



Antioxidant Activity Test and Antibacterial Gel Formulation of Ethanol Extract of Black Ginger (*Curcuma aeruginosa* Roxb.) Rhizome

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Abstract

Curcuma aeruginosa Roxb, commonly known as black ginger or wild turmeric, contains several natural compounds that have demonstrated remarkable pharmacological properties. Consequently, considering the information presented above, the purpose of the current study was to analyze the antioxidant potential, create a topical ointment of *C. aeruginosa* ethanol extract (CAEE), and evaluate the antibacterial activities of the ointment. Research methods included the extraction of *C. aeruginosa* through maceration with 96% ethanol as the solvent, phytochemical screening, antioxidant measurement using the DPPH assay, formulation (0-15%), and evaluation of the ointment, and testing of the ointment's antibacterial activity using the disc diffusion method. According to the findings of the phytochemical screening, CAEE was found to contain a number of phytochemical substances, including flavonoids, alkaloids, tannins, and steroids. The researchers measured CAEE's antioxidant activity, finding it to possess an IC₅₀ value of 6.03 µg/ml. The outcomes of the organoleptic test, the homogeneity test, and pH test were consistent with the parameters for ointment formulations. Furthermore, the antibacterial properties of 15% CAEE ointment (F4) were found to be the most effective against *Staphylococcus aureus* and *Escherichia coli*, respectively. The research revealed that CAEE and its ointment formulation have the potential to be utilized as possible antioxidant and antibacterial agents, as well as resources for the development of novel medications.

Keywords: Antibacterial; Antioxidant; *Curcuma Aeruginosa*; Ethanol Extract

Introduction

Curcuma aeruginosa Roxb. (CA), also known as black ginger, is an indigenous medicinal plant found in Southeast Asia. It belongs to the *Zingiberaceae* family and is well-known for its rich phytochemical composition (Elhawary, Moussa & Singab, 2024). Essential oils derived from various *Zingiberaceae* species, including *Zingiber officinale* and *Curcuma* species, have shown diverse biological activities such as antioxidant, antibacterial, and anti-inflammatory effects (Chen et al., 2025). This is further supported by studies on red ginger (*Zingiber officinale* var. *rubrum*) which demonstrated therapeutic efficacy in inflammatory conditions such as arthritis and gout (Anggraeni et al., 2023). CA has long been used in traditional medicine for its diverse health benefits, including anti-inflammatory, anticancer, wound healing, and analgesic properties. Several natural compounds have been isolated from *Curcuma* species, including curcuminoids such as curcumin, demethoxycurcumin, and bisdemethoxycurcumin, which are known for their antioxidant and anti-inflammatory properties (Li et al., 2014). The plant also contains essential oils that contribute to its aroma and may have antimicrobial properties (Akarchariya et al., 2017). Additionally, CA contains sesquiterpenes, such as curcumol, curzerenone, and curdione,

which have been studied for their various biological activities, including anti-inflammatory, analgesic, and anticancer effects (Suphrom *et al.*, 2012). These compounds, along with others found in the plant, contribute to its pharmacological properties and traditional uses in medicine.

Natural products have garnered considerable attention for their antioxidant activity, which shows promise in promoting health and preventing diseases such as cardiovascular diseases, neurological disorders, and cancer (Zehiroglu & Ozturk Sarikaya, 2019). Antioxidants are substances that inhibit the process of oxidation in other molecules, thereby protecting cells from damage caused by free radicals (Chaudhary *et al.*, 2023). *Curcuma* genus is well-known for containing compounds with antioxidant properties, however, additional research is required to assess the capacity of CA as a source of antioxidants.

Infectious diseases, especially bacterial infection pose a significant threat to global health, representing a leading cause of mortality worldwide (Salam *et al.*, 2023). One of the most pressing challenges in combating these diseases is the emergence of antibacterial resistance. The issue of antibiotic resistance is a pressing global health problem that threatens the advancements made in medicine over the past few decades (Salam *et al.*, 2023). Misuse and overuse of antibiotics in humans, animals, and agriculture have accelerated the development of antibiotic-resistant bacteria. These bacteria can survive and multiply in the presence of antibiotics, making infections caused by them increasingly difficult to treat (Prestinaci, Pezzotti & Pantosti, 2015). This phenomenon diminishes the effectiveness of existing treatments and can result in longer illness durations, higher medical costs, and early mortality, leading to significant public health repercussions. Finding new antibacterial agents is crucial to combat this problem and ensure that effective treatments remain available for bacterial infections.

Despite the potency of antioxidant and antibacterial of CA, there is limited research on its formulation into pharmaceutical dosage forms. Formulation into an ointment dosage form can enhance the stability and applicability of the extract for topical applications. Therefore, this study aims to evaluate the antioxidant activity of ethanol extract of CA (CAEE) rhizome and formulate it into an ointment for potential use as an antibacterial agent. The findings of this study could aid in creating new natural products for preventing and treating diseases related to oxidative stress and bacterial infections.

Material and Methods

Material

Ascorbic acid, amoxicillin, and DPPH (2,2-diphenyl-1-picrylhydrazyl) were acquired from Sigma-Aldrich. All solvents and reagents used in the analysis were of analytical grade. Fresh rhizomes of CA were sourced from the local market in Medan, Indonesia. The plant specimen was identified and verified by the Herbarium Medanense (MEDA) at Universitas Sumatera Utara (No: 826/MEDA/2022).

Plant extraction

The extraction process is carried out using the maceration method. A total of 1000 grams of CA rhizomes are first cleaned, sliced, dried, and ground into powder by using mechanical blender. The powder is then soaked in 70% ethanol for 3x24 h and filtrated to obtain CAEE. Subsequently, the filtrates are combined and subjected to evaporation through a rotary evaporator, operating at a temperature range of 40-50°C, in order to yield a concentrated extract of CAEE.

Phytochemical screening

Qualitative phytochemical screening was conducted on the crude CAEE to identify various phytochemicals. Alkaloids were tested using Mayer/Bouchardat/Dragendorff reagents, flavonoids with Shinoda test, tannins using ferric chloride, saponins with a foam test, and steroids using the Liebermann-Burchard test. Positive results were indicated by the formation of precipitates, colored complexes, or stable foam, indicating the presence of these compounds in the extract.

Determination of antioxidant activity of CAEE

The antioxidant activity of CAEE was assessed using the DPPH radical scavenging test. Various concentrations of CAEE (0-8 µg/mL) were prepared in ethanol. A 0.5 mM DPPH solution in ethanol was also prepared. Each CAEE dilution was mixed with the DPPH solution and incubated in darkness at room temperature for 60 minutes. Subsequently, the UV-Vis spectrophotometer was used to measure the absorbance of the reaction mixture at a wavelength of 516 nm. A standard curve using ascorbic acid was generated, and the percentage of DPPH radical scavenging activity was calculated. The IC50 value, representing the concentration of CAEE required to scavenge 50% of DPPH radicals, was determined by plotting scavenging activity (%) against concentration (µg/mL). Control samples without CAEE were included.

Formulation of ointment containing CAEE

CAEE was formulated as ointment by different concentrations 5%, 10%, and 15%. The entire material is weighed, then Adeps lanae and vaselin album were placed into a crucible and melted. Next, the melted mixture is transferred to a mortar and homogeneously ground. All the formulas can be seen in Table 1.

Table 1: Ointment formulation formula

Material	F1	F2	F3	F4
CAEE	-	2.5 g	5 g	10 g
Adeps Lanae	3 g	2.62 g	2.25 g	1.5 g
Vaseline album	17.g	14.88 g	12.75 g	8.5 g
m.f. Unguenta	20 g	20 g	20 g	20 g

Physical evaluation of the formulation

Physical organoleptic testing was conducted to observe the physical appearance of the preparation by examining its appearance, color, odor, and texture as previously described (Togatorop, *et al.*, 2018).

Homogeneity testing

Homogeneity testing was conducted to determine whether the prepared formulation was homogeneous. This was done by spreading the ointment on a transparent glass surface and taking samples from three different sections: the top, middle, and bottom. The formulation was considered homogeneous if no coarse particles were observed in the ointment.

pH measurement

The pH value was measured using a pH meter. The pH meter was immersed in a solution of 0.5 grams of ointment diluted with 5 ml of distilled water (aquadest). An acceptable pH range for the ointment is 5.6-7.0, which corresponds to the pH of the oral mucosa.

Antibacterial investigation of CAEE ointment

The antibacterial effect of CAEE was assessed using the disk diffusion technique, following the procedure outlined by Sinaga *et al.* (2022). *Staphylococcus aureus* and *Escherichia coli* suspensions were adjusted to a 0.5 McFarland concentration to achieve a bacterial count of 10^5 to 10^8 colony-forming units per milliliter (CFU/mL). The standardized bacterial solution was then evenly applied onto Mueller-Hinton Agar plates. Disks, free from microorganisms, were soaked with different concentrations of CAEE and amoxicillin, which served as a positive control. These disks were placed on the surface of the agar. The plates were then incubated at 37°C for 24 h. The antibacterial activity of CAEE was evaluated by measuring the diameter of the clear zones of inhibition after incubation.

Statistical analysis

The statistical analysis was performed using one-way ANOVA, and multiple comparisons between data sets were made using Tukey's honest significant difference test. The software used for the analysis was GraphPad Prism 8, and a significance threshold of $p < 0.05$ was set.

Results

Preliminary Secondary Metabolites Analysis

Several chemical assays were performed to detect secondary metabolites. The results are summarized in Table 2. CAEE was found to contain flavonoids, alkaloids, saponins, tannins, and steroids, as indicated by the presence of these secondary metabolites.

Table 2: Phytochemical screening results from CAEE

No	Class of secondary metabolite	Methods/Reagents	Results
1	Flavonoids	Shinoda Test	Positive
2	Alkaloids	Mayer	Positive
		Bouchardat	Positive
		Dragendorff	Positive
3	Saponins	Foam Test	Positive
4	Tannins	Ferric Chloride	Positive
5	Steroids	Liebermann-Burchard	Positive

Antioxidant Activity

The UV-Visible spectrophotometer was used to measure the maximum absorbance of a 10 µg/mL DPPH solution in methanol, which was found to be 1.09502 at a wavelength of 516 nm. This wavelength is situated within the visible light spectrum, which ranges from 400 to 800 nm, and specifically falls within the absorption range of DPPH, which is between 515 and 520 nm. The solution exhibited a dark violet color, indicative of the presence of DPPH in its methanol solvent. This color and absorbance measurement are critical for assessing the antioxidant activity, as they reflect the extent to which DPPH radicals are quenched by the sample.

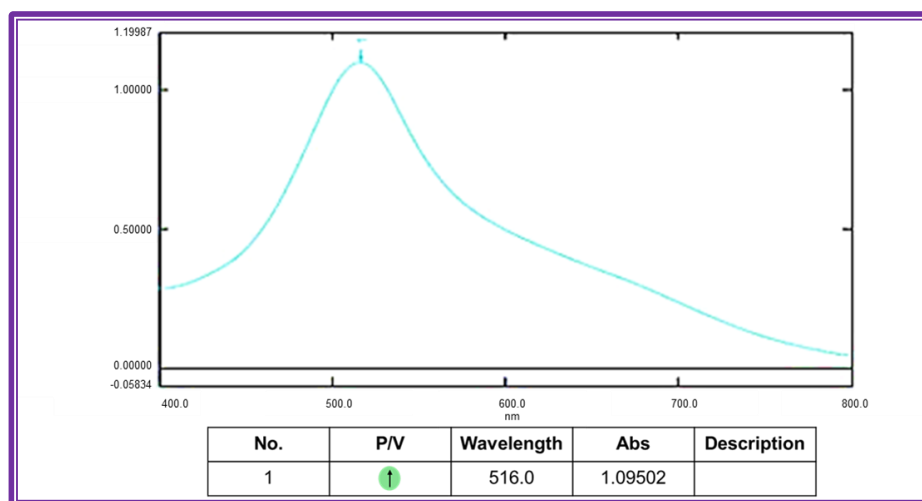


Figure 1: Maximum Wavelength Curve of DPPH Solution

The results of the DPPH scavenging percentage against CAEE and ascorbic acid as shown in Table 2 indicate that the higher the concentration of the test solution, the greater the percentage of scavenging. This is due to a decrease in absorbance during measurement. At a concentration of 6 µg/mL, the scavenging percentage of the test solution against DPPH reached 49.75%. Table 3 also shows an increase in the scavenging percentage by vitamin C with each increase in concentration. At a concentration of 6 µg/mL, the scavenging percentage of ascorbic acid against DPPH reached 64.72%. A decrease in absorbance values indicates greater antioxidant activity. CAEE showed a lower scavenging percentage of DPPH compared to ascorbic acid. Interestingly, both CAEE and ascorbic acid showed a strong IC₅₀ with 6.03 and 5.46 µg/mL, respectively.

Table 3: DPPH Scavenging Results and IC₅₀ Values

Groups	Concentration (µg/mL)	Average Percentage Inhibition	IC ₅₀ (µg/mL)
CAEE	0	0.00	6.03
	2	22.88	
	4	36.48	
	6	49.75	
	8	60.43	
Ascorbic acid	0	0.00	5.46
	2	25.91	
	4	43.71	
	6	64.72	
	8	90.28	

Evaluation of CAEE ointment

The organoleptic properties of the CAEE ointment formulation were evaluated based on appearance, color, odor, and texture. The CAEE ointment exhibited a smooth and uniform appearance, with a consistent dark brown color indicative of the presence of CAEE. The odor was mild and pleasant, and the texture was non-greasy and easily spreadable. All organoleptic parameters met the required criteria. The homogeneity test results demonstrated that the CAEE ointment formulation maintained consistent color, texture, and uniform distribution of CAEE across all sampled locations within the batch. This indicates that the formulation process effectively produced a homogeneous product. The pH of the CAEE ointment formulation was measured to ensure it falls within the acceptable range for topical applications. The pH values of all formula of CAEE ointment samples were all within the acceptable range of 4.5 to 6.5, suitable for topical applications, indicating that the formulation is gentle on the skin. The results are summarized in Table 4.

Table 4: Physicochemical evaluation of the formulated CAEE ointments

Parameters	Formula			
	F1	F2	F3	F4
Appearance	Smooth	Smooth	Smooth	Smooth
Color	Clear	Light brown	Brown	Brown
Odor	Mild and pleasant	Mild and pleasant	Mild and pleasant	Mild and pleasant
Texture	Non-greasy	Non-greasy	Non-greasy	Non-greasy
Homogeneity	Consistent and uniform	Consistent and uniform	Consistent and uniform	Consistent and uniform
pH	6.07	5.08	5.8	5.96

Antibacterial testing of CAEE ointment

The study investigated the antibacterial activity of ointments containing CAEE against the growth of *Staphylococcus aureus* and *Escherichia coli*. The assays were conducted by incubating media mixed with a bacterial suspension of *Staphylococcus aureus* and *Escherichia coli*. at 37°C for 24 h, followed by the observation of clear zones around paper discs placed on the media. Results indicated that all concentrations of the CAEE ointment exhibited antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*. The findings revealed that clear zones formed around each paper disc treated with the ointment with CAEE concentrations of 5% (F2), 10% (F3), and 15% (F4). The antibacterial results are summarized in Figure 2.

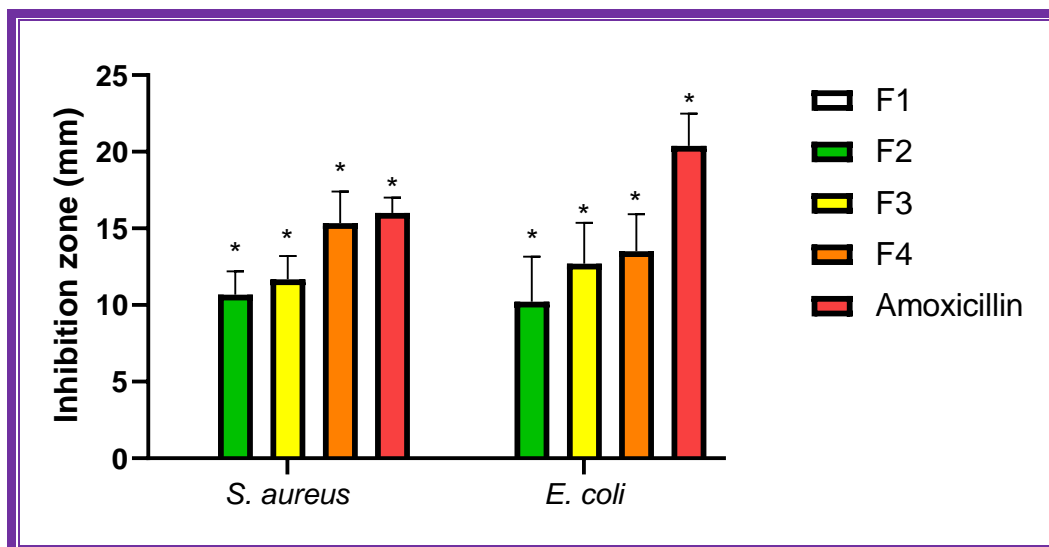


Figure 2: The antibacterial results of the CAEE ointment against *Staphylococcus aureus* and *Escherichia coli*. * $p < 0.05$ vs F1.

Discussion

The phytochemical screening of the CAEE ointment formulation unveiled a rich composition of bioactive compounds essential for therapeutic efficacy. Alkaloids, known for their analgesic properties, offer pain relief and anti-inflammatory effects, making them valuable in soothing skin irritations and promoting healing (Jiang *et al.*, 2022). Flavonoids, potent antioxidants with anti-inflammatory properties, contribute to reducing oxidative stress and protecting skin cells from damage caused by free radicals (Čižmárová *et al.*, 2023; Hasnat *et al.*, 2024). Tannins, recognized for their astringent qualities, aid in skin tightening and provide a protective barrier against infections, thereby enhancing the ointment's wound-healing capabilities (Chokocho & van Hasselt, 2005). Additionally, the presence of steroids complements these effects by further reducing inflammation and swelling, supporting the formulation's overall anti-inflammatory action (Semwal *et al.*, 2022). CAEE demonstrated robust antioxidant activity, evidenced by a DPPH scavenging percentage of 6.03, closely comparable to ascorbic acid at 5.46. This high antioxidant capacity underscores its ability to combat oxidative stress, a key contributor to skin aging and various dermatological conditions. Such potent antioxidant properties position the CAEE ointment as a promising product for maintaining skin health and preventing premature aging. Recent studies have highlighted similar trends, such as the use of natural compounds from *Curcuma amada*, *Curcuma caesia*, *Curcuma longa*, and *Curcuma zedoaria* in green nanotechnology-based formulations, which demonstrated enhanced bioactivity including antioxidant and antimicrobial properties (Gangal *et al.*, 2025). These findings reinforce the therapeutic relevance of *Curcuma* species Gangal and support the use of plant-based strategies in modern pharmaceutical applications.

In terms of formulation quality, all variants of the CAEE ointment met stringent criteria for appearance, color, odor, texture, homogeneity, and pH. The ointment exhibited a smooth, uniform texture with a consistent light green color indicative of its natural ingredients, complemented by a mild and pleasant eucalyptus fragrance suitable for topical application. Its non-greasy and easily spreadable texture ensures user comfort and convenience during application, enhancing its practicality as a daily skincare regimen. Furthermore, the ointment demonstrated substantial antibacterial efficacy, as indicated by inhibition zones ranging from 10 to 16 mm against tested bacterial strains. This potency can be attributed to the presence of bioactive compounds such as curcuminoids and essential oils found in *Curcuma* species, which are known for their antimicrobial properties (Amalraj *et al.*, 2017; Wu *et al.*, 2024). These compounds likely disrupt bacterial cell membranes, interfere with cellular processes (Shome, Talukdar & Upadhyaya, 2022), or induce oxidative stress (Dai *et al.*, 2022), thereby compromising bacterial viability. In line with this, Yadav *et al.* (2025) have also shown that plant-based

bioactive formulations, such as Jumli Marsi rice extract, exhibited promising antioxidant, antibacterial, and antidiabetic activities, further validating the role of functional natural ingredients in combating microbial threats. The broad-spectrum activity observed suggests that CAEE-based formulations could serve as valuable alternatives or adjuncts to conventional antibacterial treatments, particularly in contexts where antibiotic resistance poses a significant challenge. Future research should focus on isolating and characterizing specific active compounds to optimize CAEE formulations for enhanced antibacterial effectiveness and therapeutic applications. Building on our findings, future investigations could explore the potential of CAEE ointment in wound healing and managing oxidative stress-related skin disorders, further expanding its therapeutic value.

Conclusion

This study provides evidence that CAEE contains a diverse array of phytochemical metabolites known for their potent antioxidant and antibacterial properties. When formulated into topical ointments, CAEE demonstrates promising capabilities in inhibiting bacterial growth. This suggests that CAEE-based ointments could potentially offer effective therapeutic benefits for various dermatological applications requiring antioxidant and antibacterial interventions.

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgment

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