3	*Moellerella wisconsensis*	R	R	R	R	R	R
4	*Eikenella corrodens*	R	R	R	R	R	R
5	*Chromobacterium violaceum*	R	R	R	17	17	20

R: Resistance, CFC: Toothpaste brand

human and food pathogens; *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Staphyloccocus aureus* (bacteria) as well as *Trichophyton rubrum* and *Candida albicans* (fungi).

The effect of the extracts was dependent on the concentrations of extracts used; it was observed that high concentrations (50-100 mg/mL) of the leaves extract had more inhibitory effect on the bacteria identified. The effect of the extracts was also dependent on solvents used for the extraction, as the leaves and bark interaction with the solvents may have contributed to the effects on the bacteria. The extracts of both the leaves and bark of *Murraya paniculata* when compared with the antibacterial effects of commercially available toothpaste showed that two of the toothpastes had inhibitory effects on the isolates that ranged from 14-21 mm (Tables 2 & 3), while others had none.

Chryseobacterium meningosepticum was susceptible to the CUP toothpaste while the other bacteria were resistant to it (Table 2), *Chryseobacterium meningosepticum* and *Chromobacterium violaceum* were also susceptible to a high concentration (50-100 mg/mL) of the CFT toothpaste (Table 3). The bacteria were resistant to the CUH, DHM, DHO toothpastes.

In comparison, the methanolic extracts of the leaves of *Murraya paniculata* had more inhibitory effects on the isolates from the oral cavity than the commercially available toothpastes (Tables 1, 2 & 3).

Table 4 showed the antibiotic susceptibility pattern of the bacteria. All of the bacteria were inhibited by at least one antibiotic. They were all resistant to cefuroxime (CXM), ampiclox (ACX), cefotaxime (CTX), imipenem/cilastatin (IMP), and nalidixic acid (NA). They were all susceptible to ofloxacin (OFX) and levofloxacin (LBC). *Chryseobacterium* species has been found to exhibit resistance to tetracyclines and chloramphenicol [33], while they exhibited susceptibility to fluoroquinolones [34].

The phytochemical analysis of the leaf and bark extracts revealed the presence of seventeen compounds and 99.99% as the percentage composition for the leaf extracts, while twenty-two constituents were revealed for the bark extracts and 99.75% as the percentage constituents (Tables 5 & 6). The chemical composition of the leaves extract revealed the highest value of 29.16 % in 2-pyrrolidinone,1-methyl with retention index of 3.96 and the least of 0.59% in hepasiloxane, 1,1,3,3,5,5, 7,7,9,9,11,11,13,13-tetradecame-thyl which had

Table 4. Antibiotic susceptibility pattern of bacteria isolated from the oral cavity

S/N	Isolates	Concentration of extract (mg/mL)											
		CXM	ACX	CTX	IMP	OFX	GN	NA	LBC	CRO	AUG	ZEM	NF
		Zone of inhibition (mm)											
1	*Chryseobacterium meningosepticum*	R	R	R	R	S	S	R	S	R	R	R	S
2	*Ochrobactrum anthropi*	R	R	R	R	S	S	R	S	R	R	R	R
3	*Moellerella wisconsensis*	R	R	R	R	S	S	R	S	S	R	S	S
4	*Eikenella corrodens*	R	R	R	R	S	S	R	S	R	R	R	R
5	*Chromobacterium violaceum*	R	R	R	R	S	R	R	S	R	R	S	S

R: Resistance; S: Susceptible; CXM: Cefuroxime; ACX: Ampiclox; CTX: Cefotaxime; IMP: Imipenem/Cilastatin; OFX: Ofloxacin; GN: Gentamycin; NA: Nalidixic acid; LBC: Levofloxacin; CRO: Ceftriaxone/Sulbactam; AUG: Amoxicillin/Clavulanate; ZEM: Cefexime; NF: Nitrofurantoin.

Table 5. Chemical composition of *Murraya paniculata* leaves extract

S/N	Compounds	Retention Index	Composition (%)
1	Benzene, 1, 2, 4- trimethyl	3.276	4.65
2	Benzene, 1, 2, 3-trimethyl	3.322	9.33
3	Cyclotetrasiloxane, octamethyl	3.442	2.59
4	2-Pyrrolidinone, 1-methyl	3.963	29.16
5	cis-4-Nonene	4.804	0.67
6	Cyclopentasiloxane, decamethyl	5.290	8.64
7	Cyclohexasiloxane, dodecamethyl	7.361	6.70
8	Cycloheptasiloxane, tetradecamethyl	9.238	8.38
9	Diethyl phthalate	10.280	6.51
10	2,5-Dihydroxybenzoic acid, 3TMS derivative	10.903	3.92
11	2-(2′, 4′, 4′, 6′,6′, 8′, 8′- Heptamethyltetrasiloxan-2′-yloxy)-2, 4, 4, 6, 6,8, 8, 10, 10 nonamethylcycllopenta siloxane	12.345	1.48
12	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,1,3,15,15hexadecamethyl	13.633	0.67
13	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradeca methy-	14.806	0.59
14	Cyclotrisiloxane, hexamethyl-	15.721	0.60
15	Trans-2,3-Methylenedioxy-b-methyl-b-nitrostyrene	15.927	0.74
16	Cyclononasiloxane, octadecamethyl-	17.324	14.28
17	Methyl 2-[1-(4-methylphenyl) hydrazione] propanote	19.017	1.08
		Total (Percentage)	**99.99**

retention index of 14.81 (Table 5). However, for the bark extract, it was 15.9 and 0.79, respectively with retention index of 8.07 and 15.09 for 1- tridecene and 2- methyl-z, z-3,13-octadecadienol (Table 6). This diversity of phytochemicals may likely have antimicrobial properties, suggesting their potency in the treatment of pathogenic diseases in the oral cavity. Phenolic compounds, highly oxygenated flavonoids and flavanones have been found to be present in extracts of *Murraya paniculata* leaves [35–37].

4. Conclusions

This study has shown that high concentrations (50-100 mg/mL) of *Murraya paniculata* leaves extract have the potential of being used in the treatment of oral diseases. This study also serves as a confirmatory test that *Murraya paniculata* is a potential plant for treatment of toothache and merit scientific attention towards the discovery of possibly novel drugs. The results of this study showed that there is not much bacterial activity difference in 'ordinary' toothpastes and herbal toothpastes. The antimicrobial activities of

Table 6. Chemical composition of *Murraya paniculata* bark extract

S/N	Compounds	Retention Index	Composition (%)
1	Dimethyl bicyclo [2.2.1]-2,5-heptadiene-dicarboxylate	3.213	0.81
2	[1,2,3,4] Tetrazolo [1,5-a] pyridine-6- carboxylic acid	3.265	1.48
3	Preg-4-en-3-one, 17. alpha-hydroxy-17 beta-cyano-	3.293	1.36
4	2-Amino-4-(2-methylpropeny1)-pyrimidin-5-carboxylic acid	3.333	2.60
5	Cyclopropane, octyl-	5.748	8.80
6	1-Tridecene	8.066	15.87
7	9-Octadecene, (E)-	10.143	14.44
8	5-Octadecene, (E)-	12.008	8.58
9	6,7-Dimethyl-triazolo (4, 3-b) (1,2,4)-triazine	12.729	0.84
10	Pentadecanoic acid, 14-methyl-methylester	13.152	3.07
11	1,2-Benzenedicarboxylic acid, butyl 2- ethylhexyl ester	13.513	5.86
12	Pentafluoropropionic acid, tetradecylester	13.696	7.29
13	1-Hexadecanol	14.434	1.12
14	9,12-Octadecadienoic acid, methyl ester,(E, E)-	14.514	5.14
15	7-Hexadecenoic acid, methyl ester, (Z)-	14.554	9.73
16	Methyl 8-oxoctanote	14.743	1.48
17	2-Methyl-Z, Z-3, 13-octadecadienol	15.029	0.79
18	E-11-Hexadecenoic acid, ethyl ester	15.064	3.16
19	5-Bromovaleric acid, tetradecyl ester	15.235	4.44
20	Cyclohexanemethanol, chlorodifluroacetate	16.208	1.15
21	13-Octadecanal, (Z)-	16.626	0.94
22	Chloromethyl 3-chlorononanoate z-(13,14-Epoxy) tetradeca-11-en-1-ol acetate	19.286	0.80
	Total (Percentage)		**99.75**

Murraya paniculata have proven that its leaves can be used as herbs in the production of commercial toothpastes alongside other potent herbs as well as a mouth wash.

The present economic situation globally and the high cost of living and location have affected the prices of different commodities, even toothpastes. However, people are encouraged to use *Murraya paniculata* bark as chewing stick for oral hygiene since the plant is an evergreen and available all year round.

Authors' contributions

Conceptualization, data curation, supervision and editing, O.O.K.; Investigation, formal analysis and writing, M.A.O.; Supervision, project administration, methodology and validation, F.O.A.

Availability of data and materials

All relevant data are within the paper and its supporting information files. Additional data will be made available on request according to the journal policy.

Conflicts of interest

There is no conflict of interest from any source concerning this publication.

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