



Chemometric analysis of ketogenic diet formulated from low-cost dietary fibers

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ABSTRACT

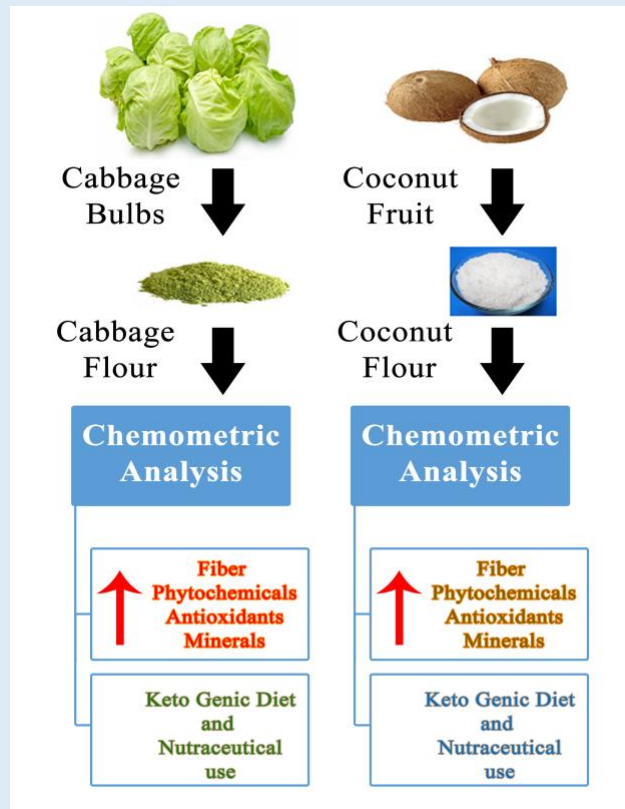
Background: Ketogenic diet (KD) is a beneficial nutritional plan consisting of low carbohydrate, high fat, and moderate protein levels and aids in amelioration of some metabolic disorders. The objective of this study is to develop a ketogenic diet model using cheap and readily available fiber sources.

METHODS: Cabbage head and coconut fruits were obtained and processed into fiber and ketogenic diet chow. They were further analyzed using standard methods for proximate, mineral, and heavy metals, phytochemicals, and DPPH radical scavenging assay.

RESULTS: Carbohydrate content of the samples were (3.35, 4.00, 3.16 and 2.08%) for cabbage feed, coconut feed, coconut fiber, and cabbage fibers, respectively. This conforms to the maximal 4% carbohydrate required for ketogenic diet daily allowable limit. Other nutrients such as lipids and proteins were in high and moderate amounts respectively. Phytochemicals were also present in varied proportions in the samples.

CONCLUSION: The developed cabbage and coconut fiber is an appropriate fiber source for ketogenic diet preparation. They are rich in nutrients based on their mineral content. They may be positioned as a nutraceutical for therapeutic and disease prevention action due to their inherent bioactive chemicals and radical scavenging activity. They may pose negligible toxicity risks as the few detected heavy metals are within permissible limits.

KEYWORDS: Fibers, ketogenic diet, cabbage, coconut, chemical analysis, and antioxidant



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INTRODUCTION

Ketogenic diet (KD) is a specialized diet that is classically composed of high fat, moderate protein, and low carbohydrate mix, of which the carbohydrate content is as low as four percent [1-3]. It has been adopted for treatment of several metabolic conditions [4]. Consumption of KD is marked by generation of ketone bodies such as acetone, acetoacetate, and beta-hydroxybutyrate by the hepatic cells and these serves to provide energy to muscle, heart, and brain tissues [1,5]. Recent studies have linked KD to the treatment of certain

metabolic disorders such as obesity, type II diabetes mellitus, epilepsy, and cancers [6-9]. A few studies had raised concerns over prolonged consumption of KD as it may cause reduced skeletal muscle uptake of glycogen, but this may still serve as a treatment strategy for resistance to increased physical activity [10]. KD may therefore pose no obvious threat to life when ingested for short term but may rather contribute to extension of lifespan and general wellness of consumers.

Despite the several benefits of KD, it is not popular in many African regions due to cost implication of obtaining

imported low carbohydrate fiber, especially for the preparation of the diet. The aim of this research therefore is to develop nutritious low carbohydrate fibers from cheap and readily available (indigenous) sources.

METHODS

Fiber preparation: Cabbage heads weighing 2.5kg and coconut fruits weighing 1.5kg were obtained from Omu Aran Market, Nigeria. The cabbage heads were rinsed in distilled water and diced with sharp knife on a vegetable cutter. The diced portion (100g) was blended in 1 liter of clean water using mechanical laboratory blender. The mixture obtained was poured into a white cotton flannel sieve to separate the water from the fiber. The wet fiber obtained as residue on the sieve was air dried at room

temperature for 48 hours and thereafter milled into fine flour using a dry mill. The cabbage flour obtained (750g) was stored in an airtight polythene bag prior to analysis and feed preparation. The coconut fruits were de-shelled, and the succulent fruit was cut into small cubes. A portion of 50g was blended in 400 mL of clean water per time until all the whole bulk is done. The fiber was separated from the milk through sieving with cotton flannel and thereafter air dried for twenty-four hours at room temperature prior to milling into fine flour. Total yield was 530g of flour which was stored in an airtight polythene bag.

Cabbage and coconut flour were used to compound ketogenic diet feed using the specification in Table 1, according to the method of Kayode et al. [11].

Table 1. Cabbage and coconut fiber feed composition

Cabbage based feed composition		Coconut based feed composition	
COMPONENT	WEIGHT(g)	COMPONENT	WEIGHT(g)
Cabbage fiber	500g	Coconut fiber	500g
Protein	100g	Protein	100g
Fat	250g	Fat	250g
Vitamin/Minerals	100g	Vitamin/Minerals	100g
Food Binder	50g	Food Binder	50g
TOTAL	1000g	TOTAL	1000g

Proximate studies: This was determined by the method described by on dry weight basis [12]. Crude fat was extracted by Soxhlet method with petroleum ether (40-60°C) for 6 hours. Total nitrogen was determined using the micro-Kjeldahl method and converted to crude protein content by multiplying with a factor of 6.25. Carbohydrate content was determined by percentage difference of the other proximate parameters summed together. The results are expressed as averages of percentage values on dry weight basis.

Mineral element determination: The mineral contents were analyzed after incineration in a muffle furnace and

the ash obtained dissolved in 2.0M HCl and diluted to 100mL with deionized water. The resulting extract was used for the determination of sodium and potassium by Flame Emission Photometry method. Calcium, magnesium, iron, copper, zinc, and manganese by using Atomic Absorption Spectrophotometer (model 400 Perkin Elmer Analyst) and phosphorus by the Vanadomolybdate colorimetric method of Pearson [13] as described by Kayode and Yakubu [14].

Phytochemical analysis: Qualitative and quantitative phytochemicals of the fibers were determined for the presence of alkaloids, saponins, tannins, glycosides,

flavonoids, phenols, and steroids using standard methods of Harborne [15], as reported by Kayode and Yakubu [14].

Radical scavenging activity: Estimation of the antioxidant activity of the fibers was done following the DPPH radical quenching assay method as reported by Sarker and Oba [16]. The absorbance was taken at wavelengths 517 nm for DPPH. The antioxidant capacity was measured according to the following equation:

$$AC (\%) = (A_b - A_s / A_b) \times 100.$$

Where, AC = antioxidant capacity, A_b = absorbance of the blank sample [10 μ L methanol], and A_s = absorbance of the test compound. The results were calculated as μ g TEAC g^{-1} DW

Hydrogen peroxide scavenging potential: The method described by Jayaprakash et al [17]. was adopted. Briefly, a solution of hydrogen peroxide (20 mM) was prepared in sodium phosphate buffer (pH 7.4). Various concentrations of 1 ml of the extracts or ascorbic acid (reference antioxidant) in methanol were added to 2 ml (20 mM) of the hydrogen peroxide. The absorbance was measured at 230 nm, after 10 min against a blank solution that contained extracts in sodium phosphate buffer without hydrogen peroxide.

Hydroxyl radical scavenging activity: The hydroxyl radical scavenging activity was measured according to the method of Halliwell et al. [18] Briefly, the reaction mixture contained 1.0 ml of reagent (3.0 mM deoxyribose, 0.1 mM EDTA, 2 mM H_2O_2 , 0.1 mM L-ascorbic acid, 0.1 mM $FeCl_3 \cdot 6H_2O$ in 10 mM phosphate buffer, pH 7.4) and various concentrations of the extract (50–350 μ g/ml). The reaction mixtures were incubated at 37 °C for 1 h, and followed by the addition of 1.0 ml of 1% (w/v) TBA (in 0.25 M HCl) and 1.0 ml 10% (w/v) trichloroacetic acid (TCA). The reaction mixture was adding 1 ml of PMS solution (60 μ M) to the mixture. The reaction mixture was incubated at 25°C for 5 minutes,

heated in a boiling water bath at 100 °C for 20 min and pink chromogen (malondialdehyde-(TBA) adduct) was extracted into 1.0 ml of butan-1-ol and absorbance (Abs) was read at 532 nm against reagent blank. BHT (butylated hydroxytoluene) served as positive control. The percentage inhibition was calculated using the following expression:

$A_{control}$ = absorbance of control (containing all reagents except the test compound) and A_{sample} = absorbance of samples (containing all reagents including the test compound).

Determination of total antioxidant capacity: Total antioxidant capacity was determined according to method described by Prieto et al. [19] Briefly, to 0.1 ml (0.25 mg/ml) of the extract or standard solutions of ascorbic acid (20–100 μ g/ml) was added 1 ml of the reagent solution which consisted of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The tubes containing the reacting mixture were incubated in a water bath at 95 °C for 90 min. The mixture was then allowed to stand and cool to room temperature and the absorbance measured at 695 nm against a blank which consisted of the reacting mixture a distilled water in place of the extract. The antioxidant activities of the extracts were expressed as an ascorbic acid equivalent.

Superoxide radical scavenging activity: The superoxide anion scavenging activity was measured based on the described method [20]. Superoxide radicals were generated in a PMS-NADH system by oxidation of NADH and assayed through reduction of NBT. In this experiment, the superoxide radicals were generated in 3 ml of sodium phosphate buffer (100 mM, pH 7.4) containing 1 ml of NBT (150 μ M) solution, 1 ml of NADH (468 μ M) solution, and different concentrations of the CRE (25–250 μ g/ml) in water. The reaction started by and the absorbance was measured against the corresponding blank solution. L-Ascorbic acid was used as

the positive control. The decrease in the extent of NBT reduction, measured by the absorbance of the reaction mixture, correlates with the superoxide radical scavenging activity of the NJE extract. The percentage of superoxide radical scavenging was calculated using the following formula: Superoxide radical scavenging activity (%) = $[(A_0 - A_1)/A_0 \times 100]$, where A_0 is the absorbance of the control and A_1 is the absorbance of NJE or the standard sample.

Statistical analysis: Data was expressed as mean \pm SEM of five replicates. Analysis of Variance complemented with Duncan post hoc was used for the data analysis. IBM SPSS statistics, version 22.0 (SPSS Inc; Chicago USA) was used for the study. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Coconut flour has significantly low levels ($P < 0.05$) of moisture, total protein, fat, and calorific value but significantly higher level ($P < 0.05$) of crude fiber compared to all the other analyzed samples, while

cabbage flour has significantly high levels ($P < 0.05$) of moisture and lowest levels of calorific value (Table 2).

The mineral content analysis shown in Table 3 reveals significantly higher level ($P < 0.05$) of sodium in cabbage samples compared to coconut, while all the other minerals were in comparable amount within and between the groups.

Heavy metal analysis data was reported in Table 4. Coconut and cabbage samples revealed significantly low levels ($P < 0.05$) of heavy metals while AS, Pd and I were not detected at all in the coconut samples. Results of the qualitative phytochemical screening are presented in table 5a. Phenolics, glycosides, triterpenes, flavonoids, and alkaloids were detected in the two flour samples while coumarins and steroids were further detected in cabbage flour. Anthocyanins and terpenoids were only present in coconut but not cabbage flours. Furthermore, the quantification of these phytochemicals (Table 5b) reveals flavonoids has the highest value ($P < 0.05$) amongst all the phytochemicals whilst anthocyanins and glycosides were least.

Table 2. Proximate analysis of cabbage feed, coconut feed, cabbage flour and coconut flour

Sample	Moisture (%)	Ash (%)	Chow (%)	Total protein (%)	Crude fat (%)	Crude Fiber (%)	Calorific Value (kj)
Cabbage Feed	5.51 \pm 0.80 ^a	8.50 \pm 0.63 ^a	3.35 \pm 0.00 ^b	17.28 \pm 0.06 ^d	38.81 \pm 0.02 ^c	26.55 \pm 0.20 ^a	1806.85 \pm 0.19 ^c
Coconut Feed	5.17 \pm 0.12 ^a	3.16 \pm 0.13 ^a	4.00 \pm 0.08 ^b	13.28 \pm 0.23 ^c	49.25 \pm 0.36 ^d	25.15 \pm 0.20 ^a	2143.60 \pm 8.36 ^d
Coconut Flour	3.66 \pm 0.20 ^a	0.61 \pm 0.24 ^a	3.16 \pm 0.37 ^{ab}	2.02 \pm 0.13 ^a	24.71 \pm 0.38 ^b	65.84 \pm 0.83 ^b	1017.15 \pm 22.54 ^b
Cabbage Flour	11.48 \pm 0.00 ^b	9.27 \pm 3.33 ^a	2.08 \pm 0.02 ^a	7.40 \pm 0.02 ^b	8.77 \pm 0.65 ^a	61.00 \pm 3.98 ^b	488.87 \pm 24.45 ^a

Results were expressed as Mean \pm SEM

Table 3. Mineral content of coconut feed, cabbage feed, coconut flour and cabbage flour

Sample	Mn (mg/kg)	Ca (mg/kg)	K (mg/kg)	Cl (mg/kg)	P (mg/kg)	Mg (mg/kg)	Na (mg/kg)	Fe (mg/kg)
Coconut Feed	0.042±0.02 ^a	15.042±0.01 ^a	2.880±0.12 ^a	1.340±0.02 ^a	1.315±0.01 ^a	0.27 ±0.02 ^a	13.636±3.02 ^a	0.096±0.00 ^a
Cabbage Feed	0.019±0.00 ^b	13.664±2.02 ^a	2.995± 0.11 ^a	1.213±0.42 ^a	0.385±0.02 ^b	1.031±0.02 ^b	40.000±2.02 ^b	0.045±0.01 ^b
Coconut Flour	0.040±0.02 ^a	15.603±3.01 ^a	2.865± 0.01 ^a	1.165±0.11 ^{ab}	1.450±0.21 ^a	0.145±0.12 ^a	7.727± 0.12 ^c	0.090±0.02 ^a
Cabbage Flour	0.033 ^a ±0.02	13.080±4.02 ^a	2.935± 1.00 ^a	1.043±0.01 ^b	0.010±0.00 ^c	1.772±0.03 ^{ab}	39.091±3.02 ^b	0.075±0.01 ^{ab}

Table 4. Heavy metals content of coconut feed, cabbage feed, coconut flour and cabbage flour

Sample	Cu (mg/kg)	I (mg/kg)	Se (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	As (mg/kg)	Cr (mg/kg)
Coconut Feed	0.581 ± 0.02 ^a	Nil	1.531 ± 0.01 ^a	0.750 ± 0.02 ^a	Nil	Nil	0.442 ± 0.00 ^a
Cabbage Feed	0.004 ± 0.01 ^b	0.599 ± 0.02 ^a	1.480 ± 0.11 ^a	0.777 ± 0.01 ^a	0.072 ± 0.02 ^a	Nil	0.150 ± 0.00 ^b
Coconut Flour	0.582 ± 0.02 ^c	Nil	1.461 ± 0.02 ^{ab}	0.485 ± 0.00 ^b	Nil	Nil	0.440 ± 0.02 ^a
Cabbage Flour	0.003 ± 0.00 ^b	0.587 ± 0.02 ^a	1.412 ± 0.02 ^b	0.410 ± 0.02 ^b	0.123 ± 0.01 ^b	Nil	0.435 ± 0.02 ^a

Results were expressed as Mean ± SEM

Table 5a. Qualitative phytochemical screening of coconut flour and cabbage flour

PHYTOCHEMICALS	COCONUT	CABBAGE
*Saponin	-	-
Tannins	-	-
Phenolics	+	+
Phlobatanin	-	-
Steroids	-	+
Flavonoids	+	+
Anthrocyanin	+	-
Terpenoids	+	-
Glycosides	+	+
Triterpenes	+	+
Alkaloids	+	+
Coumarin	-	+

Where - ; absent +; present

Table 5b. Quantitative phytochemical screening of coconut flour and cabbage flour

PHYTOCHEMICALS	COCONUT	CABBAGE
Phenolics mg/100g	49.63 ± 0.11	5.69 ± 0.13
Flavonoids mg/100g	116.07 ± 4.24	167.10 ± 1.15
Anthocyanin µg/100g	0.33 ± 0.02	Nil
Terpenoids µg/100g	27.23 ± 1.06	Nil
Glycosides µg/100g	7.88 ± 0.32	5.21 ± 0.24
Triterpenes µg/100g	255.18 ± 3.33	215.85 ± 0.10
Alkaloids µg/100g	43.14 ± 0.10	34.21 ± 0.12
Steroids µg/100g	Nil	361.52 ± 33.00
Coumarins µg/100g	Nil	51.93 ± 1.94

Results are expressed in Mean ± SEM

The DPPH radical scavenging activity of the flours are presented in Table 6 and Figure 1. Inhibition of DPPH by cabbage fiber is significantly higher ($P < 0.05$) at 20, 40, 60, 80 and 10mg/ml compared to that of coconut flour at the same concentrations. Superoxide scavenging activity

presented in table 7 reveals significantly higher ($P < 0.05$) superoxide scavenging activity by coconut flour compared to cabbage fiber at same concentration.

Table 6. DPPH radical scavenging activity of the cabbage and coconut flour

DPPH	% inhibition @20mg/ml	% inhibition @40mg/ml	% inhibition @60mg/ml	% inhibition @80mg/ml	% inhibition @100mg/ml
COCONUT FLOUR	8.37 ± 0.03	9.23 ± 0.01	26.88 ± 0.15	37.87 ± 0.18	47.72 ± 0.03
CABBAGE FLOUR	87.96 ± 0.75	87.86 ± 0.12	88.70 ± 0.18	87.96 ± 0.09	87.60 ± 0.03
ASCORBIC ACID	89.12 ± 1.01	90.21 ± 0.00	91.00 ± 2.21	91.13 ± 1.02	93.02 ± 1.02

Results are expressed in Mean ± SEM

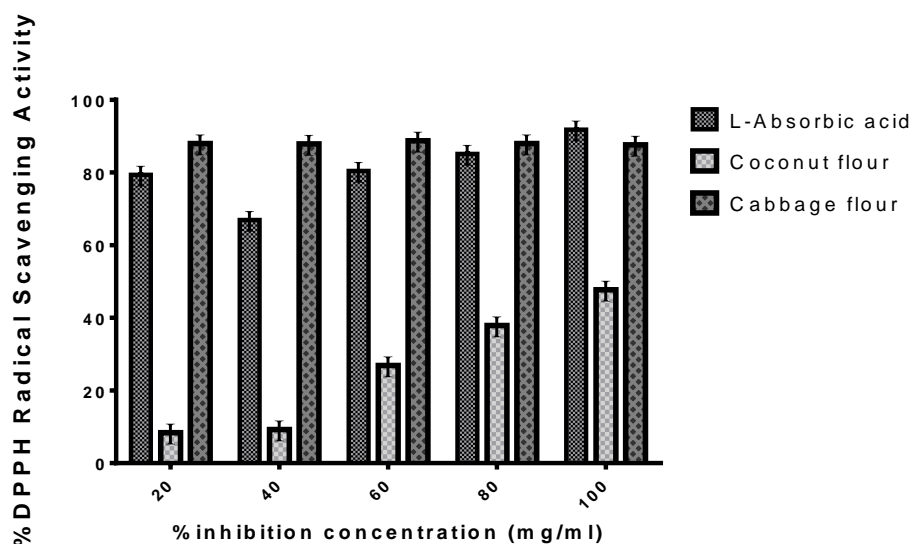


Figure 1. %DPPH Radical scavenging activity of L-Ascorbic acid, coconut flour and cabbage flour. Results are expressed in Mean ± SEM ($P < 0.05$)

Table 7. Superoxide, Hydroxyl, Hydrogen Peroxide Scavenging and Total Antioxidant Capacity Carried Out On Coconut Flour And Cabbage Flour

SUPEROXIDE	OH radical scavenging activity @100mg/ml	H ₂ O ₂ @50mg/dl	TAC @50mg/dl	O ₂ [*] scavenging activity @100mg/ml
COCONUT FLOUR	205.17 ± 1.12	10.61 ± 0.02	804.48 ± 0.00	2.38 ± 0.28
CABBAGE FLOUR	25.52 ± 0.63	7.65 ± 0.03	641.96 ± 1.96	5.03 ± 0.28
ASCORBIC ACID	30.28 ± 2.15	24.75 ± 1.57	970.21 ± 2.73	28.30 ± 2.31

Results are expressed in Mean ± SEM

DISCUSSION

Proximate analysis: The high fiber content obtained in the two samples may be important in preparing a low carbohydrate diet for populations that cannot afford refined and imported fibers with an assurance of reduced cost and yet with optimum quality. In our previous work, we reported the use of the coconut and cabbage fibers in compounding feed for laboratory animals for the management of metabolic disorders with success [2, 21]. The protein content of the feeds may enhance overall protein availability when ingested. This may enhance vital body functions such as growth, maintenance of fluid balance, formation of hormones, enzymes, and sustenance of strong immune function [22]. The moisture content in the cabbage flour, however, indicates it is more prone to deterioration by microorganisms and hence reduction in shelf life compared to the coconut-based fiber [23].

Mineral analysis: Minerals are essential for the regular maintenance of human health when consumed in adequate proportions of recommended dietary allowances (RDA). The presence of myriads of minerals in the developed fibers makes it an interesting sample for dietary inclusion for various feed formulations in nutritional studies and especially the ketogenic diet [24]. The significant elevation of sodium in the cabbage samples, however, may alter electrolyte balance and, as such, may require moderation in consumption especially for individuals with heart-related health issues [25]. The heavy metals content measured in the samples were

within permissible limits and may not predispose consumers to any related toxicity.

Phytochemicals: Phytochemicals in plants possess medicinal benefits which includes antioxidant, antipyretic, and anti-inflammatory activities amongst others [26]. They therefore support protection against and treatment of several diseases such as cancer, inflammation, obesity, diabetes, and other metabolic disorders [27]. The presence of diverse phytochemicals in the fibers (especially flavonoids) makes it of interest to nutraceutical and pharmafoods researcher's community as candidates for further investigations.

Antioxidant: Plant flavonoids and steroids have exhibited affinity for hydroxyl, peroxide and superoxide radicals and help in improving health. The cabbage flour acts as better and excellent radical scavenger compared to the coconut flour. The total antioxidant activity of the fibers may be due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers since phytochemicals like flavonoids and tannins were able to discolor DPPH solution by their hydrogen donating ability [28-29]. This inherent antioxidant ability of the fibers when ingested will help combat several health conditions whose occurrence and progression had been linked to the generation of free radicals in the biological system [30].

CONCLUSION

The fibers developed in this study are suitable for use in ketogenic diet formulation as evidenced by the rich

mineral and required proximate analysis content. They could be positioned as a nutraceutical for therapeutic action due to their inherent phytochemicals and radical scavenging activity. The heavy metals detected were also within permissible limits hence qualifying the fibers as not only nutritious but safe for dietary inclusions.

List of abbreviations: KD: Ketogenic diet, DPPH: 1,1-diphenyl-2-picrylhydrazyl, ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), TEAC: Trolox equivalent antioxidant capacity, IBM: International Business Machines Corporation, SPSS: Statistical Package for the Social Sciences, RDA: Recommended Dietary Allowances

Conflict of Interest: The authors declare there are no conflicts of interest.

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Authors Contributions: K-O.T. conceptualization, supervision, and design of experiment. K-O.T and O-T.P. conducted the research. K-O.T., O-T.P and K-A.A.A. analyzed the data. O-T.P., K-O.T and K-A.A.A. Writing – Original Draft Preparation. K-O.T,K-A.A.A and O-T.P. Writing – Review & Editing. O-T.P, K-O.T and K-A.A.A provided essential reagents and materials. All authors read and approved the final manuscript.

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