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A *SENNA PODOCARPA* AND TITANIUM FERRITE NANOPARTICLE COMPLEX ELICITS ACTIVITY AGAINST FOUR ORAL BIOFILM-PRODUCING BACTERIAL ISOLATES

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ABSTRACT

The emergence of antimicrobial resistant strains of oral cavity residence bacterial due to their ability to form biofilms is a serious global threat to healthcare. The situation has become worrisome among children population because of the undeterred evolutionary mechanisms of invasion of the emerging strains. To address this problem, this study conducted a genomic identification of oral cavity sourced bacterial among school children in selected two states in Nigeria and further developed a *Senna podocarpa* stem bark aqueous extract incorporated titanium ferrite ($TiFe_2O_4$@SPSBAE) as antimicrobial for mitigation. Only 4 among the sequenced isolated strains (*Pseudomonas stutzeri* and *Stenotrophomonas maltophilia*) were susceptible to the actions of a $TiFe_2O_4$@SPSBAE. The scanning electron microscopy (SEM) image of $TiFe_2O_4$@SPSBAE revealed a regularly patterned heterogeneous surface while and the X-ray diffraction showed a crystallite size that is < 26 nm. The antimicrobial resistance genes (ARGs) includes detected were *aph(6)*, *smeF*, *blaL2*, *blaL1*, *emrB*, *emrA*, *emrC*, *qnrD1* and *sul2*. These ARGs suggest resistance to antibiotics belonging to aminoglycoside, beta-lactam, carbapenem, efflux, quinolone and sulfonamide subclasses. Biofilm-producing genes found in the genomes of the isolates are *algC* and *rpoS*. Interestingly, the biochemical and histology assays showed non-toxic effects of $TiFe_2O_4$@SPSBAE at the effective dose used. The present results confirmed the presence of AMR genes among the isolates obtained from the children population and further suggests the chemotherapeutic potential of $TiFe_2O_4$@SPSBAE as a promising agent for combating oral cavity sourced AMR genes of pathogenic microbes.

Keywords: nanoparticles, biofilms, antimicrobial, bacteria, plant extract

INTRODUCTION

Bacteria in the mouth exhibit various modes of survival and a diversity of ecology modes. The expression of their activities could be synergistic and also antagonistic. Bacteria in the oral cavity could compete with other bacteria for nutrients that ensure their survival **(Okahashi *et al*., 2022)**. This antagonism is seen in *Pseudomonas* and *Lactobacilli* strains and is a feature that distinguish them for selection as biocontrol and probiotic agents respectively **(Clough *et al*., 2022; Un-Nisa *et al*., 2022)**. *Pseudomonas* is an opportunistic pathogen, complicating diseases like cystic fibrosis, possibly leading to a life-threatening condition in humans, especially children **(Behroozian *et al*., 2022)**. Another closely related bacterium is *Stenotrophomonas maltophilia*, even though pathogenic with high prevalence of drug-resistance, may contain a bacteriophage (Phage StM171) which may inhibit biofilm formation **(Mojica *et al*., 2022; Jdeed *et al*., 2023)**. Similar and different species of bacteria are also shown to complement activities, in mutualism, and may work together to prevent the adherence of pathogens to the mucosal surface of the mouth.

Oral bacteria can also form closely knit communities composed of similar or different species of bacteria in the oral cavity called biofilms with often deleterious effects. Effects may be localized in the mouth, or systemic when disseminated to internal organs of the body and other parts of the body. Diseases caused by oral biofilms include dental caries and periodontal disease **(Woelber *et al*., 2022; Bertolini *et al*., 2022)**. Children and adults are at risk of succumbing to the negative effects of oral biofilms which include fevers of unknown origin and previously uncharacterized illnesses arising from infections linked to oral biofilm-producing microorganisms **(Omotuyole *et al*., 2022)**. Presently, the growing need to unveil the identity of these oral biofilm-producing microorganisms among pupils in Nigeria is unprecedented. This need forms the aim of this study. Southwestern Nigeria, being the most children congested region of the most populous black nation in the world and being rife with poverty, face the need to find affordable alternatives to oral health problems. Previous studies have reported the presence of oral biofilms in African children from pre-school age to 18 years **(Kiros *et al*., 2022)** which is of great concern to the population of children within the region. Unfortunately, such information is rare from the southwestern region of Nigeria.

Mouthwash infused with antimicrobials are the common modes of control of less severe forms of these diseases **(Jiang *et al*., 2022)**. However, mechanical removal of diseased oral biofilms is compulsory in severe instances. In Nigeria, Available efforts to control the negative effects of oral biofilms include gargling or brushing with charcoal or salt water and use of mouthwashes **(Brookes *et al*., 2020; Collins *et al*., 2021; Thakur *et al*., 2020)**. This method helps the initial stages but does not completely eliminate biofilm producing microorganisms from the oral cavity. It also does not fully modify the mouth or its microbiome, to prevent future attacks by the pathogens. Also, rural populations are known to use chewing sticks from plants guided by folk literature and one example of such plants is *Senna podocarpa*. The use of Senna in oral care is not only for oral hygiene due to its anti-infectious and anti-inflammatory properties **(Alshehri *et al*., 2022)**, but also to control the effects of toxic chemicals that may have been ingested, especially in mining communities. The potential of *Senna* to reverse the effects of lead toxicity has been studied by **Akinsomisoye *et al*., (2020)**. This demonstrates the use of the plant in biological treatment or removal of lead in lead poisoning cases or as preventative method. However, there is the concern that the plant in itself, may contain other chemicals, which may present a negative situation on the health of the individual. Another negative situation includes the impact on pregnant women and impact in the development of the oral environment of children **(Anderson *et al*., 2020; Al-Mutairi *et al*., 2020)**. Also, unpublished reports show ethanol extracts of *Senna podocarpa* to be toxic to Brine shrimp (*Artemia salina*) cells. The limitations advise the coupling of the plant extracts with Titanium ferrite nanoparticles as these nanoparticles have been shown to attenuate toxicity **(Katiyar *et al*., 2020)** while also being safe to humans **(Chen *et al*., 2023)**. Nanoparticles are particles with diameters <100 nm. They have various shapes and characteristics which can be modified invitro to produce desired or expected

results. They could also be coupled with other compounds and in this case, they have been coupled with plant extracts to test against oral biofilm-producing bacteria. Due to the limitations of the archaic methods of control of the effects of oral biofilms, there is need to synthesize novel, active antimicrobials for biofilm prevention and control.

Properties of antimicrobial agents that aid in the control of bacterial biofilms lean on the origin or chemistry of such agents. The chemical should be composed of molecules which are active against the biofilms but less harmful to the host **(Yazdanian *et al*., 2022)**. The antimicrobial activities of extracts from *Senna podocarpa* against biofilm-producing microorganisms have been reported **(Sitarek *et al*., 2020; Malmir *et al*., 2017)**. However, the inclusion of $TiFe_2O_4$ nanoparticle in its extract for activities against biofilm producing organisms is a new concept which to the best of our knowledge is yet to be reported. Therefore, this study aims at immobilizing the aqueous and ethanolic extracts of *Senna podocarpa* on $TiFe_2O_4$ to produce $TiFe_2O_4$ doped *Senna podocarpa* aqueous extract which includes $TiFe_2O_4$ doped *Senna podocarpa* stembark aqueous extract ($TiFe_2O_4$@SPSBAE) and $TiFe_2O_4$ doped *Senna podocarpa* leaf aqueous extract ($TiFe_2O_4$@SPLAE). The $TiFe_2O_4$ dopped *Senna podocarpa* ethanol extract are $TiFe_2O_4$ dopped *Senna podocarpa* stembark ethanol extract ($TiFe_2O_4$@SPSBEE) and $TiFe_2O_4$ dopped *Senna podocarpa* leaf ethanol extract ($TiFe_2O_4$@SPLEE) as antimicrobial agents for mitigating the activities of biofilm producing microorganisms found in the oral cavity. Furthermore, this study aims to conduct the genomic identification of oral cavity sourced biofilm producing bacterial among school children in 3 selected schools in Lagos and Osun states for better characterization and taxonomy for future referencing. These schools were selected based on high population index and vulnerability of the children to contagion.

MATERIALS AND METHODS

Ethical Approval

Ethical approvals were obtained from Redeemer's University Directorate of Research Innovation and Partnerships (DRIPs) with reference number: REC/30/08/2021/RUN/06, the Osun State Ministry of Education, and the Lagos State Local Government Education Authority (LGEA).

Oral Sample Collection And Transport, Obtaining Pure Bacterial Cultures And Testing For Biofilm Formation

Gargled water samples were obtained from primary school pupils for the purpose of the study **(Buzalaf *et al*., 2020)**. School aged children (n=121) were enrolled from 3 schools in Lagos and Osun states. Sterile, potable drinking water in a cup was given to each participant. After gargling for 5 seconds, they were asked to spit into sterile sample bottles which were subsequently corked, stored on ice and transported to the laboratory. These bottles were stored in a refrigerator and were cultured subsequently by pour plate method on nutrient agar to enumerate and obtain pure cultures. Pure isolates were then grown on brain-heart infusion agar with a red dye which turned black if such sample produced biofilm **(Omeike *et al*., 2022)**. Biofilm-positive samples were sub-cultured into bijou bottles containing nutrient agar to store for DNA extraction and molecular characterizations. All procedures were performed in aseptic manner.

Morphological And Biochemical Characterisation Of Isolates

The phenotypical and morphological attributes of colonies were observed and recorded. The properties studied were size, shape, colour, opacity, surface, elevation and margin. Pure cultures were subjected to sugar fermentation and basic biochemical tests **(Abebe, 2021)**. The utilization of sugars by oral biofilm producing bacteria is not only a useful feature for identification, but also demonstrates its power to break down enamel and cause tooth decay. The tests included glucose, lactose, mannitol and galactose fermentation along with the detection of acid and gas production. Biochemical tests included oxidase, catalase, methyl red, vogues-proskauer test, and hydrogen sulphide production test.

Library Preparation, Whole Genome Sequencing And Bioinformatic Analysis

DNA was extracted from the biofilm producing bacterial isolates using the Zymo Research Quick-DNA^{tm} Miniprep Plus Kit following the manufacturer's protocol. DNA purity was determined using a NanoDrop One Spectrophotometer (ThermoFisher). DNA quantification was subsequently carried out using Qubit dsDNA HS kit and measured with a Qubit Flex fluorimeter (ThermoFisher). DNA size distribution was determined using an Agilent 2100 BioAnalyzerwith High Sensitivity DNA chip (Agilent Technologies). Fragmentation (amplification and indexing) and library preparation was carried out followed by tagmentation to add indexes. Library preparation was carried out using NexteraXT library preparation kit **(Matranga et al., 2016)**. Water was used as negative control and recorded identifiers (IDs) on sample sheets after which concentration with sample IDs were obtained and labelled. Whole genome sequencing (WGS) was conducted on an Illumina NextSeq 2000 system using Illumina's NextSeq 2000 P3 Reagents (300 cycles).

Raw FASTQ reads were processed with the TheiaProk Illumina pipeline (https://theiagen.notion.site/TheiaProk-Workflow-Series-cc66a9dc42a144a789990935465bc9ff) for quality assessment, *de novo* genome assembly, genome annotation, taxonomic characterization, and antimicrobial resistance prediction of the bacterial genomes. Isolates that did not pass the quality assessment criteria were excluded from further analyses as they were deemed contaminated or mixed isolates.

In-silico detection of biofilm-production genes was determined using Geneious prime. This was done by mapping biofilm-production genes to reference sequences of bacterial genomes. The genes responsible or up-down regulated in bacterial biofilm formation downloaded from NCBI were *algC*, *comD*, *comE*, *icaA*, *icaC*, *icaD*, *rpoS* and *scaA*. These genes were then mapped to reference sequences of each of the genomically identified bacteria downloaded in fasta format from the NCBI database **(Almamoori *et al*., 2023)**.

Preparation Of $Tife_2O_4$ Particles

$TiFe_2O_4$ particles were prepared as previously reported **(Adewuyi and Oderinde, 2022)**. Briefly, 200 mL solutions of $Ti(NO_3)_4$ (0.2 M) and $FeCl_3.6H_2O$ (0.4 M) were stirred in a conical flask for 1 h at 70ºC. During the stirring, oleic acid (10 mL) was added as a capping agent to control the particle growth. Ammonia solution (10 mL) was added after 1 h of stirring to bring the reaction solution to a pH range of 9-11 and to precipitate the $TiFe_2O_4$ particles from solution. This was further stirred for 30 min before the reaction was terminated. The $TiFe_2O_4$ particles in the reaction solution were filtered (using Whatman paper) and washed severally with deionized water. The filtrate was air-dried overnight and later transferred to a muffle furnace for 12 h at 600ºC.

Harvesting Plants And Obtaining Plant Extracts

Fresh leaves and stem barks of *Senna podocarpa* were obtained from the Redeemer's University environment and taken to the herbarium of the University of Lagos for identification and storage (Guill. & Perr. Luck.; Family: Fabaceae; LUH: 9425; Determinant: Dr. Niaza George; Date: 27th May, 2022). were obtained by air-drying the plant parts in the shade at ambient temperature and blending to obtain fine ground powder **(Kumar *et al*., 2021)**. The plant was obtained from Redeemer's University environment and samples were taken to the Herbarium of the University of Lagos for identification and storage. These powders were then separately extracted using ethanol or distilled water via cold extraction process by soaking them in a 2 L conical flask in the dark for 24 h. The extracts obtained were concentrated using a rotary evaporator. Extracts obtained were then labelled and stored in a refrigerator for further use.

Preparation Of $Tife_2O_4$ Dopped *Senna Podocarpa* Extracts

$TiFe_2O_4$@SPSBAE and $TiFe_2O_4$@SPSBEE were prepared by dispersing $TiFe_2O_4$ (1 g) in *Senna podocarpa* aqueous stembark extract (100 mL) and *Senna podocarpa* ethanol stembark extract (100 mL), respectively. Similarly, $TiFe_2O_4$@SPLAE and $TiFe_2O_4$@SPLEE were prepared by dispersing $TiFe_2O_4$ (1 g) in *Senna podocarpa* aqueous leaf extract (100 mL) and *Senna podocarpa* ethanol leaf extract (100 mL), respectively. The $TiFe_2O_4$ dispersed solution was kept at 60ºC while stirring for 1 h. The resulting product was filtered and washed severally with extraction solvent (ethanol for $TiFe_2O_4$@SPSBEE or $TiFe_2O_4$@SPLEE and distilled water for $TiFe_2O_4$@SPSBAE or $TiFe_2O_4$@SPLAE). The resulting $TiFe_2O_4$@SPSBAE, $TiFe_2O_4$@SPSBEE, $TiFe_2O_4$@SPLAE and $TiFe_2O_4$@SPLEE were air-dried overnight.

Characterisation Of $Tife_2O_4$, $Tife_2O_4$@Spsbae, $Tife_2O_4$@Spsbee, $Tife_2O_4$@Splae And $Tife_2O_4$@Splee

The functional groups present in $TiFe_2O_4$, $TiFe_2O_4$@SPSBAE, $TiFe_2O_4$@SPSBEE, $TiFe_2O_4$@SPLAE and $TiFe_2O_4$@SPLEE were determined by FTIR (Shimadzu FTIR-8400S). The surface morphology was determined using SEM (JOEL Co Japan) to understand the surface structure while the X-ray diffraction pattern of the particles was analysed on X-ray diffractometer (in the range 5 to 90º) at 2θ.

Antimicrobial Susceptibility And Minimum Inhibitory Concentration

The antimicrobial susceptibility screening was conducted on the *Senna podocarpa* aqueous extract (SPD@Aqua), *Senna podocarpa* ethanol extract (SPD@Etha), $TiFe_2O_4$, $TiFe_2O_4$@SPSBAE, $TiFe_2O_4$@SPSBEE, $TiFe_2O_4$@SPLAE and $TiFe_2O_4$@SPLEE. An initial concentration of 2.50 mg/L of the test samples was used for the susceptibility study via the agar well diffusion method of the 0.5 MacFarland standard of suspensions of corresponding bacteria as previously reported by **Jensen *et al*. (2019)**. The MacFarland standard exhibits the optical density of a 1.5 x 10^8CFU/mL of bacteria cells. Zones of inhibition were recorded in triplicates and subsequently, minimum inhibitory concentration (MIC) was detected using 96 well microplates and recording of the optical densities at 6 h intervals during incubation at 35ºC for 24-48 h of the bacterial suspensions in each

well-treated with antimicrobial, using an ELISA reader and by visual inspection **(Belanger and Hancock, 2021)**. The MIC is given as the lowest concentration of the active test sample which can inhibit the growth of the biofilm-producing microorganisms.

Animal Care Management, Sacrifice Of Animals And Obtaining Blood Samples

Adult rats of the Wistar strain, weighing 80 – 120 g were obtained from the animal house, Redeemer's University, Ede Osun State. Rats were fed on a commercial pelleted diet (Ladokun Feeds Ibadan, Nigeria) and drinking water ad libitum, maintained under standard laboratory conditions and subjected to a natural photoperiod of 12 h light/12 h dark cycle Rats were sacrificed by cervical dislocation and blood samples were collected by cardiac puncture into centrifuge tubes; centrifuged at 3000 g for 10 min in a laboratory centrifuge to obtain the plasma.

Testing The Effects Of $Tife_2O_4$@Spsbae On Blood And Growth Parameters

Only $TiFe_2O_4$@SPSBAE was used for the animal experiment because it exhibited the best antimicrobial activity among the test samples. Wistar rats (28) distributed into four groups of seven animals each were treated intraperitoneally, once daily, for 14 days: Group A: Control, received normal saline; Group B: 5 mg/kg $TiFe_2O_4$@SPSBAE; Group C: 10 mg/kg $TiFe_2O_4$@SPSBAE; Group D: 20 mg/kg $TiFe_2O_4$@SPSBAE. At the end of treatment, samples were collected for biochemical analyses.

Determination Of Metabolite Concentrations

Using Randox® reagents, spectrophotometer was used to determine the concentration of plasma concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), Alkaline Phosphatase (ALP), gamma-glutamyl transferase (GGT), albumin, total cholesterol, urea, and creatinine, bilirubin, uric acid, triglycerides (TG), high-density lipoprotein (HDL), sodium and potassium were determined **(Habibu *et al*., 2023)**.

Histological Assessment

Livers and kidneys from rats of all the groups were fixed in 10% formaldehyde, dehydrated in graded alcohol and embedded in paraffin. Fine sections were obtained, mounted on glass slides and counter-stained with hematoxylin-eosin (H&E) and Periodic Acid Schiff (PAS) for light microscopic analyses. The slides were coded and examined by a histopathologist.

RESULT

A total of one hundred and twenty-one (121) schoolchildren aged 4-13 were sampled in this study comprising sixty-four (64) males, and fifty-seven (57) females. Forty-four (44) pupils were sampled from Splendid Steps Nursery and Primary School, Lekki Lagos and seventy-seven (77) pupils were sampled from two schools in Ede, Osun state (Baptist Day Nursery and Primary School n=73; and Ogbon Oluwa Nursery and Primary School n=4). A total of 142 bacterial isolates belonging to 19 biochemically distinct groups were obtained and 40 tested positive for the formation of biofilms. The biofilm-forming bacteria which were sequenced were subjected to antimicrobial susceptibility testing. Our results showed that 4 out of the 23 successfully sequenced bacteria were susceptible to the antimicrobial actions of the nanoparticle-plant extract complex ($TiFe_2O_4$@SPSBAE).

Morphological And Biochemical Characterisation Of Biofilm Producing Isolates

We determined the phenotypic diversity of the bacterial colonies from which isolates were obtained and biochemically characterized (Table 1). The parameters observed were shape, size, surface, colour, opacity, elevation and margin. Table 2 shows the biochemical characteristics of the various isolates. Bergey's Manual of Determinative Bacteriology was used to obtain schemes for tentatively placing the bacteria into distinct taxa. However, molecular genomics tools were used as the gold standard for identifying and classifying the biofilm-producing isolates.
All isolates were found to be Gram-negative and aerobic except C28 which was found to be Gram-positive. There was no observed reduction of hydrogen sulphide by C28. All isolates formed a black colouration when grown on brain-heart infusion agar with congo-red as an indicator, indicating biofilm production.

Table 1 Morphology of the various oral bacterial isolates

S/N	Shape	Size	Surface	Colour	Opacity	Elevation	Margin
1	Punctiform	small	Rough	Orange	Translucent	Umbonate	Curled
2	Punctiform	small	Glistening	Creamy-white	Opaque	Convex	Filamentous
3	Irregular	large	Smooth	green	Translucent	Flat	Even
4	Irregular	large	Rough	White	Opaque	Flat	Lobate
5	Filamentous	Small	Rough	White	Transparent	Flat	Wavy
6	Irregular	Small	Smooth	Creamy-white	Opaque	Flat	Wavy
7	Irregular	Large	Rough	Creamy-white	Translucent	Flat	Filamentous
8	Punctiform	Small	Glistening	Yellow	Translucent	Raised	Lobate
9	Irregular	Large	Rough	White	Opaque	Umbonate	Filamentous
10	Circular	Large	Rough	White	Opaque	Umbonate	Filamentous
11	Irregular	Small	Glistening	White	Opaque	Convex	Filamentous
12	Irregular	Small	Wrinkled	Creamy-white	Opaque	Umbonate	Wavy
13	Circular	Small	Smooth	Creamy-white	Opaque	Flat	Even
14	Punctiform	Small	Smooth	Creamy-white	Opaque	Umbonate	Even

Table 2 Biochemical characterization of the isolates uncovered in this study

S/N	Gas	H_2S	Indole	Maltose	Sucrose	Glucose	Lactose	Mannose	Citrate	MR	VP	Urease	Motility	Oxidase	Catalase	Gram	Shape
1	+	+	-	-	-	+	-	-	+	+	-	+	+	-	+	-	Bacillus
2	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	-	Bacillus
3	-	+	-	+	-	-	-	+	-	-	+	NA	-	+	+	+	Coccus
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Coccus
5	+	-	-	+	+	+	+	+	+	+	+	+	-	-	+	+	Coccus
6	+	+	+	NA	-	+	+	+	-	+	-	-	+	-	+	-	Bacillus
7	-	+	NA	+	+	+	+	+	+	-	+	-	+	+	+	+	Bacillus
8	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	Coccus
9	+	-	NA	NA	NA	-	NA	NA	NA	NA	NA	-	+	+	+	-	Bacillus
10	+	-	-	+	+	+	+	-	-	-	-	-	+	-	-	+	Bacillus
11	-	+	-	+	+	+	+	+	NA	+	-	-	-	NA	-	-	Bacillus
12	+	+	-	+	+	+	+	+	+	+	-	+	+	-	+	-	Bacillus
13	-	-	+	-	-	+	-	+	-	+	+	-	+	-	+	-	Bacillus
14	-	-	-	+	-	NA	-	+	-	+	-	-	-	-	+	-	Bacillus
15	-	-	-	-	-	+	-	+	-	+	-	-	-	-	+	-	Bacillus
16	-	NA	+	NA	-	+	-	+	NA	NA	-	NA	+	+	+	-	Bacillus
17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Spiral
18	+	+	+	NA	+	+	+	+	+	NA	+	+	+	+	-	-	Bacillus
19	-	NA	-	NA	+	+	+	+	NA	-	-	+	-	+	+	-	Coccus

+/-: positive or negative reaction; NA: not applicable; MR: Methyl red; VP:

Vogues-proskaeurGenomic Identification Of Biofilm-Forming Isolates

Using Next-Generation Sequencing (NGS), we identified and classified all biofilm-producing isolates based on their entire genetic make-up. BUSCO (Benchmarking Universal Single-copy Orthologs) results with C (Complete) and S (Single-copy) scores of greater than 90% indicated sequencing success and completeness. Other parameters important for the determination of completeness were also taken into consideration with details in an unpublished pipeline. We determined the presence of 9 genes indicated in biofilm-formation including *algC*, *comD*, *comE*, *icaA*, *icaC*, *icaD*, *rpoS* and *scaA* and we also screened their genomes for the presence of antimicrobial resistance genes, virulence genes and plasmids. The genomic identification is shown in **Table 3**. The identification match for all the identified strains is higher than 98 % confirming the presence of these biofilm-producing pathogens from the studied population. Bacterial sequencing files that passed with predicted taxonomy out of 7 isolates obtained during this study are *Stenotrophomonas maltophilia* (n=3) and *Pseudomonas stutzeri* (n=1) (**Table 3**). Other isolates failed most likely due to contamination during culture. C2, C33 and C38 were identified as *Stenotrophomonas maltophilia*. Table 3 shows the names, assembly lengths and BUSCO results of the 4 successfully sequenced biofilm-forming isolates susceptible to $TiFe_2O_4$@SPSBAE (C2, C28, C33 and C38). **Table 4** shows the antimicrobial resistance genes present and their subclasses. The biofilm-producing (or biofilm-forming) bacteria, successfully sequenced and susceptible to $TiFe_2O_4$@SPSBAE were composed of *Stenotrophomonas maltophilia* (C2, C33, and C38) and *Pseudomonas stutzeri* (C28).

Genes responsible for biofilm production determined in silico include *algC, comD, comE, icaA, icaB, icaC, icaD, rpoS, scaA*. Results from this characterisation showed isolate C28 to contain *algC* and *rpoS* genes. Other isolates (C2, C33 and C38) did not contain any of the genes.

Isolate C2 had an assembly length of 4.4 Mb (mega base pairs) C28 had an assembly length of 4.3 Mb, C33 had an assembly length of 4.4 Mb while C38 had an assembly length of 4.5 Mb. These observations reveal that C38 has a larger assembly length than C33 and C2 despite being the same organism. These differences, coupled with the reality that isolate C28 was sampled from Lagos state while C2, C33 and C38 were obtained from test subjects in Osun state, therefore advising the study of the genes present in the genome of each isolate. C2 had the highest percentage of genes identified in the assembly with a total overall score of 99.6% followed by C38 (99.5 %). C33 had a score of 99.4% while C28 had the lowest overall score of 98.6%. However, these findings show that they all pass the criteria (>95%) for further genomic analysis. The DNA fingerprints of the isolates show *Stenotrophomonas maltophilia* as having the highest number of single-copy control at 99.2% (C2 and C38) and 99.0% (C33) with the higher number of genes used (1152) while *Pseudomonas stutzeri* showed a single-copy control of 98.6% with the lowest number of genes used (n=782).

The antimicrobial resistance gene expressions of the isolated strains are presented in **Table 4**. C2, C33 and C38 exhibited antimicrobial resistance potentials except C28 strains. C2 was found to contain antimicrobial resistance genes (n=8). They are *aph(6), smeF, blaL2, blaL1, emrB, emrA, emrC, qnrD1* (**Table 4**) with AMR subclasses of aminoglycoside, beta-lactam, carbapenem, efflux and quiolone. C33 was found to contain AMR genes (n=8) including *blaL1, aph(6), blaL2, emrB, emrA, emrC, qnrD1, smeF*, with subclasses aminoglycosides, beta-lactam, carbapenem, efflux and quinolone. C38 was found to contain AMR genes (n=8) including *sul2, aph(6), emrB, emrA, emrC, blaL2, smeF, blaL1* with subclasses including aminoglycoside, beta-lactam, carbapenem, efflux and sulfonamide. However, 2 of the isolates (C28 and C33) did not harbour any AMR genes. There were no virulence genes detected in the characterisation. There were no virulence genes detected in the characterisation, however, C2 contained the Col3M plasmid. The resistance observed is connected to the isolates' resistance to ethanol and aqueous extracts of *Senna podocarpa* to varying degrees and their resistance to $TiFe_2O_4$. C2 and C28 exhibited resistance to the SPD@Aqleaf, SPD@Aqstem and $TiFe_2O_4$ as seen in **Table 5**.

Table 3 Genomic identification of biofilm-forming isolates

Sample ID	Organism	Assembly length	BUSCO Results
C2	*Stenotrophomonas maltophilia*	4461841	C:99.6%[S:99.2%,D:0.4%],F:0.3%,M:0.1%,n:1152
C28	*Pseudomonas stutzeri*	4355763	C:98.6%[S:98.6%,D:0.0%],F:0.0%,M:1.4%,n:782
C33	*Stenotrophomonas maltophilia*	4469327	C:99.4%[S:99.0%,D:0.4%],F:0.5%,M:0.1%,n:1152
C38	*Stenotrophomonas maltophilia*	4569838	C:99.5%[S:99.2%,D:0.3%],F:0.3%,M:0.2%,n:1152

Table 4 The antimicrobial resistance genes of the isolates

Sample ID	Antibiotic Resistance Genes (ARGs)	AMR Subclasses
C2	*aph(6), smeF, blaL2, blaL1, emrB, emrA, emrC, qnrD1*	Aminoglycoside, Beta-Lactam, Carbapenem, Efflux, Quinolone
C28	No AMR genes detected	No AMR genes detected
C33	*blaL1, aph(6), blaL2, emrB, emrA, emrC, qnrD1, smeF*	Aminoglycoside, Beta-Lactam, Carbapenem, Efflux, Quinolone
C38	*sul2, aph(6), emrB, emrA, emrC, blaL2, smeF, blaL1*	Aminoglycoside, Beta-Lactam, Carbapenem, Efflux, Sulfonamide

Preparation And Characterization Of $Tife_2O_4$, $Tife_2O_4$@Spsbae, $Tife_2O_4$@Spsbee, $Tife_2O_4$@Splae And $Tife_2O_4$@Splee

Figures 1 and 2 show the characterization of $TiFe_2O_4$, $TiFe_2O_4$@SPSBAE, $TiFe_2O_4$@SPSBEE, $TiFe_2O_4$@SPLAE and $TiFe_2O_4$@SPLEE. Figure 1 shows the FTIR and XRD of $TiFe_2O_4$, $TiFe_2O_4$@SPSBAE, $TiFe_2O_4$@SPSBEE, $TiFe_2O_4$@SPLAE and $TiFe_2O_4$@SPLEE while Figure 2 shows the SEM images of $TiFe_2O_4$, $TiFe_2O_4$@SPSBAE, $TiFe_2O_4$@SPSBEE, $TiFe_2O_4$@SPLAE and $TiFe_2O_4$@SPLEE.

The FTIR result comparing the functional groups present in $TiFe_2O_4$, $TiFe_2O_4$@SPSBAE, $TiFe_2O_4$@SPSBEE, $TiFe_2O_4$@SPLAE and $TiFe_2O_4$@SPLEE is presented in **Fig. 1a**. On a closer look, all spectrum revealed signals at 3405 cm^{-1}, which may be attributed to the O-H stretching group. The Fe-Ti vibrational appeared at 1580 cm^{-1} while the Ti-O vibrations appeared at 653 cm^{-1}. The C-H stretching of alkane corresponding to methylene (2845 cm^{-1}) and methyl group (2934 cm^{-1}) appeared in $TiFe_2O_4$@SPSBAE, $TiFe_2O_4$@SPSBEE, $TiFe_2O_4$@SPLAE and $TiFe_2O_4$@SPLEE except in $TiFe_2O_4$ which may be attributed to the organic molecules emanating from the ethanol and aqueous extracts from the leaf and stembark. The C=O stretching of acid and C-O-C vibration of the ester functional group appeared at 1522 and 1107 cm^{-1}, respectively suggesting the presence of these groups of molecules in all the composite structures. The aromatic stretch appeared at 1502 cm^{-1}.

The XRD diffraction patterns are compared in **Fig. 1b**. The most intense peak for $TiFe_2O_4$, $TiFe_2O_4$@SPSBAE, $TiFe_2O_4$@SPSBEE, $TiFe_2O_4$@SPLAE and $TiFe_2O_4$@SPLEE was at $2\theta = 35.25°$ with plane spacing of (101) and subsequent planes of (121), (103), (004), (233), (111), (200), (220), (222), (400), (422) and (511). The average crystallite sizes (D) of $TiFe_2O_4$, $TiFe_2O_4$@SPSBAE, $TiFe_2O_4$@SPSBEE, $TiFe_2O_4$@SPLAE and $TiFe_2O_4$@SPLEE were calculated from Debye-Scherrer's expression **(Kumar *et al*., 2016)**:

$$D = \frac{K\lambda}{\beta\, Cos\theta} \quad (1)$$

K (0.89), β (width of diffraction line), θ (Bragg's angle) and λ (1.5406 Å) were substituted in Equ. 1 to calculate the value of D which is 19.22 nm for $TiFe_2O_4$ while those of $TiFe_2O_4$@SPSBAE, $TiFe_2O_4$@SPSBEE, $TiFe_2O_4$@SPLAE and $TiFe_2O_4$@SPLEE ranged from 23-26 nm. The increase in the average crystallite size may be attributed to the presence of the extracts immobilized on $TiFe_2O_4$. The SEM images (**Fig. 2**) of all the $TiFe_2O_4$ composites revealed the presence of irregularly shaped particles on their surfaces. The surfaces of $TiFe_2O_4$, $TiFe_2O_4$@SPSBEE and $TiFe_2O_4$@SPSBAE showed a regularly patterned heterogeneous surface. Surfaces of $TiFe_2O_4$@SPLAE and $TiFe_2O_4$@SPLEE revealed an irregularly patterned heterogeneous surface with $TiFe_2O_4$@SPLAE having flaked structures.

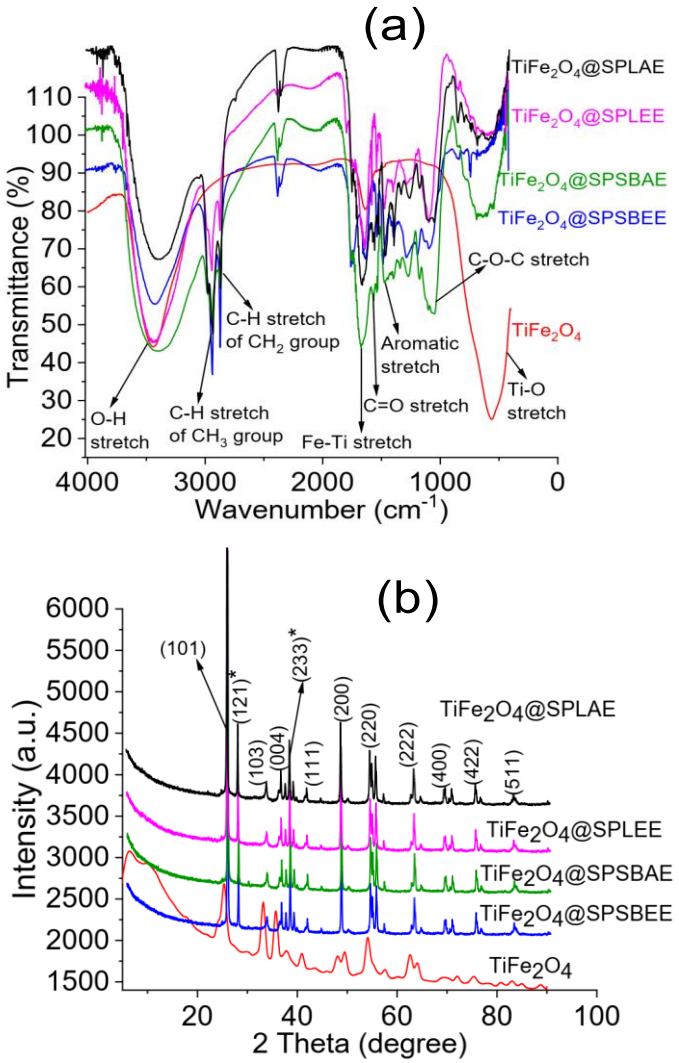

Figure 1 FTIR and XRD of $TiFe_2O_4$, $TiFe_2O_4$@SPSBAE, $TiFe_2O_4$@SPSBEE, $TiFe_2O_4$@SPLAE and $TiFe_2O_4$@SPLEE

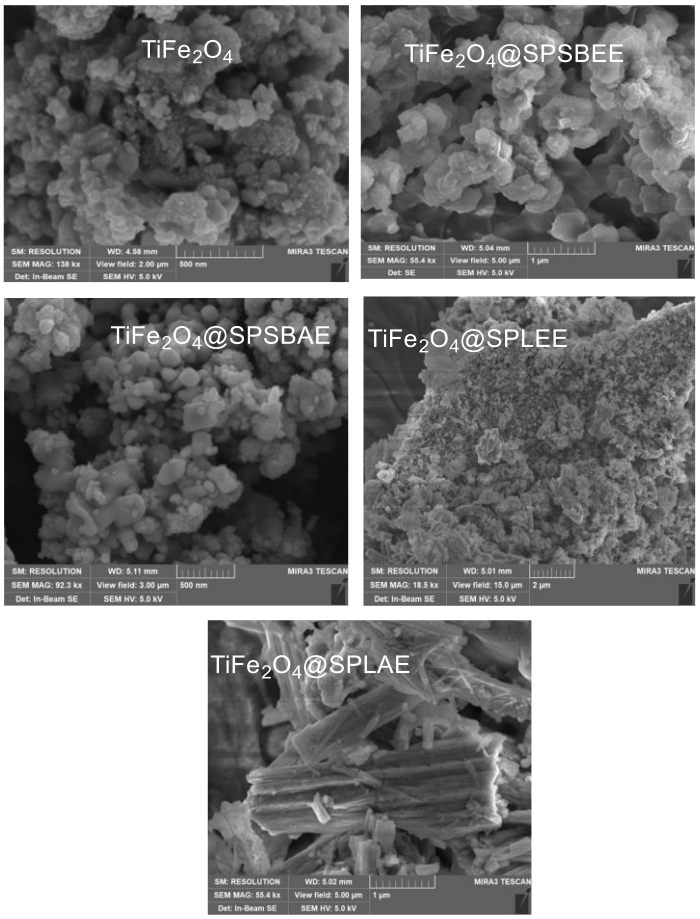

Figure 2 SEM images of $TiFe_2O_4$, $TiFe_2O_4$@SPSBAE, $TiFe_2O_4$@SPSBEE, $TiFe_2O_4$@SPLAE and $TiFe_2O_4$@SPLEE

Antimicrobial Susceptibility And Minimum Inhibitory Concentration

Table 5 details the outcome of the susceptibility of the four biofilm-producing bacteria isolated and sequenced in this study. MICs were recorded in **Table 6** for complexes that exhibited zones of inhibition greater than 15 mm. $TiFe_2O_4$@SPSBAE had the overall highest zones of inhibition against the three isolates and hence, was selected for the toxicology study. MIC plays an important role in drug potency. Low MIC is required which indicates that low active concentration can achieve the required results or efficacy. $TiFe_2O_4$@SPSBAE was selected for toxicological studies because it elicited the most effective inhibitory activity against the biofilm-producing bacteria at low concentrations. **Table 7** shows Comparison of MIC of most active complex, $TiFe_2O_4$@SPSBAE, with previous studies. The antimicrobial capacity by demonstrated zone of inhibition exhibited by the pristine aqueous and ethanol extracts of the leaf and stembark were enhanced when doped with $TiFe_2O_4$. The highest zone of inhibition was 26.5±0.60 mm for $TiFe_2O_4$@SPLEE against C2 while the least was found to be 7.5±0.3 mm against C33 by $TiFe_2O_4$.

The result demonstrates that $TiFe_2O_4$@SPSBAE, having the largest diameter zones of inhibition against the four isolates and the lowest MIC, is the most effective of all 9 test compounds against the biofilm-producing bacteria isolated in this study. The effectiveness of an antimicrobial agent at low concentrations provides an advantage in combating infections as lower concentrations are required, to eliminate pathogens and also save costs of treatment.

Table 5 Antimicrobial susceptibility screening

Samples	Microorganisms with zone of inhibition			
	C2 (mm)	C28 (mm)	C33 (mm)	C38 (mm)
Control (-)	-	-	-	-
SPD@Eth.leaf	13.0±0.19	8.2±1.18	9.6±0.17	17.7±0.09
$TiFe_2O_4$@SPLEE	26.5±0.60	20.8±0.22	12.8±0.55	25.0±0.10
SPD@Ethstem	13.1±0.60	8.5±0.10	9.3±0.26	15.5±0.17
$TiFe_2O_4$@SPSBEE	21.8±1.1	15.8±1.24	13.3±0.0	18.7±0.45
SPD@Aqleaf	-	8.0±1.15	15.5±0.16	9.3±0.06
$TiFe_2O_4$@SPLAE	21.5±2.96	15.0±0.21	20.4±0.18	20.4±0.02
SPD@Aqstem	-	10.1±0.0	16.4±0.45	8.0±0.80
$TiFe_2O_4$@SPSBAE	25.0±0.18	20.6±0.27	15.2±1.25	20.7±1.76
$TiFe_2O_4$	13.5±0.36	-	7.5±0.3	10.6±0.25

Zone diameter mean value ± SEM including cork borer with diameter 6.0 mm. Results differ significantly from the control ($P<0.05$). Note: '-' signifies no zone of inhibition

Table 6 Minimum inhibitory concentration of test samples

Samples	Microorganism with zone of inhibition			
	C2 (mg/L)	C28 (mg/L)	C33 (mg/L)	C38 (mg/L)
SPD@Ethleaf	-	-	-	0.63
$TiFe_2O_4$@SPLEE	0.16	0.16	-	1.25
SPD@Eth.stem	-	-	-	0.31
$TiFe_2O_4$@SPSBEE	>2.5	0.63	-	2.5
SPD@Aqleaf	-	-	>2.5	-
$TiFe_2O_4$@SPLAE	0.63	1.25	>2.5	0.31
SPD@Aqstem	-	-	>2.5	-
$TiFe_2O_4$@SPSBAE	0.94	0.16	0.156	0.16
$TiFe_2O_4$	-	-	-	-

Our results (Table 7) show $TiFe_2O_4$@SPSBAE compared favourably with previously reported antimicrobial agents against the studied pathogens.

Table 7 Comparison of MIC of $TiFe_2O_4$@SPSBAE with previous studies

Agent	Organism	MIC (mg/L)	Reference
Minocycline	*Stenotrophomonas maltophilia*	0.5	**Fratoni *et al*., 2022**
Delafloxacin	*Stenotrophomonas maltophilia*	0.5	**Vialichka *et al*., 2022**
Chlorohexidine	*Stenotrophomonas maltophilia*	64	**Anari *et al*., 2022**
Imipenem	*Stenotrophomonas maltophilia*	32	**Sarhan *et al*., 2018**
Amoxicillin clavulanic acid	*Pseudomonas stutzeri*	2-8	**Molina-Menor *et al*., 2023**
Azithromycin	*Pseudomonas stutzeri*	1.5	**Molina-Menor *et al*., 2023**
Chloroxylenol	*Pseudomonas stutzeri*	300-500	**Maillard, 2022**
green SMZnO-NPs compound	*Pseudomonas stutzeri*	200	**Subramanian *et al*., 2022**
$TiFe_2O_4$@SPLAE	*Stenotrophomonas maltophilia* *Pseudomonas stutzeri*	0.31 1.25	This study.

Effects Of $Tife_2O_4$@Spsbae On Growth Parameters

Table 8 shows the results for the organ and relative organ weights of rats exposed to **$TiFe_2O_4$@SPSBAE** at different concentrations.

Based on the results, there were no significant differences in liver and kidney weights and the same trend was observed in the relative weights of the liver and kidney when compared to the control. Organ and relative organ weights are used as measures of growth to determine the toxicological effects of medicinal plants or compounds from other sources on living systems. *Senna podocarpa* titanium ferrite nanoparticles ($TiFe_2O_4$@SPSBAE) did not cause any significant changes on organ and relative organ weights of the liver and the kidney.

Effects Of $Tife_2O_4$@Spsbae On Liver Function Parameters

Plasma liver function parameters including AST, ALT, ALP, and total bilirubin were not significantly altered in rats that received **$TiFe_2O_4$@SPSBAE** at different concentrations. Meanwhile, plasma albumin level was found to significantly increase ($p<0.05$) in rats that received 20 mg/kg **$TiFe_2O_4$@SPSBAE** as shown in figure 2.

The effects of $TiFe_2O_4$@SPSBAE on liver function were evaluated by determining the levels of plasma parameters including AST, ALT, ALP, bilirubin and albumin. There was no significant alteration in the levels of AST, ALT and bilirubin. Contrarywise a significant elevation was observed in albumin levels in the group that received 20 mg/kg **$TiFe_2O_4$@SPSBAE**.

Table 8 Effects of $TiFe_2O_4$@SPSBAE on organ and relative organ weights of liver and kidney

Group	Liver weight (g)	Relative liver weight (g/100g body weight)	Kidney weight (g)	Relative kidney weight g/100g body weight)
Control	5.94±0.29	3.28±0.22	1.24±0.07	0.69±0.05
5 mg/kg $TiFe_2O_4$@SPSBAE	5.96±0.23	3.20±0.13	1.23±0.03	0.66±0.02
10 mg/kg $TiFe_2O_4$@SPSBAE	5.81±0.26	3.36±0.24	1.19±0.04	0.69±0.04
20 mg/kg $TiFe_2O_4$@SPSBAE	6.13±0.12	3.36±0.12	1.26±0.03	0.69±0.02

Each value represents the mean ± SEM of 7 animals. [a] Values differ significantly from the control ($P<0.05$).

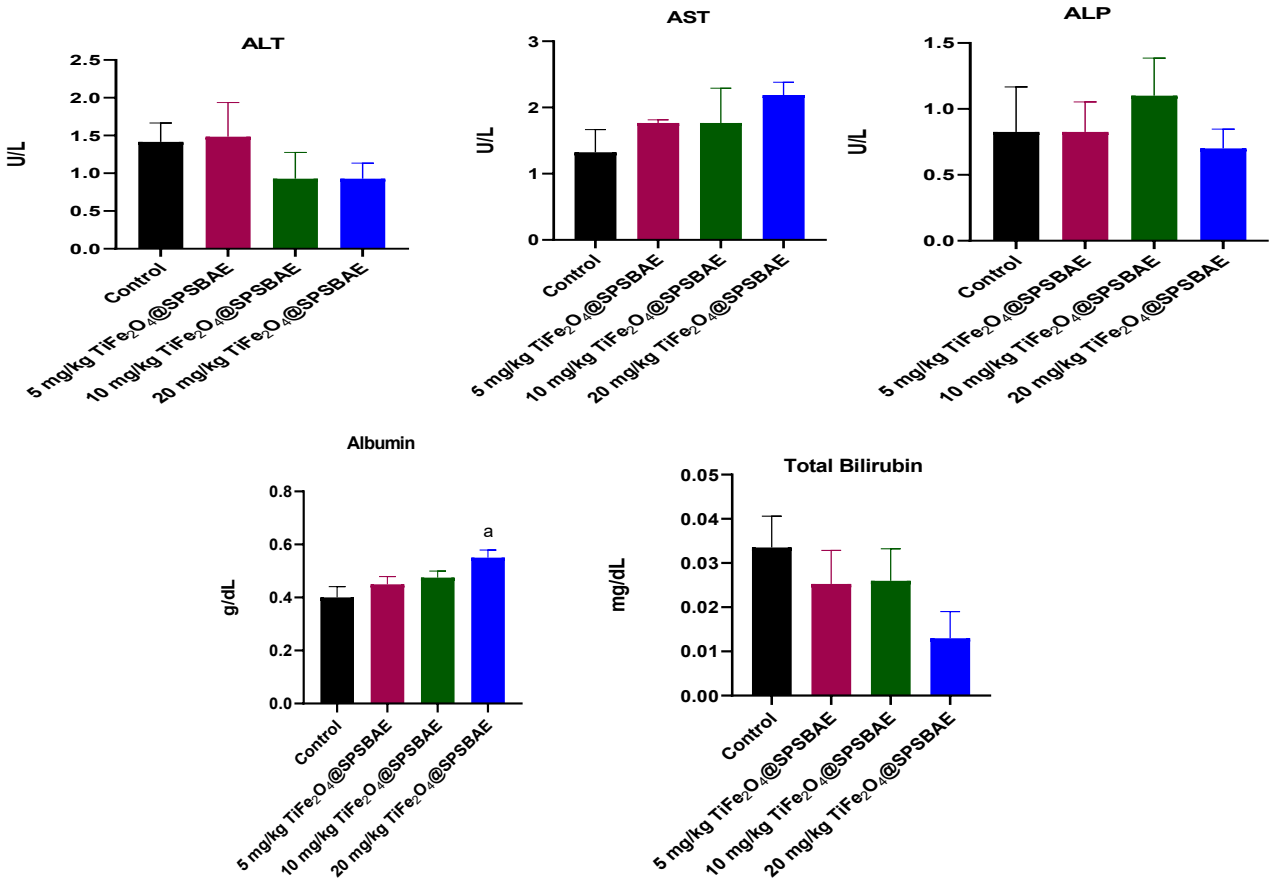

Figure 2 Effects of $TiFe_2O_4$@SPSBAE on plasma liver function parameters. Each value represents the mean ± SEM of 7 animals. [a] Values differ significantly from the control ($P<0.05$). AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase.

Effects Of $Tife_2O_4$@SPSBAE On Kidney Function Parameters

The results displayed in Figure 3 showed that administration of**$TiFe_2O_4$@SPSBAE** did not elicit any significant changes in plasma creatinine and urea levels in rats. However, a significant rise in uric acid levels was observed in rats that received 5, 10, and 20 mg/kg dosages of **$TiFe_2O_4$@SPSBAE**. Moreover, plasma electrolyte levels including sodium and potassium were not significantly altered via the administration of **$TiFe_2O_4$@SPSBAE** in rats.

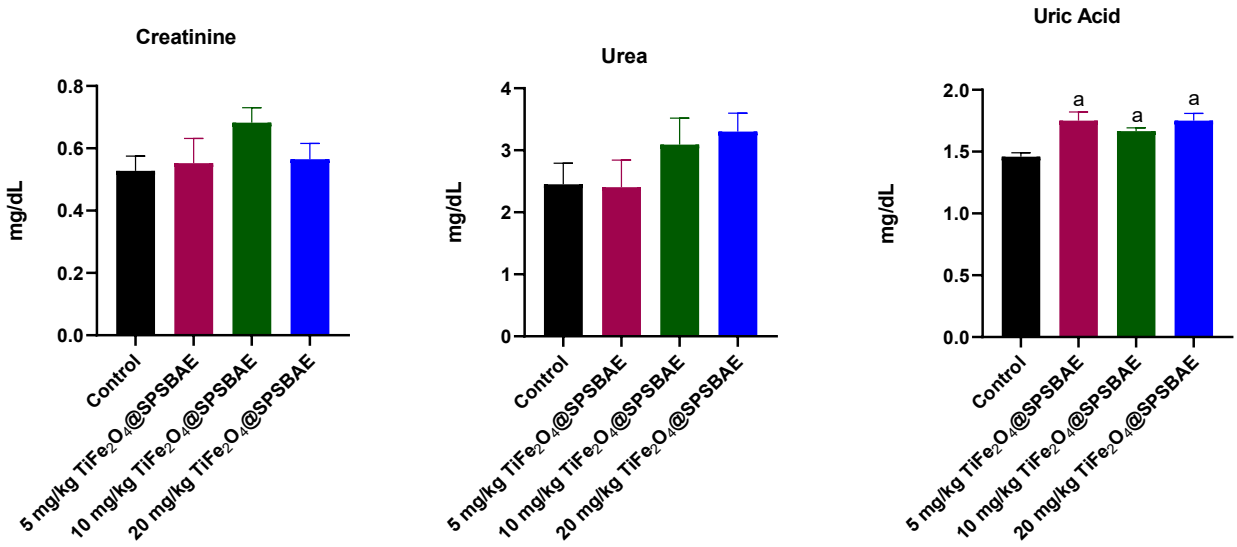

Figure 3 Effects of **$TiFe_2O_4$@SPSBAE** on plasma creatinine, urea and uric acid levels. Each value represents mean ± SEM of 7 animals. [a] Values differ significantly from the control (P<0.05).

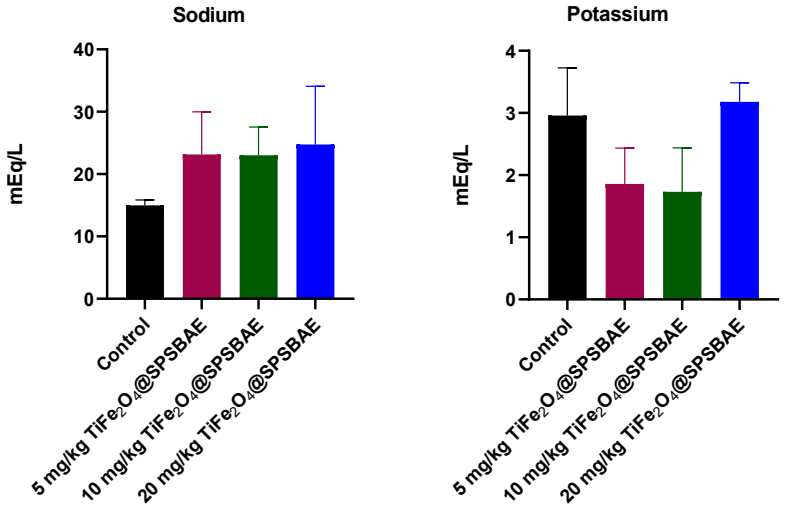

Figure 4 Effects of **$TiFe_2O_4$@SPSBAE** on plasma electrolytes. Each value represents mean ± SEM of 7 animals. [a] Values differ significantly from the control (P<0.05).

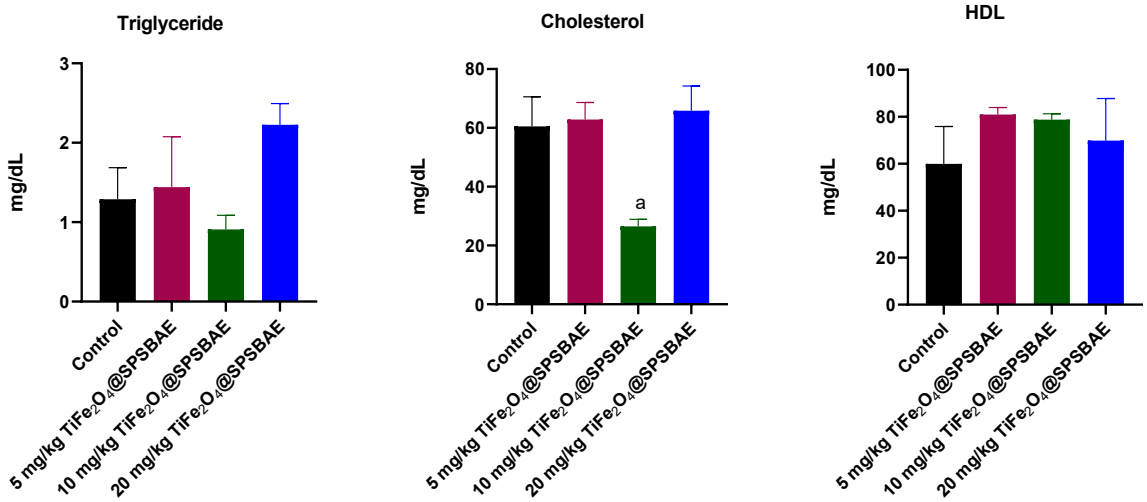

Figure 5 Effects of $TiFe_2O_4$@SPSBAE on serum lipid profile indices. Each value represents mean ± SEM of 7 animals. [a] Values differ significantly from the control (P<0.05). HDL: high-density lipoprotein.

Effects Of $Tife_2O_4$@SPSBAE On Serum Lipid Profile Indices

The effects of $TiFe_2O_4$@SPSBAE on plasma lipid profile including triglyceride, cholesterol and HDL are shown in Figure 5. Rats that received varied concentrations of $TiFe_2O_4$@SPSBAE did not demonstrate any significant changes in their triglyceride, cholesterol and HDL levels when compared to the control group.

Histology

Plate 1 shows liver sections photomicrographs of rats treated with $TiFe_2O_4$@SPSBAE and stained with periodic acid Schiff.

Plate 1 shows liver sections photomicrographs of rats treated with $TiFe_2O_4$@SPSBAE and stained with periodic acid Schiff. The control group shows normal histoarchitecture with central vein and sinusoids. The 5 mg/kg, 10 mg/kg and 20 mg/kg group did not show any significant change in liver histoarchitecture.

Plate 2 shows kidney section photomicrographs of rats that received $TiFe_2O_4$@SPSBAE and stained with periodic acid Schiff. The control and 5 mg/kg groups showed normal kidney histoarchitecture with well-delineated glomerulus, distal and proximal convoluted tubules. However, the 10 mg/kg and 20 mg/kg groups demonstrated slightly congested glomerulus (red arrow) which might be due to mild inflammation.

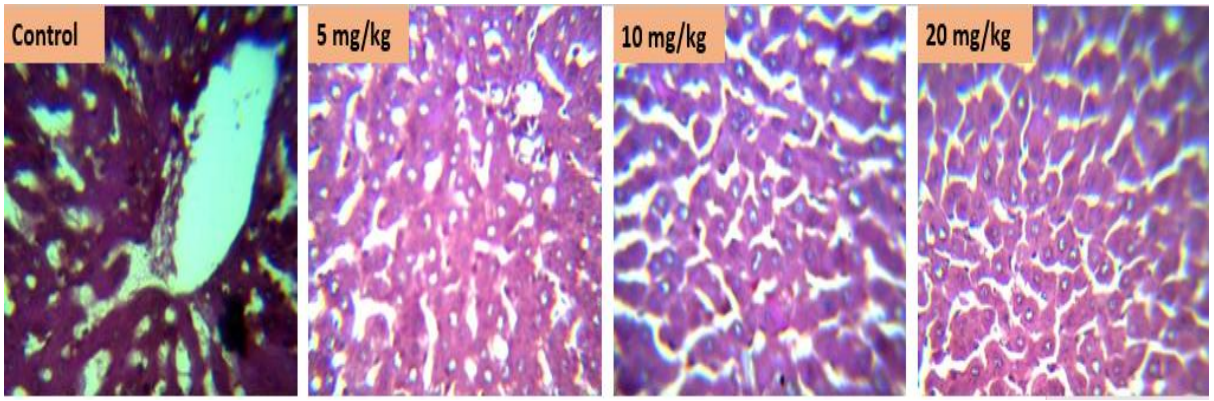

Plate 1 Photomicrographs of liver sections of Wistar rats exposed to $TiFe_2O_4$@SPSBAE. PAS x40

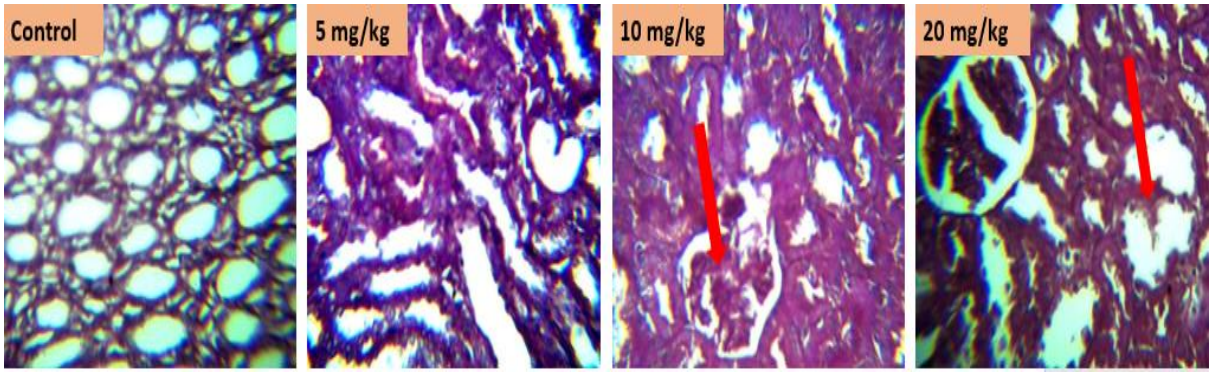

Plate 2 Photomicrographs of kidney of Wistar rats exposed to **$TiFe_2O_4$@SPSBAE**. The red arrow indicates a congested glomerulus.

DISCUSSION

This study investigated and characterized antimicrobial *Senna podocarpa* plant extracts along with titanium ferrite nanoparticles against oral biofilm-producing bacteria found to possess antimicrobial resistance genes (ARGs) in a bid to discover a safe new drug. This study revealed the presence of ARGs in isolates obtained from the oral cavity of the children. In addition, biofilm producing genes were also detected in the isolates. These isolates were also observed to be susceptible to extracts of *Senna podocarpa* in combination with titanium ferrite nanoparticles ($TiFe_2O_4$@SPSBAE). The plant extracts with the nanoparticles were also observed to be safe as they do not confer any form of toxicity. This study therefore confirms the possibility of its use for the treatment of oral bacteria infection.

The occurrence of gram-positive and gram-negative biofilm-producing bacteria with various biochemical features gives an insight on the bacterial diversity in the oral cavities of pupils in the test locations. These results can advise interventions in the conditions caused by such microorganisms in resource-limited communities However, next-generation sequencing presents a more powerful tool in studying oral pathogenic bacteria. It has been reported that the production of hydrogen sulphide can help determine whether a bacterial isolate is harmful or not **(Mendes *et al*., 2021)**. Hence enzymes involved in hydrogen sulphide production can be targeted in the control of such bacteria **(Seregina *et al*., 2022)**. Growth on different culture media, including brain-heart infusion agar with congo-red as an indicator is also a useful feature that formed the foundation of this study in the detection of biofilm-production with in-silico detection of the genes responsible for biofilm-production being possible after identification of *Pseudomonas stutzeri* and *Stenotrophomonas maltophilia* and uncovering the genes that may be responsible for the formation of biofilms by these bacteria.

The *algC* gene is involved in bacterial resistance observed in previously reported clinical isolates **(Feng *et al*., 2023; Forti *et al*., 2023)**. This double-dominance of pathogen clearance evasion (biofilm production and drug resistance) would benefit from further studies especially if observed in bacteria that are becoming global threats. Similarly, *rpoS* contributes to the ecological profile of bacteria and to their ability to evade treatment by therapeutic agents. However, they may confer an antagonistic advantage in bacteria by making them useful in drug targets identification **(Shang *et al*., 2021; Zhang *et al*., 2021)**.

Not only do these pathogens form biofilms, but also, they have been linked to the cause of respiratory diseases **(Alwazzeh *et al*., 2020)**. The presence of these pathogens may be associated with the respiratory tract diseases commonly found among pupils in the South-Western part of Nigeria **(Agwu *et al*., 2015; Folayan *et al*., 2021)**. This suggests the urgent need to develop efficient and sustainable therapy for combating these pathogens. Unfortunately, the presence of antimicrobial resistance genes in *Stenotrophomonas maltophilia* and *Pseudomonas stutzeri* makes them further entrenched as drug-resistant pathogens of concern and agrees with research by **Han *et al*., (2022)**. Infections with this microorganism are notorious for resisting antibiotics, making them difficult to treat **(Brooke, 2021; Kullar *et al*., 2022)**. The presence of antimicrobial resistance genes underlies the need to find more effective therapy against pathogenic bacteria. Although previous studies have reported compounds with the capacity to mitigate the challenge posed by *Stenotrophomonas maltophilia* **(Vidigal *et al*., 2014; Shestivska *et al*., 2015; Esposito *et al*., 2020)** they are limited in completely combatting drug resistance by biofilm production. The mechanisms of susceptibility of these two multi-drug resistant bacteria to the antimicrobial activity of the compounds used in this study would hence benefit from further investigations.

Moreso, *Pseudomonas stutzeri* is also an ecologically important bacterium, found in both soil and in clinical samples and previously believed to be a less toxic microorganism. In soil, it is believed to be involved in nitrogen fixation. It has also been shown to contain enzymes that may prevent the growth of other bacteria. However, it is being studied as a growing health concern due to its resistance to antimicrobials and its implication in more severe disease progressions. There is

scanty data demonstrating its susceptibility to antimicrobials **(Fu *et al*., 2022; Midhat and Abed., 2023; Alwazzeh *et al*., 2020)** and this research provides data to guide further studies. The use of crude antimicrobials in the control of oral bacteria may also eliminate ecologically important strains of microorganisms in the oral cavity and potentially the gut.

The antimicrobial capacity by demonstrated zone of inhibition exhibited by the pristine aqueous and ethanol extracts of the leaf and stembark were enhanced when doped with $TiFe_2O_4$. Our observations in this study may be due to the fact that the $TiFe_2O_4$ particles were able to penetrate the biofilm and cells of the pathogens due to their small size giving opportunities for the extracts to access the inner systems of the organisms for antimetabolic activities **(Ozdal and Gurkok, 2022)**. The observed performance suggests the promising capacity of $TiFe_2O_4$ doped extracts as antimicrobial agents for combating biofilm-producing pathogens. Interestingly, the antimicrobial activities exhibited by $TiFe_2O_4$@SPLAE and $TiFe_2O_4$@SPLEE are better than the activities reported by **Adebayo *et al*. (2014)**.

Stenotrophomonas maltophilia was observed to be more susceptible to $TiFe_2O_4$@SPSBAE in lower doses than *Pseudomonas stutzeri*. Higher MIC values against *Pseudomonas stutzeri* further support the position, that this bacterium is a growing health concern. There is a paucity of data on reports against this bacterium. Hence, our study provides more data to guide research. The observation suggests $TiFe_2O_4$@SPSBAE as a promising antimicrobial agent with a capacity which is better than previously reported agents (Table 7). Results available suggest that mainly extremely high concentrations of bioactive agents will achieve noticeable inhibition of the pathogens.

Our results on effects of $TiFe_2O_4$@SPSBAE on growth parameters agree with a similar study involving the toxicological evaluation of *Senna alata,* a different species of the *Senna* genus, in which the aqueous extract of the plant did not cause any significant changes in the organ weights of the rats **(Ugbogu *et al*., 2016)**. This non-toxic potential of $TiFe_2O_4$@SPSBAE to organ and relative organ weights in this study is a useful feature for drug design.

Gebrezgi *et al*., 2020 reported significant elevations in liver function enzymes only at a dosage above 400 mg/kg when *Senna occidentalis* seeds were administered in mice. This supports the possibility that $TiFe_2O_4$@SPSBAE might not be toxic at lower doses. Moreover, **Gasmalbari *et al*., (2020)** reported that administration of *Senna obtusifolia*, a member of the *Senna* genus, did not produce any significant changes in serum creatinine and urea levels as also observed in this study in which no significant alteration was observed in the levels of kidney function parameters including creatine, urea, uric acid, sodium and potassium.

Administration of $TiFe_2O_4$@SPSBAE did not cause any significant elevation in lipid profile indices including triglycerides, cholesterol and high-density lipoprotein. *Senna alata* was reported to reduce lipid profile levels in male diabetic rats **(Uwazie *et al*., 2020)**.

$TiFe_2O_4$@SPSBAE did not elicit any derangements in the histoarchitecture of the liver and kidney, especially at the doses of 5 mg/kg. However, care must be taken at the higher dose levels. A similar study on the toxicity of *Senna podocarpa* pods, reported a similar result in which the aqueous infusion of the plant in rodents did not cause any changes in normal histoarchitecture of liver and kidney tissues **(Akanmu *et al*., 2005)**. This further corroborates the report of this study on the potential safe application of $TiFe_2O_4$@SPSBAE for microbial and biochemical indications.

CONCLUSION

The successful use of less-toxic, *Senna podocarpa* extracts coupled with titanium extract nanoparticles in antimicrobial chemotherapy against drug resistant oral biofilm-producing bacteria can be studied using a multi-pronged approach involving the use of tools from various inter-related disciplines including microbiology, molecular biology, bioinformatics, biochemistry and chemistry. Further research will involve taking each aspect a step further and investigating specific mechanisms of bioactivity, a metagenomic approach towards oral microbiome characterization and concentration of the compound for use in the drug design process.

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