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Production and Physiochemical Characterization of Biodiesel from Nile cabbage grown in Pager River, Kitgum Municipality, Northern-Uganda

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Abstract: Nile cabbage (Pistia stratiotes) represents a promising source of biodiesel which has been garnered due to good biomass yield. This study aims to determine the possibility for biodiesel production from Nile cabbage plants collected from a seasonal Pager River. The bio-oil extraction from Nile cabbage leaves was done using soxhlet apparatus. The biodiesel was produced by transesterification of lipids and characterized by GC-FID and the physio-chemical parameters of the produced biodiesel were also performed. The obtained results indicate that, Nile cabbage sample showed a yield of biodiesel (30.30±0.01) %. The biodiesel obtained possess the following fuel properties: density (880.00±0.00)Kg/m^3, pH (5.70±0.00), saponification value (137.10±0.09) mg KOH/g, acid value (1.00±0.00) mg KOH/g, iodine value (63.41±0.01) mg I^2/100g, flash point (120±0.00)℃, pour point (-6.20±0.03) ℃, colour (brown), moisture content (50.00±0.00)%, and ash content (0.003±0.00)%, and cetane number (49.53±0.05). The GC-FID analysis of the obtained biodiesel showed the presence of C16:0, C18:0, C18:1 and C18:2 as the major constituents of fatty acids (FAs) detected. Therefore, all physiochemical parameters were within the allowable limits, accept only acid value which was slightly higher than the standard limits issued by the American and European (ASTM D 6571:12 and EN 14214:2012) standards. Aquatic weeds are considered a global threat in the aquatic ecosystem, which invoked a lot of attention from the general public and the scientific community. Our findings showed that Nile cabbage biomass could give a significant yield of biodiesel, with desired fuel properties which will initiates a cleaner energy.

Keywords: Nile Cabbage, Pistia stratiotes, Transesterification, FAME and Biodiesel.

1. Introduction

Uganda is a fairy-tale, with enriched biodiversity of beautiful faunas and floras, vast water resources from open water, swampy areas, rivers and lakes [1, 2]. Uganda is the source of the longest river in Africa, the source of the Nile started from L. Victoria which occupies about 69,484 km2 to Red sea through Egypt. River Nile has it tributes and considering those from Northern region; Aswa, Okok, and Pager [3]. Pager River is located in Kitgum district, Northern Uganda and its origin was traced to have started from Kitgum Matidi and joined to Aswa. The river has a length of 194.79 kilometres. As a result of over flooding in 2020, the river has been invaded by a dangerous aquatic weed (Nile cabbage). Today, there are blown trumpet and tremendous issues on climate change that calls for holistic global approaches to solve them, these include rise in temperature, rise in sea level, and climate change, accumulation of organic wastes, and excess toxic pollutants [4-8]. As a result, there are such uncontrollable phenomena due to outbreaks of growth of various aquatic macrophytes like water cabbages, water hyacinth, which have become more disastrous in the Uganda's aquatic ecosystems [3]. Water cabbage has been recognised as one of the untold dangerous alien aquatic species in freshwater, seawater, soil, hot spring [9].

Pistia stratiotes belongs to the genus pistia, it is a perennial monocotyledon with thick, soft leaves that form a rosette, its native distribution is not clear but is probably tropical; it was first discovered from the Nile near Lake Victoria in Africa [10, 11]. In Uganda, Pistia stratiotes is commonly called water cabbage or Nile cabbage. It has been termed as one of the major disastrous aquatic species which rapidly multiply and cover the surface of the open water sources and results to serious problematic issues in the environment since it destroys aquatic biodiversity by blocking the penetration of light and continuous reduction in the supply of oxygen into water bodies, also causes hypoxia in marine animal species [12-14]. From the literatures of some recent studies, it shows that there has been an increasing number of studies on the applications of renewable bioenergy sources such as agricultural wastes, microalgae as a substitute of food material for production of biofuels, antibiotics, plant growth promoters, fertilizer, bio-pesticides, and medicines [15, 16], vegetable oils like soybean (27%), palm oil (31%), and oilseed rape (20%) with their different conversion methods like transesterification to form biodiesel (Figure 1).

In the western world production of biodiesel is mainly from food sources such as oilseed rape (44%), palm oil (29%), used cooking oil (15%), and soybean oil (5%) [17, 18]. As a result of an increasing demand and technologies for biofuels, it is quite good to take the issue of food security as one of the priority points. Therefore, the use of non-food-based crops are tapped for biodiesel production since they are cost effective than food-based feedstocks as there is little or no market demand for these plants. This present study aimed at investigating the possibility to obtain a comparable quality biodiesel from the dried biomass of Nile cabbage leaves grown in Pager River, Kitgum Municipality, Northern-Uganda.

2. Materials and Methods

2.1 Sample Sites and Sample Collection

The study was carried out in the month of September 2021. Sampling of aquatic weeds was carried out in Kitgum municipality, Northern-Uganda from Pager River. This river is located at 3°09'41"N, 32°30'39"E (Figure 2). Samples of aquatic weeds were collected and were packed into sterilized poly ethene bag, marked and transported within 12 hrs to the Chemistry Laboratory, Uganda Industrial Research Institute (UIRI), plot 42A, Mukabya road, Nakawa industrial area, Kampala, Uganda.

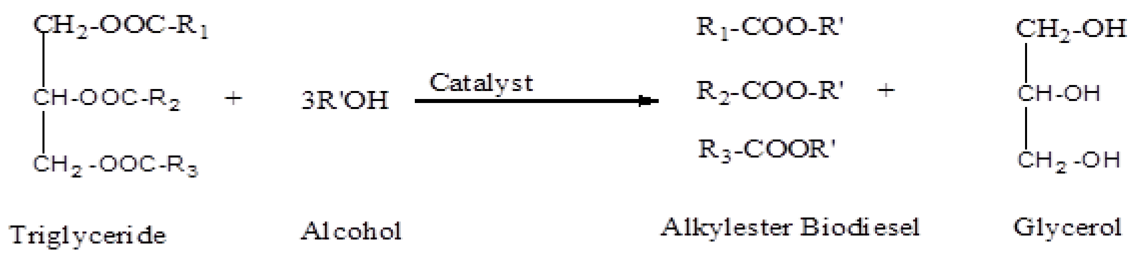

Figure 1. Transesterification of Triglyceride

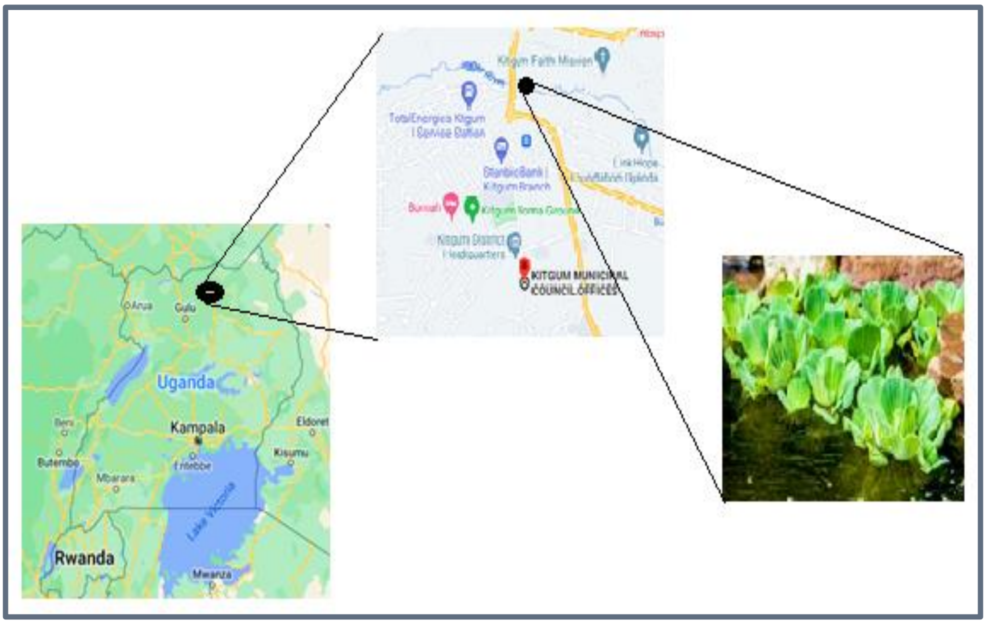

Figure 2. Map of Pager River, Kitgum Municipality, Northern-Uganda showing the location of the sampling site

2.2 Sample Preparation and Bio-Oil Extraction

Only fresh plants (5 Kg) were picked, washed with demineralized water to remove debris and dried at 105 °C for 24 hrs. The samples were ground into powder and kept in an air tight bottle for further analysis. Soxhlet extraction method was used for the extraction of Crude bio-oil from the dried biomass using the Soxhlet extractor (ST 243 Soxtec™ solvent extraction system) [19]. An extracting flask (250 ml) was dried in an oven at 105°C, cooled in a desiccator for 20 minutes and the empty flask was weighed and the mass was noted. The samples (50g) were weighed into the porous thimbles. 150 ml of petroleum ether and n-hexane mixture (ratio; 1:1) were measured and then added to the dried 250 ml capacity flask. The porous thimble containing the samples were covered with cotton wool, placed in the condenser of the Soxhlet extractor machine to perform the extraction for 5 hrs [20]. The extraction flask with the oil were removed and placed in an oven at 105°C for the period of 1hr (Figure 3). The flasks with their contents were cooled in the desiccator for 15 minutes and the weights of the extracted oils were measured.

2.3 Biodiesel Production and Fatty acid esters (FAME) Analysis from dry Biomass of Nile Cabbage

A two-neck flat bottomed flask containing methanol (350 ml), oil (25 g), and 1 wt % catalyst concentration was connected to a reflux condenser and the temperature adjusted to 60 °C. The flask was then shaken vigorously for 5 hrs. After, the mixture was filtered to separate the catalyst and the filtrate was concentrated using rotary evaporator to remove methanol. The biodiesel obtained was left in a separating funnel overnight to settled and formed two distinct layers, the biodiesel obtained was weighed and expressed as percentage weight as shown in equation (1). After transesterification, the obtained biodiesel was subjected to GC-FID for FAME analysis [21, 22].

$$\%age\ yield = \frac{weight\ of\ the\ bio-oil\ obtained}{weight\ of\ the\ dried\ biomass\ used}\ x\ 100 \qquad (1)$$

2.4 Analysis of Physicochemical Parameters

The following are the physicochemical parameters analysed on biodiesel obtained from the dried biomass of Nile cabbage; density, pH, iodine value, acid value, moisture content, ash content, saponification value, flash point, cloud point, freezing, point pour point, colour, and cetane number [20, 23].

The Density

An empty dried beaker with a known weight was taken and about 25 cm^3 of the oil sample was poured into the beaker and weight of the 25 cm^3 of the sample was obtained [23]. Thus, density was calculated by the equation (2) below;

$$Density\ of\ oil\ sample = \frac{weight\ of\ oil\ sample}{volume\ of\ the\ oil\ sample} \qquad (2)$$

The pH

The biodiesel sample (2 g) was weighed and poured into 15 ml hot distilled water in a dry clean beaker with continuous stirring, and cooled at 25 ^{0}C. The pH meter was inserted while stirring the mixture and the pH value was noted as an average value.

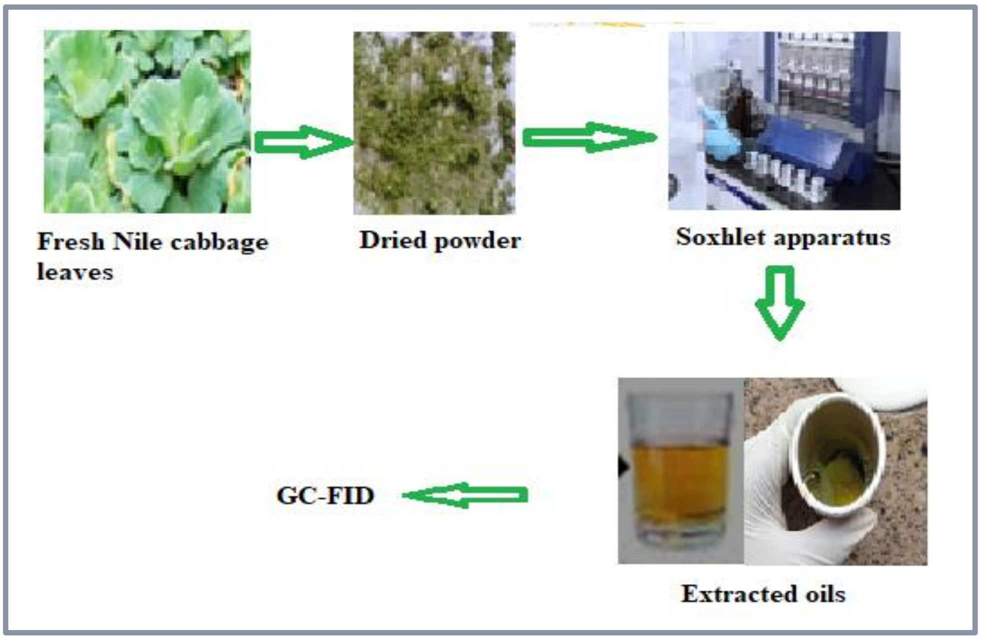

Figure 3. Experimental flow chart

The Iodine Value of Biodiesel Obtained

In a flask was added the biodiesel sample (0.5 g) and dissolved with a mixture of 10 ml of CCl_4 and 20 ml of Wij's solution and shaken properly and placed in dark to stand for 30 minutes at 37 ^{0}C. KI (10%; 15 ml) and distilled water (100 ml) were later added to the flask. Resulting mixture containing iodine was titrated against 0.1 N $Na_2S_2O_3$ using starch solution as an indicator for the titration, the colour of the solution changes to a blue-black coloration which marked the end point. Blank test was done using with 10 ml CCl_4. The equation (3) below was used to calculate the iodine value.

$$Iodine\ Value = \frac{12.69\ x\ N\ \ x\ (B-S)}{weight\ of\ sample} \quad (3)$$

Where, B = 0.1 N $Na_2S_2O_3$ needed (ml) by blank, S = 0.1 N $Na_2S_2O_3$ needed (ml) by sample, and N = Normality of $Na_2S_2O_3$

The Acid values

In the Erlenmeyer flask (250 ml) was added biodiesel sample (0.5 g) and 50 ml ethanol (95%), followed by 3 drops of 0.5% phenolphthalein indicator and allowed to boil. The solution was titrated using 0.1N NaOH solution, shaken vigorously until a pale pink colour was obtained. At the endpoint all the free fatty acid has been neutralised by 0.1N NaOH solution, the equation (4) was used to calculate the acid value.

$$Acid\ value = \frac{56.1\ x\ N\ x\ A}{W} \quad (4)$$

Where, A = Volume of Alkali, N = Normality of NaOH, and W = Weight of sample (g)

The Moisture Content

Approximately 1.0 g of the biodiesel samples were weighed and placed in the pre-weight crucibles, transferred into an oven at 105 °C for 24 hrs. The samples were removed and cooled in a desiccator for 15 minutes and the weight taken, the equation (5) below was used to obtain the moisture content.

$$Moisture\ content(\%) = \frac{Initial\ weight - Final\ weight}{Initial\ weight}\ x\ 100 \quad (5)$$

The Ash Content

Approximately 1.0 g of the samples were then placed in a pre-weight crucible and transferred to a muffle furnace with a set ramp function from 20 °C to 550 °C in 2 hrs, and the temperature maintained at 550 °C for 5 hrs. The samples were removed and cooled in a desiccator for 15 minutes and the weight taken, the equation (6) below was used to calculate the ash content.

$$Ash\ (\%) = \frac{Initial\ weight - Final\ weight}{Initial\ weight}\ x\ 100 \quad (6)$$

The Saponification values

The standard method for determination of saponification value as described in ISO 3657:2013 was followed [24]. The value was obtained by using the equation (7) below.

$$Saponification\ Value = \frac{56.1\ x\ N\ x\ (A-B)}{W} \quad (7)$$

Where, A = Volume of HCl (ml) for the sample, B = Volume of HCl used (ml) for blank titration, N = Normality of KOH, W = Weight of sample taken (g), and Equivalent weight of KOH = 56.1

The Flash Points

The method was described by Aliyu *et al*., with some modifications. The biodiesel sample was filled in an open cup and heated with continuous stirring for a given intervals [23]. A flame was introduced over the surface and distinct kinds of flame were obtained. The temperature was noted which signifies the flash point of biodiesel.

The Pour Points

Using ASTM D6571 as described by Aliyu *et al*., with some modifications. At 2°C intervals, the jar was placed horizontally on the biodiesel sample for some seconds before taking it to cool [23]. Therefore, the lowest temperature at which biodiesel obtained remained in a vertical position for a few seconds was denoted as the pour point.

The Cetane Number

The method used was described by Gopinath *et al*., with some modifications [25]. Hence, the number of carbon atoms (NC) was considered from the GC-FID results obtained which characterised the different components of fatty acid esters, and was calculated by the equation (8) below:

$$NC = \sum \frac{WaCa}{100} \quad (8)$$

where *a* is the corresponding type of fatty acid ester, *Ca* is the corresponding carbon number of fatty acid ester *a*, and *Wa* is the corresponding weight percentage of the fatty acid ester *a*,

2.5 Statistical Analysis

All data obtained were expressed as a mean ± standard deviation, and the analyses were done in triplicates. One-way analysis of variance (ANOVA) followed by student t-tests was used to determine the statistical significance. P-value ≤ 0.05 was applied as significant.

3. Results and Discussions

The result from lipid extraction of Nile cabbage leaves using petroleum ether in a soxhlet apparatus gave 30 % yields and was compared with other sources (Table 1). From the result obtained soxhlet extraction method is more efficient to extract oils from aquatic weeds as described by Tadesse *et al*. But, this method has got its main problem since it cannot operate with large samples because the thimble cannot hold large samples [26, 27].

Table 2 shows the analysed fuel properties of Nile cabbage biodiesel which includes; density, pH, saponification value, acid value, iodine value, flash point, pour point, colour, cetane number, moisture content, ash content and Cetane number. From the results, the analysed fuel properties obey the biodiesel standards described in both American and European (ASTM D 6571:12 and EN 14214:2012) standards [28, 29].

From table 2, it shows that obtained biodiesel from dried biomass of Nile cabbage has a density of 880 Kg/m^3 which is within the limit standard as compared with 860-900 Kg/m^3 described in European standard (EN 14214:2012).

Table 1. Comparison of Nile cabbage biodiesel with other biodiesel

Feedstock	Oil content (% Dry weight)	Biodiesel productivity (Kg/ha year)	Reference(s)
Soya bean	18	562	[27]
Sunflower	40	946	[27]
Palm oil	36	4747	[27]
Water hyacinth	30	51927	[27]
Nile cabbage	30	N/A	N/A

Table 2. Comparison of Nile cabbage oil with US and EU Biodiesel specification [28, 29]

Property		Value	
	Nile cabbage oil	ASM D6571:12	EN 14214:2012
Density (Kg/m^3)	880 (20℃)	(20℃)	860-900 (15℃)
pH	5.70±0.00	-	-
Saponification (mg KOH/g)	137.10±0.09	-	-
Acid value (mg KOH/g)	1.00±0.00	<0.8	< 0.5
Iodine value (mg I_2/100g)	63.40±0.01	-	120
Flash point (℃)	120.00±0.00	>130	>120
Pour point (℃)	-6.20±0.03	-	-17
Colour	Brown	-	-
Cetane Number	49.50±0.05	≥47	>51
Moisture content (mg/g)	50.00±0.00	500	500
Ash content (%)	0.003±0.00	≤0.01	≤0.01

Table 3. Fatty Acid Constituents of Nile Cabbage Biodiesel

Composition	Weight (%)		
	Nile cabbage oil	Soy bean oil	Algae oil
Oleic acid	13.20	30	36
Palmitic acid	29.15	10.4	15
Stearic acid	5.6	6.5	11
Linoleic acid	35.6	47.8	7.4
ISO	8.6	11.42	8.4

Biodiesel from different sources have different densities and this difference may be due to variation of agro ecological zone Biodiesel has higher density than conventional diesel fuel [30]. Thus, they can be blended together and use in fuel engine [31]. The pH of Pager Nile cabbage biodiesel was 5.7±0.00, which is similar to the findings by Luo *et al*. [32], in the studies they found that the pH of Nile cabbage is in the range of 5.2-6.9. The saponification value of Pager Nile cabbage biodiesel was 137.1±0.09 mg KOH/g, which shows that the corresponding fatty acid components present in biodiesel obtained from Nile cabbage are of lower molecular weight (saturated and unsaturated). The mean acid value of the biodiesel in the study was 1.00±0.00 mg KOH/g, which shows small deviation from the standards that is 0.8 mg KOH/g (ASTM D6571:12) and 0.5 mg KOH/g (EN 14214:2012) standards, respectively. Since, the acid value in our findings is slightly high, it might be suitable for use in engines but with cautions due to its corrosiveness [30]. The iodine value of Pager Nile cabbage biodiesel 63.40±0.01 mg I_2/100g was lower than 120 reported in European standard (EN 14214:2012). From the findings, the iodine value help to know the tendency at which the oil has undergone oxidative rancidity [33]. The flash point of Pager Nile cabbage biodiesel was 120.00±0.00℃, the flask point obtained in this study is within the recommended value in both US and EU Biodiesel specification. The flash point values affect the ignition of fuel in the engine that is the higher the value, the lower the possibilities for premature ignition. From Table 3, the pour point of Pager Nile cabbage biodiesel was -6.20±0.03℃ is within the limit as compared to -17℃ reported in European standard (EN 14214:2012). The average moisture content of Pager Nile cabbage biodiesel obtained was 50.00±0.00 mg/g, which is within the allowable amount of moisture content for all biodiesels by comparison with the American and European standards (<500 mg/g) [34]. Therefore, the obtained biodiesel will have low solvency properties and making it difficult for the microbial slime to detach and clog fuel filters [31]. The mean ash content of Pager Nile cabbage biodiesel was 0.003 g/100g, which is within the limited as compared to US and EU Biodiesel (0.01 g/100g) specification. The contents of the biodiesel were characterized by GC-FID and compared with soy bean and algae biodiesel, respectively [27]. The results in Table 3 showed majorly three saturated constituents (palmitic, stearic acid, and ISO) and two unsaturated constituents (linoleic, and oleic acid) of methyl esters characterised in biodiesel.

Table 3 showed that the constituents of fatty acid methyl esters of the Nile cabbage biodiesel have different quantities from both Soy bean and Algae biodiesel, respectively [35, 36]. The GC-FID result shows that the fatty acid constituents from Nile cabbage biodiesel were in the order; Linoleic acid > Palmitic acid > Oleic acid > Stearic acid, which does not correlate with the previous findings from some researchers who had reported similar findings but in the order; Palmitic acid > Linoleic acid > Oleic acid > Stearic acid on the same plant species [12]. In comparison palmitic acid methyl ester values were higher in both Nile cabbage (29.15%) and Soy bean (10.4%) biodiesels. This indicates that biodiesel obtained from Nile cabbage biomass can be a good-quality alternative feedstock for biodiesel production [31]. Therefore, it has been realised that there was increased in carbon chain length from C16:0 to C18:3 [37]. This result shows an interesting cetane numbers (49.5±0.05) which is highly affected by each component of fatty acid ester as compared to 51 of the US and EU Biodiesel specification. In the previous studies reported by Gopinath *et al.* [25], indicated that the cetane number of biodiesels was higher due to an increasing carbon chain length and lowers due to increasing unsaturation. The cetane number is an importance parameter when analysing fuel property of biodiesel by delaying the ignition period hence good quality combustion. In this study, the result indicates that the transesterification reaction was absolutely completed and the quality biodiesel was obtained from the extracted bio-oil from the dried biomass of Nile cabbage grown in Pager River, Kitgum Municipality.

4. Conclusions

Aquatic weed (Nile cabbage) is an invasive and disastrous plant species to the aquatic micro-environments. In our study, Soxhlet method was exploited for the extraction of the bio-oil from the dried biomass of Nile cabbage. This study suggested that the alkaline catalytic pre-treatment ease the transesterification process to produce biodiesel. And also, the method of extraction used is inferred as the most efficient technique due to less time and solvents consumed. The fatty acid esters characterised from the biodiesel shows great similarities in term of fuel properties when compared to the standards of biodiesel in United States and Europe. Therefore, we recommend and encourage that the obtained biodiesel from Nile cabbage biomass could be an excited alternative source to diesel in future and also will help Kitgum municipal councils to make policies on how to conserve Pager River.

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Author contributions

Conceptualization, P.O and E.N; methodology, P.O., E.N and A.D.O.; resources, P.O., E.N., I.O., J.K., I.B., W.A., and A.D.O; investigation, P.O., E.N and A.D.O; writing-original draft preparation, P.O and T.B.J; review and editing, E.N., I.O., J.K., I.B, and A.D.O. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

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