Antimicrobial and antioxidant properties of *Centella asiatica* and *Moringa oleifera* nanoparticle hydrogel: In vitro approach

Antimicrobial and antioxidant properties of *Centella asiatica* and *Moringa oleifera* nanoparticle hydrogel: In vitro approach

B Muchtaromah[1]*, A F Majid[1], S N 'Azizah[1], H Aqila[2], A Hayati[3], and A N M Ansori[4]

[1] Biology Department, Maulana Malik Ibrahim Islamic University, Malang, Indonesia
[2] Faculty of Medicine, Brawijaya University, Malang, Indonesia
[3] Biology Department, Airlangga University, Surabaya, Indonesia
[4] Postgraduate School, Universitas Airlangga, Surabaya, Indonesia

*E-mail: bayyinatul@bio.uin-malang.ac.id

Abstract. Extracts of *Centella asiatica* and *Moringa oleifera* contain bioactive compounds that contribute to wound healing through their antioxidant and antibacterial properties. Developing these extracts in the form of nanoparticle-based hydrogels can improve skin penetration and optimize therapeutic effects. This study aimed to evaluate the antimicrobial and antioxidant activities of hydrogel formulations containing different concentrations of combined *Centella Moringa* nanoparticles. The formulations were prepared with varying concentrations: F0 (0%), F1 (2.5%), F2 (5%), F3 (10%), and K+ (Bioplacenton, as a commercial comparator). Antimicrobial activity was assessed by the well diffusion method against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, while antioxidant activity was determined using the DPPH free radical scavenging assay. The results showed that the hydrogel exhibited significant antibacterial activity against *S. aureus*, with inhibition zones ranging from 10.76 ± 0.43 to 11.82 ± 1.70 mm. However, the effect against *P. aeruginosa* was weaker, with inhibition zones between 4.08±0.33 and 4.68±1.09 mm. Antioxidant testing revealed that the 10% extract hydrogel demonstrated the strongest activity, with an IC_{50} of 7,849.47 ppm, compared to 259,423 ppm for the commercial gel. In conclusion, centella moringa nanoparticle hydrogels demonstrate promising antioxidant and antimicrobial potential, supporting their role as a natural and sustainable topical agent for burn wound treatment.

Keyword: Antimicrobial, Antioxidant, *Centella*, Hydrogel, Moringa

1. Introduction

Burn wounds represent a major global health concern, often referred to as a silent epidemic due to their significant social, economic, and personal impacts [1]. According to the World Health Organization (WHO), approximately 180,000 deaths occur annually due to burn related injuries [2]. These wounds are complex, typically involving irregular tissue damage, necrosis, and high susceptibility to infection [3]. When not managed properly, burn wounds can develop into chronic conditions due to bacterial colonization and delayed healing responses [4].

The loss of the skin protective barrier in burn injuries increases vulnerability to microbial invasion, particularly by pathogens such as Staphylococcus aureus and Pseudomonas aeruginosa. These bacteria not only trigger excessive inflammatory cytokines but also contribute to elevated reactive oxygen species (ROS), disrupting macrophage metabolism and impairing the healing process [5-6]. Persistent oxidative stress and uncontrolled infection are key barriers that hinder wound progression to the proliferation and remodeling phases [7]. Therefore, investigating the antioxidant capacity to neutralize ROS and the antimicrobial activity to suppress pathogen growth is essential for developing effective wound care formulations.

Natural products, especially medicinal plants, have gained attention as promising alternatives to synthetic drugs due to their lower toxicity and biocompatibility [8-9]. *Centella asiatica* and *Moringa oleifera* are two widely available plants known for their wound-healing, antimicrobial, and antioxidant properties [10-12]. *C. asiatica* contains active compounds such as asiaticoside and madecassoside, which support fibroblast proliferation and angiogenesis, while *M. oleifera* provides bioactive alkaloids and flavonoids, including moringyne, known for their potent antibacterial and ROS-scavenging properties [13-15]. Their combination may provide synergistic effects to promote tissue regeneration and prevent infection in burn wounds [16].

Recent advances in green extraction technologies, such as microwave ultrasonic assisted extraction (MUAE) and pulse electric field (PEF) pre-treatment, have improved the extraction efficiency and bioactivity of plant-derived compounds [17]. These methods promote plant cell wall disruption, reduce particle size, and increase solubility, resulting in greater antioxidant and antimicrobial potential [18-19]. Preliminary studies reported significant increases in flavonoid content, IC_{50} reduction, and antibacterial efficacy against *S. aureus* using MUAE-treated *C. asiatica* and *M. oleifera* extracts [20-21].

To ensure efficient topical delivery, these plant extracts were incorporated into a hydrogel base. Hydrogels, composed of hydrophilic polymers with high water content, exhibit excellent spreadability, cooling sensation, and enhanced drug penetration compared to creams or ointments [22-23]. Furthermore, they offer a favourable environment for wound healing without disrupting skin physiology.

Despite prior studies utilizing *C. asiatica* or *M. oleifera* in topical preparations, few have investigated their synergistic potential in nano-sized hydrogel formulations specifically for burn care [24]. Therefore, this study aims to evaluate a hydrogel containing nanoparticle extracts of *C. asiatica* and *M. oleifera*, focusing on the assessment of antioxidant and antimicrobial properties is central to understanding its potential as a multifunctional wound healing agent.

2. Methods

2.1 Research design

This study employed a true experimental design comprising three stages: hydrogel formulation, antimicrobial evaluation (well diffusion), and antioxidant activity testing (DPPH). The first used a completely randomized design with five treatments and four replications, while the seccond stages were exploratory.

2.2 Preparation of Centella and Moringa simplisia

This study employed a total of 176 grams of *Centella asiatica* and *Moringa oleifera* simplicias, which were procured from the Tawangmangu-based Research and Development Center for Traditional Herbal Medicine, Central Java.

2.3 Extraction of nanoparticle Centella and Moringa combination

The extraction process was conducted using a continuous combination of Pulse Electric Field (PEF), microwave, and ultrasonic methods. The PEF extraction was performed for 3 min at a frequency of 1000 Hz and a voltage of 2000 V [25]. Subsequently, microwave extraction was carried out by dissolving 176 g of Centella and Moringa extracts in 8000 mL of distilled water and processing for 4 min at 300 W [26]. Finally, ultrasonic extraction was applied at 120 W and 40 kHz for 9.5 min. The obtained extracts were freeze-dried, ground using a blender, and sieved through a 100-mesh sieve. In a preliminary study by Muchtarohmah et al. [19], the combined extraction of *Centella asiatica* and *Moringa oleifera* using PEF–MUAE produced particle sizes ranging from 125 to 129 nm.

2.4 Preparation of nanoparticle Centella and Moringa hydrogel

The optimal hydrogel formulation was adapted from Muchtaromah et al. [19], with modifications on the concentration of *Centella asiatica* and *Moringa oleifera* nanoparticle extracts. Formula F0 was prepared as a control without extract, while F1, F2, and F3 contained 2.5%, 5%, and 10% extract, respectively. All formulations consisted of 0.6% Carbopol-940 as a gelling agent, 0.4% triethanolamine (TEA) as a neutralizer, 1% propylene glycol as a humectant, and 0.5% sodium benzoate as a preservative. Distilled water was added up to 40 mL to complete the formulations.

2.5 Antimicribial activity

The antimicrobial activity of hydrogel formulations containing *Centella asiatica* and *Moringa oleifera* nanoparticle extracts was evaluated using the well diffusion method. Hydrogel concentrations of 2.5% (F1), 5%, (F2), and 10% (F3) were tested, while extract-free hydrogel (F0) served as the negative control and a commercial product (Bioplacenton®) was used as the positive control. The assays were performed against Gram-positive Staphylococcus aureus and Gram-negative Pseudomonas aeruginosa.

A total of five treatments (F0, F1, F2, F3, and positive control) were prepared in quadruplicate, following the minimum replication requirement of $t(n-1) \geq 15$. Antimicrobial activity was categorized based on inhibition zone diameter: ≥20 mm (very strong), 10–20 mm (strong), 5–10 mm (moderate), and <5 mm (weak) [2]. Muller Hinton Agar (MHA) medium was prepared according to standard procedures and sterilized at 121 °C for 15 min. Test bacteria were rejuvenated on MHA plates and incubated at 37 °C for 24 h. Microbial suspensions were adjusted to 0.5 McFarland standard (1–2 × 108 CFU/mL, OD = 0.132 at λ = 600 nm). For the assay, 15 mL of sterile MHA was poured into petri dishes, and 50 µL of bacterial suspension was spread evenly. Wells were loaded with 60 µL of each hydrogel formulation. Plates were incubated at 37 °C for 24 h, and inhibition zones were measured in both horizontal and vertical directions.

2.6 Antioxidant activity

The antioxidant activity was determined using the DPPH method as described by Molyneux [28] and Nurmazela et al. [29]. A 0.5 mM DPPH stock solution was prepared by dissolving 20 mg of DPPH in 100 mL of methanol. Hydrogel samples containing *Centella asiatica* and *Moringa oleifera* nanoparticles were prepared by weighing 100 mg of each formulation, dissolving in 10 mL of methanol (10,000 µg/mL), and incubating in the dark at room temperature for 30 min with vortex mixing [30].

For the assay, 2 mL of 200 µg/mL DPPH solution was mixed with hydrogel sample solutions at final concentrations of 2000, 4000, 6000, and 8000 µg/mL, adjusted to a total volume of 10 mL with methanol. The mixtures were homogenized and incubated in the dark for 30 min, and absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Antioxidant activity was

expressed as % inhibition of DPPH radical, and IC50 values were determined from the regression equation of concentration (x-axis) versus % inhibition (y-axis). The IC50 value represents the concentration required to inhibit 50% of the DPPH radicals, where lower IC50 indicates stronger antioxidant activity.

2.7 Data analysis

Data were analyzed qualitatively and quantitatively. Antioxidant activity was described and compared with a commercial burn gel reference following the Blois method. Antimicrobial activity was analyzed using SPSS v25 through Shapiro–Wilk and Levene's tests, followed by one-way ANOVA and Duncan's Multiple Range Test (DMRT) to determine significant differences among treatments.

3. Results and Discussion

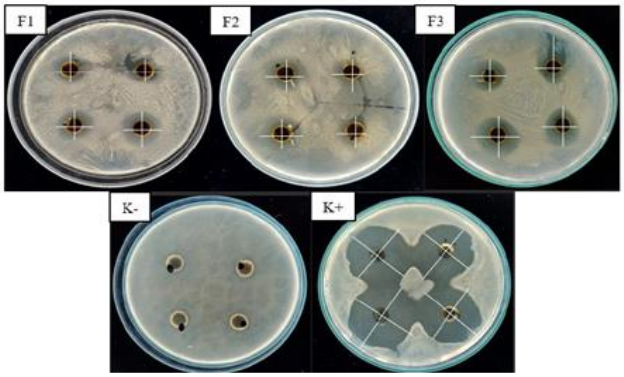
3.1 Antimicrobial activity of Centella asiatica and *Moringa oleifera hydrogel formulation*

The hydrogel formulations exhibited antimicrobial activity against both *Staphylococcus aureus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative). The 10% extract formulation (F3) showed the strongest inhibition, with zones of 11.82 ± 1.70 mm for *S. aureus* and 4.68 ± 1.09 mm for *P. aeruginosa* (Table 1). All extract-based hydrogels showed strong inhibition against *S. aureus* (≥10 mm) but only weak inhibition against *P. aeruginosa* (<5 mm). In comparison, the commercial product Bioplacenton showed inhibition zones of over 22 mm for both bacteria.

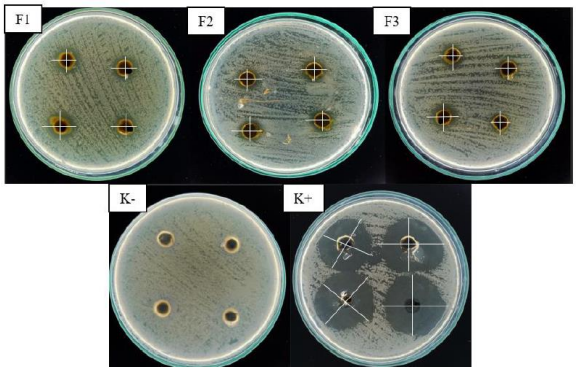
Table 1. Average inhibition zone diameters of hydrogel formulations.

Formula	Inhibition Zone Diameter (mm) ± SD			
	S. aureus		*P. aeruginosa*	
	Average	Category	Average	Category
K-	0,00	None	0,00	None
F1	11,10±1,33	Strong	4,08±0,33	Weak
F2	10,76±0,43	Strong	4,31±0,72	Weak
F3	11,82±1,70	Strong	4,68±1,09	Weak
K+	23,58±1,77	Very Strong	22,96±1,40	Very Strong

The extract-containing hydrogels (F1, F2, F3) did not differ significantly from the commercial product against *S. aureus*, indicating comparable efficacy. This result is based on statistical analysis using SPSS, where one-way ANOVA followed by a post hoc test revealed no significant difference ($p > 0.05$) between the extract-containing hydrogels (F1, F2, F3) and the commercial product (Bioplacenton) against *S. aureus*. The greater effectiveness against Gram-positive bacteria is explained by their simpler cell wall structure, allowing easier penetration of bioactive compounds (31). Gram-negative bacteria, protected by an outer membrane with β-barrel proteins, are more resistant to external agents (32).



(a)



(b)

Figure 1. Inhibition zone diameter of hydrogels with three variations of extract concentration and control against *S. aureus* (a) *and P. aeruginosa* (b).

Centella asiatica contains multiple bioactive compounds that contribute to its antibacterial potential. Chonsut et al. [33] identified three major constituents, namely madecassic acid, madasiatic acid, and asiatic acid, which are associated with strong antimicrobial properties. Asiatic acid, a pentacyclic triterpenoid uniquely found in C. asiatica, has been extensively reported for its antibacterial activity [2]. Madecassic acid (MA), another terpene compound, exhibits multiple biological effects, including antibacterial potential [34]. Wei et al. [35] demonstrated that MA increases extracellular galactosidase and alkaline phosphatase activity, indicating cell wall damage and disruption of membrane permeability in *S. aureus*. Furthermore, MA inhibits key metabolic enzymes such as succinate dehydrogenase (SDH) and malate dehydrogenase (MDH), thereby reducing bacterial energy metabolism [36]. DNA interaction assays revealed that MA can bind plasmid DNA (pET-28a), causing relaxation of supercoiled structures and destabilization of bacterial genetic material [37]. These findings suggest a dual antibacterial mechanism: disruption of cell membranes and metabolic inhibition, combined with direct genetic interference.

Moringa oleifera leaves also contain bioactive compounds with antibacterial potential. Nugraha et al. [38] identified glycosides, benzoic acid, palmitic acid, and linolenic acid in nanosuspension extracts, which contribute to antioxidant and antibacterial activity. Begum and Jayawardana [39] reported that pterygospermin, a compound found in *M. oleifera* leaves, readily

degrades into benzyl isothiocyanate, an established antimicrobial agent. Abdallah et al. [40] confirmed that benzyl isothiocyanate disrupts nucleic acid synthesis, damages cell membranes, and interferes with energy metabolism by inhibiting ATPase and phospholipase activity.

The higher concentration in F3 suggests enhanced antibacterial efficacy. Phytochemical screening further demonstrated that *M. oleifera* contains flavonoids, terpenoids, tannins, and alkaloids, which are associated with antimicrobial and antioxidant activities [41]. In this study, the hydrogel formulation exhibited stronger antibacterial activity against Gram-positive bacteria (*S. aureus*) compared with Gram-negative bacteria (*P. aeruginosa*). This difference may be attributed to the structural complexity of Gram-negative bacterial cell walls. Gram-positive bacteria possess teichoic acids embedded within the peptidoglycan layer, which increase the negative charge of the cell wall and enhance cationic interactions, thereby facilitating lysis [33]. Conversely, Gram-negative bacteria possess an additional outer membrane stabilized by β-barrel porin proteins, reducing permeability and conferring intrinsic resistance. The peptidoglycan layer serves as a critical site of attack in Gram-positive bacteria, where its loss or damage compromises cell wall integrity and increases susceptibility to antibacterial agents [42].

3.2 Antioxidant activity of Centella asiatica and *Moringa oleifera hydrogel formulation*

The antioxidant activity of the nanoparticle hydrogel formulations was assessed using the DPPH radical scavenging method. As shown in Table 2., all extract-containing formulations exhibited weak antioxidant activity. The F3 formula (10%) demonstrated the lowest IC_{50} value (7,849.47 ppm), which was significantly better than F2 (11,900.71 ppm) and F1 (20,161.29 ppm). In contrast, the negative control (0%) and the commercial comparator Bioplacenton hadextremely high IC_{50} values (173,514.3 ppm and 259,423 ppm), indicating negligible antioxidant activity.

Table 2. IC50 by DPPH radical scavenging assay of tested hydrogel formulations.

Sample	IC_{50} (ppm)
K- (0 %)	173,514.3
F1 (2,5 %)	20,161.29
F2 (5 %)	11,900.71
F3 (10 %)	7,849.47
K+ (Bioplacenton)	259,423

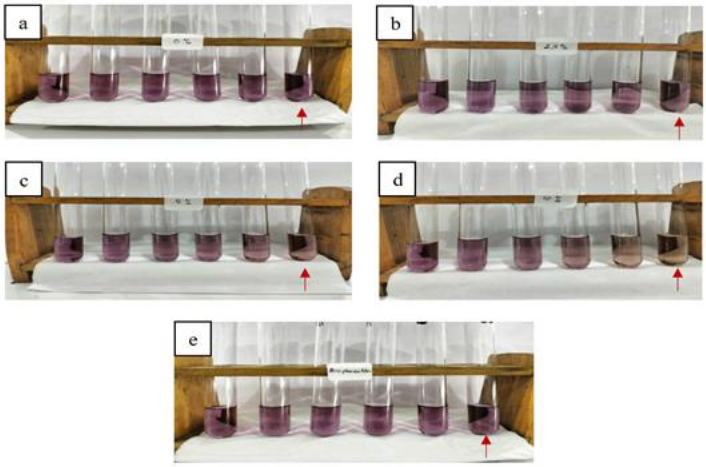


Figure 2. Antioxidant activity of hydrogel formulations: (a) F0 (0%, K–), (b) F1 (2.5%), (c) F2 (5%), (d) F3 (10%), and (e) positive control (K+).

The antioxidant assay revealed that the F3 hydrogel formulation containing 10% extract demonstrated a significantly higher activity, 33 times greater than the commercial control, highlighting the potential of *Centella asiatica* and *Moringa oleifera* extracts as natural antioxidant agents. The results confirmed a concentration-dependent effect, where higher extract content enhanced the radical scavenging activity of the hydrogel formulations. This finding is consistent with previous studies showing that asiatic acid, madecassic acid, and asiaticoside from *C. asiatica*, along with quercetin, kaempferol, rutin, and moringin from *M. oleifera*, contribute to strong antioxidant capacity through hydrogen donation, ROS inhibition, lipid peroxidation suppression, and activation of endogenous antioxidant pathways [14,43]. Additionally, *M. oleifera* extracts were reported to accelerate wound healing by reducing oxidative stress and upregulating enzymatic antioxidants such as SOD and CAT [44].

Despite these promising results, the overall antioxidant activity of the hydrogel formulations containing 2.5%, 5%, and 10% extracts was still classified as weak, likely due to the limited proportion of active compounds incorporated in the gel matrix. A similar observation was reported by Kabiru et al [45], where hydrogels containing 10% *M. oleifera* extract displayed higher activity than lower concentrations, but remained less potent compared to pure extracts. Bioplacenton was selected as the reference formulation because it represents a standard topical hydrogel widely used in clinical practice for wound healing. Bioplacenton, the commercial comparator containing placental extract and neomycin sulfate, exhibited no notable antioxidant activity as its mechanism of action is primarily antibacterial and tissue-regenerative [46-47]. However, preliminary extraction studies using PEF-MUAE demonstrated very strong antioxidant potential of combined *C. asiatica* and *M. oleifera* extracts (IC_{50} = 34.40 ppm) [47], indicating that optimization of extract loading could further enhance the antioxidant efficacy of the hydrogel formulations. Although the IC_{50} values categorize the activity as weak, the F3 hydrogel was shown to be 33 times more potent than the commercial product. This is consistent with Muchtaromah et al. [9], who demonstrated strong radical scavenging activity from a PEF-MUAE extracted blend of *Centella asiatica* and *Moringa oleifera* leaves.

4. Conclusion

The hydrogel formulation combining *Centella asiatica* and *Moringa oleifera* demonstrated promising antioxidant and antimicrobial properties. Antimicrobial testing revealed strong inhibition against *Staphylococcus aureus* (10.76±0.43 to 11.82±1.70 mm) and weaker activity against *Pseudomonas aeruginosa* (4.08±0.33 to 4.68±1.09 mm). The 10% extract hydrogel showed the highest antioxidant activity with an IC_{50} value of 7,849.47 ppm, which is about 33 times more effective than the commercial gel (IC_{50} 259,423 ppm). The use of plant-derived ingredients in this formulation supports the development of safer and more sustainable alternatives for topical wound treatment.

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