**RESEARCH ARTICLE**

# Anti-aging activity of tetrahydrocurcumin, *Centella asiatica* extract, and its mixture

Ika Yuni Astuti[1] · Ani Yupitawati[1] · Nunuk Aries Nurulita[1]

## Abstract

Tetrahydrocurcumin has potent antioxidant activity and depigmentation of the skin. *Centella asiatica* extract can stimulate the formation of collagen, thus reducing wrinkles in aging skin. The combination of tetrahydrocurcumin (THC) and *Centella asiatica* extract (CAE) is expected to improve various parameters of premature aging. This study used pre-test post-test control group design. Mice were randomized into 5 test groups. The dorsal part of the mice was shaved, and then various anti-aging parameters, including skin sensitivity, moisture content, collagen levels, elasticity, and large pores, were measured using a skin analyzer. Furthermore, the dorsal was smeared with a cream base for the negative control (NC) group, a cream containing ethyl ascorbyl ether for the positive control (PC) group, a cream containing THC for THC group, a cream containing CAE for the CAE group, and a cream containing THC and CAE for the Combination group, each with a dose of 2 mg/cm2 before and after UV irradiation. After treatment for four weeks, the anti-aging parameters were measured again. The combination of THC and CAE could increase the collagen and elasticity levels compared to THC and CAE itself, but the increase was not significant. Based on all anti-aging parameters scores, THC and CAE's combination did not provide more significant anti-aging activity than single THC and single CAE.

**Keywords** Preclinical · Anti-aging · Tetrahydrocurcumin · *Centella asiatica*

## Introduction

The incidence of premature aging increases, partly because of the increasing exposure to UV rays and air pollution produces more free radicals that act as mediators of oxidative damage to body cells. UVB exposure to the skin causes expression of MMP 1 (matrix metalloproteinase 1), which further degrades collagen so that the skin loses its elasticity (Kim et al. 2014). Another mechanism of aging is inflammation. A low-grade chronic inflammation causes the collagen and elastin fibers to break down, and undergo remodeling, makes the skin thin and sagging. The decreased skin appearance, such as wrinkles, brown spots, and uneven skin color, are signs of aging.

The premature aging signs can be delayed using anti-aging products. In general, anti-aging products contain antioxidants as an antidote to free radicals and cell regulators that directly affect the degradation or synthesis of collagen.

Curcumin has been widely known as a good antioxidant. Among the curcumin derivatives, tetrahydrocurcumin has shown the most potent antioxidant properties. It also inhibits collagen degradation and shows anti-inflammatory and skin brightening properties (Bylka et al. 2013; Xiang et al. 2011). Thus, THC has good potential to be used as an anti-aging active ingredient.

*Centella asiatica* is often used as an antioxidant and anti-aging because it can repair cell damage by stimulating collagen synthesis (Bylka et al. 2013). The anti-aging cell regulatory of CAE is different but complements with the THC's. So, the combination of CAE and THC was expected to produce a synergistic effect for an anti-aging agent.

This research aims to get information about the THC's anti-aging properties, CAE, and its mixture. A vanishing cream base was used as a carrier for the tested active ingredient. The collagen levels, sensitivity, moisture content,

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elasticity, and large pores were used as anti-aging parameters (Kim et al. 2018; Badenhorst et al. 2017).

## Materials and methods

### Materials

*Centella asiatica* extract was obtained from the Pharmacy Faculty, UMP, Indonesia. Tetrahydrocurcumin was purchased from Xi'an Tianxingjian Natural Bioproduct Co. Ltd (Shaanxi, China). Spectrophotometric grade reagents of concentrated HCl, Magnesium, ethanol, AlCl3, and methanol (Merck). Ethyl Ascorbyl Ether was kindly provided by PT Tissan Nugraha Globalindo (Jakarta, Indonesia). The pharmaceutical grade of excipients of sodium benzoate, stearic acid, triethanolamine, glycerin (Bratachem). UV A and UV B lamp Repti Glo 13 W (Exoterra), and skin analyzer EH 900 U were used to anti-aging study.

## Experimental animals

This study used male mice of Balb/C strain with a weight of 20–25 g and the age of 6–8 weeks. Each group's mean of weight and age should be distributed normally, homogenous, and not different significantly. They were housed in an animal cage at 25 °C under a cycle of 12 h-light/dark and given a standard diet and free access to distilled water before the research. The experimental procedures were approved by the Animal Care Committee of Medical Faculty, Jenderal Soedirman University (Purwokerto, Indonesia).

## Methods

### Cream Preparation

A simple vanishing cream base was used as the carrier for all of the treatment groups. The formulation of each group can be seen in Table 1.

The oil phase of the stearic acid and water phase (triethanolamine, glycerin, sodium benzoate, and aquadest) each melted in a porcelain dish over 70 °C. The oil phase is poured into the water phase, stirred in warm to the cold mortar. Each treatment's active ingredients were poured into another mortar, then added the cream base bit by bit, stirring until homogeneous.

## Anti-aging activity test

The anti-aging activity test approach was Pretest-Posttest Control Group Design, referring to anti-aging test conducted by Nazliniwaty (2016) and Rahmawati (2019) with modification (Nazliniwaty et al. 2016; Rahmawati et al. 2019). The mice were randomly selected, grouped into five treatment groups, then weighed. All of the mice were acclimatized for seven days for adaptation to the experimental environment conditions.

Measurements of the mice conditions before and after treatments were performed by Skin Analyzer EH 900 U. The parameters of anti-aging measurement were skin sensitivity, moisture content, collagen levels, elasticity, and large pores.

The UVA and UVB irradiation of all mice was performed for 10 minutes daily for four weeks. UVA irradiation dose was 630 μW / cm$^2$ and UVB 105 μW / cm$^2$. The dorsal skin leather distance with a UV lamp was 15 cm.

The mice's entire group was shaved off on the back of the $2 \times 2$ cm$^2$ area using electric shavers and manual shavers, then smeared with the cream on the shaved area. The basting

**Table 1** Cream formulation

| Ingredient | Negative Control | Positive Control | THC | CAE | THC-CAE |
|---|---|---|---|---|---|
| Stearic acid | 15 | 15 | 15 | 15 | 15 |
| Triethanolamine | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Glycerine | 10 | 10 | 10 | 10 | 10 |
| Sodium benzoate | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Ethyl ascorbil ether | – | 3 | – | – | – |
| Tetrahydrocurcumin | – | – | 2 | – | 2 |
| Centella asiatica extract | – | – | – | 10 | 10 |
| Aquadest | ad 100 | ad 100 | ad 100 | ad 100 | ad 100 |

Treatment group (%)

dose is 2 mg/cm$^2$ administered at 2 h before and 15 min after the irradiation daily for four weeks.

Weight and animal health control were performed by weighing the mouse every two weeks during the study to ensure that the mouse's weight did not rise or decrease too far from the specified weight range. The health of mice was also always monitored by observing the liveliness of movement and the mouse's appetite.

After four weeks of treatment, the parameters of anti-aging were evaluated. The evaluation was done on the mice in an active state because the skin analyzer does not hurt the skin and is not invasive. At the end of the study, it was likely that the shaved animal skin experienced premature aging. The mice were restored to normal conditions, given nutrition and drinking water.
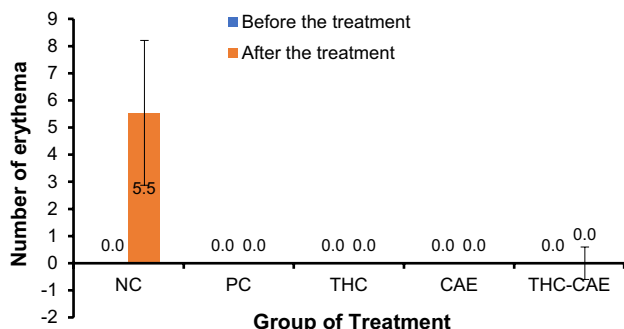
### Data analysis

Data of anti-aging activity was analyzed by SPSS 17. The data were tested for normality with Shapiro-Wilk, and its homogeneity was tested with Levene's test. When the data were normal and homogeneous, the data then tested with One Way Anova, followed by the Pos-hoc test with LSD test. If the data are not normal, use Kruskal nonparametric analysis -Wallis test followed by the Mann–Whitney test.

### Result

The anti-aging activity was evaluated based on sensitivity, water content, collagen content, elasticity, and pore diameter. The *number of erythema* before and after the treatment is shown in Fig. 1.

Before the treatment, the sensitivity of all test groups was 0.00 (no sensitivity). After four weeks of treatment, the negative control group showed the most significant sensitivity, while the lowest was a positive control and tetrahydrocurcumin group.

The sensitivity diameter is shown in Fig. 2. Before the treatment, the area of all of the test groups' erythema was 0.00 mm (there was no sensitivity). After the treatment for four weeks, the negative control group had the largest area, followed by the CAE group.

Before the treatment, all test groups did not experience sensitivity. After treatment for four weeks, the NC and THC-CAE group underwent sensitivity. The sensitivity shown by the NC group was much more significant than that of the other groups. Otherwise, the PC, THC, and CAE group did not experience sensitivity. Sensitivity is indicated by red spots on the surface of the skin (erythema) (Fig. 3).

The water content before and after treatment can be seen in Fig. 4. The negative control group showed the lowest water content after the treatment.

The collagen levels before and after treatment are shown in the Fig. 5. Collagen levels before and after treatment are shown in the following Fig. 5. The THC-CAE group had the most significant collagen level compared to the other test groups.

It can be seen from Fig. 5 that, after the treatment, the groups given the active substances showed more excellent elasticity than the negative control group. The THC-CAE group showed the greatest elasticity.
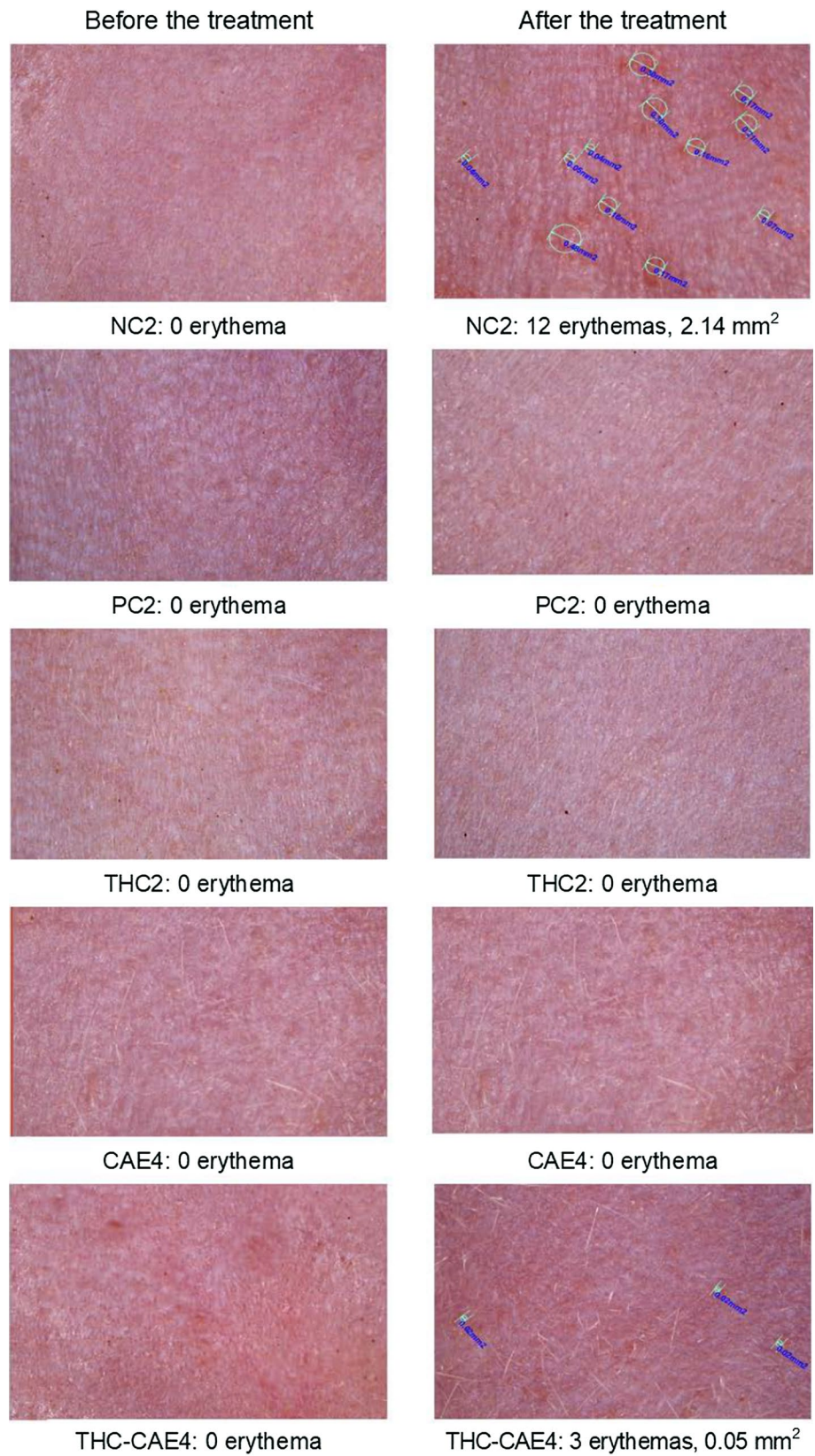
Figure 6 shows the diameter of pores before and after the treatment. The NC group, which had the smallest elasticity, had the largest pore diameter. The CAE group pore diameter was more significant than the THC and THC-CAE.

After summarizing all the values of anti-aging activity parameters of each treatment group, the total score was obtained. The total score of anti-aging activity for each test group was as follows: NC = -14; PC = +7; THC = +7; CAE = − 5; THC-CAE : + 5.



**Fig. 1** The comparison of number of erythema before and after the treatment
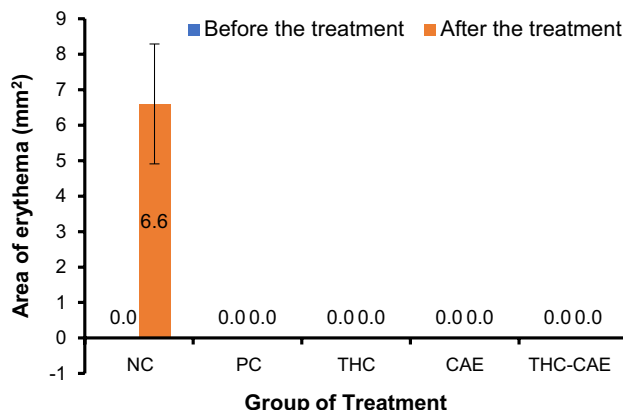


**Fig. 2** The comparison of area of erythema before and after the treatment

**Fig. 3** Comparison of rat skin sensitivity (number and area of erythema) of representative of each test group before and after the treatment viewed using a skin analyzer
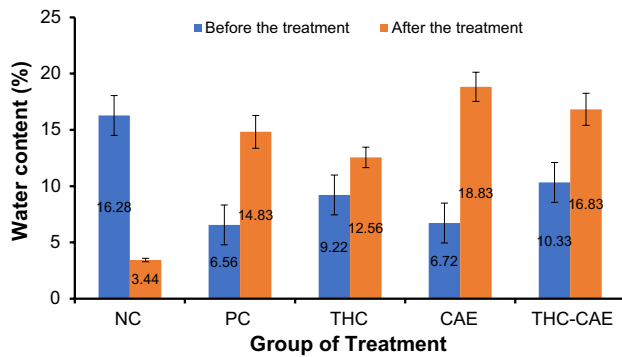


Before the treatment

After the treatment

NC2: 0 erythema

NC2: 12 erythemas, 2.14 mm$^2$

PC2: 0 erythema

PC2: 0 erythema

THC2: 0 erythema

THC2: 0 erythema

CAE4: 0 erythema

CAE4: 0 erythema

THC-CAE4: 0 erythema

THC-CAE4: 3 erythemas, 0.05 mm$^2$

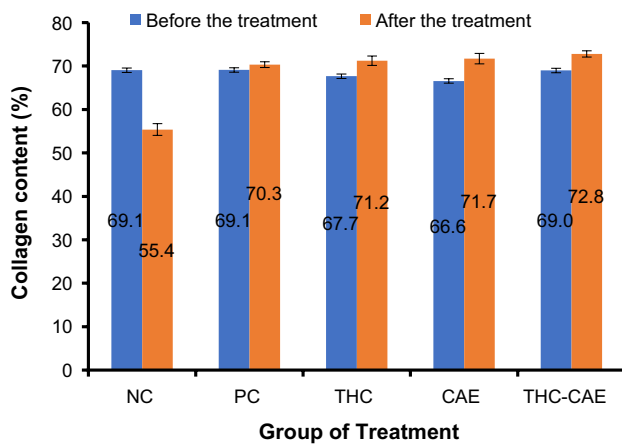**Fig. 4** The comparison of water content before and after the treatment



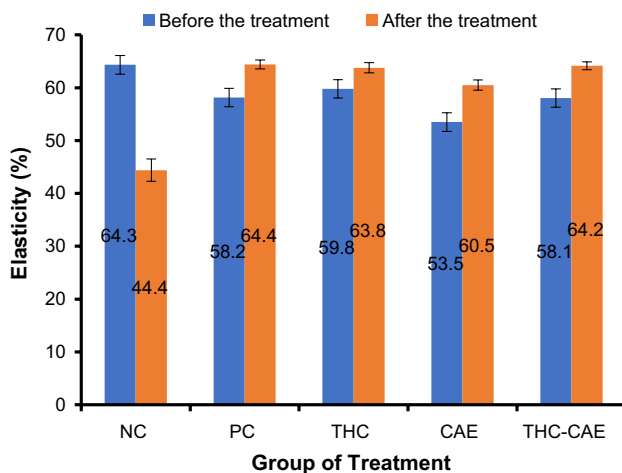**Fig. 5** The comparison of collagen content before and after the treatment



**Fig. 6** The comparison of elasticity before and after the treatment

## Discussion

In this research, the animal skin was exposed by UV solar irradiation that simulated with a UV lamp to approach daily reality. Thirty mice were selected according to the inclusion criteria. Mice of Balb / c strain have a skin structure similar to the human skin structure to be used as a topical anti-aging test model. The age of mice used is still young, between 6 and 8 weeks, so hopefully, the results obtained purely by treatment, not affected by the aging process of mice (Rahmawati 2019). Male mice have lower estrogen levels than female mice, so the effect is less on collagen measurement results. Estrogen has been shown to affect skin thickness by stimulating the synthesis and maturation of collagen (Wend et al. 2012).

The UV irradiation for 10 minutes is enough to slowly make the skin aging and do not cause severe acute erythema. After 28 days of treatment, the anti-aging activity was evaluated by a skin analyzer that performed on the mice under operational conditions (without anesthesia) because the test apparatus is not invasive nor painless. Also, the anesthesia process also affects the temperature and blood flow in skin tissue (Díaz and Becker 2010), so the possibility of anesthesia can affect the results of superficial measurements. The data obtained were not normally distributed and homogeneous, so the data were processed further using the Kruskal-Wallis test and Mann–Whitney test.

It can be seen from Fig. 1 that after four weeks of UV irradiation treatment, the most incredible sensitivity was found in the negative control group. In contrast, in the other groups, the sensitivity was found relatively small, with the smallest sensitivity found in the THC and PC group. The exposure to ultraviolet radiation from sunlight or the other UV sources for long or short but often periods can lead to free radical reactions. Large numbers of UV B cause oxidative injury in the epidermal layer, thus activating inflammatory mediators such as cytokines, vasoactive, and neuroactive. Then, ultimately, the skin undergoes an inflammatory response by sunburn or erythema. Sunburn may occur acutely, i.e., several minutes (less than 30 min) after exposure to UV, may also have delayed reactions. In general, sunburn is seen 24–48 h after exposure to UV rays (D'Orazio et al. 2013).

The use of natural antioxidants such as flavonoids or other phenolic compounds either orally or topically can serve as radical scavenging, thereby reducing inflammation due to the radical UV rays of the sun (Petruk et al. 2018). The negative control group was undergone the most significant skin sensitivity since it did not contain antioxidants. The smallest sensitivity was shown in the THC and PC group, which proved an effective antioxidant and

antiinflammation activity of THC and ethyl ascorbyl ether. Combining CAE with THC did not provide a synergistic inflammatory protective effect.

From the statistical analysis of the data in Fig. 2, the NC group's number and area of erythema after UV irradiation were significantly larger than the THC group, which means the UV irradiation method in this study can be used simulate the photoaging due to sun exposure. Otherwise, PC, THC, CAE, and THC-CAE sensitivity after the treatment were not significantly different compared to the sensitivity before the treatment. Therefore, the addition of active ingredient (ethyl ascorbyl acid, THC, CAE) in the given dose can protect the test animal's skin from the photosensitivity symptom. The erythema's number and area of the THC group were not different significantly from the PC group and significantly smaller than the negative control group.

From the results shown in Fig. 3, it is clear that exposure to UV light every day for 10 minutes for four weeks can cause signs of aging. Giving antioxidants to the PC, THC, CAE, and THC-CAE groups was able to protect the mouse skin from the sensitivity shown by the presence of erythema.

Antioxidants also affect the skin's water content, as evidenced by Fig. 4. The low water content in the negative control group was due to the absence of antioxidants, which protects the stratum corneum lipid in the epidermis from oxidation by free radicals of UV radiation. The oxidative damage causes the decrease of cell cohesion and the stratum corneum mechanical integrity, followed by increasing water transepidermal loss (WTL), and finally, the water content of the stratum corneum becomes decreased. The water content of CAE after treatment was the greatest compared to the other test group. Ratz-Lyko reported a similar result. The application of 2.5% and 5% cream and hydrogel *Centella asiatica* extract on 25 female volunteers aged 18–55 years twice daily for four weeks without sun, or artificial UV light exposure decreased the TWL and increased skin hydration significantly compared to the cream and hydrogel base (Ratz-łyko et al. 2016).

The high collagen content in the THC-CAE group indicated that the combination of THC and CAE functioning as a synergistic antioxidant that prevents dermal degradation of the dermis by inhibition of expression of MMP-1 and serves to stimulate collagen synthesis due to the triterpenoid from *Centella asiatica* extract. Collagen levels before and after treatment are shown in the following Fig. 5. The THC-CAE group had the most significant collagen level compared to other test groups. This increase in collagen levels is in line with the Trivedi report (2017). In the Trivedi study, the presence of 1 μg/mL THC in HFF-1 cells that were undergo UVB-induced stress improved level of collagen, elastin, and hyaluronic acid significantly (Trivedi et al. 2017). While Rahmawati reported that the application of *Centella asiatica* extract for four weeks did not result in elevated levels of collagen after treatment in histologic measurements, it could restrain a decrease in collagen levels better than negative controls (Rahmawati 2019).

As shown in Fig. 6, the elasticity after the treatment of groups given the antioxidant was higher than the negative control group. This is because the antioxidants protect the skin exposed to the sun's UV rays from damage by free radicals. Without antioxidants, free radicals in the dermis layer will directly induce the expression of MMP-2 and MMP-9 or gelatinases enzymes that can break down elastin fibers. The elastin fibers that rupture then clot irregular, forming elastosis tissue, so that the skin structure in the epidermis layer appears slack or lose elasticity.

The THC-CAE's most significant elasticity is caused by the synergistic activity of radical scavenging between THC and CAE. Besides, elasticity is also influenced by collagen levels because elastin fibers are in the vicinity and crosslinked with collagen tissue. The THC-CAE has the most widely collagen content, so it also has the most excellent elasticity (Hachiya et al. 2009; Langton et al. 2010).

In this study, the increase in skin elasticity was seen after four weeks of treatment. A similar result was also reported by Haftek (2008). The increase of elasticity was seen after six months of application of 0.1% madecasoside, i.e. triterpenoid derivative of *Centella asiatica*. According to Haftek, the triterpenoid of madecasocide has an essential role in improving skin elasticity, since madecasocide can induce collagen expression through activation of the SMAD signal pathway (Haftek et al. 2008).

The diameter of the pores is shown in Fig. 7. The simulation of UV rays in this study affected the larger pores in the experimental animals. Large pores are also affected by elasticity. The decreased skin elasticity makes the skin integrity and structural support around the perifollicular become weaker and looser, so the pore appears enlarged (Lee et al. 2016). Therefore, the diameter of the pores of CAE was higher than THC and THC-CAE.
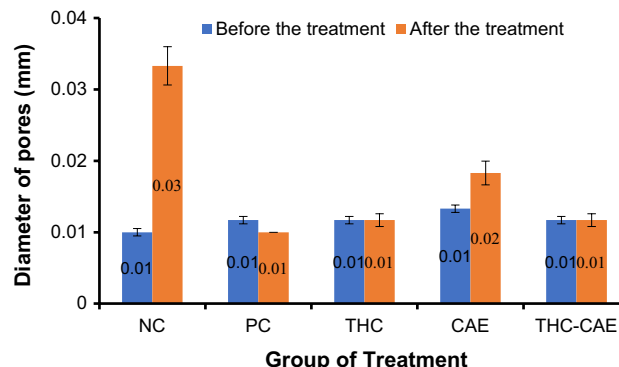


**Fig. 7** The comparison of diameter of pores before and after the treatment

To determine which treatment group (THC, EP, THC-CAE) has the largest anti-aging activity, each anti-aging parameter is scored by comparing each anti-aging parameter value between all the test groups (control group NC and KP; treatment group THC, CAE, THCCAE). Score values are $-1$, $0$, and $+1$. When the comparison of parameters has a worse and significant value, given a score of -1. When comparing parameters has a better value and significance, given a score of $+1$, and when the parameter comparison is worse or better significant, then given 0. Then all scores of each test group are summed. From the summarizing results, it can be seen that THC had the greatest anti-aging activity, while the CAE had the least anti-aging activity.

## Compliance with ethical standards

**Ethical statement** The Research Ethics Committee of Jendral Soedirman Medical Faculty states that the above protocol meets the ethical principle outlined in the Declaration of Helsinki 2008 and therefore can be carried out. All applicable institusional and/or national guidelines for the care and use of animals were followed. The Ethical Reference Number is 1751/KEPK/V/2017.

**Conflict of interest** Ika Yuni Astuti has no conflict of interest. Ani Yupitawati has no conflict of interest. Nunuk Aries Nurulita has no conflict of interest.

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