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Anti-Acne Activity Evaluation Of *Cyperus rotundus* L. Rhizome Fraction Gel Against *Staphylococcus aureus* And *Propionibacterium acnes*

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ABSTRACT
Acne is an inflammatory skin condition often caused by bacteria such as *Propionibacterium acnes* and *Staphylococcus aureus*. The increasing incidence of antibiotic resistance encourages the search for alternatives from natural ingredients. Nut grass rhizome (*Cyperus rotundus* L.) is known to have antibacterial compounds. This study aimed to formulate a gel from the fractions of nut grass rhizome, test its activity against acne-causing bacteria, and identify the most potent fraction. This study employed a laboratory experimental design. The nut grass rhizome was extracted with 70% ethanol and then fractionated by liquid-liquid partitioning using n-hexane, ethyl acetate, and water as solvents. Each fraction was formulated into a gel at a 5% concentration, and its antibacterial activity was tested using the well diffusion method. All gel formulas met the physical quality evaluation requirements. The ethyl acetate fraction (F2) exhibited the strongest antibacterial activity, with an average inhibition zone of 19.48 ±0.99 mm (moderate category) against *Staphylococcus aureus* and 25.86 ± 0.65 mm (strong category) against *Propionibacterium acnes*. The nut grass rhizome fraction gel preparation possesses anti-acne activity. The ethyl acetate fraction proved to be the most potent, showing efficacy against *Propionibacterium acnes* (inhibition zone 25.86 mm) that was statistically equivalent to the positive control ($p = 0.767$).

Keywords : *Cyperus rotundus* L., Gel,Fractionation, Anti-acne, *Propionibacterium acnes*, *Staphylococcus aureus*

INTRODUCTION

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous unit and one of the most common dermatological problems worldwide. Its pathogenesis is complex, but a central role is played by keratinocyte hyperproliferation, excess sebum production, and bacterial colonization, *Propionibacterium acnes* which triggers an immune and inflammatory response in the skin (Dréno et al., 2018). In addition to *Propionibacterium acnes*, opportunistic bacteria such as *Staphylococcus aureus* are also often involved in exacerbating the inflammatory condition of acne lesions (Nuralifah et al., 2019).

Conventional therapeutic approaches often involve the use of topical and systemic antibiotics to suppress bacterial populations. However, long-term antibiotic use has raised global concerns regarding the increasing prevalence of resistant bacterial strains, which can reduce treatment efficacy and trigger relapses (Dréno et al., 2018). This clinical challenge underscores the urgency to find alternative antibacterial compounds, particularly from natural sources that can offer diverse mechanisms of action with a better safety profile.

In the Indonesian herbal pharmacopeia, nutgrass rhizome (*Cyperus rotundus* L.) is a plant that has long been used empirically. Phytochemical studies show that nutgrass rhizome is rich in

secondary metabolites such as alkaloids, flavonoids, tannins, and saponins, which are known to have various biological activities (Wirdatul, J., 2019). Its relevant antibacterial potential for acne has been demonstrated in previous studies, where a nutgrass tuber extract at a concentration of 11-19% was proven to completely inhibit the growth of *Propionibacterium acnes* (Nurjanah et al., 2018).

Although the crude extract shows promising activity, it is a highly complex mixture of various compounds with different levels of polarity. To identify the group of compounds most responsible for the antibacterial activity, a further separation process is required. Stepwise liquid-liquid fractionation is an effective method to simplify the composition of the extract by separating it into fractions based on polarity (e.g., non-polar, semi-polar, and polar). This step is crucial for isolating and concentrating the active compounds, thus facilitating the identification of the most potential fraction for further development (Windi, W., 2020).

For topical application, the formulation is key to ensuring optimal delivery of the active ingredient. A gel preparation was chosen for its pharmaceutical advantages, such as its water base that provides a cooling sensation, is non-sticky, and is easy to spread and wash off the skin (Larasati, 2020). Therefore, this study was designed to formulate a gel from the fractions of nutgrass rhizome extract and to evaluate its antibacterial activity *in vitro* against *Staphylococcus aureus* and *Propionibacterium acnes*, with the aim of identifying the most potent fraction as a candidate for a herbal-based anti-acne agent.

METHODS

Tools and Materials

The materials used included *Cyperus rotundus* L. rhizome sourced from Wates, Kediri, East Java, 70% ethanol, n-hexane, ethyl acetate, Carbopol 940, Triethanolamine (TEA), methyl paraben, propylene glycol, and purified water. Bacterial cultures of *Staphylococcus aureus* ATCC 25923 and *Propionibacterium acnes* ATCC 6919 were also used. A commercial clindamycin gel (Cindala Gel) served as the positive control. Key instruments included a rotary evaporator, analytical balance, hotplate-stirrer, Brookfield viscometer, pH meter, and laboratory glassware.

Plant Material and Extraction

The plant was authenticated at UPT Materia Medica Batu (Specimen number: 000.9.3/8191/102.20/2024). The cleaned, dried, and powdered rhizomes were extracted by maceration, a process suitable for extracting heat-sensitive compounds (Endah, S. R. N., 2017). A total of 100 g of powdered rhizome was soaked in 1000 mL of 70% ethanol at room temperature with occasional stirring. The resulting liquid extract (maserat) was then concentrated using a rotary evaporator to obtain a thick ethanolic extract.

Fractionation and Phytochemical Screening

The concentrated ethanol extract underwent liquid-liquid fractionation using a separatory funnel to partition compounds based on their polarity (Kristanti et al., 2017). A 10 g sample of the thick extract was first dissolved in 100 mL of purified water. This aqueous solution was then partitioned sequentially, first with 100 mL of n-hexane (non-polar). The mixture was gently shaken for 5-10 minutes, with periodic venting to release pressure. After allowing the layers to separate, the n-hexane fraction was collected. This process was repeated on the remaining aqueous layer using 100 mL of ethyl acetate (semi-polar) to obtain the ethyl acetate fraction. The final remaining aqueous layer constituted the polar fraction. Each fraction was concentrated to dryness. Preliminary phytochemical screening of the crude extract was performed to identify major secondary metabolite groups (Handayani et al., 2017). The resulting fractions were then analyzed using Thin-Layer Chromatography (TLC) to detect the presence of flavonoids, with quercetin used as a standard reference.

Gel Formulation

Three gel formulas were prepared, each containing a 5% concentration of a different fraction: F1 (n-hexane), F2 (ethyl acetate), and F3 (water). The gel base was formulated with Carbopol 940 (2%) as the gelling agent, TEA (2%) as a neutralizing agent, propylene glycol (15%) as a humectant, and methylparaben (0.2%) as a preservative. The preparation method involved two parts. First, Carbopol 940 was dispersed in hot purified water (70°C) in a mortar and stirred until a gel was formed, after which TEA was added to neutralize the polymer. Separately, methylparaben was dissolved in propylene glycol. This solution was then incorporated into the Carbopol base with continuous stirring. Finally, the respective 5% fraction was added to the base gel and mixed until a homogeneous gel was obtained. A gel base without any active fraction was used as a negative control (K-), and the commercial clindamycin gel was used as a positive control (K+).

Physical Evaluation of Gel Formulations

The prepared gels were evaluated for their physical properties:

1. **Organoleptic and Homogeneity:** Assessed visually for color, odor, consistency, and the absence of coarse particles, which indicates uniform dispersion of components (Kemenkes RI, 2020).
2. **pH:** The pH of the gel dispersion in purified water was measured using a standardized pH meter. The acceptable range for topical preparations is 4.5-6.8 to prevent skin irritation (Anggraeni et al., 2019).
3. **Spreadability:** The ability of the gel to spread upon application was measured. A good spreadability range is considered to be between 5-7 cm, ensuring easy application without being too runny (Voigt, R., 2018).
4. **Adhesion:** The time (in seconds) the gel adheres to a surface under a specific weight was measured. A time greater than 1 second is considered good, ensuring adequate contact time with the skin (Garg et al., 2015).
5. **Viscosity:** Measured using a Brookfield viscometer. An acceptable viscosity for a topical gel is typically between 2000-5000 cP to ensure it is thick enough to stay on the skin but still spreadable (Voigt, R., 2018)

Antibacterial Activity Assay

The antibacterial activity was evaluated using the agar well diffusion method. Nutrient Agar plates were uniformly inoculated with standardized suspensions of *S. aureus* and *P. acnes*. Wells (6 mm diameter) were made in the agar, and 20 µL of each test gel (F1, F2, F3, K-, K+) was placed into the wells. The plates were incubated at 37°C for 24 hours. The antibacterial effect was determined by measuring the diameter of the clear zone of inhibition around each well (Permatasari, D. A., 2020).

Data Analysis

All experiments were performed in triplicate. The inhibition zone data were analyzed using SPSS software. The normality of the data was checked with the Shapiro-Wilk test, and the homogeneity of variances was assessed with the Levene test. A One-Way Analysis of Variance (ANOVA) followed by a Post-Hoc Least Significant Difference (LSD) test was used to determine significant differences between the groups. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Physical Evaluation of Gel Formulations

All formulated gels (F1, F2, F3) were semi-solid, homogeneous, and showed no coarse particles. The evaluation results of other physical properties are summarized in Table 1.

Table 1. Physical Evaluation Results of Gel Formulations

Test	Formula F1 (n-Hexane)	Formula F2 (Ethyl Acetate)	Formula F3 (Water)
pH	5.82 ± 0.03	4.68 ± 0.03	6.35 ± 0.03
Spreadability (cm)	5.83 ± 0.11	5.13 ± 0.11	6.72 ± 0.10
Adhesion (s)	3.22 ± 0.05	3.87 ± 0.05	3.62 ± 0.05
Viscosity (cP)	382.6	4483.55	4102.45

Antibacterial Activity

The ethyl acetate fraction (F2) showed the most significant antibacterial activity against both test bacteria. The well diffusion test results are presented in Table 2.

Table 2. Inhibition Zone Diameter (mm) of Nut Grass Rhizome Fraction Gel

Treatment	*Staphylococcus aureus* (mm)	*Propionibacterium acnes* (mm)
F1 (N-Hexane)	7.43 ± 0.30	8.62 ± 0.55
F2 (Ethyl Acetate)	19.48 ± 0.99	25.86 ± 0.65
F3 (Water)	9.84 ± 0.83	14.02 ± 0.35
K+	25.51 ± 0.52	26.03 ± 1.18
K- (Gel Base)	0.0 ± 0.00	0.0 ± 0.00

One-Way ANOVA analysis showed a statistically significant difference in antibacterial activity among the treatment groups for *S. aureus* and *P. acnes* ($p < 0.001$). The Post-Hoc LSD follow-up test showed that for *P. acnes*, there was no significant difference between the activity of the ethyl acetate fraction (F2) and the positive control, Cindala Gel ($p = 0.767$). Against *S. aureus*, the activity of F2 was significantly different from the positive control ($p < 0.05$).

DISCUSSION

The study successfully formulated stable and physically acceptable gels from different fractions of *C. rotundus* rhizome. All formulations met the established pharmaceutical criteria for topical preparations, including pH (4.68-6.35), which is compatible with the skin's physiological pH to avoid irritation (Anggraeni et al., 2019). The gels also demonstrated good homogeneity, spreadability (5.13-6.72 cm), adhesion (3.22-3.87 seconds), and viscosity (3852.6-4483.55 cP), indicating their suitability for practical application (Voigt, R., 2018; Garg et al., 2015). These results confirm that the incorporation of the rhizome fractions did not negatively impact the physical quality of the gel base.

The key finding of this research is the superior antibacterial performance of the ethyl acetate fraction (F2). This enhanced activity can be directly linked to its chemical composition. Our phytochemical analysis identified the presence of flavonoids in *C. rotundus*, a finding consistent with other studies (Jios et al., 2020). Fractionation effectively separated these compounds, with the semi-polar ethyl acetate solvent concentrating the flavonoid aglycones, identified as quercetin through TLC. In contrast, the more polar aqueous fraction (F3) likely contained flavonoid glycosides like rutin. This is significant because the aglycone form (quercetin) is generally more biologically active than its glycoside counterpart (rutin). The simpler structure of the aglycone facilitates better interaction with microbial targets, leading to a stronger antibacterial effect (Kapešová et al., 2019).

The ethyl acetate fraction produced a significant inhibition zone of 19.48 mm against *S. aureus* and a remarkable 25.86 mm against *P. acnes*. This result is more potent when compared to a similar study by Astuti & Reny (2023), who reported an inhibition zone of 14.3

mm against *S. aureus* using essential oil from the same plant. This suggests that the compounds extracted by ethyl acetate may possess higher antibacterial potency than the volatile components obtained through distillation. The most striking result was that the antibacterial activity of F2 against *P. acnes* was statistically comparable to that of the clindamycin control ($p = 0.767$). This suggests that the compounds concentrated in this fraction have a potent effect against the primary bacterium involved in acne inflammation. The mechanism of action of quercetin involves multiple pathways, including the disruption of bacterial cell membrane integrity and the inhibition of crucial enzymes, alongside possessing significant anti-inflammatory properties that are beneficial in acne treatment (Li et al., 2016; Dréno et al., 2018).

In contrast, the non-polar n-hexane fraction (F1) showed the weakest activity, indicating that the primary antibacterial compounds in *C. rotundus* are not lipids or other highly non-polar substances, but rather semi-polar compounds like flavonoid aglycones. The moderate activity of the water fraction (F3) can be attributed to the presence of less potent glycosides and other water-soluble compounds like tannins, which are also known to possess antibacterial properties (Park et al., 2014). This study clearly demonstrates that fractionation is an effective strategy to concentrate the bioactive compounds and identify the most promising fraction for developing a targeted therapeutic agent.

CONCLUSION

Gels formulated using fractions of *Cyperus rotundus* L. rhizome possess significant antibacterial activity against the acne-causing bacteria *Staphylococcus aureus* and *Propionibacterium acnes*. The gel containing the 5% ethyl acetate fraction demonstrated the most potent activity, particularly against *P. acnes*, where its efficacy was found to be statistically equivalent to the commercial antibiotic gel used as a positive control. This study identifies the ethyl acetate fraction of *C. rotundus* rhizome as a promising natural candidate for the development of a new topical agent for the treatment of acne.

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