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PARTIAL PURIFICATION AND CHARACTERIZATION OF ANGIOTENSIN CONVERTING ENZYME INHIBITORY PEPTIDE FROM SCARLET RUNNER BEANS (*PHASEOLUS COCCINEUS*) SEEDS

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ABSTRACT

*In the present study, an angiotensin converting enzyme (ACE) inhibitory peptide from fresh seedpods of scarlet runner beans (*Phaseolus coccineus*) was partially-purified via a two-step process: acetone precipitation and gel filtration chromatography. The ACE inhibitory activities of all fractions were determined by a spectrophotometric method. The amino acid composition, effect of temperature, pH, digestive enzymes and metal ions on the activity of the partially-purified peptide were determined. From the results, the crude proteins from seedpods of *Phaseolus coccineus* had a specific inhibitory activity of $0.046 \pm 0.002 \mu\text{mol}/\text{min}/\text{mg}$, which increased to $0.064 \pm 0.004 \mu\text{mol}/\text{min}/\text{mg}$ after acetone precipitation. Gel filtration chromatography with sephadex G-50 further increased the specific inhibitory activity to $0.169 \pm 0.021 \mu\text{mol}/\text{min}/\text{mg}$ at a purification fold of 3.40 and yield of 12.8%. The peptide contained seventeen amino acids with - Glutamic acid at 13.63g/100g protein being highest. The optimum pH and temperature of the peptide was 7.0 and 40°C, respectively. The activity of the ACE inhibitory peptide was strongly inhibited by Na⁺, Mg²⁺, K⁺, and Ca²⁺ but enhanced by EDTA. Pepsin and trypsin also significantly ($p < 0.05$) decreased the activity of the partially-purified peptide. In conclusion, this study has shown that angiotensin-converting enzyme inhibitory peptide can be isolated from *Phaseolus coccineus*, but the purification steps needs to be modified to obtain a peptide with higher yield.*

Keywords: *Phaseolus coccineus*, Angiotensin converting enzyme, peptide,

INTRODUCTION

Hypertension is a common and often progressive disorder that poses a major risk for cardiovascular diseases, diabetes, stroke and renal disorders. Recent data have revealed that the global burden of hypertension is growing at an alarming rate with an estimated 25 % increase in the next ten years (Mittal and Singh, 2010). It is well documented that hypertension could have many causes, and a prominent factor among all is over-activation of the renin angiotensin aldosterone system (RAAS) (Schmieder et al., 2007; Persson et al., 2009). Angiotensin converting enzyme (ACE) plays a key role in RAAS by producing angiotensin II, which is a potent vasoconstrictor (Ottaviani et al., 2006). Therefore, in the past decade, inhibition of ACE has become a key therapeutic target in the treatment of hypertension. Common ACE inhibitors currently in use include captopril, ramipril, lisinopril, enalapril etc. all of which have undesirable side effects (Schmieder et al., 2007). As a result an increased interest in finding non-toxic and effective ACE inhibitors from natural food source is on the increase. These alternatives include medicinal plants known to be of great importance to the health of individuals and communities, many of which are used as spices and food plants. In recent years ACE inhibitory peptides have been isolated from

hydrolysates of many food materials (Chia-Ling et al., 2012; Abdulazeez et al., 2015).

Phaseolus coccineus also known as Scarlet Runner beans originated from Mexico around 900 AD (Freytag and Debouck, 2002), is now cultivated all round the world. It belongs to the family '*Leguminosae*' and genus, '*Phaseolus*' and occupies the third position in the economic ranking among the different *Phaseolus* species (Westphal, 1974). The stem, leaves and seedpods are nutritious and widely consumed as food, while the bark and the roots are used in folk remedies. It is an unconventional legume that contains nutritionally useful quantities of most of the essential amino acids and adequate amounts of the limiting amino acids and also a good source of essential minerals (Aremu et al., 2006). Scarlet runner bean is known for its high starch and protein content, which can be carboxymethylated into products with economic, health and medicinal purpose. In the pharmaceutical industry, carboxymethylated starch (CMS) is used as disintegrant in tablets and it has also been reported that CMS is preferably used as osmotic agents for adjusting the toxicity of drugs especially medicinal solutions for parenteral administration based on the swelling and solubility properties, and hence, its use in the treatment of dialysis (Sutie, 1969; Akinterinwa et al., 2014).

In vivo and *in vitro* studies indicate that the fresh seedpods, seeds and stem extracts of *P. coccineus* possess anti-inflammatory, antibacterial, anti-fungal and anti-hypertensive properties. They also promote wound healing (Figen, 2006). *P. coccineus* contains the peptide coccinin, in the seed and stem of scarlet runner beans, which has a strong antifungal activity. Previous phytochemical studies on *P. coccineus* have reported the presence of carotenoids, flavonoids, thiamin, niacin, glucosinate, glycosides, isothiocyanates saponins, free anthraquinones, alkaloids, flavonoids and phenolics (Mizutani et al., 1984), however, literature on its ACE inhibitory activity is scarce. The present study is aimed at partially-purifying an ACE inhibitory peptide from the fresh seedpods of scarlet runner bean (*Phaseolus coccineus*) characterize, and determine its inhibition pattern.

MATERIALS AND METHODS

Chemicals and reagents

Hippury-histidine-leucine (HHL), ACE from rabbit lung, Silica gel, Sodium Chloride, Sodium Diphosphate, Sodium Monophosphate, Sodium Hydroxide, Hydrochloric Acid, Biuret Reagent, Bovine Serum Albumin, Acetone, Ethyl Acetate, Potassium Diphosphate, Potassium Monophosphate (Sigma Chemical Co., St Louis, MO, USA). All other reagents and chemicals used were of analytical grade.

Plant material

Fresh pods of *Phaseolus coccineus* were obtained from Sabon-Gari market, Zaria, Kaduna State Nigeria, and authenticated by a botanist at the herbarium of the Department of Biological Science, Ahmadu Bello University Zaria, Kaduna State, Nigeria.

Partial-purification of ACE-inhibitory proteins from *Phaseolus coccineus*

The fresh pods were washed under running water, macerated with phosphate buffer at pH 7.4 at a ratio of 1:10. The mixture was then centrifuged at 4°C and 10000×g for 15 minutes to obtain the supernatant, which was bottled and stored in a cold room until when required.

Cold acetone precipitation

The supernatant was precipitated using cold acetone at a ratio of 1:4. The tube containing the precipitated protein was vortexed and incubated at -20°C for one hour after which it was centrifuged for 10 minutes at 4°C and 10000×g. The supernatant was discarded leaving the pellet. Acetone was allowed to evaporate from the uncapped test-tube. The pellet was reconstituted in 5ml phosphate buffer (pH 7.4) to determine the ACE-inhibitory activity and protein content.

Gel filtration using sephadex G-50

The reconstituted protein was subjected to gel filtration chromatography with sephadex G-50. The gel was soaked overnight in phosphate buffer (pH 7.4). The column was packed with the gel and washed with the same buffer. The protein was then poured into the column and allowed to elute at a flow rate of 0.5ml/min. Thirty(30) fractions of the sample was collected, 5mls each. The protein content and ACE-inhibitory activity of each fraction was determined and fractions with the highest ACE-inhibitory activity pooled together.

Protein estimation

Protein content of the fractions was determined according to Biuret method using serum bovine albumin (BSA) as the standard and the concentration was expressed in milligram per millilitre.

Determination of ACE-Inhibitory Activity

The inhibitory activity of ACE was determined as described by Cushman and Cheung (1971). Briefly, the inhibitor solution (protein-rich fraction) was added to a test-tube containing 5mM hippuryl-histidine-leucine (HHL) previously dissolved in 0.1M potassium phosphate buffer containing 0.3M NaCl (pH 8.3). Then the enzyme, ACE was added to the mixture and incubated at 37°C for 30 minutes. The reaction was terminated by adding 0.25ml of 1M HCl, and 1.5ml ethyl acetate was added to extract the hippuric acid formed by the action of ACE. The ethyl acetate was removed by heat evaporation, and residual hippuric acid (HA) dissolved in 3ml of deionized water. The absorbance of the solution was taken at 300nm to determine the hippuric acid concentration.

Inhibition was expressed as the concentration of inhibitor that inhibits 50% of ACE-activity (IC_{50}), and was calculated using a non-linear regression from a plot of activity versus inhibitory concentration of at least five separate determinations. Each assay was performed in triplicate. One (1) unit was expressed as the potency showing 50% ACE-inhibition under these conditions, which corresponds to 1 μ mol of hippuric acid, released by hydrolysis of HHL per minute per millilitre of inhibitor.

Determination of Inhibitory Pattern and Measurement of Kinetic Constants

To investigate the ACE inhibitory pattern of the partially-purified peptide, various concentrations of the substrate (0, 1, 2, 3, 4, and 5 mM) were treated in the assay system. The ACE activity was determined both in the presence and absence of 0.5 and 1mg/ml of the partially-purified peptide at 37°C. The Michaelis Menten constant, K_m , the inhibition constant, K_i , and the V_{max} were obtained from the Lineweaver–Burk plots.

Characterization of the Partially Purified Peptide from Seedpods of *P. coccineus*

Effect of pH and Temperature on ACE Inhibitory peptide

The effect of pH on the ACE-inhibitor was determined by varying the pH of reaction mixture from 3 to 10, while the influence of temperature on inhibitory activity was analyzed by varying the incubation temperature from 10 to 70°C.

Effect of some metal ions on the ACE inhibitory peptide

ACE activity was determined in the presence of the monovalent and divalent metal ions of Chloride, Sodium, Potassium, Magnesium and Calcium as well as EDTA.

Effect of some digestive enzymes on the ACE inhibitory peptide

The effect of trypsin and pepsin on the activity of the ACE inhibitory peptide was determined as described by Choi *et al.* (2001).

Briefly, digestive enzymes (pepsin and trypsin) were added to the partially purified ACE inhibitor. The reaction mixture was incubated at 37°C for one hour and the reaction stopped by standing in boiling water bath for 10mins. After centrifugation at 2800xg, the supernatant was assayed for ACE inhibitory activity.

Determination of Amino Acid Profile

The Amino Acid profile of the partially purified peptide was determined using methods described by Benitez (1989). The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon sequential Multi-Sample Amino Acid Analyzer (TSM).

RESULTS

Partial purification of ACE inhibitory peptides

The steps involved in the purification of Angiotensin converting enzyme inhibitor are summarized in table 1

below. The crude enzyme inhibitor had a specific activity of $0.046 \pm 0.002 \mu\text{mol}/\text{min}/\text{mg}$ at 100% yield. The precipitated proteins after undergoing purification 1.3 times emerged with a percentage purity of 48.4 % and a specific activity of $0.064 \pm 0.004 \mu\text{mol}/\text{min}/\text{mg}$. On further purification, the specific activity increased to $0.169 \pm 0.021 \mu\text{mol}/\text{min}/\text{mg}$ with a percentage yield of 12.8% and purification fold of 3.40.

From the gel filtration chromatography elution profile, there was a gradual decrease and increase in ACE activity with no specific rhythm, with an increase in percentage up to fraction 6. Fractions 23 and 26 were pooled together as they had the highest inhibitory activity and protein content (figure 1).

Table 1: Purification table of the partially purified ACE inhibitory peptide from seedpods of *P. coccineus*

Purification Step	Protein Content (mg/ml)	Inhibitory Activity ($\mu\text{mol}/\text{min}$)	Specific Activity ($\mu\text{mol}/\text{min}/\text{mg}$)	Inhibitory Purification Fold	Yield (%)
Crude	11.40	0.556	0.046	1	100
Acetone Precipitation	4.02	0.269	0.064	1.30	48.4
Gel Filtration	0.40	0.071	0.169	3.40	12.8

1 U of inhibitory activity was defined as the amount of the inhibitor that decreased the ACE activity by 50%

Effect of pH and Temperature on the partially-purified ACE inhibitory peptide

The highest ACE inhibitory activity of the partially-purified protein was obtained at pH 7.0 and temperature of 40°C and then subsequent decrease. The activity of the peptide inhibitor increased as the temperature increased from 20° C through to 40 ° C and then decreased when temperature was further increased.

Effect of Gastrointestinal enzymes on the partially-purified ACE inhibitory peptide

Treatment of the partially-purified ACE inhibitor with pepsin and trypsin, significantly ($P < 0.05$) decreased its inhibitory activity compared to the control ($0.071 \pm 0.17 \text{ mg}/\text{ml}$). The ACE inhibitory effect of captopril ($0.127 \pm 0.14 \text{ mg}/\text{ml}$) was significantly ($P < 0.05$) lower than the control ($0.071 \pm 0.17 \text{ mg}/\text{ml}$) and peptides treated with pepsin ($0.305 \pm 0.024 \text{ mg}/\text{ml}$) and trypsin ($0.242 \pm 0.015 \text{ mg}/\text{ml}$) (Figure 1).

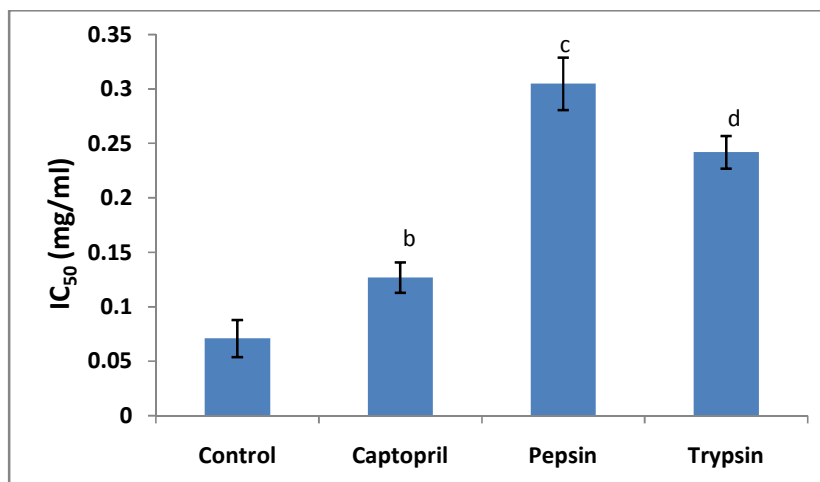


Figure 1: Effect of some gastrointestinal enzymes on the ACE inhibitory activity of partially purified peptide from *P. coccineus*

a, b, Mean values having different superscript letters are significantly different ($P < 0.05$)

IC₅₀ defined as the concentration that inhibits 50% of the angiotensin converting enzyme activity

Effect of some metal ions on the partially-purified ACE inhibitory peptide

Figure 2 below shows that Na⁺, Mg²⁺, K⁺, and Ca²⁺ decreased the activity of the partially-purified peptide,

while EDTA enhanced it. This shows that the partially purified protein may be a good metal chelator. This is consistent with the result obtained by Dorrer *et al.* (1970).

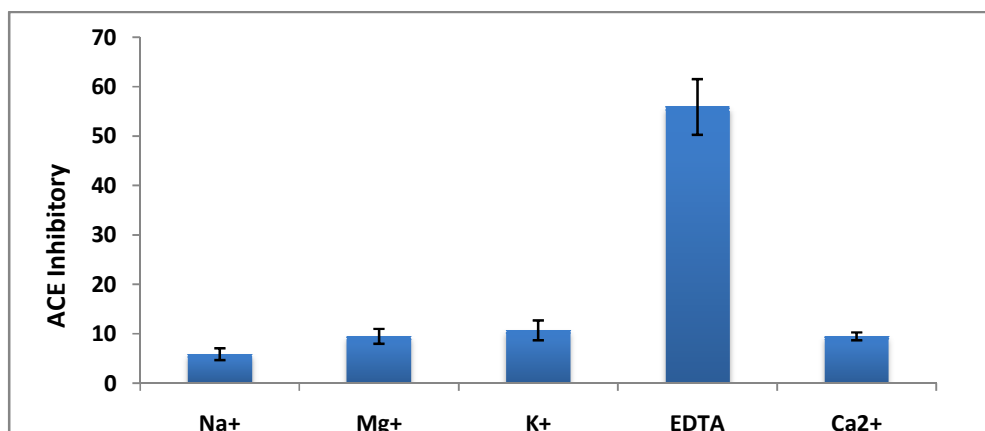


Figure 2: Effect of some metal ions on the ACE inhibitory activity of partially purified peptide from *P. coccineus*

Amino Acid Analysis of the Partially purified ACE Inhibitory Peptide

As shown on table 2, the partially-purified ACE inhibitory peptide from *P. coccineus* contained

seventeen (17) amino acids having Glutamic acid (13.63 g/100g protein) at the highest concentration, and Cysteine (0.79 g/100g protein) the least.

Table 2: Amino acids present in the partially purified ACE inhibitory peptide from *P. coccineus*

Amino Acids	Concentration (g/100g protein)
Lysine	6.51
Histidine	2.52
Arginine	5.01
Aspartic acid	9.00
Threonine	3.86
Serine	3.69
Glutamic acid	13.48
Proline	4.17
Glycine	5.96
Alanine	5.24
Cystine	0.79
Valine	4.20
Methionine	2.08
Isoleucine	3.20
Leucine	6.55
Tyrosine	2.86
Phenylalanine	3.88

DISCUSSION

Hypertension is recognized as major risk factor for the development of many chronic diseases (Kearney et al., 2005) and since RAS plays a significant role in the pathogenesis of hypertension, ACE inhibitors have long been known for their beneficial effects in reducing high blood pressure (Brown and Hall, 2005). ACE inhibitors reduce blood pressure by preventing the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor. They also degrade bradykinin, either by binding to the active site as a substrate for the enzyme or as a non-substrate, hence, inhibiting the activity of this enzyme ultimately results to low blood pressure (Viernes et al., 2012). Green tea, blueberry, *Hibiscus sabdariffa* and *Senecioina equidens* extracts were reported to inhibit ACE with

IC₅₀ values of 125 µg/ml, 46 µg/ml, 91µg/ml and 192µg/ml, respectively. Although hypotensive properties of some plant bioactive constituents have been widely reported (Loizzo et al., 2007; Ojeda et al., 2010), very little has been done to determine such properties in *P. coccineus*.

In the present study, using the spectrophotometric method of Angiotensin converting enzyme inhibitory activity by Cushman and Cheung (1971), an angiotensin converting enzyme inhibitory peptide was partially purified from *Phaseolus coccineus* (Scarlet runner bean). The ACE inhibitory activity of the partially-purified inhibitor was lower when compared to that of *Hibiscus rosasinensis* (0.43± 0.04 U/ml) and *Vinica* leaves (1.37±0.06 U/ml) (Aprilita et al., 2013).

This might be as a result of differences in assay condition, source of ACE, plant source and substrate used.

Phaseolus coccineus has some medicinal uses, which have long been recognized in the Ayurvedic and Unani systems of medicine (Gupta et al., 2012), but there is little or no information on the ACE inhibitory effects of its bioactive constituents. This study shows that inhibitory peptides are found in fresh pods of *P. coccineus* and inhibit ACE *in vitro*. It is well established that *in vitro* analysis offers the best opportunity and insight to widen analysis in different directions.

The partially-purified peptide showed maximum ACE inhibitory activity at the pH 7 and the temperature of 40°C. This result conforms to reports by Bernstein *et al.* (2013) that the optimum pH and temperature of most ACE peptides falls within a pH range of 7 to 8 and temperature range of 37 to 40°C.

The effect of gastrointestinal enzymes on ACE inhibitory peptides is important because after ingestion, their activities may either increase or decrease as the enzymes may break up peptides. Though it is often expected that the peptides are able to resist gastrointestinal hydrolysis after oral intake, this is not always the case. This may be attributed to variations in the type of ACE-inhibitory peptides liberated after treating with different enzymes (Barbana and Boye, 2011). Studies have shown that a decrease in ACE inhibitory activity of a peptide after treatment with gastrointestinal enzymes does not make it ineffective, as they may still maintain their

REFERENCES

- Abdulazeez, M.M., Ndubuisi, T.C., Mohammed, I., Abdullahi, S.A., Chintem, W. and Wudil, A.M. (2013). Partial-purification and Characterization of Angiotensin Converting Enzyme Inhibitory Proteins from the Leaves and Seeds of *Moringaoleifera*. *International Journal of Biochemistry Research and Review*, 5(1): 39-48.
- Akinterinwa, A., Osemeahon, S.A., Akinsola, A.F. and Reuben, U. (2014). Physicochemical and Pasting Characterization of Carboxymethylated Scarlet Runner Bean (*Phaseoluscoccineus*) Starch. *Journal of Agriculture and Food Technology*, 4(2):13-20.
- Aprilita, R., Maksun, R., Abdul, M. and Suyatna, F.D. (2013). Screening Angiotensin Converting Enzyme (ACE) Inhibitor Activity of Antihypertensive Medicinal Plants from Indonesia. *International Journal of Pharmacy Teaching and Practices*. 4(1): 527-532.
- Aremu, M.O., Olaofe, O. and Akintayo, E.T. (2006). A comparative study on the chemical and amino acid composition of some Nigerian underutilized legume flours. *Pakistan Journal of Nutrition*, 5: 34-38.
- Barbana, C. and Boye, J. (2011). Angiotensin I-converting enzyme inhibitory properties of lentil protein hydrolysates: Determination of the kinetics of inhibition. *Food Chemistry*, 127: 94-101.
- inhibitory activity (Choi et al, 2001). Fujita and Yoshikawa (1999) attributed this to the fact that other factors such as route of administration, food matrix and intestinal absorption play a significant role in the activity of the peptide. Also, several ACE inhibitory peptides with weak *in vitro* activity produce a strong antihypertensive effect *in vivo*. Therefore, even if the enzymes affected the partially purified inhibitory peptide obtained from *Phaseoluscoccineus*, they may still be effective in reducing blood pressure.
- The ACE inhibitory activity of the partially purified peptide was significantly decreased by sodium, calcium, magnesium, and potassium ions, but addition of EDTA to the inhibitor did not significantly affect its activity when compared to the control. This shows that the partially purified protein may be a good metal chelator. This is consistent with the result obtained by Dorer *et al.* (1970).

Conclusion

In conclusion, this study has established that ACE inhibitory proteins can be purified from scarlet runner beans, and thus, may be beneficial as nutraceuticals or drugs for treatment of hypertension.

Author's contributions

Abdulazeez, A.M. designed the study, wrote the protocols and managed the statistical analysis. Kurfi, B.G. wrote the first draft of the manuscript. Osumaje, G. carried out the laboratory work. All authors read and approved the final manuscript.

Conflict of Interest

There are no conflicts of interests

Benitez, L.V. (1989). Amino Acid and fatty acid profiles in aquaculture nutrition studies. in S.S. De Silva (ed.) *Fish Nutrition Research in Asia*. Proceedings of the Third Asian Fish Nutrition Network Meeting. Asian fish. Society Special Publication. Asian Fisheries Society, Manila Philippines. 1989; 23- 35.

Bernstein, K.E., Frank, S.O., Wendell-Lamar, B.B., Kandarp, H.S., Jorge, F.G., Gonzalez-Villalobos, R.A., Xiao, Z.S. and Sebastien F. (2003). A modern understanding of the traditional and nontraditional biological functions of angiotensin-converting enzyme. *Pharmacological Reviews*, 65:1-46

Brown, B. and Hall, A.S. (2005). Renin-Angiotensin System Modulation: The Weight of Evidence. *American Journal of Hypertension*, 18(9): 127-133.

Chia-Ling, J., Shih-Li, H. and Kuo-Chiang, H. (2012). Angiotensin I-converting enzyme inhibitory peptides: Inhibition mode, bioavailability, and antihypertensive effects. *BioMedicine*, 2: 130-136.

Choi, H.S., Cho, H.Y., Yang, H.C., Ra, K.S. and Suh, H.J. (2001). Angiotensin I-converting enzyme inhibitor from *Grifolafrondosa*. *Food Research International*, 34: 177-182.

Cushman, D.W. and Cheung, H.S. (1971). Spectrophotometric assay and properties of angiotensin converting enzyme from rabbit lungs. *Biochemical Pharmacology*, 20(7): 1637-48.

- Dorrer, F.E., Skeggs, L.T., Khan, J.R., Lentz, K.E. and Levine, M. (1970). Angiotensin converting enzyme. *Analytical Biochemistry*, 33: 102.
- Figen, M.T. (2006). Saponin versus plant fungal pathogens. *Journal of Cell and Molecular Biology*, 5:13-17.
- Freytag, G. and Debouck, D.G. (2002). Taxonomy, distribution and ecology of the genus *Phaseolus* (Leguminosae-Papilionoideae) in North America, Mexico and Central America. Botanical Institute of Texas, Fort Worth, Texas, United States. 300 pp.
- Fujita, H. and Yoshikawa, M. (1999). LKPNM: A prodrug-type ACE-inhibitory peptide derived from fish protein. *Immunopharmacology*, 44: 123-127.
- Gupta, R., Mathur, M., Bajaj, V.K., Katariya, P., Yadav, S. and Kamal, R. (2012). Evaluation of antidiabetic and antioxidant activity of *Moringa oleifera* in experimental diabetes. *Journal of Diabetes*, 4(2):164-171.
- Kearney, P.M., Whelton, M., Reynolds, K., Muntner, P., Whelton, P.K., He, J. and Whelton, H. (2005). Global burden of hypertension: analysis of worldwide data". *Lancet*, 365 (9455): 217-223.
- Loizzo, M.R., Said, A., Tundis, R., Rashed, K., Statti, G.A., Hufner, A. and Menichini, F. (2007). Inhibition of angiotensin converting enzyme (ACE) by flavonoids isolated from *Ailanthu excels* (Roxb) (Simaroubaceae). *Phytotherapy Research*, 21:32-36.
- Mittal, B.V. and Singh, A.K. (2010). Hypertension in the developing world: challenges and opportunities. *American Journal of Kidney Disease*, 55:590-598.
- Mizutani, K.K., Othani, J.X.W. and Tanaka, O. (1984). Saponins from *Anemone rivularis*. *Planta Medica*, 51:327-331.
- Ojeda, D., Jiménez-Ferrer, E., Zamilpa, A., Herrera-Arellano, A., Tortoriello, J. and Alvarez, L. (2010). Inhibition of angiotensin converting enzyme (ACE) activity by the anthocyanins 10 delphinidin- and cyanidin-3-O-sambubiosides from *Hibiscus sabdariffa*. *Journal of Ethnopharmacology*, 127(1):7-10.
- Ottaviani, J.I., Actis-Goretta, L., Villordo, J.J. and Fraga, C.G. (2006). Procyanidin structure defines the extent and specificity of angiotensin I converting enzyme inhibition. *Biochimie*, 88:359-365.
- Persson, I.A.L, Persson, K. and Andersson, G.G. (2009). Effect of *Vaccinium myrtillus* and its polyphenols on angiotensin-converting enzyme activity in human endothelial cells. *Journal of Agricultural and Food Chemistry*, 57:4626-4629.
- Schmieder, R.E., Hilgers, K.F., Schlaich, M.P. and Schmidt, B.M. (2007). Renin-angiotensin system and cardiovascular risk. *Lancet*, 369:1208-1219.
- Suttie, J.M. (1969). The butter bean (*Phaseolus coccineus* L.) in Kenya. *East African Agricultural and Forestry Journal*, 35: 211-212.
- Viernes, L.B.G., Garcia, R.N., Torio, M.A.O. and Angelia, M.R.N. (2012). Antihypertensive peptide from vicilin, the major storage protein of mung bean. *Journal of Biological Sciences*, 12:393-399.
- Westphal, E. (1974). Pulses in Ethiopia, their taxonomy and agricultural significance. Agricultural Research Reports 815. Centre for Agricultural Publishing and Documentation, Wageningen, Netherlands. 276 pp.
- Sebastien F. (2003). A modern understanding of the traditional and nontraditional biological functions of angiotensin-converting enzyme. *Pharmacological Reviews*, 65:1-46