Original Article

Study on hydrogel eye mask with Centella asiatica L and Aloe vera L extract

Bheta Sari Dewi[1], Silvia Surini[2]*

[1]Division of Pharmaceutical and Pharmaceutical Technology Development, Faculty of Pharmacy, University of Indonesia, Depok, 16424, West Java, Indonesia. [2]Faculty of Pharmacy, University of Indonesia, Depok, 16424, West Java, Indonesia.

Correspondence: Silvia Surini, Faculty of Pharmacy, University of Indonesia, Depok, 16424, West Java, Indonesia. silvia@farmasi.ui.ac.id

ABSTRACT

Centella asiatica and Aloe vera can be useful as anti-aging agents. This research aims to evaluate the in vitro anti-collagenase, safety, and anti-aging effects of hydrogel eye masks containing Centella asiatica extract and Aloe vera extract. The anticollagenase activity was determined based on Collagenase Activity Colorimetric Assay Kit. Hydrogel eye mask containing 5% w/w C. asiatica extract and 3% w/w A. vera extract. Then a double-blind clinical trial was carried out to investigate its anti-aging effect based on collagen fiber, hydration, and skin elasticity in 30 healthy women volunteers. The hydrogel eye mask was simultaneously rubbed just beneath the right eye and the placebo rubbed beneath the left eye. The safety of the formulation was evaluated with Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) and patch test. The efficacy of the formulation was evaluated with a Skin Analyzer EH-900U. The combination of C.asiatica extract and A. vera extract showed an IC50 value was 21.912 µg/mL higher than C. asiatica and A. vera extract alone. The HET-CAM test results on the 300 mg Hydrogel eye mask showed no sign of alteration on CAM. The irritation test results indicated that the hydrogel eye mask did not cause any skin irritation and increased collagen fiber, elasticity, and moisture ($p<0.05$) after 4 weeks of use. Hydrogel eye mask had good characteristics, safe and effective as skin aging cosmetics.

Keywords: Hydrogel eye mask, Centella asiatica, Aloe vera, Anti-collagenase, Anti-aging, HET-CAM

Introduction

The skin aging process can be divided into two factors: Intrinsic aging and extrinsic aging. The former or intrinsic aging is caused by the elasticity of the skin changes over time, while extrinsic aging is especially a result of constant exposure to UV radiation [1, 2]. The exposure to UV light for a long period will damage the skin. The skin starts to stretch and slack [3]. Changes of collagen and elastin fibers which keep the elasticity of the skin become stiff, not elastic leads to this condition, therefore, loses its elasticity [4]. Exposure to UV light the skin changes physically, due to alterations occurring in the connective tissue through the enzymes, cell contents, formation of lipid peroxides, and Reactive Oxygen Species (ROS). Lipid peroxides may be metabolized to form by-products that damage the Extracellular Matrix (ECM). Meanwhile, with involvement in the loss of skin elasticity, ROS are credited [1, 5]. The very first place where aging became visible is the eyes. In this area, the skin is extremely thin and the fat tissue has little subcutaneous fat. At this age, the dermis, the inner layer of skin begins to thin, and slowly the skin cells are divided. The collagen fiber tissues and elastin are beginning to break down, leading to degradation on the surface. The elasticity of the skin also loses and is then unable to retain moisture. Furthermore, the efficiency of oil-secreting glands has deteriorated, and the healing of the skin is becoming slower. All of the contributing factors described above are what make wrinkles develop [3, 6].

Collagenase enzyme is a metalloproteinase (MMP) that enables debase molecules, like fibronectin, elastin, aggrecan, collagen, laminin, and gelatine. For those reasons, an activity that inhibits collagenase by the agents may have valuable effects on preventing dermal matrix degradation, thus maintaining healthy skin [6].

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The safety of the formulation was assessed with the Test of Hen's Egg– Chorioallantoic Membrane (HET-CAM) and patch test. Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) is an in vitro test acknowledged for identifying the potential substance that tends to be irritative or non-irritative (formulation and ingredient). This method used a chorioallantoic membrane (CAM) from a Hen Embryo. CAM is a complete tissue, comprising Arteries, capillaries, and veins. Through this method, a chemical was placed on the chorioallantoic membrane (CAM) from a hen egg. On the 9th day of embryonase, the neural tissue and pain perception were not yet developed. A difference in the injury of veins on the 9th day of embryonase could demonstrate Potential for chemicals to damage mucous membranes [7].

Studies showed that C. asiatica extract can improve skin health and help prevent dark circles and aging. Four weeks study with two applications per day, evaluating the effect of a cosmetic formulation containing various concentrations of Centella asiatica extract (2.5 and 5%) on skin hydration, Transepidermal Water Loss (TEWL), and micro-inflammation in human skin using in vivo techniques (corneometer, tewameter, and chromameter) [8].

Studies on Aloe vera extracts that Aloe vera gel significantly reduces wrinkles and improves skin elasticity by increasing collagen production in protected skin and decreasing the expression of the MMP-1 gene reducing collagen with a maximum level of 3% [9]. We investigate the inhibitory activity of formulation combined extract of hydrogel eye mask containing 5% w/w C. asiatica extract and 3% w/w A. vera extract against collagenase enzyme A double-blind clinical trial was also carried out to investigate its anti-wrinkling effect based on collagen fiber, hydrating and skin elasticity on 30 healthy women volunteers.

Materials and Methods

Material and chemicals

C. asiatica extract and A. vera extract were purchased from Xi'an Geekee Bio-Tech Co., Ltd. (China). Collagenase Activity Colorimetric Assay Kit was purchased from Sigma Aldrich (Singapore). For cosmetic formulation, sodium alginate (Qingdao, China), xanthan gum (Qingdao, China), propylene glycol (Qingdao, China), glycerine (Clariant, Germany), sodium metabisulfite (Qingdao, China), methylparaben (Clariant, Germany), propylparaben (Clariant, Germany), and calcium chloride (Clariant, Germany) were used. For the clinical trial, Finn Chambers on Scanpor patch test was purchased from Smart Practice, Canada, Ethic agreement from The Health Research Ethics Committee, Faculty of Medicine, University of Indonesia in Depok, Indonesia (clinical trial registration number: 19-12-1414; date of approval: January 06, 2020).

Collagenase inhibition activity

Collagenase inhibition activity was conducted based on the protocol of the Collagenase Activity Colorimetric Assay Kit (Sigma-Aldrich). Briefly, a dilution series (different concentrations of 12.5, 25, 50, 100, and 150 µg/ml extract) was prepared. Two µL of the extract was added to 96 well plates, and the volume to 100 µL with Collagenase Assay Buffer was adjusted. For negative control, 10 µL of provided Collagenase (0.35 U/ml) was added. For positive control, 10 µL of provided Collagenase (0.35 U/ml) and 2 µL of inhibitor (1,10-Phenanthroline) were added into desired well (s). The volume of control wells to 100 µL was adjusted with Collagenase Assay Buffer. For reagent background control, 100 µL of Collagenase Assay Buffer was added [10, 11].

To determine collagenase activity (U/Ml), equation 1 was used:

$$([(-\Delta A\_(345\ Test))/(\Delta T) - (-\Delta A\_(345\ Reagent\ Background))/\Delta T] \times (0,2) \times DF)/(0,53)V \quad (1)$$

Where ΔA_(345 Test): difference between A2 and A1; ΔT : difference between T2 and T1;0.2: reaction volume (mL); DF: Dilution Factor; 0.53: millimolar extinction coefficient of FALGPA; V: Enzyme volume (Ml)

For the inhibitor screen, percent inhibition was calculated using equation 2:

$$\%\ Inhibition = Activity\_((Enzyme) - Activity\_((Inhibitor)))/Activity\_((Enzyme)) \times 100 \quad (2)$$

Preparation of hydrogel mask formulation

The composition and concentration of the hydrogel mask formulations are listed in **Table 1**, as follows:

Table 1. Hydrogel mask formulation

Composition	Concentration (g)
Gotu kola extract	5
Aloe vera extract	3
Sodium alginate	2
Xanthan gum	0.5
Propylene glycol	2.5
Glycerin	5
Sodium metabisulfite	0.02
Methylparaben	0.02
Propylparaben	0.10
Distilled water	81.68

Referring to the table, Sodium alginate was dispersed in distilled water and homogenized for 10 minutes. Next, xanthan gum was added to glycerin, and the mixture of xanthan gum and glycerin was added to alginate gel to form the hydrogel base [12, 13]. Sodium metabisulfite was poured into 20 mL of distilled water. A mixture of C. asiatica and A. vera extracts was then added to the hydrogel base and homogenized. Propylparaben and methylparaben were poured into propylene glycol, added to the hydrogel base, and homogenized until a semi-solid gel was formed. Semi-solid gels were cast into glass molds and soaked in 2 µl of the chloride mixture for 30 minutes [14].

Physicochemical evaluation and stability testing

The physicochemical evaluation and stability of physical state of the hydrogel eye mask were evaluated at temperatures 4 ± 2 oC, 25 ± 2 oC, and 40 ± 2 oC. Organoleptic properties, hydrogel mask weight and size, swelling index, and consistency were determined every 2 weeks from week 0 to week 12.

Organoleptic properties

The hydrogel eye mask was observed in size, shape, color, and smell every 2 weeks from week 0 to week 12.

Hydrogel mask size and weight

Five hydrogel eye masks were weighed using an analytical balance. Her 5 sides of the mask (the middle of the mask and her 4 sides) were then measured with a micrometer gauge. Calculations were repeated up to 3 times and average results were used every 2 weeks from week 0 12.

Swelling index

The hydrogel eye mask turned to pieces measuring 1.2 cm x 1.2 cm x 0.04 cm. The initial weight of the debris was recorded and submerged in 30 ml of distilled water. The weight was measured in intervals of 0,3,9, and 12 hours every 2 weeks from week 0 to week 12. The Formula to Calculate The swelling index of the hydrogel eye masks was calculated from measured values as described in equation 3 [12, 14]:

$$\text{Swelling index} = (W_n - W_o)/W_O \times 100\% \quad (3)$$

Where Wn: hydrogel eye mask weight after hydration; Wo = hydrogel eye mask weight before hydration

Consistency

The hydrogel eye mask was put in a penetrometer (Herzoo, Germany). The value of penetration evaluates the consistency five secs following the mask was penetrated by the cone When the hydrogel mask was made every 2 weeks from week 0 to week 12, consistency rates were measured.

Clinical study

A double-blind, placebo-controlled trial was conducted to evaluate the clinical effects of the use of a hydrogel eye mask containing 5% w/w Centella extract and 3% w/w Aloe extract on under eye for 1 month. This study was conducted in agreement with The Health Research Ethics Committee, Faculty of Medicine, University of Indonesia in Depok, Indonesia (clinical trial registration number: 19-12-1414; date of approval: January 06, 2020).

There were thirty healthy Indonesian female volunteers, aged 20-40 years, who signed informed consent. All of them were not allowed to use eye cosmetic products for one month of treatment. The exclusion criteria were pregnancy and breastfeeding, using drugs that could affect hormonal conditions, undergoing anti-aging medication, experiencing chronic disease, having a history of allergies to the substances used, taking oral anti-aging supplements, smoking, and drinking. They applied a hydrogel eye mask beneath the right eye and a control, consisting of a base beneath the left eye simultaneously. The effect and safety of the formulations were assessed with the Skin Analyzer EH-900U. The instrument measured collagen fibers, moisture, and elasticity [6].

Skin aging safety testing

The eggs were incubated in an incubator. The temp was 370 C and with 60-70% of relative humidity, further to prevent the egg embryo from being attached to one side of the egg, they were rotated for 8 Days. The eggs were candled and within day 9, the non-viable ones were discarded, Viable eggs is replaced in the incubator with the large end facing upwards. On day 10, the eggs were prepared for analysis. Some parts of the shell above the air cell were removed carefully with scissors. The membrane was precisely moistened with 0.9% Sodium Chloride solution and then, in the incubator, the eggs were replaced until it is ready for analysis. With tapered forceps tools, the membrane was carefully removed. The chorioallantoic membrane was added with 300 mg of Sodium Chloride and then observed for 300 seconds for effects. As the investigated parameters, hemorrhage, vascular lysis, and coagulation were evaluated [15]. Each reaction was recorded in seconds (sec). Irritation index can be counted:

$$RI = \{[(301-secH))/300 \times 5]\}\{[(301-secL))/300 \times 7]\}\{[(301-secC))/300 \times 9]\} \quad (4)$$

Where H: hemorrhage; L: vascular lysis; C: coagulation; RI: irritation index, and sec: start second.

The irritation categories can be seen in **Table 2** [15].

Skin irritation tests were conducted on 30 volunteers using a single application closed patch epicutant test with the occlusion or semi-occlusion method. The test products, which included masks containing extracts, and control products, which were masks without extracts, were attached to the upper arm and left in place for 24 hours. The Finn Chambers® AQUA from SmartPractice, USA, were used to ensure proper occlusion. Control products were also attached to the upper right and left arms for comparison. Skin irritation reactions were evaluated by an experienced doctor at 30 minutes, 24 hours, and 48 hours after patch removal. The degree of skin irritation reaction was assessed, and an Irritation Score (R) was calculated based on the evaluated skin response, using a formula that takes into account the grade and number of respondents. This score provides a standardized measure for assessing human skin irritation.

Skin aging efficacy testing

Initially, the volunteers explained the study's purpose, and then they gave written consent. Throughout the trial period, the

volunteers were advised not to use any other type of preparation except that under the study every night to reduce the possible experimental errors. Voluntary withdrawal from the study was allowed if they experience discomfort during the study. They were given one week before the start of the study for consideration.

The prepared hydrogel eye mask was put in a jar labeled A and the control mask was placed in jar B. Thirty volunteers were randomly chosen and in a double-blind manner; they were advised to apply the mask in jar A under the right eye and B under the left eye or vice versa for forty-five minutes every night, for four weeks. Before the day of application, all women were assessed for collagen fiber, hydration, and elasticity value. They were followed up for four weeks and after a visit at each 2-week follow-up. At the end of the fourth week, the overall performance of the anti-wrinkle and dark circle was evaluated.

The effect and safety of the formulations were evaluated with the Skin Analyzer EH-900U. First of all, adjust the tester's lens under the eye. Then, click the button "Collagen Fiber", "Elasticity", or "Moisture" on the left side of the analysis operation area and the button "Shooting", and then click "Freeze". The picture will be fixed at the chief shooting area. Next, click "Analysis" to analyze the skin. The analysis results will be shown in the left corner of the "Analysis Results Showcase". The collagen fibers value is loose skin 25-35%, weak 35-50%, normal 50-65%, better 65-70%, and best 70-80%. The elasticity value is loose skin 15-35%, weak 35-50%, normal 50-65%, better 65-70%, and best 70-71%. The moisture value is dry at 3-4%, aging at 4-10%, normal at 10-15%, higher at 15-30%, and shiny moist at 30-65% [6, 16].

Statistical analysis

All statistical analyses were performed using SPSS 24.0 for Windows. When checked by using the Shapiro-Wilk test, the scores were found to be skewed at $p < 0.05$ significance level. Accordingly, for non-parametric tests, Friedman tests were used to probe the differences between independent and dependent variables within and between the groups before and after the interventions respectively. The Wilcoxon signed-rank test was used for pre-and post-comparison within the groups. Post hoc analysis with Wilcoxon signed-rank test was used to find where the significant differences occurred in the group at the new p-value of ≤0.005. A significance level of ≤0.05 was used in all analyses.

Results and Discussion

Asiaticoside content

Assay analysis of asiaticoside in *C.asiatica* extract was performed at Biofarmaka Laboratory Bogor using High-Performance Liquid Chromatography (HPLC) of 0.91 mg/g ± 0.012 with retention times at 14.4 min and 206 nm wavelength **(Figure 1)**.

Table 2. Results of Asiaticosides Analysis in Centella asiatica Extract and Aloin A in Aloe Vera Extract.

Sample Name	Result	Unit	Method
Centella asiatica extract	0.91	mg/g	HPLC
	0.89	mg/g	HPLC
	0.92	mg/g	HPLC
Aloe vera extract	0.1771	mg/L	Spectro 267 nm

Table 2 Shows the analysis of aloin a content in avera leaf extract carried out at the Department of Chemistry, University of Indonesia, Depok using UV-VIS spectrophotometry of 0.1771 mg/L ± 0.00012 with a wavelength of 267 nm.

Collagenase inhibition activity

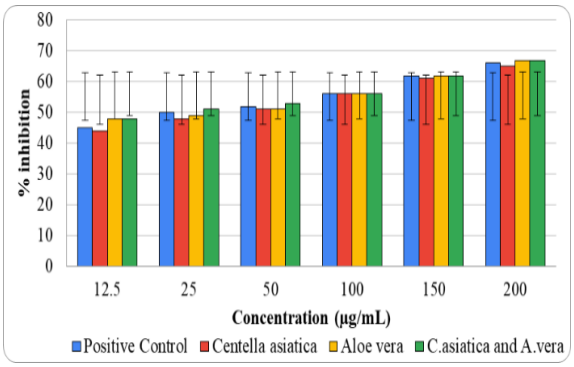

Figure 1. Result from collagenase inhibitory activity test from a Combination of *C.asiatica* extract and *A.vera* extract, *A.vera* extract alone and *C.asiatica* extract alone

Based on **Figure 1** it is known that the combination of C. asiatica and A. vera extracts showed an lC50 value of 21.912 µg/mL which was higher than the lC50 value of the A.vera extract of 34.166 µg/mL and the C.asiatica extract of 176.945 µg/mL.

Stability of the formulation

Organoleptic properties

At storage at 4±2°C and 25±2°C for 12 weeks, there was no change in organoleptic odor, color, shape and taste. At storage at an extreme temperature of 40±2°C for 12 weeks, organoleptic changes occurred in color and taste when used at 8-12 weeks, the shape changed a little bit and the color turned very dark brown and the taste was different.

Hydrogel mask weight and size

The Hydrogel mask at 4°C and 25°C did not change in length, width, and thickness. However, at 40°C, the longer the hydrogel eye mask was stored, the thicker the mask and the smaller its length and width.

The ability to swell from weeks 0 to 12 at a temperature of 4°C and 25°C had a slight decrease of about 10% compared to before storage, while at a temperature of 40°C had a quite large decrease was around 25%.

Consistency

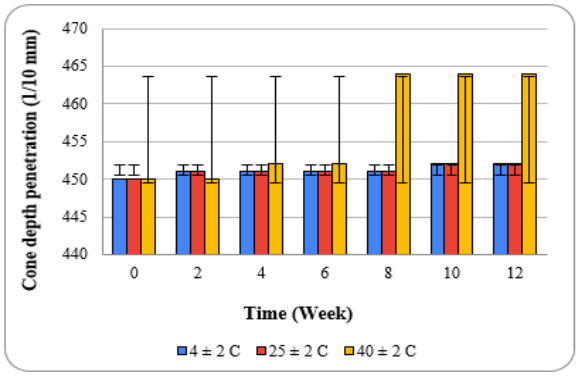

Figure 2. Consistency hydrogel eye mask at 4±2ºC,25±2ºC, 40±2ºC

The table shows that the longer it is stored, the deeper the penetration, especially at high temperatures because the mask is getting thicker. This indicates that the gel has become denser. The decrease in the depth of penetration is inversely proportional to the viscosity of the preparation which increases/thickens.

Safety study

In the cosmetic industry, safety studies are paramount. Using HET-CAM and patch tests, this study has investigated the skin irritation potential of the product.

The results of the study were through the HET CAM test on the 300 mg Hydrogel eye mask anti aging (test control), where there was no sign of change in the CAM. The average irritation index for the control test is 0, so it is equivalent to the "no irritation" classification. On administration of 0.9% NaCl (negative control) there were no signs of change with CAM. The average irritation index for the negative control was 0, so it was classified as "non-irritating". In the administration of 1% NaOH (positive control) there are differences with CAM namely bleeding, lysis, and coagulation. The average value of the irritation index in the positive control was 11.49 so it was classified as severe irritation. None of the volunteers experienced skin irritation after the skin patch test. The results showed that none of the products caused skin irritation in all of the volunteers. The primary human skin irritation index and irritation scores are shown in **Table 3**.

Table 3. The result of skin irritation in all of the volunteers

Product	N	Irritation score (R)				Primary irritation index
		30 minutes	24 hours	48 hours	Mean	
Control	30	0.00	0.00	0.00	0.00	No/slight irritation
Test	30	0.00	0.00	0.00	0.00	No/slight irritation

Note: Control mask without extract; Test-mask with extract

Efficacy study

Based on **Table 3**, it is known that this product is safe to use because there is no skin irritation reaction. The average intensity of the collagen fiber, elasticity, and moisture for every volunteer before using the product was significantly different ($p < 0.05$). The result showed that the collagen fiber, elasticity, and moisture in the test group after the application were significantly higher than before the application. The control product was insignificantly different before and after the application. The change of the collagen fiber, elasticity, and moisture in the test group was significantly increased than the control group in week 4. In vivo, the clinical study revealed significant skin changes in the collagen fiber, hydration, and elasticity of the stratum corneum. For the test group, the increase in collagen fiber was 46.87%, the hydration increased by 71.98% and the elasticity increased by 36.14% from baseline in collagen fiber was 16.60%, whereas hydration and elasticity increased from baseline, 44.97%, and 16.99%, respectively.. The subjective self-assessment of the volunteers indicated that, after 4 weeks of mask application, wrinkles become less defined and less depressed. In the case of the control, the increase

In this case, the findings show the value of relative change during the study (mean ± standard deviation), where the average increase in collagen fibers in the control group and the test group ($n=30$, $P<0.05$), the mean increase in skin elasticity in the control and test groups ($n=30$, $P<0.05$), and the mean increase in skin moisture in the control and test groups ($n=30$, $P<0.05$).

Asiaticoside and aloin a content

The results of the table above also show that the asiaticoside content of *C.asiatica* extract was 0.91 mg / g ± 0.012. Based on the literature, all of the above-ground parts of *C.asiatica* extract. The Apiaceae family contains not less than 0.07% asiaticoside [17]. The asiaticoside levels in *C.asiatica* herb extract using the High-Performance Liquid Chromatography (HPLC) method were obtained in the range of 1.0 - 3.0 mg/ml [18]. The highest asiaticoside content in herbs/leaves, with values of 0.79 ± 0.03% dry mass using the High-Performance Liquid Chromatography (HPLC) method [19].

Collagenase inhibition activity

Combined Hydrogel Eye Mask *C. asiatica* and *A.vera* extract was synergistic as an anticollagenase. Aloin A is an effective inhibitor of stimulated granulocyte matrix metalloproteinase (MMP). Aloin A can bind and inhibit degraded collagen in a reversible and non-competitive manner [20]. Reversible inhibitors are inhibitors whose chemical reactions go two-way or reversible and are unstable, when the inhibitor binds to the active side of the enzyme, then this inhibitor can be separated again from its bonds. Non-competitive inhibitors are those that can bind to both the enzyme and the enzyme-substrate complex. If the inhibitor attaches to the enzyme, the active site structure of the enzyme will change but the substrate can still stick to the active site, but the enzyme cannot work [21].

Stability of the formulation

Physical stability test plays an important role in the development and improvement of formulations, it is to ensure the quality, safety, and efficacy of the product, determining the validity and monitoring of physical and chemical characteristics [22].

Temperature can affect emulsion stability significantly. These, in turn, affect the stability of the emulsion [23, 24].

This happens because the extreme temperature changes make the propylenglycol solution in the mask evaporate quickly so that the OH hydroxyl group that can bind to air molecules can easily escape from the hydrogel structure causing the hydrogel to dry out and shrink. This propylenglycol solution functions as a humectant to keep water in the hydrogel.

Asiaticosides are triterpenoid saponin glycosides, while Aloin A is also an anthraquinone glycoside compound. Glycosides are compounds of natural ingredients which consist of a combination of two parts of a compound, namely sugar and not sugar [25].

Safety study

Actual usage of cosmetic products that are used on human skin is the criteria for the classification of primary irritation index [26-28].

The HET CAM test results on 300 mg Hydrogel anti-aging eye mask (test control) showed no sign of alteration on CAM. The average irritation index on test control was 0, in line with the classification of "no irritation". The composition of the hydrogel eye mask did not contain any irritating ingredients and both extracts were considered safe. On giving NaCl 0,9% (negative control), there was no sign of change with CAM. The average irritation index on negative control was 0, in line with the classification of "no irritation". On giving NaOH 1% (positive control), there were differences with CAM, which were bleeding, lysis, and coagulation. The irritation index average value on positive control was 11.49, in line with the classification of "severe irritation".

Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) is an *in vitro* test acknowledged for identifying the potential substance that tends to be irritative or non-irritative (formulation and ingredient) [7]. This method used a chorioallantoic membrane (CAM) from a Hen Embryo. CAM is a complete tissue, including Arteries, capillaries, and veins. Through this method, a chemical was placed on a chorioallantoic membrane (CAM) from a hen egg. This method has several advantages i.e. fast, simple, sensitive, easy, and relatively inexpensive. The disadvantage is that the evaluation results are subjective [29]. Test assessment depends on visual evaluation, and the results may vary among individuals [30]. HET-CAM only evaluates one eye segment (conjunctiva) and must be completed by a corneal model [7]. In this test, fresh white leghorn chicken eggs were used for no more than 7 days, clean, fertile, weighing 50-60 g totaling 12 grains, and then possible reactions, including bleeding (hemorrhage), blood vessel lysis (hyperemia) and coagulation (denaturation of intra and extravascular protein) were observed.

Efficacy study

The application hydrogel eye mask containing *C.asiatica* and *A.vera* extract resulted in a significant enhancement of collagen fiber, hydrating, and elasticity of the skin ($p < 0.05$) after 4 weeks of use. Asiaticoside in *C.asiatica* extract induces collagen type I synthesis in human skin fibroblast cells [31]. Aloin can inhibit the collagenase enzyme [20, 32]. *C.asiatica* extract inhibits the activity of the elastase enzyme and inhibits MMP-1 activity to increase skin elasticity [33]. A.vera gel that has been done in vivo also significantly reduces wrinkles and increases elasticity in the skin by increasing collagen production in protected skin and decreasing the expression of the MMP-1 gene which reduces collagen with a maximum level of 3% [9]. The results of other in vivo studies, emulsions and hydrogels with a concentration of 5% C.asiatica extract not only have a significant effect on anti-aging but also increase skin hydration (decreased Transepidermal Water Loss value and decreased skin pH value) [8, 34]. *A.vera* gel is an effective moisturizer because of its hygroscopic nature which aims to prevent water loss in the skin so as keep the skin moist [35, 36].

Conclusion

The combination of C. asiatica extract and A. vera extract showed an lC50 value of 21.912 µg/mL which was higher than C. asiatica and A. vera extract alone. The volunteers did not obtain any skin irritation and showed an enhancement of collagen fiber, hydrating and elasticity of the skin ($p < 0.05$) after 4 weeks of use.

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Conflict of interest: None

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Ethics statement: All animal treatment was ethically approved by The Ethics Committee of The Faculty of Medicine, University of Indonesia (No. KET-23/UN2.F1/ETIK/PPM.00.02/2020).

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