Research Article

Effects of chemical treatments on the bioethanol yield and composition of *Isoberlinia doka* waste

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Abstract
Isoberlinia doka sawdust; an abundant waste material was pre-treated using $FeCl_3$, HCl, NaOH, sequential HCl and NaOH treatments at 121 °C for 15 min to mitigate the challenges of its utilisation as a carbon source for bioethanol production. The effects of these treatments on the biomass were evaluated using Fourier transform infrared (FTIR) spectroscopy and gravimetric methods. The capability of the pre-treated residue to produce fermentable sugars and bioethanol were also assessed. The treated biomass were saccharified using cellulase mix from *Aspergillus niger* and *Trichoderma reesi* and fermented using *Saccharomyces cerevisiae*. The result shows that the chemical treatments significantly ($p < 0.05$) diminished the lignin contents and improved cellulose content of the treated samples; evident from decreased FTIR spectral intensities related to lignin. The cellulase mixture efficiently digested the treated biomass and resulted in significant ($p < 0.05$) sugar yield compared to the untreated biomass. Although the sequential HCl and NaOH treatment had the highest reducing sugar yield (280.1 mg/g), its ethanol yield (172.2 mg/g) was low, possibly due to formation of inhibitory bye-products. The alkali treatment reduced the lignin content and resulted in the highest ethanol yield (230.7 mg/g). *Isoberlinia doka* sawdust demonstrated great potential to be used as a sustainable feedstock for bioethanol production.

Keywords Lignocellulosic biomass · *Isoberlinia doka* · Chemical pre-treatment · Bioethanol and biofuel

1 Introduction

Bioconversion of forest based agro wastes and other non-edible materials to renewable fuel are receiving great attention universally. Many nations have continuously fund researches seeking to utilize available agro wastes as sustainable substitute to fossil fuels or as primary feedstock for chemicals industries [1]. This initiative is anchored on meeting the energy needs of increasing population especially in transportation and other industrial sectors. Other reasons for these increased funding include: participating in a global transition to clean low-carbon energy systems, environmentally friendly nature of renewable fuels as well as its non-competition with human food sources [2–4]. However, a major challenge hindering the effective production of biofuels from these sources is the recalcitrance of these waste to degradation by enzymes and microorganisms [5]. Therefore, significant research effort to enhance biofuel production from sources that are economically feasible and competitive with fossil fuels have increased [1, 3, 6].

Agro wastes are among the most abundant and readily available wastes materials that can be exploited in the production of biofuels. Utilization of these cellulosic wastes via conversion to biofuels and other value-added compounds is a potential measure to having a clean and sustainable environment and reducing the production cost of biofuels [1, 7]. Bioconversion of these waste residues by the natural process of microbial degradation has been documented over the years and is a promising approach for efficient biomass utilization [8, 9]. Harnessing and optimizing this natural bioconversion processes would

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SN Applied Sciences (2019) 1:216 | https://doi.org/10.1007/s42452-019-0223-z

Received: 4 November 2018 / Accepted: 30 January 2019 / Published online: 6 February 2019

be of enormous advantage; as the quantity and quality of products obtained from these natural processes are not economically viable or feasible for industrial application [8, 10, 11].

The crystalline structure of lignocellulosic materials consists of lignin chains, cellulose, and hemicellulose entangled in a complex structure, linked by non-covalent forces as well as covalent crossed connections. This structure physically seals the surrounding cellulose fibres and makes enzymatic hydrolysis challenging by preventing contact between the cellulose and the enzyme [12, 13]. Most plant biomass contains lignocellulose and requires pre-treatment prior to bioconversion to reduce lignin component as it is considered a leading contributor of biomass recalcitrance. This pre-treatment also makes the biomass responsive to other processing procedures [14–16]. To be successful as a commercially viable source for bioethanol production, understanding of the complex structures of lignocellulose feedstock, their chemical compositions, and development of economically feasible processes that diminishes the recalcitrance in the use of lignocellulosic biomass materials is essential [17]. Hence, knowledge of treatment methods that can adequately improve the susceptibility of chemical components like cellulose and hemicellulose in plant cell walls to hydrolysis are required [14, 18–23].

Pre-treatment alters the chemical and structural properties of plant biomass resulting in transformed physical characteristic that makes it more amenable to enzymes and microbial degradation as different treatment procedures have varying effects on the physiochemical composition of lignocellulosic biomass [15]. Therefore, several physical, chemical and biological treatments or their combination are being scrutinized for their effectiveness in improving the susceptibility of lignocellulose biomass to biodegradation and their ability to give rise to the desired end product. Hence, effective pre-treatment methods will result in increased digestibility, permeability and enhance biomass hydrolysis and subsequent conversion to the anticipated fuels [23–25].

Isoberlinia doka is one of the most prevalent hardwood timbers used in Yola metropolis Nigeria, for making furniture. Its waste is used for numerous processes ranging from fuel for domestic cooking, to its use as quilt materials in poultry farms. The prevalence in use of *I. doka* makes the sawdust from this wood available in abundance from wood mills. This waste can be regarded as a potential feedstock for bioethanol production owing to its cellulose composition. Sawdust is composed mainly of cellulose bond within a complicated entwined lignocellulose structure, the cellulose component can be utilized as a carbon source by microorganisms for the production of bioethanol under appropriate conditions [26, 27]. Therefore, this study investigated the effects of chemical treatments (sodium hydroxide (NaOH), hydrochloric acid (HCl), ferric chloride ($FeCl_3$) and sequential HCl and NaOH treatments) on the yield of bioethanol produced from *I. doka* sawdust.

2 Materials and methods

2.1 Chemical reagents

Glucose, K_2PO_4, HCl, NaOH, $FeCl_3$, potassium sodium tartrate, magnesium sulfate, calcium chloride, sodium acetate, dinitrosalicylic acid (DNSA), *Trichoderma reesei* ATCC 26921 (endoglucanase activity ≥ 700 units/g) and cellobiase from *Aspergillus niger*; cellobiase activity ≥ 0.3 unit/mg were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium acetate and acetic acid were from Fisher Scientific UK. All chemicals used for this work were of analytical grade and obtained from reputable vendors.

2.2 Sample collection and pre-treatment

Isoberlinia doka sawdust samples were obtained from wood mill in Katako Market, Yola Town, Adamawa State, Nigeria. Samples were subjected to different treatments namely: 1% $FeCl_3$, HCl, NaOH as well as sequential HCl and NaOH treatment at 121 °C for 15 min. The biomass was loaded at 10% w/v loading and pre-treated using Astell autoclave (Model number AMA260). For the sequential treatment, the method described by Guilherme et al. [28] was used. The acid treatment preceded the NaOH treatment. All samples were washed to neutral pH after the respective treatments using tap water and dried at 70 °C for 48 h in a hot air oven.

2.3 Compositional analysis of *Isoberlinia doka* biomass

The effects of each pre-treatment condition on the composition of the treated *I. doka* biomass were evaluated by sequential chemical extraction and gravimetric method described by Sridevi et al. [29]. The hot water soluble extracts, cellulose, hemicellulose and lignin contents were determined.

FTIR analysis were carried out on a Buck Scientific FTIR model 530 to determine the structural changes that occurred in each pre-treated biomass. Two milligram of dried samples were mixed with 250 mg of dried KBr, pressed to pellets and scanned over the range 4000–400 cm^{-1} at a resolution of 4 cm^{-1} [30, 31]. The spectra produced were used to evaluate structural changes that occurred with each pre-treatment condition.

2.4 Saccharification of pre-treated sawdust

In a 250 ml Erlenmeyer flask, three grams of the pre-treated and untreated sawdust were loaded into 45 ml of 0.1 M sodium acetate buffer solution, pH 5.0 consisting of 5 g/l KH_2PO_4; 5 g/l $(NH_3)_2SO_4$; 1 g/l $MgSO_4.7H_2O$; 1 g/l NaCl 1.5 g/l yeast extract; the slurry were sterilized using an autoclave at 121 °C for 15 min and were allowed to cool to room temperature. The saccharification was carried using commercial cellulase enzyme cocktail from *T. reesei* ATCC 26921 (endoglucanase activity ≥ 700 units/g) and cellobiase from *A. niger*; cellobiase activity ≥ 0.3 unit/mg solid using the methods described by Corbin et al. [32] with some modification. The enzymes were mixed in ratio 1:1 and loaded into the flasks. The flasks were incubated at 45 °C and 70 rpm in an orbital shaker for 18 h. Aliquot of each sample was collected in Eppendorf tubes and centrifuged at 8500 rpm for 10 min, the supernatants were subsequently filtered using Puradisc 0.2-µm PES filter (Whatman GE Healthcare) and utilised for sugar analysis.

2.5 Sugar analysis of saccharified pre-treated biomass

The reducing sugars contents were estimated as glucose equivalent, measured using 3,5 dinitrosalicyclic acid (DNSA) colorimetric method [33]. Absorbance was taken at 540 nm using 6850 Jenway UV–visible spectrophotometer.

2.6 Bioethanol production

The supernatants from the cellulase saccharified biomass from all the treatment were supplemented with fermentation media described by Sun and Tao [34] and used for the bioethanol production. Samples were subjected to fermentation using commercial *S. cerevisiae*. Two millilitres of the *S. cerevisiae* having optical density of 0.5 were used to inoculate each flask; conditions of fermentation were pH 5, temperature 32 °C and agitated at 70 rpm for 48 h. Fermentation was carried out in 250-ml Erlenmeyer flask in triplicates. At the end of the fermentation, samples were collected to quantify the ethanol content.

2.7 Quantification of bioethanol produced

Fermented samples were centrifuged at 8500 rpm for 15 min, and the supernatant was filtered through a Puradisc 0.2 µm PES filter (Whatman GE Healthcare), the filtrate was diluted twice before direct injection into the HPLC. The analysis was performed at 60 °C using 0.01 M sulphuric acid pH 2 as mobile phase at a flow rate of 0.6 mL/min on an Agilent HPLC 1200 Series equipped with isocratic pump, degasser, auto sampler, column oven and refractive index detector. Other conditions for analysis are: Eclipse XDB-C18 RP, 5 µm, 4.6 × 150 mm column; injection volume, 1 µl of samples and standards; and the detection was by refractive index detector Agilent G1362A at 35 °C. Ethanol yield was based on the external standard method using calibration curve.

2.8 Statistical analysis

For the gravimetric analysis and bioethanol quantification, experiments were carried out in triplicates; the results obtained are presented as mean ± standard error of mean and analysed using one way analysis of variance (ANOVA) to evaluate the differences between the means, values with $p < 0.05$ were considered significant. All statistical analyses were done using GraphPad Prism 5.01.

2.9 Results and discussion

The biomass recalcitrance and enzymatic saccharification play important role in the conversion of lignocellulose biomass to fuels. This study, investigated the effects of chemical treatments on the conversion of *I. doka* wood waste, an abundant lignocellulose feedstock in Nigeria for bioethanol production. Table 1 shows the results of the hemicellulose, cellulose and lignin composition of *I. doka* wood wastes before and after chemical pre-treatments. The chemical pre-treatments resulted in further breakup of the samples, it also decreased the lignin content of the biomass; the NaOH treatment being the most effective (with over 10% reduction in the lignin content of the treated samples compared to the untreated). The removal of lignin and other water extract resulted in improved

Table 1 Chemical composition of both the pre-treated and untreated *I. doka* wood biomass

Treatments	Untreated	Acid	Base	$FeCl_3$	Sequential HCl/NaOH
Hot Water Extractives	7.63 ± 0.69	5.58 ± 0.09	6.28 ± 0.15	5.50 ± 0.74	6.53 ± 0.22
Lignins	22.59 ± 0.72	15.94 ± 0.09	12.76 ± 0.29	17.24 ± 0.12	13.17 ± 0.46
Hemicellulose	32.72 ± 2.07	12.62 ± 2.14	10.21 ± 0.83	10.01 ± 0.54	13.66 ± 1.07
Cellulose	51.14 ± 0.74	69.94 ± 3.84	70.45 ± 4.57	66.42 ± 0.96	70.00 ± 4.57

Values in the table are presented as Mean ± Standard Error of mean of the percentage dry weight of each treatment

Table 2 Prominent and minor peaks of cellulose, hemicellulose and lignin in raw and treated samples and their peak assignment

Wavenumber (cm^{-1})	Raw	Acid Treatment	Base treatment	$FeCl_3$ treatment	Sequential treatment	Spectral assignment of prominent peaks
1750–1650	1737					According to Prats-Mateu et al. [43], the peak intensities in the range of 1750-1600 cm^{-1} are ascribed to C=O in hemicellulose. The peak intensity around 1730 cm^{-1} was found only in the raw sample
1650–1600	1607, 1638	1605	1603	1602	1603	This spectral region represents C-H aromatic vibration and have been attributed to coniferyl alcohol and C=O bonds in coniferaldehyde, and ring-conjugated C=C bonds in coniferaldehyde lignin [44, 45]. This spectral intensity was most prominent in the raw compare to other treated samples
1550–1500	1505		1501	1506	1507	Spectral intensities around 1500 cm^{-1} have been assigned to guaiacyl and syringyl C=O and aromatic linkages/skeletal vibration in lignin units [44]
1450–1400	1425	1446	1427	1448	1439	These peaks have being associated with C–O deformation in lignin
1400–1350					1363	
1350–1300	1326	1327	1323	1364		The spectral in this region have been associated with syringyl moiety in lignin [46]
1300–1250				1248	1260	Absorption spectral below 1300 cm^{-1} have been attributed to the presence of phenol and aryl–alkyl ethers from the breakdown of lignin [44]
1250–1200	1227		1223	1235	1223	These peaks have been ascribed to C-O in syringyl ring [47]
1150–1100				1108	1109	1120 cm^{-1} was attributed to the ring stretching frequency of cellulose
1100–1050	1061	1057	1054	1051	1054	The spectral region 1060-1040 cm^{-1} have been associated with C=O stretch in cellulose
950–900	905		903			
850–800				821	812	Glycosidic linkage in cellulose
800–750	790					
650–600	647			651	652	

cellulose composition of the treated samples. Information on the lignin, hemicellulose and cellulose contents of wood are important in the conversion processes of lignocellulose biomass to biofuels. It is well known that the presence of lignin is responsible for the recalcitrance of wood biomass and constitute a challenge in the utilization of cellulose present in these biomass. Therefore, its removal enhances cellulose and hemicellulose digestibility in the biomass [14, 35]. Effective and economical methods of enhancing the utilization of lignocellulose carbon resource are continually been investigated but not much progress have been made, partly due to the difficulties in the characterization of the structures of native lignocelluloses and lignocellulose-based materials [17]. Knowledge of chemical alterations that occurs during pre-treatment of biomass is fundamentally important to achieving the goal of transforming lignocellulose biomass to fermentable sugars and renewable energy thereby replacing petroleum-derived fuels and chemicals with biofuels and biochemicals [14, 22, 36, 37].

Utilization of biomass feedstock for renewable fuel production requires a reliable selection process through valid methods for compositional and structural characterization of materials [38]. The Fourier transform infrared (FTIR) in the mid infrared region (4000–600 cm^{-1}) have been widely utilized for the characterization of lignocellulose and other

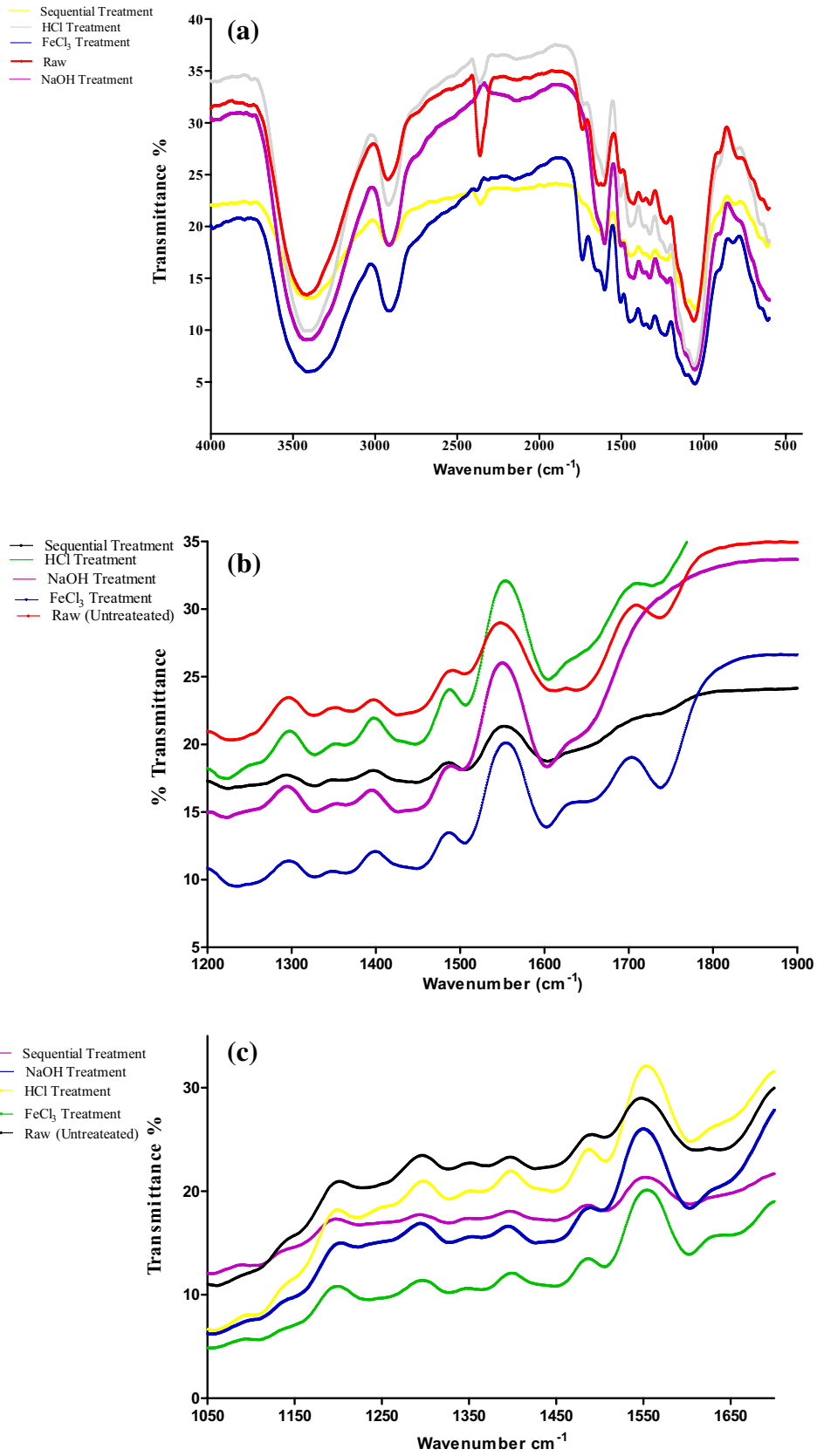

Fig. 1 **a** Showing the complete FTIR spectra of raw and treated samples **b** the FTIR spectral of the 1900–1400 region and **c** the FTIR spectral of 1000–1670 cm^{-1} region

Table 3 Reducing sugar contents of samples after Saccharification (mg/g)

Treatments	Untreated	Acid	Base	$FeCl_3$	Sequential HCl–NaOH
	187.51 ± 15.74	271.4 ± 10.07*	279.8 ± 4.38*	279.7 ± 9.52*	280.1 ± 0.67*

Results are presented as Mean ± standard error of mean of starting materials. Values with * are significant ($p < 0.05$) compared to the untreated

Table 4 Ethanol contents of samples from various pre-treatment (mg/g) of starting biomass

Treatments	Untreated	Acid	Base	$FeCl_3$	Sequential HCl-NaOH
	97.4 ± 7.70	155.0 ± 5.00	230.7 ± 2.53*	171.1 ± 1.15	172.2 ± 1.09*

Results are presented as mean ± standard error of mean of starting materials. Values with * are significant ($p < 0.05$) compared to the untreated

biomaterials. This method offers both qualitative and semi quantitative information on the functional groups of lignocellulose in biomass and many researchers have successfully used this tool to study the chemical structure and spatial distribution of cellulose, hemicellulose and lignin in various agricultural biomasses [21, 30, 39–41]. This study utilized the FTIR technique to evaluate the effects of chemical treatments on the lignins and celluloses contents of *I. doka*. Table 2 shows the prominent peaks in the raw and treated samples and their peak assignment while Fig. 1 shows the FTIR spectra of each pre-treatment on the *I. doka* biomass. The FITR spectra for the untreated and chemical treated (NaOH, $FeCl_3$, HCl and sequential HCl and NaOH treatments) samples of *I. doka* biomass showed broad peak between 3371 and 3414 cm^{-1} in all samples which relates to the hydroxyl group (OH) of the cellulose components. Strong peak was also observed between 2911 and 2923 cm^{-1} corresponding to C–H stretching in hemicellulose and cellulose [41, 42]. The chemically treated samples showed considerable alterations in the region 1800–800 cm^{-1} of FTIR spectra clearly indicating the varying efficacies of the various treatments in removing lignin from *I. doka* biomass.

During the saccharification of lignocellulose biomass, β-1,4 glucosidic linkages in cellulose present a formidable barrier to enzymatic hydrolysis even after chemical treatments have provided increased access to these bonds [48]. A combination of *Tricoderma reesi* and *A. niger* cellulases was used in the saccharification of the biomass due to the structural composition of *I. doka* biomass. Monitoring and quantifying hydrolysis and fermentation products like glucose and ethanol are necessary for effective evaluation of the feasibility of plant biomass for biofuel production [32]. Table 3 shows the quantity of reducing sugar measured as glucose equivalent produced and Table 4 presents the ethanol produced from *I. doka* from both the raw and treated sample. The sequential pre-treatment method adequately increased the digestibility of these biomass for the cellulases as it gave rise to the highest reducing sugars (280 mg/g of sample) but this yield was not significantly ($p > 0.05$) higher than those of the acid, alkali and $FeCl_3$ pre-treatments.

The bioethanol yield from the NaOH and the HCl-NaOH sequential treatments varied significantly compared to the untreated biomass. Although all the treated biomass produced similar amount of the reducing sugars, the amount of ethanol obtained varied from each other. This could be as a result of differences in chemical composition and bye products of resulting residues from distinct pre-treatment conditions. The ethanol yield compared to the reducing sugars produced indicates that the pre-treatments may have produced inhibitory substances, which may have hindered the *Saccharomyces cerevisiae* from efficient utilization of sugars produced during saccharification for bioethanol production. Treatment of *I. doka* biomass with NaOH supported bioethanol production and resulted in the highest yield (230 mg/g). Although the sequential acid and alkaline treatment gave the highest reducing sugar yield, the ethanol yield was lower compared to the alkali treatment. This may have been as a result of high-temperature acidic treatment that transforms pentoses to furfural and hexoses to hydroxymethyl furfural resulting in unwanted use of biomass carbon [12, 36]. These compounds, along with lignin-derived phenolics and hemicellulose- derived acetic acid, are inhibitors of cell metabolism that reduces the overall ethanol yield and makes comparison of data difficult [49–51].

From the preceding results, chemical pre-treatment with sodium hydroxide proved to be the most effective chemical treatment in the reduction of lignin content from the *I. Doka* sawdust of the treatments investigated. The reducing sugars and ethanol produced from this treatment were comparatively high compared to other treatments used in this work.

Acknowledgements We are grateful to the American University of Nigeria for generously providing all the materials needed for this work.

Author contributions ABO conceived the research, and revised the manuscript. MJO performed the research, analysed the data and wrote the first manuscript. FTE carried out the experiments and revised the manuscript draft. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest We have no conflict of interest to declare.

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