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ANTIOXIDANT ACTIVITY AND INHIBITION OF α -AMYLASE AND α -GLUCOSIDASE IN *VERNONIA AMYGDALINA* DEL AND FISH OIL-ENRICHED BISCUITS

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ABSTRACT

Vernonia amygdalina (VA) and Fish oil (FO) are important functional foods that play important roles in disease prevention and management. Incorporating them into biscuits will provide alternative and healthier snack options for consumers. This study aimed to evaluate the *In vitro* antioxidant potential and the inhibitory capacity of biscuits enriched with *Vernonia amygdalina* Del and fish oil (VFEB) against α -amylase and α -glucosidase. Four biscuit samples were produced, and a conventional biscuit (B1) purchased commercially served as control. Biscuits without VA and FO (B2), and biscuits with 1 g, 2 g, and 3 g of VA and FO (B3, B4, B5). Alpha amylase and α -glucosidase inhibitory activities, and *In vitro* antioxidant capacity assessments of experimental biscuits using various assays were carried out. Results showed that the inhibitory activities against α -amylase and α -glucosidase increased in a dose-dependent manner. Similarly, the antioxidant capacity of B3, B4, and B5 showed a dose-dependent increase compared to B1 and B2. In conclusion, VFEB exhibited substantial antioxidant potential and possessed the ability to inhibit α -amylase and α -glucosidase respectively. Hence, VFEB would be a healthier snack option whose consumption would likely improve postprandial glycemic responses.

Keywords: Antioxidants; Biscuits; α -amylase; *Vernonia*; Dietary

INTRODUCTION

In recent years, the study of functional foods with enhanced health benefits has gained significant attention within the realm of nutritional science (Shaikh, 2022; Dixit *et al.* 2023). Among these, *Vernonia amygdalina* Del, commonly known as bitter leaf, stands out for its rich phytochemical content and traditional medicinal uses (Soji-Omoniwa, *et al.* 2023). *Vernonia amygdalina* Del is a green leafy vegetable widely consumed in Nigeria and some African countries, has been recognized for its antioxidant, anti-inflammatory,

and antidiabetic properties (Farombi and Owoeye, 2011; Adedapo *et al.*, 2014). Additionally, its high polyphenol content, including flavonoids and phenolic acids, has been associated with potential health-promoting effects (Obboh *et al.* 2012; Edo *et al.*, 2023). Concurrently, the integration of fish oil into food matrices has been advocated for its potential to confer various health advantages owing to its omega-3 fatty acid composition (Siscovick *et al.*, 2017; Santos *et al.* 2023). Fish oil, derived from fatty fish species, is renowned for its omega-3 fatty acids, particularly

eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids have been linked to cardiovascular health, anti-inflammatory effects, and cognitive function (Kris-Etherton *et al.*, 2002; Swanson *et al.*, 2012). *Heteroclaris* catfish is an intergeneric hybrid crossbred between *Heterobranchus longifilis* and *Clarias gariepinus*. Previous study had shown that consumption of heteroclaris in human diet may contribute to reducing the risk of coronary heart disease and cancer due to its fatty acids' composition (Sobczak *et al.*, 2022). The by-products of catfish processing consist of frames and viscera which are often thrown away during processing. Catfish viscera could be used as a source of lipids rather than waste.

Despite the individual recognition of *Vernonia amygdalina* Del and fish oil for their health benefits, limited research has explored their combined effects in a food product. Biscuits, as a widely consumed and convenient snack, serve as an ideal vehicle for incorporating these bioactive compounds into the diet. Additionally, understanding the inhibitory potential of these biscuits against α -amylase and α -glucosidase is crucial for elucidating their impact on postprandial glycemic responses.

This study aimed to evaluate the *in vitro* antioxidant potential and the inhibitory capacity against α -amylase and α -glucosidase of biscuits enriched with *Vernonia amygdalina* Del and *Heteroclaris* viscera oil. The outcomes of this research could shed light on the potential health-promoting effects of *Vernonia amygdalina* Del and fish oil-enriched biscuits, providing valuable insights for the development of functional foods with enhanced health-promoting profiles.

MATERIALS AND METHODS

Plant collection and preparation

Fresh leaves of *Vernonia amygdalina* were harvested from a garden at Ajowa Street, Sango Road, Ilorin, Kwara state, Nigeria (8°31'37.12116''N 4°35'19.21848''E) in January, 2023. The identification and authentication of the plant was carried out at the herbarium unit of the Department of Plant biology, University of Ilorin, Ilorin, Kwara State. A voucher specimen no UILH/001/1324/2023 was assigned to the *Vernonia amygdalina* sample.

Vernonia amygdalina leaves collected were rinsed with tap water and air-dried to constant weight at room temperature. The dried leaves were then pulverized using an electronic blender and kept in air-tight container prior to usage.

Heteroclaris viscera collection and oil extraction

The viscera of *Heteroclaris* spp catfish was used for this experiment. The viscera were purchased from the local market at Unity Road catfish Market (8.4809375N, 4.5619375E), Ilorin, Kwara State, Nigeria. The fish viscera were excised from the fish manually by the market vendors. They were bought fresh and washed to remove any blood residue. The viscera oil was extracted according to the wet (boiling) extraction method as described by Vidotti *et al.* (2011) with slight modification by skipping the centrifugation step. A weight of 1000 g chopped viscera was placed into a cooking bowl with 1000 ml of water and cooked at 100 °C for 50 minutes. The cooked viscera were left to cool down for 3 hours and the oil allowed to float on the surface of the cooked mixture. The floating oil was then collected and placed in a separating funnel in order to separate the oil from the cooked mixture. The clear oil was decanted into a reception container, sieved and heated to remove moisture in the oil.

Formulation of *Vernonia amygdalina* Del and Fish Oil-enriched Biscuits

The biscuits were formulated following the recipe of Whitley (1970) with slight modifications in the percentages of the ingredients. Four biscuits samples were formulated, namely; biscuit without *Vernonia amygdalina* and fish oil (B2), biscuit with 1 g of *Vernonia amygdalina* and fish oil (B3), biscuit with 2 g of *Vernonia amygdalina* and fish oil (B4) and biscuit with 3 g of *Vernonia amygdalina* and fish oil (B5). The control biscuit labelled B1 (a product of Yale Foods Industrial LTD, plot 1B, Block1, Oluyole Estate. P.O. Box 2033, Ring Road Ibadan, Oyo State, Nigeria) was purchased from a local vendor in Ilorin.

B2 was prepared by mixing 240 g of wheat flour with 15 g of sweetener, 1 g of salt, 4 g of baking powder, 60 g of butter and 60 g of milk, to form a dough. The dough was kneaded and rolled carefully with a rolling pin, cut into round shapes and baked in the oven for 15 minutes at 175 °C.

B3 was prepared by mixing 240 g of wheat flour with 15 g of sweetener, 1 g of salt, 4 g of baking powder, 69.5 g of fish oil, 60 g of milk and 1 g of pulverized *Vernonia amygdalina* leaves, to form a dough. The dough was kneaded and rolled carefully with a rolling pin, cut into round shapes and baked in the oven for 15 minutes at 175 °C. The above process used for the formulation of B3 was repeated for the formulation of B4 and B5 with variation in the quantity of *Vernonia amygdalina*. Two (2 g) of *Vernonia amygdalina* was used for the formulation of B4 while 3g was used for the formulation of B4. Afterwards, the biscuits were cooled at room temperature and kept in zip lock bags.

In vitro Antioxidant Assay

Determination of Ferric Reducing Antioxidant Power (FRAP)

The antioxidant capacity of the biscuit was assessed using a spectrophotometric method based on the procedure developed by Benzie and Strain (Benzie and Strain, 1996). To prepare the Ferric reducing antioxidant power (FRAP) reagent, a mixture of 300 mM acetate buffer, 10 ml TPTZ in 40 mM HCl, and 20 mM FeCl₃.6H₂O in a 10:1:1 proportion was made at 37 °C. The freshly prepared working FRAP reagent (3.995 ml) was then added to 5 µl of the suitably dissolved biscuit sample using a 0.2-1.2 mg variable micropipette (total volume 3.995 ml + 5 µl) and thoroughly mixed. The reduction of the Fe³⁺ TPTZ complex to its ferrous (Fe²⁺) form resulted in the formation of an intense blue-coloured complex. After incubating the mixture at 37° for 30 minutes, the absorbance at 593 nm was measured against a reagent blank (3.995 ml FRAP reagent + 5 µl distilled water). The experiment was performed in triplicate to ensure accuracy, and a calibration curve was constructed by plotting the absorbance at 593 nm against various concentrations of FeSO₄. Finally, the FRAP values were calculated from the calibrated and expressed as milligrams FeS equivalent per gram of the sample.

Determination of Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide scavenging activity was according to the method described by Borquaye *et al.* (2020). Ferrous ammonium sulfate 0.5 ml of 1 mM was added to the test tube, followed by

0.13 ml of 5 mM H₂O₂ and 3 ml of sample extract or standard drug at varying concentrations. Each test tube was incubated in the dark for 5 min at room temperature. After this, 1, 10-phenanthroline (1 mM, 3 ml) was added to each mixture and the test tube was shaken to ensure uniform mixing. The reaction mixture was then incubated at room temperature for 10 min. The absorbance of the reaction mixture was taken at 510 nm. Water was used in place of sample extract for the blank. The amount of hydrogen peroxide scavenged was obtained from the following equation:

Calculation:

$$\% \text{ hydrogen peroxide scavenged} = A(\text{test}) \div A(\text{control}) \times 100$$

Where A_{test} is the absorbance of the test sample and A_{control} is the absorbance of the blank

Determination of 2,2-DiPhenyl-1-Picrylhydrazyl Free Radical Scavenging Activity

The free radical scavenging ability of the formulated biscuits against DPPH (2,2-diphenyl-1-picrylhydrazyl)-free radical was determined as described by Gyamfi *et al.* (1999). The extracts (1 ml) were mixed with 1 ml of 0.4 mM/L methanol solution containing DPPH radicals, and the mixture was left in the dark for 30 minutes. The absorbance was measured at 516 nm using a spectrophotometer, and the DPPH-free radical scavenging ability was calculated with respect to the reference containing all the reagents without the test sample.

Determination of α-Glucosidase Inhibitory Activity of Vernonia amygdalina Del and Fish Oil-enriched Biscuits

The α-Glucosidase inhibitory activity assay was performed following the method described by Kazeem *et al.* (2013) with slight modification. Briefly, the reaction mixture contained 250 µL of different concentrations of the sample (50-250 µg/mL), add 500 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-glucosidase solution. The mixture was incubated at 25 °C for 10 min after which 250 µL of 3.0 mM p-nitrophenyl-α-d-glucopyranoside in phosphate buffer was added. This was incubated again at room temperature for 20 min after which 1.0 ml of 0.1 sodium carbonate

was added to stop the reaction. Blank was prepared by using distilled water in place of sample while all reagent without sample and distilled water served as the control and acarbose as standard. The absorbance of the mixture was taken at 405 nm with the spectrophotometer against the reagent blank. The inhibitory activities of the samples were calculated as follows:

$$\text{Inhibitory activity (\%)} = [(A_c - A_s) / A_c] \times 100$$

Where: A_s is the absorbance in the presence of test substance or standard and A_c is the absorbance of control.

Determination of α - Amylase Inhibitory Activity of *Vernonia amygdalina* Del and Fish Oil-enriched Biscuits

The effective alpha amylase scavenging activity was according to the method described by (Giancarlo *et al.* 2006). Starch solution was prepared using 0.1 g of starch dissolved in 100 ml of sodium phosphate buffer (pH 6.9). 300 ml of distilled water was poured in a beaker then 35 g of sodium phosphate dibasic and 15 g of sodium phosphate monobasic was added, distilled water was added up to 500 ml and the pH was adjusted using 0.1 M HCl. The colouring agent was prepared by withdrawing 8 ml of NaOH from 1 M of NaOH then 12 g of sodium potassium tartrate was then dissolved in the 8 ml of NaOH. 438 g of 3,5 dinitrosalicylic acid was dissolved in 20 ml of deionized water and heated in water bath at 50 °C-70 °C, the solution was stirred continuously under maintained temperature within the range of 50 °C -70 °C. The sample extracts (20 μ l) and 1 ml of starch solution were mixed in a tube and incubated at 20 °C for 20 minutes. To this mixture, 1 ml α -amylase was added, incubated at 20 °C for 3 minutes, and placed in a water bath at 75-80 °C after the addition of 1 ml coloring reagent. After 15 minutes, the reaction mixture was removed from the water bath and cooled. The absorbance value was determined at 540 nm using a spectrophotometer. A similar study was conducted without the plant extracts and taken as a control reaction. Commercially available antidiabetic drug, metformin was taken as a positive control and evaluated similarly. A triplicate study was performed to ascertain the accuracy of the results.

Calculation:

$$\alpha\text{-Amylase Inhibition} = \left[\frac{A(\text{control}) - A(\text{sample})}{A(\text{control})} \right] \times 100$$

Where A is the absorbance

Statistical Analysis

Data were expressed as a mean of 3 replicates $\pm$ standard deviation (SD). The data were subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS) software version 20. All significant differences were determined by one-way analysis of variance (ANOVA). Post hoc multiple comparisons were done using Duncan's multiple range test. The level of significance was set at $p < 0.05$ (confidence level = 95 %).

RESULTS

Ferric Reducing Antioxidant Power (FRAP) of *Vernonia amygdalina* Del and Fish Oil-enriched Biscuits

Figure 1 shows the result of the ferric-reducing power of *Vernonia amygdalina* Del and Fish Oil-enriched Biscuits. The result shows a dose-dependent increase in the FRAP of the experimental biscuits with B5 (biscuit with 3 g *Vernonia amygdalina* and fish oil) having the highest FRAP activity.

Hydrogen Peroxide Scavenging activity of *Vernonia amygdalina* Del and Fish Oil enriched Biscuits

Figure 2 shows the hydrogen peroxide scavenging activity of biscuit sample B5 was significantly ($p < 0.05$) higher when compared to the other samples (B1, B2, B3 and B4). However, a dose-dependence increase was recorded for samples B1, B2 and B3.

2, 2-DiPhenyl-1-PicrylHydrazyl (DPPH) Free Radical Scavenging Activity of *Vernonia amygdalina* Del and Fish Oil-enriched Biscuits

Table 1 shows the result of DPPH free radical Scavenging Activity of *Vernonia amygdalina* Del and fish oil-enriched Biscuits. Result shows a consistent increase in the DPPH scavenging activity as the concentration of the sample increases. In addition, B3, B4 and B5 were significantly higher ($p < 0.05$) than B1 and B2. Results of B4 at 10 and 100 mg/ml, DPPH scavenging activity of B4 and B5 was not significantly different compared to ascorbic acid.

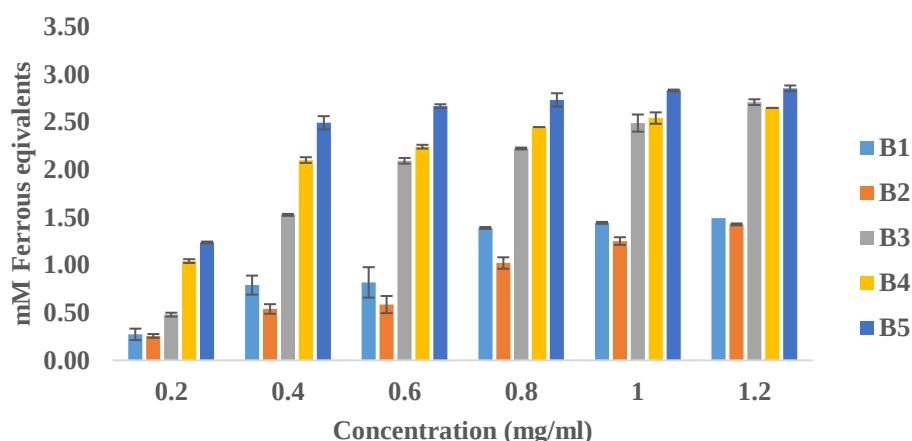


Figure 1: Ferric Reducing Antioxidant Power (FRAP) of *Vernonia amygdalina* Del and Fish Oil-enriched Biscuits

Values are expressed as mean of 3 replicates $\pm$ SD; B1 - Conventional biscuit; B2 - Biscuit without *Vernonia amygdalina* and fish oil; B3 - Biscuit with 1 g *Vernonia amygdalina* and fish oil; B4 - Biscuit with 2 g *Vernonia amygdalina* and fish oil; B5 - Biscuit with 3 g *Vernonia amygdalina* and fish oil

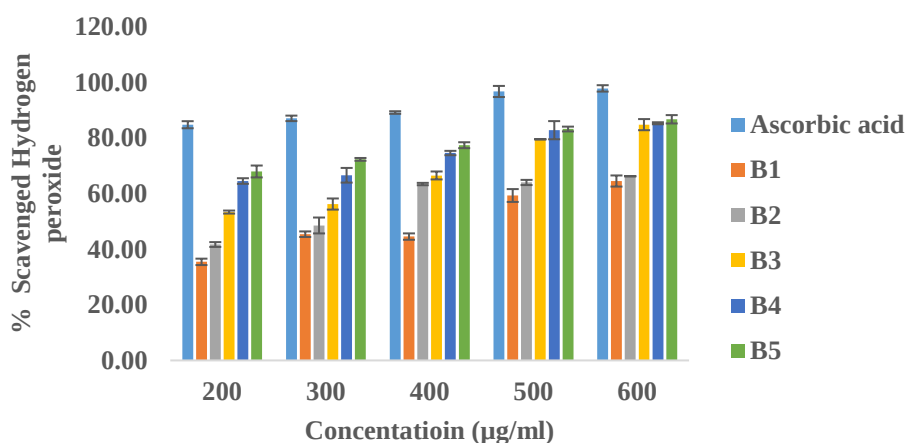


Figure 2: Hydrogen Peroxide-scavenging Activity of *Vernonia amygdalina* Del and Fish Oil-enriched Biscuits

Values are expressed as mean of 3 replicates $\pm$ SD; B1 - Conventional biscuit; B2 - Biscuit without *Vernonia amygdalina* and fish oil; B3 - Biscuit with 1 g *Vernonia amygdalina* and fish oil; B4 - Biscuit with 2 g *Vernonia amygdalina* and fish oil; B5 - Biscuit with 3 g *Vernonia amygdalina* and fish oil

Table 1: 2, 2-DiPhenyl-1-PicrylHydrazyl (DPPH) free radical Scavenging Activity of *Vernonia amygdalina* Del and Fish Oil-enriched Biscuits

Conc. (mg/ml)	B1	B2	B3	B4	B5	Ascorbic acid
10	24.27 $\pm$ 0.85 ^a	25.22 $\pm$ 0.07 ^a	46.19 $\pm$ 0.04 ^b	63.62 $\pm$ 0.30 ^c	64.18 $\pm$ 0.03 ^c	64.25 $\pm$ 0.05 ^c
100	24.59 $\pm$ 0.54 ^a	24.15 $\pm$ 0.01 ^a	48.53 $\pm$ 0.29 ^b	64.83 $\pm$ 0.10 ^c	65.18 $\pm$ 0.03 ^c	66.14 $\pm$ 0.01 ^c
200	24.71 $\pm$ 0.43 ^a	24.21 $\pm$ 0.02 ^a	48.90 $\pm$ 0.11 ^b	65.48 $\pm$ 0.26 ^c	66.16 $\pm$ 0.03 ^c	71.47 $\pm$ 0.36 ^d
300	24.34 $\pm$ 0.22 ^a	24.46 $\pm$ 0.34 ^a	50.06 $\pm$ 0.06 ^b	66.15 $\pm$ 0.05 ^c	67.23 $\pm$ 0.04 ^c	73.28 $\pm$ 0.01 ^d
400	23.06 $\pm$ 0.74 ^a	24.19 $\pm$ 0.04 ^a	50.54 $\pm$ 0.02 ^b	67.18 $\pm$ 0.05 ^c	68.09 $\pm$ 0.02 ^c	75.63 $\pm$ 0.35 ^d

Values are expressed as mean of 3 replicates $\pm$ SD. and values with different alphabets across the rows are statistically different ($p < 0.05$); B1 - Conventional biscuit; B2 - Biscuit without *Vernonia amygdalina* and fish oil; B3 - Biscuit with 1 g *Vernonia amygdalina* and fish oil; B4 - Biscuit with 2 g *Vernonia amygdalina* and fish oil; B5 - Biscuit with 3 g *Vernonia amygdalina* and fish oil

Alpha-glucosidase Inhibitory Activity of *Vernonia amygdalina* Del and Fish Oil-enriched Biscuits

As shown in Figure 3, effect of acarbose (the positive control), against α -glucosidase increased in a concentration-dependent manner, with an IC_{50} at 4.59 mg/ml (Table 2). At concentrations up to 8 mg/ml, the inhibitory effect increased near linearly, thereafter its increase slowed obviously, reaching a final inhibitory effect of 79.00% at 10 mg/ml. Effect of the *Vernonia amygdalina* Del and fish oil-enriched Biscuits also rose in a concentration-dependent manner with an IC_{50} value at 6.36, 5.55 and 5.29 mg/ml for B3, B4

and B5 respectively (Table 2), was significantly higher than that of acarbose at all concentrations (Figure 3).

Alpha-amylase Inhibitory Activity of *Vernonia amygdalina* Del and Fish Oil-enriched Biscuits

The result of α -amylase inhibitory activity of *Vernonia amygdalina* Del and fish oil-enriched Biscuits is shown in Figure 4. In a similar trend as α -glucosidase, percentage inhibition peaked at 10 mg/ml for B3, B4, B5 and acarbose against α -amylase. IC_{50} values for B3 (7.92 mg/ml), B4 (7.31 mg/ml), and B5 (6.03 mg/ml) increased significantly compared to acarbose.

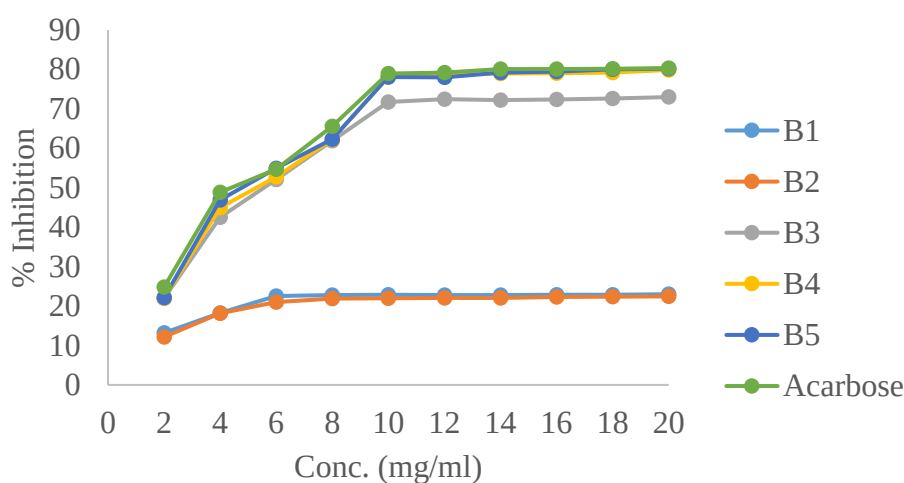


Figure 3: α -Glucosidase Inhibitory Activity of *Vernonia amygdalina* Del and Fish Oil-enriched Biscuits

Values are expressed as mean of 3 replicates $\pm$ SD; B1 - Conventional biscuit; B2 - Biscuit without *Vernonia amygdalina* and fish oil; B3 - Biscuit with 1 g *Vernonia amygdalina* and fish oil; B4 - Biscuit with 2 g *Vernonia amygdalina* and fish oil; B5 - Biscuit with 3 g *Vernonia amygdalina* and fish oil

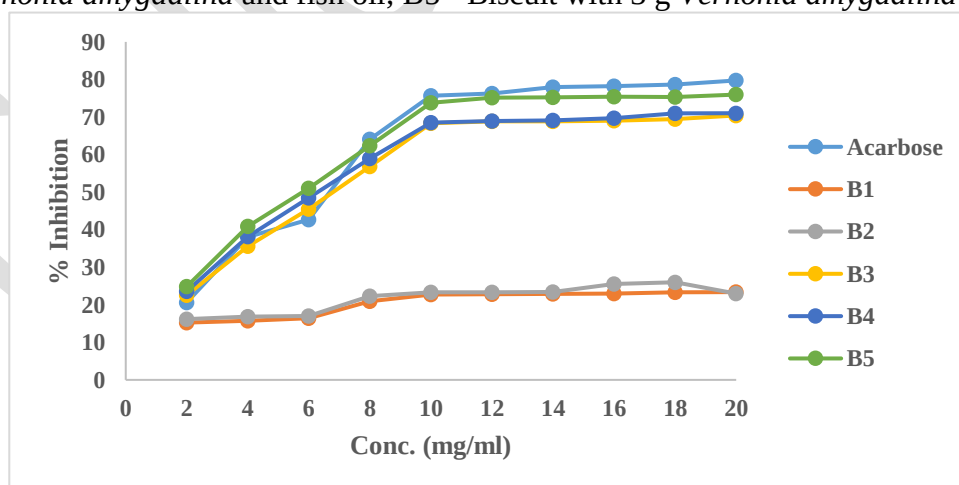


Figure 4: α - Amylase Inhibitory Activity of *Vernonia amygdalina* Del and Fish Oil-enriched Biscuits

Values are expressed as mean of 3 replicates $\pm$ SD.; B1 - Conventional biscuit; B2 - Biscuit without *Vernonia amygdalina* and fish oil; B3 - Biscuit with 1 g *Vernonia amygdalina* and fish oil; B4 - Biscuit with 2 g *Vernonia amygdalina* and fish oil; B5 - Biscuit with 3 g *Vernonia amygdalina* and fish oil

Table 2: IC₅₀ Values for α -Glucosidase and α -Amylase Inhibitory Effects of *Vernonia amygdalina* and Fish Oil-enriched Biscuits and Acarbose

Groups	IC ₅₀ (mg/ml)	
	α -glucosidase	α -amylase
Acarbose	4.59±0.24 ^a	6.63 ± 0.22 ^a
B1	87.98±2.33 ^b	72.32 ± 3.12 ^b
B2	85.76±2.22 ^b	65.38 ± 2.28 ^b
B3	6.36±0.01 ^c	7.92 ± 0.22 ^c
B4	5.55±0.12 ^d	7.31 ± 0.08 ^d
B5	5.29±0.06 ^d	6.03 ± 0.01 ^e

Values are expressed as mean of 3 replicates $\pm$ SD; Values with different superscripts down the column are statistically significant at $p < 0.05$; B1 - Conventional biscuit; B2 - Biscuit without *Vernonia amygdalina* and fish oil; B3 - Biscuit with 1 g *Vernonia amygdalina* and fish oil; B4 - Biscuit with 2 g *Vernonia amygdalina* and fish oil; B5 - Biscuit with 3 g *Vernonia amygdalina* and fish oil

DISCUSSION

Biscuits are one of the best-selling food products in many countries. They have a long shelf life and, because a wide variety of ingredients can be incorporated into their formulation, they can be a useful tool to improve nutritional quality in man's diet as well as promote health (Guiné *et al.*, 2020). Dietary guidelines worldwide recommend to increase the consumption of fruits and vegetables. These foodstuffs are rich sources of antioxidants and dietary fibre that could reduce the risk of human diseases such as cancer, atherosclerosis, heart diseases, osteoporosis, and obesity. *Vernonia amygdalina*, a vegetable consumed in Nigeria has been reported to contain phytochemicals with strong antioxidant capacity. Its incorporation into biscuits in our study showed that the biscuits elicited antioxidant capacities *in vitro* as shown by our findings. The presence of antioxidants in the samples caused the reduction of the Fe²⁺/Ferricyanide complex to the ferrous form (Pulido *et al.*, 2000). A similar trend was observed for DPPH and hydrogen peroxide with their activity compared to standard antioxidant, ascorbic acid. The antioxidant capacity elicited by the biscuits might be attributed to the *Vernonia amygdalina* and fish oil incorporated into the dough. Previous studies on *Vernonia amygdalina*

had reported that the plant is rich in flavonoids, tannins and saponins (Farombi, 2011) and these may play important roles in the anti-oxidant capacity observed in samples B3, B4 and B5. Flavonoids are known to be good antioxidants, and luteolin, a flavonoid found in *Vernonia amygdalina* has been reported as a strong antioxidant. Igile *et al.* (1994) confirmed that luteolin was more potent as an antioxidant than Butylated hydroxytoluene (BHT), and reported that its glucosides – luteolin 7-O- β -glucuroniside and luteolin 7-O- β -glucoside also have anti-oxidant activities. Similarly, previous studies had reported that fish oil supplementation may attenuate oxidative stress by increasing antioxidant defense as well (Popović *et al.*, 2012; Avramovic *et al.*, 2012). Fish oil is a rich source of omega-3 polyunsaturated fatty acids, especially eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) which have been linked to improve cardiovascular health, cognitive function and shown anti-inflammatory effects (Kris-Etherton *et al.* 2002; Swanson *et al.* 2012).

The dietary carbohydrate digestive enzymes, α -glucosidase and α -amylase were inhibited by VFEB, which suggest that the test agent *V. amygdalina* might be contributing to the observed effect. The ability of a drug or diet to delay the production or absorption of glucose by inhibiting carbohydrate hydrolyzing enzymes such as α -amylase and α -glucosidase is one of the therapeutic approaches for decreasing postprandial hyperglycemia. Postprandial hyperglycemia plays an important role in the development of T2DM. Regulating plasma glucose level is vital for delaying or preventing T2DM.

Vernonia amygdalina had earlier been reported to contain vernoamyoside, a steroid saponin, which can inhibit α -glucosidase and α -amylase activities respectively (Anh *et al.*, 2021; Djeujo *et al.*, 2021). Similarly, the use of *Vernonia amygdalina* leaves in lowering blood sugar levels in type 2 diabetes mellitus (T2DM) has been reported (Asante *et al.*, 2016).

CONCLUSION

The biscuits fortified with *Vernonia amygdalina* and fish oil (B3, B4 and B5) have significant antioxidant potential and inhibit the activities of

α -glucosidase and α -amylase. Therefore, VFEB is a healthy snack option whose consumption would improve postprandial glycemic responses and serve as a good source of antioxidants.

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