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Aduwa (*Balanites aegyptiaca Del.*) hydrolysates as a novel source of in vitro and in vivo ACE-inhibitory peptides

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ABSTRACT

Background: Nut protein is gaining recognition worldwide due to its encryptable amino acid sequences. This study investigates the inhibitory potential of Aduwa hydrolysates from Aduwa protein concentrate, produced by selected proteases or hypertensive-modulated activities with the aim of enriching the database of bioactive peptides that could serve as a potential ingredient in lowering hypertension.

Objective: This study reports on the *in vitro* and *in vivo* ACE-inhibitory activity of Aduwa (*Balanites aeqyptiaca Del*) protein hydrolysates to evaluate their potency as anti-hypertensive agents.

Methods: Aduwa protein concentrate (APC) sample was digested with pancreatin-pepsin (PP) combined protease, Alcalase (Alca), and Flavourzyme (Flav) hydrolytic enzymes. Samples were subjected to hypertension-lowering ability in spontaneous hypertensive rats (SHRs).

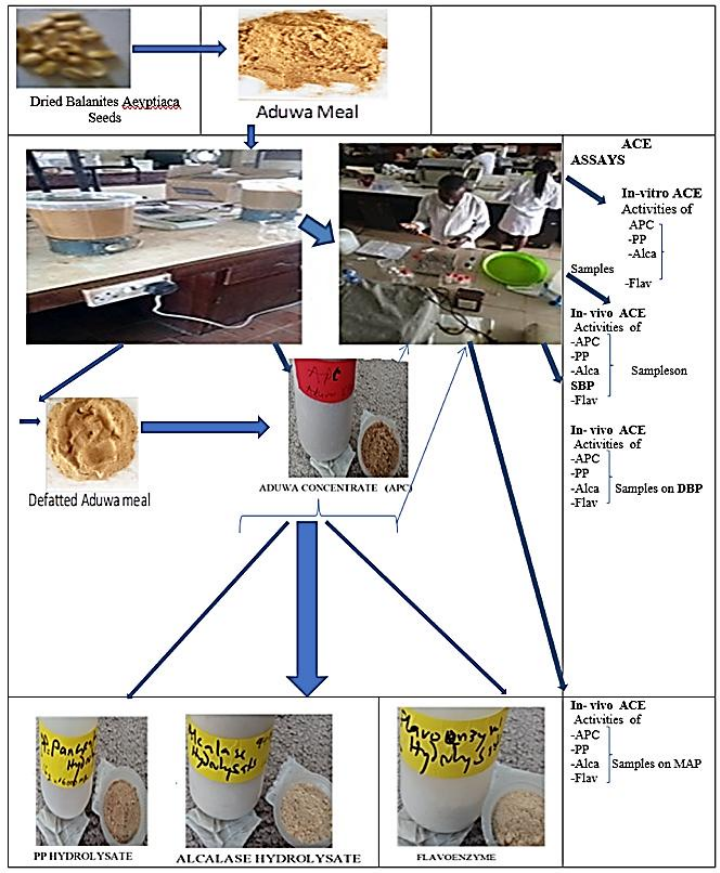
Results: The Alcalase hydrolysate had better angiotensin-converting enzyme *in vitro* and *in vivo* lowering activity than APC and Flav samples. The angiotensin converting enzyme *in vitro* and *in vivo* lowering activity at 200mg/kg and 500mg/kg revealed enhanced hypertension, lowering potentials by PP and Flavourzyme; SBP (maximum~ -9, ~ -7) mmHg, dystonic blood pressure; DBP (maximum~ -6.96, -4.3) mmHg and mean arterial blood pressure MAP (maximum ~ -9, ~ -6) mmHg, respectively in SHRs. This study provides the first report of the *in vitro* and *in vivo* antihypertensive potential

of PP and Flav hydrolysates. These results suggest that Aduwa hydrolysates are effective functional ingredients or raw materials for the production of ACE inhibitors and antihypertensive agents.

Practical Implication: Functional foods derived from nut proteins such as soybean and moringa oleifera can be successfully incorporated into various food matrices, including beverages, baked goods, dairy products, and dietary supplements, without a significant effect on palatability or nutritional quality. Plant peptides are adaptable to diverse climatic conditions and have the potential to support sustainable agricultural production, thereby enhancing their commercial viability as functional food ingredients.

Conclusion: This is the first study to report the antihypertensive potential of Aduwa (*Balanites aegyptiaca)* protein hydrolysates as ACE inhibitors. The peptides from APC, PP, and Alcalase hydrolysates are capable of lowering SBP, DBP, and MAP to levels comparable to captopril. These peptides appear to resist gastrointestinal degradation, retaining ACE-inhibitory activity after oral gavage. Overall, PP and Alca peptides show strong potential for development into functional foods, nutraceuticals, and natural antihypertensive agents. APC and PP hydrolysates could serve as useful bioactive raw materials to promote human health as antihypertensive ingredients in functional food formulations, helping manage high blood pressure.

Keyword: *Balanites aegyptiaca* (Aduwa), protein hydrolysates, ACE-inhibitory peptides, antihypertensive peptides, spontaneously hypertensive rats (SHR), blood pressure reduction, enzymatic hydrolysis, functional food ingredients



Graphical Abstract: Aduwa (*Banalities aeqyptiaca del*) hydrolysates: A new source of *in-vitro* and *in-vivo* ACE inhibitory peptides

INTRODUCTION

Nut protein is gaining recognition worldwide for its potential encrypted active amino acid sequences [1-2]. Food-sourced protein usually carries bioactive peptides or amino acid sequences that can be released by enzyme hydrolysis either *in vivo* or *in vitro* [3]. A bioactive compound (BC) is a broad term that encompasses a wide range of chemical structures with diverse biological activities. This association study was identified through epidemiological research, which shows a correlation between high intake of fruits, vegetables, and whole grains, foods rich in BC, could improve health outcomes [4-5]. Diets rich in these BC are associated with a lower risk of chronic diseases such as cardiovascular disease, cancer, and diabetes [5]. A food's BCs are often categorized by origin. These items may come from plants, animals, or microorganisms. Bioactive peptides derived from protein sources can enhance enzyme activities, improve immune function, and stabilize metabolic health [6-7].

Enzymatic hydrolysis of nuts has been demonstrated using endogenous and exogenous proteases to release efficient bioactive peptides with demonstrated positive effects on health as antihypertensive, therapeutic agents [3,8,11]. Hydrolysates from pea, beans, and mushrooms have been reported to influence α-amylase or α-glycosidase inhibition activities and to demonstrate peptide antihypertensive activity [12,14]. Cardiovascular disease is one of the leading causes of death and disabilities, and it is projected that in twenty-five years, over 23.3 million people may die annually from cardiovascular disease [15]. Cardiovascular disease is often attributed to hypertension, which is usually defined by an increase in systemic arterial pressure above a given threshold value [16-17]. Mild hypertension was grouped by the European Society of Hypertension (ESH) into systolic blood pressure (SBP, 140−159 mmHg) and diastolic blood pressure (DBP, 90−99 mmHg). Mammalian blood pressure is regulated by the renin-angiotensin system, in addition to renin and chymase. But ACE plays a major role by converting angiotensin I to angiotensin II, which is a potent vasoconstrictor [4]. ACE activities have been linked to the pathogenesis of high blood pressure; therefore, their inhibitory potential is used as a therapeutic agent to lower blood pressure [4]. The ACE-inhibitory peptides have been reported from various plant food-derived hydrolysates, such as canola [18-21], and from almond, cashew, and flaxseed, which also have potent *in vitro* ACE activities. The effects of oxidative stress on the onset of chronic diseases and their relationship have been well documented [22-24].

Functional food proteins from plants can be used as matrices in beverages, baked goods, or dairy products, for product development, without altering nutritional quality [25-26]. Peptides from plant sources could be modulated to different geotropic conditions and could sustain agricultural or food production chains as a commercially viable functional food ingredient [27-28].

Therefore, the use of hydrolysates from Aduwa concentrates by PP, Alca, and Flav proteases could inhibit ACE activity, aiding the prevention and management of hypertension. It is a recognized fact that natural proteins and hydrolysates are cheap, safe, and have a lower risk and no negative side effects compared to the synthetic inhibitor drugs such as orlistat, ramipril, and Lisinopril [29-30]. Researchers have identified several plants and tree seed nut proteins as potential sources of antihypertensive bioactive peptides [9-10]. However, no such peptides have been reported from *Balanites aeyptiaca Del*. This work aims to study the inhibitory potential of Aduwa hydrolysates from Aduwa protein concentrate by selected proteases on ACE or hypertensive-modulated activities. There is limited information on the ability of Aduwa hydrolysates from Aduwa meal to have multi-functional properties by

limiting the activity of ACE enzymes, blood SBP, and blood DBP-lowering potentials.

The successful extraction of bioactive compounds from Aduwa protein using a conventional method suggests that large-scale production of functional food ingredients is feasible. Moreover, our results indicate that Aduwa peptides retain their bioactivity under standard processing protocols, which is crucial for maintaining functional food efficacy [31-32]. The potential applications of the Aduwa peptide in functional food products could be diverse. It can be incorporated into beverages, baked goods, dairy products, nutritional bars, and dietary supplements, providing flexibility to meet diverse consumer preferences, health conditions, and market needs [24-25].

The use of active hydrolysate from Aduwa concentrate could lower the activities of these enzymes and provide a more effective means of controlling hypertension. Thus, the objective of this study was to produce hydrolysates of Audwa protein with multi-functional ACE inhibitors and blood pressure-lowering activities that can also be used as a functional ingredient in nutraceuticals.

MATERIALS AND METHODS

Source of raw material: Mature seeds of *Balanites aegyptiaca* (Aduwa) used for this study were purchased from Gashua Market in Yobe State, Nigeria. The seeds were transported to the Department of Biological Sciences for lot identification and then to the Biochemistry Laboratory of the Federal University Gashua. The samples were packaged and transported to the Department of Food and Human Nutritional Sciences, University of Manitoba, Canada, for analysis. Pancreatin-pepsin (PP), Alcalase (Alca), and Flavourzyme (Flav) were obtained from Sigma-Aldrich (St. Louis, MO, USA). ANG-converting enzyme (ACE; Sigma) and its substrate N-(3-[2-furyl] acryloyl)-phenylalanylglycylglycine (FAPGG), as well as Tris-HCl and NaCl, were also purchased from Sigma-Aldrich. Additional equipment and materials included a Synergy H4 microplate reader (BioTek Instruments, Winooski, VT, USA), Data Sciences International (DSI) HD-S10 telemetry transmitters (St. Paul, MN, USA), six-week-old male spontaneous hypertensive rats (SHRs) from Charles River (Montreal, PQ, Canada), and a Mouse/Rat Tail Cuff Blood Pressure System (IITC Life Sciences, Woodland Hills, CA, USA). All chemicals and reagents used were of analytical grade, including hydrochloric acid, glass wool, NaCN, dimethyl sulfoxide (DMSO), formic acid (FA), acetonitrile (ACN), ACE inhibitor (captopril), ethanol, and trichloroacetic acid (TCA), all obtained from Sigma-Aldrich.

Preparation of *Balanites aegyptiaca* protein concentrate using isoelectric precipitation [23,33]: Defatted Aduwa seed meal was mixed with double-distilled water at a 1:50 (w/v) ratio and solubilized at pH 10 using 1 M NaOH. The mixture was stirred for 1 h and centrifuged at 31,000 × g for 30 min. The supernatant was filtered through cheesecloth and adjusted to pH 4.5 with 1 M HCl, stirred for 30 min, and centrifuged again. The precipitate obtained was washed with running water to remove non-protein materials. After a final centrifugation, the precipitate was resuspended in distilled water, adjusted to pH 7.0, and freeze-dried to obtain Aduwa protein concentrate (APC).

Preparation of *Balanites aegyptiaca* protein hydrolysate using pepsin and pancreatin (combined enzymes) (PP) by [34-35]: A 1:2 (w/v) slurry was prepared from 40 g APC and incubated at 37°C. The pH was adjusted to 2.0, after which pepsin (3.04% w/w based on protein content) was added. Digestion proceeded for 2 h at constant pH 2, maintained with 1 M HCl. Pancreatin digestion is followed by adjusting the temperature to 40°C and the pH to 7.5. Pancreatin (4%

w/w) was added, and digestion continued for 2 h at constant pH 7.5, maintained with 1 M NaOH. The reaction was terminated by adjusting the pH to 4.5 and heating the mixture in a boiling water bath at 95°C for 15 min to inactivate enzymes. After cooling, the digest was centrifuged, and the supernatant was collected and freeze-dried.

Preparation of *Balanites aegyptiaca* protein hydrolysate using Alca [34]: A 1:2 (w/v) slurry of 40 g APC was incubated at 50°C and adjusted to pH 9.0 using 2 M NaOH. Alca (3.04% w/w) was added, and digestion proceeded for 4 h at constant pH 9.0, maintained with 2 M NaOH. The reaction was terminated by adjusting the pH to 4.0 and placing the mixture in a boiling water bath (95°C) for 15 min. After cooling, the mixture was centrifuged, and the supernatant was collected and freeze-dried.

Preparation of *Balanites aegyptiaca* protein hydrolysate using Flav [34]: A 1:2 (w/v) slurry of 30 g APC was incubated at 50°C and adjusted to pH 6.5 using 2 M NaOH. Flav (3.04% w/w) was added, and digestion was carried out for 4 h at constant pH 6.5. The reaction was terminated by adjusting the pH to 4.5 using 2 M HCl and heating at 95°C for 15 min to inactivate the enzyme. After cooling, the mixture was centrifuged, and the supernatant was collected and freeze-dried.

In Vitro ACE Inhibition Assay [35-36]: ACE-inhibitory activity of the hydrolysates was measured following the method of [35-36] with modifications. A 0.5 mM solution of FAPGG was prepared in 50 mM Tris-HCl buffer containing 0.3 M NaCl, and the solution was adjusted to pH 7.5. Samples were dissolved in the same buffer. For each assay, 170 µL of 0.5 mM FAPGG was mixed with 10 µL ACE (0.5 U/mL; final activity 25 mU) and 20 µL of hydrolysate sample (0.125–1.0 mg/mL final concentration). The decrease in absorbance at 345 nm was monitored for 30 min at 37°C using a Synergy H4 microplate reader. Control reactions contained a buffer instead of a sample. ACE activity was expressed as the rate of reaction (ΔA/min), and percent inhibitory activity was calculated as:

$$\text{ACE inhibition (\%)} = [(A_{blank\,1} - A_{sample})/(A_{blank\,1} - A_{blank\,2})] \times 100$$

(change A/min) (blank) = ACE activities in the absence of samples but presence of (blank)

(change A/min) (sample) = ACE activities in the presence of samples.

Assessment of the antihypertensive effect of APC and hydrolysate from PP, Alca, and Flav at 200mg/kg and 500mg/kg -

***In vivo* experimental procedures [35]:** All *in vivo* experiments were conducted in accordance with protocols approved by the University of Manitoba Animal Care Protocol and Management Review Committee and followed the Canadian Council on Animal Care guidelines. Male SHRs (2 weeks old), weighing 270–299 g, were housed individually in stainless-steel cages at the Animal Care Facility of the Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba. The animal room was maintained under a 12 h light/dark cycle, at 21 ± 2 °C, and 60% relative humidity. The SHRs were randomly divided into four groups (n = 3 per group) and administered the following treatments:

a)Aduwa protein concentrate (APC) and APC hydrolysates (PP, Alca, Flav) dissolved in phosphate-buffered saline (PBS; pH 7.2) at 200 mg protein/kg body weight

b) APC, Alca, and Flav hydrolysates dissolved in PBS at 500 mg protein/kg body weight

c) Positive control: captopril dissolved in PBS at 20 mg/kg body weight

d) Negative control: PBS only

Captopril, a standard antihypertensive drug, has been used previously as a positive control in SHR studies at a single oral dose of 10 mg/kg body weight. APC and hydrolysate samples were dissolved in PBS, centrifuged, and the supernatant was administered by oral gavage (1 mL per rat). Prior to blood pressure measurement, each rat was placed in a 40 °C warm chamber with 4% isoflurane for 3 min to minimize stress-related blood pressure fluctuations.

***In Vivo* ACE-inhibition assessment:** Evaluation of antihypertensive activity followed the method of [37-38]. Six-week-old male SHRs from Montreal, PQ, Canada was housed under a 12 h light/dark cycle at 22 °C and 50–56% humidity, with regular chow and tap water provided ad libitum. After a 1-week period, SHRs were surgically implanted with a Data Sciences International telemetry transmitter (HD-S10) under anesthesia. All surgical procedures were conducted under sterile conditions. Rats were allowed a two-week recovery period before oral gavage. Aduwa concentrate (APC) and hydrolysates (PP, Alca, and Flav) were dissolved in 1 mL PBS and administered at 200 mg/kg or 500 mg/kg body weight. Captopril (20 mg/kg body weight) served as the positive control, while saline served as the negative control. Each rat received 1 mL of the sample hydrolysate by oral gavage. Real-time systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) were continuously recorded at 10-min intervals over 24 h using Ponemah 6.1 data acquisition software, with each cage placed on the appropriate receiver unit (RPC-1, DSI). An APR-1 atmospheric-pressure monitor was used to normalize transmitted pressure values. Results are presented as changes in SBP, DBP, and MAP at two-hour intervals for 24 h relative to the baseline of zero hour (0 h).

Statistical Analysis: All assays were performed in triplicate. Results are expressed as means ± standard deviation. ANOVA was used to assess differences among treatments. Statistical analysis was performed using SPSS Version 16.0.

RESULTS AND DISCUSSION

***In Vitro* ACE-Inhibitory Properties of Aduwa Concentrate and Hydrolysates:** The in *vitro* ACE-inhibitory activity of APC and its hydrolysates is shown in Figure 1. All hydrolysates exhibited a dose-dependent increase in ACE inhibition but were significantly different from Flav. Captopril nearly completely inhibited ACE activity (~99.9%), whereas APC inhibited ~80% ACE activity at the same concentration (1 mg/mL). No significant differences were observed between APC and Alca at 0.5mg/mL tested dosage. The ACE-inhibitory potential of a protein hydrolysate depends on the protease used, as different enzymes cleave peptide bonds at different amino acid sites, producing peptides with varying bioactivities [8], because ACE-inhibitory activities are strongly influenced by peptide sequence. Hydrolysates produced from the same protein but digested with different enzymes often differ in potency [8]. From these results, APC and Alca hydrolysates show stronger ACE-inhibitory activity at increasing concentrations than Flav, consistent with earlier findings that Alca hydrolysates exhibited strong antioxidant properties [38]. This may be attributed to the combined endo- and exopeptidase activities of Alca, which generate peptides with superior ACE-inhibitory properties. Despite high *in vitro* potency, few studies have reported the blood pressure–lowering effects of Aduwa hydrolysates *in vivo.* Validating this ACE-inhibitory activity in animal models is critical in establishing these peptides as potential therapeutic ingredients for functional food.

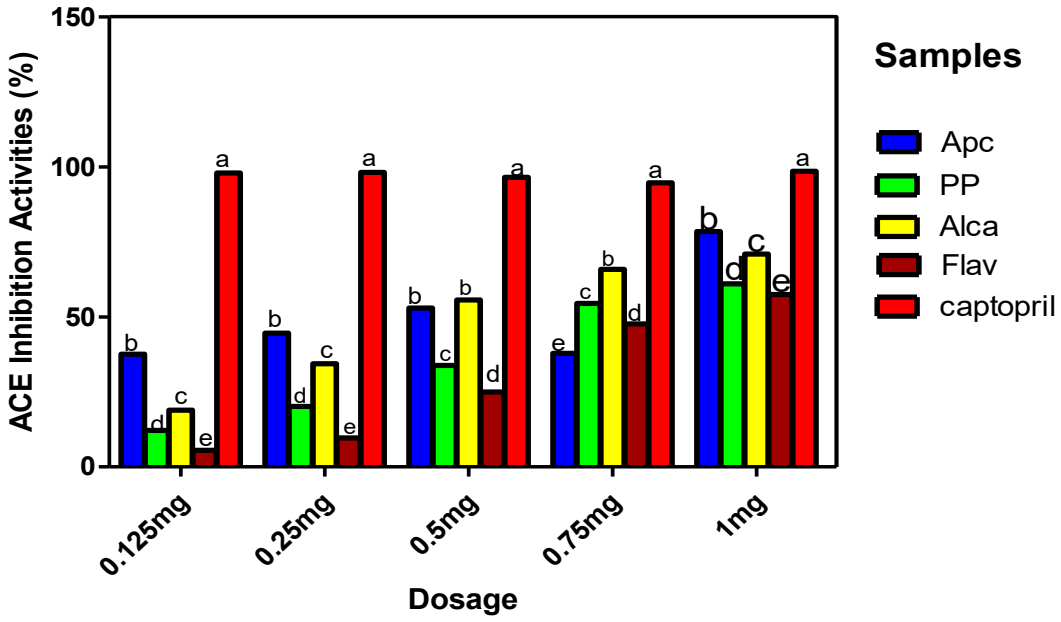


Figure 1. In vitro ACE Activity graph of APC = Aduwa protein concentrate (Aduwa concentrate), PP = pancreatin and pepsin combined hydrolysate. Bars with similar superscripts are not significantly different at $p<0.05$ Keys: APC = Aduwa protein concentrate, PP =pancreatin and pepsin combined hydrolysate, Alca = Alcalase hydrolysate, Flav = Flavourzyme

Systolic blood pressure (SBP) of the *In vivo* ACE activity of APC and Aduwa hydrolysates samples at 200 mg/kg gavage concentration using SHR: The antihypertensive effects of APC and hydrolysates (PP, Alca, Flav) in SHRs at 200 mg/kg are presented in Figure 2. All animals were hypertensive at baseline. Significant change in SBP occurred over time for all treatments except in the APC sample. The SBP reduction between 2–4 h and 8–24 h showed no significant differences at ($p<0.05$) as observed in rats administered APC and PP hydrolysates. Similar trends were observed for Flav at 2 h and 8 h, but they differed at ($p<0.05$). The samples APC, PP, and Alca differed less from captopril during the 2–8 h periods, despite captopril being administered at a much lower dose (20 mg/kg). These findings indicate that APC, PP, and Alca hydrolysates may also have short-term antihypertensive effects similar to captopril. This aligned with earlier studies showing that captopril and certain food-derived peptides had similar blood pressure-lowering effects in SHRs [39].

The maximum SBP reductions using Aduwa hydrolysates were:

APC (2–4 h): –0.5 to –0.9 mmHg

Flav (2–4 h): –15 to –9.5 mmHg

PP (8–24 h): –0.45 to –0.95 mmHg

Alca (8–24 h): –5 to –0.9 mmHg

These results suggest that APC and PP hydrolysates showed no significant differences and produced rapid, sustained antihypertensive effects, similar to previous reports on hemp seed hydrolysates [35].

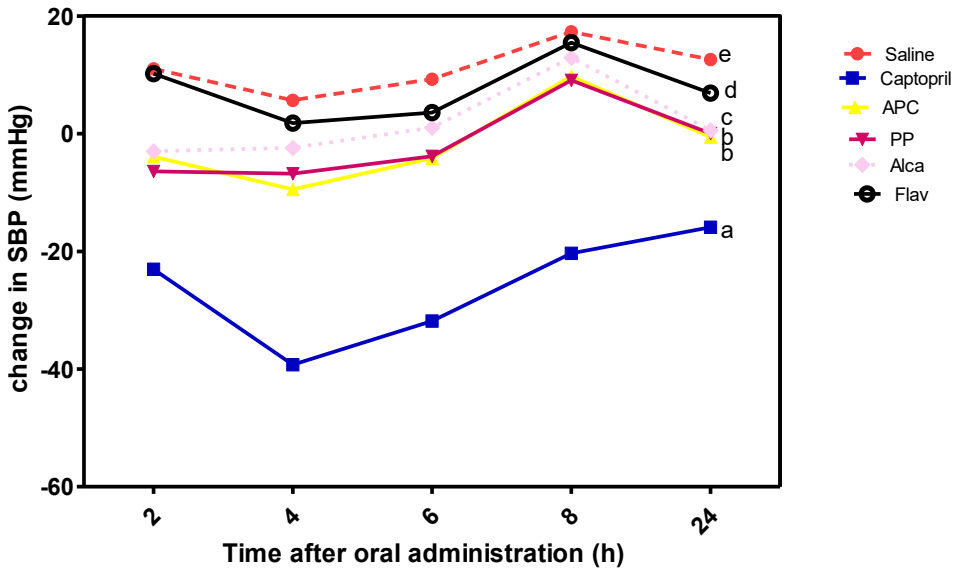


Figure 2. Systolic Blood Pressure (SBP) of In vivo ACE Activity graph of APC and hydrolysates samples at 200mg/kg/body weight. Line graphs with similar superscripts are not significantly different at $p<0.05$ Keys: APC = Aduwa protein Concentrate, PP = pancreatin and pepsin combined hydrolysate, Alca = Alcalase hydrolysate, Flav = Flavourzyme

Diastolic blood pressure (DBP) of the *in vivo* ACE activity of APC and Aduwa hydrolysates samples at 200 mg/kg gavage concentration using SHR: Results demonstrated that during the 2-4 h and 8-24 h periods, the DBP group receiving saline solution showed a drop in SBP, whereas the APC, PP, Alca, and Flav hydrolysates showed a drop in SBP (Figure 3). The APC, PP, Alca, and Flav samples did show significant change between 8-24 h analyzed times in this study, but APC and PP were near similar to the animals receiving captopril. The APC, PP, Alca, and Flav hydrolysates, and standard or captopril showed significant differences in lowering DBP in SHRs, suggesting the potential of the generated hydrolysates to lower DBP in the short term. The captopril action had a similar effect to APC and PP, which were not significantly different ($p<0.05$), but differed towards the 8th hour of administration with Alca samples, lowering the DBP of the SHRs at 200mg/kg body weight. This suggests the potential of the generated peptide samples to lower DBP on a 2-4 h and 8- 24 h term at 200mg/kg/body weight. This observation may have come from the nature of the Aduwa sample, peptide chain length, and less interference from intestinal mucosal enzyme action on the intact peptide samples [40]. The maximal reduction of DBP between 2-4 h by APC appeared between (0.4 to 8.5) mmHg and 8-24h periods by APC and PP, while Alca and Flav appeared between (5 to 9.5) mmHg, respectively. The observed change in SBP at 8 to 24 h after oral gavage with Aduwa APC sample and PP peptide samples would have been an inherent antihypertensive effect of the samples against SHRs [29-39]. It was also observed that peptides generated from the Aduwa APC sample and PP hydrolysates showed no significant differences in (DBP) diastolic blood lowering potentials at long time compared to Alca and Flav samples, which differ.

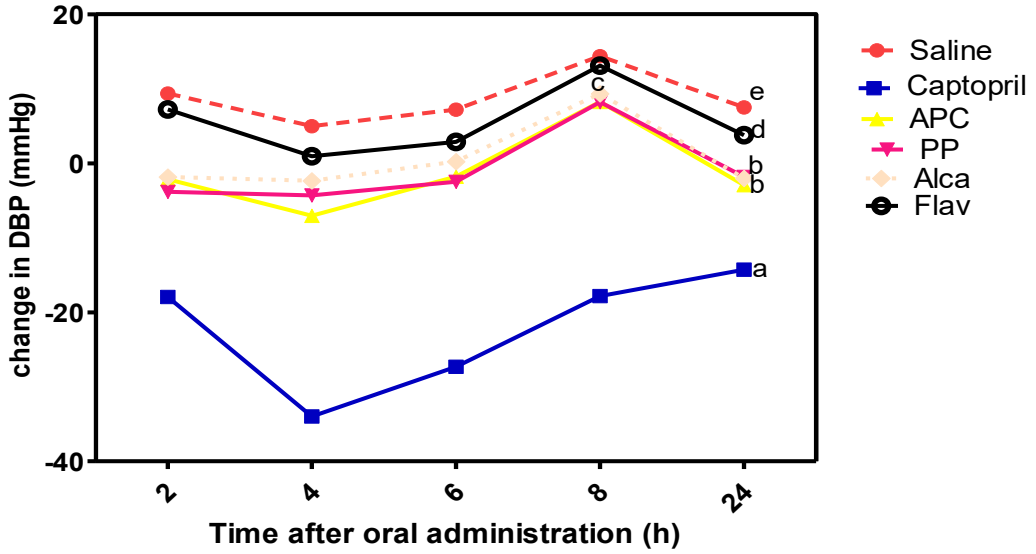


Figure 3. Diastolic Blood Pressure (DBP) of *in vivo* ACE activity graph of APC and hydrolysate samples at 200mg/kg/body weight. Line graphs with similar superscripts are not significantly different at p<0.05
Keys: APC = Aduwa protein Concentrate, PP = pancreatin and pepsin combined hydrolysate, Alca = Alcalase hydrolysate, Flav = Flavourzyme

Mean arterial pressure (MAP) of *In vivo* ACE activity of APC and Aduwa hydrolysates samples at 200 mg/kg gavage concentration using SHR: Figure 4 shows the MAP graph of APC, PP, Alca and Flav at 200mg/kg/body weight. The MAP reduction by the samples was significantly greater ($p < 0.05$) between 2-4 h and 8-24 h. Results demonstrated that the MAP of the group that received saline solution varied throughout the period. Between 2-4 h and 8-24 h, a drop in MAP was observed in the rat groups administered APC, PP, Alca, and Flav hydrolysate samples, respectively. APC, PP, Alca, and Flav samples did not show significant differences during the 8-24 h period in this study compared with those from animals that received captopril. The APC and PP samples showed no significant differences in the trend of lowering the MAP of the SHRs, in short-term (8-24 h terms), suggesting the potential of the generated APC and PP-hydrolysate to lower MAP on a long-term basis. The captopril had similar ability with APC and PP samples but significantly differed at ($p<0.05$) in lowering MAP activities in SHRs at 500mg/kg body weight, confirming the potential ability of the generated samples to lower MAP on a 2-4 h and 8- 24 h term at 200mg/kg/body weight far above the saline samples. This observation may have come from peptide chain length and less interference from intestinal mucosal enzymes on intact peptide samples [38]. The maximal reduction of MAP between 2-4 h by APC appeared at 0.5 mmHg - 9.5 mmHg, while PP and Alca at 8-24 h appeared between 5.5 mmHg to 10.5 mmHg. The observable changes in MAP activity at 8 to 24 h after oral gavage with Aduwa peptide samples would have indicated an antihypertensive effect of the Aduwa samples in SHRs. It was also observed that peptides generated from the Aduwa concentrate sample have a greater long-term mean arterial blood pressure-lowering potential compared to the hydrolysate samples.

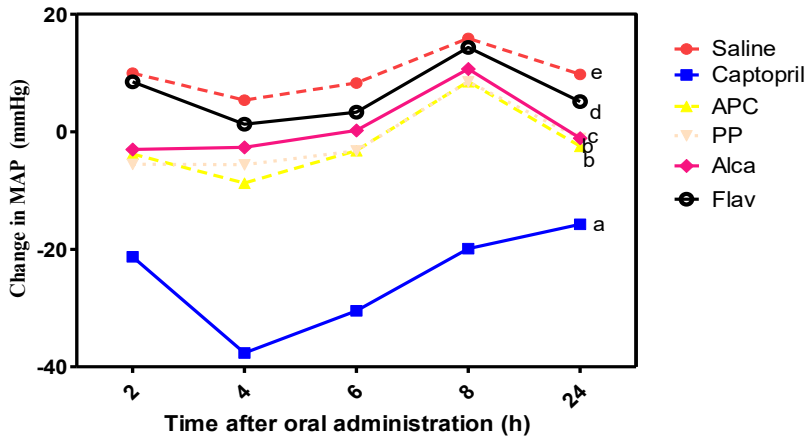


Figure 4. Mean Arterial Pressure (MAP) graph of *in vivo* ACE activity graph of APC and hydrolysates samples at 200mg/kg/body weight. Line graphs with similar superscripts are not significantly different at p<0.05
Keys: APC = Aduwa protein Concentrate, PP = pancreatin and pepsin combined hydrolysate, Alca = Alcalase hydrolysate, Flav = Flavourzyme

Systolic blood pressure (SBP) of *in vivo* ACE of APC and Aduwa hydrolysates at 500mg/kg/body weight: At 500 mg/kg (Figure 5), saline produced no significant SBP reduction. The Alca hydrolysate produced the longest SBP-lowering effect (2–6 h; –5.1 to –10.8 mmHg). PP and Flav also demonstrated short-term reductions (2–4 h; –4.1 to –9.5 mmHg), above the saline negative control. At 8–24 h, PP, Alca, and Flav hydrolysates produced SBP reductions between (5.2–7.5 mmHg), lower than those of captopril. Alca and PP hydrolysates are not significantly different at (p<0.05) and showed the strongest and most sustained SBP inhibition, likely due to enzyme specificity and its ability to generate peptides resistant to gastrointestinal degradation.

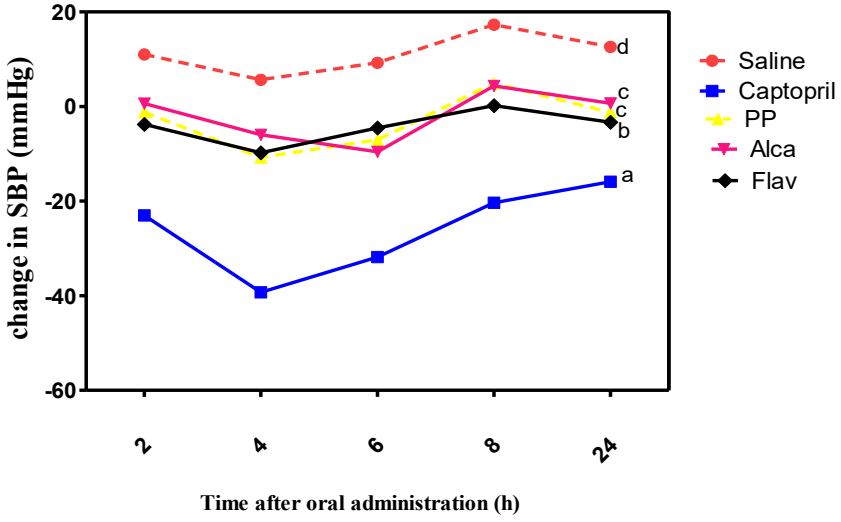


Figure 5. Systolic Blood Pressure (SPB) of in vivo ACE Activity graph of PP = pancreatin and pepsin combined hydrolysate at 500mg/kg/body weight. Line graphs with similar superscripts are not significantly different at p<0.05 Keys: APC = Aduwa protein Concentrate, PP = pancreatin and pepsin combined hydrolysate, Alca = Alcalase hydrolysate, Flav = Flavourzyme

Diastolic blood pressure (DBP) of *in vivo* ACE of APC and Aduwa hydrolysates at 500mg/kg/body weight: As shown in Figure 6, saline had no antihypertensive effect. Flav and PP hydrolysates produced short-term reductions in DBP (2–4 h; –4 to –10.2 mmHg). The Alca hydrolysate produced the longest reductive effect (2–6 h; –4.5 to –9.5 mmHg). During the 8–24 h period, the DBP reductions (5.1–8.5 mmHg) from the hydrolysates were lower than those from captopril and higher than those from the negative control saline. Alca showed the most stable long-term DBP reduction activity but differs from Flav and PP hydrolysates, which are not significantly different ($p<0.05$) in lowering DBP activities in SHR

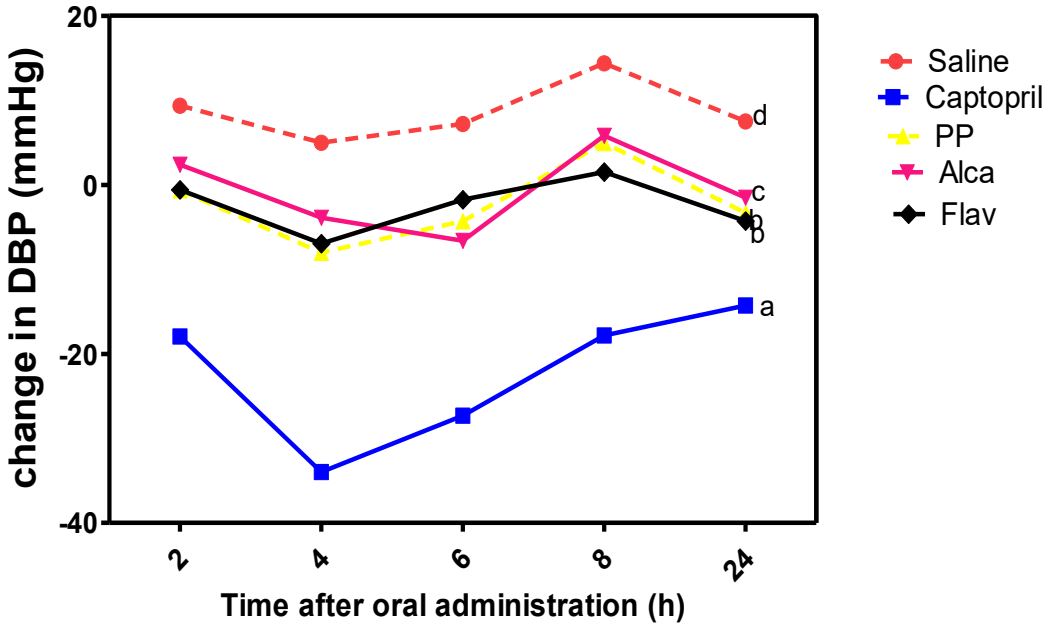


Figure 6. Diastolic Blood Pressure (DBP) of *in vivo* ACE activity graph of PP = pancreatin and pepsin combined hydrolysate at 500mg/kg/body weight. Line graphs with similar superscripts are not significantly different at $p<0.05$.
Keys: Alca = Alcalase hydrolysate and Flav = Flavourzyme hydrolysates

Mean arterial pressure (MAP) of *in vivo* ACE activity of APC and Aduwa hydrolysates at 500mg/kg/body weight: Figure 7 shows that saline and Flav had no significant effect on MAP. Aduwa PP produced short-term in MAP reductions (4 to –10.2 mmHg), while Alca again produced the highest and most sustained MAP-lowering effect (8–24 h; –4.5 to –9.5 mmHg), which differs significantly but is similar to that of referral, captopril. While some samples showed enhanced blood pressure-lowering potency, these effects were not time-dependent. No significant variations in activity were observed between the different hydrolysates across the recorded hourly intervals. The Alca, Flav, and PP hydrolysate activities likely resulted from their broad external and internal cleavage specificity and ability to generate stable hypotensive peptides, thereby affecting MAP-lowering potentials. The saline, a negative control, shows a significant difference from the positive control and the hydrolysate samples.

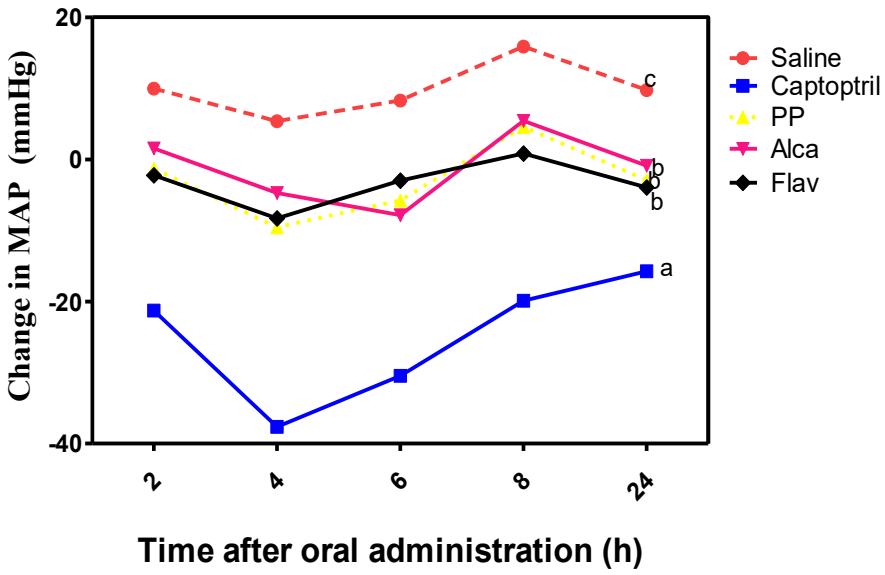


Figure 7. Mean Arterial Pressure (MAP) of *in vivo* ACE activity graph of PP = pancreatin and pepsin combined hydrolysate, at 500mg/kg/body weight. Line graphs with similar superscripts are not significantly different at $p<0.05$.
Keys: Alca = Alcalase hydrolysate and Flav = Flavourzyme hydrolysates

CONCLUSION

This study is the first to report the antihypertensive potential of Aduwa protein concentrate and its enzymatic hydrolysates. The hydrolysates produced bioactive peptides with ACE-inhibitory activity, which varied according to peptide size and enzyme specificity. Alca generated the most potent hydrolysate, exhibiting both short- and long-term ACE-inhibitory effects. PP hydrolysates also demonstrated strong inhibitory activity. The *in vivo* results suggest that peptides from APC, PP, and Alca hydrolysates are capable of lowering SBP, DBP, and MAP, with effects in some cases comparable to captopril. These peptides appear to resist gastrointestinal degradation, retaining ACE-inhibitory activity after oral administration. Overall, Aduwa-derived peptides show strong potential for development into functional foods, nutraceuticals, and natural antihypertensive agents. Future clinical studies in humans are recommended to validate their therapeutic potential.

Abbreviations: Angiotensin-converting enzyme (ACE), Acetonitrile (ACN), Aduwa protein concentrate (APC), Alcalase (Alca), Analysis of variance (ANOVA), Bioactive compound (BC), Diastolic blood pressure (DBP), Dimethyl sulfoxide (DMSO), Flavourzyme (Flav), Formic acid (FA), Mean arterial blood pressure (MAP), N-(3-[2-furyl]_acryloyl)-phenylalanylglycylglycine (FAPGG), Pancreatin-pepsin (PP), Phosphate-buffered saline (PBS), Spontaneously hypertensive rats (SHRs), Systolic blood pressure (SBP), Trichloroacetic acid (TCA).

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