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The Effect of Formulation on The Activity of Catechins as a Sunscreen and Skin Lightening

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ABSTRACT

Catechins, a natural compound found in many plants like gambier, are affected by factors such as pH, temperature, and stability in the dosage form, all of which affect their activity. This study aims to evaluate the effect of catechin formulations in the form of gel and nanoemulsion on sunscreen and skin-lightening activities. In vitro tests were conducted to assess antioxidant activity using the DPPH method and B16F0 cell viability, Sun Protection Factor (SPF), Protection grade UVA (PA), the inhibition of tyrosinase, and melanogenesis. All were measured through the spectrophotometric method. The results showed that catechins had very strong antioxidant activity with an IC_{50} of 16.421µg/mL and were not toxic to B16F0 cells up at a concentration of 200µg/mL. Both catechin gel and nanoemulsion formulations demonstrated good stability during storage. Moreover, the formulation had a significant effect ($p<0.05$) on the value of SPF, PA, and the percentages of tyrosinase and melanogenesis inhibition. Notably, catechins in the nanoemulsion formulation provided better sunscreen and skin-lightening activity than those in gel dosage forms.

Keywords: gel, nanoemulsion, catechin gambier, SPF, lightening agent

INTRODUCTION

Catechins are flavonoid compounds of the flavan-3-ol class, commonly found in various berries, tea, gambier, and nuts. These compounds have strong antioxidant properties, which are highly beneficial for treating skin damage. The antioxidant helps counteract free radicals generated by radiation and ultraviolet (UV) light exposure (Biang, 2022). Many scholars have investigated plants containing catechins to protect the skin. For example, the use of green tea containing catechins provides protection against damage caused by UV radiation and helps prevent erythema inflammation of the skin (Kapoor et al., 2021). Additionally, catechin compounds isolated from the root bark of Ulmus davidiana var. japonica can reduce the expression and secretion of proinflammatory cytokines, thereby improving both intrinsic and extrinsic skin aging (Lee et al., 2020). Furthermore, catechins found in the leaves of Graptophyllum pictum (L.) Griff exhibits sunscreen activity (Masyita et al., 2022). In comparison, catechins isolated from Gambir demonstrate stronger antioxidant activity than vitamin E which was tested in experimental animals (Musdja et al., 2018). Several polyphenolic and flavonoid compounds exhibit antioxidant activity and are characteristic as photoprotective on the skin against the effects of Reactive Oxygen Species (ROS) induced by UV radiation (Stevanato et al., 2014).

While catechins and other antioxidants help protect the skin from UV damage, managing the effects of UV radiation on pigmentation requires a different approach. UV radiation from the sun stimulates melanogenesis, the process by which melanin is produced in the skin. Melanin serves as a natural photoprotector, shielding the skin from harmful UV radiation. However, when melanin is unevenly distributed, it causes the formation of dark spots on the skin, reducing the aesthetic appearance of the skin. To address this, depigmenting agents are needed to inhibit excessive melanin formation. One mechanism for inhibiting the formation of melanin is inhibiting the tyrosinase enzyme (Ando, 2017). Hydroquinone was the first depigmenting agent used, working by inhibiting the action of the tyrosinase enzyme. However, when tyrosinase oxidizes hydroquinone, it produces benzoquinone, causing contact leukoderma, which is toxic to skin cells. Therefore, the use of hydroquinone as a depigmenting agent

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must be under the supervision of a dermatologist (Matsumoto et al., 2016).

It is important to note that catechins found in various sources, such as tea and *Vitis*, have demonstrated the ability to inhibit melanin synthesis in B16 cells by affecting tyrosinase activity so that they can be used as depigmenting agents. B16 cell viability at 20 μM catechin concentration ranges from 60 – 80% (Sato & Toriyama, 2009). This evidence supports the idea that catechins can effectively contribute to skin-lightening strategies by targeting the enzymatic pathways involved in melanin production (Fujimaki et al, 2018). Additionally, avocado seed extract, which also contains catechins, has shown activity as a skin lightener by inhibiting tyrosinase activity (Laksmiani et al., 2020).

Furthermore, catechins isolated from Gambir have been made into lotion dosage forms and have demonstrated activity as sunscreens (Kamal & Rusdi, 2018). Gambir catechins in liposome gel dosage forms exhibit better absorption than those in regular gel forms, as shown in a vitro diffusion system (Verawaty et al., 2019). Additionally, the bioavailability of catechins in serum can be increased if catechins are prepared as nanocapsules rather than in the form of free drugs (Samanta et al., 2016). Nanocapsulated catechins not only increase bioavailability but also increase their antioxidant activity (Monika et al., 2017). Notably, epigallocatechin gallate (EGCG) made in nanoformulation can increase its effectiveness in inhibiting cancer cells in the skin (Sudha et al., 2021).

In conclusion, the low bioavailability of catechins is a factor causing inconsistencies observed between in vitro and in vivo tests. After being formulated into dosage forms, the stability of catechins is affected by pH and temperature, which also affect the activity of catechins in dosage forms. Differences in excipients in the preparation affect the activity of catechins. Additionally, variations in excipients used in the preparations can also impact the activity of catechins. To enhance bioavailability, preparations can be developed in the form of nanoparticles, involve molecular modifications, or incorporate co-administration with other bioactive ingredients. The choice of catechin formula affects drug release and absorption rate, thereby affecting the bioavailability of catechins. Increasing the permeability of catechins in the skin is necessary if catechins are formulated in cosmetic dosage forms (Cai et al., 2018). Therefore, a test is required to examine the effect of catechin formulations in the form of gel and nanoemulsion on their activity as sunscreens and skin lightening agents.

MATERIALS AND METHODS

This section outlines the key ingredients utilized in this research, highlighting their sources and relevance to studying catechins and their effects. The Ingredients used in this research were catechin 98% isolated from Uncaria gambir (Andalas Sitawa Fitolab), B16F0 cells (ECACC), kojic acid (K3125), cell media, 3-isobutyl-1-methylxanthine (IBMX), mushroom tyrosinase 3,4-dihydroxy-L-phenylalanin (L-DOPA), 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT), triethanolamine (TEA), ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), phosphate buffer pH 6,5 from Sigma- Aldrich, Carbopol 940 from Lubrozol, hydroxy propyl methyl cellulose (HPMC) from Making Cosmetics, polyvinyl alcohol (PVA), propylene glycol, tween 80, PEG-400, phenoxyethanol from bratachem, virgin coconut oil (VCO), virgin olive oil (VOO), virgin avocado oil (VAO), and vanilla oil from Happy Green.

Antioxidant activity test

The antioxidant activity of catechins and vitamin C was assessed using the method described by Widowati et al., (2017). Initially, catechins and vitamin C were dissolved in distilled water at various concentrations (10 – 50 μg/mL). Then, the test solution was added to each well, followed by the addition of 0.077 mM DPPH solution. Subsequently, the mixture was stored at room temperature in a dark place for 30 minutes. After this incubation period, the absorbance was measured at a wavelength of 517 nm using a microplate reader (xMark™). To quantify the results, the inhibition percentage was calculated using a blank solution. Furthermore, a graph was made to illustrate the relationship between concentration and the percentage of inhibition. Finally, the IC_{50} values for catechins and vitamin C were calculated, with vitamin C serving as a positive control

Viability test

The viability of B16F0 cells in the presence of catechin and kojic acid was assessed using the MTT method. B16F0 cells were cultured with a density of 5 x 10^3 cells/well and incubated for 24 hours at 37 °C in 5% CO_2. Next, catechin and kojic acid were dissolved in distilled water at concentrations ranging from 25 to 500 μg/mL).

Table I. Formulation of gel and nanoemulsion dosage forms

Ingredients	Formula (%)											
	gel						nanoemulsion					
	B1	B2	B3	F1	F2	F3	B4	B5	B6	F4	F5	F6
Catechin	-	-	-	0.1	0.1	0.1	-	-	-	0.1	0.1	0.1
HPMC	3	-	-	3	-	-	-	-	-	-	-	-
Carbopol-450	-	1	-	-	1	-	-	-	-	-	-	-
TEA	-	0.5	-	-	0.5	-	-	-	-	-	-	-
PVA	-	-	10	-	-	10	-	-	-	-	-	-
(VCO)	-	-	-	-	-	-	5	-	-	5	-	-
(VOO)	-	-	-	-	-	-	-	5	-	-	5	-
(VAO)	-	-	-	-	-	-	-	-	5	-	-	5
Tween 80	-	-	-	-	-	-	40	40	40	40	40	40
PEG-400	-	-	-	-	-	-	20	20	20	20	20	20
Propylene glycol	10	10	10	10	10	10	5	5	5	5	5	5
Phenoxyethanol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Oleum vanilla	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Aquadest ad	100	100	100	100	100	100	100	100	100	100	100	100

Following this, each test solution was put into the well, and the cells were incubated for an additional 72 hours. After incubation, MTT reagent was added to each well, including the blank, and the samples were incubated for 2–4 hours. Subsequently, a stopper was added, and the samples were incubated overnight. Finally, the absorbance was measured using a microplate reader (Benchmark Biorad) at 595 nm. Using the following equation, the percentage of cell viability B16F0 was calculated (Kamiloglu et al., 2020):

$$\% \text{ Viability} = \frac{Mean\ OD_{blank} - Mean\ OD_{sample}}{Mean\ OD_{blank}} \text{ x } 100\%$$

Additionally, a graph was created to illustrate the relationship between concentration and the percentage of cell viability, and the IC_{50} values of catechins and kojic acid were calculated as a positive control.

Gel and nanoemulsion formulations

Initially, gel dosage forms (F1 – F6) were prepared by developing a gelling agent (Table I). Then, catechins, propyleneglycol, and phenoxyethanol were added, with distilled water included to achieve the desired gel weight. In contrast, nanoemulsion dosage forms (F7 – F12) were prepared by heating the oil phase and water phase, using a magnetic stirrer at 1500 rpm for 120 minutes. After this, the water phase was added to the oil phase, and the mixture was mixed homogeneously.

Evaluation of catechin gel dosage forms included assessments of organoleptic, homogeneity, pH (using an ISTEK pH meter), viscosity (measured with a Brookfield KU-3 Viscometer), and storage stability through a freeze-thaw method with six cycles. Similarly, the evaluation of catechin nanoemulsion dosage forms included organoleptic, homogeneity, pH, type of emulsion, washability, density, viscosity (assessed using an Oswald Viscometer), centrifugation test (Onemed), storage stability, freeze-thaw method with six cycles, particle size and distribution using a Particle Size Analyzer (SHIMAZU SALD-2300).

Sunscreen activity test

This section details the methodology used to evaluate the sunscreen activity of catechins through spectrophotometric analysis. Initially, the catechins were weighed and dissolved using distilled water to prepare a series of concentrations ranging from 50 to 800 µg/mL. Subsequently, each formula was weighed and also dissolved in distilled water, ensuring that the concentration was equivalent to 50 µg/mL catechins. Then, the absorption of the test solution was measured using a UV Vis spectrophotometer at a wavelength of 290 – 400, with an interval of 5 nm.

The sun protection factor (SPF) value was determined using the following formula (Khunkitti et al., 2014):

$$\text{SPF} = \frac{\sum_{290}^{320} E(\lambda)\, S(\lambda)}{\sum_{290}^{320} E(\lambda)\, S(\lambda)/T(\lambda)} = \int_{290}^{320} \frac{1}{T} \,.\, E\lambda \,.\, S\lambda \,.\, d\lambda$$

where E(λ) is the spectral irradiance of the light spectrum at wavelength λ nm, E(λ) is the erythemal action spectrum at wavelength λ nm, and T(λ) is the spectral transmittance of the sunscreen. Additionally, the persistent attenuation (PA) value was calculated using the formula (Khunkitti et al., 2014):

$$\frac{UVA}{UVB} = \frac{\int_{320}^{400} \frac{1}{T} \,.\, E\lambda \,.\, S\lambda \,.\, d\lambda}{\int_{290}^{320} \frac{1}{T} \,.\, E\lambda \,.\, S\lambda \,.\, d\lambda}$$

Tyrosinase inhibition activity test

In this test, the dosage forms were weighed and dissolved with distilled water to achieve a concentration equivalent to 50 µg/mL catechins. Next, 90 µL of the test solution was combined with 30 µL of mushroom tyrosinase (200 U/mL in phosphate buffer pH 6.5) and 60 µL of L-DOPA solution (10 mM in phosphate buffer pH 6.5) in each well. Following this, the mixture was allowed to incubate for 60 min in a dark place. This process was repeated for the blank solution (without tyrosinase) and the control solution (without test solution). Finally, the absorbance was measured using a microplate reader at 475 nm. The inhibition capacity of the tyrosinase was calculated for each dosage form (Widyastuti et al., 2023).

Melanogenesis inhibition activity test

The test began by adding 100 µL of B16F0 cells into the well at a density of 5 x 10^3 cells/well. It was incubated for 24 h, and the media was replaced. The dosage forms were weighed and dissolved with distilled water to obtain a concentration equivalent to 50 µg/mL catechins. Next, 100 µL of the test solution and 50 µL of IBMX solution (0.1 mM) were added into each well. The cells were then incubated for 24, 48 and 72 h. Following incubation, the absorbance was measured at 400 nm using a microplate reader.
Finally, the percentage of inhibition of melanogenesis for each preparation was determined using this formula (Chung et al., 2019):

$$= \frac{abs\ cells_{untreated} - abs\ cells_{treated}}{abs\ cells_{untreated}}\ x\ 100\%$$

Statistical analysis

In this section, we outlined the statistical analysis performed to evaluate the experimental data. In this research, all measurements were presented as averages of replicated groups. Next, the data was processed using one-way ANOVA to assess the overall differences among the groups. Following this, Tukey's post hoc test was applied to examine whether the differences in group means were significant at a threshold of $p<0.05$.

RESULTS AND DISCUSSION

Antioxidant activity

UV radiation on the skin causes reactive oxygen species (ROS) in the form of free radicals; thus, protecting the skin from UV rays is essential. To address this, this research icluded antioxidant testing of catechins to evaluate their activity in scavanging free radicals. From the conducted experiments, a linear regression equation was established between the concentration of catechins and the percentage of inhibition of DPPH, yielding equations of y = 1.5718x + 24.189 for catechins and y = 3.4941x + 26.988 for vitamin C. From the equation, it was found that the IC_{50} values of catechins and vitamin C were 16.421 and 6.585 µg/mL, respectively, categorizing both as very strong antioxidants (<50 µg/mL). These results indicate that the antioxidant activity of catechins was found to be superior to that reported by Anggraini et al. (2011).

B16F0 cell viability

The objective of cell viability testing is to determine the IC_{50} value, which allows for the assessment of the potential toxicity of the test compound. In this research, a logistic regression equation was established correlating concentration with the percentage of B6F0 cell viability, where catechins and kojic acid have the equation y = -0.1050x + 71.471 and y = -0.1001x + 50.585. The calculated IC_{50} values were 204.486 for catechins and 5.844 µg/mL for kojic acid. Based on these IC_{50} values, catechins are considered not toxic to cells, especially when compared to kojic acid. Catechins demonstrated non-toxic effects on B16F0 cells up to a concentration of 200 µg/mL, indicating their potential for topical application. Moreover, the viability of B16F0 cells observed in this study was better than that of Sato and Toriyama (2009), where catechins derived from green tea at 20 µM (~5.8 µg/mL) had viability against B16 cells of 60 – 80% over a 5-day treatment period. Additionally, catechin isomers (EGCG), at a concentration of 100 µg/mL, were found to have a B16 cell viability of 67.09% (Liang et al., 2014). These findings suggest that catechins derived from Gambir have lower toxicity compared to compounds or isomers of catechins derived from other plants.

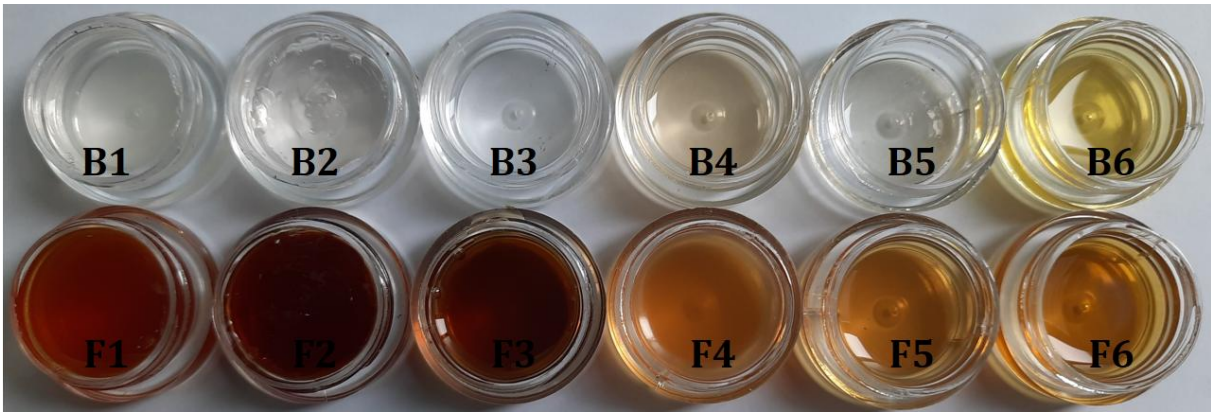

Figure 1. Gel and nanoemulsion dosage forms

Table II. Evaluation of gel and nanoemulsion dosage forms

test	gel					
	B1	**B2**	**B3**	**F1**	**F2**	**F3**
homogeneity	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous
pH	5.545 ± 0.538	6.690 ± 0.176	5.515 ± 0.170	5.527 ± 0.229	6.578 ± 0.192	5.090 ± 0.312
viscosity (cP)	2561.000 ± 244.305	2610.333 ± 281.958	2541.333 ± 167.025	2736.667 ± 250.273	2711.667 ± 206.500	2277.333 ± 189.813
storage stability	stable	stable	stable	stable	Stable	stable
freeze thaw	stable	stable	stable	stable	stable	stable
test	**nanoemulsion**					
	B4	**B5**	**B6**	**F4**	**F5**	**F6**
homogeneity	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous
pH	5.416 ± 0.025	5.403 ± 0.016	5.391 ± 0.022	5.420 ± 0.025	5.399 ± 0.025	5.348 ± 0.011
type of emulsion	o/w	o/w	o/w	o/w	o/w	o/w
washability (mL)	9.933 ± 0.115	9.867 ± 0.115	9.533 ± 0.058	9.667 ± 0.115	9.967 ± 0.058	9.967 ± 0.058
density (g/mL)	1.066 ± 0.021	1.066 ± 0.012	1.065 ± 0.015	1.063 ± 0.016	1.064 ± 0.029	1.064 ± 0.024
viscosity (cP)	523.333 ± 2.887	511.667 ± 2.887	521. 667 ± 2.887	507.667 ± 6.807	521.667 ± 5.774	516.333 ± 2.887
centrifuge test	stable	stable	stable	stable	stable	stable
storage stability	stable	stable	stable	stable	stable	stable
freeze thaw	stable	stable	stable	stable	stable	stable

Gel and nanoemulsion formulations

In this study, physical evaluation was carried out on gel and nanoemulsion dosage forms for 6 weeks. Organoleptic observations indicated that there were no changes in shape, color, and smell throughout the evaluation period. It was noted that the color variations among the dosage forms containing catechins were dependent on the base used. Consequently, the six formulations exhibited different colors (Figure 1). All dosage forms demonstrated homogeneity and maintained a pH suitable for catechin (Table II). Furthermore, all formulations remained stable in their respective stability tests. Importantly, the nanoemulsion dosage forms met the required particle sizes for nanoparticles, from 20 – 200 nm, and are transparent (Gupta et al., 2016 and Gurpreet & Singh, 2018) (Figure 2).

This study also explored the formulation of catechin dosage forms in the form of nanoparticles, focusing on the stability of catechins in the dosage forms. However, comparisons of the activity of catechins in various dosage forms have not been carried out, especially as formulations for sunscreens and skin lightening. Therefore, the present study aimed to examine the effects of catechin activity in gel dosage forms using different gelling agents and nanoemulsion dosage forms using different oils.

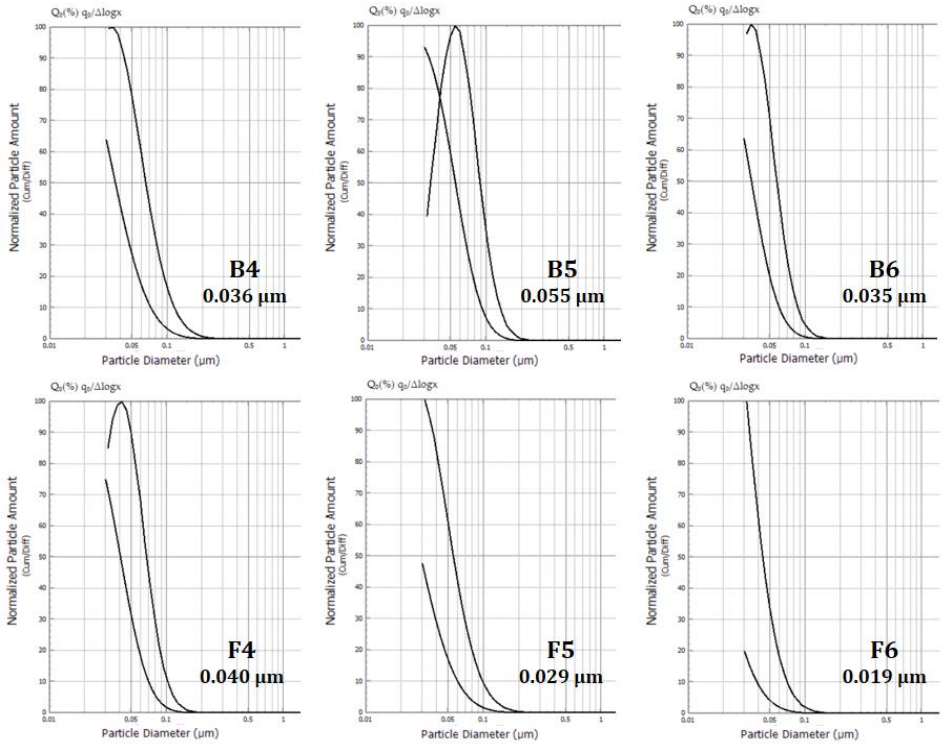

Figure 2. Graph of distribution and particle size of nanoemulsion formula

Table III. Sunscreen activity of catechin, gel and nanoemulsion

Sample	SPF		PA	
	value	category	value	star rating category
catechin (µg/mL)				
50	2.159 ± 0.390	minimal protection	0.658 ± 0.110	+++
100	4.656 ± 2.521	minimal protection	0.405 ± 0.181	no rating
200	7.130 ± 2.323	minimal protection	0.299 ± 0.132	no rating
400	9.256 ± 4.249	minimal protection	0.291 ± 0.115	no rating
800	20.864 ± 5.253	good protection	0.155 ± 0.039	no rating
gel				
B1	1.753 ± 0.020	minimal protection	0.907 ± 0.007	+++++
B2	1.763 ± 0.073	minimal protection	1.193 ± 0.064	+++++
B3	1.638 ± 0.014	minimal protection	0.988 ± 0.005	+++++
F1	2.425 ± 0.043	minimal protection	0.627 ± 0.007*	+++
F2	2.703 ± 0.043	minimal protection	0.549 ± 0.004	no rating
F3	2.701 ± 0.382	minimal protection	0.580 ± 0.056	no rating
nanoemulsion				
B4	4.264 ± 0.126	minimal protection	0.450 ± 0.006	no rating
B5	5.505 ± 0.315	minimal protection	0.476 ± 0.021	no rating
B6	3.475 ± 0.275	minimal protection	0.507 ± 0.033	no rating
F4	7.037 ± 0.795	minimal protection	0.267 ± 0.022	no rating
F5	7.247 ± 0.909	minimal protection	0.229 ± 0.024	no rating
F6	10.043 ± 0.920*	minimal protection	0.232 ± 0.041	no rating

* formula with high value

Sunscreen activity of dosage forms

The sunscreen activity of the dosage form is evaluated based on the values of SPF and PA. The SPF value is the product's protection against UVB radiation, while the PA value indicates the product's protection against UVA. According to Donglikar and Deore (2016), a good sunscreen formulation should have an SPF value greater than 15 (good protection) and a PA value greater than 0.2 (moderate protection). In this research, the results showed that as the concentration of catechins increased, the SPF value also rose significantly. At a concentration of 800 µg/mL, the SPF value of catechins was 20.864 ± 5.253. The Gambir catechins in this study had a better SPF value than catechins derived from Graptophyllum pictum (L.) Griff, where at a concentration of 100 µg/mL, the SPF values were 4.656 ± 2.521 and 2.324 (Masyita et al., 2022).

Although none of the dosage forms had an SPF value above 15, the highest SPF value was for F6 (the nanoemulsion dosage forms with an oil phase using VAO), which measured approximately 10.043 ± 2.120. This indicates that the formulation significantly affected the sunscreen activity of the extract, showing a significant increase in SPF values ($p<0.05$) between the extract and the dosage forms. Additionally, catechins made in nanoemulsion dosage forms had a greater SPF value than in gel dosage forms (Table III).

The gel base has an SPF value of less than 2, which is considered negligible. Conversely, the nanoemulsion base has a value of greater than 2, confirming its activity as a sunscreen. While the PA values for all bases were higher than those of the dosage forms containing catechins, the PA value of catechins was greater in the dosage forms. Further research is needed to explore the formulation of catechins when used to protect against UVA. Based on statistical analysis, no significant difference was observed in the SPF values on the gel base and nanoemulsion base and F3 on the resulting SPF value ($p>0.05$).

Skin lightening activity of catechin gel and nanoemulsion dosage forms

Melanogenesis in melanocyte cells primarily involves the enzyme tyrosinase, which catalyzes the conversion of L-tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and then to dopaquinone. Tyrosinase catalyzes the first two rate-limiting syntactic reactions, namely the hydroxylation of tyrosine to form DOPA and the subsequent oxidation of DOPA to produce dopaquinone. The subsequent reactions are more or less spontaneous in the formation of melanin. In addition to tyrosinase, several factors regulate melanogenesis, including dopachrome tautomerase, peroxidase, and certain metal ions, such as copper and iron. Therefore, tyrosinase activity is an important factor in melanogenesis, although it is not the sole factor in the level of melanin production in the skin (Hu, 2008).

In this study, the skin lightening activity was marked by a high percentage of tyrosinase inhibition and a reduction in melanogenesis. The high percentage of inhibition indicates the inhibitory power of the tyrosinase enzyme and the large process of melanin formation. The process of forming melanin is called melanogenesis (Pillaiyar et al., 2018).

Tyrosinase inhibition testing was carried out *in vitro* using mushroom tyrosinase, where the measurement used a spectrophotometry method. To evaluate melanin content, two parameters can be used: extracellular melanin determination and intracellular melanin determination. This study focused on extracellular melanin determination, where the melanin content was determined by comparing the density of melanin that was given treatment with the density of melanin in cells without treatment (Chung et al., 2019). Melanogenesis inhibition testing was performed using *in vitro* using B16F0 melanoma cells. This approach allowed us to examine the melanin content extracellularly through a simple method by measuring the absorbance using a microplate reader (D'Ischia et al., 2013) (Table IV).

In this research, the results showed that catechin formulations in different dosage forms significantly affected tyrosinase inhibition ($p<0.05$). Both base gel and nanoemulsion showed tyrosinase inhibitory activity; however, it is still below 10%. Hence, this can be neglected. While catechins did not show a significant difference in inhibition compared to F3 ($p = 0.082$), they did differ significantly from other formulations. The best formula was F5, which had a tyrosinase inhibition value of 87.820 ± 0.764%.

The determination of melanogenesis inhibition revealed that the exposure time to the dosage forms affects the inhibition of melanogenesis from catechin formulas. In this study, the use of IBMX stimulated the process of melanogenesis so that the amount of B16F0 cells produced was greater than that of B16F0 cells without IBMX.

Table IV. Skin lightening activity of gel and nanoemulsion

Sample	tyrosinase inhibition (%)	inhibition of melanin extracellular on B16F0 (%)		
		24 h	48 h	72 h
catechin 50 µg/mL	72.312 ± 0.946	24.744 ± 0.778	35.633 ± 1.694	36.938 ± 1.118
gel				
B1	6.869 ± 0.609	19.228 ± 2.253	29.725 ± 2.874	20.244 ± 2.919
B2	7.786 ± 0.820	19.490 ± 0.765	14.044 ± 4.322	16.353 ± 2.112
B3	4.608 ± 0.362	17.717 ± 1.132	24.407 ± 4.673	22.429 ± 2.859
F1	81.933 ± 1.225	38.272 ± 2.700*	42.951 ± 2.048	59.538 ± 1.118
F2	81.890 ± 1.171	33.543 ± 1.040	38.133 ± 1.100	55.271 ± 1.240
F3	71.118 ± 0.796	28.815 ± 0.913	36.179 ± 1.488	53.428 ± 0.984
nanoemulsion				
B4	5.610 ± 0.871	6.554 ± 2.373	12.408 ± 1.380	25.024 ± 1.417
B5	4.309 ± 0.796	9.312 ± 1.077	10.090 ± 1.377	23. 044 ± 1.151
B6	4.480 ± 0.330	12.333 ± 0.748	13.590 ± 1.669	26.936 ± 1.092
F4	76.067 ± 0.330	32.624 ± 0.694	50.132 ± 5.007	68.858 ± 0.992*
F5	87.820 ± 0.764*	33.937 ± 1.984	53.313 ± 4.326*	66.708 ± 0.653
F6	83.468 ± 0.568	36.302 ± 1.153	46.223 ± 1.533	65.786 ± 1.227

* formula with high value

At 24 hours, F1 had greater melanogenesis inhibition activity than other formulas, about 38.272 ± 2.700%. Melanogenesis inhibition test at 24 hours found a significant difference between catechins with gel and nanoemulsion formulas ($p<0.05$). At 48 hours, the best formula was F5, which inhibits melanogenesis by 53.313 ± 4.326%. Based on statistical tests, there was a significant difference ($p<0.05$) in the inhibition of melanogenesis between catechins with gel and nanoemulsion formulas. At 72 hours, the best formula was F4, achieving an inhibition of melanogenesis of 68.858 ± 0.992%. There was a significant difference ($p<0.05$) between catechins in the form of pure compounds and those that had been formulated in the form of gels and nanoemulsions.

Based on the results obtained, the nanoparticle dosage form of catechins had greater melanogenesis inhibition activity than the gel dosage form, particularly with extended duration of treatment of B16F0 cells. A similar finding was also found in tests conducted by Sudha et al. (2021), which showed catechins in the form of EGCG isomers, after being formulated in the form of nanoformulations, can increase the efficiency and time of treatment. Overall, the catechin formulations in gel and nanoemulsion dosage forms can enhance the melanogenesis inhibition activity of catechins.

CONCLUSION

In conclusion, the research on catechins reveals that they possess a very strong category, with an IC_{50} value of 16.421 µg/mL. Importantly, catechins are non-toxic to B16F0 cells, where in testing for 72 hours, an IC_{50} value of 204.486 µg/mL was obtained. F6 had the highest SPF value, ranging from 10.043 ± 2.120. F5 was the most effective tyrosinase inhibition, ranging from 87.820 ± 0.764%. For instance, F4 had the greatest melanogenesis inhibition activity at 72 hours, 68.858 ± 0.992%. There was a significant difference ($p<0.05$) between catechins in the form of pure compounds and those in the form of gel and nanoemulsion formulations. Overall, catechin nanoemulsion dosage forms had a better effect than gel dosage forms in their activity as sunscreens and skin-lightening agents

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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