Research in Education, Technology, and Multiculture

ISSN: 3025-6763 | Journal's DOI: 10.61436/rietm

Homepage: https://imrecsjournal.com/journals/index.php/rietm

Development of Herbal Cosmetics Based on Local Resources from Eastern Indonesia: Formulation of an Antioxidant and Antibacterial Mask from Wojalaka Black Rice

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ARTICLE INFO

Article History:

Received: 27 August 2026

Accepted: 17 October 2026

Published: 19 November 2026

Keywords:

Antioxidant;
Citronella essential oil;
Facial mask;
Propionibacterium acnes;
Wojalaka black rice.

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ABSTRACT

Acne (acne vulgaris) is a complex skin condition caused by inflammation and infection with the bacterium *Propionibacterium acnes*. This study aimed to formulate and evaluate a facial mask combining black rice (*Oryza sativa* L.) extract from the local *Wojalaka* superior variety from Sumba as an antioxidant agent, together with citronella essential oil as an antibacterial agent. *Wojalaka* black rice extract was obtained through maceration in 70% methanol and was tested for its phytochemical content both qualitatively and quantitatively. Citronella essential oil was obtained through steam distillation. Three mask formulations (F1 30%, F2 40%, F3 50% black rice extract, each containing 0.3% citronella essential oil) were tested for physical stability (organoleptic properties, homogeneity, pH, spreadability), irritation (patch test), preference (hedonic test), antioxidant activity (DPPH), antimicrobial resistance, in vitro antibacterial effectiveness against P. *acnes*, and an *in vivo* clinical trial on 15 subjects over 6 weeks. Phytochemical screening showed that Wojalaka black rice extract contains flavonoids (332.58 mg/100 g QE), tannins (137.89 mg/100 g TAE), phenolics (49.33 mg/100 g GAE), saponins, triterpenoids, and alkaloids, with very strong antioxidant activity (IC50 37.561 mg/L; AAI 5.32). Formulation F3 proved to be the best preparation, meeting the physical quality criteria (homogeneous, pH 6.0, spreadability 5.0 cm), being the most preferred by the subjects, and retaining very strong antioxidant activity (IC50 48.9 mg/L; AAI 4.08). In addition, F3 showed strong-category antibacterial activity against *P. acnes* (inhibition zone 10.30 ± 1.08 mm) and antimicrobial resistance (inhibition zone 18.61 ± 6.21 mm). *In vivo* testing confirmed that the most noticeable improvement in the subjects' skin condition occurred from week 2 of use onward. This combination proved effective and holds strong potential for development as an anti-acne herbal cosmetic preparation based on the local wisdom of Eastern Indonesia.

To cite this article:

Ghello, J. M., Bani, G. A., Laalobang, S., & Bani, M. D. (2026). Development of Herbal Cosmetics Based on Local Resources from Eastern Indonesia: Formulation of an Antioxidant and Antibacterial Mask from Wojalaka Black Rice. *Research in Education, Technology, and Multiculture*, *5*(4), 437-456. doi: https://doi.org/10.61436/rietm/v5i4.pp437-456

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■ INTRODUCTION

Skin health, particularly in the facial area, significantly influences a person's psychological condition and quality of life (Ozcan et al., 2023). Disturbed or unhealthy facial skin can reduce self-confidence and trigger negative stigma in social interactions (Zhou et al., 2023). One of the most common facial skin problems is acne (*acne vulgaris*), estimated to affect up to 80% of adolescents and young adults (Guguluș et al., 2025). The pathogenesis of acne is closely related to inflammation, hyperseborrhea, and infection by the bacterium *Propionibacterium acnes* as the main microorganism responsible for inflammation and comedone formation (Niedźwiedzka et al., 2024). The impact of acne is not limited to physical appearance but can also trigger profound psychological disturbances such as anxiety, depression, and reduced self-esteem (Woźna et al., 2026).

To address acne, researchers have developed various synthetic cosmetic products (Li et al., 2024). However, continuous use of synthetic chemical ingredients often causes side effects such as skin irritation, redness, and

increased sensitivity (Alnuqaydan, 2024; Wirtu, 2024). This phenomenon has driven a shift in public trends toward natural-ingredient-based personal care products ("back to nature"), which are considered safer, more environmentally friendly, lower in side effects, and more economical (Baba et al., 2026). One local natural ingredient from eastern Indonesia with substantial potential is black rice (*Oryza sativa L.*) of the local superior *Wojalaka* variety from Sumba Island, East Nusa Tenggara (NTT) Province. *Wojalaka* black rice is rich in phenolic compounds, flavonoids, and anthocyanins that function as free-radical-scavenging antioxidants (Ghello & Bani, 2025).

Although the antioxidant potential of black rice is well recognized, preparations based solely on black rice extract have limitations (Das et al. 2023). In addition, organic cosmetic ingredients derived from black rice show no significant antibacterial activity against *P. acnes* and are also prone to microbial contamination during storage (Nemati et al., 2024). Therefore, a combination formulation strategy is needed using a distilled natural ingredient with strong antimicrobial properties, such as citronella essential oil (Zhang et al., 2022). Citronella essential oil is rich in volatile active components (such as citral, citronellal, citronellol, and geraniol) that can damage bacterial cell membranes, reduce inflammation, and control acne (Mangalagiri, Panditi, & Jeevigunta, 2021). Preliminary studies indicate that combining black rice extract and citronella essential oil can produce optimal synergistic antioxidant capacity (Ghello, Arpiwi, & Sukmaningsih, 2025).

This study's novelty lies in using the local *Wojalaka* black rice variety from Sumba, which has a unique secondary metabolite profile compared with other black rice varieties, combined synergistically with citronella essential oil in a facial mask. This combination approach of two natural ingredients based on local wisdom presents a comprehensive skin care solution, in which black rice extract acts as a cell-protective antioxidant agent, while citronella essential oil serves as an antimicrobial agent inhibiting *P. acnes*. The urgency of this study covers three main aspects: (1) Local Economic Empowerment: encouraging value addition of agricultural commodities and improving the economy of *Wojalaka* black rice and citronella farmers on Sumba Island; Market Response and Green Trends: meeting increasing consumer demand for natural skin care products that are safe, stable, and environmentally friendly; and (3) Development of Science and Technology Based on Local Wisdom: providing scientific contributions in the form of quality standardization of the preparation (organoleptic properties, pH, spreadability, homogeneity), safety testing (patch test irritation), preference testing (hedonic), and validation of biological effectiveness both *in vitro* (DPPH antioxidant activity and *P. acnes* inhibition zone) and *in vivo* on subjects' skin. This effort aligns with the Indonesian Government's 2025 Asta Cita agenda to promote the green and creative economy, as well as Sustainable Development Goal (SDG) Number 3 on Good Health and Well-Being.

Based on this background, this study aimed to evaluate the effectiveness of a facial mask formulation combining *Wojalaka* black rice extract and citronella essential oil. The evaluation was carried out comprehensively, covering the physical stability of the preparation, free-radical-scavenging effectiveness (DPPH antioxidant activity), inhibitory ability against *P. acnes* bacteria, resistance to microbes, hedonic acceptance level, and its direct effect on improving the subjects' facial skin condition. The results of this study are expected to provide solid scientific evidence supporting the downstream development of natural cosmetic products based on local resources of Eastern Indonesia that are safe, stable, and efficacious.

■ METHOD

Phytochemical Extraction of *Wojalaka* Black Rice

Wojalaka black rice was ground into powder, then macerated using 800 mL of 70% methanol for 24 hours. Analytical-grade methanol was employed strictly for in vitro analytical procedures and laboratory assays (i.e., quantitative phytochemical characterization and DPPH radical scavenging assays). Methanol was neither utilized in the topical formulation matrix nor directly applied to the subjects' skin. After maceration, the mixture was filtered using filter paper. The resulting filtrate was then evaporated using a rotary evaporator (Janke & Kunkel RV 06-ML) to remove the solvent and obtain a concentrated extract. The concentrated extract was subsequently used for qualitative phytochemical testing, covering alkaloids, saponins, triterpenoids, steroids, flavonoids, tannins, and phenolics (Ndezo Bisso et al. 2022). The antioxidant activity of the black rice extract was then evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method.

Qualitative and Quantitative Phytochemical Testing

Qualitative phytochemical testing was performed to identify the presence of several phytochemical compound groups in the *Wojalaka* black rice extract, including alkaloids, saponins, triterpenoids, steroids, flavonoids, tannins, and phenolics. Based on the qualitative identification results, quantitative analysis was then carried out on flavonoids, phenolics, and tannins to determine the total content of each compound group in the extract. The total content of flavonoids, phenolics, and tannins was determined using quercetin, gallic acid, and tannic acid as standards, respectively. All extract analyses were performed at the Integrated Services Laboratory, Faculty of Agricultural Technology, Udayana University, Bali.

Alkaloids, Saponins, Triterpenoids, and Steroids

Alkaloids. A total of 4 g of *Wojalaka* black rice extract was redissolved in 10 mL of a chloroform–ammonia mixture. The mixture was then filtered, and a few drops of 1 M sulfuric acid (H2SO4) were added to the filtrate, followed by shaking until two layers formed. The acidic layer was separated and used for alkaloid testing with Mayer's and Dragendorff's reagents. The presence of alkaloids was indicated by the formation of a white precipitate with Mayer's reagent and an orange to reddish-brown precipitate with Dragendorff's reagent (Gunjkar & Ghiware, 2023).

Saponins. The presence of saponins was tested by mixing *Wojalaka* black rice extract with 10 mL of distilled water in a beaker, then heating the mixture to boiling. After cooling, the mixture was transferred to a test tube and shaken vigorously until foam formed. Foam persisting for at least 10 minutes indicated stable foam formation. Subsequently, a few drops of concentrated hydrochloric acid (HCl) were added, and the mixture was left to stand for 15 minutes. The presence of saponins was confirmed if the foam remained stable after adding HCl (Hussen & Endalew, 2023).

Triterpenoids and steroids. A total of 4 g of extract was mixed with 10 mL of diethyl ether. The diethyl ether-soluble fraction was separated and used for the Liebermann–Burchard test. Glacial acetic acid (10 mL) and concentrated sulfuric acid (H2SO4) were then added to this fraction, gently shaken, and left to stand for 10 minutes. The presence of steroids was indicated by the formation of a blue or green color, while the presence of triterpenoids was indicated by the formation of a red or purple color (Wutsqa, Suratman, & Sari, 2021).

Flavonoids

Qualitative flavonoid test. A total of 4 g of *Wojalaka* black rice extract was dissolved in 10 mL of boiling water. The mixture was then filtered using filter paper, and the resulting filtrate was used for the Willstätter test. A small amount of magnesium powder and 1 mL of concentrated HCl were added to the filtrate, and the mixture was shaken vigorously. The formation of a red, yellow, or orange color indicated a positive result for flavonoids (Thi et al. 2020).

Determination of total flavonoids. Total flavonoid content was determined using a spectrophotometric method with quercetin as the standard. A quercetin stock solution was prepared by dissolving 100 mg of quercetin in 100 mL of methanol to obtain a 1000 ppm stock solution. Standard solutions with concentrations of 6, 8, 10, 12, and 14 ppm were prepared from this stock solution. A 100 µL aliquot of each standard solution was mixed with 100 µL of 2% AlCl3 solution and 100 µL of 120 mM potassium acetate (KCH3COO) solution. The mixture was vortexed and incubated for 30 minutes at room temperature in the dark. Absorbance was measured at a wavelength of 415 nm using a UV-Vis spectrophotometer (Shimadzu UV-2250). A standard curve was constructed by plotting quercetin concentration against absorbance, and the resulting linear regression equation was used to determine the total flavonoid content of the samples.

For sample analysis, 15 mg of *Wojalaka* black rice extract was dissolved in 10 mL of methanol to obtain a stock solution with a concentration of 1500 ppm. The stock solution was then diluted 10-fold with methanol to obtain an intermediate solution with a concentration of 150 ppm. A 100 µL aliquot of this sample solution was mixed with 100 µL of 2% aluminum chloride (AlCl3) solution and 100 µL of 120 mM KCH3COO solution. The mixture was vortexed and incubated for 30 minutes at room temperature in the dark. Absorbance was measured at 415 nm using a UV-Vis spectrophotometer (Shimadzu UV-2250). Total flavonoid content was calculated based on the linear regression equation of the quercetin standard curve and expressed as mg Quercetin Equivalent (QE) per gram of extract. All measurements were performed in triplicate (Lisdiani, Susanto, and Manurung 2022).

Tannins

Qualitative tannin test. *Wojalaka* black rice extract was mixed with 10 mL of distilled

water, and the mixture was then filtered using filter paper. The resulting filtrate was diluted with distilled water as needed. The presence of tannins was tested by mixing 2 mL of filtrate with 1–2 drops of 1% ferric chloride (FeCl3) solution. The formation of a blackish-blue or green color indicated a positive result for tannins (Seck et al. 2021).

Determination of total tannins. Total tannin content was determined using the Folin-Ciocalteu method with tannic acid as the standard. A tannic acid stock solution was prepared by dissolving 100 mg of tannic acid in 100 mL of distilled water to obtain a 1000 ppm stock solution. A working solution of tannic acid at 100 ppm was prepared through a 10-fold dilution. Standard solutions with concentrations of 6, 8, 10, 12, and 14 ppm were then prepared from this working solution. For the 6 ppm standard, 60 µL of the 100 ppm tannic acid working solution was mixed with 40 µL of distilled water and 100 µL of Folin-Ciocalteu reagent, followed by the addition of 5% sodium carbonate (Na2CO3) solution to a final volume of 1 mL. Standards of other concentrations were prepared using the same principle with the appropriate volume of working solution. The mixtures were vortexed and incubated for 40 minutes at room temperature in the dark. Absorbance was measured at a wavelength of 760 nm using a UV-Vis spectrophotometer (Shimadzu UV-2250). A standard curve was constructed by plotting tannic acid concentration against absorbance and used to obtain a linear regression equation.

For sample analysis, 0.5 g of *Wojalaka* black rice extract was mixed with 50 mL of hot distilled water at 80 °C and left to stand for 30 minutes. The mixture was then filtered, and the filtrate was used for analysis. A 100 µL aliquot of the filtrate was mixed with 100 µL of Folin-Ciocalteu reagent and 800 µL of 5% Na2CO3 solution. The mixture was vortexed and incubated for 40 minutes at room temperature in the dark. Absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Shimadzu UV-2250). Total tannin content was calculated based on the linear regression equation of the tannic acid standard curve and expressed as mg Tannic Acid Equivalent (TAE) per gram of extract. All measurements were performed in triplicate (Sharma et al. 2021).

Phenolics

Qualitative phenolic test. *Wojalaka* black rice extract was mixed with 10 mL of hot water, and the mixture was then filtered using filter paper. A few drops of 1% FeCl3 solution were added to the resulting filtrate. The formation of a green, blue, or purple color indicated a positive result for phenolic compounds.

Determination of total phenolic content. Total phenolic content was determined using the Folin-Ciocalteu method with gallic acid as the standard. A gallic acid standard solution was used to construct a calibration curve at various concentrations. A gallic acid stock solution was prepared by dissolving 100 mg of gallic acid in distilled water to a final volume of 100 mL. A working solution was then prepared through dilution to obtain a concentration of 1 mg/mL. To prepare the 10 ppm standard solution, 10 µL of the working solution was mixed with 90 µL of distilled water, 100 µL of Folin-Ciocalteu reagent, and 5% Na2CO3 solution to a final volume of 1 mL. Standard solutions with concentrations of 20, 40, 60, and 80 ppm were prepared using the appropriate volume of working solution and the same procedure. All standard solutions were shaken and incubated for 60 minutes, and their absorbance was then measured at a wavelength of 760 nm using a Shimadzu UV-Vis spectrophotometer (UV-2250). A standard curve was constructed by plotting gallic acid concentration against absorbance and used to obtain a linear regression equation.

For sample analysis, 20 mg of *Wojalaka* black rice extract was dissolved in 10 mL of methanol, then vortexed and filtered to obtain a sample stock solution with a concentration of 2000 ppm. A 1 mL aliquot of the sample stock solution was diluted with 19 mL of methanol to obtain a working solution with a concentration of 100 ppm. A 100 µL aliquot of the sample working solution was mixed with 100 µL of Folin-Ciocalteu reagent and 800 µL of 5% Na2CO3 solution. The mixture was vortexed and incubated for 30 minutes at room temperature. Absorbance was measured at a wavelength of 760 nm using a UV-Vis spectrophotometer (Shimadzu UV-2250). Measurements were performed in triplicate (Sharma et al., 2021).

Antioxidant Activity Test of Black Rice Extract

The antioxidant activity of *Wojalaka* black rice extract was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. A DPPH stock solution was prepared by dissolving 20 mg of DPPH (MW 394.326 g/mol) in 70% methanol to a final volume of 100 mL, yielding a DPPH solution at 0.507 mM (200 ppm). A DPPH working solution with a concentration of 40 ppm was then prepared by

diluting the stock solution with 70% methanol.

To determine the maximum wavelength of DPPH and assess possible interference from phenolic and flavonoid compounds, a spectral scan of the 40 ppm DPPH solution was performed over 400–800 nm. The maximum wavelength was determined based on the highest absorbance. Subsequently, the absorbance stability of the DPPH solution at the maximum wavelength was monitored for 0–30 minutes to determine an appropriate measurement time. Based on the measurement results, the maximum wavelength used for analysis was 517 nm.

To determine the antioxidant activity of *Wojalaka* black rice extract, a 1000 ppm stock solution was prepared by dissolving 25 mg of extract in 70% methanol to a final volume of 25 mL. Sample solutions with final concentrations of 50, 100, 200, and 300 ppm were prepared by taking 1.25, 2.5, 5.0, and 7.5 mL of the stock solution, respectively, and diluting to a final volume of 25 mL with 70% methanol. Each sample solution was then mixed with 0.5 mL of the 40 ppm DPPH working solution and diluted with 70% methanol to a final volume of 25 mL. The mixture was incubated for 30 minutes at room temperature (25 °C), then its absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-2250).

Ascorbic acid (vitamin C; C6H8O6) served as the positive control. An ascorbic acid stock solution with a concentration of 1000 ppm was prepared by dissolving 25 mg of ascorbic acid in 25 mL of 70% methanol. Working solutions with final concentrations of 2, 4, 6, and 8 ppm were prepared from this stock solution. Each working solution was mixed with 0.5 mL of the 40 ppm DPPH working solution and diluted with 70% methanol to a final volume of 25 mL. The mixture was incubated for 30 minutes at room temperature (25 °C), then its absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-2250) (Baliyan et al., 2022).

DPPH free-radical-scavenging activity was determined by the percentage inhibition of the DPPH radical. The IC50 value was defined as the extract concentration required to produce 50% inhibition of the DPPH radical. The percentage of inhibition was calculated using Equation (1).

% inhibition = (blank abs − sample abs) / blank abs × 100% ... (1)

A curve relating sample concentration to the percentage of inhibition was then constructed to determine the linear regression equation. The IC_{50} value was obtained from this regression equation by determining the sample concentration that produced 50% inhibition. The Antioxidant Activity Index (AAI) was then calculated using Equation (2).

AAI = DPPH concentration (ppm) / IC_{50} of the sample (ppm) ... (2)

Distillation of Citronella Essential Oil

Citronella essential oil was obtained by steam distillation. A total of 4 kg of freshly harvested citronella leaves were first dried in a shaded area for 12–24 hours until reaching a semi-dry condition. The citronella leaves were then placed in a distillation kettle filled with water to a predetermined level. The kettle was heated to generate steam. During the initial heating stage, the steam-flow valve from the kettle to the material chamber was kept closed. Once the steam pressure reached the level indicated by the kettle's pressure gauge, the steam-flow valve was opened, allowing steam to flow into the chamber containing the citronella leaves (Elwina, Rihayat, and Hafid 2024). The water vapor carrying the essential oil was then channeled to a condenser. Inside the condenser, the mixture of water vapor and essential oil was cooled until it condensed into a liquid phase and was subsequently collected in a separator. The mixture of essential oil and hydrosol was then separated using a dropper pipette and filtered through Monel cloth. The essential oil obtained was collected in glass bottles. The essential oil yield was calculated using Equation (3).

Yield = (weight of oil produced (g) / weight of sample (g)) × 100% ... (3)

Formulation of a Facial Mask Combining Black Rice and Citronella Essential Oil

The facial mask developed in this study was formulated using black rice as the main active ingredient and citronella essential oil as an additional active ingredient. Citronella essential oil was added because of its potential antimicrobial activity. Three mask formulations were prepared with varying concentrations of black rice: F1 at 30% (w/w), F2 at 40% (w/w), and F3 at 50% (w/w). Each formulation

Table 1. Composition of the black-rice-based facial mask formulations

Component	Function	F1 (% w/w)	F2 (% w/w)	F3 (% w/w)
Black rice	Active ingredient	30.0	40.0	50.0
Citronella essential oil	Active ingredient	0.3	0.3	0.3

HPMC	Thickener	0.1	0.1	0.1
TEA	pH adjuster	0.1	0.1	0.1
Distilled water	Solvent	69.5	59.5	49.5
Final weight		100	100	100

contained 0.3% (w/w) citronella essential oil, 0.1% (w/w) HPMC as a thickening agent, and 0.1% (w/w) TEA as a pH adjuster. Distilled water was added to reach a final weight of 100 g. The composition of each formulation is presented in Table 1 (Yap et al., 2024).

The mask formulation process began with preparing the HPMC and TEA base. A total of 100 mg of HPMC was dispersed in a portion of hot distilled water (approximately 30 mL) and stirred until fully hydrated. Next, 100 mg of TEA was incorporated until homogeneous. The active ingredients (black rice and citronella essential oil) were subsequently incorporated, and the remaining distilled water was added quantitatively to achieve a final formulation weight of 100 g in accordance with Table 1. After the mixture reached room temperature, black rice was added according to each formulation (30%, 40%, or 50% w/w) and stirred until homogeneous. Subsequently, 300 mg of citronella essential oil was added to each formulation and mixed until homogeneous. Distilled water was then added according to each formulation's composition. The resulting product was a paste-form mask, which was then used for evaluation of its physical characteristics and application (Nemati et al., 2024).

Facial Mask Quality Testing: Organoleptic Properties, Homogeneity, Spreadability, and pH

Physical quality testing was carried out to evaluate the characteristics of the three facial mask formulations based on black rice and citronella essential oil. The parameters observed included organoleptic properties, homogeneity, spreadability, and pH. This evaluation was performed to determine the physical characteristics of each formulation prior to antioxidant activity testing. Organoleptic testing was carried out by observing the physical characteristics of each mask formulation, including color, aroma, and texture. This observation was used to provide an initial description of the physical characteristics of each formulation. Homogeneity testing was then carried out by placing 0.1 g of each formulation on a glass slide and observing it visually. A formulation was considered homogeneous if its components were evenly distributed and no separation or particle aggregation was observed. Spreadability testing was carried out by placing 0.5 g of each formulation on a 20 cm × 20 cm glass plate. The sample was then covered with a second glass plate of the same size and loaded with a weight of 125 g on top. After 1 minute, the diameter of mask spreading was measured. A spreadability considered suitable for facial application was 5–7 cm (Syakri et al., 2021). pH was measured to evaluate the formulation's suitability for skin pH conditions. The mask pH was measured using a pH meter calibrated with buffer solutions of pH 4 and pH 7. The electrode was thoroughly cleaned with distilled water before and after each measurement. The pH value of the formulation was then compared with the range of 4.5–6.5 used as the acceptance criterion in this study.

Skin Irritation and Allergic Sensitivity Testing

Before testing, prospective subjects received information about the test procedure, potential risks, and product benefits, and then provided written informed consent. The test material was prepared at a predetermined concentration and applied to a clean, hairless area of skin using the human patch test method. The sample was applied to the back area or the inner forearm of the subject. After 24 hours, the application area was observed to evaluate possible irritation or sensitivity reactions, including erythema (redness), edema (swelling), and itching. Observations were repeated 24 hours after the first observation to detect any possible delayed reactions. The observation results were recorded based on the presence and intensity of the observed skin reactions.

***In Vivo* Testing of Facial Mask Formulation Use on Subjects**

An *in vivo* test was conducted on volunteers (subjects) to evaluate changes in skin condition after using the facial mask. A total of 15 women aged 15 – 40 years were enrolled as subjects. Inclusion criteria included women with acne-prone skin conditions who had not undergone facial treatments such as facials or peeling in the previous three months and who did not use any other cosmetic products during the mask-use period. Before mask application, the subjects' faces were cleansed using a mild soap, then rinsed with water and dried with a towel. The mask was

applied evenly to the face using a mask brush and left to dry for approximately 15 – 30 minutes. After the mask had dried, the face was cleansed with warm water until all mask residue was removed (Wang et al., 2024). Treatment was given for six weeks. The subjects' facial condition was documented using a DSLR camera at weeks 0, 2, 4, and 6. The condition at week 0 was used as the baseline for comparison. Changes in skin condition at weeks 2, 4, and 6 were then compared with the baseline condition at week 0.

Collection and Analysis of Subject Photographic Data

Photographs of the subjects taken at weeks 0, 2, 4, and 6 were used to evaluate changes in skin condition during the period of mask use. The photographs were randomized before assessment by five observers. The randomization process was carried out by another party (person 2) based on a design determined by the researcher (person 1). The randomized photographs were then observed by five observers. Assessment was carried out by observing the presence or absence of the most visible change compared with the baseline condition. The observation results from the five observers were then compiled and analyzed qualitatively. This is far more consistent with the *in vivo* method described above (Draelos et al., 2021).

Hedonic Test

The hedonic test was conducted to determine the subjects' level of preference for the facial mask used. The test was carried out on 15 subjects using three assessment categories: 1 = dislike (D), 2 = like (L), and 3 = strongly like (SL). Each subject rated the mask preparation based on their level of preference. The assessment results were recorded in the D, L, and SL categories, and the percentage of each category was then calculated based on the number of subjects who rated each category (Colbert et al., 2025).

Antioxidant Activity Test of the Facial Mask Formulation

Based on the physical quality test results, the hedonic test, and subject evaluations, the formulation that met the criteria was used for antioxidant activity testing using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. This testing was performed to evaluate the free-radical-scavenging activity of the black rice and citronella essential oil combination formulation. The selected formulation sample was prepared as a stock solution at 1000 ppm by dissolving 25 mg of mask paste in 70% methanol to a final volume of 25 mL. From this stock solution, working solutions with final concentrations of 50, 100, 200, and 300 ppm were prepared by taking 1.25, 2.50, 5.00, and 7.50 mL of the stock solution, respectively, and diluting to a final volume of 25 mL with 70% methanol. Each sample working solution was then mixed with 0.5 mL of the 40 ppm DPPH working solution and diluted with 70% methanol to a final volume of 25 mL. The mixture was homogenized and incubated in the dark for 30 minutes at room temperature (25 °C). Absorbance was then measured at a wavelength of 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-2250) (Chanthathamrongsiri et al., 2022).

Ascorbic acid (vitamin C) served as the positive control. An ascorbic acid stock solution with a concentration of 1000 ppm was prepared by dissolving 25 mg of ascorbic acid in 25 mL of 70% methanol. Working solutions with concentrations of 2, 4, 6, and 8 ppm were prepared by diluting the stock solution. Each working solution was then tested using the same DPPH procedure, i.e., mixed with 0.5 mL of the 40 ppm DPPH working solution and diluted with 70% methanol to a final volume of 25 mL. The mixture was incubated for 30 minutes in the dark at room temperature before its absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-2250). Antioxidant activity was determined based on the percentage of DPPH radical inhibition. The IC50 value was defined as the sample concentration required to produce 50% inhibition of the DPPH radical. The percentage of inhibition was calculated using Equation (1). A curve relating sample concentration (ppm) to percentage of inhibition was constructed to obtain the linear regression equation $y = bX + a$, where y is the percentage of inhibition and x is the sample concentration. The IC50 value was determined by substituting $y = 50$ into the linear regression equation. The Antioxidant Activity Index (AAI) was then calculated using Equation (2) (Arivo et al., 2023).

Screening of Anaerobic Antimicrobial Activity against *Cutibacterium acnes*

The resistance-to-microbial-growth test was performed using the well diffusion method on blood agar medium, applied to the formulation selected based on the results of the quality test, hedonic test, and subject evaluation. The treatments consisted of black rice (P1), citronella essential oil (P2), and the selected mask formulation (P3). Distilled water was used as the negative control (−), while the antibiotic clindamycin was used as the positive

control (+) (Sharma & Ruhela, 2026).

Five wells with a diameter of 6 mm were made in each Petri dish using a sterile cork borer. The distance between wells was set at a minimum of 24 mm to prevent overlapping of inhibition zones. Each well was filled with 50 µL of test sample. The plates were then incubated at 37 °C for 48–72 hours under anaerobic conditions using an anaerobic jar equipped with a gas pack.

Testing was performed to observe microbial growth during the incubation period and to evaluate the sample's ability to inhibit microbial growth, as indicated by the formation of an inhibition zone around the well. Inhibition-zone measurement was performed using a digital caliper in three measurement directions, namely 0°, 120°, and 240°, for each zone. The inhibition-zone diameter was determined based on the diameter of the clear zone formed around the well, minus the well diameter (6 mm).

Each treatment was repeated seven times. All observation results were documented using a DSLR camera, recording the sample code, observation time, inhibition-zone diameter in the three measurement directions, the average value, and special notes if any anomalies or other important findings were observed. After incubation was complete, the number of colonies growing on the agar medium was counted as part of the microbial growth evaluations (Kebede & Shibeshi, 2022).

In Vitro Antimicrobial Activity Test Method against *Propionibacterium acnes*

In vitro testing was conducted to determine the antimicrobial activity of the selected formulation against the bacterium *Propionibacterium acnes* (Pacheco et al., 2025). Testing was performed using the well diffusion method, involving all treatments, namely P1, P2, and P3. A pure culture of *P. acnes* was inoculated into Mueller-Hinton broth and incubated under anaerobic conditions. Once the bacterial suspension reached the desired turbidity level, it was inoculated evenly onto the surface of Mueller-Hinton agar medium (Salar-Vidal et al., 2022). Wells were then made in the agar medium, and each well was filled with the test sample as well as the positive and negative controls.

After the incubation process was complete, the diameter of the inhibition zone formed around each well was measured. The inhibition-zone diameter was then divided by two to obtain the radius. This radius value was used to calculate the inhibition-zone area using the circular area equation, $\pi r2$, where π = 3.14159 and r is the radius of the inhibition zone. The inhibition-zone area of each treatment was then compared with the positive control to evaluate its antimicrobial activity. In addition to the well diffusion test, Time-Kill Kinetics measurements were performed to evaluate changes in bacterial numbers over a certain period following treatment. Samples were taken at time intervals of 0, 15, 30, 60, and 120 minutes after treatment, and the number of bacterial colonies was then counted at each observation time interval (Rathnayake et al., 2020).

Data Analysis

Inhibition-zone diameter data obtained from antimicrobial activity testing were statistically analyzed to determine differences in antimicrobial activity between treatments. The data were first tested for normality using the Shapiro–Wilk test to determine whether the data were normally distributed. Next, the homogeneity of variance was tested before parametric analysis was performed. If the data met the assumptions of normality and homogeneity of variance, the analysis was continued using analysis of variance (ANOVA) at a significance level of 1% ($p < 0.01$). If the ANOVA results showed significant differences between treatments, the analysis was continued with Duncan's Multiple Range Test (DMRT) at a 99% confidence level to determine differences between treatments. Observational data on the subjects were analyzed according to the type of data obtained. Data on changes in skin condition obtained through visual observation and photographic documentation were analyzed descriptively/qualitatively by comparing conditions at weeks 0, 2, 4, and 6. Hedonic test data were analyzed based on the distribution and percentage of the subjects' level of preference for the tested formulations (Pereira et al., 2023).

■ RESULT AND DISCUSSION

Phytochemical Test Results

Qualitative phytochemical testing was carried out to identify the groups of secondary metabolite compounds present in *Wojalaka* black rice extract. The test results showed that the extract gave positive results for flavonoids, phenolics, tannins, saponins, triterpenoids, and alkaloids, while steroids were not detected. Subsequently, quantitative analysis was carried out on flavonoids, phenolics, and tannins because these three compound groups are known to have antioxidant activity and have the potential to contribute to the biological activity of the extract (Medda et al., 2021). The phytochemical test results are presented in Table 2.

Table 2. Phytochemical test results of Wojalaka black rice extract

Compound Class	Reagent	Observed Result	Test Result	Total Content
Flavonoids	Mg and HCl	Orange color	+	332.58 mg/100 g QE
Phenolics	1% $FeCl_3$	Blackish-blue color	+	49.33 mg/100 g GAE
Tannins	1% $FeCl_3$	Blackish-blue color	+	137.89 mg/100 g TAE
Saponins	Concentrated HCl	Persistent foam	+	–
Triterpenoids	Liebermann–Burchard	Brown ring	+	–
Steroids	Liebermann–Burchard	Colorless	–	–
Alkaloids	Mayer/Dragendorff	Precipitate	+	–

Note: QE = Quercetin Equivalent; GAE = Gallic Acid Equivalent; TAE = Tannic Acid Equivalent

The qualitative test results showed the presence of flavonoids in the *Wojalaka* black rice extract, indicated by the formation of an orange color after adding magnesium and HCl. This result indicates that the extract contains compounds with structural characteristics capable of giving a positive response in the flavonoid test. Quantitative analysis using a spectrophotometric method with quercetin as the standard showed that the total flavonoid content of the extract was 332.58 mg/100 g QE. This value indicates that flavonoids are one of the fairly dominant compound groups in the analyzed extract. The presence of flavonoids, including the anthocyanin group commonly found in black rice, may be one of the factors contributing to the extract's free-radical-scavenging ability (Palungwachira et al., 2019). This result forms an important basis for testing the antioxidant activity of the extract and mask formulation in the subsequent stage. Thus, the presence of flavonoids was confirmed qualitatively and supported by quantitative results. This value can then be compared with the DPPH test results to determine whether flavonoid content relates to the free-radical-scavenging activity obtained.

Phenolic testing gave a positive result, indicated by the formation of a blackish-blue color after the addition of FeCl3. This reaction indicates the presence of phenolic compounds capable of interacting with Fe3+ ions to form a colored complex. Based on analysis using the Folin–Ciocalteu method with gallic acid as the standard, the total phenolic content of the *Wojalaka* black rice extract was 49.33 mg/100 g GAE. Phenolic compounds are known to be capable of donating electrons or hydrogen atoms, allowing them to play a role in the free-radical-scavenging mechanism (Ahmad et al., 2025). Thus, the phenolic content obtained provides a basis for suspecting a contribution of phenolic compounds to the extract's antioxidant activity. This relationship needs to be further confirmed through the DPPH test results, so that the discussion is based not only on the presence of the compounds but also on the measured antioxidant activity.

Wojalaka black rice extract gave a positive result for tannins, indicated by the formation of a blackish-blue color after the addition of FeCl3. This result indicates the presence of tannin compounds in the extract. Quantitative analysis using the Folin–Ciocalteu method with tannic acid as the standard showed a total tannin content of 137.89 mg/100 g TAE. The presence of tannins in the extract may contribute to the extract's biological activity, including its antioxidant activity, because these polyphenolic compounds have hydroxyl groups capable of reacting with free radicals (Fraga-Corral et al., 2021). This tannin content value, together with the flavonoid and phenolic content, forms the initial chemical characterization of the *Wojalaka* black rice extract. These three parameters are subsequently important to relate to the antioxidant activity test results, in order to determine whether the extract with these bioactive compound contents shows a corresponding free-radical-scavenging ability.

The qualitative test results also showed the presence of saponins, triterpenoids, and alkaloids in the *Wojalaka* black rice extract, while steroids were not detected. Saponins were indicated by the formation of persistent foam, while triterpenoids gave a positive response in the Liebermann–Burchard test. *Alkaloids* also showed a positive result based on the formation of a precipitate in testing with alkaloid reagents. The presence of these compound groups indicates that *Wojalaka* black rice extract has a diverse secondary metabolite composition. Overall, the phytochemical screening results show that *Wojalaka* black rice extract contains several groups of secondary metabolites, with flavonoids, phenolics, and tannins being the groups subsequently analyzed quantitatively. The total flavonoid content was the highest value among the three parameters

measured, at 332.58 mg/100 g QE, followed by tannins at 137.89 mg/100 g TAE and phenolics at 49.33 mg/100 g GAE.

These results provide a chemical basis for proceeding with antioxidant activity testing using the DPPH method. If the extract shows good free-radical-scavenging activity, the presence of flavonoids, phenolics, and tannins can be discussed as factors that may contribute to this activity. However, the cause-and-effect relationship between the content of each compound and antioxidant activity cannot be determined solely based on these phytochemical results. In addition, the presence of several secondary metabolites detected qualitatively provides a basis for evaluating the biological potential of the mask formulation in the subsequent stage. Therefore, these phytochemical results serve as baseline data before discussing antioxidant activity, antimicrobial activity, and formulation evaluation in subjects.

Antioxidant Activity Test Results of Black Rice Extract

The antioxidant activity of *Wojalaka* black rice extract was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. Linear regression analysis between extract concentration and the percentage of DPPH radical inhibition produced the equation y = 1201.51x + 4.87013, with a coefficient of determination (R^2) of 0.98198, as shown in Figure 1. The test results showed that inhibition increased with increasing extract concentration. The inhibition percentages were 0%, 29.358%, 42.752%, 61.468%, and 77.248%. Based on this regression equation, the IC50 value of

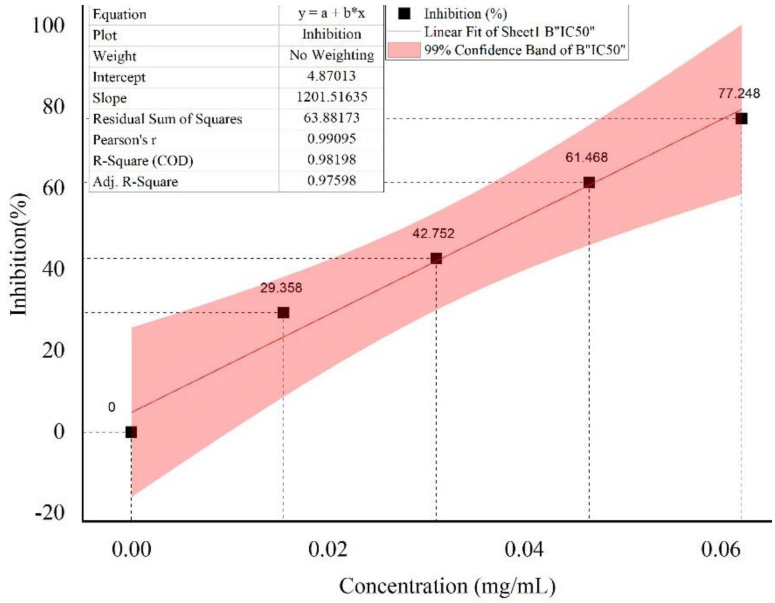


Figure 1. Linear regression graph of the antioxidant activity of Wojalaka black rice extract

Wojalaka black rice extract was 37.561 mg/L. The Antioxidant Activity Index (AAI) value obtained was 5.32.

YThe DPPH method was used to evaluate the extract's ability to scavenge free radicals. The results showed that *Wojalaka* black rice extract exhibited increasing DPPH-radical-scavenging activity with increasing extract concentration. This is reflected in the increase in inhibition percentage from 0% to 77.248% over the concentration range used. An R^2 value of 0.98198 indicates that the relationship between extract concentration and percentage of inhibition, within the tested concentration range, fits the linear regression model very well. An IC50 value of 37.561 mg/L indicates that *Wojalaka* black rice extract has a high capacity to scavenge DPPH radicals. A lower IC50 value means a lower extract concentration is needed to achieve 50% inhibition of the DPPH radical, indicating higher radical-scavenging activity. Based on the classification used in this study, an IC50 value below 50 mg/L is included in the very strong antioxidant activity category (Akullo et al., 2023).

An AAI value of 5.32 also indicates very strong antioxidant activity based on the AAI classification used in this study (Sulistiarini et al., 2021). This result reinforces the finding from the IC50 value that *Wojalaka* black rice extract has a high radical-scavenging capacity under the DPPH test conditions. These antioxidant activity results can be related to the phytochemical screening results, which showed

the presence of flavonoids, phenolics, and tannins in *Wojalaka* black rice extract. A total flavonoid content of 332.58 mg/100 g QE, phenolics of 49.33 mg/100 g GAE, and tannins of 137.89 mg/100 g TAE provide the basis that the extract contains phenolic compound groups with the potential to contribute to free-radical-scavenging activity. Thus, the phytochemical and DPPH test results together indicate that *Wojalaka* black rice extract has a secondary metabolite content relevant to antioxidant activity and shows high radical-scavenging activity. This result serves as the basis for the next stage of research, namely formulating black rice extract with citronella essential oil and evaluating the formulation's antioxidant activity.

Facial Mask Quality Test Results: Organoleptic Properties, Homogeneity, pH, and Spreadability

The black rice extract mask formulations showed different physical characteristics among the three formulations (F1, F2, and F3). Based on organoleptic observation, F1 had a liquid consistency, F2 had a thick but still somewhat runny consistency, while F3 had a thicker consistency. Color intensity also increased with increasing black rice extract concentration, ranging from light purple in F1 and F2 to dark purple in F3. These differences in organoleptic characteristics are presented in Table 3.

Based on the homogeneity test results, only F3 showed a homogeneous component distribution, while F1 and F2 still showed non-uniformity. The formulations' pH values were in the range of 5.0 – 6.0, while the spreadability diameter was in the range of 5.0–5.5 cm. The homogeneity, pH, and spreadability test results are presented in Table 4.

Table 3. Organoleptic test results of the facial mask

Formulation	Consistency	Color	Aroma
F1	Liquid	Light purple	Aroma of citronella essential oil and black rice
F2	Thick but somewhat runny	Light purple	Aroma of citronella essential oil and black rice
F3	Thick	Dark purple	Aroma of citronella essential oil and black rice

Note: F1 = mask formulation 1; F2 = mask formulation 2; F3 = mask formulation 3.

The differences in physical characteristics observed among the three formulations may be related to the difference in black rice extract concentration used. The increase in extract concentration from F1 to F3 was accompanied by an increase in the viscosity and color intensity of the formulation. F3, which contained the highest extract concentration, showed the thickest consistency and the darkest color. This result indicates that extract concentration is one of the factors influencing the physical characteristics of the mask (Magwere et al., 2026). The homogeneity test results showed that F3 was the only formulation classified as homogeneous, while F1 and F2 were not homogeneous. Homogeneity is an important parameter in topical preparations because it relates to the uniformity of component distribution within the formulation (Manian et al., 2022). The non-uniformity in F1 and F2 may indicate that the formulation components had not been evenly dispersed or that the mixing process had not produced a sufficiently stable system. Therefore, homogeneity is an important consideration in determining the formulation to use in the subsequent stage of research.

Table 4. Homogeneity, pH, and spreadability test results

Formulation	Homogeneity	pH	Spreadability (cm)
F1	Not homogeneous	5.0	5.5
F2	Not homogeneous	6.0	5.3
F3	Homogeneous	6.0	5.0

The pH values of the three formulations ranged from 5.0 to 6.0. This range falls within the range established in this study as suitable for skin application, namely 4.5–6.5. Thus, based on pH, all three formulations met the established criteria (Slavkova et al., 2023). The difference in pH values between formulations was relatively small, with F1 having a pH of 5.0 and F2 and F3 having a pH of 6.0. This result indicates that increasing the extract concentration within the formulation range used did not result in a large change in pH. The spreadability test results showed spreading

diameters of 5.5 cm for F1, 5.3 cm for F2, and 5.0 cm for F3. All these values fall within the range of 5 – 7 cm used as the spreadability criterion in this study. Thus, all three formulations met the spreadability criterion (Al-Barghouthy et al., 2025). The decrease in spreadability diameter from F1 to F3 is consistent with the increase in formulation viscosity. The thicker F3 formulation produced a slightly smaller spreading diameter compared with F1 and F2.

Overall, the physical evaluation results show that F3 has an advantage in the homogeneity parameter, while all three formulations met the established pH and spreadability criteria. Therefore, based on the physical quality test results alone, F3 is the candidate formulation that best meets the quality parameters, although determination of the best formulation still needs to take into account the results of further testing, including irritation testing, hedonic testing, evaluation by the subjects, and the biological activity parameters used in the study.

Mask Formulation Most Preferred by the Subjects

Based on the hedonic test results, formulation F3 was the formulation most preferred by the subjects. This preference is related to the physical characteristics of F3, which had a thicker, more homogeneous consistency than F1 and F2. The earlier physical quality test results showed that F3 was the only formulation classified as homogeneous, while F1 and F2 still showed non-uniformity. In addition, F3 had a pH value of 6.0 and a spreadability of 5.0 cm, so it still met the quality criteria established in this study.

The high preference for F3 indicates that the preparation's physical characteristics influenced subjects' acceptance of the mask. The thicker consistency of F3 compared with F1 and F2 still allowed the mask to be applied well to the facial surface. This result supports the selection of F3 as the formulation used for further testing.

In addition to physical characteristics and level of preference, F3 also showed antioxidant activity that needs to be considered in formulation selection. The higher black rice extract content in F3, namely 50%, is also one of the characteristics that distinguishes F3 from F1 and F2. Based on the earlier phytochemical results, *Wojalaka* black rice extract contains flavonoids, phenolics, and tannins, with total contents of 332.58 mg/100 g QE, 49.33 mg/100 g GAE, and 137.89 mg/100 g TAE, respectively. The DPPH test results for black rice extract also showed high antioxidant activity, with an extract IC50 value of 37.561 mg/L. Thus, the higher extract content in F3 provides a basis for evaluating whether the increased amount of extract is also related to the formulation's antioxidant activity (Al-Barghouthy et al., 2025).

Effect of Mask Use on the Subjects' Skin Condition

After physical quality testing and the hedonic test, the three mask formulations were applied to the subjects to observe changes in skin condition over a six-week period. A total of 15 subjects were divided into three groups of five subjects each, receiving treatment F1, F2, or F3. Observations were made at the baseline

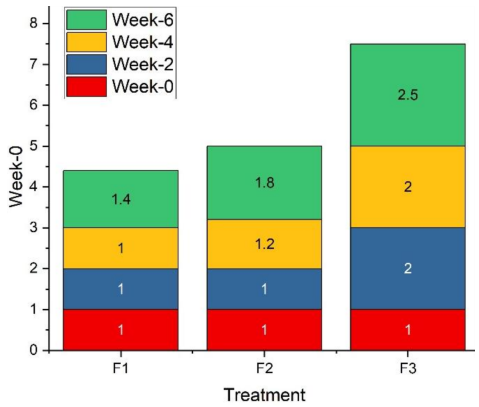


Figure 2. Average observation scores for changes in the subjects' skin condition during mask use. Note:

F1	F2	F3
Wrinkles	Dark spots fading	Firm skin
Dry	Skin appears moist	Clean, bright skin
Dark spots/blemishes	Skin appears clean and bright	Soft, smooth skin
Dull	Skin slightly firmer	Moist, healthy skin
Acne	Acne drying up	Acne fading

condition (week 0), and then at weeks 2, 4, and 6. Changes in skin condition were documented using photographs and subsequently assessed qualitatively based on established observation criteria (Marin-Gomez et al., 2020). The observation results are shown in Figure 2.

Based on the observation results, the baseline condition at week 0 was used as the baseline before mask use. At baseline, some subjects showed skin characteristics such as wrinkles, dryness, dullness, dark spots or blemishes, and acne. Changes in skin condition began to be observed in some subjects after two weeks of mask use and continued to be observed up to week 6. In the F3 group, visual changes began to appear at week 2. Based on the observation data, the average F3 score increased from 1.0 at week 0 to 2.0 at weeks 2 and 4, then to 2.6 at week 6. This increase in score indicates a change in skin condition based on the assessment criteria used in the study. By the end of the observation period, the F3 group showed skin that appeared firmer, cleaner, brighter, smoother, softer, and more moisturized, along with reduced blemishes or acne in some subjects. In the F2 group, changes in skin condition began to be observed at week 4. At that stage, 2 of 5 subjects showed visual changes, including fading dark spots, skin appearing more moisturized, cleaner and brighter, and appearing slightly firmer. By week 6, the number of subjects showing changes increased to 3 of 5. This finding indicates that visual changes in the F2 group appeared later than in the F3 group. Meanwhile, in the F1 group, the visual changes observed were relatively more limited. Based on the results, 1 of 5 subjects showed changes at week 6, mainly skin appearing slightly brighter and a reduction in the appearance of acne. Thus, based on visual observation over six weeks, the F3 group showed more and earlier changes than F1 and F2. Overall, the results show a tendency toward more pronounced changes in skin condition in the F3 group during the mask-use period. This finding may be related to F3's characteristics, which had the highest black rice extract concentration (50%), as well as the physical quality test results showing that F3 was a homogeneous formulation with a pH of 6.0 and a spreadability of 5.0 cm.

Antioxidant Activity Test Results of the Facial Mask Formulation

Based on the physical quality test results, formulation F3 was selected for antioxidant activity testing because it showed the best physical characteristics compared with F1 and F2. F3 had homogeneous properties, a pH of 6.0, and a spreadability of 5.0 cm, thus meeting the quality criteria established in this study. Antioxidant activity testing was then carried out on F3 using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The test results showed that formulation F3 had DPPH-radical-scavenging activity that increased with increasing sample concentration. Linear regression analysis produced the linear regression equation y = 917.2604x + 5.1454, with a coefficient of determination (R^2) of 0.9640 (Pearson's r = 0.9819). An R^2 value close to 1 indicates a strong linear relationship between sample concentration and the

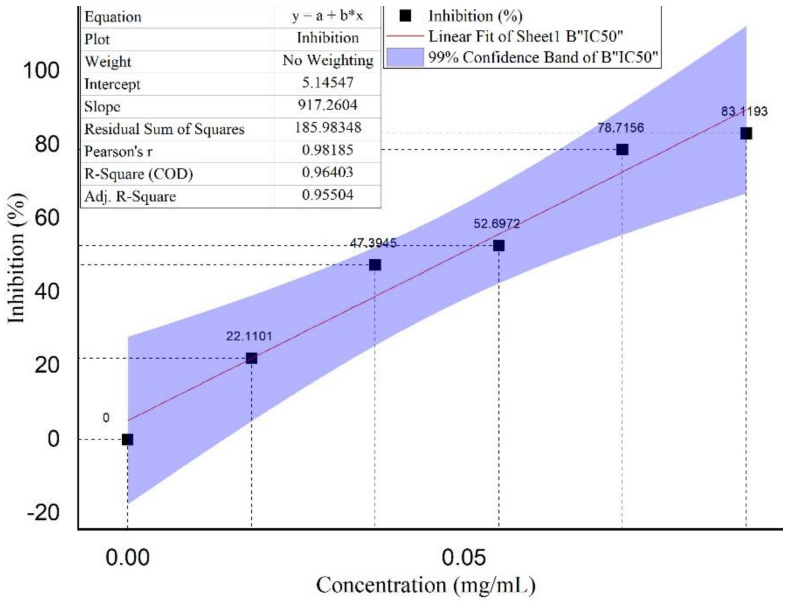


Figure 3. Linear regression graph of the antioxidant activity of formulation F3

percentage of DPPH radical inhibition within the tested concentration range. The percentage of inhibition increased to 83.1193% at the highest concentration used.

Based on this regression equation, the IC50 value of formulation F3 was 48.9 mg/L. The IC50 value indicates the sample concentration required to produce 50% inhibition of the DPPH radical. The lower the IC50 value, the higher the sample's ability to scavenge free radicals. Based on the classification used in this study, an IC50 value below 50 mg/L falls into the very strong antioxidant activity category. Thus, formulation F3 showed very strong antioxidant activity. An Antioxidant Activity Index (AAI) value of 4.08 also indicates very strong antioxidant activity based on the criterion of AAI > 2.0 (Sulistiarini et al., 2021). This result supports the DPPH test findings, showing that formulation F3 has a good ability to scavenge free radicals. This activity likely relates to bioactive compounds in the black rice extract, particularly the phenolic and flavonoid compounds identified during the phytochemical characterization stage.

In this study, F3 contained 50% black rice extract, the highest extract concentration compared with F1 (30%) and F2 (40%). Earlier tests on black rice extract also showed strong antioxidant activity, with an IC50 value of 37.561 mg/L. Therefore, the antioxidant activity observed in F3 can be attributed to antioxidant compounds in the black rice extract. Overall, this result is one basis for selecting F3 for the next stage of research, along with the physical quality and hedonic test results. However, in vitro antioxidant activity does not directly prove antioxidant effectiveness on human skin. Therefore, the DPPH test results should be interpreted alongside observations in subjects and the antimicrobial testing conducted in the subsequent stage.

Screening of Anaerobic Antimicrobial Activity against *Cutibacterium acnes*

The assay was performed using a standardized bacterial suspension of *Cutibacterium acnes* (ATCC 6919 / clinical isolate) adjusted to a 0.5 McFarland standard turbidity (1.5 x 108 CFU/mL) prior to lawn inoculation onto blood agar plates. Antimicrobial resistance testing was carried out on five treatments: the positive control (K+, clindamycin), the negative control (K−, deionized water), black rice extract (P1), citronella essential oil (P2), and the selected mask formulation (F3) containing black rice extract and citronella essential oil (P3). Each treatment was applied to wells in the agar medium and incubated for 24 hours. Testing

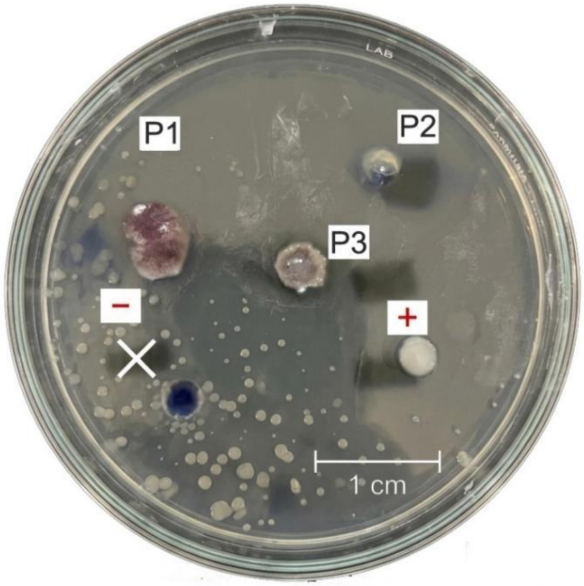


Figure 4. Antimicrobial resistance test results on agar medium

was carried out with seven replications to obtain more representative data. One of the test results is shown in Figure 1.

Antimicrobial activity was indicated by the formation of a clear zone around the well. Inhibition-zone diameter was used as an indicator of each treatment's ability to inhibit the growth of microorganisms under the test conditions. The larger the inhibition-zone diameter, the greater the sample's ability to

Table 5. Antimicrobial activity analysis results based on inhibition-zone diameter

Code	Treatment Description	Mean Inhibition Zone (mm) ± SD	Relative Antimicrobial Activity
P1	Wojalaka Black Rice Extract	0.00 ± 0.00	Inactive
P2	Citronella Essential Oil	36.31 ± 1.45	Strong
P3	Mask Formulation F3	18.61 ± 6.21	Very Strong / Synergistic
K+	Clindamycin	25.27 ± 0.53	Positive Control (Optimal)
K-	Deionized / Distilled Water	0.00 ± 0.00	Negative Control (Inactive)

Note: Values are presented as mean ± standard deviation. Different superscript letters indicate significant differences based on the LSD test at a 1% significance level.

inhibit the growth of the tested microorganisms (Bubonja-Šonje, Knezević, and Abram 2020). The analysis results showed differences in antimicrobial activity between treatments. Based on one-way analysis of variance and the LSD (Least Significant Difference) follow-up test at a 1% significance level, the results are shown in Table 5.

Table 5 shows that citronella essential oil had the highest inhibitory activity against the tested microorganisms under the conditions of this study. The activity of F2 even produced a larger inhibition zone than the positive control used. In contrast, black rice extract alone showed no measurable inhibitory activity under the method and conditions used for testing. Interestingly, formulation F3, which is a combination of black rice extract and citronella essential oil, produced an inhibition zone of 18.61 ± 6.21 mm. This value was lower than that of citronella essential oil alone, but still showed significantly different inhibitory activity compared with black rice extract and the negative control. This result indicates that combining black rice extract with citronella essential oil does not automatically produce higher antimicrobial activity than the essential oil alone. The decrease in inhibition-zone diameter in F3 compared with F2 may be related to differences in the effective concentration of essential oil in the formulation, the mask preparation matrix, or limitations in the diffusion of active components from the paste preparation into the agar medium.

The difference in activity between black rice extract and citronella essential oil also shows that the presence of bioactive compounds in a material does not always result in the same antimicrobial activity (Mangalagiri et al., 2021). Black rice extract contains various phytochemical compounds, including phenolics, flavonoids, and tannins, whereas the essential oil contains volatile components that can interact with the cell membranes of microorganisms. Differences in chemical composition, active compound concentration, and diffusion ability in the agar medium can influence the size of the inhibition zone formed. Thus, these test results support the use of citronella essential oil as a component with the potential to provide antimicrobial activity in the mask formulation.

Antibacterial Activity Test Results Against *Propionibacterium acnes*

In vitro antibacterial activity testing against *Propionibacterium acnes* was carried out as a follow-up test to evaluate the ability of black rice extract, citronella essential oil, and the selected mask formulation to inhibit the growth of the bacteria responsible for acne. Testing was performed using the well diffusion method. A suspension of *P. acnes* was inoculated evenly onto the agar surface; wells were then made and filled with a test sample. The treatments used consisted of P1 (black rice extract), P2 (citronella essential oil), and P3 (mask formulation of black rice extract and citronella essential oil (F3)). Each treatment was performed with seven replications to increase the reliability and consistency of the measurement results. After 24 hours of incubation under conditions suitable for *P. acnes* growth, antibacterial activity was observed based on the formation of a clear zone around the well. Inhibition-zone diameter was measured using a digital caliper in three measurement directions, and the average value was then calculated. The test results are presented in Figure 5 and Table 6.

The analysis of variance (ANOVA) results showed that the treatments had a highly significant effect on the *P. acnes* inhibition-zone diameter ($P < 0.001$). This result indicates that the type of test material produced a different antibacterial response against *P. acnes*. Based on the follow-up LSD test at the 1% level, the treatments of citronella essential oil (P2), mask formulation (P3), and black rice extract (P1) showed different inhibitory responses. Citronella essential oil produced the largest inhibition-zone diameter (30.31 ± 1.45 mm), while the mask formulation produced



Figure 5. Antibacterial activity test results against P. acnes

Table 6. Average inhibition-zone diameter of P. *acnes* for each treatment

Treatment Code	Sample Description	Mean Inhibition Zone Diameter (mm) ± SD	Statistical Grouping (DMRT $p < 0.05$)
P1	Wojalaka Black Rice Extract	0.00 ± 0.00	c
P2	Citronella Essential Oil	30.31 ± 1.45	a
P3	Mask Formulation F3	10.30 ± 1.08	b

Note: Values represent the mean ± Standard Deviation of three biological replicates. Different superscript letters within the grouping column indicate statistically significant differences according to Duncan's Multiple Range Test ($p < 0.05$).

10.30 ± 1.08 mm. Black rice extract did not produce a measurable inhibition zone (0.00 ± 0.00 mm).

The high antibacterial activity of P2 indicates that citronella essential oil is the most dominant component in providing inhibitory activity against *P. acnes*. This activity is thought to be related to the essential oil's main components, such as citronellal, citronellol, and geraniol, which are lipophilic and can interact with bacterial cell membranes (Mráz et al., 2025). This interaction can increase membrane permeability and cause leakage of intracellular components, thereby disrupting bacterial growth. Earlier microbial resistance test results showed the same pattern: citronella essential oil provided the highest inhibitory activity. Thus, both tests showed consistent results: essential oil is the main component contributing to the formulation's antimicrobial activity (Falleh, 2025).

Mask formulation P3 produced an inhibition zone of 10.30 ± 1.08 mm and was classified as strong based on the criteria used. Although this value was lower than citronella essential oil alone (30.31 ± 1.45 mm), it indicates that the essential oil retained its antibacterial activity after formulation into the mask base. The decrease in inhibition-zone diameter may be related to the lower concentration of essential oil in the formulation and limitations in the release and diffusion of active components from the mask matrix into the agar medium. Thus, the decrease in inhibition zone does not directly indicate a loss of antibacterial activity, but may reflect differences in test-material conditions between free essential oil and essential oil within the formulation matrix (Hulankova, 2024).

Black rice extract (P1) did not produce a measurable inhibition zone against *P. acnes*. This result indicates that although black rice extract has strong antioxidant activity based on the DPPH test, this activity is not always followed by antibacterial activity detectable through the well diffusion method (Khalid et al., 2022). Thus, based on these results, the main contribution of black rice extract in the formulation is antioxidant activity, while antibacterial activity mainly originates from citronella essential oil. These antibacterial findings should be considered alongside the antioxidant results. Black rice extract showed very strong antioxidant activity (IC50 = 37.561 mg/L), while formulation F3, selected based on physical characteristics, subject acceptance,

and mask-use observations, showed antioxidant activity (IC50 = 48.9 mg/L). Thus, F3 has two complementary functional characteristics: antioxidant activity mainly supported by black rice extract and antibacterial activity mainly supported by citronella essential oil. Overall, based on the quality, hedonic, antioxidant, and antibacterial test results, formulation F3 has the potential to be further developed as a natural-ingredient-based facial mask.

■ CONCLUSION

Wojalaka black rice extract contains secondary metabolites in the form of flavonoids (332.58 mg/100 g QE), tannins (137.89 mg/100 g TAE), phenolics (49.33 mg/100 g GAE), saponins, triterpenoids, and alkaloids. This polyphenol content gives the extract very strong antioxidant activity, with an IC50 value of 37.561 mg/L and an Antioxidant Activity Index (AAI) of 5.32. Formulation F3 (50% black rice extract concentration and 0.3% citronella essential oil) proved to be the best physical formulation. This formulation was the only preparation that was homogeneous, had a pH of 6.0 (meeting the skin pH standard of 4.5–6.5), a spreadability of 5.0 cm (meeting the criterion of 5–7 cm), and was the preparation most preferred by the subjects in the hedonic test. Formulation F3 retained very strong free-radical (DPPH) scavenging capacity, with an IC50 value of 48.9 mg/L and an AAI of 4.08, mainly contributed by the *Wojalaka* black rice extract. Formulation F3 produced strong-category antibacterial activity against *Propionibacterium acnes*, with an inhibition zone of 10.30 ± 1.08 mm, and showed antimicrobial resistance (inhibition zone of 18.61 ± 6.21 mm), mainly driven by the role of citronella essential oil. Routine use of the F3 preparation for 6 weeks showed the most noticeable and earliest visual improvement in skin condition (beginning from week 2) compared with the other formulations, including cleaner, brighter, more moisturized, and firmer skin, along with a reduction in the appearance of blemishes and acne in the subjects. Overall, formulation F3 demonstrates a synergistic effect between *Wojalaka* Sumba black rice extract (as an antioxidant agent) and citronella essential oil (as an antibacterial agent against *P. acnes*), making it highly promising for further development as a safe, stable, and efficacious cosmetic product for acne-prone skin care based on local natural ingredients from Eastern Indonesia.

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