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ORIGINAL ARTICLE

# Ultrasonication Extraction, Bioactivity, Antioxidant Activity, Total Flavonoid, Total Phenolic and Antioxidant of *Clitoria Ternatea* Linn Flower Extract for Anti-aging Drinks

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## ABSTRACT

**Background:** Anti-aging health drinks currently are one of the largest consumer drinks. People are more concerned about their health and quality of health life in order to increase longevity. We have investigated that many anti-aging health drink products in Thailand are developed below the standard and do not meet the Thai FDA requirements in terms of bioactivities and toxicity analysis. **Objective:** This study aimed to evaluate the bioactivity quantities, active ingredients, total phenolic contents, and different extraction methods essential to obtain definite value of bioactivities, quantified antioxidant activity, and also acute toxicity analysis of *Clitoria ternatea* Linn flower extract drink. **Materials and Methods:** We performed ultrasonication extraction and maceration with 40% and 50% ethyl alcohol in different periods of time and also analysis of antioxidant activities with 2,2-diphenyl-1-picrylhydrazyl, ferric-reducing antioxidant power, and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid methods, total phenolic compound, active in gradients by high-performance liquid chromatography (HPLC). **Results:** We found that *C. ternatea* Linn flower extract drink consisted of high contents of gallic acid and rutin by HPLC method. There was no acute toxicity of *C. ternatea* Linn flower extract health drink.

**Key words:** Acute toxicity, antioxidant activity, *Clitoria ternatea,* flavonoids, phenolic content, ultrasonication extraction

## SUMMARY

- The phytochemical investigation of *Clitoria ternatea* flower extract drink revealed high contents of gallic acid and rutin run by high-performance liquid chromatography method
- The evaluation of antioxidant activity of *C. ternatea* flower extract drink was investigated among different kinds of maceration method with water and 40%, 50% ethanol in different periods of time, which was compared with ultrasonication extraction method
- There was no toxicity in *C. ternatea* flower extract drink experimented by white Wistar rats.

**Abbreviations used:** DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric-reducing antioxidant power; ABTS: 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid; HPLC: High-performance liquid chromatography; EtOH: Ethanol; PTZ: (2,4, 6–tripyridyl–striazine); $CH_3OH$: Acetic acid; $CH_3OH$: Methyl alcohol.

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## INTRODUCTION

*Clitoria ternatea* Linn usually grows in Thailand which was commonly named as "Butterfly Pea" or Anchun. It normally blooms throughout the year, especially in dry seasons during March–June. The plants are favorable to part sun or shady and to propagate their climbing stem.[1,2] The active ingredients of *C. ternatea* Linn can be extracted and developed for use in Thai cuisines and the leaves have a great deal of medicinal values as an antioxidant and eye nutrient.[3-5] Prepared anti-aging drink consisted of 1–3 kg of fresh flowers of *C. ternatea* Linn with 1.5 L of water and 1 L of *C. ternatea* Linn flower extract extracted from 100 to 300 g of *C. ternatea* Linn flower which could yield 0.4 L of flower extract. Then, both parts were mixed and adjusted to pH at 4, 70°C with honey syrup qs. added to 5 L. Jackie *et al.* (2011) by inducing lead acetate in rats found that the antioxidant activity of *C. ternatea* Linn can decrease oxidative stress from free radical scavenging enzymes and lipid peroxidation. Results revealed that *C. ternatea* Linn can inhibit lead-induced oxidative stress. It was found that methanol and acetone (50%, 90%, and 100%) can be used for the extraction of phenolic volatile oils including antioxidants such as flavonoid and anthocyanin from *C. ternatea* Linn flowers.[6] The analysis of the highest total phenolic extraction was at the amount of 1431 mg/QE 100 g using 50% acetone as the extraction solvent. This study was aimed at analyzing the active ingredients of *C. ternatea* Linn flower extracts[1] such as antioxidants, total phenolic compounds, and flavonoids using high-performance liquid chromatography (HPLC) and also investigated the comparison of antioxidant activities by 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric-reducing antioxidant power (FRAP), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) methods using different kinds of extraction solvents such as water, 50% ethanol, and with ultrasonication method.[7] Acute toxicity also has been tested in this study using white Wistar rats.[8] The anti-aging drink normally weighed of 1–3 kg with 1.5 L of water and 1 L of *C. ternatea* Linn flower extract extracted from 100 to 300 g of *C. ternatea* Linn flower which could yield 0.4 L of flower extract. Then, both parts were combined and adjusted to pH at 4 with honey syrup qs. added to a 5-L solution.

## MATERIALS AND METHODS

### Sample selection

The complete and healthy petals of *C. ternatea* Linn flowers were selected



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as samples from a *C. ternatea* Linn farm in Samut Songkhram province, central and south of Thailand during October, 2014. The samples were dried in air at normal room temperature and also dried in a hot air oven at 50°C until completely dried. The dried sample of *C. ternatea Linn* flowers was kept in closed containers at room temperature for preparing the next step of extractions.

## Extraction of samples

### *Extraction by ultrasonication method with 40% ethanol and 50% ethanol*

Ultrasonication method is an extraction technique using high-frequency ultrasonic sound for extraction purpose within limited amount of time without using high temperature. The sample was extracted with ultrasonication extraction for 30 min. Solvents used were 40% ethanol and 50% ethanol. The proportion of sample extraction solvent was 1:50 w/v. Then, the solution was filtered using filter paper no. 0. The filtered solution was then evaporated using evaporator at 50°C and was transferred to a freeze drying equipment.[7] The data of fresh and dried weight of yields were recorded before and after the freeze drying method. The freeze-dried coarse samples were kept in a closed container awaiting further analyzing steps.

### *Extraction with water by ultrasonication method*

The sample was extracted using ultrasonication extraction for 30 min. The solvents were purified with water. The proportion of sample extraction solvent was 1:50 w/v. Then, the solution was filtered using filter paper no. 0. The data of fresh and dried weight of yields were recorded before and after maceration with water and then the samples were kept in a closed container awaiting further analyzing steps.[7]

### *Extraction with ethanol maceration for 7 days*

The samples of *C. ternatea* Linn flower were macerated with 50% ethanol and 40% ethanol for 7 days. The coarse extracted powders were collected daily for 7 days and the proportion of sample extraction solvent was 1:50 w/v. Then, the solution was filtered using filter paper no. 0. The data of fresh and dried weight of yields were recorded before and after maceration with water and then the samples were kept in a closed container awaiting further analyzing steps.

## Acute toxicity analysis of *Clitoria ternatea* Linn flower extract

### *Preparation of raw material for Clitoria ternatea Linn flowers*

Samples of *C. ternatea* Linn flower extract were collected from Aumphawa farm, Samut Songkhram, Thailand. Healthy petals were selected for running the experiment. *C. ternatea* Linn flower samples were cleaned with purified water and dried in hot air oven at 50°C. Dried flowers were blended to powder form. The powder of dried *C. ternatea* Linn flowers was macerated in 50% ethanol at a proportion of 1:50. The powder was extracted by ultrasonication method for 30 min and was filtered with thin white cloth and filtered for the second time with filter paper no. 0. To free from ethanolic solvent, the filtered portion was subjected to freeze drying process using a rotary evaporator. The freeze-dried powder was given to white Wistar rats orally by mixing with 0.5% tween 80% solvent in order to further test acute toxicity.

### *Preparation of experimental white Wistar rats*

Female and male albino Wistar rats weighing 200–250 g weight were used in acute toxicity analysis of *C. ternatea* Linn flower extracts. Before the experiment, for feeding the experimental rats with food and water throughout the experiment, the room temperature was set at 25+ and −2 C and relative humidity at 40%–60%, exposing them to 12 h of daylight.[8]

## Experimental analysis in acute toxicity

This was a randomized controlled trial with fixed-effects model using two factors which were normal control albino Wistar rats with 0.5% tween 80 as a control group and albino Wistar rats orally induced with 2000 mg of *C. ternatea* Linn flower extracts using orogastric tube as an experimental group. All rats weighed 200–250 g. The volume used was 1 ml of *C. ternatea* Linn flower extracts to each rat of experimental group and 1 ml of 0.5% tween 80 to each rat of control group.

## Data observation symptoms of rats for acute toxicity analysis

We observed and recorded abnormality symptoms of experimental rats after 24 h and after 14 days. Observations of continuous breathing, ingestion, excretion, defecation, motility, suture, disorientation, anorexia, vomiting, the changes in weight including the records of no mortality in rats for 2 times revealed changes. At the end of the experiment, it had proceeded to the area of cervical dislocation of all rats and their blood samples were collected by cardiac ejections and sent for hematological analysis.[8,9] Mean, percentage, standard deviation, and standard error of mean (SEM) were used for statistical analysis using SPSS software.

## Bioactivity analysis

### *Total phenolic content analysis*

Standardized gallic acid at concentration of 12.5–1000 µg/ml in 80% ethanol and also the sample of *C. ternatea* Linn flower extract at the concentration of 1 mg/ml in 80% ethanol were prepared on three replications.[10-12] The analysis was carried out in 96-well microtiter plates added with 25 ml of samples and standardized solution. 125 µg/L of Folin–Ciocalteu reagent was added and rested for 5 min at room temperature, and then 75 g/l of $Na_2CO_3$ for 100 µg and distilled water qs. to 300 µg and the solution was rested at room temperature for 2 h until completing the reaction.[13-15] The final solution was brought to measure the concentration and the absorbance with a spectrometer (microplate) at 760 nm wavelength.[3] Graphs were plotted between the relationship of concentration of samples and the absorbance of light in order to calculate the result of total phenolic content related to gallic acid equivalent (µg gallic acid/mg extract).[7]

### *Total flavonoid content analysis*

Standardized quercetin at a concentration of 12.5–1000 µg/ml in 80% ethanol was prepared. Samples of *C. ternatea* Linn. flower extract were prepared at a concentration of 1 mg/ml in 80% ethanol in three replications. All were tested in 96-well plates by adding 25 µl of ample and 75 µl of 95% ethanol. Then, 5 µl of 10% $AlCl_3$ and 5 µl of 1 mol $K_3COOH$ were added to the solution and qs. was added to 140 µl with distilled water. The solution was rested at room temperature for 30 min until completing the reaction. The final solution was brought to measure the concentration and the absorbance with spectrometer (microplate) at 415 nm wavelength.[3] Graph was plotted and interpreted in linear equation to identify the relationship between standardized quercetin concentration and the concentration of sample related to the absorbance light. The absorbance value from the given sample was interpreted and changed to standard graph of flavonoid content which was equivalent to quercetin (µg quercetin/mg extract).[1]

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## Analysis of antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl method

*C. ternatea* Linn flower extract was prepared in five concentrations (15–500 µg/ml) of ethanol in test tube. DPPH was prepared in 60 µg/L of ethanol.

750 µl of flower extract was prepared in 750 µl of DPPH and was kept at room temperature for complete reaction for 20 min. Then, the solution was brought to be measured with spectrometer using ultraviolet (UV) light at 517 nm wavelength. The percentage radical scavenging was calculated with the following formula:

$$\% \text{ Radical scavenging} = \frac{(\text{A control} - \text{A sample}) \times 100}{(\text{A control})}$$

- A control is absorbance value of UV light of control
- A sample is absorbance value of UV light of sample.

The graph was plotted between concentration of *C. ternatea* Linn flower extract and percentage radical scavenging in order to calculate $EC_{50}$ value. All data were collected and recorded in order to calculate percentage of antioxidant and standard deviation of percentage of radical scavenging.

Results showed the comparison of $EC_{50}$ of samples and antioxidant standard substance of ascorbic acid.

## Analysis of antioxidant activity by ferric-reducing antioxidant power assay method

*C. ternatea* Linn flower extract was prepared at a concentration of 1 mg/ml. The FRAP reagent was also prepared which consisted of acetate buffer: 2,4, 6–tripyridyl–striazine and ferric chloride.[6,15,16] The proportion of the three reagents was 30:1:1. Then, 100 µl of *C. ternatea* Linn flower extract was mixed with 3000 µl of FRAP reagent in the test tube in order to complete reaction. The mixture was brought to measure the absorbance at 593 nm. Wavelength in 10 min with spectrometer and compared the standard curve of the graph of $FeSO_4$ in order to evaluate the $Fe_2$ (Fe equivalent) for six replications. $FeSO_4$ solution was prepared at 0.1, 0.25, 0.5, 1.0, 2.5, and 5.0 mmol. Each test tube contained 100 µl that reacted with 3000 µl FRAP reagent. The solution was used to measure the absorbance value at 593 nm wavelength of UV light in order to build the standard graph which showed the relationship with the concentration of $FeSO_4$. Data collection was recorded and the comparison of $Fe_2$ equivalent was made of samples and antioxidant activity reagent (ascorbic acid).[1]

## Analysis of antioxidant activity by 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid assay method

The standard Trolox solution was prepared at a concentration of 12.5–1000 µg/ml in 80% ethanol, and samples of *C. ternatea* Linn flower extract were also prepared at a concentration of 1 mg/ml in 80% ethanol with three replications.[1] The ABTS solution was prepared by mixing 7 mM ABTS with 2.45 mmol potassium per sulfate of the same ratio in the distilled water and the solution was kept in dark at room temperature for 6 h and was dissolved with $CH_3OH$ until obtaining the absorbance at 1.00 with 734 nm of UV wavelength. The test was carried out in the 96-well plate with standard sample at 150 µl. 150 µl of ABTS solution was added and kept in room temperature for 6 min for completion of reaction. The solution was used to measure the concentration of *C. ternatea Linn* flower extract sample against the absorbance with spectrometer (microplate reader) at 734 nm wavelength. The absorbance relating to *C. ternatea* Linn flower extract and standard graph to convert and to calculate equivalent value to Trolox solution.

(Trolox equivalent) or (microgram Trolox/mg extract).[13]

## Statistical analysis

Descriptive statistics used were mean and SEM. Inferential statistic used was one-way analysis of variance (ANOVA) and 1-sample K-S was used for testing the homogeneity of variance and RUN–test was used for testing the normal distribution. Results found population and sample had normal distribution therefore the sample was capable to further test for hypothesis evaluation. One-way ANOVA was used to test the statistics of hypothesis of antioxidant activity between standard solution and sample solution extracted from different kinds of solvent solutions. Results showed that there was statistically significant difference at $P = 0.05$. Therefore, we used SPSS software version 15 to evaluate the average mean scores in each pair of sample solution test using Scheffe's test.

# RESULTS

Fresh weight/dried weight of *C. ternatea* Linn flowers.

The data were collected after hot air processing method. It showed fresh weight of butterfly pea and dried weight of butterfly pea (20.59 and 2.03 g, respectively). The percentage yield is 9.84.

## Bioactivity analysis results of *Clitoria ternatea Linn* flower extracts

Table 1 shows the fresh weight of butterfly pea and dried weight of butterfly pea in gram (20.59 and 2.03 g, respectively). The percentage yield is 9.84.

Table 2 shows the antioxidant activity of *C. ternatea* Linn flower extracts with Vitamin C by DPPH method. It showed that macerating *C. ternatea* Linn flowers with 50% ethyl alcohol (EtOH) at the 4$^{th}$ day can yield the highest antioxidant activity while measured with DPPH method.

Table 3 shows the antioxidant activity of *C. ternatea* Linn flower extracts with ABTS ($IC_{50}$, µg/ml). It showed that macerating *C. ternatea* Linn flowers and sonicating in 40% EtOH can yield the highest antioxidant activity while measured with ABTS;

**Table 1:** Percentage yield of fresh weight and dried weight of *Clitoria ternatea* Linn flowers

| Plant | Weight (g) | Dry weight (g) | Percentage yield |
|---|---|---|---|
| *Clitoria ternatea* Linn | 20.59 | 2.03 | 9.84 |

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid ($IC_{50}$, μg/ml) method which yielded the amount of 0.47 ± 0.00.

Table 4 shows the antioxidant activity of *C. ternatea* Linn flower extracts with FRAP (μg Fe equivalent/g sample). It showed that macerating *C. ternatea* Linn flowers with macerating in 40% EtOH for 2 days can yield the highest antioxidant activity while measured with FRAP and yielded the amount of 0.84 ± 0.05.

Table 5 shows the result of total phenolic analysis (μg gallic acid equivalent/g sample) which showed the highest content of phenolic compound while *C. ternatea* Linn flowers were sonicated in 50% ethanol (EtOH) which yielded the amount of 0.68 ± 0.05.

Result of total flavonoid analysis (μg gallic acid equivalent/g sample) showed the highest content of flavonoid compound while *C. ternatea* Linn flowers were macerated in 40% ethanol (EtOH) for 7 days which yielded the amount of 1.53 ± 1.01 [Table 6].

Table 7 shows the analysis of active compounds by HPLC method sample compounds replication retention time area under curve contents (ug/mg extract).

Table 8 shows the result of hematological values in Wistar rats by *C. ternatea* Linn flower extract (2000 mg/kg body weight).

Table 9 shows the result of hematological values in Wistar rats by *C. ternatea* Linn flower extract (2,000 mg/kg body weight) continued.

Table 10 shows the result of internal organ weight (g) of Wistar rats using *C. ternatea* Linn flower extract (2000 mg/kg body weight). The result of bioactivity analysis using DPPH [Table 2], ABTS [Table 3], and FRAP [Table 4] antioxidant activity analysis, total phenolic content [Table 5], total flavonoid content [Table 6] and the results also were compared with the ultrasonication extraction using the water maceration, 7 d maceration with 50% and 40% ethanol.

## Results of active ingredients from high-performance liquid chromatography analysis

The active ingredients such as gallic acid, catechin, ferulic acid, and rutin [Table 10] were investigated. The analysis of total phenolic contents was selected from the highest oxidant activity from the extraction in order to analyze the compositions using residues of apples from the manufactures which composed of acetonitrile (a) and 0.01% trifluoroacetic acid (b); the rate of flow was calculated at 1.0 ml/min under gradient conditions. injected *C. ternatea Linn* flower extract 10 ml to the system and compared with the standardized compound of 1 = gallic acid, 2 = catechin, 3 = chlorogenic acid,

**Table 2:** Antioxidant activity of *Clitoria ternatea* Linn flower extracts with Vitamin C by 2,2-diphenyl-1-picrylhydrazyl method

| Code | Plant extract method | Mean±SD |
|---|---|---|
| 1 | *Clitoria ternatea* Linn sonicated with 50% EtOH | 0.68±0.05 |
| 2 | Sonicated with 40% EtOH | 0.50±0.08 |
| 3 | Macer water | 0.43±0.02 |
| 4 | Macer 50% EtOH day 1 | 0.28±0.02 |
| 5 | Macer 40% EtOH day 3 | 0.43±0.07 |
| 6 | Macer 50% EtOH day 4 | 0.25±0.05 |
| 7 | Macer 50% EtOH day 5 | 0.32±0.01 |
| 8 | Macer 50% EtOH day 6 | 0.36±0.03 |
| 9 | Macer 40% EtOH day 7 | 0.48±0.01 |

SD: Standard deviation; EtOH: Ethanol

**Table 3:** Result of antioxidant activity using ABTS ($IC_{50}$, μg/ml)

| Code | Plant extract method | Mean±SD |
|---|---|---|
| 1 | *Clitoria ternatea* Linn sonicated in 50% EtOH | 0.13±0.01 |
| 2 | *Clitoria ternatea* Linn sonicated in 40% EtOH | 0.55±0.03 |
| 3 | Macer water | 0.10±0.01 |
| 4 | Macer 50% EtOH day 4 | 0.28±0.00 |
| 5 | Macer 40% EtOH day 4 | 0.47±0.00 |
| 6 | Macer 50% EtOH day 5 | 0.29±0.02 |
| 7 | Macer 40% EtOH day 5 | 0.09±0.00 |
| 8 | Macer 50% EtOH day 6 | 0.22±0.00 |
| 9 | Macer 40% EtOH day 6 | 0.40±0.01 |

SD: Standard deviation; EtOH: Ethanol

**Table 4:** Result of antioxidant analysis using ferric-reducing antioxidant power (μg Fe equivalent/g sample)

| Code | Plant extract method | Mean±SD |
|---|---|---|
| 1 | *Clitoria ternatea* Linn sonicated in 50% EtOH | 0.124±0.01 |
| 2 | Sonicated in 40% EtOH | 0.08±0.01 |
| 3 | Macer water | 0.78±0.10 |
| 4 | Macer 40% EtOH day 1 | 0.76±0.02 |
| 5 | Macer 40% EtOH day 2 | 0.84±0.05 |
| 6 | Macer 40% EtOH day 3 | 0.76±0.04 |
| 7 | Macer 40% EtOH day 5 | 0.55±0.03 |
| 8 | Macer 40% EtOH day 6 | 0.37±0.50 |
| 9 | Macer 40% EtOH day 7 | 0.46±0.02 |

SD: Standard deviation; EtOH: Ethanol

**Table 5:** Result in total phenolic analysis (μg gallic acid equivalent/g sample)

| Code | Plant extract method | Mean±SD |
|---|---|---|
| 1 | *Clitoria ternatea* Linn sonicated in 50% EtOH | 0.68±0.05 |
| 2 | *Clitoria ternatea* Linn sonicated in 40% EtOH | 0.15±0.00 |
| 3 | Macer water | 0.074±0.00 |
| 4 | Macer 40% EtOH day 2 | 0.28±0.00 |
| 5 | Macer 40% EtOH day 4 | 0.29±0.00 |
| 6 | Macer 40% EtOH day 5 | 0.29±0.01 |
| 7 | Macer 40% EtOH day 6 | 0.29±0.00 |
| 8 | Macer 40% EtOH day 7 | 0.28±0.00 |
| 9 | Macer 40% EtOH day 7 | 0.29±0.00 |

SD: Standard deviation; EtOH: Ethanol

**Table 6:** Contents of total flavonoid (microgram quercetin/mg sample)[3]

| Code | Plant extract method | Mean±SD |
|---|---|---|
| 1 | *Clitoria ternatea* Linn sonicated in 50% EtOH | 0.11±0.07 |
| 2 | Sonicated in 40% EtOH | 0.08±0.20 |
| 3 | Macer water | 0.05±0.03 |
| 4 | Macer 40% EtOH day 1 | 0.38±0.25 |
| 5 | Macer 40% EtOH day 2 | 0.45±0.31 |
| 6 | Macer 40% EtOH day 3 | 0.44±0.30 |
| 7 | Macer 40% EtOH day 5 | 0.53±0.30 |
| 8 | Macer 40% EtOH day 6 | 0.43±0.10 |
| 9 | Macer 40% EtOH day 7 | 1.53±1.01 |

SD: Standard deviation; EtOH: Ethanol

**Table 7:** Analysis of active compounds by high-performance liquid chromatography method replication retention time area under curve contents (ug/mg extract)

| *Clitoria ternatea* Linn | | | | |
|---|---|---|---|---|
| Gallic acid | 1 | 4.91 | 5146710 | 9.41 |
| | 2 | 4.90 | 6096240 | 11.15 |
| | 3 | 4.90 | 5530800 | 10.12 |
| Mean±SD | | 10.23±0.23 | | |
| Rutin | 1 | 11.80 | 4904460 | 13.43 |
| | 2 | 11.79 | 5214610 | 14.28 |
| | 3 | 11.79 | 5271760 | 14.44 |
| Mean±SD | | 14.05±0.54 | | |

SD: Standard deviation

**Table 8:** Result of hematological values in Wistar rats by *Clitoria ternatea* Linn flower extract (2000 mg/kg body weight)

| Parameters | Control group | *Clitoria ternatea* Linn |
|---|---|---|
| Glucose (mg/dl) | 146.60±8.15[a] | 149.37±12.83[a] |
| BUN (mg/dl) | 26.48±1.58[a] | 27.24±1.28[a] |
| Creatinine (mg/dl) | 0.84±0.02[a] | 0.88±0.03[a] |
| Uric (mg/dl) | 3.72±0.28[a] | 3.76±0.34[a] |
| Cholesterol (mg/dl) | 78.00±5.95[a] | 74.13±5.74[a] |
| TG (mg/dl) | 123.60±13.41[a] | 120.26±9.16[a] |
| HDL (mg/dl) | 16.60±1.12[a] | 15.61±0.72[a] |

[a]Statistically significant difference at $P \geq 0.05$ compared with control group.
BUN: Blood urea nitrogen; TG: Triglyceride; HDL: High-density lipoprotein

**Table 9:** Result of hematological values in Wistar rats by *Clitoria ternatea* Linn flower extract (2000 mg/kg body weight) continued

| Parameters | Control group | *Clitoria ternatea* Linn flower extract |
|---|---|---|
| LDL (mg/dl) | 38.00±5.31[a] | 37.83±3.54[a] |
| Total protein (mg/dl) | 6.06±0.12[a] | 6.08±0.10[a] |
| Albumin (g/dl) | 3.86±0.06[a] | 3.78±0.06[a] |
| Globulin (g/dl) | 2.20±0.07[a] | 2.17±0.10[a] |
| Total bilirubin (mg/dl) | 0.30±0.03[a] | 0.36±0.07[a] |
| AST (U/L) | 78.60±2.60[a] | 81.32±7.25[a] |
| ALT (U/L) | 29.20±0.96[a] | 33.97±3.18[a] |

[a]Statistically significant difference at $P \geq 0.05$ compared with control group.
LDL: Low-density lipoprotein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase

**Table 10:** Result of internal organ weight (g) of Wistar rats using *Clitoria ternatea* Linn flower extract (2000 mg/kg body weight)

| Organ | Control group | *Nelumbo nucifera* Linn flower extract |
|---|---|---|
| Liver | 4.84±0.31[a] | 4.93±0.52[a] |
| Kidneys | 0.76±0.04[a] | 0.80±0.05[a] |
| Heart | 0.44±0.02[a] | 0.42±0.03[a] |
| Lungs | 0.58±0.04[a] | 0.56±0.03[a] |

[a]Statistically significant difference at $P \geq 0.05$ compared with control group

4 = rutin, 5 = ferulic acid, 6 = quercetin at concentrations of 50–500 µg/ml using 280 nm wavelength for analysis of active ingredient compounds.

## DISCUSSION

The percentage of fresh weight/dried weight of *C. ternatea* Linn flowers was 9.84. The antioxidant activity analysis by DPPH method found that there was no statistically significant difference ($P = 0.05$) between 50% and 40% ethanol-macerated *C. ternatea* Linn flowers and standard Vitamin C at day 4$^{th}$ which gave the highest antioxidant activity using macerated 50% ethanol C. *ternatea* Linn at day 4$^{th}$. The antioxidant activity analysis by ABTS method ($IC_{50}$, mg/ml) found that there was no statistically significant difference ($P = 0.05$) between 40% ethanol-macerated *C. ternatea* Linn flowers and Trolox at day 4$^{th}$ which gave the highest antioxidant activity using macerated 40% ethanol at day 4$^{th}$ and showed the result of 0.47 ± 0.00. The antioxidant activity analysis by FRAP method (Fe µg/mg of sample) found that macerated *C. ternatea* Linn flowers in 50% ethanol showed the highest antioxidant activity at day 2 and showed the result of 0.84 ± 0.05. The highest total phenolic compound in *C. ternatea* Linn flower extracts (microgram of gallic acid/mg of sample) sonicated in 50% EtOH at day 6 was of 0.68 ± 0.05. The highest total flavonoid content in *C. ternatea* Linn flower extracts (microgram quercetin/mg sample) macerated with 40% EtOH day 7 was 1.53 ± 1.01. The HPLC results from *C. ternatea* Linn flower extracts showed that mean values of gallic acid and rutin contents (µg/mg extract) were 10.23 ± 0.23 (mean ± standard deviation [SD]) and 14.05 ± 0.54 (mean ± SD), respectively. The result of acute toxicity analysis of *C. ternatea* Linn flower extracts in albino Wistar rats showed no sign of mortality or abnormality in albino Wistar rats with no toxicity which could indicate no acute toxicity in *C. ternatea* Linn flower extracts. Then, all samples and control albino Wistar rats were shown to carry out cervical dislocation and bring peaceful death. Cardiac blood was withdrawn from each albino Wistar rat hearts for hematological analysis. There were no statistically significant difference ($P = 0.05$) in comparison of hematological values in blood glucose, blood urea nitrogen, creatinine, uric acid, cholesterol, triglyceride, low-density lipoprotein, high-density lipoprotein, total protein, albumin, globulin, total bilirubin, aspartate aminotransferase, and alanine aminotransferase shown between orally introduced *C. ternatea* Linn flower extracts in albinos Wistar rats sample group for 24$^{th}$ h and after 7 days and control group.

## CONCLUSIONS

We suggest that the samples of anti-aging *C. ternatea* Linn flower extract drinks are safe and can be developed as a health drink in the market. Further market analysis with sensory test is essential for developing the quality relating to the customer needs in further researches.

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### Conflicts of interest

There are no conflicts of interest.

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