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Isolation, Elucidation, and Molecular Docking Studies of Active Compounds from *Phyllanthus niruri* with Angiotensin-Converting Enzyme Inhibition

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ABSTRACT

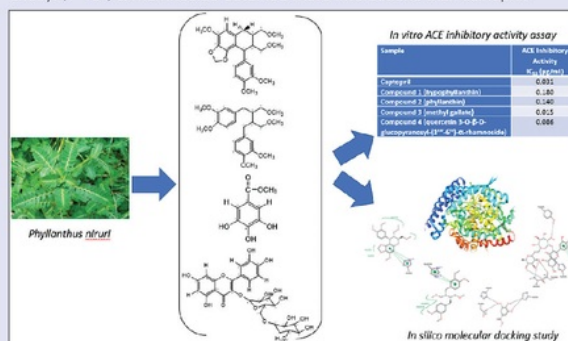
Background: *Phyllanthus niruri*, in Indonesia, is known as "Meniran" has a long history of use in ethnic or traditional medicine worldwide, mainly as an antihypertensive agent. **Objective:** The present study was designed to isolate and identify active compounds with angiotensin-converting enzyme (ACE) inhibition activity from *P. niruri* herb and confirm the mechanism of action, affinity, and domain specificity interactions of the isolated compounds. **Materials and Methods:** Some fractions of *P. niruri* methanolic extract were subjected to column chromatography and preparative thin-layer chromatography to get active compounds. Structural elucidation was determined via spectroscopic methods. ACE inhibition activity was measured using hippuryl-L-histidyl-L-leucine as a substrate *in vitro* assay. Furthermore, confirmation of the mechanism of action, affinity, and domain specificity interaction of the isolated compounds on ACE complex macromolecule (protein database id: 1O86) was performed by *in silico* molecular docking studies. **Results:** In this work, four active compounds were isolated from aerial part of *P. niruri*, including hypophyllantin (50% inhibition concentration [IC₅₀] = 0.180 µg/mL), phyllantin (IC₅₀ = 0.140 µg/mL), methyl gallate (IC₅₀ = 0.015 µg/mL), and quercetin 3-O-β-D-glucopyranosyl-(1''-6'')-α-rhamnoside (IC₅₀ = 0.086 µg/mL). *In silico* molecular docking method emphasizes ligand-residue interactions, thereby predicting the inhibitory activity of these compounds. After docking to an ACE complex macromolecule, quercetin 3-O-β-D-glucopyranosyl-(1''-6'')-α-rhamnoside obtained more interactions than lisinopril. **Conclusion:** The results were obtained from *in silico* and *in vitro* experiments and confirm the potential active compound is an ACE inhibitor and a new antihypertensive agent.

Key words: Angiotensin-converting enzyme inhibitors, angiotensin-converting enzyme, antihypertensive agent, molecular docking, *Phyllanthus niruri*

SUMMARY

- Meniran (*Phyllanthus niruri*) has a long history of the use in ethnic or traditional medicine worldwide
- Four active compounds were isolated from an aerial part of *Phyllanthus niruri* include hypophyllantin, phyllantin, methyl gallate, and quercetin 3-O-β-D-glucopyranosyl-(1''-6'')-α-rhamnoside

- 50% inhibition concentration value of these compounds, such as 0.180 µg/mL, 0.140 µg/mL, 0.015 µg/mL, and 0.086 µg/mL, respectively
- In silico* molecular docking method emphasizes ligand-residue interactions, thereby predicting the inhibitory activity of these compounds
- After docking to an ACE complex macromolecule, quercetin 3-O-β-D-glucopyranosyl-(1''-6'')-α-rhamnoside obtained more interactions than lisinopril.



Abbreviations Used: *P. niruri*: *Phyllanthus niruri*; ACE: Angiotensin-converting enzyme; HHL: Hippuryl-L-histidyl-L-leucine; HA: Hippuric acid; PDB: Protein database; IC₅₀: 50% inhibition concentration; FH: N-hexane fraction; FE: Ethyl acetate fraction; TLC: Thin layer chromatography; UV-VIS: Ultraviolet-visible; NMR: nuclear magnetic resonance; FTIR: Fourier-Transform infrared; MS: Mass spectrometry; HMQC: Heteronuclear Multiple-Quantum Correlation; HMBC: Heteronuclear multiple bond correlation; TADOK: Tugas Akhir Mahasiswa Doktor.

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INTRODUCTION

Hypertension is one of the most common diseases worldwide and causes about 12% of deaths each year.^[1] It is a risk factor for heart disease, mainly myocardial infarction, atherosclerosis (i.e., hardening of the arteries), heart failure, and stroke.^[2] The angiotensin-converting enzyme (ACE) plays an essential role in hypertension management. It converts angiotensin I into angiotensin II in the kidneys (renin-angiotensin-aldosterone system) and activates

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bradykinin.^[3] Skeggs *et al.* first explained ACE's mechanism of action in 1953–1956.^[4,5] Some kinds of synthetic ACE inhibitors, such as captopril, lisinopril, ramipril, enalapril, fosinopril, and zofenopril, have been widely used for the treatment of hypertension. However, studies of ACE inhibitor active compounds from natural products (mainly plant resources) continue because they have potential secondary metabolite content and fewer side effects.^[5–7] *In vitro* ACE inhibitory activity assay is a valid assay method for discovering drugs from active compounds found in natural products.^[8]

Some studies have reported medicinal plants that exhibit potential antihypertensive effects.^[2,5,7,9–11] Therefore, advanced research into finding other sources of natural products is needed. Various plants have been conventionally used to control blood pressure or hypertension. One of them is *Phyllanthus niruri* from the Euphorbiaceae family, known locally in Indonesia as “Meniran.”^[12] This plant can be found in tropical and subtropical climates including Indonesia and has a long history of ethnomedicinal or traditional use worldwide. Some studies have reported that aerial part of this plant has various pharmacological properties as follows: hepatoprotective,^[13] antiplasmodial,^[14] antihyperalgesic,^[15] antioxidant,^[16] anti-inflammatory,^[16,17] anti-gastric ulcer,^[17] immunostimulator,^[18] diuretic,^[19] prostate cancer treatment,^[20] and hair growth stimulant.^[21] Scientific information related to the phytochemical constituents of this plant was described in detail by Kaur *et al.* in 2017; it contains alkaloids, polyphenols, tannins, flavonoids, lignans, terpenoids, anthocyanins, saponins, and coumarins.^[22] Methanol, n-hexane, and aqueous extracts of *P. niruri* were reported to have the inhibitory activity on ACE.^[23] However, an active compound from this plant that can be used as an ACE inhibitor has not been reported. In this study, four active compounds which could potentially act as ACE inhibitors were isolated and identified from *P. niruri*, and a molecular docking study was performed to predict the mechanism of action and interactions between the ligands (i.e., active compounds) and a macromolecule.

33 MATERIALS AND METHODS

Plant samples and collection

The aerial parts of *P. niruri* were purchased in Banten Province, Indonesia and were authenticated by the Indonesia Institute of Sciences, Research Center of Biology, Cibinong-Bogor, West Java, Indonesia. The voucher specimen was deposited at the Laboratory of Pharmacognosy–Phytochemistry, Faculty of Pharmacy, Universitas Indonesia, Depok, West Java, Indonesia.

Chemical reagents

Some chemicals used in this study, including n-hexane, ethyl acetate, chloroform, n-butanol, and acetone, were purchased from PT SmartLab Indonesia, Indonesia. ACE and hippuryl-L-histidyl-L-leucine (HHL) substrates were purchased from Sigma Aldrich, USA. Captopril was acquired from PT Kimia Farma, Indonesia. Hydrochloric acid, sodium carbonate, potassium dihydrogen phosphate, sodium hydroxide, silica gel 60 H, silica gel thin-layer chromatography (TLC) plate GF₂₅₄, and preparative TLC plates were purchased from Merck, Germany. Sephadex LH-20 was obtained from Amersham Bioscience, Sweden.

Apparatus

Apparatuses used in the study include a rotary vacuum evaporator (Buchi, Germany), micropipettes 100–1000 μ l (Eppendorf, Germany), a ultra violet visible (UV-VIS) spectrophotometer (Shimadzu, Japan), a UPLC-Qtof-HR-mass spectrometry (MS) XEVOtm mass spectrometer (Water, Milford, MA, USA), a Fourier-transform infrared (FT-IR) (Waltham, MA, USA), an ¹H-nuclear magnetic

resonance (NMR) and ¹³C-NMR (JEOL JNM, Japan), a microplate reader (BioTek Elx 808, USA), and multichannel pipettes (Thermo Scientific).

Extraction and isolation

The powdered herb was macerated with 80% methanol and filtered. The same procedure was repeated three times. The organic layer was combined and concentrated using a rotary vacuum evaporator and then dried to give 476 g of methanolic extract. The extract (450 g) was dispersed in warm water and n-hexane was added. The n-hexane layer was collected and dried using a rotary vacuum evaporator to obtain the n-hexane fraction (FH). Partitioning was continued by adding ethyl acetate and then n-butanol. The organic layers were evaporated to give ethyl acetate and n-butanol fractions. The n-hexane (19.28 g) and ethyl acetate (5 g) fractions were subjected to column chromatography using silica gel 60 as a stationary phase. FH and ethyl acetate fraction (FE) were eluted with n-hexane: Ethyl acetate with a polarity gradient from 100% to 10%, followed by ethyl acetate: Methanol with a gradient from 100% to 10%. Eighty-one 100 ml fractions were collected. The FE was subjected to silica gel column chromatography and eluted with n-hexane: Ethyl acetate eluent (5:5), and 24 fractions were collected every 100 ml for each fraction. The n-butanol fraction was subjected to column chromatography with Sephadex LH-20. The sample was eluted with methanol: Water with several comparison gradients from 50% to 100%. Two hundred and twenty fractions of 20 ml for each fraction were collected. Elution and fractionation were controlled by TLC under UV light at 254 nm and 366 nm. Similar fractions were combined according to their purities to give 15 for an (FH_A–FH_O), 5 for ethyl acetate (FE_A–FE_E) fraction, and 6 for an n-butanol fraction (FB_A–FB_E). Compound 1, Compound 2, Compound 3, and Compound 4 were obtained from FH_E, FH_O, FE_O, and FB_O, respectively, after recrystallization.

Structure elucidation of isolated compounds

The structure of the isolated compounds was determined by analyzing spectroscopic data from UV-VIS, FT-IR, MS, ¹H-NMR, ¹³C-NMR, and NMR-two-dimensional (2D) that covered Heteronuclear Multiple-Quantum Correlation (HMQC) and Heteronuclear Multiple-Bond Correlation (HMBC) spectra.

Angiotensin-converting enzyme inhibition assay

The inhibitory effect was analyzed *in vitro* via spectrophotometry, which measured the formed hippuric acid (HA). HHL was catalyzed with ACE to form HA and L-histidyl-L-leucine. The sample's ACE inhibitory activity was determined according to Kurniawan *et al.* which was based on the strategy developed by Cushman and Cheung.^[24,25] Briefly, 50 μ l of the sample solution was added to 50 μ l of 5 mM HHL as the substrate, it was vortexed for a few seconds to achieve homogenization, 100 μ l ACE solution (0.025 U/ml) was added, and then it was incubated at 37°C for 90 min. The reaction was stopped by the addition of 250 μ l 1N HCl. Formed HA was extracted with 1.5 ml ethyl acetate followed by centrifugation for 10 min and evaporated. HA was dissolved in 3 ml of ionized water, and the absorbance was measured at 228 nm.

Molecular docking studies

In silico molecular docking studies were conducted using a computer with core i7 processor, and AutoDockZn was applied based on the previous study.^[26] The crystal structure of the human ACE in complex with native ligand lisinopril (protein database (PDB) id: 1O86) was downloaded from <http://www.rcsb.org/pdb/explore.do?structureId=1O86>. Ligands were created manually in a 2D format

in Marvin Sketch. AutoDockZn was employed for calculations in the molecular docking studies using a Lamarckian genetic algorithm between the flexible ligand and rigid receptor, a population size of 300, a maximum of 2,50,000 generations, and 2,500,000 evaluations for 100 GA runs. The root means square deviation was tolerated to below 2.0 Å for the clustering of docking results. The ligands' 2D structure was visualized by LigandScout. Ligand-residue interactions were observed by LigPlot software.

RESULTS AND DISCUSSION

One cardiovascular risk for diabetic patients is hypertension. In this study, ACE inhibition as a hypertension mechanism was analyzed by measuring the absorbance of HA as the product of the reaction between HHL and ACE that was not inhibited by the sample. The positive result of ACE inhibition analysis is consistent with a previous study which found that a methanol extract of *P. niruri* inhibits the activity of ACE.^[23]

Isolation and identification of compounds from *Phyllanthus niruri*

Each fraction was isolated using column chromatography; analyzing its inhibitory effect on ACE resulted in four compounds [Table 1]. Specific results regarding each compound are as follows: 203.1 mg of Compound 1 and 78.5 mg of Compound 2 contained hexane fractions while 182.1 mg of Compound 3 was from the ethyl acetate fraction, and 10.9 mg of Compound 4 was from n-butanol.

Compound 1: The FTIR spectrum of Compound 1 (crystal-like white needle) showed asymmetric = C-H-(2901.04 cm^{-1}), C-O ester (1259.56, 1026.16 cm^{-1}), and a H-C=C-H aromatic ring bond (3055.3, 1639.5, 1506.4, 1425.4 cm^{-1}). The molecular weight [M+] = 430.2 and molecular formula $\text{C}_{24}\text{H}_{30}\text{O}_7$, were obtained using GC-MS. Next, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ were performed using CD_3OD , and the proton signals were obtained at 3.3 ppm (6H, s), 3.84, 3.86 (6H, s), and 3.80 ppm (3H, s).

Compound 2: The FTIR spectrum of Compound 2 (crystal-like white needle) had a similarity to Compound 1 because it also showed the asymmetric = C-H-(2868.24 cm^{-1}), C-O ester (1236.41, 1141.9, 1167.18, 1026.16 cm^{-1}), and H-C=C-H aromatic ring bond (1587.47, 1508.38 cm^{-1}). Via the GC-MS study, the molecular weight (M+) 418 and the molecular formula $\text{C}_{24}\text{H}_{34}\text{O}_6$ was obtained. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ analyses were performed using CDCl_3 , and the proton signals were received at 6.74 ppm (2H, d), 6.64 ppm (2H, dd), 6.61 ppm (2H, d), 3.85 ppm (6H, s), 3.8 ppm (6H, s), 3.2–3.3 ppm (10H, m), 2.6–2.7 ppm (4H, m), and 2.03 ppm (2H, m).

Compound 3: (solid creamy-colored amorphous) showed O-H (3365.9 cm^{-1}), C = O (1693.59), aromatic C = C (1616.4 cm^{-1}), and C-O bond (1263.42 cm^{-1}) based on the FTIR study. The GC-MS data showed that the molecular weight (M+) was 184 with a molecular formula of $\text{C}_8\text{H}_8\text{O}$. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ were subsequently performed using CD_3OD ; the proton signals were obtained at 3.81 ppm (3H, s) and 7.04 (2H, s).

Compound 4: (yellow crystal) showed O-H (3431.48, 3319.6 cm^{-1} , broad), C = O (1694.98 cm^{-1}), aromatic C = C (1597.11 cm^{-1} , 1506.46 cm^{-1} ,

Table 1: IUPAC name and chemical structures of isolated compounds from *Phyllanthus niruri*

Compound	IUPAC name	Structure
Hypophyllanthin	9-(3,4-dimethoxyphenyl)-4-methoxy-7,8-bis(methoxymethyl)-6,7,8,9-tetrahydronaphtho[1,2-d][1,3]dioxole	
Phyllanthin	4,4'-(2,3-bis(methoxymethyl)butane-1,4-diyl)bis(1,2-dimethoxybenzene)	
Methyl gallate	Methyl 3,4,5-trihydroxybenzoate	
Quercetin 3-O-β-D-glucopyranosyl-(1"6")-α-rhamnoside	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-(((3S,4S,5R,6S)-3,4,5-trihydroxy-6-(((1S,2S,3S,4S,5R)-2,3,4-trihydroxy-5-methylcyclohexyl)oxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-4H-chromen-4-one	

1548.23 cm⁻¹), and C-O bond (1203.62, 1062.81, 1012.66 cm⁻¹) from the FTIR study. The LC-MS data showed that the molecular weight (M + Na) was 633 with a molecular formula of C₂₇H₃₀O₁₆. Then the ¹H-NMR and ¹³C-NMR analyses were performed, and the proton signals were obtained at 7.67 ppm (1H, d), 7.64 ppm (1H, dd), 6.8 ppm (1H, d), 6.4 ppm (1H, d), 6.21 ppm (1H, d), 5.10 ppm (1H, d), 4.5 ppm (1H, d), 3.4 ppm (1H, m), 1.12 ppm (3H, d), and 3.27–3.80 ppm (glucose proton).

This study found four potential compounds to be developed as antihypertension. Table 2 shows a comparison of all compounds with literature compound based on previous studies.^[16,22,27] Compound 1 was identified as hypophyllanthin with (C₂₄H₃₄O₇) [Table 2a], Compound 2 was identified as phyllanthin (C₂₄H₃₄O₆) [Table 2b], Compound 3 was identified as methyl gallate (C₈H₈O₅) [Table 2c], and Compound 4 was identified as quercetin 3-O-β-D-glucopyranosyl-(1^{'''}-6^{'''})-α-rhamnoside [Table 2d].

Angiotensin converting enzyme inhibitory assay

Value of ACE inhibitory activity (as % inhibition) from methanolic extract was 48.32%, FH of 43.54%, FE of 56.55%, and an n-butanol fraction of 76.99%, at each concentration of 125 μg/ml. Analysis of the inhibitory effect of ACE was performed to methyl gallate and quercetin

Table 2a: Chemical shift comparison of hypophyllanthin and compound 1 (CDCl₃, 125 MHz)

Atom	¹³ C-NMR hypophyllanthin	¹³ C-NMR compound 1
C-1	131.96	131.8
C-2	106.55	106.5
C-3	143.52	142
C-4	133.75	133.2
C-5	147.08	147
C-6	115.25	115.0
C-7	33.3	33.2
C-8	35.62	36.6
C-9	75.47	75.4
C-1'	138.15	138
C-2'	111.84	111.8
C-3'	148.85	148.5
C-4'	147.08	147.1
C-5'	110.79	110.6
C-6'	120.6	120.4
C-7'	42.08	41.8
C-8'	45.59	45.3
C-9'	71.87	71.7
C-9a & 9'a	58.98; 59.12	58.8
C-1''	101.33	101
C-3'a & 3a	55.63	55.8; 55.7
C-4'a	56.92	56.3

NMR: Nuclear magnetic resonance

Table 2b: Chemical shift comparison of phyllanthin and compound 2 (CDCl₃, 125 MHz)

Atom	¹³ C-NMR phyllanthin	¹³ C-NMR compound 2
C-3, 3'	148.7	148.8
C-4, 4'	147.1	147.2
C-1, 1'	133.6	133.7
C-6, 6'	121.0	121.2
C-2, 2'	112.2	112.2
C-5, 5'	111.0	111.0
C-9, 9'	72.6	72.7
C-9, 9'-OCH ₃	58.7	58.9
C-3, 3'; and 4, 4'-OCH ₃	55.8	56.0 and 55.8
C-8, 8'	40.7	40.9
C-7, 7'	34.9	35.0

NMR: Nuclear magnetic resonance

3-O-β-D-glucopyranosyl-(1^{'''}-6^{'''})-α-rhamnoside compared to captopril as the positive control [Table 3].

Hypophyllanthin and phyllanthin are marker compounds for *P. niruri*, and the inhibition effect of ACE enzymes for these two compounds was less potent than captopril as a control. Among these four compounds, methyl gallate and quercetin 3-O-β-D-glucopyranosyl-(1^{'''}-6^{'''})-α-rhamnoside were the most likely to inhibit the activity of ACE enzymes actively.

Molecular docking studies

Table 4 demonstrates the interaction of the ACE complex with the ligand (native ligand, positive control, and isolated compounds), free energy binding, and inhibition constant (μM) from the molecular docking studies results using the AutoDockZn program. Glu384, Glu162, Lys511, Tyr520, Val518, Asp377, Ala354, Tyr523, Glu411, His513, His353, His383, and His387 are binding sites of ACE. Lisinopril as a native ligand performs hydrogen bonding with Glu384 and Glu162 and has a hydrophobic interaction with Val518. The C-terminal carboxylate of lisinopril interacts with positively charged ions toward Lys511 and Tyr520 [Figure 1].

Figure 2a shows the interaction of the ACE complex with native ligand lisinopril (PDB: 1O86) visualized by LigPlot software. Lisinopril performs hydrogen bonding with Glu384 and Glu162 and indicates a hydrophobic interaction with Val518. C-terminal carboxylate of lisinopril interacts with positively charged ions toward Lys511 and Tyr520.^[28]

Table 2c: Chemical shift comparison of methyl gallate and compound 3 (CD₃OD, 125 MHz)

Atom C	δ methyl gallate	δ senyawa 3
C-1	121.6	121.5
C-2, 6	110.2	110.1
C-3, 5	146.7	146.6
C-4	139.9	139.8
C-7 (C=O)	169.2	169.1

Table 2d: Chemical shift comparison of quercetin 3-O-rutinoside and compound 4 (CD₃OD, 125 MHz)

Atom	¹³ C-NMR quercetin 3-O-rutinoside	¹³ C-NMR compound 4
C-2	158.5	159.4
C-3	135.6	135.7
C-4	179.4	179.5
C-5	163.0	163.1
C-6	100.0	100.0
C-7	166.1	166.2
C-8	94.9	94.9
C-9	159.4	156.6
C-10	105.6	105.7
C-1'	123.6	123.6
C-2'	116.1	116.1
C-3'	145.8	145.9
C-4'	150.0	149.9
C-5'	117.7	117.7
C-6'	123.1	123.1
Glu C-1''	102.7	104.5
Glu C-2'' and 3''	75.0; 77.4	75.8 and 77.3
Glu C-4'', 5'' and 6''	70.8; 76.7; 67.9	71.4; 78; 68.6
Rha C-1'''	101.7	102.5
Rha C-2'''	71.2	72.1
Rha C-3'''	71.5	72.3
Rha C-4'''	72.8	74.0
Rha C-5'''	69.1	69.8
Rha C-6'''	18.6	17.9

NMR: Nuclear magnetic resonance

Figure 2b shows the interaction of captopril as a ligand or positive control with the ACE complex. This ligand gives free energy binding (-5.17 ΔG Kcal/mol) and performs hydrogen bonding with Glu384, has a hydrophobic interaction with Val518 and interacts with positively

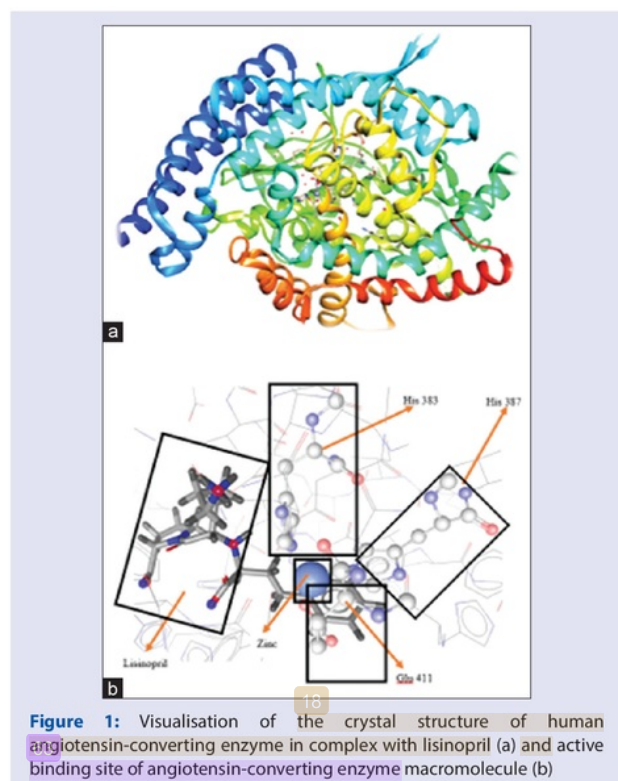


Figure 1: Visualisation of the crystal structure of human angiotensin-converting enzyme in complex with lisinopril (a) and active binding site of angiotensin-converting enzyme macromolecule (b)

Table 3: Angiotensin-converting enzyme inhibition effect of isolated compounds from *Phyllanthus niruri*

Sample	ACE inhibitory activity IC_{50} ($\mu\text{g/mL}$)
Captopril	0.031
Compound 1 (hypophyllanthin)	0.180
Compound 2 (phyllanthin)	0.140
Compound 3 (methyl gallate)	0.015
Compound 4 (quercetin 3-O- β -D-glucopyranosyl-(1 ^m -6 ^m)- α -rhamnoside)	0.086

ACE: Angiotensin-converting enzyme

Table 4: Recapitulation of angiotensin-converting enzyme binding site docked, free energy binding, inhibition constant using Auto Dock Zn

Ligand	Interaction of ACE binding site											Free energy binding (ΔG Kcal/mol)	Inhibition constant (μM)
	Glu384	Glu162	Lys511	Tyr520	Val518	Asp377	Ala354	Tyr523	Glu411	His513	His353		
Lisinopril (native)	√	√	√	√	√	√	√	√	-	-	√	-8.53	556.25×10^{-3}
Captopril	√	-	√	√	√	-	√	√	-	-	√	-5.17	162.82
Hypophyllanthin	-	-	-	-	√	-	-	-	-	-	-	-7.19	5.38
Phyllanthin	-	-	-	-	√	-	-	√	-	-	-	-6.30	24.02
Methyl gallate	-	-	-	-	-	-	-	-	-	-	-	-4.81	229.35
Quercetin 3-O- β -D-glucopyranosyl-(1 ^m -6 ^m)- α -rhamnoside	-	-	-	-	-	-	-	√	-	√	√	-6.52	16.66
Quercetin (aglycone of Compound 4)	-	√	√	√	-	√	√	-	-	-	-	-6.86	9.35

ACE: Angiotensin-converting enzyme

charged ions on Lys511 and Tyr520. The positive control acts like the native ligand.

In Figure 2c, hypophyllanthin gives the lowest free energy binding while demonstrated fewer contacts with the potential binding site. This compound exhibits hydrogen bonding with Lys511 (NZ atom, 2.84 Å). Tyr520 is an essential binding site for C-terminal carboxylate by performing an ionic interaction. In this study, Tyr520 performed a hydrophobic interaction with Compound 1 was rather than an ionic interaction. Ala354, which is known for carrying out hydrogen bonding, showed a hydrophobic interaction instead. His383 and His387 might also be accompanied by Glu411, are residues that bind to Zinc, and have zinc-coordinating behavior.^[28] These residues performed hydrophobic interactions toward the ligand. Zinc forms a strong bonding with histidine and ligand. It is important to remember that zinc is material for the catalytic activity of ACE.^[29]

Phyllanthin also binds to Lys511 through hydrogen bonding (NZ atom, 3.32 Å) as shown in Figure 2d. This ligand performed hydrophobic contact with His383, His387, His353, and Glu411. Histidine, accompanied by Glu411, appeared in this interaction of ligand-residues. These residues bind to zinc, and zinc binds to the ligand. Essential residues that bind to the ligand were very limited in hypophyllanthin and phyllanthin.

Figure 2e demonstrates that methyl gallate has hypoglycemic activity by inhibiting the aldose reductase enzyme, thus reducing cardiovascular risk (e.g., hypertension) in diabetic patients. Compared to the two previous compounds, methyl gallate demonstrated more H-bonding with residues. One of the essential residues, His353, interacted with two oxygen atoms of methyl gallate (O atom, 3.00 Å, and O1 atom 2.93 Å). Glu384 was also involved in this hydrogen bonding interaction (OE2 atom, 2.74 Å). Ala354 displayed hydrophobic contact instead of binding to the nitrogen atom as shown on lisinopril. His383, His387, and Glu411 appeared once more in the visualization.

In Figure 2f, quercetin 3-O- β -D-glucopyranosyl-(1^m-6^m)- α -rhamnoside showed the most interactions compared to the other structures and scored the best affinity. The residue of protein receptor mainly Tyr520 and Glu162 bound with the O8 atom (3.17 Å), OE atom (2.34 Å), and the OE1 atom (2.93 Å). These bindings were also demonstrated by the native ligand (lisinopril).^[28] In this compound, zinc bound to His387, His383, Glu411, and the ligand.

Figure 2g, unlike other compounds, the aglycone of quercetin (Compound 4) did not bind to zinc, although the histidine and Glu411 bound to Zn. Aglycone of Compound 4 created several interactions with essential residues of 1O86. Aglycone slightly had less interaction compared to quercetin 3-O- β -D-glucopyranosyl-(1^m-6^m)- α -rhamnoside and placed as the second lowest free energy binding. It performed hydrogen bonding with Lys511 (NZ atom, 2.99 Å, and 2.78 Å), Tyr520 (OH atom, 2.98 Å) which also demonstrated in lisinopril interaction. Ala354, Glu162, and Tyr523

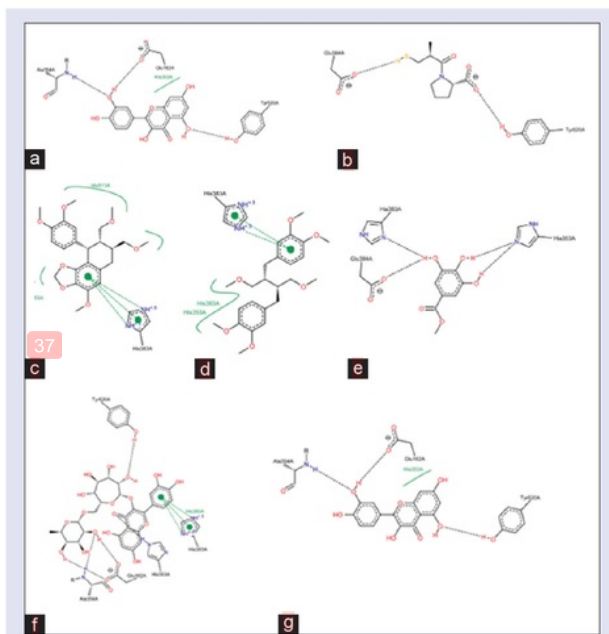


Figure 2: Interactions of the angiotensin-converting enzyme complex (protein database ID: 1O86) with ligand visualized by PoseView. Where (a) is Lisinopril; (b) is Captopril; (c) is Compound 1; (d) is Compound 2; (e) is Compound 3; (f) is Compound 4; and (g) is Compound 4 (aglycone)

showed hydrophobic interaction rather than bound to electronegative atoms (oxygen or nitrogen) as shown on the native ligand.

In silico molecular docking studies provides additional data on ACE inhibitors mainly by predicting interactions and binding sites. The *in-silico* method emphasizes ligand-residue interactions, thereby predicting the inhibitory activity of these compounds. Residues that played a vital role in this docking study were less involved than what we expected from the literature. After docking to an ACE complex macromolecule (PDB: 1O86), quercetin 3-O- β -D-glucopyranosyl-(1^{'''}-6^{'''})- α -rhamnoside demonstrated more interactions than lisinopril. ACE is a chloride-dependent zinc metalloendopeptidase. The abolition of zinc alters the activity toward chromophoric substrates furan acryloyl-Phe-Gly-Gly.^[30] ACE activity was lost below pH 7.5 without the presence of zinc. In the presence of zinc, ACE remains active at pH 6.^[31] Zinc plays a vital part as a catalytic component of ACE; it binds to His383, His387, Glu411, and oxygen atoms from the carboxylate group of Lisinopril.^[28]

CONCLUSION

This study showed that Compound 3 (methyl gallate) and Compound 4 (quercetin 3-O- β -D-glucopyranosyl-(1^{'''}-6^{'''})- α -rhamnoside), are potential active compounds as ACE inhibitors from *P. niruri* herb. Furthermore, molecular docking studies predicted that there are similar action mechanism and domain specificity interaction between native ligand and this compound with the ACE complex macromolecule.

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Conflicts of interest

There are no conflicts of interest.

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