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Comparative Potential of Cream Preparations As Tyrosinase Inhibition Between Ethanolic Extracts of Kepel Leaves (Stelechocarpus burahol (Blume) Hook f. & Thomson*) and Pulasari Bark (Alyxia reinwardii* Blume*)*

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ABSTRACT

The problem of skin hyperpigmentation due to excessive exposure to ultraviolet rays is a primary concern in the development of cosmetic preparations based on natural ingredients. The enzyme tyrosinase plays a crucial role in the melanin formation process, making it a key target in the formulation of skin-lightening agents. This study evaluated the potential of cream preparations containing ethanol extract of Kepel leaf (Stelechocarpus burahol) and pulasari bark (Alyxia reinwardii) as tyrosinase inhibitors. The extract is obtained through the maceration method using a 96% ethanol solvent and then formulated into a cream preparation with concentrations of 1.25% and 2.5%. Physical characteristics tests (viscosity, pH, dispersibility, adhesion, homogenity) and tyrosinase enzyme inhibition activity tests were performed in vitro using ELISA instruments or microplate readers. The results showed that increased concentration of the extract affected the physical properties of the preparation and increased inhibition ability against tyrosinase, but statistical tests showed insignificant differences in results. Formulations with a concentration of 2.5% showed the highest activity in inhibiting tyrosinase, with pulasari bark extract being more effective than kepel leaves. These findings strengthen the potential of both plants as active ingredients in natural-based skin lightening cosmetic products

Keywords: *Antioxidant, Cucumber Leaves, Hyperpigmentation, Pulasari Wood, Topical Cream, Tyrosinase.*

INTRODUCTION

High exposure to ultraviolet (UV) rays in tropical regions, such as Indonesia, is a primary factor that triggers increased melanin production in the skin. The process of melanin biosynthesis is triggered by the activation of the enzyme tyrosinase, which catalyzes the oxidation of L-tyrosine into L-DOPA and subsequently into dopaquinone. Excessive tyrosinase activity leads to hyperpigmentation, characterized by the appearance of dark or uneven patches on the skin's surface. This condition is often a significant aesthetic problem, especially in the context of skin care and cosmetics. (*Nailufa dan Najih, 2020*).

Traditional Indonesian medicinal plants offer great potential as a source of natural bioactive agents. Kemp leaves (*S. burahol*) and pulasari bark (*Alyxia reinwardii*) obtained from B2P2TOOT, Tawangmangu, are known to contain flavonoids, which act as antioxidants as well as have the potential as tyrosinase inhibitors. This compound can interfere with tyrosinase activity by binding to the enzyme's active sites or inhibiting the formation of substrate-enzyme complexes. (*Alvina et al., 2023*). At the concentration of extract used, which is 1.25% and 2.5%, considering the increased flavonoid content in the cream, it can be said that the high flavonoid content concentration of extract has antioxidant activity. Inhibition of the enzyme tyrosinase increases with the antioxidant potential of the sample (*Furi, et.al, 2022*).

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Topical formulations, such as creams, are an effective method for delivering active substances to the skin's surface. Not only is the cream easy to use and absorb, but it can also maintain the stability of the active compounds in the formulation. Evaluation of the physical characteristics of the preparation, such as pH, viscosity, dispersibility, and adhesion (*Safitri et al., 2016)*. This study aims to evaluate the inhibitory activity of the tyrosinase enzyme from cream preparations containing an ethanol extract of kepel leaf (*S. Burahol*) and pulasari bark (*Alyxia reinwardii* Blume), as well as to examine the effect of concentration variations on the physical characteristics of the preparation. It is hoped that the results of this study can provide a scientific basis for the development of cosmetics based on natural ingredients as a safer and more effective alternative in treating hyperpigmentation.

RESEARCH METHODS

Tools and Materials

The tools used are maceration vessels, glass funnels, stirring rods, drip pipettes, dips, cola cloth, rotary evaporator (Heidolph Laborota 4001), porcelain cups, mortar and stamper, water bath, hot plate, digital balance (Shimadzu ATX224), analytical balance, measuring cup, beaker glass (Pyrex), spatula, watch glass, object glass, filter paper, test tube, microscope, magnifying glass, pH meter, Brookfield Viscometer (DV-1 Prime), dispersion test kit, adhesion test kit, ELISA microplate reader (Agilent Multi-Mode Reader SYNERGY HTX), micropipette (DLAB multi-channel and single-channel sizes 5–50 µL, 10–100 µL, 50–300 µL), ultrasonic, stopwatch, UV lamp (254 nm and 366 nm)

The ingredients used are kepel leaf powder (*S. Burahol*), pulasari bark powder (*Alyxia reinwardii*), 96% ethanol, aquadest, cetyl alcohol, Virgin Coconut Oil (VCO), tween 80, span 80, glycerin, stearic acid, methyl paraben, propyl paraben, $FeCl_3$ 1%, H_2SO_4, HCl 1%, NaCl 1%, magnesium powder, amyl alcohol, Dragendorff reagent, Bouchardat reagent, reagent Lieberman-Burchard, anisaldehyde-sulfuric acid reagent, vanilla-sulfuric acid reagent, glacial acetic acid, chloroform, ammonia, toluene, tyrosinase enzyme (Sigma-Aldrich), L-Tyrosine substrate (Sigma-Aldrich), kojic acid (positive control), DMSO, KH_2PO_4 and NaOH buffer solutions.

Research Methods

Preparation

Extraction was carried out on kepel leaf powder (*S. burahol*) and pulasari bark (*Alyxia reinwardii* Blume), each weighing as much as 500 grams. The material was extracted using the maceration method with 96% ethanol solvent in a 1:6 ratio for 3 × 24 hours, followed by one 1 × 24-hour maceration. The filtrate is filtered using a cola cloth, then concentrated with a rotary evaporator at a temperature of ±55°C and concentrated utilizing a water bath to obtain a viscous extract (Ananto et al., 2015).

The cream is formulated in two concentration variations: FI (1.25%) and FII (2.5%) extracts, respectively. The composition of the formula is compiled and refers to the modification of the cream formulation as described by Geraldine and Hastuti (2018) As shown in Table 1.

Table 1. Formula of Kepel Leaf and Pulasari Bark Ethanol Extract Cream

Material	FI	FII
Thick Extract of Kepel Leaves/Pulasari Bark (% w/v)	1.25	2.5
VCO (% w/v)	10	10
Cetyl alcohol (% w/v)	4	4

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Material	FI	FII
Tween 80 (% w/v)	2.204	2.204
Glycerol (% w/v)	12	12
Span 80 (% w/v)	2	2
Methyl paraben (% w/v)	0.2	0.2
Propyl paraben (% w/v)	0.1	0.1
Stearic acid (% w/v)	3.796	3.796
Aquadest (% w/v) until	60	60

The cream is separated into its oil and water phases, then heated to 70°C. The oil phase consists of VCO, cetyl alcohol, stearic acid, span 80, and propyl parabens. The water phase consists of tween 80, glycerin, methyl parabens, and aquadest. Once both phases reach the same temperature, the water phase is slowly added to the oil phase while stirring until an emulsion is formed. Once the temperature drops to 40°C, the extract is added and homogenized. (*Geraldine and Hastuti, 2018*).

Evaluate Cream Preparations

Organoleptic

Organoleptic tests include texture, color, and odor of preparations. The results will affect how comfortable the preparation is when used. (Tari and Indriani, 2023).

Homogenates

In this test, the cream is applied to the glass of the object, and the surface is then observed to ensure it is smooth and even. The preparation requirements must be homogeneous and not have visible coarse grains. (*Tungadi et al., 2023*).

pH

The ideal pH of the skin is between 4.5 and 6.5. pH is measured with a pH meter after the electrodes are dipped in a cream preparation (*Pratasik et al., 2019*).

Dispersion

The 0.5-gram cream is weighed on the watch glass, and then the glass is placed back on the scale. The load is added and left for one minute. Then, the load is added gradually, starting from 50 grams, 100 grams, 150 grams, 200 grams, and 250 grams, until the cream side becomes stable. The ideal spreadability is 5-7 cm (*Tungadi et al., 2023*).

Adhesive

A cream sample weighing up to 0.5 grams is applied to a glass plate and then held for five minutes under a load of 100 grams. The load is lifted, and the two glass plates are released. The time it takes for both plates to be released is recorded. The ideal adhesion of the cream is more than 4 seconds. (*Tungadi et al., 2023*).

Viscosity

The viscosity test is measured using the Brookfield Viscometer. The tool is switched on and set to 100 rpm with a 64-spindle. The requirement for a good viscosity of a cream is 4000–40,000 cP. (*Tungadi et al., 2024)*.

Cream Type Test

Methylene blue gives the emulsion a blue color because it is water-soluble and provides color to the water phase. The w/o emulsion falls under the o/w category. *(Suwandi, et.al, 2023*)

Tyrosinase Inhibition Test

The preparation of cream extract of kepel leaf (*S. Burahol*) and the bark of the pulasari tree (*Alyxia reinwardii* Blume) was weighed according to the calculation of needs, with a

concentration of 2000 ppm, namely FI = 4 grams, FII = 2 grams, then put into a centrifuge tube along with 25 mL of phosphate solution with a pH of 6.5. Centrifuge for 10 minutes until the supernatant separates from the cream base. It is then diluted to 1000 ppm in the supernatant using a 2.5 mL pipette and transferred into a 5 mL measuring flask. The supernatant obtained was pipetted and measured using a microplate reader or ELISA. (*Furi et al., 2022*).

Samples, culture 96-well plates, and controls must be prepared before inhibition testing using the ELISA method. Each cell was filled with 70 µL of the test solution, 110 µL of phosphate buffer solution, and 30 µL of the enzyme tyrosinase. The enzyme tyrosinase was incubated for five minutes in an ELISA device at 25°C. The L-tyrosine solution was also incubated for 30 minutes in an ELISA device at 37°C, and then the absorbance was read at a wavelength of 510 nm.

Data analysis

The results of the absorbance test for the enzyme inhibition activity of tyrosinase cream, kepel leaf extract, and pulasari bark cream were analyzed to obtain data. The data results were statistically analyzed using SPSS version 23. The normality and homogeneity tests were carried out at the 0.05 significance level. If the data shows normal and homogeneous distribution using ANOVA One Way, while if the data shows an abnormal or homogeneous distribution using Kruskal-Wallis.

RESULTS AND DISCUSSION

Result

Phytochemical Screening & Extract Yield

In the phytochemical screening test, the results were obtained for kepel leaf and pulasari stem bark extract. The extract contains chemical compounds such as flavonoids, alkaloids, tannins, saponins, steroids, and triterpenoids. The remaceration extraction method was chosen because the tool is easy to use and the process is straightforward. Extracting all polar and non-polar compounds from the sample, kepel leaf powder and pulasari bark were extracted with 96% ethanol. Pulasari bark gets a % yield of 22.80%, and kepel leaves get a % yield of 10.10%.

Ethanol-Free Test

To ensure that the extract of kepel leaves and pulasari bark is not overlooked, which could endanger the preparation's safety, the condensed extracts obtained are then tested for ethanol content. The results showed that kepel leaf extract and pulasari bark were free from ethanol odor.

Thin-Layer Chromatography Test

The results of the thin-layer chromatography test showed that the extracts of kepel leaf (S. burahol) and the bark of the pulasari tree (*Alyxia reinwardii* Blume) contained alkaloid compounds, flavonoids, steroids, triterpenoids, and saponins. The addition of ammonia patches and Dragendorff patches caused yellow stains in the flavonoid compound. (*Astuti, et.al, 2023*) The addition of 1% of the $FeCl_3$ reagent to the phenolic test resulted in a black stain, which indicates that the pectin extract contains phenolics. Saponin tests (*Ngginak, et.al, 2021*). Show that positive extracts contain saponin compounds if red, yellow, brown, blue, or purple stains are formed. Steroid and triterpenoid tests on the extract yielded purple stains, indicating the presence of triterpenoid compounds, as shown in Table 2.

Table 2. Results of Thin-Layer Chromatography Test of Kepel Leaf and Pulasari Bark Extract

Compound		UV 254 nm	Rf	UV 366 nm	Rf	Spotting Sighting	Rf	Rf (Reference)	Conclusion
Flavonoid	Kepel Leaf Extract	Blackish Green	0.16	Blackish Blue	0.68	Yellow	0.16	0.1-0.75 (*Astuti, et.al, 2023*)	(+)
	Pulasari Bark Extract	Greenish Yellow	0.63	Greenish Yellow	0.46	Orange	0.50		(+)
Tanin	Kepel Leaf Extract	Green	0.65	Blue	0.75	Green	0.65	0.62-0.65 (*Raihan, et.al, 2020*)	(+)
	Pulasari Bark Extract	Blackish Blue	0.69	Blue	0.62	Blue Purple	0.65		(+)
Alkaloid	Kepel Leaf Extract	Green	0.63	Blue	0.72	Orange	0.75	0.05-0.75 (*Fahrurroji and Riza, 2020*)	(+)
	Pulasari Bark Extract	Blue	0.34	Blue	0.58	Purple	0.71		(+)
Saponin	Kepel Leaf Extract	Blackish Green	0.73	Blackish Blue	0.85	Brown	0.80	0.70-0.84 (*Ngginak, et.al, 2021*)	(+)
	Pulasari Bark Extract	Blackish Green	0.69	Brown	0.80	Brown	0.85		(+)
Steroid	Kepel Leaf Extract	Green	0.60	Blue	0.63	Purple	0.67	0.52-0.66 (*Raihan, et.al, 2020*)	(+)
	Pulasari Bark Extract	Blue	0.50	Blue	0.60	Blue	0.65		(+)

The difference in the concentration of kepel leaf extract in this formulation is to determine the quality of the cream preparation. Physical characteristics, including organoleptic, homogenity, pH, adhesion, dispersion, viscosity, and emulsion type, and measurement of tyrosinase inhibiting activity. The concentrations chosen are 1.25% and 2.5% for both extracts. The first test of the physical characteristics of the cream is an organoleptic test, which involves visual observation and includes the colour, smell, and texture of the cream.

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Kepel Leaf Extract Cream Concentration 1.25%

Kepel Leaf Extract Cream Concentration 2.5%

Pulasari Bark Extract Cream Concentration 1.25%

Pulasari Bark Extract Cream Concentration 2.5%

Figure 1. Preparation of Kepel Leaf and Pulasari Bark Extract Cream

Based on Figure 1, both creams are obtained in a semi-solid form; the distinctive smell of the extract and the colour produced are different depending on the concentration added. Higher concentrations of kepel leaf extract in the cream result in a dark brown colour, while lower concentrations yield a brown colour. For the bark cream, pulasari is the colour produced, ranging from bone white to brownish white. The homogeneity test is the next characteristic test used to determine the homogeneity of the mixed preparation. Homogeneous preparations indicate that the active substance is evenly distributed throughout the cream base, ensuring an appropriate dosage when used. The results showed that creams with concentrations of 1.25% and 2.5% produced the same outcomes in both kepel leaf extract cream and pulasari bark cream.

Cream Type Test

Further testing evaluated the types of Pussari bark extract cream and Kepel leaf extract cream in oil-in-water or water-in-oil emulsions. This test (Figure 2) is essential for determining how well the cream preparation functions in terms of spreadability, adhesion, and viscosity, depending on the type of cream being made (*Hidayatu et al., 2013*).

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Kepel FI (1.25%)	Kepel FII (2.5%)	Pulasari FI (1.25%)	Pulasari FII (2.5%)

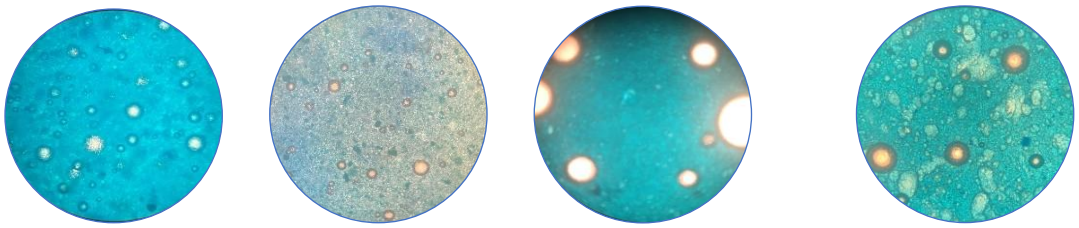

Figure 2. Test Cream-Type of Kepel Leaf and Pulasari Bark Extract

When methylene blue is evenly distributed throughout the cream in this test, it indicates the presence of a continuous (water) phase. The scattered phase (oil) indicates small droplets inside the outer phase. The test results showed that both creams, at concentrations of 1.25% and 2.5%, contained oil-in-water emulsions.

pH Test

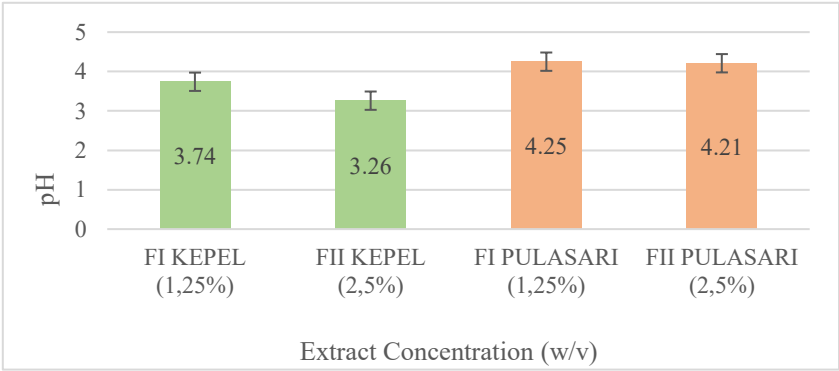

Figure 3. pH Test of Kepel Leaf and Pulasari Bark Extract Cream

The pH test is conducted to determine the acidity level of the cream; the optimal range for skin pH values is 4.5-6.5. In both cases, as the concentration increases, a more acidic pH is obtained. This is because the pH of kepel leaf extract and pulasari stem bark has acidic properties (Figure 3), so it is necessary to pay attention to the potential for irritation due to a pH that is too acidic. The decrease in pH of the cream is due to the acidic properties of some phenolic active compounds, including flavonoids and tannins; the more acidic the extract is, the more H^+ ions it contains. The higher the concentration of extracts added, the lower the pH value of the cream preparation *(Rikadyanti et al., 2021)*; (*Ulandari and Sugihartini, 2020*). The results of the pH test were obtained using the SPSS test with Sig 0.000 < 0.05, indicating a significant difference in the extract's concentration on the pH.

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Viscosity Test

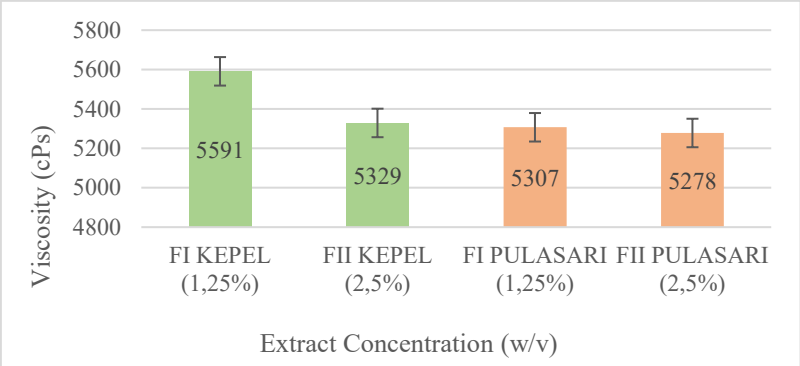

Figure 4. Viscosity Test Results of Cream Preparations

Adhesive Strength Test

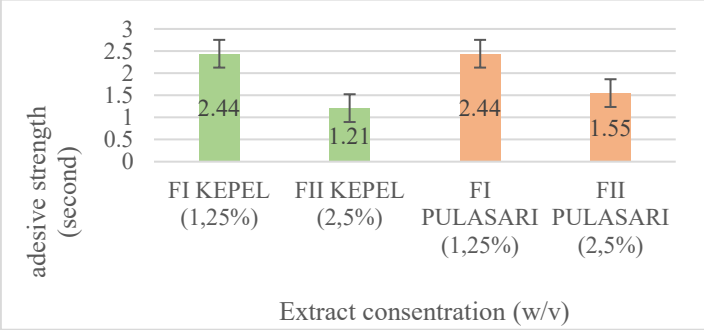

Figure 5. Results of Adhesive Test of Cream Preparations

Based on Figure 5, the higher the concentration of the extracts added, the greater the decrease in adhesion. The results of the cream's adhesion test depend on how long the preparation remains attached to the skin's surface and how comfortable it is to use. The reasonable adhesion requirement for topical preparations is > 4 seconds. *(Pratasik et al., 2019)*. The adhesion test results showed Sig. (2-tailed) <0.0, so that it can be concluded that there is a significant difference in adhesion for all formulas.

Dispersion Test

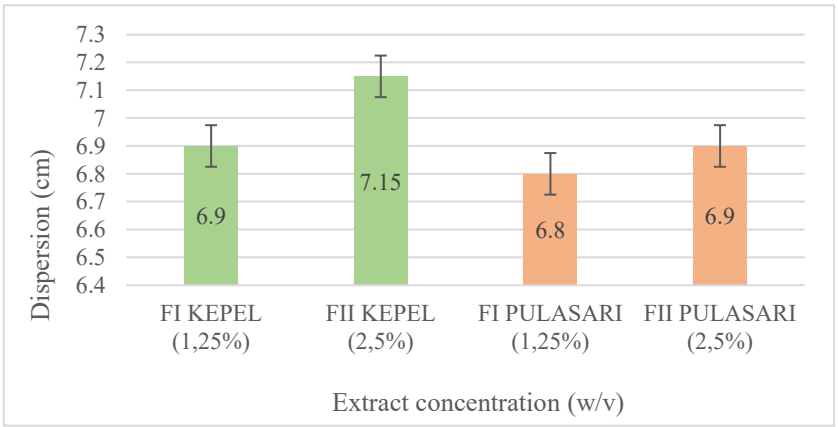

Figure 6. Results of Cream Preparation Dispersion Test

The higher the extract concentration, the greater the dispersion of the preparation and the lower its viscosity, which affects the spreading power of the cream (Figure 6). The dispersion requirement is 5-7 cm (*Tungadi et al., 2024*). The results of the dispersion test were obtained with a significance value of Sig 0.000 < 0.05, indicating an effect of the concentration difference in kepel leaf extract and pulasari stem bark on the dispersion test.

Tyrosinase Inhibition Test

Tyrosinase inhibition test using an ELISA instrument or microplate reader. To assist the sample pipetting process in this test, it is necessary to do well plate mapping. At pH 6.5, enzymes, substrates, kojic acid, and even phosphates are required. The presence of phenolic compounds causes kepel leaf extract and pulasari bark extract to inhibit tyrosinase, which is responsible for producing melanin. (*Sholikha, et.al, 2021*). As a reactive compound that can spontaneously polarize to produce melanin, the hydroxylation of L-tyrosine to 3,4-dihydroxyphenylalanine (L-DOPA) and the oxidation of L-DOPA to dopamine are the first two reactions involving melanin synthesis. *(Syahputra et al., 2022*). This is due to the abundance of phenolic active compounds in the extract, which interact with enzymes and compete with L-tyrosine substrates.

The use of kojic acid as a positive control to ensure that the method used is correct by comparing the inhibition values. The Shapiro-Wilk normality test indicated that the data were normally distributed ($p > 0.05$) for all cream formulas tested. In addition, the results of the homogeneity test of the cream formula showed that the data were inhomogeneous ($p = 0.005$; < 0.05), indicating that the differences between groups were not uniform. Therefore, the analysis was followed by a non-parametric *Kruskal-Wallis test*. The results of yielded a p-value of 0.192 (>0.05). This shows that there is no statistically significant difference in the percentage of tyrosinase inhibition between the cream formulas. To examine further differences between groups, the Mann-Whitney test was performed. The inhibition ability of creams with a concentration of 2.5% is greater than that of creams with a concentration of 1.25% in kepel leaf extract and pulasari bark. Furthermore, due to saturation, other factors that affect the activity of the tyrosinase enzyme in the extract cream formula do not change significantly. Kepel leaf extract and pulasari bark contain phenolic compounds that are highly bound to the enzyme tyrosinase. Therefore, adding additional extracts cannot result in significant tyrosinase inhibition. (*Furi et al., 2022)*.

CONCLUSION

The formulation of the cream preparation containing ethanol extract of kepel leaf (*Stelechocarpus burahol*) and pulasari bark (*Alyxia reinwardii* Blume) at concentrations of 1.25% and 2.5% showed potential as an inhibitor of the tyrosinase enzyme. Creams with a concentration of 2.5% showed a higher inhibition effectiveness compared to 1.25%. Physical evaluation showed that the increased concentration of the extract affected the viscosity, pH, spreadability, and adhesion of the cream, with results still meeting the criteria of good topical preparation.

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