Medical Sains : Jurnal Ilmiah Kefarmasian
Vol. 8 No. 3, July – September 2023
https:/ojs.stfmuhammadiyahcirebon.ac.id/index.php/iojs

SOLID EYELINER FORMULATION CONTAIN CLITORIA TERNATE L. EXTRACT IN PH ACID AND ALKALI AND IN VIVO IRRITATION TEST

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Submitted: 20 May 2023 ***Revised: 23 August 2023*** ***Accepted: 23 August 2023***

ABSTRACT

Clitoria ternatea flowers contain blue anthocyanin that has potential as natural coloring. The purpose of this study was to obtain an eyeliner product containing natural coloring that is safe, stable, and can replace hazardous chemicals. *Clitoria ternatea* flowers were extracted by kinetic maceration with 70% ethanol. The obtained extract was subjected to standard quality tests, phytochemical screening, and determination of total anthocyanin content and antioxidant activity (DPPH assay). Eyeliner-containing extracts were prepared in three pH variations (acidic, neutral, and alkaline). These three types of eyeliner preparations were subjected to an irritation test on shaved male albino white rabbit skin. stability test using the cycle test method, hardness, break point, and melting point were also performed. The results showed that *Clitoria ternatea* extract contains 0.2187% anthocyanin, has an antioxidant activity IC_{50} of104.7 µg/mL, and is free of heavy metals. The eyeliner contained *Clitoria ternatea* extract was dark blue when applied to the skin. Eyeliner preparations were stable in three stability cycle tests. The irritation test on the male albino rabbit skin showed no irritation. *Clitorial ternatea* extract can be used as natural coloring in eyeliner preparation.

Keywords: Butterfly pea, *Clitoria ternatea* L., Anthocyanin, Antioxidant, Eyeliner.

INTRODUCTION

Aside from being decorative plant, the *Clitoria ternatea* L., commonly known as ‘Butterfly pea’ has been widely used traditionally by the locals to heal eye irritations, blain, digestive problems to anti-depressants. Furthermore, it is believed that the *Clitoria ternatea* L. flower can improve visions and has been applied to infants (Budiasih, 2017). In addition to its medical benefits, *Clitoria ternatea* L. has been used as a natural and attractive blue color for food and traditional ice popsicles (Budiasih, 2017).

Clitoria ternatea L. contains saponin, alkaloid, flavonoid, ca-oxalat and sulfur . The flower, as shown in figure 1, is a source of the natural dark blue color derived from the petals. The source of the indigo color was extracted from blue and purple flower petals. The indigo color of *Clitoria ternatea* L. is categorized as anthocyanin. Anthocyanins and anthoxanthin are water-soluble flavonoid pigments (Khoo et al., 2017). Anthocyanins are derived from aglycones and are esterified with one or more glucose molecules (Khoo et al., 2017). Aside from its function as a natural coloring for edible food, *Clitoria ternatea* L. can also be used in cosmetics, such as the eyeliner (Khoo et al., 2017).

The number of eyeliners produced locally in Indonesia is relatively small. The ingredients used in the production of eyeliners are imported from abroad. The eyeliners sold in the market use synthetic artificial coloring. This offers great potential for the natural coloring of dark blue eyeliners produced from *Clitoria ternatea* extract.

The scope of this study was to determine whether eyeliner preparations with natural pigment from *Clitoria ternatea* extract are stable during storage and have characteristics of eyeliner preparations that are easy to use. In addition to the stability and characteristics of the preparation, the eyeliner with *Clitoria ternatea* extract has clinical safety in that it is not irritating and causes an allergic reaction to the eye skin.

RESEARCH METHODS

Equipment and Materials

Materials

Clitoria ternatea L. petals are obtained from cultivation in soiled vase, Ethanol 70%, Carnauba wax, microcrystal wax, castor oil, Paraffin, Vaseline, Lanolin, Citric Acid, Triethanolamine (PT. Sumber Berlian Kimia), Soy wax and Surfactant (Jebsen & Jessen), Rabbits (local white male New Zealand, obtained from Bogor Agricultural University, 5 months old and approximately 1 to 1.5 kilograms body weight) were used for in vivo assay.

Instruments

A rotary evaporator (Buchi, RII), eyeliner mold (property of PT. Kemas Indah Maju), Fudoh Rheometer, Meihoh Sharp Melting Pointer, Spectrophotometer (Shimadzu UV 1800), Refrigerator (LG), kinetic macerator, Oven (Memmert), caliper (Mitutoyo).

Research Procedure

1. Plant Determination
   Plant determination was performed at the Herbarium Bogoriense Biology Research Center-LIPI, Cibinong (certificate No. 2252/IPH.1.01/If.07/IX/2018). The performance of the Clitoria ternatea flower is described in Figure 1.

Figure 1. *Clitoria ternatea* Flower

2. Extraction Process
   Clitoria ternatea petals were extracted by kinetic maceration, and the filtrate was evaporated using a rotary evaporator to obtain a concentrated extract (Khoo et al., 2017).
3. Quality Parameter Test on Extract
   The specific quality test parameters of the extract included organoleptic testing of the form, color, scent, taste, and phytochemical screening (identifying the content of alkaloids, flavonoids, anthocyanins, phenols, tannins, saponins, and quinones). A phytochemical analysis was performed to determine the presence of major secondary metabolites. Organoleptics are used to describe shape, color, scent/smell, and taste. The consistency and color of the ethanol extract were visually observed, and smell was determined using sensory organs (nose and tongue). Nonspecific quality parameters included the test of total ash, moisture content (Karl Fischer method), level of soluble extract in ethanol, and heavy metal contamination (determined using the Atomic Absorption Spectrophotometry method) (BPOM, 2014).
4. Total Anthocyanin Content
   The total anthocyanin content was measured by the pH differential method at pH 1.0 and 4.5. This is a rapid and simple spectrophotometric method based on anthocyanin structural transformation that occurs with a change in pH (colored in pH 1.0 and colorless in pH 4.5) (Zheng et al., 2011).
5. Antioxidant Activity with DPPH (Free Radical Scavenging Activity Determination)
   Antioxidant activity was tested as the release of the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. In the radical form, DPPH maximum absorption at λ515 nm. One mL of 0.5 mM DPPH solution in methanol was placed into vials. Sample solutions prepared in 0 ppm; 40 ppm; 60 ppm; 80 ppm; 100 ppm; and 150ppm in methanol were

added to vials contain DPPH 0.5 mM solution. Methanol was then added to the sample solution until 5mL. DPPH absorption measured at λ 515 nm. Vitamin C was used as the comparison standard (Alamsyah et al., 2016).

6. Eyeliner Formulation

Eyeliner formulation was described in Table I.

All waxes and oil-based materials were weighed and placed in a hot water bath at 90 °C for melting. The surfactant was weighed and added to the oil base and stirred in a water bath (70 – 80^{0}C) (bulk). After blending, the extract was weighed, added to the mixture, and stirred well in a water bath (70 – 80^{0}C). The bulk material was poured into a mold and cooled in the chiller. Remove content in the containers. Citric acid was added to the *Clitoria ternatea* extract to obtain acidic pH (purple color) and triethanolamine under alkaline conditions (green). The performance of the finished eyeliner is shown in Figure 2.

Table I. Eyeliner Formulation

Component		Quantity (% w/w)			
		F1 (Blank)	F2	F3	F4
Oil Base	Carnauba wax	6.0	6.0	6.0	6.0
	Ozokerite wax	9.0	9.0	9.0	9.0
	Microcrystalline wax	12.1	12.1	12.1	12.1
	Soy wax	21.2	21.2	21.2	21.2
	Paraffin	6.0	6.0	6.0	6.0
	Castor oil	6.0	6.0	6.0	6.0
	Vaseline	6.0	6.0	6.0	6.0
	Lanoline	3.0	3.0	3.0	3.0
	Cetyl alcohol	3.0	3.0	3.0	3.0
Surfactant	Poligliseril-2-stearate-Gliseril Stearate- Stearil alcohol	6.0	6.0	6.0	6.0
Pigment	Clitoria Ternatea extract	-	21.2	21.2	21.2
	Citric acid	-	-	1.5	-
	Triethanolamine	-	-	-	1.5

Figure 2. Eyeliner Finished Product

RESULTS AND DISCUSSION

Extract Quality Parameter Test

The results of the specific extract quality parameters, organoleptic, are shown in Table II. Phytochemical screening of *the Clitoria ternatea* extract, Table II respectively, showed flavonoid, anthocyanin, and phenol contents. Therefore, this flower can be used as a pigment for products, as it has been confirmed to have anthocyanin. The results of the non-specific quality parameters are shown in Table III.

The test trial met the regulation of (BPOM 2014) that moisture content in the extract ≤ 10% and moisture content of *Clitoria* ternatea extract met the requirement (0.5%). The total Ash content is used to acknowledge the inorganic or mineral residue after the ash process. The standard parameter from the Health Department, 2008 Indonesia Herbal Pharmacopoeia should not more than 2%. Therefore, the content of *Clitoria ternatea* extract also met this requirement. The level of the soluble extract in ethanol was 19.81%, indicating that the extract dissolved in ethanol was 19.81%. The extract was not contaminated with heavy metals.

Table II. Specific Parameter Organoleptic Result Of *Clistoria ternatea* Extract

Specific Parameter Organoleptic	Result
Form	Thick extract
Color	Dark Blue
Smell	Has not specific aromatic odor
Taste	Bitter

Table III. Phytochemical Screening Result Of *Clistoria ternatea* Extract

Compound Group	Screening Result
Alkaloid	
- Dragendroff reagent	-
- Mayer reagent	-
- Wagner reagent	-
Flavonoid	+
Saponin	-
Anthocyanin	+
Tannin	-
Phenol	+
Quinone	-

Notes : - is negative reaction
+ is positive reaction.

Table IV. Nonspecific Quality Parameter Of *Clitoria ternatea* Extract

Nonspecific parameter	Result
Moisture content	0.5 %
Total Ash	3.29%
Level of soluble extract in the ethanol	19.81%
Pb metal contamination	-
Hg metal contamination	-
Cd metal contamination	-
As metal contamination	-

Notes : - negative reaction

+ positive reaction.

Total Anthocyanin Content

The total anthocyanin content of *the Clitoria ternatea* extract was 0.2187±0.011%. As research by (Sangadji et al., 2017). The total anthocyanin content in various flowers (using quantitative methods and analysis of anthocyanin content by the explorative method), *Hibiscus rosa sinensis* L. (0.739%), *Mirabilis jalapa* L. (0.977%), *Hibiscus sabdariffa* L. (0.795%), and *Rosa sherardi* (0.925%) (Sangadji et al., 2017). These flowers have a color contrast to *Clitoria ternatea*, so the total anthocyanin content of *Clitoria ternatea* is lower than that of the flowers.

Table V. Total Anthocyanin Content In *Clitoria ternatea* Extract

Sample	Kadar (%)
Antosianin 1	0,2031
Antosianin 2	0,2073
Averange (%)	0,2052±0,02%

Antioxidant Activity with DPPH (Free Radical Scavenging Activity Determination) Method

Antioxidant activity was determined using the DPPH method with vitamin C as the comparison standard. The parameter used to represent the DPPH Assay result was the IC_{50} value. The smaller the IC_{50} value, the higher the antioxidant activity. The IC_{50} value of *Clitoria ternatea* extract was 104,71% and compared to Vitamin C, with an IC_{50} value of 5.98% for Vitamin C. The antioxidant activity of *Clitoria ternatea* extract is moderate, and Vitamin C has a high level of antioxidant activity. As research by (Djamil et al., 2017), the antioxidant activity of 70% ethanol extract of Bungur Leaves (*Lagerstroemia speciose* L. Pers) was 26.5% (Djamil et al., 2017). Bungur leaves are purple flowers and are a nutritious plant used in medicine in Indonesia. Bungur leaves contain chemicals similar to those in *Clitoria ternatea*, so a comparison was made between these two flowers. Based on these results, the 70% ethanol extract of Bungur leaves had a very strong antioxidant activity (26.5%). The antioxidant activity of the 70% ethanol *Clitoria ternatea* extract was 104.71%, indicating moderate activity. The flower colors of *Clitoria ternatea* in blue and Bungur leaves in purple have different antioxidant activities: *Clitoria ternatea* 70% ethanol extract has lower antioxidant activity than Bungur leaves.

Table VI. Antioxidant Determination Category

IC_{50} Value (ppm)	Category
< 50	Very strong
50 – 100	Strong
100 – 150	Middle
150 – 200	Weak
>200	Very Weak

Table VII. Antioxidant Activity Test Result (IC_{50}) In *Clitoria ternatea* Extract

Sample	IC_{50} (µg/mL)	IC_{50} Averange (µg/mL)
Vitamin C Repeat 1	5,01	5,98±1,37
Vitamin C Repeat 2	6,96	
Clitoria ternatea Extract Repeat 1	107,23	104,71±3,56
Clitoria ternatea Extract Repeat 2	102,19	

Eyeliner Evaluation

There are three variant colors of eyeliners that have been made. The formulation with the original extract was dark blue, the acidic pH was purple, and the alkaline pH was green, as shown in Figure 3.

Figure 3. Eyeliner Color Variants

Organoleptic Test

Eyeliner content is made as a solid stick with dark blue, purplish blue, and green colors. The content still has the signature scent of the *Clitoria ternatea* flower extract (Alamsyah et al., 2016).

Homogeneity Test

The results show that the bulk can disperse evenly, and there is no granulized bulk (Alamsyah et al., 2016).

Emulsion Test

The emulsion test results from the microscope showed that the methylene blue added to the bulk content was evenly dispersed, causing a blue color. It is O/W emulsion. The results of the emulsion-type test are presented in Table IV (Alamsyah et al. 2016).

Bulk pH Test

Bulk pH tested by using pH meter as shown in Table V.

The bulk pH test result was the color determination of the extract due to changes in pH.

The color of anthocyanins depends on the pH of the solution. Under acidic conditions, some anthocyanins appear red (pH >3), purple at pH 6, purple to blue at neutral pH, greenish to yellow in alkaline solution (pH >7), and colorless in very alkaline solution, where the pigment is completely reduced (Alamsyah et al., 2016)(Khoo et al., 2017).

Table VIII. Bulk pH and Emulsion Test Result

Bulk Sample	pH	Emulsion type
Blue	8.46	O/W
Purple	5.17	O/W
Green	8.81	O/W

Uniformity of Weight

The eyeliner bulk content weighed 0.20 – 0.22 ±0,007 gram. This means that all materials mixed in the making of the content were well blended. In Indonesia Pharmacopoeia, third edition, the deviation of weight in the solid bulk content less than 25 mg weight is not more than 2 pieces with weight deviation higher than 15% and not even 1 piece higher than 30%. The eyeliner weight produced is still within the standard range (Ditjen, 2013).

Hardness Test, Break Point and Melting Point

Hardness and break points were tested using a Fudoh Rheometer instrument. The melting point was tested using a Meihoh Sharp Melting Pointer, as shown in Table VI. It is clear that the above test results show stability for the three differences of acid, alkaline, and normal conditions; therefore, in any condition, the hardness, breakpoint, and melting point of the eyeliner.

Table IX. Hardness, Break Point, and Melting Point Test Results

Sample	Hardness (gram/cm)	Break Point (gram/cm)	Melting Point (^oC)
Blue	55,5 – 76	42 - 76	53 - 60
Purple	51 – 69	42 – 51	52 - 60
Green	51 - 70	42 – 53	52 - 60

Irritation Test on Rabbit Skin (In Vivo)

The irritation test on rabbit skin (in vivo) was carried out with ethical approval no. B/2144/VIII/2019/KEPK.

The results showed no Edema or Erythema after 72 hours observation on rabbit skin, as shown in Table VII. The results of irritation test (in vivo) on white rabbit skin were positive. There was no erythema or edema reaction after the three colors of eyeliner were applied to the rabbit skin after 24, 48, and 72 hours.

Table X. Skin Irritation Test Result

Rabbit	Edema and Erythema Observation on Rabbit Skin																	
	24 Hours						48 Hours						72 Hours					
	Blue		Purple		Green		Blue		Purple		Green		Blue		Purple		Green	
	E	Er	E	Er	E	Er	E	Er	E	Er	E	Er	E	Er	E	Er	E	Er
I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Notes : E = Edema
Er = Erythema
0 = no found negative reaction

Stability Test

A stability test after 3 cycle time trial showed that organoleptic tested on eyeliners (3 color variants) remained stable with no changes in scent, shape, and color. One cycle lasted 48 hours (2 days) (Cosmetics Europe, 2004). Eyeliner preparation was evaluated for organoleptic characteristics in each cycle test. The characteristics required remained stable with no changes in the scent, shape, or color after each cycle test. The Eyeliner preparations were tested for hardness, break point, and melting point after 3 cycles of stability testing (Table IX). If the product passes three cycles, it has a good degree of confidence in the stability of the product. This puts the emulsion under tremendous stress, and if it passes the test, it indicates that it will have a stable product at room temperature. The product may be stable for 1-2 years at room temperature (Djamil et al., 2017).

Table XI. Hardness, Break Point and Melting Point Test After 3 Cycle Tests

Sample	Hardness Test (gram/cm)	Break Point Test (gram/cm)	Melting Point (^oC)
Blue	25 – 32	25 - 35	52 - 63
Purple	22 – 30	25 - 33	50 - 59
Green	25 - 30	27 - 38	42 - 49

The stability test of eyeliners using the cycle testing method showed a stable result and passed the organoleptic test. However, during application after three cycle tests, an agglomerated bulk was observed. Most probably, it is caused by waxes based on eyeliner ingredients that have changed chemically because of the high temperature over a long period of time. The changes caused by the waxes are not stable at high temperatures for a period of time. The wax was heated for no longer than 7-8 hours at 54 °C and 72 h at 45 °C during cycle testing, it is 72 hours in 45^oC.

The hardness after the cycle test decreased by approximately 26 – 30 gram/cm, breakpoint test decreased by approximately 15 – 17 gram/cm. Heating for a long period often causes problems and affects the appearance of the finished products, changes the chemistry of waxes, and may cause the wax to separate. The changes are the reduction in stiffness and bulk break point. Other causes are also the small size of the eyeliner content, which is only 3 mm in diameter compared to the bulk in lipstick or other cosmetic products. The melting points of the eyeliners did not show significant changes. The changes in the hardness, break point, and melting point after cycle testing are shown in Table VIII. The eyeliner product should not exposure at the temperature higher than room temperature. It should keep in room temperature to avoid chemically changes of the eyeliner ingredients. Regarding to the cycle test result, the eyeliner should be used within 1 year. It is not suggested to keep in room temperature and use the eyeliner more than 1 year after manufacture date (Djamil et al., 2017).

CONCLUSION

The Eyeliner content derived from the three color variants became blue, purple, and green. The three color variants were stable for the three-cycle test. According to the organoleptic test, the eyeliner bulk content showed no changes in shape, color, and scent. The eyeliner preparation is wax-based, and heating for a long period will often cause problems, affect the appearance of the finished products, and change the chemistry of the waxes. It is possible that the hardness and break point decreased after 3 stability cycles. The eyeliner was maintained at room temperature during usage. It is not recommended to maintain room temperature and use the eyeliner for more than 1 year after the manufacturing date. In the in vivo irritation test, the three color variants showed a positive response with no irritation reaction or edema on the rabbit skin after 72 hours of application. In this case, we can conclude that eyeliner preparation with *Clitoria ternatea* L. flower extract is safe for use and application.

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