

# Evaluation of pancreatic lipase and angiotensin-converting enzyme inhibitors from ethanolic extract of butterfly pea (*Clitoria ternatea* L.) flowers

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## ABSTRACT

*Clitoria ternatea* L. (CT) contains flavonoids, saponins, tannins, coumarins, steroids/triterpenoids, and essential oils. CT flowers have been used for ages to maintain good health in the Indonesian community. Based on the empirical use of CT, scientific studies are needed to establish its therapeutic values. The objectives of this research were to examine the quality parameters and analyze the activities of CT as antihyperlipidemic and antihypertensive *in vitro*. The total phenolic contents (TPC) and total flavonoid contents (TFC), as well as specific (identity, organoleptic examination, and the percentage of soluble compounds in certain solvents) and non-specific (water content, loss on drying, ash content, residual solvent, heavy metal, and microbial contamination) quality parameters were also evaluated. The antihyperlipidemic and antihypertensive activities were determined using lipase enzyme inhibition and angiotensin-converting enzyme (ACE) inhibition activity. The TPC and TFC of ethanolic extract of CT (EECT) were  $38.30 \pm 4.50$  mg gallic acid equivalent/g extract and  $29.379 \pm 0.31$  mg quercetin equivalent/g extract. The determined parameters satisfy the quality and safety requirements of the medicinal herb standard. The  $IC_{50}$  values of the lipase enzyme and ACE inhibition of EECT were  $131.79 \pm 5.27$   $\mu$ g/mL and 84.9653 ppm, respectively. CT extract inhibits both the lipase and ACE enzymes. The EECT has potential as an antihyperlipidemic and antihypertensive agent.

## 1. INTRODUCTION

Hyperlipidemia and hypertension are coronary heart disease risk factors [1]. Prior research has demonstrated a correlation between plasma lipid levels and blood pressure [2]. Hyperlipidemia is associated with coronary heart disease (high blood pressure, atherosclerosis, ischemia shock), stroke, and mortality [3]. The release of angiotensin II can be negatively affected by lipid metabolism, which is linked to cardiovascular disease [4]. Treating hyperlipidemia and elevated blood pressure concurrently can reduce the circulatory effects of metabolic syndrome [5]. Since the majority of medications must be taken for an extended period of time, the treatment of hyperlipidemia and hypertension continues to rely heavily on synthesized drugs, which can result in the development of

undesirable side effects. For this reason, numerous studies have been conducted to develop antihyperlipidemic and antihypertensive drugs derived from medicinal plants to avoid adverse effects [6].

There are obvious concerns with alternative medicines, such as the fact that scientists know little about how they function. However, phytomedicine is in high demand due to its low cost and accessibility. After Brazil, the tropical forest area of Indonesia has the second-highest biological diversity in the world [7]. Various Indonesian ethnic groups have used medicinal plants in traditional medicine for centuries. *Clitoria ternatea* L.(CT) which is a member of the *Fabaceae* family, is one of numerous medicinal plants. Numerous phytochemical compounds, including flavonoids, alkaloids, tannins, and phenols, are present. Flavonoids have numerous therapeutic applications and have been used to treat hyperlipidemia and hypertension for more than a thousand years [8,9].

The CT flower has recently increased in popularity, especially in Indonesia. Multiple establishments and stores now offer an assortment of food and drink options, ranging from appetizers to

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beverages. Flowers, both fresh and dried, are increasingly traded [10]. In terms of pharmacological perspectives, the CT flower activities include antipyretic, analgesic, local anesthetic, diuretic, antidiabetic, anti-inflammatory, antimicrobial, and insecticidal activities. CT flower is frequently used in Indonesia to treat high blood pressure and aid weight loss, but its effects on high cholesterol are unknown. Consequently, the study aimed to examine the effects of CT on antihyperlipidemic and antihypertensive activities *in vitro* using lipase and angiotensin-converting enzyme (ACE). To attain the quality standards for traditional medicinal products, quality parameter tests were also conducted. According to the applicable regulations, it was anticipated that the CT extract produced would meet the requirements for a primary material in traditional medicine.

## 2. MATERIALS AND METHODS

### 2.1. Sample Collection and Plant Determination

The material used for the study was the butterfly pea petal (CT) obtained from Jogorogo, Ngawi, East Java, Indonesia, that was cultivated in January 2022. Plant determination was conducted at Herbarium Depokensis (UIDEP), Biota Collection Room, Universitas Indonesia (No. 056/UN2.F3.11/PDP.02.00/2022) to confirm the identity of the plants used. To prepare the dried CT flower, step was taken such as washing, wet sorting, cutting, drying, dry sorting, and storage. The plant was dried in a hot air-drying oven FDH-16 (Wiratech, Jakarta, Indonesia) with an internal fan to enhance the drying process at 50°C for 24 h. It was then grinded to a fine powder and passed through sieve No. 4/18 [11].

### 2.2. Chemicals and Reagent

Quercetin, Folin-Ciocalteu, sodium carbonate, and aluminum chloride anhydrous were supplied from Sigma Aldrich (Singapore). Ethanol, gallic acid, and Tris-HCl buffer were supplied from Merck (Darmstadt, Germany). Magnesium powder, hydrochloric acid, amyl alcohol, chloroform, ether, Dragendorff's reagent, Mayer's reagent, Stiasny reagent, anhydrous acetic acid, sulfuric acid, nitric acid, ammonia, gelatin solution, iron (III) chloride, sodium acetate, acetonitrile, and magnesium sulfate were purchased from Qlab Faculty of Pharmacy, Universitas Pancasila. Orlistat, captopril, pancreatic lipase enzyme solution,  $\rho$ -nitrophenyl butyrate ( $\rho$ -NPB) substrate, ACE, and hippuryl-L-histidyl-L-leucine (HHL) were supplied from Sigma-Aldrich (Missouri, United States).

### 2.3. Instruments

Hot air-drying oven (FDH-16, Wiratech, Jakarta, Indonesia). Rotary vacuum evaporator (R-206, Büchi, Switzerland). 96-well microplate reader (Versamax microplate reader, USA). UV-vis spectrophotometer (Shimadzu UV 1800, Kyoto, Japan).

### 2.4. The Extraction of Butterfly Pea Flowers

The desiccated CT flower powder was extracted by kinetic maceration using a Eurostar laboratory stirrer (IKA, Staufen, Germany) at 300 RPM for 8 h at room temperature, using 70% ethanol as a solvent at an m/V ratio of 1:5. After that, it was aged for 16 h. After vacuum filtration through a Buchner funnel with Whatman filter paper. After that, the filtrate was collected in a container. Three maceration cycles were performed, followed by concentration using a rotary vacuum evaporator to obtain an ethanolic extract of CT (EECT) [11].

### 2.5. Phytochemical Screening Analysis

The phytochemical screening test was conducted to identify qualitative phytoconstituents. Table 1 displays the identification procedures for secondary metabolites such as alkaloids, flavonoids, saponins, tannins, quinones, steroids, triterpenoids, coumarins, and essential oils from a preliminary examination in the dried powder and EECT [11].

### 2.6. Qualitative Analysis of CT extract

Several quality parameters have been determined for the CT dried flower and EECT, including the identity of the extract, organoleptic examination (appearance, color, odor, and taste), and the percentage of soluble compounds in certain solvents [11]. In addition, non-specific parameters may be determined, such as the water content and loss on drying, ash content, residual solvent, heavy metal contamination, and microbial contamination [12].

### 2.7. Determination of Total Phenolic and Flavonoid Content

The total phenolic content (TPC) was determined by combining 150  $\mu$ L of 4000 ppm EECT which was combined with 50  $\mu$ L of 10% Folin-Ciocalteu reagent and 50  $\mu$ L of 0.1 M  $\text{Na}_2\text{CO}_3$ . The solution was incubated in the dark at room temperature for 90 min. The absorbance at a wavelength of 750 nm was measured using a 96-well microplate reader (Versamax microplate reader, USA). Gallic acid was used as the standard for the determination of TPC, expressed in mg gallic acid equivalent (GAE)/g extract [11].

The total flavonoid content (TFC) was determined by combining 50  $\mu$ L of 3000 ppm EECT with 50  $\mu$ L of  $\text{AlCl}_3$  solution. The solution was allowed to stand at room temperature for 30 min. The absorbance at a wavelength of 435 nm was measured using a 96-well microplate reader (VersaMax microplate reader, USA). Quercetin served as the standard for determination of the TFC of EECT, expressed in mg quercetin equivalent (QE)/g extract [11].

### 2.8. Antihyperlipidemic Activity Assay

The  $\text{IC}_{50}$  value, which shows the amount of lipase enzyme inhibition, was used to measure the antihyperlipidemic activity. The experiments were conducted using colorimetric principles and modified versions of previous methods [13]. The modification was conducted by determining the optimal incubation time and substrate concentration at constant absorbance. The antihyperlipidemic activity was evaluated using three replicates of the sample solution: EECT, a positive control (orlistat), and a negative control (without an inhibitor). Various concentrations of each of the three stock solutions (20  $\mu$ L) were reacted with 20  $\mu$ L of pancreatic lipase enzyme solution (Sigma-Aldrich, Missouri, United States). A total of 135  $\mu$ L of Tris-HCl buffer with a pH of 7.4 were added, followed by incubation at 37°C for 15 min. The test solution was added to 20  $\mu$ L of  $\rho$ -NPB substrate (Sigma-Aldrich, Missouri, United States), followed by incubation at 37°C for 30 min. The absorbance at 405 nm was measured using a 96-well microplate reader (Versamax microplate reader, United States). The  $\text{IC}_{50}$  value was calculated by intersecting the line between the percentage inhibition and the concentration axis using the regression equation  $y = a + bx$ , where  $y = 50$  and  $x$  represents the  $\text{IC}_{50}$  value. The percentage inhibition was calculated using Equation 1:

$$\text{Percentage inhibition (\%)} = \frac{(\text{Control OD} - (\text{Sample OD}/\text{Control OD})) \times 100}{\text{Control OD}} \quad (1)$$

where control OD is the absorbance of the negative control, and sample OD is the sample absorbance. Before the calculation, both the control OD and sample OD were subtracted from blanks.

**Table 1:** Qualitative tests for phytochemical screening.

Examinations	Test	Results (indicating a positive test)
Identifying alkaloids	Dragendorff's test	A brownish-reddish precipitate
	Mayer's test	A white/yellowish creamy precipitate
Identifying flavonoids	Isoamyl alcohol test	The color-appearing upper layer
Identifying saponins	Foam test	Foam lasting for 10 min
Identifying tannins	Gelatin test	A white precipitate
	FeCl <sub>3</sub> 's test	Blue-green color
	Stiasny's test	Obtaining a precipitate shows that tannin catechins are present
	Stiasny's and FeCl <sub>3</sub> 's test	Gallic tannin is indicated by a blue-black stain
Identifying quinones	NaOH test	Intensive red color
Identifying terpenoids	Liebermann–Burchard test	Crimson color
Identifying steroids	Liebermann–Burchard test	Either blue or green color
Identifying coumarins	NaOH test	Blue-green fluorescence under UV light
Identifying essential oils	Petroleum ether test	Aromatic smell

### 2.9. Antihypertension Activity Assay

The antihypertension activity was evaluated using the ACE inhibitory activity, expressed as the IC<sub>50</sub> value. The experiment was conducted by calculating the hydrolysis rate of HHL by ACE and measuring the amount of hippuric acid (HA) using modified versions of previous methods [14,15]. The phosphate buffer solution for this assay was prepared by dissolving 177.50 g of potassium dihydrogen monophosphate, 17.50 g sodium chloride, and 48.16 g sodium hydroxide in a 1.0 L of water and adjusting the pH to 8.3. The 5 mM HHL substrate solution was prepared by dissolving 20.0 mg of HHL in 10 mL of phosphate buffer. The 4 mU/mL ACE solution was prepared by dissolving 2000 µU mg of ACE in a 1.0 mL of phosphate buffer, followed by the addition of 80 µL of the ACE solution to 5 mL of phosphate buffer to obtain the desired concentration. EECT and 25 mg of captopril were dissolved in phosphate buffer solution to produce a series of five concentrations.

It started with 50 µL of substrate solution being mixed with 50 µL of inhibitor solution (or solvent solution as a negative control). Inoculation at 37°C for 15 min was followed by the addition of 50 µL of a 4 mU mL<sup>-1</sup> ACE solution. For 30 min, the mixture was kept at 37°C. Adding 200 µL of 1 M hydrochloric acid stopped the reaction. The sample was mixed with 1.5 mL of ethyl acetate (EA) and then centrifuged at 4000 rpm for 15 min. A 1.0 mL of the supernatant was transferred into a different test tube and left at room temperature for 2 h. After being dried, the sample was mixed with 3.0 mL of aquadest. A UV-vis spectrophotometer was used to measure its absorbance at a wavelength of 228 nm. To find how much HA was present, the absorbance reading was compared to the solution comparison series' raw curve. Based on equation 1, the concentration of HA was used to find the percentage inhibition, which in turn was used to determine the IC<sub>50</sub> value.

## 3. RESULTS AND DISCUSSION

### 3.1. Plant Determination and Sample Preparation

The determination results of the Herbarium Depokensis (UIDEP), Biota collection room of the Universitas Indonesia showed that the plant used in the study was true CT from the Fabaceae family, as shown in Figure 1a. The dried flower and powder are shown in Figure 1b and c. In the sample preparation, the drying of the flowers can take anywhere from a few hours to many days, and the drying temperature should be kept below 50°C. The drying duration depends

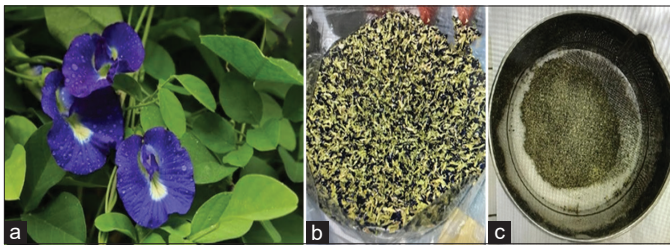
on the number of flowers dried at one time within the chamber [16]. The dried petals were ground into powder to improve the extraction process by normalizing the sample size and lowering the sample's surface area, which can cause the solvent to break down the cell wall more quickly [17]. The sample in this research was extracted by kinetic maceration to avoid the degradation of flavonoid compounds. The non-conventional extraction of Vietnam CT using ultrasound-assisted extraction and microwave-assisted extraction showed a significant effect on anthocyanins and antioxidant activities in comparison with conventional extraction [18].

### 3.2. Phytochemical Screening

The dried powder and EECT were analyzed phytochemically to identify the secondary metabolite compounds contained in butterfly pea flowers (Table 2). The result of phytochemical screening was showed that CT contained flavonoids, saponins, tannins, coumarins, steroids, triterpenoids, and essential oil. This result was similar to the previous study as that especially the petals of the CT flower contain many phytochemical compounds, such as alkaloids, phenolics, saponins, flavonoids, tannins, glycosides, resins, and steroids [19]. Flavonoids, saponins, catechutannins, and coumarins are examples of polar secondary metabolites. There were also non-polar secondary metabolites, such as steroids, triterpenoids, and essential oils. The extraction was done with ethanol, a universal solvent that can extract practically all metabolites, whether non-polar, semi-polar, or polar. This solvent can be used to extract various types of secondary metabolites. Although some compounds have limited solubility in extraction solvents at ambient temperature, the maceration technique, also known as cold extraction, allows for the extraction of numerous compounds. It also has the added benefit of reducing heat damage to thermolabile components [20].

### 3.3. Specific Quality Parameters

The specific quality parameters of the flower extract were the identity of the extract, its organoleptic properties, and its solubility in specific solvents. The identity of the extract was examined. The extract was *Clitoria ternatea* Flos Extractum Spissum from CT flower. The purpose of verifying the plant's identity is to prevent errors in the use of the sample by providing an early introduction to the plants used. Organoleptic examination serves as a preliminary identification method using the visual appearance of the extract. Organoleptic determination of the extract



**Figure 1:** The butterfly pea flower (*Clitoria ternatea* L.): (a) flower, (b) dry flower, (c) dry powder.

indicated that it was viscous and bluish purple. The odor was earthy, and the flavor was insipid. The determination of soluble contents in certain solvents was carried out to provide an overview of the percentage levels of compounds that could be extracted in ethanol and water. The levels of soluble contents in specific solvents have met the standard requirements of *Materia Medica Indonesia*. The water-soluble compounds were  $26.58 \pm 0.03\%$  (not  $<4\%$ ), and ethanol-soluble compounds were  $18.91 \pm 0.02$  (not  $<16\%$ ) [21]. Based on this data, it is evident that more compounds dissolve in water than in ethanol. This indicates that secondary metabolites in the butterfly pea flower are more attracted to polar solvents in water than in ethanol. This is due to the fact that water can dissolve polar compounds such as flavonoids, saponins, and tannins [22].

#### 3.4. Non-specific Quality Parameters of the Extract

The discovery of non-specific quality characteristics was intended to determine the extract's quality and safe limitations as a high-quality natural product material [12]. Table 3 provides a summary of the outcomes. The EECT values for water content and loss on drying meet the 10% guidelines set by the Indonesian Food and Drug Authority (BPOM RI) for the quality of extracts [21]. The total ash content, acid-insoluble ash content, and water-soluble ash content conform to the *Materia Medica Indonesia* herbal specifications. After 1 h of combustion at  $450^\circ\text{C}$ , the total ash indicated the proportion of physiological and non-physiological ash, which was assessed by a gravimetric method until the weight remained constant. Acid-insoluble ash reflects the amount of ash derived from external sources, such as sand or soil pollutants [22,23]. The residual solvent indicates the amount of solvent remaining after extraction. The results satisfy the BPOM parameters outlined in the Indonesian medicinal plant extract book, which are  $<1\%$ . Gas chromatography was unable to calculate the N/D (not detected) value of the residual solvent because the residual solvent concentration was too low. Therefore, according to the interpreted results for the residual solvent, no solvent remained after the extraction operation. The levels of heavy metals (Pb and Cd) conform to the BPOM specifications of 10 ppm (Pb) and 0.30 ppm (Cd) [21]. This demonstrates that the extract satisfies the heavy metal contamination safety requirements; consequently, it can be ingested because it is safe for the body. The study's findings could have direct ramifications for the food industry by allowing the use of EECT as a safe natural colorant [24].

#### 3.5. Total Phenolic and Flavonoid Contents

The results of the determination of the TPC and TFC were  $38.30 \pm 4.50$  mg GAE/g extract and  $29.79 \pm 0.31$  mg QE/g extract, respectively. To ascertain the number of compounds, present in the extract, the total phenolic and flavonoid contents were determined. The determination of total phenolic and flavonoid content was performed using the Folin-Ciocalteu and  $\text{AlCl}_3$  colorimetric methods, respectively, which are based on colorimetric principles [25].

**Table 2:** Phytochemical screening results of butterfly pea flowers.

Secondary metabolite	Dried CT	EECT
Alkaloids	-	-
Flavonoids	+	+
Saponins	+	+
Catechu tannins	+	+
Quinones	-	-
Steroids/Triterpenoids	+/+	+/+
Coumarins	+	+
Essential oil	+	+

(+): Possesses secondary metabolites, (-): Lacks secondary metabolites, CT: *Clitoria ternatea*, EECT: Ethanolic extract of *Clitoria ternatea*.

**Table 3:** The determined non-specific quality parameters.

Parameters	Results (%)	Standard requirements
Water content	$2.92 \pm 0.69$	$<10\%$
Loss on drying	$6.70 \pm 0.03$	$<10\%$
Total ash content	$5.19 \pm 0.00$	$<16\%$
Acid insoluble ash content	$0.27 \pm 0.00$	$<4\%$
Water soluble ash content	$3.87 \pm 0.01$	-
Residual solvent	$0.42 \pm 0.12$	$<1\%$
Pb metal contamination	Not detected	$\leq 10$ ppm
Cadmium metal contamination	Not detected	$\leq 0.30$ ppm
Total plate number	$\leq 1 \times 10^1$ colonies $\text{g}^{-1}$	$1 \times 10^4$ colonies $\text{g}^{-1}$
Yeast fungus number	$12.35 \times 10^1$ colonies $\text{g}^{-1}$	$1 \times 10^3$ colonies $\text{g}^{-1}$

Data presented with mean  $\pm$  standard deviation,  $n=3$ .

The quantity of TPC was higher than that of TFC. The TPC was expressed as mg of GAEs per gram of extract, using the equation  $y = 0.006x + 0.311$  and  $R^2 = 0.9962$ . The TFC was expressed as mg of QEs per gram of extract, using the equation  $y = 0.0052x + 0.1982$  and  $R^2 = 0.9988$  for the standard curve. Jaafar *et al.* found that the highest TPC and TFC values were  $41.17 \pm 0.5$  mg GAE/g dry sample and  $187.05 \pm 3.18$  mg quercetin/g dry sample, respectively. The extraction was conducted under optimal conditions with RSM method optimization at 37% v/v ethanol concentration for 90 min at  $45^\circ\text{C}$  [26]. Hence, it is important to conduct optimization to obtain high yields of phenolic and flavonoid contents.

Multisona *et al.* gathered the phenolic compounds found in the CT flowers from many studies. It mostly has ternatin anthocyanins and flavanol glycosides such as rutin, quercetin, kaempferol, and myricetin that are isolated in a hydrophilic extract. Phytochemical composition in relation to their hydrophobicity has been mentioned in previous studies. There are flavanol, ellagic acid, anthocyanin, anthocyanidin, and caffeoylmalic acid [19].

Previous research has revealed that these natural anti-obesity and antihypertensive compounds play important physiological roles. Phenolic compounds increased the breakdown of lipid by increasing mRNA expression of mitochondrial uncoupling proteins 3 while inhibiting alpha-amylase and pancreatic lipase [27]. Flavonoids reduced the mRNA levels of genes involved in lipogenesis, inhibited pancreatic lipase, and had an anti-adipogenic effect [27]. Different types of phenolics have been identified as potent ACE inhibitors



in addition to their other health benefits. *In vitro* test showed that phenolics and flavonoids from different plants significantly inhibited ACE [18].

### 3.6. Antihyperlipidemic Activity

The lipase inhibition test method uses  $p$ -NPB, which acts as a substrate, with the addition of a lipase catalyst to produce butyrate products and  $p$ -nitrophenolate ions with a yellow color [28]. The antilipase activity test was carried out according to the results of optimization: 30 min of incubation time, 3 mg/mL substrate concentration, and 0.025 mg/mL enzyme concentration. The antihyperlipidemic activity assay was carried out on EECT in a concentration range of 5–150 ppm. The variation was used to create a linear regression equation, which was then used to calculate the  $IC_{50}$  value, as shown in Figure 2. The lipase enzyme inhibition results demonstrated that as the concentration of the extract used increased, so did the percentage inhibition. The yellow pigment in the test solution faded with the addition of inhibitors of varying concentrations. This indicated that lipase activity was inhibited during the hydrolysis process [29]. The intensity of the inhibitory activity was indicated by the  $IC_{50}$  value, which is the sample concentration capable of inhibiting lipase enzyme activity by 50%. The lower the obtained  $IC_{50}$  value, the greater the inhibitory potential of the test sample. The  $IC_{50}$  lipase enzyme inhibition values for EECT and orlistat as the positive standard were  $131.7867 \pm 5.27$  ppm and  $79.9267 \pm 14.75$  ppm, respectively.

The *in vitro* activity assay revealed that the butterfly pea flower (CT) extract inhibits lipase with an  $IC_{50}$  value of  $131.7867 \pm 5.27$  ppm. This value was lower than that for the *in vitro* activity test of *Toona sinensis*, with an  $IC_{50}$  value of  $118.8 \pm 1.53$   $\mu$ g/mL [28], and for the methanolic extract of *Hibiscus sabdariffa* L. (*Malvaceae*), with an  $IC_{50}$  value of  $35.8 \pm 0.8$   $\mu$ g/mL, yet it was higher than the  $IC_{50}$  value of  $152.0 \pm 7.0$   $\mu$ g/mL for the methanolic extract of *Tamarindus indica* L. (*Leguminosae*) [30].

Prado *et al.* reported that the CT flower could protect against cholesterol oxidation by inhibiting the oxidation of the low-density-lipoprotein (LDL) cholesterol in humans that were caused by copper. He mentioned that phenolic compounds, mostly anthocyanins, from EECT flower, have antilipidemic and anticholesterol activities. These activities provide a defense against the oxidation of human cholesterol and LDL [31].

### 3.7. Antihypertensive Activity

The concentrations of the standard and sample solutions were determined using a linear equation obtained from a standard curve

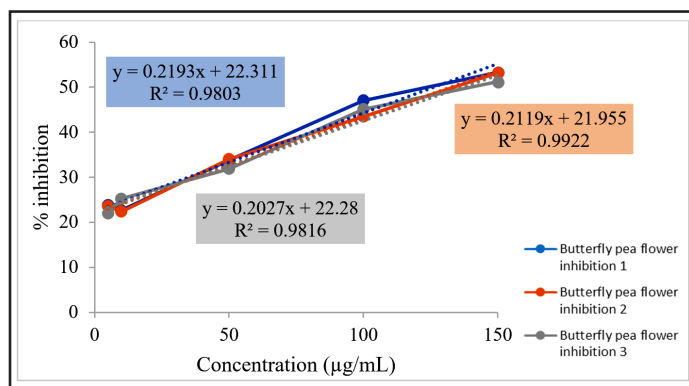


Figure 2: Lipase inhibition curve of ethanolic extract of *Clitoria ternatea* L.

of HA with  $y = 0.0489x + 0.291$  and  $R^2 = 0.9952$ . Blanks (without the inclusion of extracts) were utilized in the ACE activity inhibition test, with captopril serving as the positive control and EECT serving as the test solution. HA is produced when HHL and ACE substrates react, although both captopril and the extract utilized can block this reaction. Using a UV-vis spectrophotometer, we determined that the highest absorption wavelength for the HA produced was 228 nm. The concentrations of the standard and sample solutions were determined using a linear equation obtained from a standard curve of HA with  $y = 0.0489x + 0.291$  and  $R^2 = 0.9952$ . Blanks (without the inclusion of extracts) were utilized in the ACE activity inhibition test, with captopril serving as the positive control and EECT serving as the test solution. HA is produced when HHL and ACE substrates react, although both captopril and the extract utilized can block this reaction. Using a UV-Vis spectrophotometer, we determined that the highest absorption wavelength for the HA produced was 228 nm. The  $IC_{50}$  values for the antihypertensive activity of captopril and EECT against ACE were  $6.6696 \pm 0.74$  ppm and  $84.9653 \pm 1.70$  ppm, respectively. Captopril has greater inhibitory activity compared to EECT. The previous research on the ACE inhibitor efficacy of 10 antihypertensive medicinal herbs from Indonesia revealed that the leaves and fruits of *Phaleria macrocarpa* (Scheff.) Boerl showed significant inhibitory activity against ACE Boerl showed significant inhibitory activity against ACE [32]. The  $IC_{50}$  values for the leaf extracts were 189.13  $\mu$ g/mL in petroleum ether (PE), 157.74  $\mu$ g/mL in EA, and 101.52  $\mu$ g/mL in methanol, while the  $IC_{50}$  values for the fruits were 161.7  $\mu$ g/mL in PE, 139.11  $\mu$ g/mL in EA, and 122.38  $\mu$ g/mL in methanol [32]. According to a study that was presented by Escher *et al.*, the lyophilized extract of CT demonstrated a 61% inhibition of ACE-I activity when it was present at a concentration of 6.7 mg/mL [33]. The concentration of quercetin that was used as the positive control to demonstrate the antihypertensive activity was 1.7 mg/mL [34].

Competition for the catalytic site has been blocked by the phenolic substance gallic acid through hydrogen bonding with amino acid residues and ionic contact with zinc. Gallic acid's ACE inhibition was found to be predominantly conferred by the hydrophobicity of the benzene rings, according to *in silico* structure-activity relationship studies. In a complex involving zinc ions and amino acid residues, the flavonol molecule quercetin forms a stable combination with ACE. The interaction resembled those of ACE and lisinopril [18]. Researchers found that four flavonoids (quercetin-3-rutinoside, quercetin, kaempferol, and (-)-epicatechin) had inhibitory activity of ACE-I >42% [35], out of a total of seventeen tested. Flavonoids' antihypertensive activity is tied to the location and amount of hydroxyl groups, as well as the presence of double bonds in the rings, all of which work together to create stable complexes that chelate with the zinc that is located in the active site of ACE-I [36].

### 3.8. Development of CT as a Functional Food

By looking at certain and general parameters, phytochemical compounds, and the pharmacological activities of EECT, it was possible to show that CT could be used as a functional food. The pharmacological activities of the isolated compound that was derived from EECT have been elucidated by previous researchers. According to the findings of preclinical research involving animal testing, the extracts have the ability to demonstrate biological properties [19]. Therefore, CT flowers have the potential to be used as a powerful additive, either as a functional food incorporated into food products or as a pharmaceutical drug or supplement to improve the efficiency with which patients are treated.

#### 4. CONCLUSION

The *in vitro* activity assays revealed that the butterfly pea flower (CT) extract demonstrates potent inhibitory activity against both lipase and ACE. The determination of the specific and non-specific quality parameters satisfies the quality and safety standard requirements for medicinal herbs. This study supports further research on this plant for its potential development as a functional food or herbal substance for the treatment of hyperlipidemia and hypertension.

#### 5. ACKNOWLEDGMENT

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#### 6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

#### 7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work

#### 8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

#### 9. DATA AVAILABILITY

The authors confirm that the data supporting the findings of this study are available within the article.

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