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Comparative analysis of *Curcuma longa* (curcumin) and fusidic acid on the antimicrobial susceptibility of *Staphylococcus aureus* and *Streptococcus pyogenes* in impetigo

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Contributions: Fifa Argentina: conceptualization, data analysis and interpretation, writing – original draft; Nopriyati Nopriyati, Riri Puspa Putri Findrapase, Hasbiallah Yusuf, Monica Trifitriana: writing – review & editing; Debby Handayati Harahap: statistical analyses, histological examination, writing – review & editing. All authors approved the final version of the manuscript and have agreed to take responsibility for all aspects of the work.

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Ethics approval and consent to participate: the study protocol was reviewed by the institutional ethics committee, and formal ethical approval was waived because the study used bacterial isolates obtained during routine clinical care. Written consent to participate was obtained from all study participants.

Availability of data and materials: the datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Abstract

Impetigo is a bacterial skin infection primarily caused by *Staphylococcus aureus* or *Streptococcus pyogenes*. *Curcuma longa* contains curcumin, known for its antibacterial, anti-inflammatory, and antioxidant properties. Evaluating the susceptibility of these bacteria to *C. longa* extract can help assess its potential as an antimicrobial agent. This study aimed to determine the susceptibility of *S. aureus* and *S. pyogenes* to *C. longa* extract and fusidic acid in impetigo patients. An *in vitro* experimental study using a posttest-only control group design was conducted from August to October 2023. Bacterial isolates were obtained from 60 impetigo patients (30 *S. aureus* and 30 *S. pyogenes* isolates). The isolates were treated with fusidic acid discs and *C. longa* extracts at 20%, 40%, and 80%. Inhibition zone diameters were measured and analyzed using the disc diffusion method and SPSS 25.0. The mean inhibition zone for *S. aureus* was 10.62 mm (20%), 11.78 mm (40%), and 16.21 mm (80%). For *S. pyogenes*, it was 9.86 mm (20%), 10.99 mm (40%), and 14.91 mm (80%). The Mann-Whitney test found no significant difference in inhibition between fusidic acid 10 µg and *C. longa* 40% or 80% ($p \geq 0.05$). Kruskal-Wallis analysis showed significant differences across treatment groups ($p=0.000$). *C. longa*, 40% and 80%, showed comparable antimicrobial activity to fusidic acid, indicating its potential as an alternative treatment for impetigo.

Introduction

Impetigo is a type of pyoderma caused by *Staphylococcus aureus* or group A *Streptococcus*.[1] Approximately 12% of the global population is at risk of developing impetigo.[2] The prevalence of impetigo in developing countries ranges from 111 to 140 million people.[3] There has been an increase in impetigo patient visits from 1.6% in 2018 to 2.8% in 2022 at the Dermatology, Venereology, and Aesthetic Clinic (DVE) of the Dr. Mohammad Hoesin General Hospital (RSMH), Palembang, Indonesia. In 2021, the Indonesian Society of Dermatology and Venereology (PERDOSKI) recommended fusidic acid as one of the topical management options.[4] Fusidic acid is an effective topical treatment for impetigo with minimal side effects.[1,5] However, a major issue is the emergence of antibiotic-resistant strains, such as methicillin-resistant *S. aureus* (MRSA).[6,7] The fusidic acid resistance rate in *S. aureus* at the DVE Clinic of RSMH in 2017 was 22.2%.[8]

One of the plants from Indonesia, *Curcuma longa*, contains curcumin, a main bioactive compound.[9,10] Curcumin exhibits antibacterial, anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, and anticoagulant properties.[11,12] A study by Adamczak *et al.* showed that *C. longa* is more effective against Gram-positive bacteria.[10] In MRSA cases, curcumin can inhibit the transcription

of the *mecA* gene, leading to a reduced expression of penicillin-binding protein 2α (PBP2α). As a result, MRSA becomes sensitive to β-lactam antibiotics.[13] These findings indicate that the *fusC* gene is commonly associated with fusidic acid-resistant MRSA isolates.[14]

This experimental *in vitro* study aims to evaluate the susceptibility of *S. aureus* and *Streptococcus pyogenes* to the antimicrobial potential of *C. longa* extract and fusidic acid from impetigo patients commonly seen at RSMH Palembang.

Materials and Methods

This is an experimental *in vitro* study with a posttest-only control group design. Sampling for this study was conducted at the DVE Clinic of RSMH Palembang, Division of Infectious Dermatology. The study took place from June 1 to November 30, 2023. The population consisted of bacterial cultures from all impetigo patients visiting the DVE Clinic at RSMH Palembang during the study period. The inclusion criteria included Gram stain results showing Gram-positive cocci, bacterial cultures of *S. aureus* and *S. pyogenes*, and patient consent to participate in the study by signing an informed consent form after receiving an explanation. The exclusion criteria included the use of topical or systemic antibiotics within one week before the study and the presence of other skin diseases that may cause impetigo as a secondary condition, such as scabies, atopic dermatitis, prurigo, and others.

The independent variables were the susceptibility to *C. longa* (curcumin) extract and fusidic acid. The dependent variable was the bacterial culture results, and the covariates included age, gender, education, socioeconomic status, hygiene status, nutritional status, and antibiotic use.

Age was categorized into four groups: <1 year, 1-5 years, 6-10 years, and 11-17 years. Education was categorized as elementary school, junior high school, and senior high school. Socioeconomic status was divided into low, middle, high, and very high. Nutritional status was determined based on body mass index (BMI) calculations according to the World Health Organization (WHO) and the Centers for Disease Control and Prevention standards. Susceptibility testing was based on the measurement of inhibition zone diameters, classified as either sensitive or resistant for *S. aureus* and *S. pyogenes*. The inhibition zone diameters for *C. longa* against *S. aureus* and *S. pyogenes* were classified as follows: ineffective (<10 mm), weak inhibition (10-15 mm), moderate inhibition (16-20 mm), and strong inhibition (>20 mm).

The diagnosis of impetigo was confirmed by a dermatology and venereology physician based on anamnesis, physical examination, and supplementary tests. Supplementary tests included Gram staining, bacterial culture, and susceptibility testing. Samples were collected from lesions using two sterile cotton

swabs for Gram staining and culture. Cultures were grown on blood agar media for *S. aureus* and *S. pyogenes*. Susceptibility testing for *S. aureus* was performed using Mueller-Hinton agar, while *S. pyogenes* was cultured on blood agar. The antimicrobial agents tested were *C. longa* extract (curcumin) and fusidic acid.

The samples in this study were divided into five treatment groups for both *S. aureus* and *S. pyogenes*: a negative control group with a blank disc (Group 1), a positive control group with a 10 µg fusidic acid disc (Group 2), a *C. longa* extract 20% intervention group (Group 3), a *C. longa* extract 40% intervention group (Group 4), and a *C. longa* extract 80% intervention group (Group 5).

Data analysis was performed using SPSS 25.0 (SPSS Inc., Chicago). Descriptive data analysis determined the proportions of study participants based on sociodemographic variables (age, gender, education, nutritional status, and antibiotic use), independent variables (susceptibility test results for *C. longa* extract and fusidic acid), and dependent variables (bacterial culture results). Data normality was assessed using the Shapiro-Wilk test to determine the appropriate statistical tests. Bivariate comparisons of susceptibility test results between two groups were performed using the non-parametric Mann-Whitney test, while multivariate comparisons between the five groups were analyzed using the non-parametric Kruskal-Wallis test.

Results

An experimental (*in vitro*) study with a posttest-only control group design was conducted to assess the differences in the susceptibility of *S. aureus* and *S. pyogenes* from impetigo patients to *C. longa* (curcumin) extract and fusidic acid. A total of 60 bacterial samples were obtained from impetigo patients, consisting of 30 *S. aureus* and 30 *S. pyogenes* bacterial cultures.

In this study, most impetigo patients were female (58.3%). The mean age was 7.58±3.58 years, with the most common age groups being 1-5 years and 6-10 years (41.7% each). Fifty percent of the patients had not attended school and had low socioeconomic status, while 83.3% had a normal BMI. *S. pyogenes* was found in 50% of the samples, while *S. aureus* was found in 41.7% (Table 1).

The susceptibility of *S. aureus* and *S. pyogenes* bacterial isolates was tested using the disc diffusion method against *C. longa* (curcumin) extract and fusidic acid from impetigo patients. A total of 30 *S. aureus* isolates and 30 *S. pyogenes* isolates were tested.

The inhibition zone diameters for the five groups on *S. aureus* and *S. pyogenes* cultures are shown in Figure 1. The inhibition zone diameters were observed to be largest in the fusidic acid 10 µg

intervention group, followed by *C. longa* 80%, *C. longa* 40%, *C. longa* 20%, and the negative control (Table 2).

Based on the bivariate analysis, the results showed a significant difference in the inhibition zone diameter of *S. aureus* between the fusidic acid 10 µg group and the negative control group, as well as between the fusidic acid 10 µg group and the *C. longa* 20% group ($p<0.05$). However, no significant difference in the inhibition zone diameter of *S. aureus* was observed between the fusidic acid 10 µg group and the *C. longa* 40% or *C. longa* 80% groups ($p\geq0.05$) (Table 2). Moreover, the results showed a significant difference in the inhibition zone diameter of *S. pyogenes* between the fusidic acid 10 µg group and the negative control group, as well as between the fusidic acid 10 µg group and the *C. longa* 20% and *C. longa* 40% groups ($p<0.05$). However, no significant difference in the inhibition zone diameter of *S. pyogenes* was observed between the fusidic acid 10 µg group and the *C. longa* 80% group ($p\geq0.05$) (Table 2).

Based on the multivariate analysis using Kruskal-Wallis, the results showed a significant difference among the five groups ($p=0.000$; $p<0.050$). The inhibition zone diameters, from largest to smallest, were found in the fusidic acid 10 µg group, followed by *C. longa* 80%, *C. longa* 40%, *C. longa* 20%, and the negative control. From these analyses, it can be concluded that there was no significant difference in the susceptibility of *S. aureus* to *C. longa* 40%, *C. longa* 80%, and fusidic acid 10 µg in impetigo patients at RSMH Palembang (Table 3).

Based on the inhibition zone diameter against *S. aureus* and *S. pyogenes*, it was found that 66.7% of fusidic acid was sensitive to *S. aureus* and 100% of fusidic acid was sensitive to *S. pyogenes* (Table 4). Additionally, a weak inhibition response was observed against *S. aureus* in all groups of *C. longa* 20% and 40%, and was found in only 16.7% of the *C. longa* 80% group. A moderate inhibition response was observed in 83.3% of the *C. longa* 80% group. For *S. pyogenes*, a weak inhibition response was found in 83.3% of the *C. longa* 20% group (with 16.7% showing a less effective inhibition response), 100% in the *C. longa* 40% group, and only 33.3% in the *C. longa* 80% group. A moderate inhibition response was found in 66.7% of the *C. longa* 80% group. No strong inhibition response was observed for either *S. aureus* or *S. pyogenes* (Table 5).

Discussion

Impetigo is a superficial skin infection primarily caused by *S. aureus* and *S. pyogenes*, with risk factors including age, sex, socioeconomic status, and nutritional condition. In this study, most patients were female (58.3%), consistent with previous reports showing a higher prevalence in females.[15,16] The

majority of impetigo patients were between 1-5 years and 6-10 years old, with an average age of 7.57±3.58 years, in line with earlier studies.[1,17,18]

The majority of children in this study had no formal education and were from low socioeconomic backgrounds, which may increase infection risk due to poor hygiene and environmental conditions.[19-23] Most of the patients had a normal weight BMI (83.3%), followed by underweight (16.7%). These findings are consistent with previous studies suggesting that nutritional status was not a major contributing factor.[20,21]

According to the WHO, the main bacteria causing superficial pyoderma in tropical countries are *S. pyogenes* and *S. aureus*.[22] In this study, the most common bacteria were *S. pyogenes* (50.0%), followed by *S. aureus* (41.7%), and a mixture of both (8.3%), in agreement with epidemiological data from tropical regions.[24]

Turmeric contains natural phenolic compounds such as curcuminoids and sesquiterpenoids.[25] Curcuminoids consist of the main bioactive compound, curcumin, which functions as a strong antioxidant, anti-inflammatory, antibacterial, antifungal, and antiviral agent. Curcuminoids include curcumin (77%), demethoxycurcumin (17%), and bidemethoxycurcumin.[9,26] Sesquiterpenoid compounds include ar-turmerone, curlone, bisacumol, zingiberene, curcumene, germacrone, curcuminol, and bsabolene. The antibacterial mechanism of curcumin against *S. aureus* involves several mechanisms, including binding to the filamenting temperature-sensitive mutant Z (FtsZ) protein, thereby inhibiting the binding of FtsZ protofilaments, suppressing the formation of the Z-ring, and inhibiting cytokinesis and bacterial proliferation.[13]

In this study, *C. longa* extract showed dose-dependent antibacterial activity. Weak inhibition was observed at 20% and 40%, while moderate inhibition was observed at 80% against both *S. aureus* and *S. pyogenes*. These findings are consistent with previous studies demonstrating increased inhibition with higher extract concentrations.[27-29] *C. longa* at concentrations of 40% and 80% showed inhibition zones comparable to fusidic acid against *S. aureus*, while only the 80% concentration showed comparable results against *S. pyogenes*. However, its activity remained slightly lower than that of fusidic acid. The study concluded that *C. longa* could be considered a promising antibacterial agent due to its effectiveness against several species and strains of *S. pyogenes* (median minimum inhibitory concentration: 31.25 µg/mL) and methicillin-sensitive *S. aureus* (250 µg/mL).[10]

Fusidic acid inhibits bacterial protein synthesis by binding to elongation factor G, making it a potent antimicrobial agent.[30] The lower inhibition zones observed with *C. longa* suggest that although it has promising activity, it may not fully replace conventional antibiotics.

The mechanism of action of *C. longa* as an antibacterial agent against *S. aureus* includes interaction with the FtsZ, thereby inhibiting bacterial cell proliferation. Moreover, *C. longa* is also active against drug-resistant bacterial strains, such as MRSA. *C. longa* inhibits the transcription of the *mecA* gene, leading to a decrease in the expression of PBP2α. As a result, MRSA becomes sensitive to antibacterial actions of β-lactam antibiotics such as penicillin and methicillin. Some studies have shown that fusidic acid resistance genes, such as *fusA*, *fusB*, and *fusC*, are also found in MRSA isolates. The *fusC* gene in fusidic acid resistance cases is incorporated into the Staphylococcal cassette chromosome *mec* (SCC*mec*) region of MRSA strains. The integration of the *fusC* gene into this SCC*mec* region is believed to facilitate the rapid spread of MRSA, even from genetically diverse backgrounds. The use of *C. longa* may provide a solution for treating cases resistant to fusidic acid.[31,32]

Experimental *in vitro* studies with higher concentrations of *C. longa* for assessing its antibacterial effects and comparing the inhibition zone diameter against bacterial growth larger than that of fusidic acid, as the positive control group, are necessary for the foundation of further research. Overall, this study provides insights into the benefits of *C. longa* extract as an antibacterial agent for impetigo lesions. The results of this study can serve as a basis for future research, such as *in vivo* experimental studies on animal models and clinical trials to standardize *C. longa* as an antibacterial treatment for impetigo patients.

Conclusions

The susceptibility of *S. aureus* to fusidic acid from impetigo patients at the DVE outpatient clinic, RSMH Palembang, was 66.7%. The inhibition zone diameter for *S. aureus* against fusidic acid was below normal in 33.3% of impetigo patients. The susceptibility of *S. pyogenes* to fusidic acid from impetigo patients was 100%. No significant differences were observed in the susceptibility of *S. aureus* to *C. longa* 40%, *C. longa* 80%, and fusidic acid 10 µg from impetigo patients. Similarly, no differences were found in the susceptibility of *S. pyogenes* to *C. longa* 80% and fusidic acid 10 µg from impetigo patients.

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Table 1. Patient's characteristics.

Characteristic	Total (n)	Percentage (%)
Gender		
Male	5	41.7
Female	7	58.3
Age (years)		
1-5	5	41.7
6-10	5	41.7
11-17	2	16.7
Age		
Average (SD)	7.57 (3.58)	
Median (min-max)	5.75 (4-16)	
Education		
Not yet in school	6	50.0
Elementary	5	41.7
Junior high	0	0.0
Senior high	1	8.3
Economic status		
Low	6	50.0
Middle	4	33.3
Upper	2	16.7
BMI		
Underweight	2	16.7
Normoweight	10	83.3
Antibiotic use		
Yes	0	0.0
No	12	100
Bacteria		
S. aureus	5	41.7
S. pyogenes	6	50.0
S. aureus + *S. pyogenes*	1	8.3
Total	12	100

BMI, body mass index.

Figure 1. Diameter of the inhibition zone in (**A**) *S. aureus* culture with fusidic acid as a positive control and (**B**) *S. pyogenes* culture with fusidic acid as a positive control.

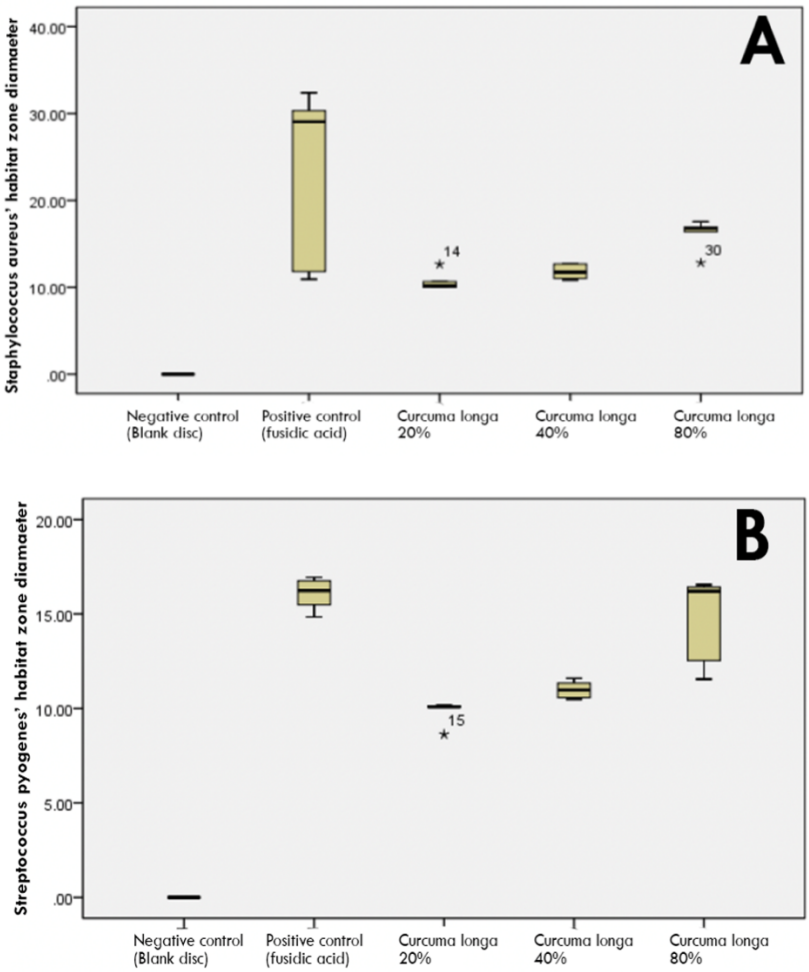

Table 2. Comparison of the susceptibility of *S. aureus* and *S. pyogenes* isolated from impetigo patients between two groups.

Bacteria	Group 1 (mean±SD)	Group 2 (mean±SD)	p-value
S. aureus	Fusidic Acid 10 µg/mL (23.93±9.83)	Negative control (0.00±0.00)	0.002[a]*
		C. longa 20% (10.62±1.02)	0.010[a]*
		C. longa 40% (11.78±0.89)	0.109[a]
		C. longa 80% (16.21±1.71)	0.337[a]
	Negative control (0.00±0.00)	*C. longa* 20% (10.62±1.02)	0.002[a]*
		C. longa 40% (11.78±0.89)	0.002[a]*
		C. longa 80% (16.21±1.71)	0.002[a]*
	C. longa 20% (10.62±1.02)	*C. longa* 40% (11.78±0.89)	0.025[a]*
		C. longa 80% (16.21±1.71)	0.004[a]*
	C. longa 40% (11.78±0.89)	*C. longa* 80% (16.21±1.71)	0.006[a]*
S. pyogenes	Fusidic Acid 10 µg/mL (16.09±0.64)	Negative control (0.00±0.00)	0.002[a]*
		C. longa 20% (9.86±0.60)	0.004[a]*
		C. longa 40% (10.99±0.45)	0.000[b]*
		C. longa 80% (14.91±2.25)	0.337[a]
	Negative control (0.00±0.00)	*C. longa* 20% (9.86±0.60)	0.002[a]*
		C. longa 40% (10.99±0.45)	0.002[a]*
		C. longa 80% (14.91±2.25)	0.002[a]*
	C. longa 20% (9.86±0.60)	*C. longa* 40% (10.99±0.45)	0.004[a]*
		C. longa 80% (14.91±2.25)	0.004[a]*
	C. longa 40% (10.99±0.45)	*C. longa* 80% (14.91±2.25)	0.006[a]*

[a]Mann-Whitney test; [b]independent *t*-test, *p<0.05; *statistically significant difference (p<0.05).

Table 3. Comparison of the susceptibility of *S. aureus* and *S. pyogenes* bacteria in impetigo patients between all groups.

Group	Mean rank	p-value
S. aureus		0.000*
Negative control	3.50	
Fusidic Acid 10 µg/mL	23.83	
C. longa 20%	10.50	
C. longa 40%	16.17	
C. longa 80%	23.50	
S. pyogenes		
Negative control	3.50	0.000
Fusidic Acid 10 µg/mL	25.50	
C. longa 20%	9.50	
C. longa 40%	15.67	
C. longa 80%	23.33	

*Kruskal-Wallis test, p<0.05.

Table 4. Inhibitory response of fusidic acid to *S. aureus* and *S. pyogenes* bacteria in impetigo patients.

Group	**Bacteria**	**Inhibition zone**	**Total**	**Percentage (%)**
Fusidic Acid	*S. aureus*	Sensitive	4	66.7
		Resistant	2	33.3
	S. pyogenes	Sensitive	6	100.0
		Resistant	0	0.0

Table 5. Inhibitory response of *C. longa* (all doses) against *S. aureus* and *S. pyogenes* bacteria in impetigo patients.

Bacteria	**Dose**	**Inhibition zone, n (%)**			
		Less effective	Weak inhibitory response	Medium inhibitory response	Strong inhibitory response
S. aureus	20%	0 (0)	6 (100)	0 (0)	0 (0)
	40%	0 (0)	6 (100)	0 (0)	0 (0)
	80%	0 (0)	1 (16.7)	5 (83.3)	0 (0)
S. pyogenes	20%	1 (16.7)	5 (83.3)	0 (0)	0 (0)
	40%	0 (0)	6 (100)	0 (0)	0 (0)
	80%	0 (0)	2 (33.3)	4 (66.7)	0 (0)