

Research Article

Irritation Test using the HET-CAM Method on A Sunscreen Formulation Containing A Combination of Tea Tree Oil and Raspberry Oil

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Abstract: Exposure to ultraviolet (UV) radiation and the risk of skin irritation are major challenges in the development of modern skincare products. Sunscreens not only serve to protect the skin from UV radiation but must also meet safety requirements and be non-irritating. Tea tree oil (*Melaleuca alternifolia*) possesses anti-inflammatory, antimicrobial, and wound-healing properties, while raspberry oil (*Rubus idaeus*) is rich in antioxidants, vitamin E, and essential fatty acids beneficial for skin health. The combination of these two oils has the potential to enhance sunscreen effectiveness while providing additional natural anti-inflammatory benefits. This study aims to evaluate the irritation effect of a sunscreen cream formulation containing a combination of tea tree oil and raspberry oil using the HET-CAM (Hen's Egg Test-Chorioallantoic Membrane) method. This method was chosen because it can mimic vascular responses similar to human skin, where parameters such as hemorrhage, coagulation, and membrane damage serve as indicators of irritation levels. The results of this study are expected to provide scientific information regarding the safety of the combined essential oils and support the development of safe, effective, and natural cosmetic products with anti-inflammatory sunscreen potential.

Keywords: HET-CAM, raspberry oil, skin irritation, sunscreen, tea tree oil

1. INTRODUCTION

Exposure to ultraviolet (UV) radiation and irritation are crucial factors in modern skincare. The function of sunscreen is not limited to protection against UV radiation alone; it must also meet safety criteria and be non-irritating. Tea tree oil (*Melaleuca alternifolia*) is known for its anti-inflammatory, antimicrobial, and wound-healing properties, while raspberry oil (*Rubus idaeus*) is rich in antioxidants, vitamin E, and essential fatty acids that are beneficial for skin health. The combination of these two ingredients has the potential to enhance the effectiveness of sunscreen while providing additional anti-inflammatory benefits.

The skin is the largest organ in the human body. It is a complex epithelial and mesenchymal tissue consisting of a multi-layered stratified epidermis; accessory structures such as hair follicles, sweat glands, and sebaceous glands; a dermis containing collagen and elastic fibers; and

subcutaneous fat [1]. Proper skin care makes the skin look healthy, well-maintained, and radiant. The skin has a complex, elastic, and smooth epithelial tissue structure, and its type and color vary according to climate, race, gender, and age [1].

Sunlight consists of UV A, B, and C spectra with wavelength ranges of 320-400 nm, 260-320 nm, and 200-260 nm, respectively. Sunlight offers various benefits, such as helping to prevent vitamin D deficiency, psoriasis, sarcoidosis, mycosis fungoides, and various other skin diseases [2].

However, in addition to providing benefits, sunlight can also cause negative effects such as photoaging and even skin cancer if excessive exposure occurs. Sunlight is known as a risk factor for three types of skin cancer. Protection against sunlight exposure can be achieved physically through clothing, hats, umbrellas, and sunglasses; however, because some body parts often cannot be physically protected, sunscreen can be utilized[3].

Sunscreen protects the skin from UV exposure through two mechanisms: absorbing UV rays or reflecting them back. Furthermore, sunscreen is available in various dosage forms that can be adjusted to meet specific consumer needs [2].

In terms of dosage form application, sunscreens can be applied topically (externally on the body) and systemically, where the preparation is ingested by the patient (oral). Systemic sunscreens are absorbed by the body and accumulate in the skin to provide protection against UV rays. Several compounds offer systemic photoprotective effects, such as β -carotene, vitamins A, C, and E, retinol, selenium, green tea polyphenols, PABA, antihistamines, aspirin, indomethacin, and corticosteroids [4].

Essential oil components exhibit significant antibacterial activity but also have the potential to cause irritation. Therefore, the selection and formulation of essential oil ingredients in topical products must consider the balance between antibacterial effectiveness and safety of use. The HET-CAM method can be used as an alternative method for evaluating the irritation potential of topical materials [5].

Essential oils, particularly tea tree oil and other natural oils, are widely used in pharmaceutical and cosmetic products because they possess antibacterial, anti-inflammatory, and antioxidant activities. However, the use of essential oils can also cause side effects such as skin irritation and allergic reactions. Therefore, evaluating the safety of natural ingredients in topical formulations is very important before they are widely used [6].

The use of Tea Tree Oil (TTO) in various cosmetic preparations, such as shampoos, facial cleansers, and face creams, is generally considered safe and non-irritating to both the skin and eyes as long as the specified concentration limits are followed. The maximum recommended concentrations are 2% for rinse-off products, 1% for facial cleansers, and 0.1% for leave-on products. Although animal testing indicates that pure TTO can be a strong irritant with a Draize irritation index of 5.0, preparations with concentrations of 25% or less have been proven not to produce such irritant effects [7].

The extraction process involves steam distillation for 2 hours at a temperature of 1000°C. The rising steam is filtered through a condenser, where it undergoes a drastic temperature drop, causing it to condense into droplets of oil and water. The oil and water then pass through a separation chamber to obtain pure tea tree oil [8].

Raspberry Seed Oil (RSO) is a valuable cosmetic ingredient because it contains high concentrations of Vitamins A and E. These vitamins are essential for the maintenance and repair of

keratinocytes, which are the main cells that make up the skin's structure. Additionally, RSO is known to possess a natural Sun Protection Factor (SPF) value, which is reported in some literature to be between 29 and 50. Because it can absorb harmful UV rays, it is often studied for its potential use in broad-spectrum sunscreen formulations [9].

Rosemary and tea tree oils have been shown to contain antioxidant compounds such as carnosol and cineole, which can combat free radicals responsible for skin aging. Although their UV protection level is relatively low (SPF 6–8), the combination of these oils has the potential to be developed as natural ingredients in sunscreen creams or anti-aging serums [10]. Both essential oils exhibit strong antioxidant activity that increases with concentration, with rosemary demonstrating higher SPF values. Despite the relatively low SPF levels, the combination of these oils shows potential as an additional ingredient in cosmetic products for UV protection and anti-aging purposes [10].

Raspberries are an extraordinary source of nutrients for the skin as they are rich in antioxidants, vitamins A, C, and E, as well as ellagic acid, which plays a vital role in preventing premature aging by protecting collagen from damage caused by free radicals and sun exposure. Beyond its ability to diminish wrinkles and fine lines, raspberry extract functions as a natural brightener capable of evening out skin tone by inhibiting the tyrosinase enzyme in the melanin formation process. On the other hand, raspberry seed oil is highly valued for its ability to deeply hydrate the skin and strengthen the protective lipid barrier through its essential fatty acid content, specifically omega-3 and omega-6. With antimicrobial and anti-inflammatory properties that effectively control acne, soothe irritation, and clear pores, raspberry serves as a safe natural ingredient for all skin types and holds great promise for the development of future beauty products [11].

Creams are water-in-lipid (oil > water) emulsions formulated for topical application on the skin or mucous membranes. Creams have a texture that is easy to spread and can serve as a vehicle for active ingredients such as moisturizers, medications, or sunscreens, containing preservatives and stabilizers to prevent phase separation. Creams are more effective at moisturizing the skin than lotions. Cream formulations have heavier lipids and can be used as nighttime moisturizers [12].

Several advantages of cream formulations include being easier to apply, comfortable to use, non-sticky, and easily washable with water. Furthermore, oil-in-water (o/w) type cream bases can enhance and improve skin moisture [13].

Sunscreen is a cosmetic preparation used to protect human skin from the unwanted effects of sunlight through specific protection mechanisms. Sunscreen preparations can be formulated in various dosage forms, such as creams, lotions, and ointments. For topically applied preparations, sunscreens are categorized into two mechanisms: those that reflect or scatter sunlight (reflecting/scattering) and those that absorb sunlight (absorbing)[14].

The Hen's Egg Test on Chorioallantoic Membrane (HET-CAM) is an *ex vivo* assay conducted using 10-day-old fertile White Leghorn chicken eggs to evaluate ocular irritation potential. In this procedure, a 0.3 ml volume of the test substance is applied directly onto the chorioallantoic membrane (CAM). Researchers then monitor the membrane for a period of five minutes to observe three specific vascular endpoints: hemorrhage (vessel bleeding), lysis (vessel disintegration), and coagulation (protein denaturation). The irritation potential is subsequently determined by calculating an irritation index (RI or IS) derived from the exact time, recorded in seconds, at which each of these reactions first appears [15].

The HET-CAM (Hen's Egg Test-Chorioallantoic Membrane) method has several key advantages over other conventional irritation testing methods, especially when compared to *in vivo* tests like the Draize rabbit eye test. The most fundamental advantage is that HET-CAM is an *in vitro/in ovo* alternative that highly upholds the 3Rs principles of animal ethics (Replacement, Reduction, Refinement), making it free from animal cruelty issues (animal cruelty-free). Biologically and anatomically, the chorioallantoic membrane in embryonated chicken eggs (typically 9 to 10 days old) has a highly complex vascular system—comprising arterial, venous, and capillary networks—that can accurately and sensitively mimic the inflammatory response in human conjunctival tissue. Compared to other irritation tests, HET-CAM offers a significantly faster evaluation process (requiring only about 5 minutes of observation time to assess toxic effects such as lysis, hemorrhage, and coagulation), more practical procedures, more economical operational costs, and is scientifically proven to have an excellent correlation with *in vivo* test results in determining the severity category of an irritant.

However, before topical application, it is important to evaluate the safety of the sunscreen formulation, particularly its irritation effect. The HET-CAM (Hen's Egg Test-Chorioallantoic Membrane) method was chosen as a testing model due to its ability to mimic vascular responses similar to human skin, where parameters such as hemorrhage, coagulation, or membrane damage can serve as indicators of irritation [10].

Halal certification in Indonesia has evolved alongside the increasing public awareness of halal products. To ensure the presence of halal products—particularly in food, cosmetics, and pharmaceuticals—the government enacted the Halal Product Assurance Law. Certification, which was initially under the authority of the Indonesian Ulema Council (MUI) through LPPOM, has since been transferred to the BPJPH, an official government body under the Ministry of Religious Affairs. This transition was accompanied by several procedural changes and adjustments to the parties involved in the certification process [16]. Synergy and collaboration among the government, business actors, and society are essential to monitor product circulation and realize comprehensive halal product assurance in Indonesia [10].

2. MATERIALS AND METHODS

2.1. Type of Research

This study is an experimental research designed to evaluate the irritation potential of a cream formulation containing tea tree oil and raspberry oil using the HET-CAM (Hen's Egg Test-Chorioallantoic Membrane) method.

2.2. Time and Place of Research

2.2.1. Research Location

The research was conducted at the Pharmaceutical Technology Laboratory, Sultan Agung Islamic University, Semarang, Indonesia.

2.2.2. Research Period

The study was carried out from July to August 2025.

2.2.3. Tools and Materials

a. Instruments

The instruments used in this study include:

The instruments used in this study consisted of equipment for cream formulation and the HET-CAM irritation test. The cream formulation process utilized a 100 mL Duran® beaker glass, a stirring rod, Pyrex® measuring cylinders (10 mL, 50 mL, and 100 mL), a mortar and pestle, a dropper pipette, a Hanna® pH meter, parchment paper, an analytical balance, a watch glass, a Memmert® water bath, a Jangkar® porcelain dish, a horn spoon, a spatula, cream containers, and a viscometer. Furthermore, the equipment required for the HET-CAM irritation test included a Mettler Toledo® analytical balance, a Memmert® IN-75 incubator (Germany), sterile scissors, a hot plate, a syringe, and tweezers.

b. Materials

The materials used in this study consisted of ingredients for cream formulation and materials for the HET-CAM irritation test. For the cream formulation, the materials included tea tree oil, raspberry oil, stearic acid, triethanolamine, glycerin, methyl paraben, propyl paraben, 96% ethanol, and distilled water (aquadest). Furthermore, the materials required for the HET-CAM irritation test comprised Leghorn chicken eggs obtained from Ayam kampung Farm, Semarang city, Central Java. sodium lauryl sulfate (SLS) solution, distilled water (aquadest), 0.9% NaCl solution, and the sunscreen formula with the best SPF value.

2.2.4. Procedures

a. Cream Formulation

The formulation base consisted of the essential components of a cream preparation, including the oil phase, aqueous phase, emulsifying agents, and additional ingredients.

Table 1. Formulation of sunscreen tea tree oil and raspberry oil

Ingredients	Concentration w/o %	Function
<i>Tea Tree Oil</i>	2.5	Active Ingredient
<i>Raspberry Oil</i>	1.5	Active Ingredient
Stearic Acid	8	Emulsifier
Triethanolamine	3	Emulsifier
Gliseryn	10	Humectant
Metyl Paraben	0.02	Preservative
Propyl Paraben	0.02	Preservative
Aquadest	Ad 100	Solvent

b. Preparation of the Formulation

The sunscreen cream was prepared by the fusion method. The oil phase, consisting of *stearic acid* and *propyl paraben*, was melted in a porcelain dish over a water bath at 70°C with constant stirring until homogeneous. The aqueous phase, containing *distilled water*, *methyl paraben*, *glycerin*, and *triethanolamine (TEA)*, was heated separately in a beaker glass on a water bath to approximately 70°C. A preheated mortar was prepared, and the aqueous phase was transferred into it. The oil phase was then added gradually while continuously stirring to form a homogeneous emulsion. The mixture was triturated until a creamy mass was obtained. The active ingredients (tea tree oil and raspberry oil) were then added slowly into the mortar and mixed until homogeneous

c. Evaluation of the Formulation

The formulated sunscreen cream was evaluated for its irritation potential in vitro using the Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM) method. This test was conducted to determine whether the cream formulation caused irritation, considering variations in the concentrations of the active ingredients, tea tree oil and raspberry oil.

HET-CAM Irritation test procedure

The HET-CAM irritation test procedure is conducted by incubating embryonated chicken eggs (typically the Leghorn breed) for 9 days at a temperature of 37°C – 38°C until the chorioallantoic membrane (CAM) is fully developed. Following the incubation period, the shell over the air space is carefully opened, and the inner white membrane is peeled back to expose the CAM vasculature, which is then treated with the test substance for a 300-second observation period. The observed irritation parameters include the occurrence of hemorrhage, lysis, and coagulation, where the time of onset for these reactions is calculated to determine the irritation index score.

The HET-CAM method was divided into several phases as follows:

- Phase I: Preparation of Eggs

Fertilized Leghorn chicken eggs were inspected for any defects and cleaned with 70% alcohol before incubation at $37 \pm 1^\circ\text{C}$ under controlled humidity 55%-70% [17]. The humidity and temperature are controlled using an automated method via a digital thermometer equipped with temperature and humidity displays. This system operates automatically, where the machine will shut down independently if the temperature or humidity levels exceed the predefined threshold.

On day 4, the eggs were candled to determine embryo viability and position. A small opening was made in the shell, and the chorioallantoic membrane (CAM) was moistened with 2–3 mL of 0.9% NaCl solution. Approximately 3.0 mL of albumin was removed from each egg to allow the embryo to detach from the shell. The opening was sealed with transparent tape, and the eggs were returned to the incubator. Daily observations were conducted. On day 10, a Teflon ring was placed on the CAM of each egg to serve as a reservoir for sample application.

- Phase II: Preparation of Test Samples and Positive Control

This procedure is performed by preparing a Sodium Lauryl Sulfate (SLS) solution at a standard concentration (typically 1% w/v in distilled water). After the eggs have been incubated for 9 days and the CAM is exposed, a 0.3 mL volume of the SLS solution is applied directly onto the membrane surface. Immediately upon contact, observation is conducted for 300 seconds to record the onset of progressive irritation reactions, as SLS typically triggers rapid hemorrhage (bleeding) and protein coagulation.

- Phase III: Test Implementation

The sample application procedure begins with preparing the test substances, which may be in the form of liquid, gel, or cream preparations, to be exposed directly to the surface of the chorioallantoic membrane (CAM). After opening the eggshell and peeling back the inner white membrane on the 10th day of incubation, a volume of 0.3 mL (for liquid preparations) or a sufficient amount to cover the membrane surface (for semi-solid preparations) is drawn using a micropipette or a sterile syringe. The sample is then slowly applied onto the observed

CAM. Immediately after the sample makes contact with the blood vessels, a stopwatch is started to initiate a 300-second observation period to monitor for hemorrhagic reactions, lysis, or coagulation. After the observation period ends, the sample may be gently rinsed with warm 0.9% NaCl solution, if necessary, to ensure the blood vessels are clearly visible for the final score assessment.

- Phase IV: Evaluation of Egg Membrane

After observation, the eggs were removed from the incubator, and the transparent tape was carefully peeled off. To facilitate visualization of the CAM, part of the eggshell was removed. The vascular effects were classified based on predetermined criteria.

- Phase V: Calculation of Irritation Score

All data obtained from the observations were analyzed using the following irritation scoring formula, allowing categorization of each sample into irritation levels (non-irritant, slight, moderate, or strong).

$$T = \frac{301-H}{300} \times 5 + \frac{301-L}{300} \times 7 + \frac{301-C}{300} \times 9$$

Explanation: T= Irritation score; H = Time required to cause hemorrhage (seconds); L = Time required to cause lysis (seconds); C = Time required to cause coagulation (seconds)

d. Data Analysis

Data analysis of the irritation test was performed descriptively, based on the irritation category. The results obtained from the above formula were then compared with the values listed in Table 2 to determine the irritation classification.

Table 2. Irritation score

HET-CAM Irritation Score	Irritation Category
≤ 0.9	Non Irritation
1.0–4.9	Slightly irritating
5.0–8.9	Moderately irritating
9.0–21	Strongly irritating







3. RESULTS AND DISCUSSION

3.1. Irritation Test Results using the HET-CAM Method on Sunscreen Formulation Containing a Combination of Tea Tree Oil and Raspberry Oil

The irritation test on the sunscreen formulation containing a combination of Tea Tree Oil and Raspberry Oil aimed to determine the potential irritant effect when applied to the CAM within a specified observation period. The following are the results of the irritation test for the sunscreen formulation containing 2.5% Tea Tree Oil combined with 1.5% Raspberry Oil, distilled water (Aquadest), and SLS, using the HET-CAM (Hen's Egg Test–Chorioallantoic Membrane) method.


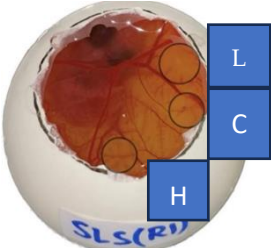

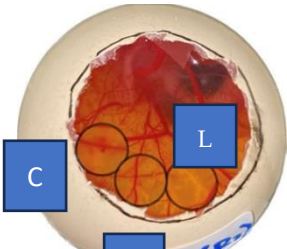
Based on the results of the irritation test, observations of the treatment using the sunscreen formulation containing 2.5% Tea Tree Oil and 1.5% Raspberry Oil in all replications showed no signs of irritation, such as lysis, hemorrhage, or coagulation, with a total irritation score of 0 (Table 3). Therefore, it can be concluded that the formulation is classified as a non-irritating preparation on the CAM.

Table 3. Observation results of sunscreen tea tree oil 2.5% dan raspberry oil 1.5 %

Treatment	Before	After	Score irritation
Replication 1			0
Replication 2			0
Replication 3			0

The test results showed that all replications of the sunscreen formulation containing 2.5% Tea Tree Oil and 1.5% Raspberry Oil had an irritation score of 0 (non-irritating). No hemorrhage, lysis, or coagulation was observed during the observation period. This finding indicates that the combination of these two essential oils at the tested concentrations is safe and does not cause irritation to membrane tissue, suggesting its potential safety for topical use

Table 4. Positive Control Results of the Irritation Test Using Sodium Lauryl Sulfat (SLS)

Treatment	Before	After	Score irritation
Replication 1			14.65
Replication 2			18.26







Notes: L=Lysis; C=Coagulation; H=Hemorrhage

Based on the results of the irritation test for the positive control, visual observation showed that in the first replication, damage to the CAM occurred with the appearance of hemorrhage at 52 seconds, lysis at 50 seconds, and coagulation at 146 seconds, resulting in an irritation score of 14.65, which is classified as strong irritation. In the second replication, CAM damage was observed with hemorrhage at 39 seconds, lysis at 50 seconds, and coagulation at 33 seconds, with an irritation score of 18.26, also categorized as strong irritation. In the third replication, CAM damage occurred with hemorrhage at 50 seconds, lysis at 120 seconds, and coagulation at 24 seconds, resulting in an irritation score of 16.71, which likewise falls into the strong irritation category [18].

The positive control using Sodium Lauryl Sulfate (SLS) showed a strong irritation response, with irritation scores ranging from 14.65 to 18.26. This was characterized by the appearance of hemorrhage, lysis, and coagulation within less than 3 minutes. SLS ($\text{CH}_3(\text{CH}_2)_{10}\text{CH}_2\text{OSO}_3\text{Na}$) is a strong anionic surfactant consisting of a hydrophilic head (water-attracting) and a hydrophobic tail (oil-attracting). SLS damages the skin barrier (stratum corneum), increases transepidermal water loss (TEWL), and causes redness or erythema, which in some cases can be severe [18].

Table 5. Negative Control Results of Irritation Test using Aquadest

Treatment	Before	After	Score irritation
Replication 1			0
Replication 2			0



Based on the results of the irritation test, observations of the negative control treatment using distilled water (Aquadest) in all replications showed no signs of irritation, such as lysis, hemorrhage, or coagulation, with a total irritation score of 0. Therefore, it can be concluded that distilled water is classified as a non-irritating substance on the CAM.

In contrast, the negative control using distilled water (Aquadest) showed no irritation reaction (score 0). No hemorrhage, lysis, or coagulation was observed in any replication. This indicates that the testing system functioned properly and that distilled water does not have any irritating effects on the CAM membrane [19].

Table 6. Results of the Irritation Test of Sunscreen Formulation Containing Tea Tree Oil Combined with Raspberry Oil using the HET-CAM Method

Treatment	Replication	Hemorrhage (seconds)	Lysis (seconds)	Coagulation (seconds)	Irritation Score	Irritation Description
Positive Control (SLS)	1	52	50	146	14.65	Strongly irritating
	2	39	50	33	18.26	Strongly irritating
	3	50	120	24	16.71	irritating
Negative Control (Aquadest)	1	-	-	-	0	Non Irritation
	2	-	-	-	0	Non Irritation
	3	-	-	-	0	Strongly Non Irritation
Formulation of Tea Tree Oil 2.5% and Raspberry Oil 1.5%	1	-	-	-	0	Non Irritation
	2	-	-	-	0	Non Irritation
	3	-	-	-	0	Non Irritation

The irritation test using the HET-CAM (Hen’s Egg Test–Chorioallantoic Membrane) method was conducted to evaluate the irritation potential of the sunscreen formulation containing a combination

of 2.5% Tea Tree Oil and 1.5% Raspberry Oil. This method observes the occurrence of hemorrhage (bleeding), lysis (blood vessel damage), and coagulation (clotting) on the chorioallantoic membrane (CAM) of chicken eggs after exposure to the sample.

The anti-irritant effect observed can be explained by the active compounds present in both oils:

a. Tea Tree Oil contains terpinen-4-ol, which exhibits anti-inflammatory activity by suppressing inflammatory mediators such as TNF- α and IL-1 β . This compound helps reduce irritation responses in tissues [20] It has been proven to inhibit the production of inflammatory mediators such as TNF- α , IL-1 β , dan prostaglandin E2 (PGE2) in *in vitro* studies. This compound works by modulating the NF- κ B and MAPK, which play a role in the inflammatory response. Additionally, α -terpineol (2-5%) exhibits antioxidant activity that helps reduce oxidative stress, a known cause of inflammation[8]. Tea tree oil is an essential oil extracted from the leaves of *Melaleuca alternifolia* and is known for its high monoterpene content, reaching levels of over 80-90%. These monoterpene compounds include critical components such as terpinen-4-ol, γ -cymene, α -terpinene, limonene, 1,8-cineole, α -terpineol, terpinolene, sabinene, and α -pinene. Owing to its monoterpene profile, tea tree oil exhibits broad-spectrum antimicrobial properties that are highly effective for acne treatment, making it a common primary active ingredient in various topical formulations designed to suppress bacterial growth and skin inflammation[20].

In terms of effectiveness, tea tree oil has been proven to possess antibacterial properties *in vitro* and has shown positive results in the treatment of acne and skin fungal infections in certain clinical trials. However, this oil has not yet received official authorization as a pharmaceutical product because the clinical evidence has not yet met the strict criteria for drug testing. Nevertheless, many consumers continue to use it independently for various issues such as skin infections, wounds, and even insect bites[21].

As a consumer protection measure, the Federal Institute for Risk Assessment (BfR) recommends limiting the concentration of tea tree oil in cosmetic products to a maximum of 1%. Furthermore, cosmetic products containing this oil must be packaged in light-protective containers and mixed with antioxidants to prevent dangerous terpene oxidation. This dilution step is considered crucial to minimize the risk of irritation and adverse skin allergic reactions [21].

b. Raspberry Oil is rich in linoleic acid, α -linolenic acid, and vitamin E (tocopherol), which act as antioxidants and skin-protective agents, strengthening the lipid layer and preventing inflammation caused by oxidative stress [20] Raspberry oil possesses anti-inflammatory, antimicrobial, and UV-protective effects, leading to its widespread use in the cosmetic industry as a moisturizer, anti-aging agent, and natural sunscreen. Although some studies suggest that Raspberry Seed Oil (RSO) has an SPF, recent research indicates its SPF values are low (0.4–2.6). Nutritionally, this oil is beneficial for cardiovascular health, brain function, and diabetes prevention. International patents demonstrate its application in cosmetic, pharmaceutical, and functional food products, with China and the US holding the highest number of patents[22].

The combination of both oils likely provides a synergistic effect as a skin protectant and inflammation suppressant, which explains the absence of irritation reactions in the HET-CAM model.

The findings of this study are consistent with those reported by [20], who demonstrated that *Tea Tree Oil* exhibits strong antimicrobial activity against skin-associated bacteria without causing irritation at low concentrations. Similarly, [20]and [23] both reported that natural oils with high antioxidant

content—such as *Tea Tree Oil*, *Tamanu Oil*, and *Raspberry Seed Oil*—are generally safe for topical application due to their protective effects against skin inflammation. In the context of the HET-CAM method, these results are further supported by [20] who found that substances with irritation scores ≤ 0.9 are classified as non-irritant, corresponding with the score of 0 obtained in the present study. Collectively, these findings reinforce that the combination formulation of *Tea Tree Oil* and *Raspberry Seed Oil* at the tested concentrations does not elicit any irritation response and is suitable for use in skin-care products such as sunscreens.

Raspberry seeds are an extraordinary reservoir of bioactive compounds, including polyunsaturated fatty acids (PUFAs) such as linoleic acid and alpha-linolenic acid, as well as vitamins A and E (tocopherols). The high content of ellagic acid provides potent anti-inflammatory, antimicrobial, and antioxidant properties for skin health. These compounds function as effective moisturizers and emollients that help reduce oxidative stress, maintain collagen structure, and form a protective lipid barrier to prevent the loss of the skin's natural moisture [24].

Overall, the present results align with previous studies investigating natural ingredients with antioxidant and anti-inflammatory potential. The combination of *Tea Tree Oil* and *Raspberry Seed Oil* not only demonstrates irritation safety but also offers additional benefits by protecting the skin from oxidative stress and inflammation. The two oils act synergistically—*Tea Tree Oil* suppresses inflammation by downregulating pro-inflammatory mediators, while *Raspberry Seed Oil* maintains skin hydration and strengthens the lipid barrier. Therefore, this sunscreen formulation containing both oils can be categorized as a safe and promising topical cosmetic preparation, particularly suitable for sensitive or irritation-prone skin.

4. CONCLUSION

The irritation test using the HET-CAM method showed that the sunscreen formulation containing a combination of 2.5% *Tea Tree Oil* and 1.5% *Raspberry Oil* was classified as “non-irritating” (score 0). The positive control (SLS) exhibited strong irritation, while the negative control (distilled water) showed no irritation, indicating that the testing method was valid.

The presence of terpinen-4-ol from *Tea Tree Oil* and unsaturated fatty acids as well as tocopherols from *Raspberry Oil* played an important role in suppressing inflammatory reactions, providing anti-irritant and anti-inflammatory effects. Therefore, the combination of these two active ingredients is considered safe for use in topical sunscreen formulations and has the potential to offer additional protection against skin irritation caused by UV exposure and environmental factors [10].

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