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# Anti-Aging and Antioxidant Activities of *Clitoria ternatea* L. Flower Extract

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## ABSTRACT

Aging is influenced by several factors, including Reactive Oxygen Species (ROS). An imbalance of higher ROS and lower antioxidants may lead to oxidative stress in cells that is manifested as wrinkles, dark spots, and fine lines. Antioxidants can prevent the effects of ROS. Safe and abundant sources of antioxidants can come from natural ingredients. One of the natural ingredients widely studied is *Clitoria ternatea* flower extract (CFE). This study aims to report the CFE potential as an anti-aging and antioxidant agent. Gallic acid and quercetin standards were used to measure the total flavonoid and phenolic content. Antioxidant activity was evaluated by hydroxyl (OH) and Nitric Oxide (NO) scavenging, while anti-aging activity was evaluated by collagenase and elastase inhibition tests. CFE showed high phenol and flavonoid content (14.49 µg GAE/mg and 9.00 µg QE/mg). CFE also has OH and NO scavenging activity with $IC_{50}$ 18.39 and 31.11 µg/mL, which exhibited antioxidant potential. CFE also showed potential to inhibit collagenase ($IC_{50}$ = 177.48 µg/mL) and inhibit elastase ($IC_{50}$ = 30.88 µg/mL). CFE may serve as a promising candidate with antioxidant and anti-aging properties.

## 1. Introduction

Skin maturation is a common process within the physiology of all living things, including humans. Skin maturation is influenced by external stressors and natural conditions, including ultraviolet (UV) radiation, pollution, and smoking habits. The combination of outside stressors and natural components increase the Reactive Oxygen Species (ROS) and results in oxidative stress (Naidoo & Birch-Machin 2017). The effects of this process include physical changes such as the appearance of lines and wrinkles, changes in skin color, widespread dryness, dull and unpleasant complexion, changes in skin tone, and decreased skin function (Papaccio *et al.* 2022).

High ROS in the body can disrupt molecular stability that is closely related to skin aging, some of which are genes that maintain the extracellular matrix, namely Nuclear Factor Kappa B (NF-κB), Metalloproteinases (MMPs), and Activator Protein 1 (AP-1) (Zhang *et al.* 2018). The increase in the expression of these genes leads to higher levels of collagenase and elastase enzymes, which influence skin aging. Both enzymes contribute to the degradation of collagen and elastin fibers within the extracellular matrix (ECM). Collagen is crucial for the tensile strength of the skin, while elastin influences its elasticity (Mehta-Ambalal 2016). Thus, these two enzymes need to be inhibited so that there is no decomposition of collagen and elastin, which can cause alteration in the structure, elasticity, and firmness of the skin (Wang *et al.* 2018).

ROS can be neutralized by the presence of antioxidants. Antioxidants refer to molecules that can prevent or retard the breakdown caused by oxidation reactions to a certain level (Nurhidayah 2020). Under normal conditions, the body naturally synthesizes endogenous antioxidants, including Glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) (Nur & Lukitaningsih

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2017). However, excessive levels of ROS in the body cannot be overcome by endogenous antioxidants alone; additional antioxidants, such as exogenous antioxidants, are needed, which can be found in natural and synthetic forms.

Synthetic antioxidants are widely used in anti-aging cosmetics due to their rapid effectiveness and cost efficiency. However, excessive use of synthetic antioxidants poses risks, including potential carcinogenicity (Kandowangko 2023). As a safer alternative, researchers have explored natural sources rich in antioxidants. One potential source is the butterfly pea flower, *Clitoria ternatea* L., which has been utilized in Indonesia for ornamental purposes, beverages, natural food coloring, and traditional medicine (Kusuma 2019; Marpaung 2020).

*Clitoria ternatea* flower extract (CFE) is known for its antioxidant properties, attributed to its high flavonoid content (Chayaratanasin *et al.* 2015; Cahyaningsih *et al.* 2019). CFE has been observed to have antidiabetic, antibacterial, anti-cataract, and anti-inflammatory effects. (Kusrini *et al.* 2017; Widowati *et al.* 2022, 2023). Previous studies reported that *C. ternatea* flower tea exhibits significant antioxidant activity using the DPPH assay. At 100% tea concentration, the total flavonoid content was 4.88 µg QE, surpassing that of pineapple tea (Widowati *et al.* 2022). Furthermore, *C. ternatea* floral extract has demonstrated significant radical scavenging activity in assays such as 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Li *et al.* 2022). Additionally, *C. ternatea* leaf extract has been found to inhibit wound-healing enzymes like hyaluronidase and MMP-1 (Li *et al.* 2022).

The antioxidant activities of CFE have been widely studied using DPPH, ABTS, and FRAP methods. Still, measurement of other specific free radical inhibition activities that are equally important, namely hydroxyl (OH) and Nitric Oxide (NO), has not been done. In addition, studies that specifically evaluate the inhibition of collagenase and elastase enzymes as anti-aging have not been widely conducted. Thus, this study aimed to assess the phytochemical composition, total flavonoid and phenolic content of CFE, and antioxidant activity with OH and NO scavenging activities, and evaluate the anti-aging activity through the collagenase and elastase inhibition activities of CFE.

## 2. Materials and Methods

### 2.1. Sample Extraction

The extraction of CFE was performed by PT FAST (Depok, Indonesia) in compliance with Good Manufacturing Practices (GMP) under CoA No. Batch 00103211072. In the extraction of *C. ternatea* using 70% ethanol, lactose functioned as an additive in the formulation (Widowati *et al.* 2023a).

### 2.2. Phytochemical Screening

Phytochemical examination of CFE extract involves a tube reaction method, which includes the identification of saponins, flavonoids, alkaloids, phenols, terpenoids, steroids/triterpenoids, and tannins (Maity *et al.* 2012; Prahastuti *et al.* 2020).

#### 2.2.1. Phenol Identification

The sample on the drop plate was added with $FeCl_3$ in 1% distilled water. Color changes to purple/blue/red/green/black indicate the presence (Maity *et al.* 2012; Prahastuti *et al.* 2020).

#### 2.2.2. Steroid/Triterpenoid Identification

A dropping plate was inserted with 10 mg of sample extract, and then acetic acid was added until the plate was submerged. Concentrated sulfuric acid ($H_2SO_4$) was administered after 10 to 15 minutes. A green or blue colour shows the existence of steroids, while the existence of an orange or red precipitate indicates the existence of triterpenoids (Prahastuti *et al.* 2020; Widowati *et al.* 2022).

#### 2.2.3. Saponin Identification

Dilute 10 mg of sample extract in aquadest, boil for 5 minutes, and then shake vigorously before adding 1N HCl. If bubbles still form after adding 1N HCl, the presence of saponins was indicated after treatment with HCl (Widowati *et al.* 2016, 2022; Prahastuti *et al.* 2020).

#### 2.2.4. Tannin Identification

After adding the 10 mg sample, 2 mL of 2N HCl was heated for 30 minutes in a water bath. After cooling and filtering, amyl alcohol was administered to the combined solution. The formation of purple colour on the surface of amyl alcohol indicated the presence of tannins (Maity *et al.* 2012; Widowati *et al.* 2018; Prahastuti *et al.* 2020).

### 2.2.5. Terpenoid Identification

Vanillin and $H_2SO_4$ were administered to 10 mg of extract. Purple colour indicated positive reaction (Jakimiuk *et al.* 2021; Widowati *et al.* 2022; Yuanda *et al.* 2023).

### 2.2.6. Flavonoid Identification

A reaction tube contained Mg and 2N HCl was added with 10 mg of the sample extract and incubated for 5 to 10 minutes. Next, amyl alcohol was added to the filtrate. The appearance of an orange to red coloration signified the presence of flavonoids (Maity *et al.* 2012; Prahastuti *et al.* 2020).

### 2.2.7. Alkaloid Identification

Two layers of chloroform were generated after adding 10% ammonia to the sample and extracting it using chloroform. One or two drops of Draggendorf's solution were added to the upper layer after it had been collected, and the lower layer had been added with 1N HCI (Maity *et al.* 2012; Prahastuti *et al.* 2020).

## 2.3. Total Phenolic Content Assay

The complete phenol substance was calculated utilizing Folin Ciocalteu reagent. The essential step was to fill the 96-well microplate with 15 µL of the test. Subsequently, 10% Folin-Ciocalteu reagent (75 µL) and 7.5% sodium carbonate (60 µL) were administered. The plate was then shaken and incubated at 50°C for 10 minutes. The absorbance was determined at 760 nm using a Multiskan GO reader. Gallic acid equivalents (GAE) in µg/100% were utilized to select the total phenolic substance. This test was replicated three times, and the information appears as mean ± standard deviation (Prahastuti *et al.* 2019; Widowati *et al.* 2022).

## 2.4. Total Flavonoid Content Assay

The colorimetric strategy was utilized to determine the full flavonoid content within the tests. The sample solution of 15 µL was administered to 75 µL of 2% $AlCl_3$. The clear well contains only solvent (150 µL) and 150 µL of the sample dissolved. The absorbance at 415 nm was recorded using a microplate reader to determine the flavonoid content. The standard straight condition of quercetin was utilized to determine flavonoid substance in Quercetin equivalents (QE) per mg of test. The test was carried out three times to guarantee the precision of the results (Prahastuti *et al.* 2020; Widowati *et al.* 2022).

## 2.5. OH Scavenging Activity Assay

Thirty microliters of the sample solution at various concentrations were administered to the reaction mixture. The following materials were combined: 10 µL of $FeCl_3$-EDTA, 5 µL of 20 mM $H_2O_2$, 5 µL of 1 mM L-Ascorbic acid, 10 µL of 28 mM Deoxyribose, and 70 µL of phosphate buffer. The mixture was heated to 37°C for 30 minutes. Afterwards, 25 µL of 1% TBA and 5% TCA was administered, and the mixture was then incubated at 80–90 °C for a further 30 minutes. Utilizing a spectrophotometer (Thermo Fisher Scientific, Multiskan GO Microplate Spectrophotometer) at 532 nm (Irwan *et al.* 2020; Widowati *et al.* 2021).

## 2.6. NO Scavenging Activity Assay

The CFE test consisted of 96-well phosphate-buffered saline (PBS) with 10 mM sodium nitroprusside. The Griess reagent was then mixed with 0.1% $C_{12}H_{16}Cl_2N_2$, 1% $C_6H_8N_2O_2S$, and 2% $H_3PO_4$. The mixture was then allowed to stand at 25°C for two hours. The maximum measurement for the test was 546 nm (Widowati *et al.* 2023b).

## 2.7. Collagenase Inhibition Assay

This assay was adapted from the method of Widodo *et al.* (2019) and Widowati *et al.* (2022). The reaction mixture was transferred into a 96-well microplate and incubated at 37°C for 20 minutes. The solution contained collagenase enzyme prepared in Tricine buffer (pH 7.5, 50 mM, supplemented with 10 mM $CaCl_2$, 6 µL, and 400 mM NaCl), *Clostridium histolyticum* (0.01 U/mL in cold water, 10 µL), and 30 µL of the sample solution at concentrations ranging from 4.69 to 150 µg/mL. Subsequently, 20 µL of FALGPA substrate (1 mM in Tricine buffer) was added. The absorbance was recorded at 335 nm using a microplate reader.

## 2.8. Elastase Assay

Elastase inhibitory activity was attempted with changes based on the protocol, followed by Widowati *et al.* (2016) and Prahastuti *et al.* (2020). The sample (2.08-66.67 µg/mL) 10 µL should be tested at room temperature for 15 minutes. Then, the mixture was supplemented with 5 µL of elastase (0.5 mU/mL in chilled $ddH_2O$) and 125 µL of Tris buffer (100 mM, pH 8). The solution was kept at room temperature for 15 minutes, followed by the addition of 10 µL of substrate

$C_{19}H_{25}N_5O_8$ (2 mg/mL in Tris buffer). Spectrophotometer set to 410 nm wavelength for measured the absorbance; the following equation was applied to calculate the percentage of elastase inhibitory activity.

### 2.9. Statistical Data Analysis

Statistical data were analyzed in the SPSS software version 20.0. The analysis of variance (One-way ANOVA) was performed, followed by Tukey's post hoc HSD test. The $IC_{50}$ value of each test (inhibition of collagenase, elastase, and OH, NO) was calculated based on the standard curve.

## 3. Results

### 3.1. CFE Quality

Table 1 presents the CFE quality test results. Based on Table 1, the quality test of CFE includes identification tests, physical characteristics, heavy metals, and microbiological tests. The results show that the identification test and physical characteristics of the CFE follow specifications. The heavy metal content test results show that CFE has an arsenic content of 0.008 ppm, lead content is 0.002 ppm, cadmium content is 0.008 ppm, and mercury content is 0.007 ppm, all within the specified limits. The microbiological tests showed that the total plate count, yeast, mold count, *E. coli*, Enterobacteriaceae count, *Clostridia, Salmonella,* and *Shigella* tests of CFE were below standard limits.

Table 1. Quality of CFE

| Item | Specification | Test result |
|---|---|---|
| Identification test | | |
| Form | Powder | Accordance |
| Color | Dark Blue | Accordance |
| Smell | Typical | Accordance |
| Flavor | Slightly Sour | Accordance |
| Extract level | 1:1 | Accordance |
| Mesh size 80 | ≥90% Passed | Accordance |
| Solubility | soluble in water | Accordance |
| Water content | < 10% | Accordance |
| Heavy metals | | |
| Arsenic (As) | ≤ 5 ppm | 0.0008 |
| Lead (Pb) | ≤ 10 ppm | 0.002 |
| Cadmium (Cd) | ≤ 0.3 ppm | 0.008 |
| Mercury (Hg) | ≤ 0.5 ppm | 0.0007 |
| Microbiological test | | |
| Total plate count | $\leq 10^5$ | $1.5 \times 10^2$ |
| Yeast mold count | $\leq 10^3$ | $9.0 \times 10^1$ |
| *E. coli* | $\leq 10^1$ | $1.0 \times 10^1$ |
| Enterobacteriaceae count | $\leq 10^3$ | $< 1.0 \times 10^1$ |
| *Clostridia* | Negative | Negative |
| *Salmonella* | Negative | Negative |
| *Shigella* | Negative | Negative |

### 3.2. Phytochemical Screening

The phytochemical screening, as a qualitative method, detected some bioactive compounds in plants, namely alkaloids, flavonoids, saponins, tannins, terpenoids, steroids/triterpenoids, and phenols. Table 2 shows the CFE phytochemical screening results. The results of CFE phytochemical screening contain flavonoids, alkaloids, phenols, triterpenoids, and terpenoids. The CFE exhibited the highest amount (++++) of phenol compounds, characterized by blackish-green color forms, serving as an indicator, whereas triterpenoids showed the lowest content (+) with brownish color forms.

### 3.3. Total Phenolic and Flavonoid Content of CFE

The research outcome confirmed that the overall level of phenolics in CFE reached 14.49±1.18 µg GAE/mg, indicating the presence of phenolic compounds, which were known for their antioxidant activities. Similarly, the flavonoid content in CFE was found to be 9.00±1.13 µg QE/mg, suggesting that flavonoids contributed significantly to the overall phytochemical profile of the extract (Table 3).

Table 2. Results of phytochemical screening test (CFE)

| Phytochemical content | Indicators | Test results of CFE |
|---|---|---|
| Flavonoids | An orange color forms in the bottom layer of amyl alcohol | (++) |
| Saponins | Foam forms, but after adding 1 drop of HCl, the foam disappears | (-) |
| Phenols | A blackish-green color forms | (++++) |
| Tannins | The amyl alcohol layer is greenish yellow (no orange/red color is formed) | (-) |
| Steroids/Triterpenoids | A brownish color forms | (-) Steroids (+) Triterpenoids |
| Terpenoids | A purple color is formed | (+++) |
| Alkaloids | An orange color forms | (+++) |

Table 3. Total phenolic and flavonoid content of CFE

| Total phenolics (µg GAE/mg) | Total flavonoids (µg QE/mg) |
|---|---|
| 14.49±1.18 | 9.00±1.13 |

*GAE: gallic acid equivalency, QE: quercetin equivalence

### 3.4. OH Scavenging Activity

The OH radical scavenging activity of CFE is demonstrated in Figure 1. The scavenging activity of CFE exhibited a significant difference in OH scavenging activity ($P<0.05$), with the highest activity observed at 13.33 µg/mL, corresponding to 36.67%. The $IC_{50}$ value of OH scavenging activity showed 18.39 µg/mL (Table 4). The OH $IC_{50}$ value presented that CFE had OH scavenging activity potential.

### 3.5. NO Scavenging Activity

The effect of CFE on NO scavenging activity is shown in Figure 2. NO scavenging activity at 66.67 µg/mL with 74.08±6.03% ($P<0.05$) showed the highest activity of CFE. The NO $IC_{50}$ value scavenging activity showed 31.11 µg/mL (Table 5). This $IC_{50}$ of NO value shows that CFE had NO scavenging activity potential.

### 3.6. Collagenase Inhibition Activity

Collagenase inhibitory activity data are shown in Figure 3. Collagenase inhibition activity showed the highest activity at 250 µg/mL with a value of 67.65±1.94% ($P<0.05$), while the lowest activity was observed at a concentration of 7.81 µg/mL. The collagenase $IC_{50}$ value inhibition activity showed 177.48 µg/mL (Table 6). This $IC_{50}$ collagenase value shows that CFE has collagenase inhibition potential.

### 3.7. Elastase Inhibition Activity

The CFE effect on elastase activity is shown in Figure 4. The elastase inhibition activity of CFE exhibited a significant concentration-dependent difference, with the highest activity observed at 66.67 µg/mL (85.73±0.52%). Increasing the CFE concentration further enhanced elastase inhibition activity significantly. The $IC_{50}$ value of elastase inhibition activity is 30.88 µg/mL (Table 7). This $IC_{50}$ elastase value showed that CFE had an elastase inhibition potential.

## 4. Discussion

Aging is a natural biological process that occurs in humans over time. Aging can be influenced by an imbalance in ROS levels in the body, which causes oxidative stress. This condition can cause degradation of elastin and collagen so that the skin becomes loose and wrinkles appear, one form of skin aging (Rinnerthaler *et al.* 2015). Excessive ROS levels require exogenous antioxidants that can come from natural ingredients. One promising source of natural antioxidants is CFE (Andriani & Murtisiwi 2020).

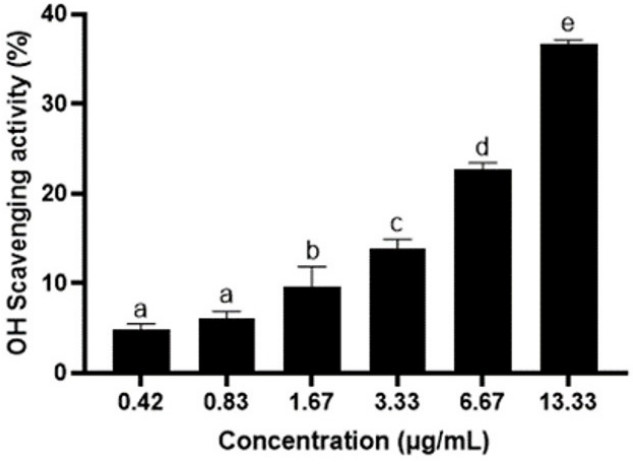


Figure 1. Effect of various concentrations of CFE on OH scavenging activity. *Data represented as the mean ± standard deviation (SD) of 3 replicates. Different letters represented the significant differences between treatments ($P<0.05$) according to the Tukey HSD post hoc test

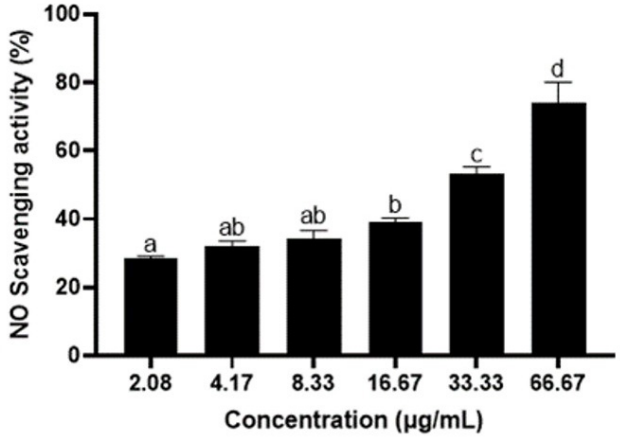


Figure 2. Effect of various concentrations of CFE on NO scavenging activity. *Data represented as the mean ± SD of 3 replicates. Different letters represented the significant differences between treatments ($P<0.05$) according to the Tukey HSD post hoc test.

Table 4. $IC_{50}$ value of OH activity in CFE

| Antioxidant activities | Equations | $R^2$ | $IC_{50}$ (µg/mL) |
|---|---|---|---|
| OH scavenging activity | y = 2.4536x + 4.874 | 0.99 | 18.39 |

Table 5. $IC_{50}$ value of NO activity in CFE

| Antioxidant activities | Equations | $R^2$ | $IC_{50}$ (µg/mL) |
|---|---|---|---|
| NO scavenging activity | y = 0.6959x + 28.352 | 0.97 | 31.11 |

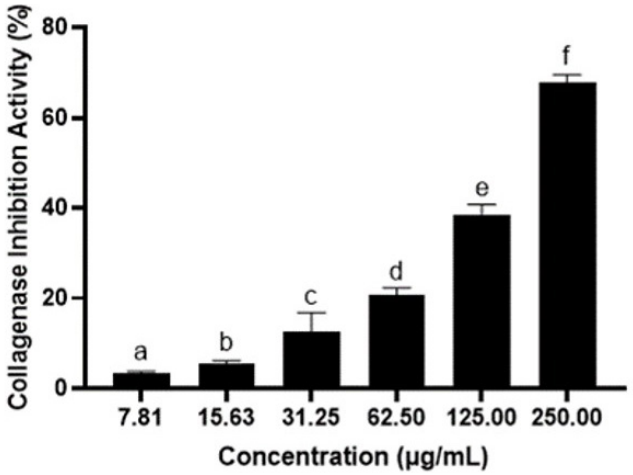


Figure 3. Effect of various concentrations of CFE on collagenase scavenging activity. *Data represented as the mean ± SD of 3 replicates. Different letters represented the significant differences between treatments (P<0.05) according to the Tukey HSD post hoc test

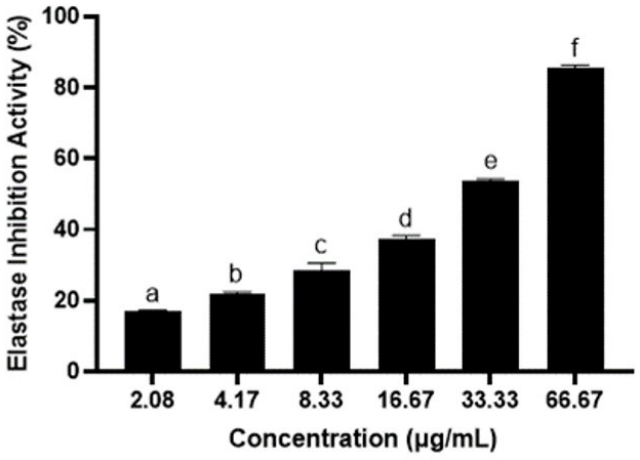


Figure 4. Effect of various concentrations of CFE on elastase inhibition activity. *Data represented as mean ± SD of 3 replicates is used to show the data. Different letters represented the significant differences between treatments (P<0.05) according to the Tukey HSD post hoc test

Table 6. $IC_{50}$ value of collagenase inhibitory activity in CFE

| Anti-aging activities | Equations | $R^2$ | $IC_{50}$ (µg/mL) |
|---|---|---|---|
| Collagenase inhibitory | y = 0.2647x + 3.0218 | 0.99 | 177.48 |

Table 7. $IC_{50}$ value of the elastase inhibitory activity in CFE

| Anti-aging activities | Equations | $R^2$ | $IC_{50}$ (µg/mL) |
|---|---|---|---|
| Elastase inhibitory | y = 1.0319x +18.133 | 0.99 | 30.88 |

In this study, *Clitoria ternatea* was extracted using the maceration method, and lactose was added to make a powder. The results of the CFE quality test showed that the CFE produced had high quality, both in terms of physical identification, physical characteristics, heavy metal content, and microbiological testing. This follows the provisions of good extract requirements according to BPOM RI (2023). The results of phytochemical screening showed that CFE contains triterpenoids, phenols, flavonoids, terpenoids, and alkaloids. These results are from other studies conducted by Torres *et al.* (2022) which showed the existence of triterpenoids, flavonoids, phenols, terpenoids, and alkaloids in CFE. Other parts of this plant, including the leaves, stems, and roots, have also been studied to contain flavonoids, phenols, triterpenoids, terpenoids, and alkaloids (Priyanga *et al.* 2019).

The research results showed that the total phenolic content in CFE reached 14.49±1.18 µg GAE/mg. The flavonoid content in CFE reached 9.00±1.13 µg QE/mg. This shows the high levels of flavonoids and phenolics in CFE. In line with this, an investigation by Patel *et al.* (2023) appeared that extracts from *C. ternatea* flowers had a total flavonoid content of 15.84±0.268 mg QE/g of extract. Several studies have shown that other parts of this plant, including roots and leaves, additionally contain high levels of flavonoids and phenolics. Nurcholis *et al.* (2023) compared the flavonoid and phenolic substances of different plant components. The results showed that the most elevated phenolic substance was found within the roots of *C. ternatea* (83.45 mg GAE/g). In contrast, the most elevated flavonoid content was found within the leaf extract (5.96 mg QE/g). Similarly, Purwanto & Aprilia (2022) reported that leaf extract contained phenolic substance of 57.51 mg GAE/g.

Reactive oxygen species (ROS) are manifested in various molecules, for example, hydroxyl (*OH), superoxide ($*O_2$), peroxyl (ROO*), singlet oxygen ($1O_2$), nitric oxide (NO*), hydrogen peroxide ($H_2O_2$), peroxynitrite (ONOO*), hypochlorous acid (HOCl), and lipid peroxidase. Superoxide is the most predominant free radical formed in the body. Within the oxidation response, this superoxide will be converted into hydrogen peroxide ($H_2O_2$), which can

be further converted into hydroxyl radicals (*OH) at the proliferation stage. These hydroxyl radicals cause lipid peroxidation within the cell membrane, leading to cell damage. If this process continues unchecked, it will cause an imbalance between free radicals and endogenous antioxidants known as oxidative stress (Purwata 2016; Kusumawati *et al.* 2021). Previous studies have shown similar results, in which CFE has antioxidant activity in assays such as ABTS, DPPH, and FRAP (Nurhayati *et al.* 2024). CFE is known to have antioxidant potential in inhibiting lipid peroxidation, with an inhibition percentage reaching 94% (Purwanto & Aprilia 2022).

Nitric oxide is a small gas molecule also known as nitrogen monoxide, with the chemical formula NO (Lancaster Jr 2015). The antioxidant impact of CFE was further assessed by its potential inhibitory effect on NO. This study demonstrated that CFE's anti-oxidant activity inhibits NO activity. Antioxidants can lower NO ability by giving up an electron. The extract contained antioxidant principles that prevent the synthesis of nitrite by competing with oxygen in their reaction with NO (Utami *et al.* 2018). This data was in line with research conducted by Purwanto & Aprilia (2022) that extracts from numerous parts of *C. ternatea*, such as flowers, leaves, and roots, have antioxidant potential with NO scavenging.

The collagenase enzyme can degrade collagen, which is the main component of the extracellular matrix (Nurhidayah 2020). Based on the research results, CFE has significant inhibitory activity against the collagenase enzyme. This inhibition is in line with the increasing concentration of CFE—studies showing that plant extracts and secondary metabolites can suppress collagenase degradation. Flavonoids, one of the compounds found in CFE, are known to have a role in inhibiting collagenase. The presence of hydroxyl and carbonyl groups in plant flavonoid particles permits them to make complexes with metal particles, allowing interaction with metalloenzymes such as collagenase. A study showed that this compound can anticipate collagen degradation by inhibiting collagenase movement (Zagórska-Dziok *et al.* 2021).

Excessive exposure to UV radiation and ROS enhances the activation of the elastase enzyme, leading to the destruction of elastin in the elastic fibers of the skin. This process is in charge of the formation of skin wrinkles (Nur & Lukitaningsih 2017). The elastase inhibitory activity of CFE increases as its concentration increases, indicating that CFE can inhibit elastase activity. Flavonoids have excellent antioxidant properties and can show their antioxidant activity by counteracting free radicals and ROS (Hassanpour & Doroudi 2023). In herbal plants, flavonoids and their derivatives are often found, which have the potential to act as elastase inhibitors (Jakimiuk *et al.* 2021). Thus, CFE possesses potential antioxidant and anti-aging agent through NO, OH scavenging activities and inhibition of collagenase and elastase. Excessive exposure to UV radiation and ROS increases the activation of the elastase enzyme, which causes damage to elastin in the skin's elastic fibers. This process plays a role in the formation of skin wrinkles (Nur & Lukitaningsih 2017).

Thus, CFE has an effective anti-aging and antioxidant mechanism through various processes. Flavonoid compounds in this extract act as antioxidants and anti-aging agents, neutralizing free radicals such as OH (hydroxyl radicals) and NO (nitric oxide) that can damage skin tissue and trigger aging. Additionally, this extract inhibits the activity of collagenase and elastase enzymes, which are responsible for the degradation of elastin and collagen proteins crucial for skin elasticity. This mechanism helps inhibit tissue damage and maintain skin health so that it can be used as an anti-aging and antioxidant in cosmetic products (Nur & Lukitaningsih 2017; Yuanda *et al.* 2023). The proposed mechanism of this research can be seen in Figure 5.

Exposure to aging factors, including UV radiation and free radicals, induces the formation of reactive oxygen species (ROS), which in turn trigger oxidative stress. This oxidative stress enhances the activity of collagenase and elastase, enzymes that degrade collagen and elastin—two key structural proteins responsible for maintaining skin strength and elasticity—thereby accelerating the aging process. Phytochemical screening has revealed that CFE is rich in flavonoids, phenols, triterpenoids, terpenoids, and alkaloids, which contribute to its ability to suppress ROS formation by scavenging hydroxyl and nitric oxide radicals, thus reducing oxidative stress. In addition, CFE protects collagen and elastin from enzymatic degradation, thereby preventing skin aging. These findings highlight the potential of CFE as an effective antioxidant and anti-aging agent.

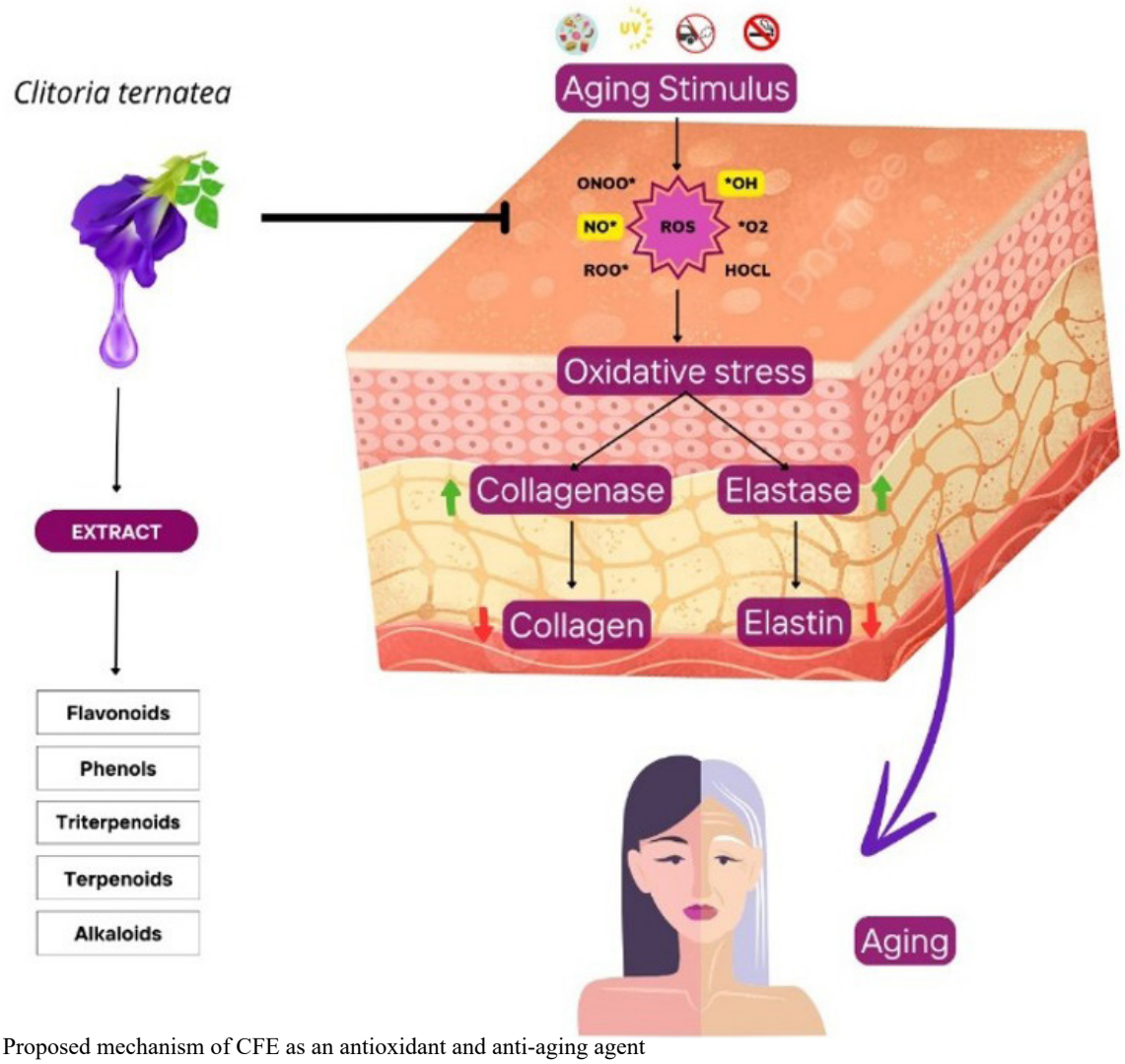


Figure 5. Proposed mechanism of CFE as an antioxidant and anti-aging agent

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