Efficacy of Indonesia Green Tea (Camellia sinensis) Leaf Extract as Natural Brightening, and Anti-acne on Human Skin: In Vitro and In Vivo Study

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Abstract

In Indonesia, beauty revolves around bright, acne-free skin, making brightening and anti-acne products a key market focus. The demand for natural ingredients in skincare is growing due to safety concerns. Indonesia's rich biodiversity offers opportunities for natural ingredients sources from plants. One prime candidate is green tea, known for its significant antioxidant effects due to catechin. This study evaluated green tea leaf ethanolic extract (GTLEE) and green tea leaf extract (GTLE) as a skincare ingredient through in vitro and in vivo tests. In the in vitro test assessing melanin content, GTLEE was evaluated at 1 and 10 ppm using the B16F10 cell line, with arbutin as positive control and α-MSH as the melanogenesis inducer over 3 days. Subsequently, in vivo tests were conducted on 22 subjects who applied 3% GTLE product twice daily for 28 days, with parameters evaluated such as skin freckles/blemishes, acne and sebum

control. The in vitro results indicated a significant efficacy of GTLE in reducing melanin content, comparable to arbutin. Moreover, in vivo tests demonstrated the 3% GTLE potential to increase skin brightness, significantly improve acne lesions, and minimize skin oil (sebum). The in vitro and in vivo study findings support its suitability for cosmetic ingredients.

Keywords: Green tea (*Camellia sinensis*) leaf extract; brightening; anti-acne; efficacy; human skin; sebum control.

Introduction

As human beings, many people are obsessed with looking beautiful. In Indonesia, having flawless, clear, bright and acne-free skin is seen as a particularly important stamp of beauty [1]. People who meet these criteria are often recognized as beautiful [2]. Many individuals invest time and money in skincare to achieve this standard. These beauty ideals have a huge impact on self-esteem and self-confidence, especially for those who do not naturally meet them [3]. The skincare industry in Indonesia has set a norm for the ideal sort by creating a beauty standard in Indonesia. It can be seen in many advertisements for beauty products in Indonesia and the most common is a skincare product that can claim as anti-dull skin to make skin brighter and solution for acne, which are prevalent skin problems in Indonesia [4].

Natural ingredients have gained much popularity and been claimed to have efficacy and intrinsic acceptability due to routine use in daily life and avoid the side effects which are commonly found in synthetic ingredients. On the other hand, compared to synthetic ingredients, natural ingredients are mild and biodegradable, exhibiting low toxicity. Natural plant molecules remain particularly interesting for new studies and several research carrying in different parts of the world on medicinal plants to developed newer natural skincare and cosmetic ingredients having lower side effects and rich sources of beneficial compounds. In the last few years, there has been an exponential growth in the field of natural skincare and cosmetic ingredients and they are gaining popularity both in developing and developed countries [5]

Globally, Indonesia is the second mega biodiversity country right after Brazil [6]. It is a tropical country that is rich in biodiversity and natural resources. There are tropical forests covering 143 million hectares, of which about 80% are indigenous medicine plants. Indonesia has 11% (38.000) of world's known flowering plant species, of which 18.700 are endemic [7]. The plant diversity in this country has many roles, such as the sources of food, medicine, cosmetics, aesthetics, and symbols of the culture. Local people in Indonesia usually take the benefits of plant diversity in their environment. The use of herbal medicine is widespread, not only for local people but also for modern society. There is a high potential of pharmaceutical and biotechnological research opportunities [8]. One of Indonesian plants that has potential to be researched as natural cosmetic ingredients is green tea (*Camellia sinensis*).

Green tea (*Camelia sinensis*) is the second most consumed beverage worldwide [9]. Indonesia is the world's seventh largest tea producing country after China, India, Kenya, Sri Lanka, Turkey, and Vietnam [10]. Green tea extract has 20 times more antioxidant activity than Vitamin C. The main attribution is supposed to be EGCG. Green tea extracts exhibit stronger antioxidant protection for the human body than vitamin C and vitamin E [11]. Indonesian tea is known for its high catechin content and approved by The International Society of Antioxidant in Health and Nutrition (ISAHN) as the highest tea catechin content in the world. Catechin, a compound that determines tea quality, is known for polyphenol derivatives that have high antioxidant properties [12]. Furthermore, tea catechin provides strong antioxidant activity, effective scavengers, and excellent electron donors to prevent the damage of cells caused by oxidative stress. The primary catechin in green tea, EGCG, has been extensively researched and shown to have positive health effects [13]. It has antibacterial activity against both gram-positive and gram-negative bacteria by inhibiting their growth and can also reduce inflammation in the body [14]. The ability of green tea polyphenol (GTP) in green tea extracts to eliminate lipid derived free radicals is noticeably stronger (almost 50 times) than that of ginkgo biloba extracts. In many experiments, green tea extracts show inhibitory effects on cancer cells [11]. In addition, in another research,

green tea (*Camelia sinensis*) has particular benefits on human skin as whitening, anti-acne, antioxidant, and anti-inflammatory thanks to flavonoids consisting of epigallocatechin gallate (EGCG) [15].

In term of environmental conditions, soil is the most important factor stimulating secondary metabolites of plants, as it controls the movement and availability of air, nutrients and water. Tea plants grow well on andisol soil. In Indonesia, the best tea plantations are also found on these soils, particularly on Java Island which is dominated by volcanic ash soils [16]. Most of the andisol soil is utilized for food and industrial estate crop production and supports some of the highest population densities in the country especially in Central Java. Temanggung is a town in Central Java, Indonesia, and one of the centers for vegetable production in the province. The soil in Temanggung is dominated by andisol which was derived from volcanic parent material from 2 mountains, Sindoro and Sumbing, and around the two mountains there are a lot of vegetables and food crops cultivated. About 42% (36,992 ha) of the area is located at an altitude between 400 to 700 m above the sea level and 54% (46,786 ha) is located in the >700 m elevation from the total area of about 87,226 ha [17]. Indonesian Andisols are thought to be very productive soils. These soils have unique and distinct properties, low bulk density, high water retention, high permeability, stable structure, high amount of active allophane (Al) and or ferrihydrite (Fe), variable charge, and high phosphate fixation. The chemical, physical, and morphological properties of these soils are closely related to the nature and behavior of non-crystalline and para-crystalline clay minerals, such as allophane, ferrihydrite, and imogolite. Allophanes are the most reactive components in volcano ash soil because of their high specific surface area. They strongly retain phosphate and organic matter [18]. Therefore, Temanggung, Central Java is one of the areas producing high-quality tea in Indonesia.

In this research, we examine the potential of green tea leaf extract (*Camellia sinensis*) from the Temanggung region of Indonesia as a natural brightening and anti-acne agent, both in vitro and in vivo. Our aim is to evaluate its effectiveness as an alternative ingredient for skincare products.

Materials and Methods

In Vitro Test Materials

Dried plants of green tea leaf. Ethanol 96% as extraction solvent. 2,2-diphenyl-1-picrihydrazyl (DPPH) reagents. Mushroom derived tyrosinase, potassium phosphate buffer, and L-DOPA. *Cutibacterium acnes* ATCC 11827, *Staphylococcus epidermidis* ATCC 12228**,** *Staphylococcus aureus* ATCC 12228 obtained from bacterial collections of the Laboratory of Microbial Analysis, School of Pharmacy, Bandung Institute of Technology.

In Vitro Test Methods

Anti-inflammatory

In the in vitro test, we used quantitative reverse transcription polymerase chain reaction (qRT-PCR) test method. The relative mRNA expression level of IL-1α/Actin and IL-1β/Actin of green tea leaf ethanolic extract (GTLEE) was tested at 1 and 10 ppm using the HaCaT (p4) cell line, dexamethasone at 1 µM as positive control, Poly I:C (10 µg/ml) + rh IL-4 (10 ng/ml) as inflammation inducer and conducted for 4 days.

Antioxidant

The assessment of antioxidative activity was accomplished through the utilization of the DPPH method. Initially, a DPPH solution was created, containing 50 µg/ml of DPPH in methanol. Subsequently, the absorbance of this DPPH solution was observed using a UV-visible spectrophotometer, employing a wavelength of 517 nm. As a standard reference, an ascorbic acid solution was meticulously prepared, containing 200 µg/ml of ascorbic acid in methanol. For the sample solution, varying volumes (12.5; 15; 20; 22.5; 25; and 30 µl) were combined with 125 µl of methanol and 750 µl of the DPPH solution. This mixture was incubated for 30 minutes in a sealed, dark environment. The absorbance was then recorded using a UV-visible spectrophotometer at 517 nm. To establish a baseline measurement, a blank solution was prepared utilizing methanol. Each concentration underwent absorbance measurements three

times. The calibration curve was generated based on the percentage of DPPH absorption in response to different concentrations of ascorbic acid solutions. The analysis of antioxidative activity in the sample solution was executed following the same procedure as the standard solution, with absorbance measurements being repeated six times for each extract. The antioxidative activity was determined through the linear regression equation derived from the ascorbic acid calibration curve and expressed as the antioxidant capacity equivalent of ascorbic acid per gram of extract (mg ascorbic acid equivalent antioxidant capacity (AEAC)/g of extract) [19].

Brightening Effect

In the in vitro test, the melanin content of green tea leaf ethanolic extract (GTLEE) was tested at 1 and 10 ppm using the B16F10 cell line, arbutin at 100 ppm as a positive control, α-MSH (100 μM) as melanogenesis inducer and conducted for 3 days.

Antibacterial Activity

Antimicrobial activity testing was conducted utilizing the broth microdilution method, which was adapted from the CLSI guidelines [20]. To prepare the inoculation suspension, the broth culture was initially diluted with a medium solution to achieve a 0.5 McFarland standard, equivalent to a concentration of 5 × 10^5 CFU/ml. This suspension was further diluted at a ratio of 1:20 with the medium. Next, 0.01 ml of this suspension was introduced into each well of a 96-well microwell plate. As a point of reference, tetracycline, nystatin, and ketoconazole were employed as positive controls. The microbial inhibition profile of the isolated substance was assessed using a microplate reader. Absorbance values were determined by measuring the reduction in microbial culture absorption in the presence of the isolate when compared to a mixture of medium and sample, at a wavelength of 625 nm.

In Vivo Test Materials

Dried plants of green tea leaf. Water, and 1,3-butylene glycol as an extraction solvent in consideration of its rapid application in cosmetics. 1,2-hexanediol as preservative.

In Vivo Test Methods

Brightening Skin Effect

The clinical brightening test was conducted by Global Medical Research Center. The subjects in this in vivo test are 22 participants (39-57 years old) with skin freckles/blemishes. 3% Green tea leaf extract (GTLE) as cream was applied twice a day for 28 days. Measurement of skin freckles/blemishes is done by using the Antera 3D CS, the face (freckles/blemishes) left and right sides were divided into a test site and a control site and measured and the affected area (mm^2) value of the Melanin-Hyperconcentration analysis mode was used as evaluation data. The measuring principle is to measure the condition of the surface images of the skin using a light-emitting diode (LED light source). The data is extracted from the three-dimensional shape image using the built-in program and the skin condition is quantified to change over time. The affected area (mm^2) value decreases as the skin freckles/blemishes are improved. Statistical analysis was conducted by using the IBM SPSS statistics 25.0 programs. The significant difference before and after the use of the test product was confirmed by the hypothesis mean difference of 5% ($p<0.05$).

Anti-acne Effect

The clinical test of anti-acne was conducted by Global Medical Research Center. The subjects in this in vivo test are 22 participants (15-32 years old) who selected for acne-prone skin (IGA grade 2-3), 3% GTLE as serum was applied twice a day for 28 days with the Mark-Vu as an instrument test. Lesions (closed comedones, open comedones, papule, pustule, nodule) on the face were counted according to each classification standard and used as evaluation data. Statistical analysis was conducted by using the IBM SPSS statistics 25.0 program. The significant differences before and after the use of the test product were confirmed by the hypothesis mean difference within 5% ($p<0.05$).

Sebum Control

The clinical test of sebum control was conducted by Global Medical Research Center. The subjects in this in vivo test are 22 participants (39-57 years), 3% GTLE as serum was applied twice a day for 28 days. Skin oil (sebum) was measured using Sebumeter SM815, and for comparison with the control product (half-test), the test and control areas (left/right cheek) were measured once each. It was used as evaluation data. The amount of oil adsorbed on the Sebumeter Cartridge is measured by the optical reflection principle. The amount of light transmitted increases as the amount of oil increases, and since the amount of transmitted light is digitized in the range of 0-350 μg/cm2, the decrease in the measured value means that the skin oil (sebum) is improved. Statistical analysis was conducted by using the IBM SPSS statistics 25.0 program. The significant differences before and after the use of the test product were confirmed by the hypothesis mean difference within 5% ($p<0.05$).

Results

Green tea leaf ethanolic extract (GTLEE) and green tea leaf extract (GTLE) was tested through in vitro and in vivo tests with several test parameters such as anti-inflammatory, antioxidant, brightening effect, antibacterial activity, and sebum control.

In Vitro Test Result

Anti-inflammatory Activity

Anti-inflammatory activity was tested through the quantitative reverse transcription polymerase chain reaction (qRT-PCR) test method to support soothing skin effect, carried out using inflammatory mediators such as IL-1a and IL-1b in green tea leaf ethanolic extract (GTLEE) 1 and 10 ppm. The results obtained showed that the Relative mRNA expression level of IL-1a/Actin at 1 ppm was 2.51 and at 10 ppm was 1.85 while in dexamethasone it was 1.22 so that between 10 ppm GTLEE and dexamethasone there was no significant difference. Then in the Relative mRNA expression level of IL-1b/Actin at 1 ppm it was 1.93 and at 10 ppm it was 2.02, while in

dexamethasone as a positive control, it was 0.78, so that between GTLEE 1 and 10 ppm compared to the positive control there was no significant difference (Figure 1).

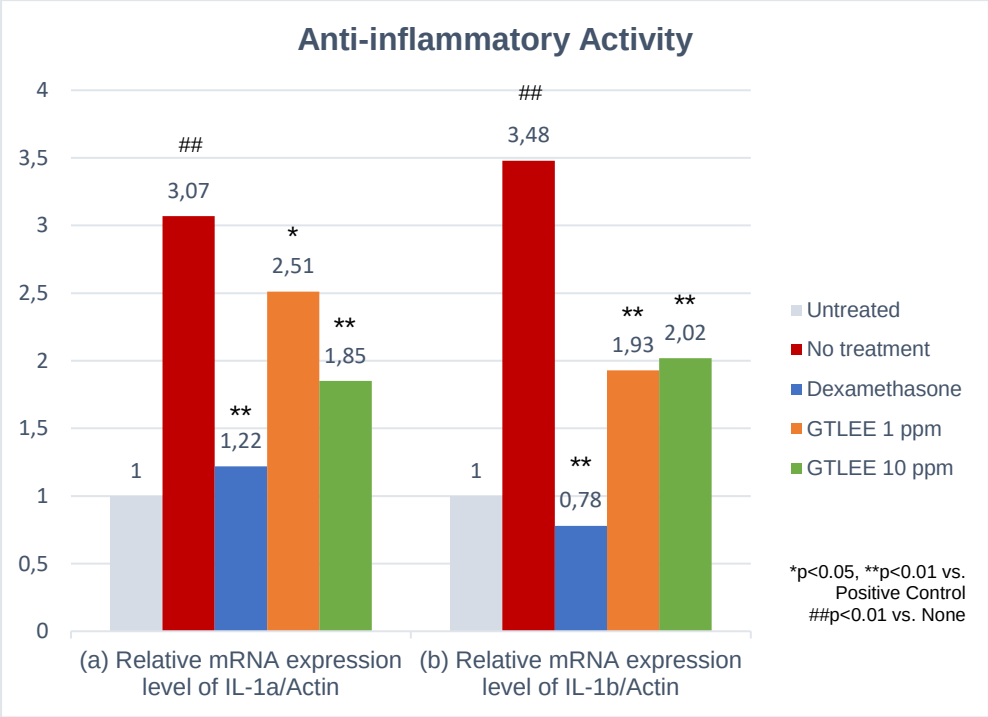

Figure 1: Anti-inflammatory Activity of Green Tea Leaf Ethanolic Extract (GTLEE): (a) Relative mRNA Expression Level of IL-1a/Actin. (b) Relative mRNA Expression Level of IL-1b/Actin

Antioxidant

In the antioxidant test through DPPH scavenging, the IC50 of green tea leaf ethanolic extract (GTLEE) was 3.56 ± 0.11 while the IC50 of ascorbic acid as a positive control was 2.54 ± 0.02 so that it can be seen that the antioxidant potential of green tea is not significantly different compared to the positive control (Figure 2).

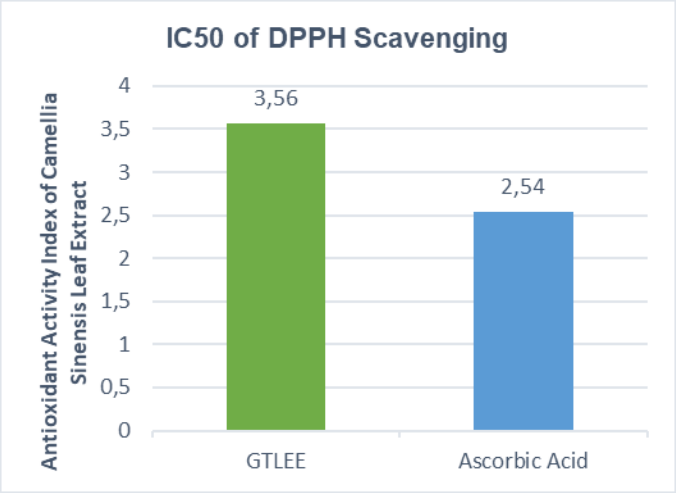

Figure 2: DPPH Scavenging IC50 Value of GTLEE Compared to Control

Brightening Effect

In the examination of melanin content, the test results showed that GTLEE at 1 ppm showed a melanin content of 112.8, while at 10 ppm, it showed a measurement result of 105.9. As a comparison, arbutin, which was used as a positive control, recorded a result of 91.9. Based on these results, it can be seen that the melanin content of GTLEE 1 and 10 ppm is not significantly different from arbutin (Figure 3).

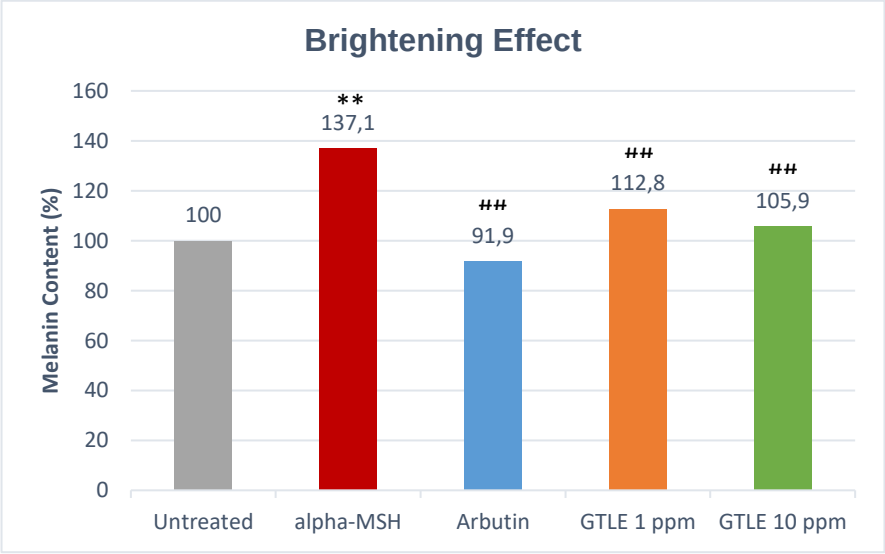

Figure 3: Melanin Content Assay

***: p<0.01 vs. None; ##p<0.01, #p<0.05 vs Negative Control*

Antibacterial Activity

Green tea ethanolic extract (GTLEE) show proper potential towards *P. acnes* with Minimum Inhibitory Concentration (MIC) of 500 µg/mL and Minimum Bactericidal Concentration (MBC) of 1000 µg/mL (Table I).

Table I. MIC and MBC Value of GTLEE Towards Several Bacteria

Microbe	MIC (µg/mL)	MBC (µg/mL)
Propionicbacterium acnes ATCC 11827	500	1000
Staphylococcus epidermidis ATCC 12228	500	500
Staphylococcus aureus ATCC 6538	500	2000

In Vivo Test Result

Brightening Skin Effect

In the clinical brightening test conducted by the Global Medical Research Center (GMRC) in South Korea, the results showed that there was a significant difference between the green tea leaf extract (GTLE) group and the control group as seen from the improvement effect on skin spots/blemishes of 16.43% after using 3% GTLE in cream, while in the control group which only increased by 9.2% ($p<0.001$) (Figure 4-5).

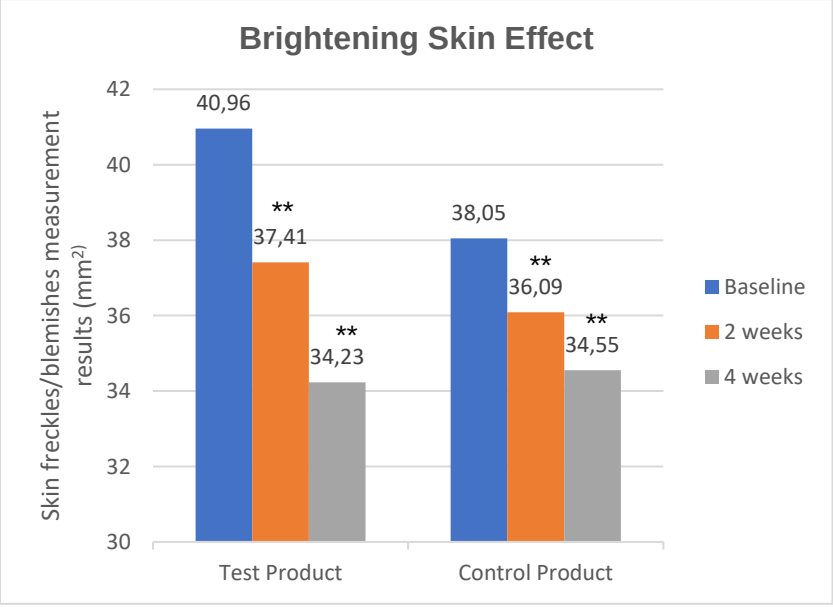

Figure 4: Skin Freckles/Blemishes Measurement Results - Comparison Within The Group

***: p<0.05 by repeated measures ANOVA*

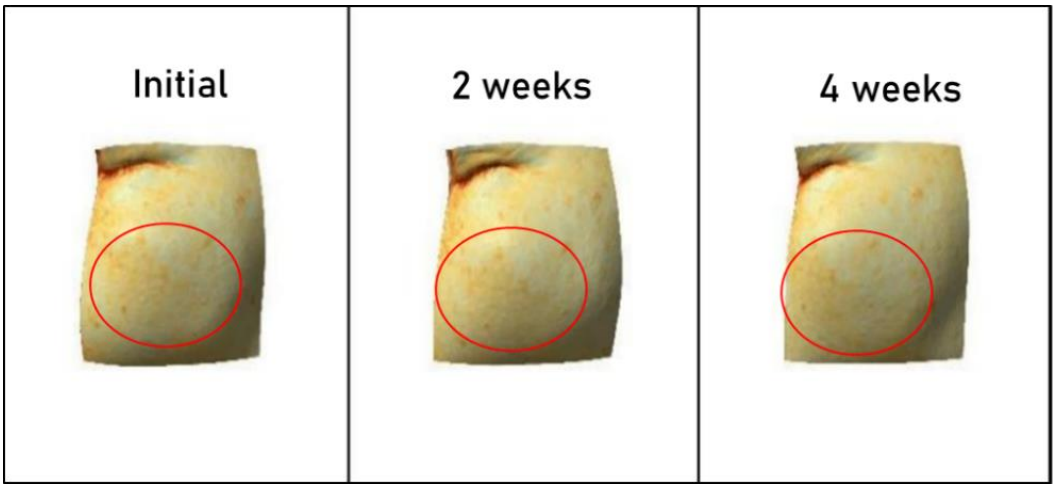

Figure 5: Measurement Image of Skin Freckles/Blemishes

Anti-acne Effect

In the anti-acne in vivo test conducted by the Global Medical Research Center in South Korea, the results showed that there was an improvement effect on all acne types such as decrease in whiteheads by 15.01%, blackheads by 20.38%, significant improvement in papules by 47%, pustules by 63%, with complete eradication of nodules by 100% after 28 days of use 3% green tea leaf extract (GTLE) in serum applied twice a day on fase (Figure 6-8).

Figure 6: High Resolution Picture of Anti-acne Effect

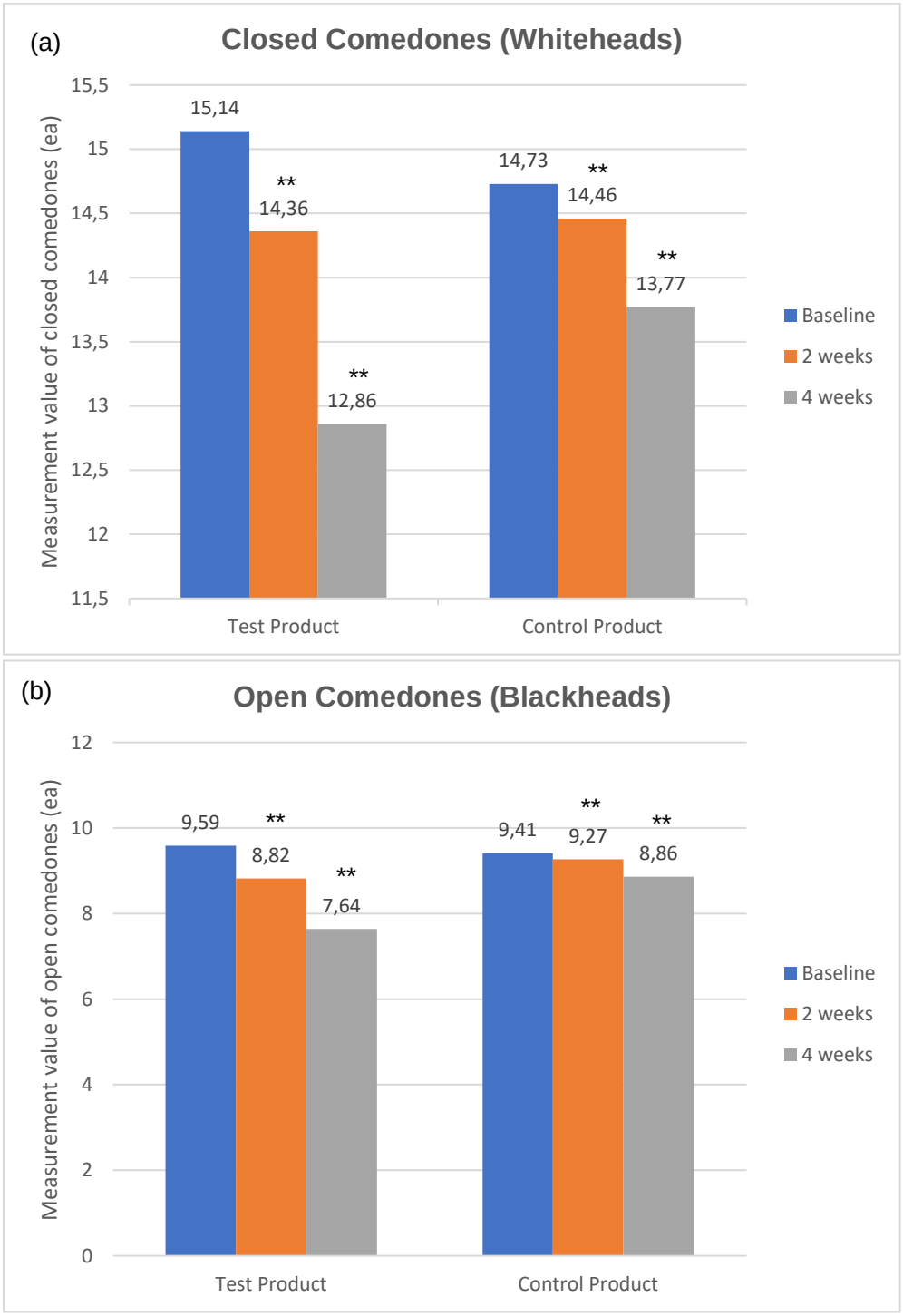

Figure 8: (a) Non-inflamed lesions - closed comedones measurement result - comparison within the groups. (b) Non-inflamed lesions - open comedones measurement result - comparison within the groups

***: p<0.05 by repeated measures ANOVA*

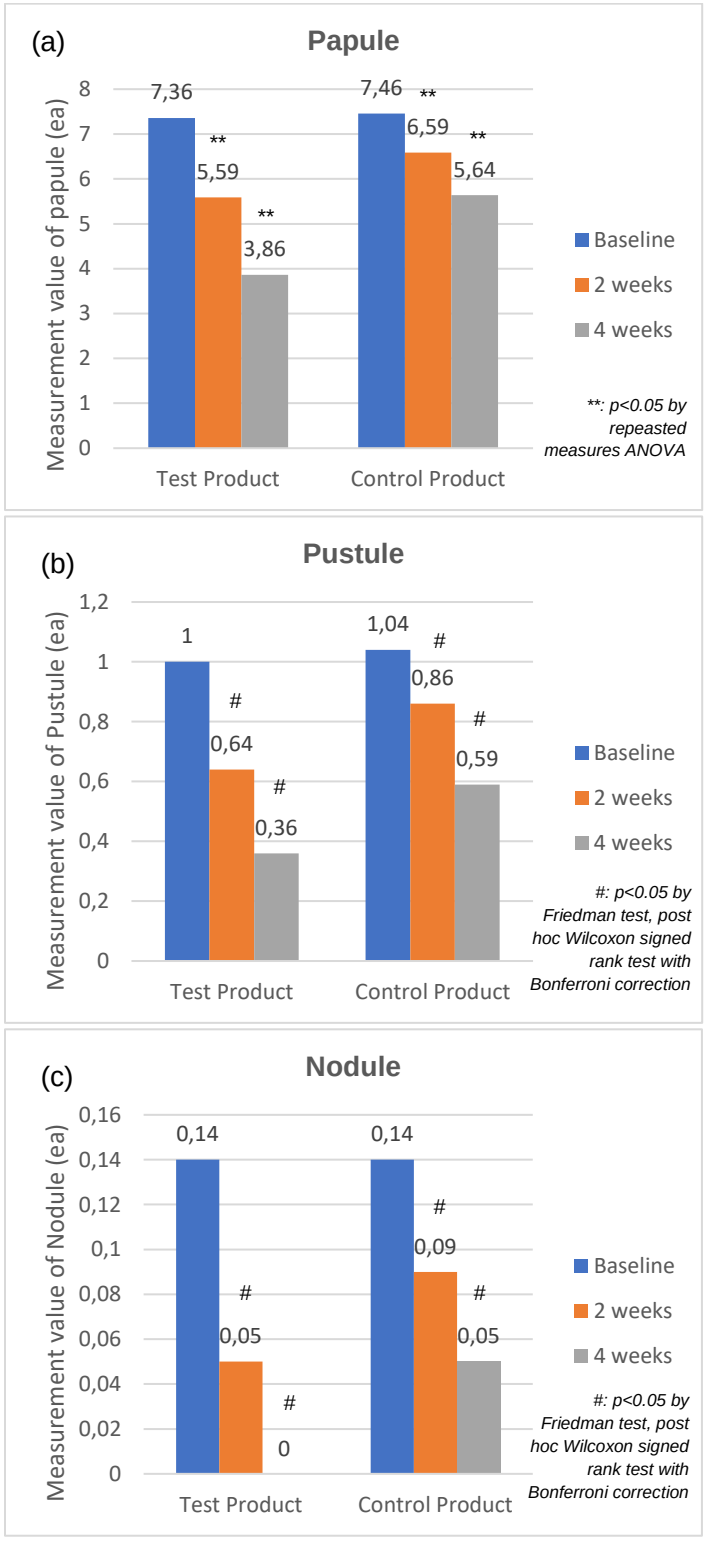

Figure 9: (a) Inflamed lesions - papule measurement result - comparison within the groups. (b) Inflamed lesions - pustule measurement result - comparison within the groups. (c) Inflamed lesions - nodule measurement result - comparison within the groups

Sebum Control

In the in vivo test of sebum control, compared to before use, 3% of green tea leaf extract (GTLE) in serum can reduced skin oil (sebum) by 76% after 28 days and the improvement rate of GTLE cream was significantly higher compared to control product ($p<0.05$) (Figure 9).

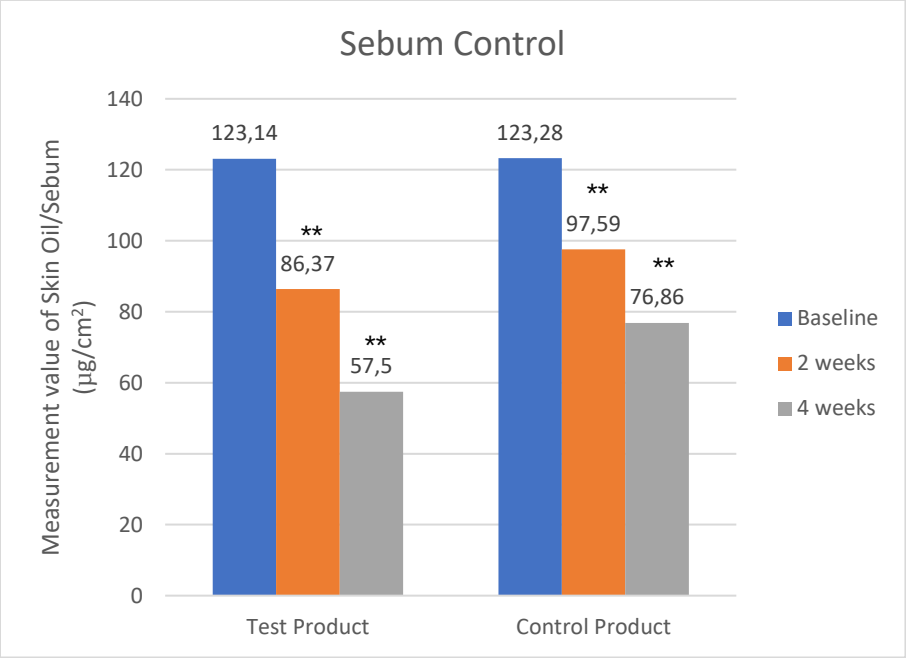

Figure 9: Sebum Measurement Result – Comparison Within The Group

***: p<0.05 by repeated measures ANOVA*

Discussion

Interleukin (IL)-1 family cytokines initiate inflammatory responses, and shape innate and adaptive immunity. They play important roles in host defense, but excessive immune activation can also lead to the development of chronic inflammatory diseases. Dysregulated IL-1 family signaling is observed in a variety of skin disorders. In particular, IL-1 family cytokines have been linked to the pathogenesis of psoriasis and atopic dermatitis. IL-1α is highly expressed in the skin and IL-1β may also be involved in the inflammatory process [21]. Oxidative stress and inflammation seem to play a critical part in the skin maturing procedure, green tea may likewise

have anti-aging impacts by diminishing inflammation, irritation, and scavenging free radicals. Scientists have discovered that the principal component in green tea, EGCG, functions admirably as a calming, oxidation-inhibiting agent, anti-inflammatory, and sunscreen [22]. Based on the in vitro anti-inflammatory test using inflammatory mediators IL-1α and IL-1β, the results showed that green tea leaf ethanolic extract (GTLEE) has anti-inflammatory activity because it is not significantly different compared to dexamethasone as a positive control. Therefore, it has a relationship to reduce signs of inflammation on the skin such as itching, redness, and irritation so it can be used as a candidate for skin soothing agent.

DPPH scavenging assay was performed to study the antioxidant potential of green tea leaf. The green tea leaf ethanolic extract (GTLEE) gave IC50 value 3.56 µg/mL. The result aligns with the IC50 value of GTLEE by [23]. The IC50 value of positive control used in the test was 2.54 µg/mL, as described in Figure 2. This value is categorized as powerful antioxidant activity, following the consideration established in the previous study by [24].

Table II. Antioxidant activity categories [24]

IC50	Antioxidant Properties
<50	Powerful
50-100	Strong
100-150	Moderate
150-200	Weak

Several studies of green tea polyphenols have reported the central role of the inhibitory effects of tyrosinase activity. [25] showed that EGCG inhibited α-MSH-induced melanin production in B16 melanoma cells. Green tea polyphenols exhibit anti-melanogenic effects by inhibiting the activity and expression of tyrosinase. Previous research has proven that Epigallocatechin Gallate could be used as a skin lightener by inhibiting melanogonesis and suppressing melanosome maturation activity. The mechanism of Epigallocatechin Gallate as an anti-acne was by inhibiting the 5 alpha-reductase type 1 enzymes in the subasea gland so it could inhibit excessive sebum production which could trigger acne growth [26]. Based on the results of melanin content assay, green tea leaf ethanolic extract (GTLEE) is not significantly different from

arbutin as positive control, thus showing that GTLEE has the potential to be used as a candidate for skin brightening. In line with the results of the melanin content assay, the clinical brightening test by the Global Medical Research Center (GMRC) showed that 3% green tea leaf extract (GTLE) could brighten the skin compared to the control group.

Antimicrobial properties of the extract were studied towards the microbes commonly found during skin inflammation, especially on the facial area. The bacteria used *are P. acnes*, *S. epidermidis*, and *S. aureus* [27]. Green tea extract exhibited antibacterial activity against *S. epidermidis*, *S. aureus*, and *P. acnes*. Phenolic compounds available from green tea extract may be beneficial for treating acne-mediated bacterial infections [28]. Propionibacterium acnes play an important role in the pathogenesis of acne by inducing certain inflammatory mediators and comedogenesis [29]. The in vitro antibacterial tests showed green tea leaf ethanolic extract (GTLEE) has the potential to be used as an anti-acne because it has antibacterial activity against acne-causing bacteria *S. epidermidis, S. aureus*, and especially *P. acnes*.

The clinical anti-acne test Global Medical Research Center (GMRC) results showed that 3% green tea leaf extract (GTLE) can improve all types of acne on the skin. This result aligns with the previous study by [30] that showed the result of topical green tea extract (GTE) application was effective in reducing total acne lesion counts in both inflammatory and non-inflammatory lesions without causing any serious adverse events than oral intake. This study provides novel insights into the respective beneficial effects of GTE in patients with acne vulgaris that may be attributed to the antimicrobial, anti-inflammatory, antioxidant, and ability to modulate sebum synthesis.

Conclusion

Based on the in vitro test that has been conducted, it can be concluded that green tea leaf ethanolic extract (GTLEE) has anti-inflammatory activity that has the potential to be used as a

skin soothing agent and has powerful antioxidant activity. In addition, the results of in vitro and in vivo tests show that 3% green tea leaf extract (GTLE) can be used as a cosmetic raw material as a natural brightening, anti-acne and sebum control.

Acknowledgments

NONE.

Conflict of Interest Statement

NONE.

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