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*Research Article*

# Antibacterial Activity of Ethanol Extract *Houttuynia cordata* Clay Mask Against *Staphylococcus aureus*

**Diah Nur Astutik[1] , Muhammad Ryan Radix Rahardhian[2*]**

[1,2] Diploma Program in Pharmacy, Stifar Yayasan Pharmasi Semarang, Semarang, Indonesia
* Corresponding Author : radixrahardhian@gmail.com

**Abstract.** Acne is a common inflammatory skin disorder frequently associated with bacterial infection, including *Staphylococcus aureus*. The increasing incidence of antibiotic resistance and potential side effects of synthetic anti-acne agents encourage the development of plant-based topical formulations. This study aimed to formulate a clay mask containing ethanol extract of *Houttuynia cordata* leaves and to evaluate its antibacterial activity against *Staphylococcus aureus* as well as its physical characteristics. The leaves were extracted using remaceration with 96% ethanol, yielding a thick extract with a rendement of 28.534%. Phytochemical screening and thin-layer chromatography confirmed the presence of alkaloids, flavonoids, tannins, saponins, and triterpenoids. Clay mask formulations were prepared at extract concentrations of 10%, 20%, and 30%, followed by antibacterial testing using the well diffusion method. The average inhibition zones were 1.612 ± 0.040 cm (10%), 1.706 ± 0.046 cm (20%), and 1.806 ± 0.039 cm (30%), categorized as strong inhibition. Statistical analysis (one-way ANOVA) showed significant differences between concentrations ($p < 0.05$). All formulations met physical evaluation requirements, including pH (4.706–6.668), spreadability (5.210–5.508 cm), adhesion (>5 seconds), viscosity, homogeneity, and no irritation response. In conclusion, the ethanol extract of *Houttuynia cordata* formulated as a clay mask exhibits concentration-dependent antibacterial activity and meets acceptable physical quality standards, indicating its potential as a natural anti-acne topical product.

**Keywords:** Antibacterial Activity; Clay Mask; *Houttuynia Cordata*; Phytochemical Screening; *Staphylococcus Aureus*.

## 1. Introduction

Acne vulgaris is one of the most prevalent inflammatory skin disorders worldwide, affecting approximately 80–85% of adolescents and a considerable proportion of adults. The condition is characterized by comedones, papules, pustules, and nodules resulting from excessive sebum production, follicular hyperkeratinization, inflammation, and bacterial colonization. Among acne-associated bacteria, *Staphylococcus aureus* plays an important role in secondary infection and exacerbation of inflammatory lesions [1]. Conventional treatments commonly involve topical or systemic antibiotics; however, prolonged use may cause skin irritation, hypersensitivity, organ toxicity, and most importantly, bacterial resistance [2]. These limitations highlight the urgent need for safer and more sustainable alternative therapies derived from natural sources.

*Houttuynia cordata* Thunb., a medicinal plant widely distributed in East and Southeast Asia, has been traditionally used for treating infections, inflammation, and skin disorders [3]. Phytochemical investigations report that this plant contains flavonoids, alkaloids, tannins, saponins, and essential oils with documented antimicrobial and anti-inflammatory properties [4]. Previous studies demonstrated that ethanol extracts of *H. cordata* exhibit antibacterial activity against Gram-positive bacteria, including S. aureus [5].

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The extraction method commonly applied is maceration or remaceration using ethanol due to its effectiveness in extracting both polar and semi-polar secondary metabolites while maintaining compound stability [6], [7], [8]. Although effective, extract-based preparations alone may present limitations in stability, user acceptability, and topical applicability.

In cosmetic and dermatological applications, clay masks composed of bentonite and kaolin are widely utilized due to their ability to absorb excess sebum, remove impurities, and enhance skin cleansing [9]. Incorporation of plant extracts into clay-based formulations offers a dual function: physical cleansing and biological antibacterial activity. However, variations in extract concentration may influence antibacterial efficacy, physicochemical stability, pH compatibility, spreadability, viscosity, and overall product performance [10]. Limited studies have systematically evaluated concentration-dependent antibacterial effects of *H. cordata* extract within a clay mask formulation alongside comprehensive physical characterization [11].

Therefore, this study aims to formulate a clay mask containing ethanol extract of *Houttuynia cordata* leaves at concentrations of 10%, 20%, and 30%, and to evaluate its antibacterial activity against *Staphylococcus aureus* using the well diffusion method. In addition, physicochemical characteristics including organoleptic properties, homogeneity, pH, spreadability, adhesion, viscosity, and irritation response were assessed to ensure product quality and safety. The main contributions of this research are: (1) development of a plant-based anti-acne clay mask formulation; (2) quantitative evaluation of concentration-dependent antibacterial activity; (3) statistical validation of antibacterial differences among formulations; and (4) comprehensive assessment of physical quality parameters. The remainder of this paper is organized as follows: Section 2 describes the materials and methods, Section 3 presents results and discussion, and Section 4 concludes the findings and outlines future research directions.

## 2. Method

### Research Design and Scope

This study employed a true experimental laboratory design to evaluate the antibacterial activity of a clay mask formulation containing ethanol extract of *Houttuynia cordata* leaves against *Staphylococcus aureus*. The research scope included plant determination, extraction, phytochemical screening, thin-layer chromatography (TLC) confirmation, clay mask formulation, antibacterial testing using the well diffusion method, physicochemical evaluation, and statistical analysis [5]. All experimental procedures were conducted at the Pharmaceutical Biology and Microbiology Laboratories, Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi Semarang, Indonesia.

The independent variable was the concentration of *H. cordata* ethanol extract in the clay mask formulation (10%, 20%, and 30%). The dependent variable was the diameter of the inhibition zone (cm) formed against S. aureus. Controlled variables included incubation temperature (37°C), incubation time (24 h), media type, and experimental procedures [5].

### Plant Material and Extraction

Fresh *Houttuynia cordata* leaves were collected from Gunungpati, Semarang, Indonesia, and taxonomically identified at the Pharmaceutical Biology Laboratory. The leaves were washed, air-dried in a drying cabinet, powdered, and sieved (mesh 30–40). Extraction was performed by remaceration using 96% ethanol (1:10 w/v). Two hundred grams of powdered leaves were soaked in 2000 mL ethanol for 3 days with occasional stirring. The filtrate was separated and the residue was re-macerated under identical conditions. Combined filtrates were concentrated using a rotary evaporator at 65°C followed by water bath evaporation to obtain a thick extract [7].

### Plant Material and Extraction

Preliminary phytochemical screening was performed to identify alkaloids, flavonoids, tannins, saponins, and triterpenoids using standard qualitative color reactions [8]. TLC analysis was carried out on silica gel GF254 plates with specific mobile phases for each compound group. Spots were visualized under UV light (254 nm) and sprayed with appropriate reagents (Dragendorff, $FeCl_3$, anisaldehyde-sulfuric acid, or ammonia vapor).

### Clay mask formulations

Clay mask formulations were prepared in four groups F0: Base (without extract), F1: 10% extract, F2: 20% extract, and F3: 30% extract. The composition included bentonite (8%), kaolin (30%), xanthan gum (1%), glycerin (5%), sodium lauryl sulfate (0.5%), titanium dioxide (2%), extract (as specified), and distilled water ad 50 g. Bentonite was dispersed in distilled water and allowed to hydrate. Xanthan gum was incorporated under continuous trituration. Kaolin, titanium dioxide, and glycerin were added gradually to form a homogeneous phase. Sodium lauryl sulfate solution was incorporated and mixed until a uniform semi-solid clay mask was obtained [12].

### Antibacterial Activity

Cultures of *Staphylococcus aureus* were rejuvenated on Nutrient Agar (NA) and incubated at 37°C for 24 h. A bacterial suspension was prepared in Nutrient Broth (NB) and adjusted to 0.5 McFarland standard (approximately $1 \times 10^8$ CFU/mL) using a UV-Vis spectrophotometer at 625 nm (absorbance range 0.08–0.10). The antibacterial activity test was performed using the pour plate technique. Mannitol Salt Agar (MSA) was used as selective medium. After inoculation with bacterial suspension, wells were created using sterile cylinder cups. Each well was filled with 100 µL of test sample (F1, F2, F3), positive control (commercial clay mask), and negative control (base formulation). Plates were incubated at 37°C for 24 h. The diameter of inhibition zones was measured using a digital caliper. Each treatment was replicated five times[13], [14].

### Physicochemical Evaluation

Physical characterization included Organoleptic Test (observation of color, odor, and consistency), Homogeneity Test (visual examination for uniform dispersion), pH Measurement (1 g sample diluted in 100 mL distilled water; measured using pH meter), Spreadability Test (0.5 g sample placed between glass plates with incremental loads 50–150 g), Adhesion Test (measured detachment time under standardized weight), Viscosity Test (Brookfield viscometer spindle 64, 100 rpm), and Irritation Test (open patch test on volunteers with observation for erythema or itching) [15].

### Statistical Analysis

Data were expressed as mean ± standard deviation. Normality was tested using Shapiro–Wilk and Kolmogorov–Smirnov tests [6]. Homogeneity was analyzed using Levene's test. If data were normally distributed and homogeneous ($p > 0.05$), one-way ANOVA was performed followed by post-hoc analysis. Statistical significance was set at $p < 0.05$. This methodological approach ensures reproducibility and comprehensive evaluation of antibacterial efficacy and formulation stability [16].

## 3. Results and Discussion

### Extraction Yield and Phytochemical Profile

Extraction of *Houttuynia cordata* leaves by remaceration with 96% ethanol produced a thick extract with a yield of 28.534%. The relatively high yield indicates efficient extraction of secondary metabolites, as ethanol is capable of dissolving polar and semi-polar bioactive compounds such as flavonoids, tannins, and alkaloids [17]. The absence of ester odor in the free-ethanol test confirmed complete solvent removal, ensuring that subsequent antibacterial activity was derived solely from plant constituents rather than residual solvent [4].

Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, and triterpenoids [5], [7]. Thin-layer chromatography confirmed these findings with Rf values within acceptable ranges (0.18–0.91), supporting the presence of semi-polar to polar compounds. These metabolites are widely reported to possess antibacterial mechanisms through membrane disruption, protein denaturation, enzyme inhibition, and interference with nucleic acid synthesis [18], [19]. The detection of multiple antibacterial-related metabolites supports the hypothesis that *H. cordata* extract exhibits inhibitory activity against *Staphylococcus aureus* [5], [20].

### Antibacterial Activity of Clay Mask Formulations

Antibacterial activity was evaluated using the well diffusion method on Mannitol Salt Agar. Hardware used included a UV-Vis spectrophotometer (λ 625 nm), incubator (37°C), autoclave, and digital caliper [11]. Statistical analysis was performed using SPSS version 23.

The inhibition zone results are presented in Table 1.

**Table 1.** Inhibition zone diameter of clay mask formulations against *Staphylococcus aureus*.

| Concentration | Mean SD (cm) | Category |
|---|---|---|
| 10% | 1.612 ± 0.040 | Strong |
| 20% | 1.706 ± 0.046 | Strong |
| 30% | 1.806 ± 0.039 | Strong |
| Negative Control | 0.000 | No inhibition |

All extract-containing formulations demonstrated concentration-dependent antibacterial activity. The 30% formulation produced the largest inhibition zone (1.806 cm), confirming that increasing extract concentration enhances antibacterial efficacy. Based on Davis and Stout classification, all concentrations were categorized as strong inhibition (10–20 mm). One-way ANOVA showed statistically significant differences among concentrations ($p < 0.05$). Post-hoc analysis confirmed significant differences between 10%, 20%, and 30% formulations. These findings validate the research hypothesis that antibacterial activity varies significantly with extract concentration [15].

The absence of inhibition in the commercial clay mask control indicates that antibacterial activity originated from *H. cordata* extract rather than the clay base itself. The mechanism of inhibition may involve synergistic interactions among flavonoids, tannins, and alkaloids, which disrupt bacterial cell walls and membranes, leading to leakage of intracellular components [1], [21], [22]. Similar antibacterial effects of *H. cordata* extracts against Gram-positive bacteria have been reported in recent studies [4].

**Physicochemical Evaluation of Formulations**

Physical characterization results are summarized in Table 2.

**Table 2.** Physicochemical properties of clay mask formulations.

| Parameter | 10% | 20% | 30% | Requirement |
|---|---|---|---|---|
| pH | 6.668 | 5.408 | 4.706 | 4.5–6.5 |
| Spreadability (cm) | 5.210 | 5.342 | 5.508 | 5–7 cm |
| Adhesion (sec) | 5 | 6 | 7 | >4 sec |
| Viscosity (cP) | 11,576 | 10,806 | 9,154 | Semi-solid range |
| Homogeneity | Homogeneous | Homogeneous | Homogeneous | Uniform |
| Irritation | None | None | None | No erythema |

All formulations met acceptable cosmetic standards. Increasing extract concentration slightly decreased pH and viscosity while increasing spreadability and adhesion. The reduction in viscosity at higher concentrations facilitated better diffusion of active compounds into the agar medium, potentially contributing to larger inhibition zones [9]. The relationship between viscosity and antibacterial activity is noteworthy. Lower viscosity enhances diffusion capacity, which may explain the superior inhibition zone observed at 30% concentration. This finding supports diffusion-based antibacterial theory, where compound mobility influences inhibition diameter [23]. Additionally, pH values remained within the physiological skin range (4.5–6.5), ensuring compatibility and minimizing irritation risk. No irritation responses were observed in volunteers, indicating safety for topical application [11].

## 4. Conclusions

This study successfully formulated a clay mask containing ethanol extract of *Houttuynia cordata* leaves and evaluated its antibacterial activity against *Staphylococcus aureus*. The extract yield obtained through remaceration with 96% ethanol was 28.534%, and phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, saponins, and triterpenoids, which are known to exhibit antibacterial properties. Antibacterial testing demonstrated concentration-dependent inhibition, with mean inhibition zones of 1.612 ± 0.040 cm (10%), 1.706 ± 0.046 cm (20%), and 1.806 ± 0.039 cm (30%), all categorized as strong inhibition. Statistical analysis using one-way ANOVA confirmed significant differences among concentrations ($p < 0.05$), supporting the hypothesis that increasing extract concentration enhances antibacterial efficacy.

All clay mask formulations met acceptable physicochemical standards, including pH compatibility with skin (4.706–6.668), adequate spreadability (5.210–5.508 cm), sufficient adhesion (>5 seconds), appropriate viscosity, homogeneity, and absence of irritation. These findings indicate that the developed formulation is not only biologically active but also physically stable and safe for topical application.

The integration of *H. cordata* extract into a clay-based delivery system provides a dual functional approach combining sebum absorption and antibacterial activity, contributing to the development of plant-based anti-acne products. However, this study was limited to in vitro antibacterial evaluation against a single bacterial strain. Future research should include minimum inhibitory concentration (MIC) determination, stability studies, broader antimicrobial spectrum testing, and in vivo clinical evaluation to further validate its therapeutic potential.

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**Data Availability Statement:** The data supporting the findings of this study are available from the corresponding author upon reasonable request. No publicly archived datasets were generated during the current study. All experimental data, including antibacterial measurements and physicochemical evaluation results, are stored at Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi Semarang and can be accessed for academic purposes with appropriate permission.

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