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*Original Research Article*

# Isolation and Characterisation of Akuammine from the Seed of *Parinari curatellifolia* and its Molecular Dynamics in Angiotensin -1- Converting Enzyme Inhibition

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ABSTRACT

Over the years, isolated compounds from medicinal plants have been lead candidates in drug discovery and they are useful therapeutic aid to patients. This work isolated a compound from the seed of *Parinari curatellifolia* and investigated its inhibitory potential and mechanism on angiotensin-1-converting enzyme (ACE1). The methanolic extract of the seed was subjected to chromatographic techniques and yielded a white, crystalline solid. Structural elucidation of the compound was carried out using NMR and MS spectroscopic techniques. The use of N-(3-[2-furyl-acryloyl]-Phe-Gly-Gly) (FAPGG) as an enzyme substrate and the Michealis-Menten method in this study showed how sensitive and fast ACE1 could be inhibited. Inhibition mechanism was explored using the Lineweaver-Burk model, and $IC_{50}$ was determined using Cheng-Prusoff empirical analysis. Molecular interaction with ACE1 was investigated computationally. NMR and MS spectroscopy showed the compound to be Akuammine, with 26 protons, 22 carbons, 2 nitrogen, and 4 oxygen atoms and chlorinated molecular ion peak at m/z 417.1593. Akuammine exhibited an $IC_{50}$ of 8.5 µM and displayed mixed type inhibition. Molecular dynamics simulations indicated strong interaction at the allosteric site of ACE1, primarily through hydrophobic interactions. Akuammine demonstrated an ability as a template for antihypertensive agent. This ability may contribute to the ethno botanical uses of *Parinari curatellifolia* seed.

***Keywords*:** Akuammine, Angiotensin converting enzyme, Antihypertension, *Parinari curatellifolia*

## Introduction

Hypertension remains a significant global health concern, posing challenges to effective management despite various treatment options. The recent surge in its incidence in Africa, attributed to factors such as stress and lifestyle changes, underscores the urgency to explore novel therapeutic avenues.[1] The regulation of blood pressure homeostasis involves complex cellular and humoral mechanisms, often compromised by stressors like oxidative stress and disease complications, leading to elevated blood pressure levels.[2]. Among the key regulators of peripheral vascular resistance, angiotensin II stands out as a potent vasoconstrictor, primarily activated by angiotensin I converting enzyme (ACE1).[3,4,5]. The inhibition of ACE has emerged as an effective strategy in hypertension management, with synthetic ACE inhibitors widely prescribed despite associated adverse effects.[6,7,8] In recent years, there has been growing interest in harnessing the therapeutic potential of natural plants for hypertension management, given their diverse bioactive compounds.

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Notably, alkaloids, nitrogen-containing compounds abundant in the plant kingdom, have demonstrated cardioprotective properties, albeit limited investigations on ACE1 inhibition.[9]. *Parinari curatellifolia*, an indigenous African medicinal plant, boasts a rich ethnopharmacological history, with documented uses spanning wound infections, cancer, and notably, hypertension. While previous studies have highlighted the pharmacological properties of *P. curatellifolia* extracts, including ACE inhibition, the active principle responsible for these effects remains elusive.[10,11]

Our previous research reported the cardioprotective (Crown *et al.*, 2010) antihypertensive potential [10] and ACE inhibition of *P. curatellifolia* extracts,[11] yet the identification of the active compound(s) driving these effects remained unexplored. In this study, we aim to isolate and characterize Akuammine, a prominent alkaloid from the seeds of *P. curatellifolia*, and investigate its potential as an ACE1 inhibitor. Through molecular dynamics simulations, we seek to elucidate the mechanistic basis of Akuammine-mediated ACE1 inhibition, shedding light on its therapeutic relevance in hypertension management. This research endeavor represents a novel approach to addressing health disparities associated with hypertension, leveraging the natural bioactive compounds present in indigenous medicinal plants like *P. curatellifolia*.

## Material and Methods

*General*

NMR spectra were measured on Bruker ARX 500 NMR spectrometer using $CDCl_3$ as solvent and internal standard. The mass spectra were determined on a Bruker Compact Q-TOF high resolution Compact mass spectrophotometer. For TLC, pre-coated silica gel $60F_{254}$ plates were

used and silica gel (70-230 mesh). Compound was detected under ultra-violet (UV) lamp (254) and further visualized by spraying with vanillin-sulphuric acid solution. Angiotensin-1 converting enzyme (ACE1) and N-(3-[2-furyl] acryloyl]-Phe-Gly-Gly) (FAPGG) were purchased from Sigma-Aldrich, MO, USA. Bradford Reagent and BSA were obtained from Bio-Rad Laboratories, Hercules, CA, USA.

*Plant material and extraction*

The seeds of *Parinari curatellifolia* (PC) were purchased at the Oba's market in Akure, Nigeria, in the month of June, 2018. Botanical identification and authentication were carried out and the voucher specimen (PC005) was deposited at the Department of Biochemistry, Federal University of Technology Akure, Nigeria.

The seeds of PC were air-dried, pulverized and extracted with 80% methanol as described by Crown *et al*., (2017). The filtrate was dried to obtain crude extract. Furthermore, the crude extract was dissolved in methanol and the solution was filtered. The methanolic solution was concentrated *in-vacuo* to obtain the methanolic extract (135 g).

*Isolation of the compound and its spectroscopic data*

The extract was subjected to column chromatography. The column was subjected to isocratic elution with chloroform/methanol (12:1). Fifty-two fractions of 250 ml each was collected and concentrated to dryness The fractions were spotted on TLC and fractions of similar RF were pooled together to obtain seven fractions. Akuammine precipitated out from fractions 6 and 7 when methanol was added. It was filtered through glass wool. It was further purified by washing the precipitate with methanol.

*Akuammine*

White amorphous solid; melting point (258 – 260° C), IR (KBr): $\upsilon_{max}$ ($cm^{-1}$) 3408 (OH), 1735 (C=O) and 1599 (C=C of the benzene ring), 1464, 1276, 1257 (C-O-C of ester). $^{1}H$ and $^{13}C$ NMR, Table 1.

*Determination of ACE1 Inhibitory Activity*

ACE1 catalyses the hydrolysis of N-(3-[2-furyl]acryloyl]-Phe-Gly-Gly) (FAPGG) to furyl-acryloyl-phenyl-alanine (FAP) and glycyl-glycine. Hydrolysis of FAPGG resulting in a decrease in absorbance was monitored at 345 nm at 25°C using Shimadzu 1800 Double beam UV-Visible Spectrophotometer. The molar extinction coefficient difference between substrate and product of 517 cm-1 M-1 was used to calculate activity. The ACE1 inhibitory activity was measured according to the method of Homoliquist *et al*., (1979)[12] with modifications reported by Crown *et al*., (2017). The ACE1 activity was expressed as ΔA 345 nm and the ACE1 inhibition (%) was calculated using the equation (1):

$$\left[1 - \frac{\Delta A\ inhibitor}{\Delta A\ control}\right] X\ 100 \qquad (1)$$

$IC_{50}$ was defined as the concentration of sample required to inhibit 50% of ACE1 activity under these conditions. The molar extinction coefficient between substrate and product was assumed to be 517 cm-1 M-1 at 345 nm.[13] Protein content was determined using Bradford method.[14]

*Determination of the Kinetic Properties of ACE1 Inhibition by extracts*

The kinetic properties of ACE1 (20 mU) without or with Akuammine (15, 20 and 30 µg/ml) in total volume of 1.22 mL were determined using different concentrations of FAPGG as substrates (0.1 - 0.5 mM). The $K_M$ and $K_M'$ were calculated from linearization of Michaelis-Menten's equation (2);

$$\frac{1}{Vo} = \left(\frac{\alpha Km}{Vmax}\right)\left[\frac{1}{S}\right] + \alpha'/Vmax \qquad (2)$$

Where V is the volumetric reaction rate, S is the substrate concentration, $K_M$ is the constant of Michaellis-Menten, $V_{max}$ is the maximum reaction rate at infinite reactant concentration.

The determination of inhibition constant (Ki) was performed using equations (3) and (4);

$$\alpha = 1 + [I]/Ki \qquad (3)$$

$$\alpha' = 1 + [I]/\ K_i' \qquad (4)$$

I is the inhibitor concentration, Ki is the inhibition constant, α is the factor by which $K_M$ changes when I occupies the enzyme, α′is the factor by which apparent $K_M$ changes when I occupies the enzyme. The determination of dissociation constant ($K_d$) was performed using the Cheng- Prusoff's equation (5):

$$K_i = \frac{IC_{50}}{1+([L]/K_d)} \qquad (5)$$

Where: Ki is the inhibition constant, $IC_{50}$ is the concentration that inhibits 50% of the enzyme, L is the free Inhibitor, $K_d$ is the dissociation constant.

*Computational methods*

Protein-ligand docking studies were carried out based on the crystal structures of angiotensin 1 converting enzyme (PDB:1O86). Prior to docking, all solvent molecules and the co-crystallized ligands were removed from the structures. Molecular docking calculations for all compounds with each of the proteins were undertaken using Molegro Virtual Docker (version 6.0, Molegro ApS, Aarhus, Denmark),[15] with a sphere (10 Å radius) large enough to accommodate the cavity centred on the binding sites of each protein structure in order to allow each ligand to search. The compound was allowed to bind randomly to the protein. Standard protonation states of the proteins based on neutral pH were used in the docking studies. Each protein was used as a rigid model structure; no relaxation of the protein was performed. Assignments of charges on each protein were based on standard templates as part of the Molegro Virtual Docker program; no other charges were necessary to be set. Akuammine was docked. Each ligand structure was built using Spartan '14 for Windows (version 1.1.8, Wavefunction Inc., Irvine, California).

**Table 1:** Proton, carbon-13 and HMBC NMR data in $CDCl_3$ (multiplicities in parenthesis)

| Position | Proton (multiplicity) | $^{13}$Carbon | HMBC ($^{1}$H to $^{13}$C) | Position | Proton (multiplicity) | $^{13}$Carbon | HMBC ($^{1}$H to $^{13}$C) |
|---|---|---|---|---|---|---|---|
| 1 | - | - | - | 13 | - | 144.9 | - |
| 2 | - | 103.5 | - | 14 | 2.00 (dd) | 27.4 | 58.0, 103.5 |
| | | | | | 2.32 (dd) | | 138.3 |
| 3 | 4.27 (s) | 52.6 | 40.4 | 15 | 3.45 (s) | 40.4 | 52.6, 73.9 |
| 4 | - | - | - | 16 | - | 58.0 | - |
| 5 | 2.69 (dd) | 50.0 | 53.4, 54.1 | 17 | 3.57 (d) | 73.9 | 53.4 |
| | 3.78 (ddd) | | 53.4, 54.1 | | 3.89 (d) | | 103.5 |
| 6 | 1.51 (dd) | 29.8 | 53.4 | 18 | 1.48 (d) | 13.0 | 120.8, 138.3 |
| | 3.36 (ddd) | | 50.0, 53.4, 58.0 | | | | |
| 7 | - | 53.4 | - | 19 | 5.44 (q) | 120.8 | 40.4, 54.1 |
| 8 | - | 139.7 | - | 20 | - | 138.3 | - |
| 9 | 6.60 (d) | 111.2 | 144.9 | 21 | 2.91 (d) | 54.1 | 40.4, 50.5, 52.6, 120.8 |
| | | | | | 3.96 (d) | | - |
| 10 | - | 150.2 | - | 22 | - | 171.6 | - |
| 11 | 6.55 (dd) | 113.7 | 144.9 | $OCH_3$ | 3.74 (s) | 52.1 | 171.6 |
| 12 | 6.43 (dd) | 110.6 | 139.7, 150.2 | 24 | 2.69 (s) | 29.6 | 103.5, 144.9 |

For each ligand, a conformational search and geometry optimization was carried out using the MMFF force field.[16] Flexible ligand models were used in the docking and subsequent optimization scheme. Variable orientations of each of the ligands were searched and ranked based on their re-rank score. For each docking simulation the maximum number of iterations for the docking algorithm was set to 1500, with a maximum population size of 30 runs per ligand. The RMSD threshold for multiple poses was set to 1.00 Å. The generated poses from each ligand were sorted by the calculated re-rank score. The homology modelling interface generates a set of putative protein structures by aligning atomic coordinates of the amino acid sequence to those of the template sequence backbone and minimising permutations of side chain orientations using the AMBER10:EHT force field with reaction field solvation.[17,18,19] The candidate structure with the lowest RMSD deviation from the template backbone is selected and optimized using a constrained minimization. Each of the sequences was aligned to its respective template and a protein backbone constructed and superposed to the reference structures using the protein alignment tool in MOE 2014.0901.

*Statistical Analysis*

The data were expressed as the Mean of the triplicate determinations and the Standard Deviation (SD) or Standard error of measurements (SEM). Statistical significance was evaluated using ANOVA followed by Tukey's test for multiple comparisons. The data were also analyzed using GraphPad Prism 5 (GraphPad software, Inc., La Jolla, CA, USA). Data were fitted to either the Eq. 1-3 or the Michaelis-Menten equation using KaleidaGraph 4.5 (Synergy Software, MA, USA).

## Results and Discussion

*Structural elucidation of the isolated compound*

The HRESIMS of the compound, Figure 1, showed a chlorinated molecular ion peak at m/z 417.1593 [M + Cl] consistent with a molecular formula $C_{22}H_{26}N_2O_4Cl$. The $^1$H NMR (500 MHz, $CDCl_3$) of the compound showed resonances at δ 3.74 (s), δ 2.69 (s) and δ1.48 (d), each integrated to be three protons indicating three methyl groups with a signal at δ 3.74 to be a methoxy. A signal at δ 5.44 (q) indicated an olefinic proton. The signals in the aromatic region at δ 6.43 d (J = 8.3), 6.55 dd (J = 2.4, 8.3) and δ 6.60 d (J = 2.4) each integrated to be one proton suggested 1, 2 and 4 tri-substitution on benzene ring. The analyses of $^{13}$C and HSQC data showed signals for twenty two carbon atoms and their respective correlations with protons (protons in parentheses) at δ 171.6, 150.2, 144.9, 139.7, 138.3, 120.8 (H (5.44 q)), 113.7 (H (6.55 dd)), 111.2 (H (6.60 d)), 110.6 (H (6.43 dd)), 103.3, 73.9 (2H (3.57 d), (3.89 d)), 58.0, 54.1 (2H (2.91 d), (3.96 d)), 53.4, 52.6 (H (4.27 s)), 52.1 (3H (3.74 s)), 50.0 (2H (2.69 dd), (3.78 ddd)), 40.4 (H (3.45 s)), 29.8 (2H (1.51 dd), (3.36 ddd)), 29.7 (3H (2.69 s)), 27.4 (2H (2.00 dd), (2.32 dd)) and 13.0 (3H (1.48 s)). The HMBC correlations were shown in Table 1. The NMR data of the isolated compound compared well with the published data by Lewin and Ménez, 1992,[20] thus the compound was identified as akuammine, Figure 2.

*Inhibitory effect of akuammine on ACE1*

The inhibitory plot of akuammine shown in Figure 3 is a sigmoidal plot. The result revealed that the ACE1 inhibition was concentration dependent. The concentration that inhibits 50% of the ACE1 was 20 µg/ml.

*Kinetics of ACE1 inhibition*

In the absence and presence of akuammine, the Lineweaver-Burk plot that the type of inhibition exhibited is a mixed type of inhibition as shown in the Figure 4. The kinetic parameters as shown in Table 2 showed that the Km decreased with the values 0.716, 0.656, 0.648 µg/ml and the $V_{max}$ increased with the values 0.34, 0.24, 17.56 ng/ml/min as the concentrations of akuammine used as inhibitors was increased from 15 to 30 µg/ml. The $K_m$' values of all the concentrations of akuammine were 2588.34, 2371.44, 2342.52 respectively and $V_{max}$' values of all the concentration were 1.518, 1.229, 63.479 µg/ml/min respectively. The inhibition constant (Ki) and the Ki′ for all concentrations of akuammine (15 to 30 µg/ml) were the same with the value of 15.03 µg/ml and 15 µg/ml respectively. The dissociation constant i.e., the $K_d$ values and $K_d$′ of all the concentration of akuammine were 0.3, 0.86, 1.44 µg/ml and 0.3, 0.64, 0.23 respectively.

*Molecular Docking Study*

Figure 5 showed the binding of pose of Angiotensin-I-Converting Enzyme {ACE1 (PDB: 1O86)} with Akuammine. It showed that the binding of Akuammine to ACE1 (1O86) was on the allosteric site. The result from Table 2 revealed that binding of Akuammine to the allosteric site was more favourable because of its low binding energy obtained while binding to the active site was not favourable.

Angiotensin-1-converting enzyme (ACE) is one of the drug targets for the management of hypertension. Despite several contraindications and multiple side effects of the ACE inhibitors, drug has remained the most used in the management of high blood pressure, and physicians are left with the management of the various side effect which also is a major challenge to the health of the patient.

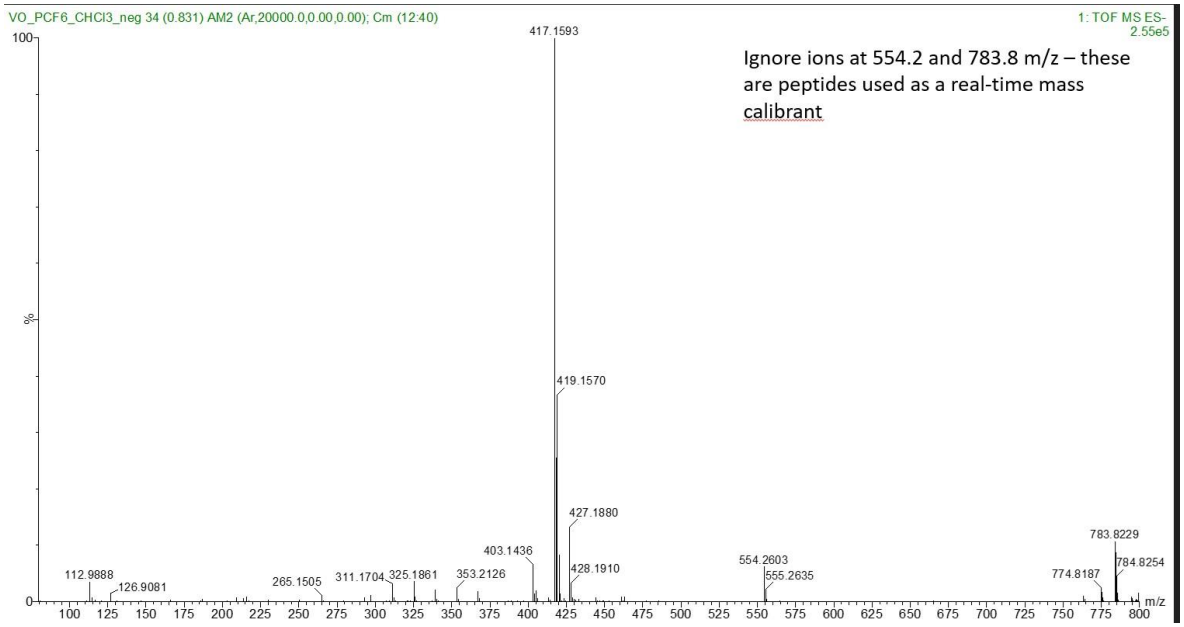


**Figure 1:** High resolution electron spray ionization mass spectrum of Akuammine

In this study, the concentration dependent inhibition (Figure 2) exhibited by Akuammine and $IC_{50}$ of 3.23 µg/ml (8.5 µM) final concentration suggested that it will be a good antihypertensive drug. Comparing with other ACE1 inhibitors, Akuammine exhibited a lower inhibition than some of the known and established antihypertensive natural products.[11,21,22] The inhibitory plot of Akuammine was sigmoidal and was fitted to a Hill's plot rather than Michaelis-Menten plot (Figure 2). Enzyme inhibition with sigmoidal plot is a clear indication that the enzyme is allosteric in nature therefore an allosteric enzyme model was used for its interpretation. This was analysed by Hill's equation (6):

$$V_o = V_{max}[S]^{n_H}/K_M^{n_H} + [S]^{n_H} \quad (6)$$

nH is a qualitative indication of the level of cooperativity. The Hill's plot for ACE binding to Akuammine gave a Hill coefficient ($n_H$) greater than 1. This indicates positive cooperativity of the inhibition at site outside the active site of ACE.

The inhibition behaviour of Akuammine on ACE1 (Figure 3) gave increasing slopes and growing Y-axis intercepts with higher inhibitor concentration indicating that Akuammine is a mixed type inhibitor. The double reciprocal plot yielded a family of lines with different slopes and intercepts, and they intersect at the 2nd quadrant. The inhibitory behaviour, from the double reciprocal plot, the decrease Km and increase Vmax denoted mixed type of inhibition, this implies that the inhibitor binds to the enzyme or enzyme-substrate complex i.e., the binding of the inhibitor did not affect the binding of the substrate, but prevented product formation. This type of inhibition is unaffected by the amount of substrate unlike competitive inhibitor where increase in concentration of the substrate prevents the binding of inhibitor. Most medicinal plants exhibited this type of inhibition. Earlier study by Crown *et al*., (2017),[11] reported crude extract of *P. curatellifolia* also exhibited mixed type of inhibition of ACE1. The factor α or α′ describes the effect of the inhibitor on the affinity of the substrate towards the enzyme and the effect of substrate on the inhibitor affinity for the enzyme. The α and α′ (Table 2) for Akuammine was not different from control which was a clear indication that the inhibitor has no effect on the affinity of substrate on inhibitor or vice-versa. In general, there is partially mixed inhibition when $\alpha < 1$, complete uncompetitive inhibition is characterized by $\alpha \ll 1$ while $\alpha > 1$ depict the enzyme binds favourably to enzyme substrate complex.

The inhibitor binding constants for enzyme (K*i*) and enzyme substrate complex (K*is*) were obtained from secondary plots; Figure 3b and c respectively. The K*is* (21.2 µg/ml) was larger than K*i* (14.3 µg/ml) indicating that the affinity for enzymes is stronger than the enzyme-substrate complex.

The Km, KI, and with enzyme-substrate complex, KIS, were obtained from the inhibition constant (Ki) was an index that defines inhibitor binding (affinity) capacity to the enzyme in order to form the enzyme-inhibitor complex and a lower value implies a higher affinity.[23] The result showed the Ki obtained was the same for the concentration of Akuammine. This suggested that the different concentrations of Akuammine had the same binding capacity to the enzyme.

The observed dissociation constant (Kd) was determined from Cheng–Prusoff's equation. Kd measured the ability of the inhibitor to dissociate from either enzyme–inhibitor or enzyme–substrate–inhibitor complex. The higher the Kd the faster the dissociation.[11] From the results, it was discovered that as the concentration increases, the dissociation constant also increases.

Molecular docking was carried out to further confirm the interaction between the Akuammine and the ACE1 and it revealed that the Akuammine bound on a separate site aside the active site, which clearly support the earlier claim in this study that the enzyme is an allosteric enzyme and that Akuammine bound to the allosteric site which favoured mixed type inhibition. The binding to the allosteric site was more favourable (Table 3) because it has very low re-rank, molecular docking score, but when Akuammine was forced to bind at the active site, it interacted with more amino acids than lisinopril but the docking/re-rank scored were very high (Table 3). This suggested that Akuammine favourably binds to the allosteric site. The hydrogen bond of Akuammine was mostly with water and at most with 2 amino acids whereas the hydrophobic bonding was with 4-8 amino acids. There was no electrostatic bonding interaction, all these depicts that the compound was highly hydrophobic. The physicochemical properties of the compound revealed that total polar surface area was 93. The total surface area measures the membrane permeability of compound.

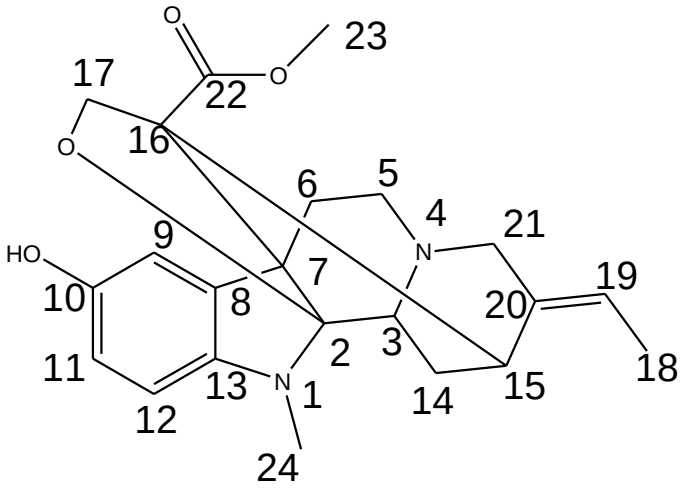


**Figure 2:** Structure of akuammine

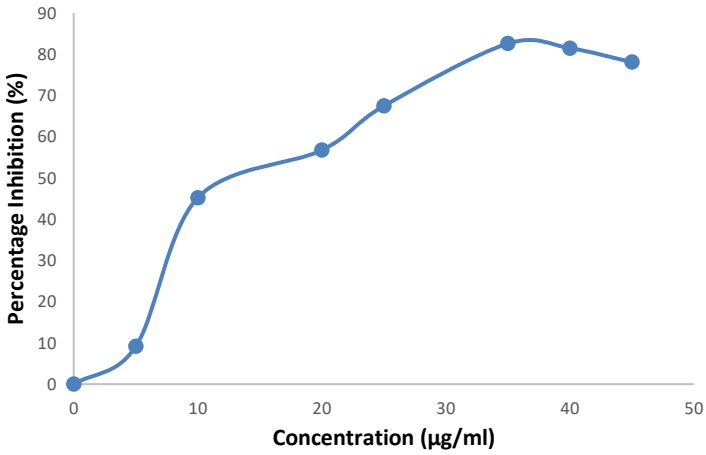


**Figure 3:** Inhibitory plot Akuammine on ACE1 activity

**Table 2:** Kinetic Parameters obtained from Lineweaver-Burk Adjustment for ACE1 Inhibition by Crude and Flavonoid-Rich Extracts

| | $V_{max}$ (µM/min) | $K_m$ (µM) | α | α′ |
|---|---|---|---|---|
| No extract | 0.21 ± 0.00[a] | 272 ± 0.02[a] | 0.002 ± 0.01[a] | 7.23 ± 0.01[a] |
| Ak (15 µg/ml) | 0.17 ± 0.08[a] | 358± 0.04[b] | 0.002 ± 2.54[b] | 7.23 ± 3.54[b] |
| AK (20 µg/ml) | 0.12 ± 0.06[a] | 328± 0.01[b] | 0.0028 ± 1.82[c] | 7.23 ± 3.73[c] |
| AK 30 µg/ml | 8.78 ± 0.92[b] | 324± 0.00[b] | 0.002 ± 0.38[b] | 7.23 ± 5.63[d] |

Values are expressed as mean ± SD. Values with different superscripts are significantly (P≤0.05) different from each other down the column.

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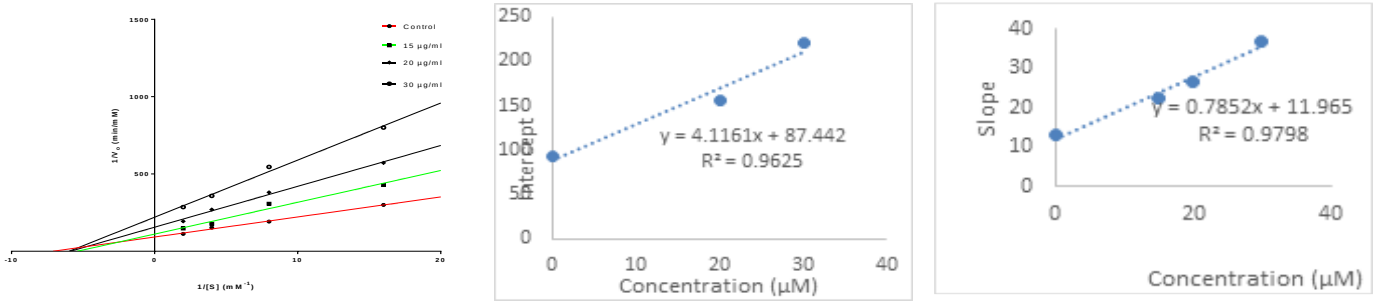
**Table 3:** The molecular interaction and Rerank score of Akuammine at the active and the allosteric site of ACE1

| Ligand | Substrate site | | | | Allosteric site | | |
|---|---|---|---|---|---|---|---|
| | RMS | Hydrogen bond | Hydrophobic bond | Electrostatic bond | RMS | Hydrogen bond | Hydrophobic bond |
| Akuammine pose 1 | 189.7 | Lys 511, Tyr 520, Gly_2000, Solvent (2473, 2569,2567,2568) | Lys 511, Tyr 520, Phe 457, Tyr 523, His 383, Ala 354, Glu 384, His 353 | | -67.6 | Arg 124, Glu 123, Thr 92, ile 88, Asn 85 | Arg 124, Solvent (2479, 2127, 2230, 2110, 2111A, 2370, 2152 |
| Akuammine pose 2 | 152.4 | Tyr 520, Asp 415, Gln 281, Solvent (2452, 2568,2569) | His 383, Tyr, 520, Phe 457, Asn 415, His 513, Tyr 523, Phe 527, Glu 384, Gln 281, Ala 354, His 363 | | -65.3 | Thr 92, Tyr 360, Glu 123, Arg 124 | Thr 92, Tyr 360, Solvent ( 2005, 2053, 2370, 2152, 2371) |
| Akuammine pose 3 | 123.8 | Asp 415, Tyr 520, Tyr 523, Gly_2000, Solvent (2569, 2568, 2569) | Asp 415, His 383, Tyr 520, His 513, Tyr 523, Glu 384, Val 380, Ala 354, His 353, Phe 457, Phe 527, | | -60.3 | Asn 85, Ala 85, Tyr 62, Ile 88, Glu 123, Thr 92, Trp 59 | Thr 92, Asn 85, Solvent (2055, 2152, 2110) |
| Lisinopril | -107.4 | Tyr 523, His 353, Tyr 520, Lys 511 solvent (2409, 2568, 2569, 2393, 2567, 2392, 2409, 2570) | Ala 354, Glu 384, Glu 411, Tyr 523, His, 353, His 513, Lys 511, Tyr 520, Gln 281, Glu 162 | Zn-701A, Glu 411, Glu 384, His 383, His 387, Glu 162, His 513, Lys 511, Asp 377 | | | |




(a) (b) (c)

**Figure 4:** Lineweaver-Burk plots (a,b,c) of angiotensin-1-converting enzyme (20 *ú*u) without or with Akuammin

Compounds with total surface area of 60 is said to be highly non polar and it can cross the blood brain barrier while those above 90 are generally too polar. Akuammine had 93.5 which made it unsuitable to cross the blood brain barrier. Solubility is also an important factor that is very paramount to drug development, Compounds with LogP below 2 is generally acceptable, Akuammine had Log octanol/water partition coefficient of 1.5, Lipinski drug like test is 1, Lipinski violation count was 0. This is a clear indication that showed that the compound is a good drug candidate because the Log P is above one and the molecular weight is below 500 g/mol.

## Conclusion

Akuammine showed inhibition on angiotensin-1-converting enzyme (ACE1), therefore Akuammine could be a lead compound in the discovery of drugs for hypertension.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

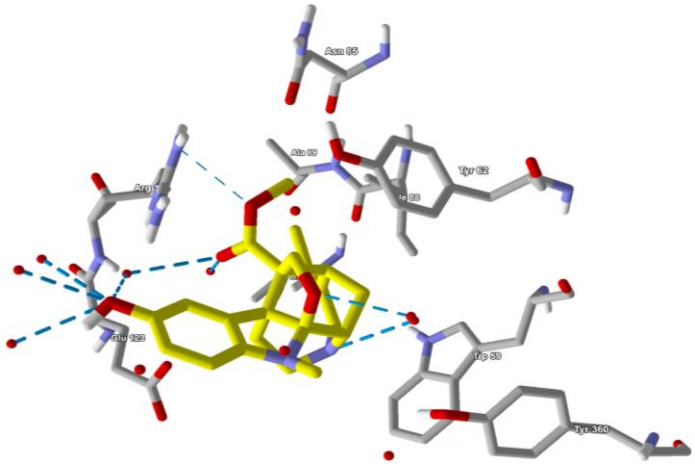



**Figure 5:** The binding of pose of Angiotensin-I-Converting Enzyme {ACE1 (PDB: 1O86)} with Akuammine (PDB: 1O8)

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