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The physical quality test formulation of ethyl acetate extract of kawista fruit peel (Limonia Acidissima L.) Ointment as an antibacterial against propionibacterium acnes

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ABSTRACT

Introduction: Using medicinal plants in Indonesia still needs to be optimized. Almost all parts of the Kawista plant have been traditionally used to treat various ailments such as antioxidants, antidiabetic, wound healing, and controlling uric acid levels. Existing research on Kawista fruit peel as an acne antibacterial includes studies on the ethyl acetate extract of Kawista fruit peel against Propionibacterium acnes, which falls into the moderate to strong category. In Indonesia, Kawista fruit is limited to the flesh and is used to make syrup and dodol (a traditional sweet). In contrast, the fruit's peel remains a waste product.

Objective: This study aims to develop a formula for an ethyl acetate extract ointment of Kawista fruit peel (Limonia acidissima L.) as an antibacterial agent against Propionibacterium acnes with good physical quality.

Methods: The fruit peel extract was obtained using the maceration method with 96% ethanol. The extract was then formulated into ointment preparations with concentration variations of 6.25% (F1), 12.50% (F2), and 25% (F3). The physical stability tests for the ointment included organoleptic parameters, homogeneity, spreadability, and pH value tests. The data were analyzed to determine the effect of the extract concentration and storage time on the ointment's physical stability.

Result: The test results showed that the ointment formulation of Kawista fruit peel extract (Limonia acidissima L.) could be successfully formulated into an ointment form and met the required evaluation criteria, including organoleptic testing, homogeneity testing, pH testing, and spreadability testing.

Conclusion: Based on the study, it can be concluded that the Kawista fruit peel extract ointment (Limonia acidissima L.) can be successfully formulated into an ointment and meets the required evaluation criteria, including organoleptic testing, homogeneity testing, pH testing, and spreadability testing. A skin irritation test is required to determine whether any irritation occurs after applying the ointment.

Keywords: ethyl acetate; extract; kawista, ointment.

INTRODUCTION

The utilization of Kawista fruit in Indonesia as a medicinal material has not been extensively studied. The existing uses are limited to the fruit pulp, commonly made into syrup, while the fruit peel is often discarded as waste (Kusuma, Jastian, and Amir, 2022). Almost all parts of the Kawista plant, including the roots, bark, leaves, sap, and fruit, have been traditionally used to treat various ailments such as antioxidants, antidiabetic, wound healing, and to regulate plasma uric acid levels as a xanthine oxidase inhibitor (Kusuma, Veryanti, Saragih, 2019). However, using Kawista fruit peel is still rare in Indonesian society (Kusuma and Adhitya, 2021). According to research conducted in India, the pulp of Kawista fruit is known to have antitumor activity due to polysaccharide compounds. The bark and fruit have antidiabetic activity due to flavonoid compounds. The leaves have anti-inflammatory, antidiarrheal, and analgesic properties from tannin, alkaloid, and flavonoid compounds (Sharma and Tenguria, 2021). Another study on the antibacterial activity of Kawista fruit in Indonesia tested the ethanol fraction of the pulp on *Staphylococcus aureus* and *Escherichia coli* bacteria (Jamil *et al.*, 2019). The ethyl acetate extract of Kawista fruit peel contains flavonoids, steroids, saponins, tannins, and alkaloids (Rahmi and Rahmadewi, 2020).

Acne vulgaris is a chronic inflammatory skin condition that affects the pilosebaceous follicles and is prevalent worldwide (Hazarika, 2021; Heath and Richard, 2021). Acne is estimated to affect 9.4% of the global population and ranks eighth among skin diseases. Acne impacts more than 85% of teenagers, and the condition can persist into adulthood, particularly in women (Tan, Schlosser and Paller, 2018). This distinctive lesion can be categorized as non-inflammatory (open/ blackheads and closed/ whiteheads) or inflammatory (papules, pustules, nodules, and cysts), often leading to scarring and pigmentation on the skin, which requires long-term and persistent treatment (Conforti et al., 2021). Typically, lesions appear on the face, neck, upper back, and chest. There are several types of acne, such as neonatal and infantile acne, occupational acne, conglobate acne, fulminant acne, mechanical acne, excoriated acne, chloracne, and acne caused by medications (e.g., anabolic steroids, corticosteroids, isoniazid, lithium, and phenytoin) (Mitchell et al., 2022; Muller et al., 2020). Acne vulgaris (AV) often causes discomfort, emotional distress, deformities, and potential permanent scarring.

Furthermore, patients may experience anxiety and shame, which can lead to mental depression (Vasam, Korutla, and Bohara, 2023). Due to these factors, the pathogenesis of acne is multifactorial, involving excessive sebum production, bacterial colonization by Cutibacterium acnes (formerly Propionibacterium acnes), abnormal keratinization of the pilosebaceous follicles, and inflammatory mechanisms. These four factors are the primary causes of acne (Kraft and Freiman, 2011). Topical therapy offers the advantage of being applied directly to the affected area, minimizing systemic absorption and enhancing the exposure of the pilosebaceous unit. Various topical formulations include creams, gels, lotions, solutions, and washes. Mild to moderate acne is typically treated with topical medications (Sevimli Dikicier, 2019; Greydanus et al, 2021). In addition to retinoids, antibiotics are used topically to treat acne patients. Skin irritation is a common side effect of topical acne medications. Topical treatments may be applied for 6-8 weeks or may continue for years (Mohamed, Gharib, and Samir, 2022). Topical retinoids should be considered as a first-line therapy for treating acne due to their role in reducing microcomedones that are important in the development of both inflammatory and non-inflammatory lesions. Systemic treatment is preferred when acne does not respond to topical treatment or presents as nodular lesions or with scarring. Systemic treatment is crucial for acne patients to prevent social stigma and psychological embarrassment. Oral antibiotics, hormonal treatments, and isotretinoin are the most commonly used systemic treatments for Acne vulgaris.

Propionibacterium acnes is a gram-positive, anaerobic bacillus bacterium that is air-tolerant and gas-forming. It is a normal flora found on the skin and mucosal tissues, typically present on the face, chest, armpits, and respiratory tract (thorax). Propionibacterium acnes primarily infects nutritionally rich synovial areas, such as the shoulder, hip, and knee after surgery, and is currently

the second most common pathogenic organism after Staphylococcus aureus (Bortman and Schefer, 2021). The management of acne includes using antibacterial agents, systemic therapy, and hormonal treatments (Sibero, Putra, and Anggraini, 2019). The administration of antibacterial agents such as tetracycline, erythromycin, and clindamycin can reduce the population of Propionibacterium acnes bacteria. However, excessive use of antibiotics can lead to increased bacterial resistance to specific antibiotics (Indarto et al., 2019).

RESEARCH METHODOLOGY

Type of Research

This research is a type of research that is conducted in an experimental laboratory.

Location and Time of Research

This research was conducted in Politeknik Sandi Karsa Makassar's pharmaceutical laboratory from September to October 2024.

Population and Sample

The population of this study consists of all parts of the Kawista fruit (*Limonia acidissima* L.) that grow and are commonly found in Woha District, Risa Village, West Nusa Tenggara Province. The sample used was the waste from the peel of the Kawista fruit (*Limonia acidissima* L.) that had previously been consumed in Woha District, Risa Village, West Nusa Tenggara Province.

Tools and materials used

The tools used in this study were porcelain cups, hot plates (Maspion 5.304), pH meters (Laqua), pH meters (Sartorius), analytical balances (Henherr JGS-K), ultrapure homogenizers (Wisd HS-5-A), a set of extraction equipment, and a set of glassware (Pyrex Iwaki). The materials used in this study include distilled water (aqua dest), ethyl acetate solvent (technical grade), 70% ethanol, 96% ethanol, menthol, methylparaben, polyethene glycol 400, polyethene glycol 4000, and propylparaben. The sample used was the peel of *Limonia acidissima* L. (Kawista fruit) collected from Risa Village, Woha District, West Nusa Tenggara Province.

Extract Manufacturing

The *simplisia* of Kawista fruit peel (*Limonia acidissima* L.) was placed in a maceration container and soaked with ethyl acetate until the *simplisia* was evenly submerged. The maceration container was covered and stored for 1 x 24 hours in a place protected from sunlight, with occasional stirring. The mixture was then filtered, separating the filtrate from the residue. The residue was re-extracted using a fresh solvent in the same amount. This process was repeated until the solvent became clear (after three extractions). The ethyl acetate extract obtained was then collected and concentrated using a rotary evaporator at 40°C. The sample extract was then separated from the ethyl acetate.

Ointment Preparation

The ointment preparation process begins with the melting of PEG 400 over a water bath. Once it has melted, PEG 4000 is poured into a warm mortar and PEG 400 is gradually added. The ointment base is then slowly added with propylene glycol after the mixture has cooled down (below 50°C). Menthol, methylparaben, and propylparaben, each dissolved in 96% ethanol, are added to the ointment base. Next, the extract is added to the base mixture. The mixture is stirred until a smooth and homogeneous ointment mass is formed. Finally, the ointment is packaged into tightly closed containers (plastic pots).

Evaluation of the Physical Stability of Ointment Supplies

The *Ointment* obtained is stored at a temperature of 25°C-30°C and tested for its physical stability, including organoleptic parameters, homogeneity, spreadability, and pH value tests.

Data Analysis

The normality analysis of the stability data of the salep Ekstrak et asetat kulit buah kawista was carried out by the Shapiro-Wilk test at a confidence level of 95%. If the p-value > 0.05, the distributed data is average; If the p-value < 0.05, the data is not normally distributed. Average undistributed data was tested with the Wilcoxon test, while average data was followed by the Paired Samples Test (p-value < 0.05 showed significant differences between formulas). Organoleptic, homogeneity, pH test, and spreadability test were performed descriptively. This should include the exact method of observation or experiment. Mathematical and statistical methods must be mentioned, and any general computer package must be specified.

RESULT

The evaluation of the physical properties of ointment preparations aims to determine the physical properties of good ointment preparations, namely semi-solid and not rancid odour. The evaluations include organoleptic parameters, homogeneity, spreadability, and pH value tests.

Table 1. The organoleptic test of the ethyl acetate extract of the kawista fruit peel ointment.

Formula	Observation					
	Before Storage			After Storage		
	Colour	Odor	Consistency	Color	Odor	Consistency
F1	Brown	Characteristic odor of the extract	Semisolid	Brown	Characteristic odor of the extract	Semisolid
F2	Brown	Characteristic odor of the extract	Semisolid	Brown	Characteristic odor of the extract	Semisolid
F3	Brown	Characteristic odor of the extract	Semisolid	Brown	Characteristic odor of the extract	Semisolid

Based on the organoleptic observation results, F1, F2, and F3 did not show any color, odor, or appearance changes from the initial preparation to storage for 4 weeks. This indicates that the ointment formulations were physically stable during the storage period.

Table 2. Results of homogeneity, ph measurement, and spreadability testing of the ethyl acetate extract of the kawista fruit peel ointment.

1. *Homogeneity Test*		
Formula	**Observation**	
	Before Storage	**After Storage**
F1	Homogen	Homogen
F2	Homogen	Homogen
F3	Homogen	Homogen
2. *pH Measurement*		
Formula	**Observation**	
	Before Storage	**After Storage**
F1	6	6
F2	6	5
F3	5	5
3. *Spreadability Test*		
Formula	**Observation (cm)**	
	Before Storage	**After Storage**
F1	5.23	5.73
F2	5.50	5.89
F3	5.81	6.13

The results of this test indicate that the ointments have a homogeneous composition, and no coarse particles were found in F1, F2, and F3. This meets the homogeneity requirement, where the ointment shows a uniform structure characterized by the absence of coarse particles. The pH

test results of the ethyl acetate extract ointment of Kawista fruit peel, both before and after storage, were within the pH 5-6 range. This aligns with expectations, as the pH range for topical preparations should ideally be between 4.5 and 6.5. Any changes in pH during storage are likely due to the influence of temperature. The spreadability test results of the ethyl acetate extract ointment of Kawista fruit peel showed a range between 5-6, with all three formulations meeting the spreadability test requirements. Formula F3 exhibited the highest spreadability value. The results of the spreadability test indicate that an increase in the extract concentration is directly proportional to the improvement in spreadability.

DISCUSSION

This study conducted physical property tests on the formulation of Kawista fruit peel extract (Limonia acidissima L.) ointment. The physical property tests were part of the evaluation carried out in this research. This evaluation aimed to determine whether the produced ointment formulation has good physical properties and stability. Good physical properties and stability are important in determining the quality of a pharmaceutical preparation and its ease of use by consumers (Farhamzah *et al*., 2022).

The Organoleptic Test

Organoleptic testing of the ointment formulation of Kawista fruit peel extract (*Limonia acidissima* L.) was conducted using the five senses. This test aimed to assess the ointment's physical properties, including its form, odor, and color (Rawung *et al*., 2020). Based on the organoleptic observation results, both before and after stability testing, no changes in form, odor, or color were observed in each formulation (F1, F2, and F3), as they showed the same results: semisolid form, brown color, and a characteristic extract odor. The brown color of the formulation is due to the addition of the extract and the mixture of excipients. These results are consistent with the literature, which states that a suitable ointment formulation should have a semisolid form, a characteristic odor similar to the sample, and a colour resembling the extract (Rawung *et al*., 2020).

Homogeneity Test

The homogeneity test was performed by applying the formulation to the top, middle, and bottom areas and placing it on a transparent glass slide. The homogeneity test results before and after stability testing for formulas 1, 2, and 3 showed a homogeneous composition, evidenced by the absence of coarse particles and clumps in the formulation. The homogeneity of the ointment indicates that mixing the ointment base with the ethyl acetate extract was done properly, ensuring no clumps or coarse particles in the formulation. A homogeneous ointment is essential to prevent irritation and ensure even distribution when applied (Novita, Munira, and Hayati, 2017).

pH Measurement

The pH test aims to assess the acidity level of a formulation to ensure that it does not cause irritation or dryness (flaking) on the skin. This is important to guarantee that the formulation is safe and suitable for topical use without harming the skin (Lasut *et al*., 2019). Based on the pH test results of the Kawista fruit peel extract ointment (*Limonia acidissima* L.), the pH values for each formula were as follows: for Formula 1, the pH was six before storage and remained six after storage; for Formula 2, the pH was six before storage and decreased to 5 after storage; and for Formula 3, the pH was five before storage and remained five after storage. The post-storage pH values indicate a decrease in pH across all three formulations. The change in pH during storage suggests a lack of stability in the formulations over time. This instability could potentially damage the product during storage or use. The decomposition of the medium could influence the change in pH due to high temperatures during manufacturing or storage, which may result in the formation of acids or bases. (Susanti *et al*., 2019). Although different pH values were obtained, the pH of all three formulas still falls within the acceptable range for normal skin pH (4.5–6.5) and is in accordance with the Indonesian National Standard (SNI) 16-4399-1996 for the quality

of skin moisturizers, which specifies a pH range of 4.5–8. The pH test results were analyzed using SPSS version 26 with the Shapiro-Wilk test to check for normal distribution of the data, with a significance value > 0.05. The pH test data showed a normal and homogeneous distribution, allowing for further analysis using ANOVA. The ANOVA results showed a significance value of 0.016 < 0.05, indicating a significant difference between the formulas. A subsequent LSD (Least Significant Difference) test was conducted to identify the differences between each formula. The LSD test revealed a significant difference between Formula 1 and Formula 2, with a p-value of 0.010 (< 0.05), as well as between Formula 1 and Formula 3, with a p-value of 0.011 (< 0.05).

Spreadability Test

The spreadability test results for the Kawista fruit peel extract ointment (Limonia acidissima L.) showed the following values: for Formula 1, the spreadability was 5.23 cm before storage and remained 5.23 cm after storage. Formula 2's spreadability was 5.50 cm before storage and increased to 5.89 cm after storage. Formula 3's spreadability was 5.81 cm before storage and increased to 6.13 cm after storage.

The spreadability test data were analyzed using SPSS version 26 with the Shapiro-Wilk test to check for normal distribution of the data, with a significance value > 0.05. The spreadability data showed a normal and homogeneous distribution, allowing for further analysis using ANOVA. The ANOVA results yielded a significance value of 0.016 (< 0.05), indicating a significant difference between the formulas.

Subsequently, a Least Significant Difference (LSD) test was conducted to determine the differences between each formula. The LSD test revealed significant differences as follows: between Formula 1 and Formula 2 ($p = 0.005 < 0.05$), between Formula 1 and Formula 3 ($p = 0.000 < 0.05$), and between Formula 2 and Formula 3 ($p = 0.003 < 0.05$).

CONCLUSION

Based on the study, it can be concluded that the Kawista fruit peel extract ointment (*Limonia acidissima* L.) can be successfully formulated into an ointment and meets the required evaluation criteria, including organoleptic testing, homogeneity testing, pH testing, and spreadability testing. A skin irritation test is required to determine whether any irritation occurs after applying the ointment. Using kawista fruit peel extract as an active ingredient in acne ointment formulations opens up opportunities for developing safe and effective cosmetic products based on natural ingredients.

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Conflict of Interest

We have no conflicting interests.

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