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Evaluation of In-vitro Angiotensin Converting Enzyme (ACE) Property of (*Balanites aeqyptiaca*. Del) Meal, Defatted Meal, Concentrate and Hydrolysates

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ABSTRACT

Edible seed proteins stand greater chance to manage chronic diseases. Balnites aegyptiaca seed is widely natural across Africa and in Asian countries. This desert three seed nut has potential nutritional properties which are been utilized at home nowadays. This study was to determine the in vitro ACE potentials of *Aduwa* protein meals and hydrolysate. *Aduwa* seeds were toasted at 70 °C, milled and oil extracted to make APM. The meal was defatted made into concentrate and finally into hydrolysates. The hydrolysate was hydrolyzed by using pepsin, pancreatin, pepsin and pancreatin combines protease. The samples were subjected to different inhibitory concentration EC_5, EC_{10} and EC_{20} invitro angiotensin converting enzyme activities. The result revealed that *Aduwa* meal protein and hydrolysate has the potential to reduce blood pressure. The resolved meals exhibited greater angiotensin converting enzyme activities at five inhibitory concentrations (EC_5) respectively, for defatted meal was (5%), concentrate (3%) pancreatin hydrolysate (4%), pepsin hydrolysate (2%) and combined hydrolysate (2%). Inhibition concentration at EC10 exhibited significant high angiotensin converting enzyme activities value in defatted meal and pancreatin hydrolysate when compared with the referral control. The results suggested that defatted meal, pancreatin hydrolysate at EC_{10} and pepsin hydrolysate at EC_5 and EC_{20} respectively had better invitro angiotensin converting enzyme inhibitory potentials, hence *Aduwa* seed protein bioactive has inhibitory potentials to act as antihypertensive agents that could be utilized as ingredients for the development of novel functional foods and nutraceuticals in future.

Keywords: *Aduwa* Seed, Meals, Protein Concentrate, Protein Hydrolysates, Inhibition Concentration, ACE Inhibition.

INTRODUCTION

Balanites aeqyptiaca is a (dry desert fruit) from the family of desert date *balanitacea* an under-utilized and neglected food tree in dry land Africa.

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It is found in most arid to subhumid tropical savannas of the Sudan Sahel region of Africa, the Arabian Peninsula and South regions [1]. In Nigeria it is located on dark cracking clays of the north east, North West and part of middle belt regions. *Balanites aeqyptiaca*, called *Aduwa* in Hausa language, its leafe, mesocarps and seeds have been used over years in rural homes as trado medical herbs and in food applications.Traditionally, the leaves, seed, root and stem have immense medicinal potentials [2].The Leafe is a browse plant and the fruit mesocarp is commonly used as an oral antidiabetic and anticancer drug in traditional medicine to treat various disorders including cancer [3], HIV/AIDS [1], malaria [4] diabetes [5]. The meal seed is a potent hypoglycemic and was effective in streptozotocin-induced diabetic mice [5,6].Their biological active compounds such a saponin, furanocoumarin and flavonoid contributes a lot to vital role in its nutraceutical applications hence, wide range of consumption of the meal and seed flour by human as food [7,8].It has been reported to have medicinal properties with a wide range of products through ethno-botanical studies as anti-helminthic, purgative, food value, fodder, shade, oil and for traditional leukoderma and emetic medicines [9,10], to meet the increasing demand for edible bioactive proteins in the future, efforts are endeavoured to source for cheap, available, and applicable protein sources. *Aduwa* seeds could serve as a suitable raw material in the manufacture of protein bioactive ingredients in food systems, food, and non-food industrial sectors. Previous work has shown the physiochemical composition, functional properties, and anti -nutrients factors from the raw seeds, roasted seed, soaked seed and fermented pre-treated seeds [8,11]. The biochemical and haematological profile meal on rabbit performance has been confirmed [12]. *Aduwa* protein concentrate by isoelectric precipitation [8] and methanolic extraction of the raw seeds have been reported to possess good protein and antioxidant properties, however, the seed used were not given toasted treatment and their report did not provide information on the relationship with angiotensin converting enzyme activities. ACE property continue to be a research domain because of the potential contribution to human health and prevention of chronic diseases [13]. Hypertension is linked to high blood pressure modulated by enzymes in the lung and liver angiotensinogen I to angiotensin II that increases Vaso constriction resulting in high blood pressure [13]. Some short chain peptides have been recognised to inhibit the rate determining steps of renin to affects the release of angiotensin I from the kidney [14].It has been reported that natural and derived peptides are safer and cheaper remedy than synthetic ACE drugs like Ramipril, lisinopril [13]. Therefore, this work was aimed at determining the in-vitro ACE properties of *Aduwa* protein meals and selected protease hydrolysates.

MATERIALS

All the reagents such as sodium phosphate, reduced glutathione (GSH), methanol, Tris-HCl buffer, EDTA, NaOH, HCl, 1,10-phenanthroline, hydrogen peroxide, $FeSO_4$, $FeCl_3$, $FeCl_2$, were all analytical grade and provided by food science department Obafemi Awolowo University, Ile Ife. Two kilogram (2 kg) of mature *B. aegyptiaca* fruits were bought from Gashua market in Yobe State of Nigeria. They were conveyed to the biochemistry laboratory of the Federal university Gashua. One and the half kilogrammes (1.5 kg) of cracked seed kernels of Balanites aegptiaaca were weighted using a weigh balance and moisture value ascertain at 10% using moisture prob (E20 USA Model 121). The weighted samples were apportioned to toasting treatments under dry heat at 70 °C for 30 minutes and allowed to cool.

Seed processing into *Aduwa* protein meal (APM)

The seed kernels were subjected to toasting pre-treatments. The cakes were made from the seed flours by mechanically expelling the oil using centrifugal screw press. semi-automated.

The cake produced were dried to 10% moisture content using the solar cabinet dryer and well packaged for analysis.

Preparation of defatted *Aduwa* protein meal (DAM)

Preparation of Defatted *Aduwa* seed protein meal were prepared from toasted seed. The flour sample was defatted with cold (4 ^{0}C) acetone using flour to solvent ratio of 1:5 w/v. The mixture was stirred over a magnetic stirred for 4 hrs. The slurry was then filtered through a Whatman No. 1 filter paper. The residue was re-extracted twice in a similar fashion. The defatted flour was de-solvent zed by drying in a fume hood at room temperature and the dried flour was finally ground in a blender to obtain homogeneous defatted *Aduwa* flour and stored in an air-tight plastic bottle for use.

Preparation of *Aduwa* hydrolysate using pepsin enzyme (APHpa)

Aduwa protein hydrolysate by pancreatin (APHpa) was prepared using pepsin enzymes in an optimum reaction condition. Acting on the isolate (Pepsin with pH 2 at 37 $_0$C), using the method of Omoni and Aluko (2006) as shown in Figure 2 & 3. A 1:20 w/v *Aduwa* protein isolate's slurry was adjusted to pH 2.0 and incubated at 37 ºC followed by addition of pepsin (4% w/w, based on protein content of *Aduwa* protein isolate), The digestion was carried out for 4 h and the pH is maintained by adding 1 M NaOH or HCl when necessary. The digestion was terminated by adjusting the pH to 4.0 and then place the mixtures in boiling water for 30 min to inactivate the enzymes which ensure complete denaturation of enzyme protein and coagulation of undigested proteins. The mixture was allowed to cool to room temperature and later centrifuged and supernatant collected and freeze dried.

Preparation of *Aduwa* Hydrolysate using Pepsin enzyme (APHpe)

Aduwa protein hydrolysate by pepsin (APHpe) was prepared using pancreatin enzyme optimum reaction conditions acting on the concentrate. Pepsin with pH 7.5 at 40^{0}C using the method of Omoni and Aluko (2006) as shown in Figure 2 & 3. A 1:20 w/v okra seed protein isolate's slurry was adjusted to pH 7.5 and incubated at 40 ºC followed by addition of pepsin (4% w/w, based on protein content of okra seed protein isolate) or pH 7.5 in and incubation at 45 °C followed by the addition of trypsin enzyme (4% w/w, based on protein content of seed protein concentrate) for pepsin. The digestion was carried out for 4 h and the pH is maintained by adding 1 M NaOH or HCl when necessary. The digestion was terminated by adjusting the pH to 4.0 and then place the mixtures in boiling water for 30 min to inactivate the enzymes which ensure complete denaturation of enzyme protein and coagulation of undigested proteins. The mixture was allowed to cool to room temperature and later centrifuged to get the supernatant and freeze dried.

Preparation of protein Concentrate (PC)

Protein concentrate (PC) from *Aduwa* was prepared by a method modified by Gbadamosi et al. [15] as shown in Figure 2. A known weight (200 g) of the defatted flour were dispersed in distilled water (2,000 ml) to give final flour to water ratio of 1:10. The dispersion will then be gently stirred on a magnetic stirrer for 10 min to form a suspension, after which the pH of the resultant slurry will be adjusted with 0.1 M HCl to pH 4.

The precipitation process will be allowed to proceed with gentle stirring for 2 h keeping the pH constant. Soluble carbohydrates (oligosaccharides) and minerals will be removed by centrifugation at 3,500 × g for 30 min using a centrifuge. The precipitate (concentrate) will afterward be washed with distilled water to remove the residual minerals and soluble carbohydrates and the pH is later adjusted with 0.1 M NaOH to 7.0 for neutralization and then centrifuged at 3,500 × g for 10 min. The resultant precipitate (concentrate) will be collected and dried in an oven at 45 ºC for 8 h and kept in air-tight container for further analysis.

***Aduwa* Protein Hydrolysate by pancreatin and pepsin enzymes combined (APHpa+pe).**

Combine enzyme hydrolysate APHpa+pe were carried out using the method of Aluko and McIntosh [16] with slight modification by Girgih et al. [17]. The API was dispersed in water (2%, w/v), and was adjusted to pH 9.0 using 1 M NaOH solution for pancreatin while pH 2.0 was used for pepsin digestion. The dispersion was heated to 60^{0}C under continuous stirring on a hotplate equipped with an electronic thermometer. The enzymes (4 % w/w) were added based on the protein content of the APC and incubated at constant temperature of 60°C for 2.5 or 10 min. An un-hydrolyzed control for each time was prepared by omitting the enzymes during thermal incubation of the API. The reaction mixture was maintained at pH 9.0 using 1 M NaOH solution or pH 2.0 with 1 M HCl. At the end of the incubation period, the hydrolysates were transferred into a boiling water bath for 5 min to inactivate the enzymes. The hydrolysates were cooled to room temperature (22±2 ^{o}C) and adjusted to pH 7.0 with 1 M HCl solution (for pancreatin) or 1 M NaOH (for pepsin digest), and finally freeze-dried.

METHODS

In-vitro Angiotensin converting enzyme (ACE) inhibition activity of Aduwa meal, defatted Aduwa meal, concentrates and hydrolysate.

Angiotensin-I Converting Enzyme (ACE) Inhibition Assay. (Cushman and Cheung 1971) Fifty microliters (50 μL) of appropriate dilution of the extracts and ACE solution (50 μL

and 4 mU) were incubated at 37 °C for 15 min. The enzymatic reaction was initiated by adding 150 μL of 8.33 mM of the substrate Bz-Gly-His-Leu in 125 mM Tris-HCl buffer (pH 8.3) to the mixture. After incubation for 30 min at 37 °C, the reaction mixture was terminated by adding 250 μL of 1 M HCl. The Gly-His bond was then cleaved and the Bz-Gly produced by the reaction was extracted with 1.5 mL ethyl acetate. Thereafter the mixture was spurned to separate the ethyl acetate layers. 1 mL of the ethyl acetate layer was transferred to a clean test tube and evaporated. The residue was redissolved in distilled water and its absorbance was measured at 228 nm. The ACE inhibitory activity was expressed as percentage inhibition using control blank

ACE inhibition was calculated using the equation:

ACE inhibition (%) = $1- (\Delta A.\ min\text{-}1_{(sample)}/\Delta A.\ min\ 1_{(blank)}) \times 100$

Where:

$A.\ min^{1}_{(sample)}$ = Reaction rate in the presence of sample.

$A.\ min^{-1}_{(blank)}$ = Reaction rate in the absence of sample.

Statistical Analysis

Antioxidant assays were conducted in duplicates and anal were analysed by one way analysis of variance ANOVA. Means were compared using Duncan multiple range test and significance difference accepted at p<0.05 using SPSS statistical software v.20

RESULTS AND DISCUSSION

In-vitro -ACE properties of Aduwa protein meal, defatted meal, protein concentrate and hydrolysates

The result on the percentage ACE inhibition at different inhibition concentrations (EC) are shown in Figure 1 with values in the 0-100% range. The defatted Aduwa protein meal DAP (4.5%) and Aduwa protein hydrolysate by pancreatin APHpa (4.3%) showed no significance (p< 0.05%) difference but had stronger ACE inhibitions, however lower than the reference standard of (6.4%). The APC (2.3%) and APHpe (2.5%) Aduwa samples exhibition effects are not significance difference at (p<0.05) compared to APC and APHpa+pe that are significantly different p<0.05 at ACE inhibition effects. DAP and APHpa inhibition effects were good ACE inhibitors when compared to reference standard (6.4%), which was significantly lower than the 77% and 83% reported for kidney bean protein hydrolysate [18], and enzymatic rapeseed protein hydrolysate [17]. Similarly, the DAP, APC,APHpa and APHpe had ACE-inhibitory activity lower than mung bean protein hydrolysate reported at 23.62% [19]. Samples resolved by enzymatic hydrolysis to make Aduwa protein hydrolysate by pepsin (APHpe), pancreatin enzyme protein hydrolysate (APHpa) and Defatted Aduwa protein meal (DAPM) had better ACE-inhibitory abilities at EC $_5$.

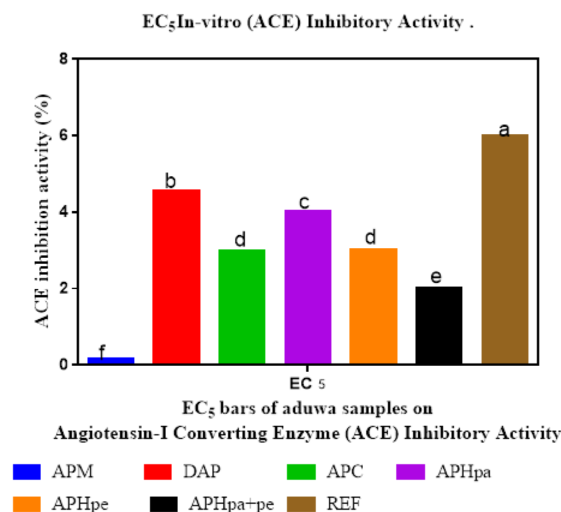

Figure 1. EC5 In-vitro-ACE properties of Aduwa protein meal, defatted meal, protein concentrate, isolate and hydrolysates.

Means are readings from duplicate determination; Means followed by the same alphabetic on the bar are not significantly different at p>0.05. Key: APM= Aduwa protein meal, DAP =defatted Aduwa protein meal, APC= Aduwa protein concentrate, APHpa= Aduwa protein Hydrolysates by pancreatin, APHpe= Aduwa protein hydrolysates by pepsin. APHpan+pe= Aduwa protein hydrolysates by pancreatin and pepsin.

Results of the percentage ACE inhibition at EC_{10} are shown in Figure 2 with values in the 0-100% range. All the sample exhibited ACE inhibition activities. There exists no significance difference (p>0.05) between DAPM (6.4%) and referral standard (6.4%). The ACE percentage value for APHpa (6.0%) compared favorably with reference but is significantly different (p<0.05). Aduwa sample of APM (4.0%), APHpa+pe (2.0%) APHpe (1.9%), API (1.5%) and APC (0.9%) ACE inhibitory activities were good but were significantly (p<0.05) lower than the reference (6.4%). At EC10 inhibition effects, DAP and APM were stronger ACE inhibitors, this could be attributed to high phenolics presence in the samples [20]. However, the APM (4.0%), APHpa+pe (2.0%) APHpe (1.9%), APC (1.5%) and APC (0.5%) low ACE inhibition effects may be due to concentration dosage.

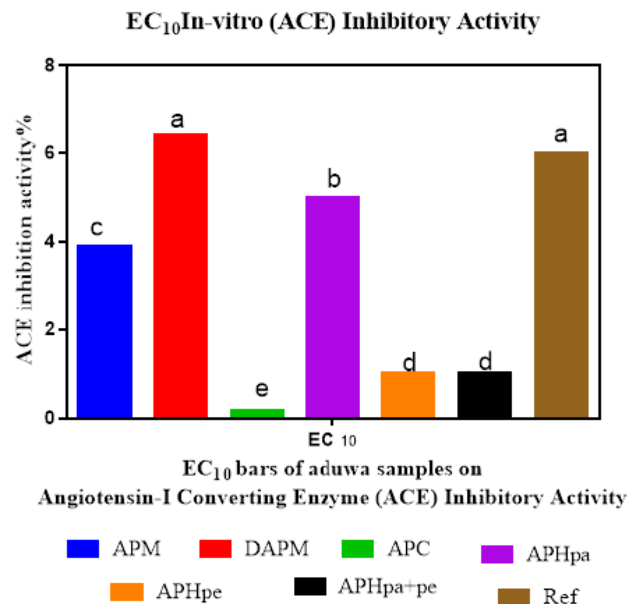

Figure 2. EC10 In-vitro-ACE properties of Aduwa protein meal, defatted flour, protein concentrate, isolate and hydrolysates.

Means are readings from duplicate determination; Means followed by the same alphabetic on the bar are not significantly different at p>0.05. Key: APM= Aduwa protein meal, DAP=defatted Aduwa protein meal, APC= Aduwa protein concentrate, APHpa= Aduwa protein Hydrolysates by pancreatin, APHpe= Aduwa protein hydrolysate by pepsin. APHpan+pe= Aduwa protein hydrolysate by pancreatin and pepsin.

Results of the percentage ACE inhibition at EC_{20} are shown in Figure 3 with values in the 0-100% range, which are lower than the control (6.4%). However, there was no significant difference between reference standard and pepsin hydrolyzed hydrolysate APHpe at (p<0.05%). At EC_{20} inhibition effects, there exist no significant difference (p<0.05) in ACE inhibition activities between sample APC (2.2%) and API (2.1%), also with APM (3.9%) and APHpa (3.8%) respectively and are lower than referral standard. DAPM (1.3%) and APHpa (0.9%) are significantly (p>0.05) lower than APHpe and reference, respectively. Within the Aduwa samples APHpe (6.4%) and APM (3.9%) were a stronger ACE inhibitor maybe due to the presences of flavonoid and phenolics [20,21]. These were significantly (p<0.05) lower than the 77% and 83% reported for kidney bean protein hydrolysate [22]. Resolved Aduwa pepsin enzyme hydrolysate (AHPpep) had higher ACE-inhibitory ability (6.4%) when compared to the unresolved APM (3.9%) at EC_{20}. This confers that choice of enzymes and enzyme activities on Aduwa sample could influence activities of ACE inhibitors [23-35].

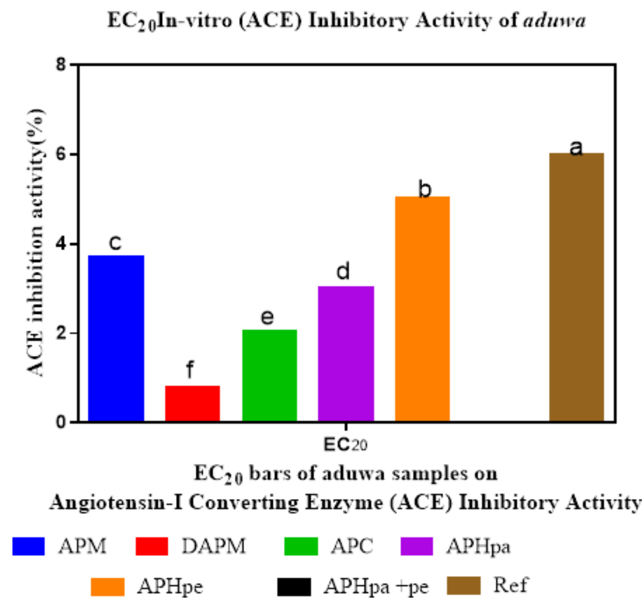

Figure 3. EC_{20} In-vitro-ACE properties of Aduwa protein meal, defatted meal, protein concentrate, isolate and hydrolysates.

Means are readings from three determination; Means followed by the same alphabetic on the bar are not significantly different at p>0.05. Key: APM= Aduwa protein meal, DAP =defatted Aduwa protein meal, APC= Aduwa protein concentrate, APHpa= Aduwa protein Hydrolysates by pancreatin, APHpe= Aduwa protein hydrolysate by pepsin. APHpan+pe= Aduwa protein hydrolysate by pancreatin and pepsin.

CONCLUSION

The results showed significant improvement in anti-hypertensive properties of protein meal upon material resolves. The invitro angiotensin converting enzyme depended on the type of material resolved, either during oil expulsion (meal), defatting (defatted meal), solubilization (concentrate) and enzymatic hydrolysis (hydrolysate) of material Aduwa sample. Defatted Aduwa protein meal, Aduwa protein hydrolysate by pancreatin and pepsin respectively at inhibition concentration of 5, 10 and 20, inhibited in vitro angiotensin converting enzyme better. The results suggest that the Aduwa protein meals and the hydrolysates have potential to manage angiotensin converting enzymes I from breaking down to angiotensin II, a potent Vasoconstrictor which could trigger hypertension and graduate death. Also, the nutritional and bioactive ingredients in the meal, concentrate and hydrolysate could serve for the formulation of health-promoting functional foods and products [36-45].

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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