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Original Research Article

Peptide Fractions from Fermented African oil bean (*Pentaclethra macrophylla*) Condiment Exhibited Antioxidant Property and Inhibited Key Enzymes Linked to Hypertension *In Vitro*

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ABSTRACT

This study aimed to fractionate water-soluble antihypertensive peptides from fermented African oil bean condiment and evaluate their antioxidant properties and inhibitory effects on enzymes associated with hypertension. The condiment was prepared using traditional methods, oven-dried, and extracted in distilled water (200 g/L) for one hour, followed by filtration through a 0.45 µm membrane filter. Subsequently, the filtrate was fractionated using molecular weight cut-off (MWCO) membranes with pore sizes of 1 kDa, 3 kDa, and 10 kDa. The resulting peptide fractions yielded 1.05% (1 kDa), 7.16% (3 kDa), 10.53% (10 kDa), and 80.36% (>10 kDa) by dry weight of the condiment. The antioxidant properties of the fractions were assessed through ferric reducing antioxidant power (FRAP), DPPH radical scavenging, $ABTS^{+\bullet}$ scavenging, nitric oxide ($NO^{\bullet}$) scavenging, and Fe^{2+}-chelating assays. Additionally, the inhibitory effects of the fractions on arginase and angiotensin-I converting enzyme (ACE) – key enzymes linked to hypertension – were evaluated *in vitro*. The results demonstrated that all peptide fractions exhibited potent radical scavenging abilities (>60%), with the 1 kDa fraction showing the highest scavenging activity for all tested radicals. However, no significant difference ($P > 0.05$) was observed in the FRAP assay among the fractions. Furthermore, the 1 kDa fraction also displayed the strongest inhibitory effects on arginase and ACE activities *in vitro*. In conclusion, this study revealed that fermented African oil bean condiment is a potential source of bioactive peptides with significant antioxidant and antihypertensive properties. Among the fractions, the 1 kDa fraction emerged as the most potent and could be incorporated into functional food formulations aimed at managing hypertension.

Keywords: Peptide fractions; fermented condiment; *Pentaclethra macrophylla*; ACE inhibition; antihypertension.

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INTRODUCTION

Hypertension, a chronic medical condition characterized by elevated blood pressure, is a leading risk factor for cardiovascular diseases (CVDs), including stroke, heart failure, and coronary artery disease (Mills et al., 2020). Globally, hypertension affects over 1.3 billion people, contributing significantly to morbidity and mortality (WHO, 2021). Despite the availability of synthetic antihypertensive drugs, their long-term use is often associated with adverse side effects, such as electrolyte imbalances, renal dysfunction, and metabolic disorders (Carey et al., 2018). This has spurred interest in exploring natural alternatives, particularly bioactive peptides derived from food sources, which are considered safer and more sustainable.

Bioactive peptides are short amino acid sequences that exhibit specific physiological functions, including antioxidant, antihypertensive, and anti-inflammatory activities (Udenigwe & Aluko, 2012). These peptides are often released during fermentation or enzymatic hydrolysis of proteins, a process that enhances their bioactivity (Sarmadi & Ismail, 2010). Fermented foods, in particular, have gained attention as rich sources of bioactive peptides due to the microbial transformation of proteins into smaller, more potent fragments (Sanchez & Vázquez, 2017). Among these, fermented African oil bean (*Pentaclethra macrophylla*), a traditional food widely consumed in West Africa, has shown promise as a source of bioactive peptides with potential health benefits.

The antihypertensive potential of bioactive peptides is primarily attributed to their ability to inhibit key enzymes involved in blood pressure regulation, such as angiotensin-I converting enzyme (ACE) and arginase. ACE catalyzes the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor, while arginase competes with nitric oxide synthase (NOS) for L-arginine, reducing nitric oxide (NO) bioavailability and impairing vasodilation (Mahdi et al., 2020). Inhibition of these enzymes can thus help restore vascular homeostasis and lower blood pressure. Additionally, the antioxidant properties of peptides play a crucial role in mitigating oxidative stress, a key contributor to endothelial dysfunction and hypertension (Chen et al., 2019).

Recent studies have highlighted the potential of plant-derived peptides in managing hypertension. For instance, peptides from fermented soybean (natto) and millet have demonstrated significant ACE inhibitory and antioxidant activities (Majid & Priyadarshini, 2019; Li et al., 2022). Similarly, peptides from African legumes, such as cowpea and Bambara groundnut, have shown promising antihypertensive effects *in vitro* (Segura Campos et al., 2010; Mune Mune et al., 2018). However, limited research has been conducted on the bioactive potential of peptides from fermented African oil bean, despite its high protein content and widespread consumption.

This study investigates the antioxidant and enzyme inhibitory properties of peptide fractions derived from fermented African oil bean (*Pentaclethra macrophylla*). Using *in vitro* assays, we evaluated the DPPH, ABTS, and NO radical scavenging abilities of the peptide fractions, as well as their reducing power and metal-chelating properties. Furthermore, we assessed their inhibitory effects on ACE and arginase, two key enzymes implicated in hypertension pathogenesis. The findings from this study aim to provide insights into the potential of fermented African oil bean peptides as natural antihypertensive agents, contributing to the growing body of knowledge on functional foods and their role in cardiovascular health.

MATERIALS AND METHODS

Materials

Sample Collection

African oil bean (*Pentaclethra macrophylla*) was sourced from farm settlements around Akure, Nigeria. The sample was identified and authenticated at the Herbarium, Centre for Research and Development (CERAD), Federal University of Technology, Akure where a voucher sample was deposited. Subsequently, the beans were dried to constant weight, sorted to remove stones and dirt prior to fermentation to traditional condiment.

Chemicals and Reagents

Except stated otherwise, all chemicals and reagents used were of analytical grade from Sigma Aldrich Chemie GmbH, Steinheim, Germany and BDH Chemicals Ltd., Poole, England. Double distilled water was also used for all analysis.

Methods

Production of Traditional Fermented Oil Seed Condiment

The production of the traditional condiment was done using modified methods of Obizoba and Atu (1993) and Ademiluyi et al., (2015). The African oil bean seed was cooked for 3hrs using a pressurized pot, dehulled and further cooked for 1hr. Thereafter, the cooked African oil bean was spread in a sterile raffia basket lined with blanched banana leaves and covered with the same banana leaves. This was allowed to ferment for 6 days at room temperature to produce the traditional condiment. Subsequently, the condiment was oven dried (45 ^{o}C) to constant weight, pulverized and stored in an air-tight container prior to analysis.

Extraction of Water-Soluble Peptides Fractions

Water extractible peptide fractions were extracted from the condiment according to Verdini et al. (2004) with slight modification. Briefly, 200g of the pulverized condiment was extracted in 1000 mL of distilled water under continuous stirring for 1hr at 40 ^{o}C. Then, the mixture was filtered through a filter paper (Whatman No. 42) and centrifuged at 5000 × g using an ultra-cold centrifuge (Sigma 3-30KS Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) at -4 ^{o}C for 30 mins. The supernatant was further filtered through 0.45 µm membrane filter to get the crude peptide fraction (extract). The extract was further partitioned using molecular weight cut-off (MWCO) membrane (Pall Corporation Macrosep Advance Centrifugal Device) yielding different fractions of 1, 3, 10, and >10 kDa. Peptide fractions so obtained were lyophilized and reconstituted in distilled water for subsequent biochemical analysis.

Determination of Antioxidant Properties of the Peptide Fractions

Antioxidant properties of the peptide fractions were determined using standardized *in vitro* methods. The radical scavenging ability of the fractions as typified by 1,1-diphenyl-2-picryhydrazyl (DPPH), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and nitric oxide (NO) radicals scavenging abilities were determined according to Gyamfi et al. (1999), Re et al., (1999) and Marcocci et al., (1994) respectively. The ferric reducing power (a measure of the reductive potential) of the peptide fraction was carried out according to Pulido et al. (2002), while the Fe^{2+} chelating property of the fraction was carried out as described by Minotti and Aust, (1987) as modified by Puntel et al. (2005).

Determination of Arginase Inhibitory Property

The inhibitory effect of the peptide fractions on arginase activity was assayed *in vitro* according to a previously reported method by Adefegha et al. (2015) in a reaction mixture containing Tris–HCl bufer (1.0 mM, pH 9.5, 1.0 mM $MnCl_2$), 0.1 M arginine solution and peptide fractions. The mixture was made to a final volume of 1.0 mL and was incubated for 10 min at 37 °C. The reaction was thereafter terminated by the addition of 2.5 mL Ehrlich reagent (2.0g of *p*-dimethylaminobenzaldehyde in 20 mL of absolute hydrochloric acid (37% purity) and made up to 100 mL with distilled water). The absorbance was read after 20 min at 450 nm. The control experiment was performed without the test sample or standard and arginase modulatory activity was calculated and expressed as percentage of control

Determination of Angiotensin Converting Enzyme (ACE) inhibitory property

The inhibitory effect of the peptide fractions on ACE inhibitory activity *in vitro* was determined according to the method of Cushman and Cheung (1971). ACE solution (50 µL, 4 mU) was preincubated with each peptide fraction (50 µL) for 15 min at 37 ^{o}C. Thereafter, enzymatic reaction was initiated by adding 150 µL of 8.33 mM Bz–Gly–His–Leu in 125 mM Tris– HCl

buffer (pH 8.3) to the mixture. After incubation for 30 min at 37 ºC, the reaction was arrested by the addition of 250 µL of 1 M HCl and the Bz–Gly produced by the reaction was extracted with 1.5 mL ethyl acetate. Thereafter the mixture was centrifuged to separate the ethyl acetate layer and 1 mL of the ethyl acetate layer was transferred to a clean test tube and evaporated. The residue was dissolved in distilled water and its absorbance was measured at 228 nm. The ACE inhibitory activity was expressed as percentage inhibition of the enzyme.

RESULT

The study explored the antihypertensive potential of peptide fractions from fermented African oil seed using in vitro assay methods. Peptide fractions were obtained using molecular cut-off filters and the following fractions were realized; 1kDa, 3kDa, 10kDa, >10kDa and the crude fraction. The result obtained revealed that all the peptide fractions exhibit strong DPPH radical scavenging ability (>60%). However, 1kDa fraction had the highest DPPH radical scavenging ability while 3kDa fraction had the least. The ABTS radical scavenging ability of the fractions also revealed that the 1kDa fraction had the highest radical scavenging ability, however, >10kDa and crude fraction exhibited the least ABTS scavenging ability. Furthermore, the NO radical scavenging ability of the peptide fractions was also determined and the result revealed that the 1kDa fraction exhibited the highest No radical scavenging ability while 3kDa and 10kDa

fractions had the least NO radical scavenging ability.

Nevertheless, the reducing power of the peptide fractions as a function of their antioxidant ability was carried out and the result revealed no significant difference in the ability of the peptide fractions to reduce Fe^{3+} to Fe^{2+}. However, the crude fraction exhibited the highest Fe^{2+}-chelating property of all the peptide fractions tested.

The effect of the peptide fractions on arginase activity in vitro was determined. The result revealed that 1kDa fraction had the highest inhibitory effect on arginase activity while 10kDa and >10kDa fractions had the least arginase inhibitory property. Likewise, the 1kDa fraction had the highest angiotensin-I converting enzyme (ACE) inhibitory property in vitro while the >10kDa and crude fraction had the least ACE inhibitory property.

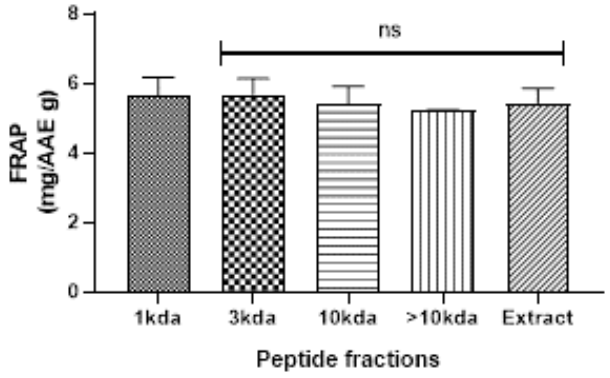

Figure 1: The Ferric Reducing Antioxidant Power (FRAP) of the Peptide Fractions from Fermented African Oil Seed Condiment.

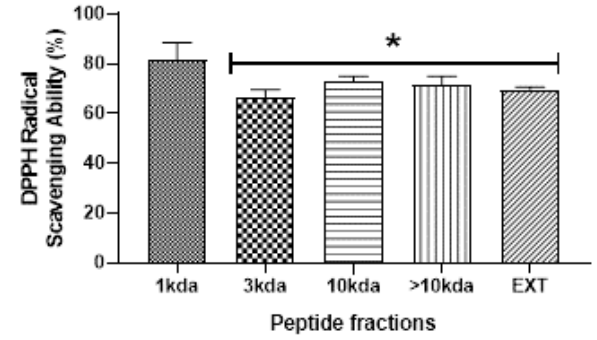

Figure 2: The DPPH Radical Scavenging Ability of the Peptide Fractions from Fermented African Oil Seed Condiment.

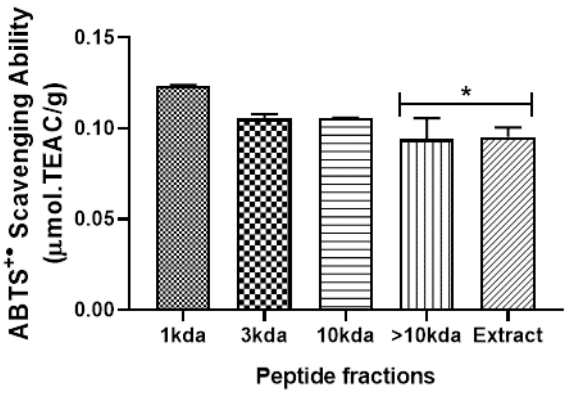

Figure 3: The $ABTS^{+\bullet}$ Scavenging Ability of the Peptide Fractions from Fermented African Oil Seed Condiment.

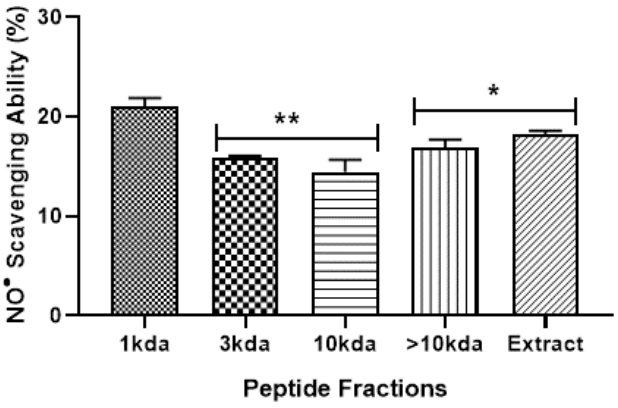

Figure 4: The NO• Scavenging Ability of the Peptide Fractions from Fermented African Oil Seed Condiment.

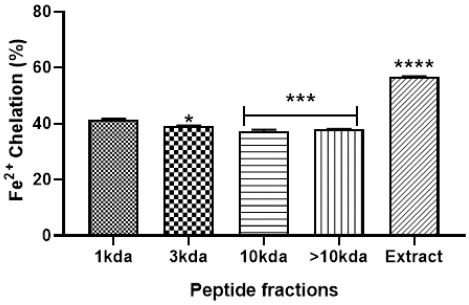

Figure 5: The Fe^{2+}-Chelating Ability of the Peptide Fractions from Fermented African Oil Seed Condiment.

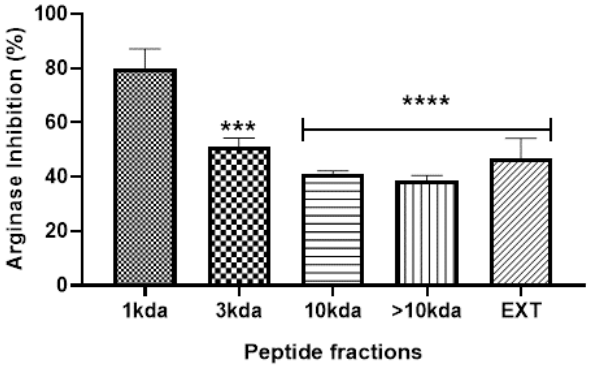

Figure 6: The Arginase Inhibitory Ability of the Peptide Fractions from Fermented African Oil Seed Condiment.

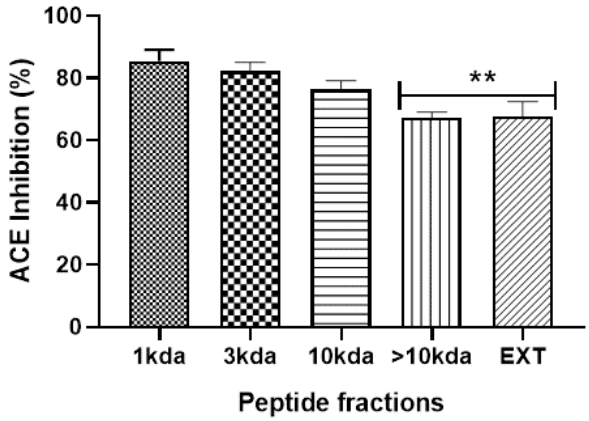

Figure 7: The ACE Inhibitory Ability of the Peptide Fractions from Fermented African Oil Seed Condiment.

DISCUSSION

Hypertension, a major risk factor for cardiovascular diseases, remains a global health challenge. The search for natural antihypertensive agents has gained significant attention due to the side effects associated with synthetic drugs. Bioactive peptides derived from food sources have emerged as promising candidates due to their multifunctional properties, including antioxidant and enzyme inhibitory activities. This study explores the antihypertensive potential of peptide fractions from fermented African oil seed, focusing on their antioxidant capacity, arginase inhibition, and angiotensin-I converting enzyme (ACE) inhibitory properties.

The antioxidant capacity of bioactive peptides is crucial in mitigating oxidative stress, a key contributor to hypertension. The results revealed that all peptide fractions exhibited strong DPPH radical scavenging ability (>60%), with the 1kDa fraction showing the highest activity. This aligns with findings by Udenigwe and Aluko (2012), who reported that low molecular weight peptides (<1kDa) often exhibit superior antioxidant activity due to their higher bioavailability and ability to penetrate cellular membranes. Similarly, the 1kDa fraction demonstrated the highest ABTS and NO radical scavenging

abilities, further underscoring its potent antioxidant properties. These results are consistent with studies by Sarmadi and Ismail (2010), who highlighted the role of small peptides in scavenging reactive oxygen and nitrogen species, thereby reducing oxidative stress. The reducing power assay, which measures the ability of peptides to donate electrons, showed no significant differences among the fractions. However, the crude fraction exhibited the highest Fe2+-chelating property, suggesting its potential in inhibiting Fenton reactions, which generate highly reactive hydroxyl radicals. This finding is supported by Chen et al. (2019), who emphasized the importance of metal-chelating peptides in preventing oxidative damage.

Arginase, an enzyme that converts L-arginine to urea and L-ornithine, competes with nitric oxide synthase (NOS) for their common substrate, L-arginine. Elevated arginase activity reduces nitric oxide (NO) bioavailability, leading to endothelial dysfunction and hypertension. The 1kDa fraction exhibited the highest arginase inhibitory activity, which correlates with its superior NO radical scavenging ability. This is consistent with the work of Mahdi et al. (2020), who demonstrated that arginase inhibition enhances NO production, thereby improving vascular function. The 10kDa and >10kDa fractions showed minimal arginase inhibition, likely due to their larger size and reduced interaction with the enzyme's active site.

ACE plays a pivotal role in blood pressure regulation by converting angiotensin I to angiotensin II, a potent vasoconstrictor. The 1kDa fraction demonstrated the highest ACE inhibitory activity, aligning with previous studies that identified low molecular weight peptides as potent ACE inhibitors (Aluko, 2015). The >10kDa and crude fractions showed the least activity, possibly due to steric hindrance limiting their interaction with the ACE active site. These findings are consistent with research by Aluko, (2015), who reported that small peptides with specific amino acid sequences, such as hydrophobic residues at the C-terminus, exhibit strong ACE inhibitory properties.

The strong antioxidant, arginase inhibitory, and ACE inhibitory activities of the 1kDa fraction suggest its potential as a multifunctional antihypertensive agent. Thus, by scavenging free radicals, inhibiting arginase, and blocking ACE, this fraction addresses multiple pathways involved in hypertension pathogenesis. The synergistic effects of these activities could enhance its therapeutic efficacy because, oxidative stress, reduced NO bioavailability, and elevated angiotensin II levels are interconnected mechanisms driving hypertension. Nevertheless, it is worth nothing that, the results of this study are consistent with recent findings on bioactive peptides from other plant sources (Li et al., 2022). Peptides derived from fermented soybean (natto) have shown similar antioxidant and ACE inhibitory properties (Yamaguchi et al., 2020). Additionally, peptides from fermented millet demonstrated significant arginase inhibition, further supporting the potential of fermented plant-derived peptides in hypertension management (Majid & Priyadarshini (2019)).

While these *in vitro* results are promising, further studies are needed to validate these findings *in vivo*. Animal models and clinical trials are essential to assess the bioavailability, pharmacokinetics, and safety of these peptide fractions. Additionally, structural characterization of the peptides within the 1kDa fraction could provide insights into the specific sequences responsible for their bioactivity.

CONCLUSION

This study highlights the potential of peptide fractions from fermented African oil seed, particularly the 1kDa fraction, as natural antihypertensive agents. Their strong antioxidant, arginase inhibitory, and ACE inhibitory activities position them as promising candidates for hypertension management. Future research should focus on in vivo validation and mechanistic studies to fully elucidate their therapeutic potential.

AUTHORS' CONTRIBUTION

AOA and GO were involved with conceptualization, fund acquisition, project administration and approval of final draft of manuscript. VAA and ORJ carried out data collection and wrote the first draft of the manuscript.

CONFLICT OF INTEREST

The authors declared that, there is no conflict of interest.

ETHICAL APPROVAL

This study was carried out in accordance with the Federal University of Technology, Akure, ethical committee

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