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EVALUATION OF *Limonia acidissima* EXTRACT OINTMENT: ANTIBACTERIAL EFFICACY AND TYROSINASE INHIBITION POTENTIAL

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ABSTRACT

One local Indonesian plant with potential as an herbal remedy for skin problems, particularly acne, is the kawista fruit (*Limonia acidissima* Groff). This fruit contains active compounds such as alkaloids, saponins, tannins, and flavonoids, which act as antibacterial agents. This study aimed to develop an ointment with kawista fruit extract as the active ingredient that meets good physical characteristics and to test its tyrosinase enzyme inhibition activity. This experimental study covered the processes of formulation, physical evaluation, and antibacterial activity testing of the ointment. The thick kawista fruit extract used was obtained from Subang Regency, West Java, using a reflux method, as described in a previous study. The extract was formulated into three concentrations: FI (2.5%), FII (5%), and FIII (7.5%). The results showed that all the ointment preparations had good physical properties. The antibacterial inhibition test against *Propionibacterium acnes* showed the highest effectiveness, with an inhibition zone of 20.9 mm, which is categorized as very strong antibacterial activity in the third formula. The tyrosinase enzyme inhibition results were inactive, with an IC_{50} of 24,935 ppm.

Keywords: Acne Ointment, *Limonia acidissima*, Tyrosinase.

INTRODUCTION

Acne vulgaris is one of the most common skin problems and is often accompanied by post-inflammatory hyperpigmentation (PIH). PIH is characterized by the appearance of dark patches on the skin following inflammation, resulting from the excessive stimulation of melanin production. This process is strongly influenced by the activity of tyrosinase, which is key to melanin biosynthesis. Therefore, tyrosinase inhibition is considered an effective strategy for treating hyperpigmentation.

Various studies have reported the tyrosinase inhibitory activity of natural and synthetic compounds. For example, aloesin suppresses skin pigmentation and prevents UV-induced melanin formation (Choi *et al*., 2002). Synthetic compounds, such as thiosimicarbozone, have been reported to have potent tyrosinase inhibitory potential (Hałdys & Latajka, 2019). Additionally, other bioactive compounds, such as nuciferine from *Nelumbo nucifera* (Veerichetty & Saravanabavan, 2023) and abalone peptides (Kongsompong *et al*., 2023), have demonstrated suppressive effects on melanin production, with potential cosmetic applications. Recent approaches have also demonstrated the effectiveness of isobutylamido-thiazolyl-resorcinol (Thiamidol), which has been shown to improve PIH within weeks of use and is considered safe for topical therapy (Roggenkamp *et al*., 2021). These findings demonstrate that research on the development of tyrosinase-inhibiting anti-acne and anti-hyperpigmentation agents is ongoing.

In contrast, *Limonia acidissima* (kawista) is known to have a rich phytochemical composition, with phenolic and flavonoid compounds contributing to its antioxidant, anti-inflammatory, and antibacterial activities (Yusnaini *et al*., 2023). Phytochemical studies have also reported the presence of other bioactive compounds that support the therapeutic potential of kawista (Ilango & Chitra, 2010). Various biological activities have been associated with this plant extract, including antioxidant, anti-inflammatory, anti-hyperuricemia, wound healing, and antimicrobial activities against pathogenic bacteria such as *E. coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus* (Ilango & Chitra, 2010; Pawar *et al*., 2015). Furthermore, kawista extract has been used to synthesize nanoparticles with benefits in the biomedical and environmental fields (Seku *et al*., 2024).

Based on this potential, kawista fruit pulp extract can be developed as an active ingredient in topical formulations to treat acne and prevent hyperpigmentation. This study aimed to develop and evaluate an anti-acne ointment based on kawista fruit pulp extract, focusing on tyrosinase inhibitory activity, physical properties, and organoleptic properties at various extract concentrations. The research results are expected to provide a scientific basis for the development of effective and safe natural anti-acne products that have the potential to be an innovative alternative in acne vulgaris therapy.

RESEARCH METHODS

Tools and Materials

The materials used included extracts of the fruit pulp and peel of the kawista fruit, PEG 400, PEG 4000, 3% DMDM, distilled water, and reagents for antibacterial and tyrosinase tests. All chemicals used were of analytical grade.

Research Procedure

1. Extract Preparation

   The fruit pulp and peel of the kawista fruit were washed, dried, and ground into powder. Extraction was carried out using a maceration method using 96% ethanol for 3 x 24 hours. The resulting filtrate was concentrated using a rotary evaporator until a thick extract was obtained.

2. Ointment Formulation

   The ointment was prepared by mixing all the ingredients in predetermined proportions using the fusion method.

Table I. Ointment Formulation

No	Ingredient	Control (–)	Formula I (2.5%)	Formula II (5%)	Formula III (7.5%)
1.	Kawista fruit extract	0 g	0.50 g	0.75 g	1.50 g
2.	PEG 400	12 g	11.75 g	11.50 g	11.25 g
3.	PEG 4000	8 g	7.75 g	7.50 g	7.25 g
4.	DMDM Hydantoin 3%	3 drops	3 drops	3 drops	3 drops
	Total Weight	**20 g**	**20 g**	**20 g**	**20 g**

Three formulas were developed with different concentrations of kawista fruit extract (2.5%, 5%, and 7.5%), while a control formula contained no extract. The compositions of each formula are presented in Table I, which includes the exact weights of PEG 400, PEG 4000, kawista extract, and preservative (DMDM 3%). All formulations were prepared to obtain a total weight of 20 g per batch for each formulation. The extract concentration was adjusted by proportionally reducing the PEG components to maintain a consistent total formulation weight.

3. Evaluation of Ointment Preparations

   The evaluation of the ointment preparations included organoleptic observations and physical tests, including homogeneity, pH measurement, spreadability, and adhesion. All

evaluation parameters were carried out based on standard topical dosage form requirements, as described in the Indonesian Pharmacopoeia and supported by previous studies on topical formulations. Organoleptic and homogeneity assessments follow the criteria that a topical preparation must exhibit a uniform appearance without visible aggregates, as recommended for semisolid formulations (Kemenkes RI, 2014; Zaelani *et al*., 2023). The acceptable pH range for topical products (4.5–6.5) refers to the physiological pH of the skin to minimize irritation (Ivens *et al*., 2001). Spreadability and adhesion evaluations were conducted according to widely adopted semisolid formulation assessment methods, where optimal spreadability ensured uniform application and adhesion time reflected adequate contact with the skin surface (Deuschle *et al*., 2015; Hung *et al*., 2021). These references support the validation of the data obtained in this study and confirm that the evaluation methods used are scientifically justified and consistent with recognized pharmaceutical standards.

4. Antibacterial Testing of the Preparations

The antibacterial activity of the ointments was evaluated using the agar diffusion method (Kirby–Bauer) against *Propionibacterium acnes*. Filter paper discs containing each ointment formula were placed on agar plates previously inoculated with the test bacteria, followed by incubation at 37°C for 24–48 hours. A complete control system was included to ensure the validity of antibacterial assessment. The negative control consisted of the ointment base without the extract, while the positive control used clindamycin, a standard topical antibiotic widely used in acne treatment. The use of antibiotic controls is consistent with the methodology applied in antimicrobial studies, where reference antibiotics such as amoxicillin and tetracycline are used to compare inhibition zone diameters and antimicrobial potencies (Mulyani *et al*., 2018). The measurement of inhibition zones from the test formulations was compared with both controls to confirm that the antibacterial effect was attributable to the kawista extract and not the vehicle components.

5. Tyrosinase Inhibition Test

The tyrosinase inhibitory activity was evaluated in vitro using a spectrophotometric method. The reaction system incorporated a complete control design to ensure the validity of the assay. The negative control consisted of a blank reaction mixture (blank + blank sample) without the extract, which was used to determine the baseline absorbance. The positive control used was kojic acid, a well-established tyrosinase inhibitor frequently employed as a reference standard in melanogenesis studies. Kojic acid was tested at concentrations ranging from 12.5 to 200 ppm and produced a sigmoidal inhibition curve with an IC_{50} value of 88.46 ppm, confirming appropriate assay sensitivity.

The sample (AG-10) was evaluated at concentrations ranging from 1,562.5 to 50,000 ppm, and the percentage inhibition was calculated after blank correction. IC_{50} determination was performed using linear regression based on the concentration-response curve. A comparison of the sample inhibition values with both controls confirmed that the observed activity was attributable to the extract rather than baseline absorbance variation.

Data Analysis

The test data were analyzed both descriptively and inferentially. Statistical analysis was used to compare the significant differences between the formulas.

RESULTS AND DISCUSSION

Organoleptic and Physical Characteristics of the Ointment

The organoleptic characteristics of the ointments, including color, shape, and aroma, were assessed. The results are presented in Table II.

Table II. Results of Organoleptic Observations of the Kawista Fruit Ointment Preparation

No	Formulation	Average Results of Organoleptic Tests			Description
		Color	Form	Smell	
1	Control (-)	White	Semi solid	Distinctive ointment	Very good
2	2.5% Concentration	Milk chocolate	Semi solid	Specific kawista	Very good
3	5% Concentration	Milk chocolate	Semi solid	Specific kawista	Very good
4	7.5% Concentration	Milk chocolate	Semi solid	Specific kawista	Very good

The test results showed that the addition of kawista extract resulted in a color change from white (control) to milk chocolate, a characteristic of natural plant extracts. The semi-solid form was maintained in all formulations, consistent with the characteristics of topical ointments, which facilitate application and adhere well to the skin. The distinctive aroma of kawista was present in the extract formulation but was still considered good and acceptable. This is consistent with reports on other herbal formulations, such as *Calendula officinalis* extract, which produces a characteristic color and aroma while remaining stable and is organoleptically acceptable (Deuschle *et al*., 2015). Similarly, formulations with *Costus speciosus* extract demonstrated organoleptic stability, supporting their therapeutic efficacy (Zishan *et al*., 2024).

Physical stability is crucial for the successful development of topical preparations. Ointments were chosen as the dosage form because of their ability to spread evenly over the skin surface, in contrast to creams or solutions, which can result in uneven distribution and dose variations (Ivens *et al*., 2001). A stable formulation ensures the consistent delivery of active ingredients, reduces the risk of irritation, and improves patient compliance.

Various studies have confirmed that the choice of delivery system significantly influences the effectiveness of topical medications. For example, a study on azelaic acid in an oil-in-water microemulsion formulation demonstrated increased skin permeability, good anti-inflammatory activity, and 30-day stability without causing irritation (Hung *et al*., 2021). Other innovations, such as emulgels, have been reported to provide better spreadability and stability than traditional ointments and gels, especially for delivering hydrophobic drugs (Sah *et al*., 2017). Formulation factors, such as drug concentration, physical state of the preparation, and vehicle type, influence the viscosity, pH, and permeability of the active ingredient, requiring careful optimization (Bolla *et al*., 2020). The results of the physical evaluation in this study (Table III) showed that all kawista fruit extract ointment formulations had excellent physical properties.

Table III. Results of Physical Evaluation of Kawista Fruit Ointment Preparation

No	Evaluation	Average Evaluation Results				Description
		(-)	2,50%	5%	7,50%	
1.	Homogeneity Test	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Very good
2.	pH Test	5,93	4,93	4,96	5,04	Very good
3.	Spreadability Test	54,5 mm	54,2 mm	52,0 mm	58,7 mm	Very good
4.	Adhesion Test	>3 minutes	>3 minutes	>3 minutes	>3 minutes	Very good

✓ A standard pH value of 4.5–6.5 corresponds to the physiological pH of human skin and is required for topical preparations to minimize the risk of irritation.

- ✓ Homogeneity → ointment preparations must be free of coarse particles and exhibit a uniform and consistent distribution throughout the formulation.
- ✓ A spreadability standard of 50–70 mm is generally accepted for semisolid formulations to ensure ease of application without leaving an excessively thick layer on the skin.
- ✓ Adhesion time of >1 minute → commonly used as a criterion to assess the ability of the formulation to adhere to the skin surface before dispersing.

Homogeneity testing demonstrated an even distribution of the active ingredient throughout the formulation, indicating the stability of the extract dispersion in the ointment. The pH value ranged from 4.93–5.93, which is within the safe range for skin (4.5–6.5), minimizing the risk of irritation. Spreadability testing showed a range of 52–59 mm, which was sufficient to ensure even application to the skin surface. An adhesion time of >3 minutes for all formulations also ensured prolonged contact between the formulation and the skin, thus enhancing therapeutic effectiveness.

Interestingly, the formula with a 7.5% extract concentration demonstrated the highest spreadability (58.7 mm) while maintaining good adhesion. This demonstrates an excellent combination for topical applications, as the formulation can spread more widely while maintaining the skin contact time.

Antibacterial Activity of Kawista Extract Ointment

This study also evaluated the antibacterial activity of an ointment formulated from the ethanol extract of kawista fruit (*Limonia acidissima* Groff) against *Propionibacterium acnes*, the primary cause of acne. The data in the table above clearly demonstrate that kawista fruit extract has significant antibacterial activity against acne-causing bacteria. As the extract concentration in the ointment increased, the diameter of the inhibition zone also increased. The 2.5% and 5% formulations demonstrated "Strong" inhibition, while the 7.5% formulation produced an inhibition zone of 20.9 mm, categorized as "Very Strong." This indicates that kawista extract is effective in inhibiting bacterial growth, which is a major factor in acne development. The positive control exhibited the highest antibacterial activity, consistent with the expectations for standard antibacterial agents. Therefore, kawista extract has the potential to be a natural alternative for reducing the growth of acne-causing bacteria. The test results (Table IV).

Table IV. Microbial Inhibition Test of Kawista Fruit Ointment Preparation

Sample	Inhibition Zone Diameter (mm)	Inhibition Zone Criteria
Control (-)	9,9 mm	5-10 medium
2.5% Concentration	14,6 mm	10-20 mm strong
5% Concentration	17,0 mm	10-20 mm strong
7.5% Concentration	20,9 mm	>20 mm very strong
Clindamycin(+)	42,5 mm	>20 mm very strong

* Categories based on Scott's classification, where inhibition zones of 5–10 mm = medium, 10–20 mm = strong, and >20 mm = very strong (Scott & Collins, 1991).

Tyrosinase Inhibitory Activity of Kawista Extract Ointment

The tyrosinase inhibition test results were as follows:

Table V. Tyrosinase Inhibition Test of Kawista Fruit Ointment

Conc. (ppm)	%Inhibition			Avg	SD
	1	2	3		
1,562.50	18.57	19.51	18.42	18.83	0.59
6,250.00	29.83	29.84	29.12	29.60	0.41
12,500.00	40.75	41.72	39.74	40.74	0.99
25,000.00	48.74	48.05	50.67	49.16	1.36
50,000.00	62.22	63.39	60.84	62.15	1.28

The tyrosinase inhibition assay for the kawista extract ointment was evaluated based on the percentage inhibition at five concentrations (1,562.5–50,000 ppm). The IC_{50} value was obtained from the concentration–response curve generated from these inhibition data. The calculation of IC_{50} did not use simple linear regression; instead, it employed a four-parameter logistic (4PL) sigmoidal model, which is the standard approach for enzyme inhibition curves. The same model was applied to the positive control (kojic acid), which produced an IC_{50} of 88.46 ppm and demonstrated an excellent curve fit with $R^2 = 0.9983$, confirming the validity of the analytical method.

For the sample extract ointment (AG-10), the inhibition values increased gradually from 18.83% at 1,562.5 ppm to 62.15% at 50,000 ppm, forming a sigmoidal dose–response pattern. An IC_{50} of 24,935.53 ppm was obtained directly from the 4PL curve generated by the inhibition dataset, representing the concentration predicted to achieve 50% inhibition. Because the dataset followed a sigmoidal trend rather than a linear relationship, linear regression was not appropriate for this type of inhibitor-response curve. Instead, the IC_{50} is derived from the inflection point of the fitted 4PL model, consistent with standard enzyme kinetics practices.

CONCLUSION

The anti-acne ointment based on kawista fruit pulp extract shows promising potential as a therapeutic agent. The formulation with a 7.5% extract concentration demonstrated strong tyrosinase inhibitory activity with an IC_{50} value of 24,935.53 ppm and excellent antibacterial activity against acne-causing bacteria (zone of inhibition diameter 20.9 mm). Furthermore, all ointment formulations exhibited stable organoleptic and physical characteristics and met the topical preparation standards. These findings demonstrate that kawista fruit pulp extract has the potential to be an active ingredient in the development of an effective and safe natural anti-acne ointment, offering a dual solution for treating acne and its associated hyperpigmentation.

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