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Research Article

Comparative evaluation of phenolic content, antioxidant, anti-inflammatory, and antidiabetic properties of *Clitoria ternatea* flowers and leaves from Vietnam

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Abstract

Clitoria ternatea L., a Southeast Asian herbal medicine and tea, is increasingly used in Vietnam as a natural food colorant and remedy, but its bioactive constituents are not well-documented. The present study evaluated the phenolic content, antioxidant activity, and potential antidiabetic and anti-inflammatory properties of *C. ternatea* flower and leaf extracts. *Clitoria ternatea* flowers and leaves were extracted using methanol and ethyl acetate. Phenolic and flavonoid contents of the extracts were quantified through spectrophotometry, while high-performance liquid chromatography connected to a diode-array detector was used to identify phenolic compounds. Antioxidant activity was assessed using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assays. α-glucosidase inhibition was determined spectrophotometrically. Anti-inflammatory effects were evaluated via albumin denaturation assay. The results demonstrated that the ethyl acetate extract of the flowers contained the highest total phenolic and flavonoid contents. The ethyl acetate flower extract exerted the strongest radical scavenging activity (IC_{50} = 474.76 μg/mL) while the methanolic leaf extract possessed the greatest capacity to quench free radicals (IC_{50} = 284.91 μg/mL). The ethyl acetate extract at 4000 μg/mL was identified as having the most potent inhibitory effect on α-glucosidase, comparable with that of 100 μg/mL acarbose. The methanolic extract of leaves exerted the strongest protective effect against albumin denaturation (IC_{50} = 120.05 μg/mL), and this may be on a par with diclofenac. The study's findings showed that flowers and leaves of *C. ternatea* are abundant in phenolics and possess high bioactivities. Therefore, they have the potential for being utilized in the production of functional food products.

Keywords: *Clitoria ternatea*, phenolic, flavonoid, anti-inflammatory, α-glucosidase

Introduction

Clitoria ternatea, also known as butterfly pea, is a plant species in the legume family. It is often used in the treatment of animals infected with parasitic worms, rheumatism, bronchitis, sexual disorders, and certain cancers [1]. Seeds and leaves of *C. ternatea* are commonly utilized as brain tonics and are thought to enhance memory [2]. A traditional remedy in Cuba involves using an infusion of the flowers to alleviate menstrual issues [1]. Pharmacological research reported that aerial parts of the plant species have anti-hyperglycemic and anti-hyperlipidemic effects. Aqueous extract was orally administered to alloxan-induced diabetic rats at a dose of 400 mg/kg body weight for 12 weeks, significantly decreasing serum glucose levels as well as total cholesterol and triglycerides [3]. Several important chemical constituents have been identified in *C. ternatea*, including delphinidin glycosides, cyanidin glycosides, pentacyclic triterpenoids, β-sitosterol, and taraxerol [4-6]. For example, a flavonol-rich fraction from crude *C. ternatea* flower extract obtained with MeOH/acetone/water (5:4:1; v/v/v) showed mild suppression of reactive oxygen species (ROS) in the lipopolysaccharides-stimulated inflammation in RAW-264.7 macrophage cells [7]. In another study, anthocyanin-rich fractions were shown to have more potent antibacterial activity compared to the

crude extract against *Bacillus cereus, Bacillus subtilis,* and *Escherichia coli* [8].

In Vietnam, *C. ternatea* flowers are harvested for use as a food colorant due to their pigments (i.e., anthocyanin). Dried flowers are often used to prepare herbal tea, which is believed to provide multiple health benefits, such as reducing stress, improving cognitive function, and promoting relaxation [1]. Despite growing recognition of the plant's phytochemicals and medicinal values, limited data about these constituents in dried aerial parts are available in the literature. This study focused on the antioxidant, antidiabetic, and anti-inflammatory activities of *C. ternatea* extracts to address key health challenges linked to oxidative stress, diabetes, and chronic inflammation. As *C. ternatea* is rich in phenolics and flavonoids, it is known for neutralizing ROS. Investigating this activity validates its potential to combat oxidative stress. By inhibiting carbohydrate-hydrolyzing enzymes like α-glucosidase, *C. ternatea* extracts could help manage postprandial hyperglycemia, offering a natural alternative to synthetic antidiabetic drugs. More so, evaluating the extracts' ability to inhibit protein denaturation supports their traditional use for managing inflammation.

The present study was conducted to determine phenolic and flavonoid contents, antioxidant potential, and inhibitory effects of *C. ternatea* on α-glucosidase and albumin denaturation. The findings of the study will hopefully provide a better understanding of the potential antidiabetic and *in vitro* anti-inflammatory properties of the plant species.

Materials and Methods

Sample collection

Leaves and flowers of *C. ternatea* were collected from a garden located in Ho Chi Minh City (latitude 10°49′49″ N, longitude 106°49′3″ E), Vietnam, in November 2021. The authentication of the specimens was performed at the Southern Institute of Ecology, Ho Chi Minh City. The collected samples were carefully washed and air-dried to a moisture content of less than 10%, then stored at 4°C with 65% relative humidity.

Sources of chemicals

Phenolic acids (chemical purity: ≥ 98%) were purchased from Sigma–Aldrich (St. Louis, Missouri, USA), while flavonoids were obtained from Chengdu Biopurify Phytochemicals (Sichuan, China). Folin-Ciocalteu's reagent was obtained from Merck KGaA (Darmstadt, Germany). Reagents used in antioxidant activity assays, including DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), were purchased from Sisco Research Laboratories (Maharashtra, India) and Sigma-Aldrich, respectively. Yeast α-glucosidase was purchased from BOSF (Hefei, China).

Preparation of crude extracts

Dried *C. ternatea* (flower or leaf) samples mixed with methanol or ethyl acetate at a ratio of 1:10 (g/mL) were placed on a horizontal shaker. After 24 h of extraction, the mixture was filtered through a Whatman filter paper (GE Healthcare, Illinois, USA), and the filtrate collected was evaporated to remove the solvent using a rotary evaporator (DLAB Scientific, Beijing, China).

Determination of total phenolic and flavonoid contents

Total phenolic and flavonoid contents in the crude extracts from the samples of *C. ternatea* flowers and leaves were determined according to a previously developed method [9]. A mixture of diluted extract (0.5 mg/mL) or standard, 2% sodium carbonate, and 50% Folin–Ciocalteu solution (1:1:1, v/v) was prepared in a screw-capped tube and incubated at room temperature for 5 minutes. The absorbance was then measured at 760 nm, with methanol serving as the blank. Gallic acid was used as the reference standard. The results were expressed as mg of gallic acid equivalent mg GAE/g extract. To determine total flavonoid content, 100 µL of extract (10 mg/1.6 mL) was mixed with 4.9 mL of distilled water and 300 µL of 5% $NaNO_2$, then incubated for 5 minutes. Next, 300 µL of 10% $AlCl_3$ was added, followed by a 6-minute incubation. Next, 2 mL of 1 M NaOH and enough water were added to bring the final volume to 10 mL. The mixture was incubated for 15 min and then measured spectrophotometrically at 510 nm. Quercetin, with concentrations ranging from 1 to 100 mg/mL, was used as the reference standard. The results were shown as mg of quercetin equivalent mg QE/g extract.

HPLC-DAD analysis of phenolic compounds

The quantification of phenolics in the extracts was carried out on a high-performance liquid chromatograph connected to a diode-array detector (HPLC-DAD). The system was equipped with a VertiSep GES C18 reverse-phase column (250 × 4.6 mm, 5.0 µm particle size) set at 40°C. The analysis was performed following a previously developed method [10].

Evaluation of antioxidant activity

The potential to scavenge DPPH and ABTS free radicals was used to evaluate the antioxidant activity of the extracts [11, 12]. Ascorbic acid was employed as a reference standard. The DPPH or

ABTS scavenging activities were determined as follows:

$$\% \text{ scavenging activity} = (A_0 - A_S)/A_0 \times 100\% \quad \text{(Eq. 1)}$$

where A_0 and A_S are the absorbance of the blank and test samples, respectively. IC_{50} was used to compare the activities among the samples.

α-glucosidase inhibition assay

A diluted solution of an extract or acarbose (positive control) was combined with 40 µL of glucosidase (0.05 U) dissolved in a 0.1 M phosphate buffer (pH 6.8). The mixture was then incubated at 37 °C for 20 min. After adding 40 µL of 5 mM p-NPG (4-nitrophenyl-β-D-glucopyrano side), another 20 min incubation at 37 °C was performed. The reaction was stopped with 130 µL of 0.2 M sodium carbonate solution and the change in absorbance was measured at 405 nm in a spectrophotometer [13]. Determination of the inhibitory activity was based on calculated IC_{50} values (µg/mL).

***In vitro* assessment of the protective effect of extracts on bovine albumin denaturation**

The extracts were tested for their protective effect on the denaturation of bovine albumin using a modified version of a previously described method [14]. A diluted extract was mixed with a 0.16% albumin solution and sodium acetate buffer (pH 5.5) in a 1:1:2 (v/v/v) ratio, then incubated at 37 °C for 45 min and heated at 67 °C for 3 min. After cooling to ambient temperature, the absorbance was measured at 660 nm using a spectrophotometer. Diclofenac, a nonsteroidal anti-inflammatory drug, served as the positive control for the assay. The inhibitory activity was estimated at IC_{50} values (µg/mL).

Statistical analysis

All the experiments were carried out in triplicate. The data were analyzed using the Microsoft Excel 365 software platform and Minitab 19 (Minitab LLC, PA, USA), and the results are expressed as mean ± standard deviation (SD). The data obtained from the experiments were statistically analyzed by one-way analysis of variance (ANOVA), followed by Tukey's HSD test at $p = 0.05$.

Results and Discussion

Total phenolic and flavonoid contents

The four crude extracts obtained were methanol flower (ME-F), methanol leaf (ME-L), ethyl acetate flower (EA-F), and ethyl acetate leaf (EA-L) extracts. **Figure 1** displays the total phenolic content (TPC) and total flavonoid content (TFC) in the methanol and ethyl acetate extracts of *C. ternatea* flowers and leaves. The results demonstrated that EA-F had the highest TPC value (15.39 ± 1.58 mg GAE/g) among the extracts, followed by EA-L (7.06 ± 0.87 mg GAE/g). The methanol extracts contained much lower amounts of phenolics, with the levels in flowers and leaves averaging 0.81 and 3.86 mg GAE/g, respectively. The extraction of phenolics appeared to be more successful using ethyl acetate compared to methanol, as the extracts produced with the former contained a significantly higher amount of phenolics. Previous research reported that the average TPC of methanol extracts of *C. ternatea* flowers ranged from 5.72 to 13.70 mg GAE/g [9, 15]. In another study, methanol extracts prepared from *C. ternatea* flowers had an average TPC value of 61.7 mg GAE/g [16]. Research also indicated that leaf extracts of *C. ternatea* were rich in phenolics, with phenolic content ranging between 12.34 and 75.21 mg GAE/g [4, 16]. Of these, methanol extract reportedly contained up to 73.03 mg of gallic acid equivalent per gram of extract.

The results (**Figure 1**) also demonstrated that the flower extracts had a significantly ($p < 0.05$) higher TFC compared to those from the leaves. Previous research reported that a methanolic extract of dried flowers was slightly richer in flavonoids than that of dried leaves [17]. A recent study revealed that extract from fresh flowers had a significantly ($p < 0.05$) higher TFC compared to fresh leaves [9]. The findings of this study are consistent with those of previous research, which indicated that *C. ternatea* flowers are a rich source of flavonoids. As observed in **Figure 1**, EA-F had an average TFC value of 306 mg QE/g, showing a 13% higher number of flavonoids than ME-F. This also indicated that ethyl acetate was more effective in extracting flavonoids compared to methanol.

Phenolic composition

Table 1 shows the concentrations of phenolics detected in the flower and leaf extracts. The results demonstrated that EA-F may be richest in phenolics among the extracts. Most of the phenolics monitored in the present study were found at higher levels in EA-F compared to the other extracts. Notably, the concentration of caffeic acid in EA-F was 16 – 69 times as high as those in the others. Of the compounds, chlorogenic and ferulic acids were observed to be the most abundant in ME-L. Previous research reported the presence of gallic and chlorogenic acids in freeze-dried extracts of *C. ternatea* flowers at an average level of 0.67 and 0.54 mg/g, respectively [18]. Recently, one study showed that methanolic extracts of *C. ternatea* fresh flowers contained 2.32 and 0.81 mg/g of gallic and chlorogenic acids, respectively [9]. Two flavonoids (i.e., rutin and quercetin) were previously detected and

quantified in the flowers, though their concentrations were very low [18].

Antioxidant activity

In this study, antioxidant activity of the extracts was conducted by determining their ability to scavenge ABTS and DPPH free radicals. As presented in **Figure 2**, EA-F exerted the strongest DPPH radical scavenging potential, with an average IC_{50} value of 474.76 μg/mL. ME-L was ranked second, followed by ME-F, though IC_{50} values of these two methanolic extracts differed insignificantly (592.29 and 641.56 μg/mL, respectively). The DPPH assay is more suitable for systems containing hydrophobic molecules. It is widely known that ethyl acetate is particularly good at extracting non-polar and moderate polar compounds. This could explain why EA-F exerted higher DPPH activity than the other extracts. Concerning the ABTS assay, ME-L was found to possess the strongest capacity to trap ABTS free radicals (IC_{50} = 284.91 μg/mL), followed by EA-F. In both assays, ME-F and EA-L had much lower potential to scavenge the free radicals compared to the other extracts. The results also indicated that EA-L may exhibit the lowest activities, with IC_{50} values greater than 2000 μg/mL. In this study, ascorbic acid, used as a reference standard, presented the DPPH and ABTS activity with IC_{50} values of 6.79 and 55.04 μg/mL, respectively.

The ABTS assay, which measures the production of blue/green $ABTS^{+}$ radicals, is well-suited for evaluating both hydrophilic and lipophilic antioxidant components, whereas the DPPH assay is more effective for analyzing hydrophobic compounds. Phenolics are well-known for their antioxidant properties due to their ability to donate hydrogen atoms or electrons to neutralize free radicals. Gallic acid, caffeic acid, p-coumaric acid, and ferulic acid, as well as flavonoids, have been shown to have strong antioxidant activity through various mechanisms, including free radical scavenging and metal chelation [19-21]. Previous research has shown that the methanolic extracts of *C. ternatea* flowers exhibited higher antioxidant activity, as measured by the DPPH assay, compared to the extracts from *C. ternatea* leaves [16]. In another study, leaf extracts from methanol had significantly ($p < 0.05$) higher scavenging activities than those from ethyl acetate [22]. This may stem from differences in plant parts and extraction solvents used.

α-glucosidase inhibitory activity

In the present study, the determination of α-glucosidase inhibitory activity was used to predict the potential antidiabetic activity of the extracts. As depicted in **Figure 3**, among the extracts, EA-F demonstrated the highest inhibitory effect, reducing enzyme activity by over 74.6%, followed by ME-F, which inhibited 33.0% of the enzyme activity. The extracts from leaves displayed a much lower inhibitory activity, with the inhibition percentages of about 8%. In comparison with acarbose used as a positive control, EA-F was on a par with that of 100 μg/mL acarbose solution (73.92%). The results also showed that the other extracts exerted a weaker activity than all the solutions of acarbose. Research has demonstrated that both rutin and quercetin can effectively inhibit α-glucosidase, an enzyme responsible for breaking down complex carbohydrates into glucose in the small intestine [23]. These flavonoids are considered to interact with the active site of α-glucosidase, preventing the enzyme from binding to its substrate (carbohydrates). This inhibition slows down the

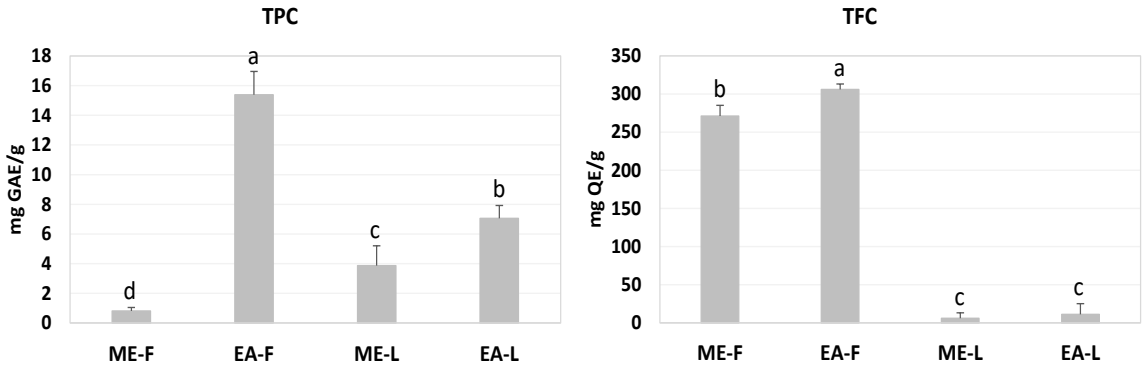

Figure 1. Total phenolic and flavonoid contents in the extracts of *Clitoria ternatea* flowers and leaves. Different letters indicate statistically significant differences among the extracts ($p < 0.05$); TPC: Total phenolic content; TFC: Total flavonoid content; ME-F: Methanolic flower extract; EA-F: Ethyl acetate flower extract; ME-L: Methanolic leaf extract; EA-L: Ethyl acetate leaf extract

rate of glucose absorption, leading to a more gradual increase in blood sugar levels. Previously, a study on *C. ternatea* revealed that IC_{50} values of its aqueous flower extracts ranged from 3150 to 4410 µg/mL [24]. Furthermore, at 100 µg/mL, the inhibition percentages were about 35%. One recent investigation showed that methanolic crude extract and its ethyl acetate fraction inhibited α-glucosidase, with IC_{50} value more than 1000 µg/mL [25]. In general, these findings are similar to those reported in the present study. Besides, one study by Verma *et al*. showed that *C. ternatea* possessed an antidiabetic potential [26].

Inhibition of bovine albumin denaturation

Exposure to heat stress or chemicals can denature proteins, altering their biological, chemical, and physical properties. This protein denaturation may serve as a marker for inflammatory diseases. The determination of albumin denaturation was used to evaluate the *in vitro* anti-inflammatory activity of the extracts of *C. ternatea* flowers and leaves. As graphically presented in **Figure 4**, the IC_{50} values of the extracts follow the order ME-L < EA-F < ME-F < EA-L. Therefore, their capacity to prevent albumin from denaturation appeared to be inversely related. In the present study, diclofenac, used as a reference standard, had an average IC_{50} value of 116.88 µg/mL. The results demonstrated that the inhibitory effect of ME-L (IC_{50} = 120.05 µg/mL) on albumin denaturation was comparable with that of diclofenac. The mechanism by which the extracts were able to inhibit the denaturation of albumin is not fully understood. Evidence suggests that this could be attributed to the interactions between the phenolics in the extracts and the aliphatic regions surrounding the lysine residue on the surface of the albumin molecules [27]. However, as ME-L was not rich in phenolics and flavonoids, its potent effect could be due to the presence of other phytochemicals. Generally, rutin and quercetin were shown to be able to interact with proteins, including albumin, through various mechanisms, such as hydrogen bonding, hydrophobic interactions, and electrostatic interactions [28, 29]. These interactions can stabilize the protein structure, preventing it from unfolding and denaturing.

Table 1. Phenolic composition of the *Clitoria ternatea* extracts

Phenolics, mg/g extract	flower		leaf	
	ME-F	EA-F	ME-L	EA-L
Gallic acid	0.57	0.86	0.07	0.17
Chlorogenic acid	0.20	0.49	1.17	0.22
Caffeic acid	0.22	4.15	0.23	0.06
p-coumaric acid	0.36	1.23	0.02	0.53
Ferulic acid	0.20	1.72	1.89	0.34
Salicylic acid	0.94	0.98	0.95	0.86
Cinnamic acid	0.12	0.29	0.02	0.22
Rutin	0.40	0.88	0.02	0.03
Quercetin	0.88	2.49	0.01	0.06
Sum of phenolics	3.89	13.08	4.38	2.49

ME-F: Methanolic flower extract; EA-F: Ethyl acetate flower extract; ME-L: Methanolic leaf extract; EA-L: Ethyl acetate leaf extract; Values are expressed as means of duplicate measurements

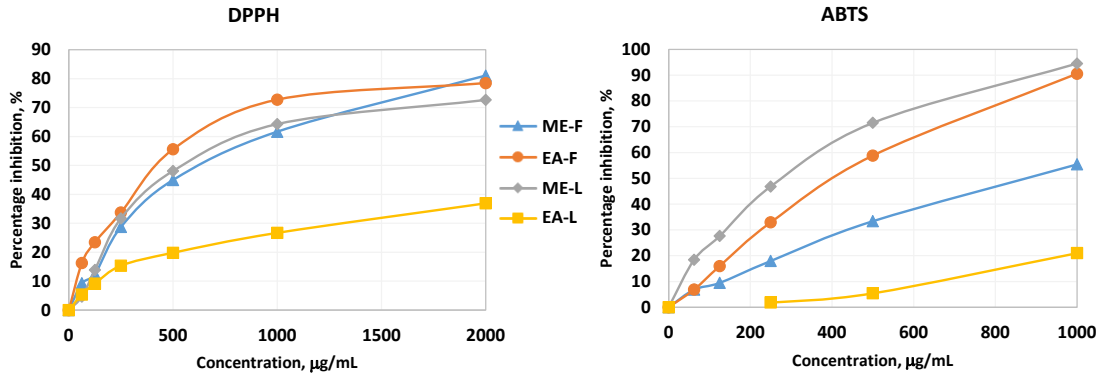

Figure 2. DPPH and ABTS free radical scavenging activities of *Clitoria ternatea* extracts. ME-F: Methanolic flower extract; EA-F: Ethyl acetate flower extract; ME-L: Methanolic leaf extract; EA-L: Ethyl acetate leaf extract; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

Research has demonstrated that extracts from various parts of the plant, including roots, leaves, and flowers, exhibit significant anti-inflammatory properties. For instance, an ethanolic root extract was found to reduce inflammation in animal models, supporting its traditional use in treating inflammatory conditions [30]. Additionally, studies on leaf extracts have reported both analgesic and anti-inflammatory effects, further validating the plant's medicinal applications [31]. These findings suggest that *C. ternatea* holds promise as a natural source of anti-inflammatory agents.

Study limitations

While the study demonstrates the antioxidant and potential antidiabetic properties of the extracts through *in vitro* assays, these findings are limited in their ability to fully predict biological effectiveness in complex living systems. To build on these findings, future research should prioritize *in vivo* validation to confirm the observed bioactivities and understand the compounds' behavior in biological systems. Additionally, isolating and characterizing individual bioactive compounds is essential to identify specific mechanisms of action and potential synergistic effects. Such approaches will provide a more comprehensive understanding of the therapeutic potential and safety profiles of these bioactive extracts, paving the way for their application in functional foods or pharmaceuticals.

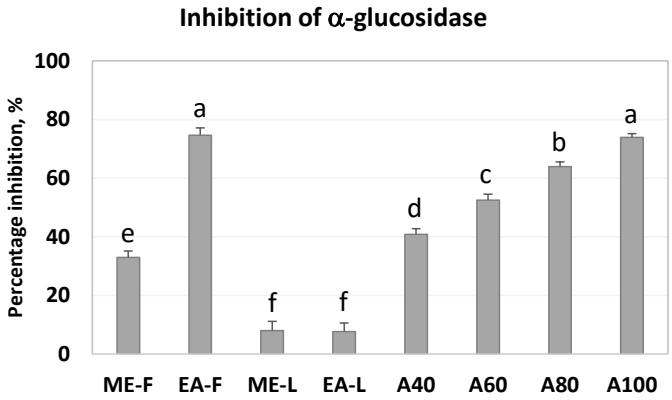

Figure 3. Inhibitory effect of *Clitoria ternatea* extracts (4000 µg/mL) and acarbose on α-glucosidase. ME-F: Methanolic flower extract; EA-F: Ethyl acetate flower extract; ME-L: Methanolic leaf extract; EA-L: Ethyl acetate leaf extract; A40, A60, A80 and A100: Acarbose solutions at concentrations of 40, 60, 80 and 100 µg/mL, respectively; Different letters indicated statistically significant differences among the test samples ($p < 0.05$)

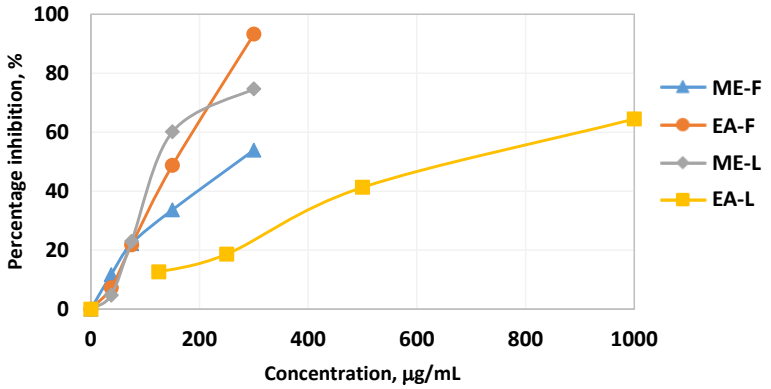

Figure 4. Inhibitory effect of the *Clitoria ternatea* extracts on albumin denaturation. ME-F: Methanolic flower extract; EA-F: Ethyl acetate flower extract; ME-L: Methanolic leaf extract; EA-L: Ethyl acetate leaf extract.

Conclusion

The extracts from *C. ternatea* flowers are highly rich in phenolics and flavonoids. The findings also showed free radical scavenging potentials estimated by DPPH and ABTS assays generally differed significantly among the extracts. The ethyl acetate extract may have the strongest activity to inhibit α-glucosidase. Moreover, its activity was shown to be as potent as diclofenac, a nonsteroidal anti-inflammatory drug. The leaf extract obtained with methanol appeared to protect albumin from denaturation most effectively. Additional research should prioritize how the bioactivities of *C. ternatea* aerial parts are influenced by phenolic profiles from the plant samples of different genotypes, maturity stages, and storage conditions.

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