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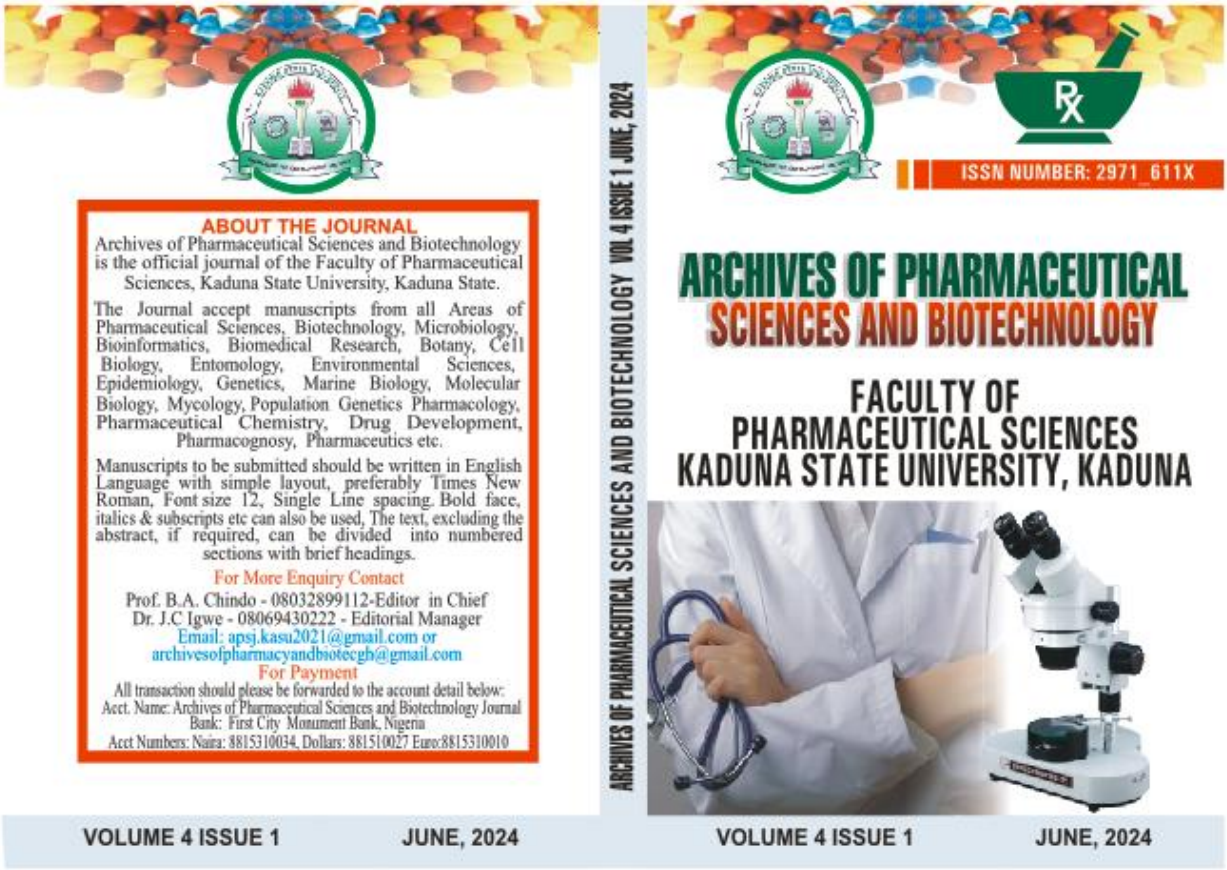

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ASSESSMENT AND CHARACTERIZATION OF ANGIOTENSIN CONVERTING ENZYME INHIBITORS ISOLATED FROM HYDROLYZED GOAT MILK USED AS PEPTIDE INHIBITOR AGAINST HYPERTENSION

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ABSTRACT

Introduction: Hypertension has been a significant risk factor for cardiovascular illness. Synthesized angiotensin converting enzyme (ACE) inhibitors are active antihypertensive medicines but they commonly cause unwanted side effects.
Aim: This study describes the characterization of an ACE inhibitory peptide from goat milk hydrolysate.
Methods: Hydrolysates containing angiotensin converting enzyme (ACE) inhibitory peptide were prepared from goat milk using pepsin and trypsin.
Results: The purified peptide has an optimum temperature of 40 °C and optimum pH of 2. The activity of the purified peptide was not significantly (P>0.05) affected after *in vitro* incubation with gastrointestinal enzymes pepsin and trypsin. The purified peptide contained 18 amino acids with glutamic acid being the most abundant (13.63g/100).
Conclusion: ACE inhibitory peptide from purified goat milk hydrolysate digested with pepsin may be a helpful component to serve as drug against hypertension.
Keywords: Hypertension; Angiotensin Converting Enzyme Inhibitory Peptide; Goat Milk Hydrolysate; Purification; Ultrafiltration Membrane

1.0. INTRODUCTION

Worldwide, hypertension affects 15-20% of adults and is the primary cause of cardiovascular illness, accounting for 7.5 million deaths annually [1]. It is linked with cardiovascular disease, insulin resistance, obesity, carbohydrate tolerance, hyperuricidemia, and atherosclerosis [2]. Hypertension disturbs the structures and functions of arterioles, other blood vessels and can cause damage at variable rates to different target organs such as the brain, eye and kidney [2].

Angiotensin converting enzyme (ACE) inhibitors are used as the primary line of treatment for hypertension [3]. ACE is crucial for the physiological control of hypertension. ACE catalyzes the conversion of the inactive decapaptide, angiotensin I into an active vasoconstricting octapeptide, angiotensin II [3]. Lisinopril, captopril, enalapril, and other common ACE inhibitors are currently been used as synthetic inhibitors in the market, however they all have undesirable side effects such as cough, skin rashes, nausea, vomiting and dizziness. As a result, interest has grown in looking for all-

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natural remedies that could reduce hypertension [4].

Proteins source such as milk, soy, fish, and other proteins have been found to contain peptides with inhibitory activity against ACE. Many of these peptides that are bioactive are found to have anti-oxidative, anti-carcinogenic, antibacterial, immunomodulatory, antithrombotic, mineral binding, and ACE inhibitory properties [5]. Milk proteins serve as rich sources of bioactive peptides, as they are very well characterized, available in large amounts at a high degree of purity and low price [6]. As a result, the aim of this study was to characterizing an ACE inhibitory peptide purified from goat milk digested by pepsin and trypsin.

2.0. MATERIALS AND METHODS

2.1. Materials

Angiotensin converting enzyme from rabbit lungs, Hippuryl-histidyl-leucine (HHL), pepsin and trypsin (Sigma-Aldrich, St. Louis, MO 63103, USA). All other reagents used in the study were of analytical grade.

2.2. Equipment

Refrigerated centrifuge (MSB005.CR2.K, MSE Ltd, U.K), HPLC Machine (Adept CECIL CE4201) and spectrophotometer (JENWAY 6300) (Bibby scientific limited, UK), Spectrophotometer CE7400 (CECIL, Cambridge England), Oven (OV/100), Incubator (MINI/50/VIS) and pH meter (Genlab, Metrohm Ltd., Herisan, Switzerland), Electrical weighing balance and Freeze dryer (LabogeneApS, Denmark).

2.3. Effect of pH and Temperature on ACE Inhibitory Activity of the Partially Purified Peptide

The lyophilized ACE inhibitory peptide was dissolved in distilled water and incubated for 2 hours at various pH values ranging from 2 to 10. The inhibitor's ACE inhibitory activity was investigated. The influence of temperature on inhibitory action was also investigated by altering the incubation temperature from 20 °C to 80 °C [7].

2.4. Effect of Digestive Enzymes on ACE Inhibitory Activity

The peptide solution was incubated with pepsin at pH 2.0 and 37°C for 2 hours to imitate the enzymatic effects in the human intestines and stomach, as well as to analyze the stability of the ACE inhibitory peptide in vitro. After standing in boiling water for 10 minutes, the reactions were halted and centrifuged at 10,000 x g for 30 minutes. After that, the supernatant was collected to test for ACE inhibitory activity.

2.5. Determination of Amino Acid Composition

The amino acid profile in the sample was determined using the method described by [8]. Before loading into the amino acid analyzer, the sample was dried to a constant weight (10ml), hydrolyzed, and evaporated in a rotary evaporator.

2.6. Statistical Analysis

Data were expressed as mean of three replicates ± standard deviation (SD). The data were subjected to analysis of variance using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA).

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3.0. RESULTS

3.1 Effect of pH and Temperature on the Partially Purified Peptide

The inhibitory activity of the peptide on ACE was optimum at 40 °C (84 %), after which it decreased with increasing temperature having the lowest activity at 80 °C (71 %) (Figure 1).

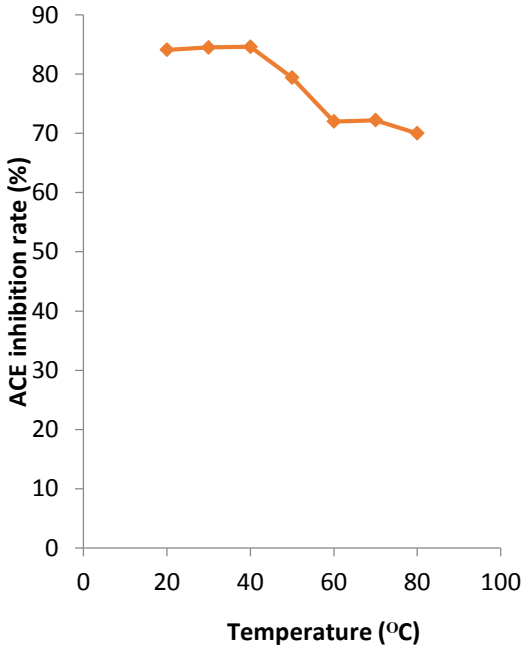

Figure 1: Effect of temperature on the ACE inhibitory activity of the partially purified peptide

The ACE inhibitory activity of the partially purified peptide was optimal at pH 2 (83 %), and remained stable between pH 3 and 4 due to slight decrease in inhibitory activity. The inhibitory activity of the peptide continues to decrease beyond pH 4 until it reached the lowest activity at pH 8 (58 %) (Figure 2).

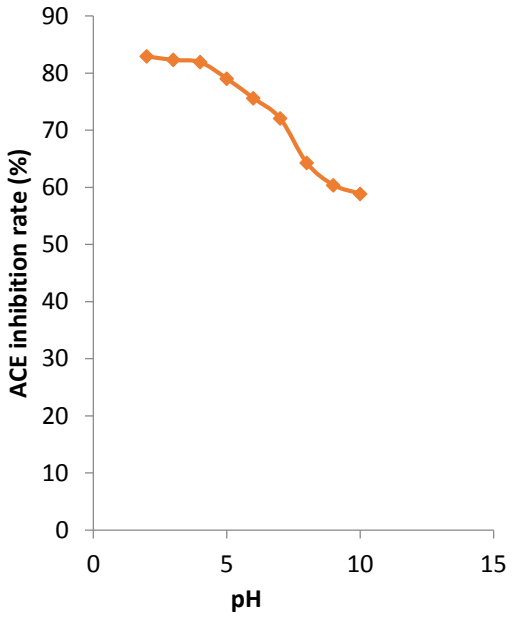

Figure 2: Effect of pH on the ACE inhibitory activity of the partially purified peptide

Figure 3 present the effect of pepsin and trypsin on the stability of the ACE inhibitory peptide. The results revealed that the ACE inhibitory activity of the partially purified peptide was not significantly ($P > 0.05$) affected after *in vitro* incubation with gastrointestinal enzymes. The value obtained for digestion with pepsin was 84.41 ± 0.06 %, while that of trypsin was 84.32 ± 0.04 % and control had 84.50 ± 0.26 %.

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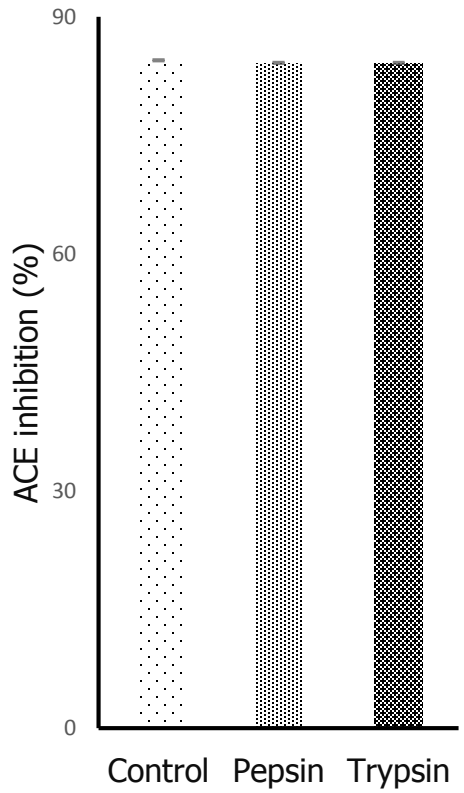

Figure 3: Stability of ACE inhibitory activity of the partially purified peptide after digestion with some gastrointestinal enzymes

Control = ACE inhibitory activity without gastrointestinal enzymes

Table 1 present the amino acid content of the partially purified peptide. The peptide contained eighteen (18) amino acids with glutamic acid having the highest percentage (13.63g/100 g), followed by leucine (10.25 g/100 g) and aspartic acid (8.20 g/100 g), while cysteine (1.33 g/100 g) was the lowest.

Table 1: Amino acids composition of ACE inhibitory peptide derived from enzymatic hydrolysis of goat milk

Amino Acids	Concentration (g/100g protein)
Leucine	10.25
Lysine	7.40
Isoleucine	7.34
Phenylalanine	5.23
Trytophan	2.36
Valine	5.44
Methionine	3.55
Proline	3.25
Arginine	6.54
Tyrosine	4.30
Histidine	3.13
Cystine	1.33
Alanine	4.02
Glutamic acid	13.63
Glycine	5.42
Threonine	4.80
Serine	4.86
Aspartic acid	8.20

4.0. DISCUSSIONS

The partially purified peptide from goat milk hydrolysate maintained their ACE-inhibitory activity after exposure to different pH values and different temperatures for 2 hours (Figures 1 and 2). The ACE inhibitory peptide was more stable at lower temperatures below 40 °C, but was more sensitive at higher temperatures, resulting in a drop in inhibitory activity. The decrease in activity of the partly purified peptide (Figure 1) indicates that they may be thermally denatured at high temperatures [9].

The effect of pH on the ACE inhibitory activity of the partly purified peptide suggests that acidic and slightly alkaline circumstances were more affirmative for

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maintaining inhibitory activity than high alkaline settings with an optimal pH of 2 (Figure 2). These findings showed that the partly purified peptide produced from goat milk hydrolyslate is thermally and pH stable. This is consistent with findings from other studies [9,10], who isolated stable ACE-inhibitory peptides from various sources, demonstrating that the peptides can be easily transported and stored at various temperatures and pH levels after being developed into a product.

For ACE inhibitory peptides to maintain their antihypertensive effect *in vivo* they must be ingested in their intact form from the intestine and further be resistant to plasma peptidase degradation to reach their target sites. This is because their activity may be affected if hydrolyzed *in vivo* [11]. The *in vitro* gastrointestinal enzyme stability studies of isolated ACE-inhibitors allow us to model their action *in vivo* following oral administration. The results showed that *in vitro* incubation with gastrointestinal enzymes had no effect on the partially-purified peptide's ACE-inhibitory action ($P>0.05$). This study is consistent with the work of [12] and [9], the results indicate that the partially purified peptide may be real inhibitor-type ACE-inhibitory peptides because it is capable of inhibiting ACE.

On assessment of the amino acids composition of ACE inhibitory peptide derived from enzymatic hydrolysis of goat milk. The findings from this study showed that partially purified peptide contain amino acid residues such as Leucine, Isoleucine, Tryptophan, Proline, and Glutamic acid were obtained; all of which have been shown to improve peptide ACE inhibitory activity [13,14], with Glutamic acid concentration being higher than other amino acids (Table 1). This is similar to an ACE inhibitory peptide isolated from *Moringa oleifera* leaves [15].

5.0 CONCLUSION AND RECOMMENDATIONS

This study showed the ACE inhibitory activity of the partially purified peptides at different pH and temperature, as well as the stability of the peptides in the presence of gastrointestinal enzyme. The amino acid composition of the peptides were also obtained and it contains 18 amino acid. The purified peptide isolated from goat milk hydrolysate possess potent ACE inhibitory activity and may be used in the development of reasonably cost, effective, and harmless antihypertensive peptides to serve as substitute to synthetic ACE inhibitory medication. Further studies are required to determine the *in vivo* activity of the purified ACE inhibitory peptide.

Authors Contribution:

Hashidu A.S., Babangida M. I. and Abdulazeez A.M. contributed to the study's conception, implementation, data analysis, and manuscript writing. Adekale I. A., Musa U. U., Efienokwu J. N. and Makeri M. S. contributed to data collection, processing and interpretation of the results. All of the writers examined the findings and approved the final manuscript version.

Conflict of Interest

The writers have all stated that they have no conflicts of interest.

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