Available online at *www.ujpronline.com*
Universal Journal of Pharmaceutical Research
An International Peer Reviewed Journal
ISSN: 2831-5235 (Print); 2456-8058 (Electronic)

RESEARCH ARTICLE

FORMULATION AND EVALUATION OF ANTIBACTERIAL ACTIVITY OF EMULGEL PREPARATION CONTAINING *MUNTINGIA CALABURA* L. LEAVES EXTRACT

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Article Info:

Article History:
Received: 3 February 2025
Reviewed: 9 March 2025
Accepted: 22 April 2025
Published: 15 May 2025

Cite this article:
Agustina L, Lailiyah M, Yuliati N, Prasongko ET, Istiqomah N, Soehartono DR, Dayoob M. Formulation and evaluation of antibacterial activity of emulgel preparation containing *Muntingia calabura* L. leaves extract. Universal Journal of Pharmaceutical Research 2025; 10(2): 7-10. ***http://doi.org/10.22270/ujpr.v10i2.1304***

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Abstract

Background and aims: Acne develops due to the obstruction of pilosebaceous follicles by accumulated sebum, keratinocytes, and microbial colonization Certain secondary metabolites from plants possess anti-acne properties, including karsen (*Muntingia calabura* L.). This study aims to formulate and evaluate the anti-acne activity of an emulgel against *Propionibacterium acnes*. The ethanolic extract from *Muntingia calabura* L. leaves was used in the formulation.
Methods: The extraction process was carried out using maceration. The ethanolic extract of karsen was utilized as the active ingredient in the emulgel formulation. The emulgel was evaluated based on its organoleptic properties, homogeneity, pH, emulsion type, spreadability, and adhesion. The antibacterial activity was assessed through an *in vitro* antibacterial assay.
Results: The extraction yield was 10.8%. The extract contained flavonoids, tannins, and saponins. The formulated emulgel had a semi-solid consistency, a brownish-green color, a distinct odor characteristic of *Muntingia calabura* L. leaves, and was homogeneous. It had a pH range of 4.87–5.40, an oil-in-water (O/W) emulsion type, a spreadability of 5.1–5.8 cm, and an adhesion time of 3.26–4.36 seconds. The formulation exhibited anti-acne activity against *Propionibacterium acnes*, with the highest inhibitory activity observed at a concentration of 12%.
Conclusion: The ethanolic extract from *M. calabura* L. leaves can be successfully formulated into a stable emulgel. The formulated emulgel exhibits antibacterial activity against *Propionibacterium acnes*.
Keywords: Antiacne, antibacterial activity, emulgel, kersen.

INTRODUCTION

The excess sebum and lipids on the skin can clog hair follicles, leading to acne formation. These deposits can combine with sweat, dust, and other impurities, leading to the formation of blackheads. Blackheads represent the initial stage of acne, characterized by an oily bump where excess oil reacts with oxygen, forming a black plug. When bacteria infect blackheads, an inflammatory response is triggered, resulting in acne[1]. Areas of the skin with a high concentration of oil glands, such as face, are more prone to acne. Individuals affected by this condition may experience pimples, nodules, blemishes, and, in some cases, scarring.

Acne can also result from bacterial infections, such as those caused by *Propionibacterium acnes*. This bacteri secretes lipase that hydrolyze sebum triglycerides into free fatty acids, provoking inflammatory responses that contribute to acne pathogenesis[2]. Antibiotics and chemical treatments, including sulfur, resorcinol, salicylic acid, benzoyl peroxide, azelaic acid, tetracycline, erythromycin, and clindamycin, are commonly used to treat acne. However, these treatments may cause side effects such as antibiotic resistance and skin irritation. As a result, traditional anti-acne remedies are gaining popularity, offering a more natural approach to treatment with fewer adverse effects. Therefore, discovering antibacterial agents derived from natural ingredients, which are known to be safe, is essential[3].

The extract of *Muntingia calabura* L. (kersen) leaves contains tannins, saponins, and flavonoids, which are effective as antibacterial agents[4]. Due to its high

content of flavonoids, steroids, alkaloids, tannins, glycerol, and saponins. This compound displays diverse therapeutic potentials, antipyretic efficacy, anti-inflammatory responses, and antioxidant capabilities[5]. *In vitro* studies indicate the antibacterial potential of *M. calabura* L. extract, exhibiting growth inhibition against *Escherichia coli* (6.45 mm) and *Staphylococcus aureus* (6.44 mm) in disc diffusion assays. Additionally, *M. calabura* L. leaves ethanolic extract of demonstrated antibacterial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*, with clear zone diameters of 20 mm, 19 mm, and 17 mm, respectively[5].

The aim of this study was to formulate karsen (*M. calabura* L.) leaf extract into an emulgel. Emulgel is considered superior to other formulations due to its high penetration ability and cooling effect[6]. The emulgel was evaluated for its organoleptic properties, homogeneity, pH, emulsion type, spreadability, and adhesion. Its antibacterial activity against *P. acnes* was also assessed.

MATERIALS AND METHODS

The materials used in this study included *M. calabura* L. (kersen) leaves, 96% ethanol, hydrochloric acid, triethanolamine (TEA), magnesium powder, 1% $FeCl_3$, Span 80, Carbopol 940, Tween 80, liquid paraffin, distilled water, propylene glycol, Mueller-Hinton Agar (MHA), Nutrient Broth (NB) medium, and Medi-Klin® gel.

Preparation extract of kersen (*M. calabura* L.) leaves

The material used in this study was *M. calabura* L. (kersen) leaf powder, which was obtained from UPT Materia Medica Batu, Malang, Indonesia. The ethanolic extract was prepared by soaking the kersen leaf powder in a 96% ethanol solution at a 1:10 ratio (powder to solvent). The mixture was agitated for 24 hours using a rotary shaker. The maceration was repeated twice. The evaporation using a rotary evaporator at 50°C for 4.5 hours, followed by further evaporation in a water bath for 2 hours. The extraction yield was calculated using formula (7):

$$\% \text{ yield} = \frac{\text{weight of extract (gm)}}{\text{weight of sample (gm)}} \text{ x } 100\%$$

Table 1: Composition of emulgel formulations.

Materials	F I (%)	F II (%)	F III (%)
Ethanol extract	4	8	12
Carbopol 940	1.5	1.5	1.5
Triethanolamine	1	1	1
Propylen glycol	15	15	15
Tween 80	1.5	1.5	1.5
Span 80	1	1	1
Paraffin liquid	5	5	5
Distilled water	Ad 30	Ad 30	Ad 30

Phytochemical screening

The flavonoid content in the extract was determined by adding 0.1 g of magnesium powder and 0.2 ml of sulfuric acid. The presence of flavonoids was indicated by the appearance of red, orange, or yellow color. To identify saponins, 1 ml of *M. calabura* L. leaf extract was added with hot distilled water (10 ml) and cooled to room temperature.

The solution was then shaken vigorously for 10 seconds, and the formation of a stable, persistent froth after adding 1 N HCl confirmed the presence of saponins. Tannins were detected by adding a diluted ferric chloride solution to 1 ml of the ethanolic extract, with a dark green or deep blue color[8]. Furthermore, thin layer chromatography (TLC) was conducted using rutin as a marker to support the identification of phytochemical compounds.

Preparation of emulgel containing kersen leaves extract

The water phase of the emulsion was prepared by dissolving the ethanolic extract, distilled water, and Tween 80, while the oil phase was prepared by mixing liquid paraffin and Span 80. Each phase was continuously stirred using a magnetic stirrer. The oil phase was then gradually added to the water phase, stirring until an emulsion was formed. This emulsion was then combined with the gel base (propylene glycol, TEA, Carbopol 940, and aquadest) to produce a homogeneous emulgel. The emulgel formulation is detailed in Table 1, with extract concentrations of 4%, 8%, and 12%. Table 1. Emulgel formula in various concentrations of kersen (*M. calabura* L.) leaves extract.

Physicochemical stability test of emulgel

The stability test was conducted to evaluate the homogeneity, organoleptic properties, emulsion type, and pH of the formulated emulgels. The organoleptic evaluation in this study included observations of taste, odor, and formulation appearance[3]. To measeured pH, we used pH meter. To assess homogeneity, the emulgel from each formulation was spread onto a glass slide or other suitable transparent material for visual inspection[9]. Spreadability and adhesion (inherent ability) were evaluated following the method described by Murtiningsih[11]. This procedure was performed in triplicate to ensure accuracy.

Antibacterial activity assay

The antibacterial activity was evaluated using the disc diffusion method. *P. acnes* was grown in NB (Nutrient Broth) and incubated for 24 hours at 37°C until reaching an optical density of 0.5 of McFarland standard. Sterile discs were immersed in the prepared emulgel at varying concentrations and left for 15 minutes. In this study, Medi-Klin® gel served as the positive control. The treated discs were then placed on Mueller Hinton Agar (MHA) plates that had been pre-inoculated with *P. acnes* suspensions. The procedure repeated in triplicate, and the plates were incubated for 48 hours at 37°C. The antibacterial efficacy was assessed by measuring the diameter of inhibition zones surrounding the test discs, with clear zones indicating bacterial growth suppression[12].

Statistical analyses

Statistical analysis was conducted on the data collected from pH, dispersion, adhesion, and antibacterial activity tests. The evaluation involved a homogeneity test, which was then followed by a One-Way ANOVA.

RESULTS

Extraction and phytochemical content of leaves extract

The leaves of kersen (*M. calabura* L.) were macerated in 96% ethanol at a 1:10 ratio. The resulting filtrate was evaporated, yielding 324 g of a concentrated extract. This maceration process produced a yield of 10.8%. The result of screening analysis showed the presence of flavonoid, tannin and saponin.

Emulgel preparation

The extract form kersen (*M. calabura* L.) leaves was formulated into emulgel in various concentrations (Table 1).

Physicochemical evaluation of emulgel formulations

Table 2 displays the physicochemical stability results, including organoleptic properties, pH, homogeneity, emulsion type, spreadability and adhesion.

DISCUSSION

The leaves from kersen (*M. calabura* L.) were extracted by maceration using 96% ethanol (1:10 ratio). The filtrate was then evaporated to produce 324 g of thick extract. In this experiment, the yield obtained from maceration as much as 10.8 % (Table 2).

The ethanol extract of *M. calabura* L. (kersen) leaves demonstrated the presence of saponins, tannins, and flavonoid in qualitative analysis. Previous studies have documented that kersen leaves contain various bioactive compounds, including steroids, flavonoids, alkaloids, saponins, glycosides, and tannins[5]. To verify these findings, thin-layer chromatography (TLC) was conducted using rutin as a reference standard. The TLC results aligned with prior research[13], showing characteristic spots visible under different conditions: yellow-brown in natural light, black under UV 254 nm, and blue rings under UV 366 nm, all exhibiting an Rf value of 0.65 (data not shown). The ethanolic extract of *Muntingia calabura* L. leaves was formulated into emulgels at different concentrations (Table 1).

The base formulation consisted of Tween 80/Span 80 emulsion system gelled with Carbopol 940. This gelling agent provides hydrophilicity, high stability, and excellent biocompatibility for natural product formulations[14]. As topical delivery vehicles, emulgels combine the benefits of gels and emulsions, offering superior stability, easy application, and a non-occlusive feel[15]. Their biphasic nature enhances the delivery of lipophilic actives through both increased skin penetration and improved drug bioavailability[6].

Table 2: Characterization of emulgel formulations.

Parameters	Emulgel Formulation		
	F I (4%)	F II (8%)	F III (12%)
Organoleptic		Semi-solid	
Color	Brownish green	Brownish green	Dark Brownish green
pH	5.23±0.31	4.87±0.15	5.40±0.10
Spreadability (cm)	5.8±0.10	5.4±0.10	5.1±0.10
Adhesion (second)	3.26±0.05	3.58±0.04	4.36±0.05
Homogeneity		Homogenous	
Emulsion Type		O/W	

The observation showed that the emulgels from all formulations were semi-solid, brownish green, and has a distinctive aroma of kersen leaves extract. Emulgel color was getting darker along with the increasing extract concentration. The pH values of the developed emulgels were measured between 4.8 and 5.4, conforming to the physiologically acceptable range (4.0-5.5) for cutaneous application on facial skin[16]. This appropriate pH range is crucial as formulations with excessively acidic or alkaline pH may lead to skin irritation[11]. The spreadability of emulgels, a key parameter of physicochemical stability, was evaluated based on the distribution area when applied to skin surfaces. Optimal spreadability ensures uniform coverage for effective active ingredient release and sustained therapeutic action. All formulated emulgels demonstrated favorable spreadability, with measured values ranging from 5.1-5.8 cm (Table 2). Furthermore, adhesion test resulted that the for over 1 second, confirming their suitable viscoelastic properties for topical application[17].

The antibacterial activity was evaluated using the disc diffusion method. A blank emulgel (without ethanol extract) served as the negative control, while commercially available Gel Medi-Klin® containing clindamycin was employed as the positive control for topical bacterial growth inhibition[18].

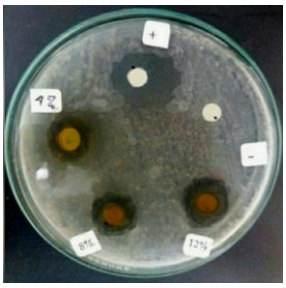

Figure 1: Antibacterial activity of emulgel formulations.

As shown in Figure 1, all formulated emulgels containing *M. calabura* L. leaf extract demonstrated dose-dependent antibacterial activity against *P. acnes*, with inhibition zones ranging from 6 to 21 mm. The 4% extract formulation (F1) produced an inhibition zone of 12.07 mm, while FII showed moderate activity (15.52 mm). Notably, FIII exhibited the strongest antibacterial effect among the test formulations with an 18.75 mm inhibition zone. The antimicrobial activity of

M. calabura L. (kersen) leaf extract likely due to its phytochemical constituents. Existing literature indicates that flavonoids, tannins, and saponins exert antibacterial effects through multiple mechanisms, including interference with nucleic acid synthesis[19].
K (-): emulgel contains no kersen (*M. calabura* L.) leaf extract as negative control. K (+): Medi-Klin® as positive control. Data were analyzed statistically with significant value $p<0.05$.

CONCLUSION

The ethanolic extract derived from *M. calabura L.* (kersen) leaves was successfully incorporated into a stable emulgel formulation. This topical preparation demonstrated dose-dependent antibacterial efficacy against the target microorganisms.

ACKNOWLEDGEMENTS

This research was fully supported by the Institut Ilmu Kesehatan Bhakti Wiyata Kediri. The authors extend their sincere gratitude to Dr. Mohamad Dayoob from the Pharmaceutical Technology Department at Mahsa University, Malaysia, for his expert guidance in manuscript preparation.

AUTHOR'S CONTRIBUTION

Agustina L: conducted antibacterial testing and drafted the manuscript. **Soehartono DR:** conducted antibacterial data analysis. **Yuliati N:** data analysis, review. **Lailiyah M:** formulation and physical quality evaluation. **Prasongko ET:** data analysis, review. **Istiqomah N:** formulation development. **Dayoob M:** review. Final manuscript was checked and approved by all authors.

DATA AVAILABILITY

The accompanying author can provide the empirical data that were utilized to support the study's conclusions upon request.

CONFLICT OF INTEREST

None to declare.

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