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Research Article
Antibacterial Activity of Curcumin in Combination with Tetracycline Against *Staphylococcus aureus* by Disruption of Cell Wall

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Abstract

Background and Objective: Curcumin has long been known to have an antibacterial activity. The main objective of this study was to evaluate the antibacterial activity of curcumin in combination with tetracycline or ciprofloxacin against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) and its underlying mechanism. **Methodology:** The antibacterial activity of the combination of curcumin with tetracycline or ciprofloxacin was determined using the checkerboard method. Furthermore, the antibacterial activity of the chosen combination was determined *in situ* by the recovery rate and wound score of *S. aureus*-infected rabbit's skin following the curcumin-antibiotic combination treatment. Lastly, imaging using scanning (SEM) and transmission (TEM) electron microscopy was done to determine the morphological changes of the curcumin combination-treated *S. aureus*. Data were analyzed by using one-way ANOVA followed by LSD and Tukey *post hoc* analysis using SPSS. **Results:** The results of this study show that combinations of curcumin with tetracycline had a synergistic interaction against *S. aureus* and *E. coli*, while a combination of curcumin with only ciprofloxacin showed a synergistic interaction on *S. aureus*. The combination of curcumin and tetracycline was then chosen for further studies. According to dermal and ocular irritation tests on rabbits, an ointment based on a combination of curcumin with tetracycline did not cause dermal or ocular irritation. Furthermore, the application of a combination of 2% curcumin with 1% tetracycline on *S. aureus*-infected rabbit's skin healed the infected skin faster than the 3% tetracycline ointment alone. This combination was found to cause a distortion and rupture of the bacterial cell wall after being treated with the combination for 18 h. **Conclusion:** Curcumin in combination with tetracycline can be an alternative topical application in the treatment of *S. aureus*-caused skin infections.

Key words: Curcumin, tetracycline, ciprofloxacin, *Staphylococcus aureus*, skin infection

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Combination antibiotic therapy is increasingly being employed to reduce the risk of emerging resistance to antibiotics in bacteria during therapy and to increase the antibacterial spectrum. Beta-lactamase inhibitor-penicillin combinations, such as ampicillin-sulbactam, amoxicillin-clavulanate, ticarcillin-clavulanate and piperacillin-tazobactam are used for beta-lactamase resistant bacteria. Of these antibiotic combinations, ticarcillin-clavulanate and piperacillin-tazobactam have been reported as having the broadest spectra of activity, including against Enterobacteriaceae and *Pseudomonas aeruginosa*[1].

A few studies have found that the antibacterial activity of antibiotics against different bacteria can be improved by combining them with crude plant extracts[2-4]. For example, Indonesian plant extracts such as *Kaempferia pandurata* have shown a synergistic activity in combination with penicillin or ampicillin against methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA)[2]. However, the exact mechanism of these interactions is still under investigation.

Curcuma longa Linn. (Zingiberaceae family), traditionally known as turmeric, is a traditional plant found in Indonesia typically used for spices, colouring, preservative and also for treating bacterial infections. A major compound found in *Curcuma longa* is curcumin, which has been reported to be responsible for most of the plant's biological activities, such as antioxidant, antimicrobial, antitumor and anti inflammatory activities[5-6]. Curcumin also possesses potent antibacterial activity against a wide range of bacteria[7-8] and has synergistic activities with various anti-microbial drugs[9-10]. However, these studies were only conducted in *in vitro* experiments. In this study, the *in situ* antibacterial activity of the combination of curcumin and antibiotics against *Staphylococcus aureus* caused skin infections was evaluated.

MATERIALS AND METHODS

This study was carried out from January, 2015-December, 2016.

Materials: Mueller Hinton Agar (MHA) (Oxoid Ltd., Hampshire, England), Mueller Hinton Broth (MHB) (Oxoid Ltd., Hampshire, England) and MacConkey agar (Oxoid Ltd., Hampshire, England) were used as growth media for the bacteria. Tetracycline HCl, ciprofloxacin HCl, ethanol 70%, NaCl, glutaraldehyde, phosphate buffer, osmium tetroxide, white vaseline and liquid paraffin were purchased from Brataco, Indonesia. Curcumin was obtained from *Curcuma longa* (Sigma Aldrich, St. Louis, MO, USA), which contains 80% curcumin. Dimethyl sulfoxide (DMSO) (Merck, Germany) was used for diluting the curcumin.

Test microorganisms and culture: *Staphylococcus aureus* and *Escherichia coli* were obtained from the culture collection of the School of Pharmacy, Bandung Institute of Technology and were cultured aerobically in MHA and MHB media.

Determination of the susceptibility of bacteria: *In vitro* susceptibility tests were performed in a 96-well microtiter plate to determine the minimum inhibitory concentrations (MICs) of the extracts and tetracycline HCl. The tests were carried out against *S. aureus* and *E. coli* using standard broth microdilution methods with a bacteria inoculum of 5×10^5 CFU mL^{-1}, according to the guidelines of the Clinical and Laboratory Standards Institute CLSI M7-A8 standard[11]. The MIC was defined as the lowest concentration of antimicrobial agents that resulted in the complete inhibition of visible bacterial growth. Furthermore, the minimum bactericidal concentrations (MBCs) were also established for each test sample. Approximately 100 µL of media from each well which showed no visible growth were spread on MHA plates. The plates were then incubated at 37°C for 24 h, or until microbial growth was seen in the growth-positive control plates. The MBC was defined as the lowest concentration of antimicrobial agents at which all bacteria in the culture are killed, or the lowest concentration of drugs that kill 99.9% of the total initially viable cells[12].

Checkerboard assay: The antibacterial activities of the combination of antibiotics and plant extracts were investigated using the checkerboard broth microdilution method. Two-fold serial dilutions of the antibiotic and the plant extracts were prepared for every combination tested and 50 µL aliquots of each component was placed into the wells of the sterile 96-well microtiter plate. The inoculum was prepared using the above-described MIC determination method. The microtiter plates were then incubated at 37°C and the MIC was determined after 18-20 h of incubation.

The fractional inhibitory concentration (FIC index) for all of the combinations was determined using the following formula[13]:

$$\text{FIC index} = \text{FIC}_A + \text{FIC}_B = \frac{[A]}{\text{MIC}_A} + \frac{[B]}{\text{MIC}_B}$$

Where, FIC_A and FIC_B are the fractional inhibitory concentration of drugs A and B, MIC_A and MIC_B are the minimum inhibitory concentration of drugs A and B and [A] and [B] are the

concentration of drugs A and B, respectively. An FIC index of ≤0.5 was interpreted as synergistic, between 0.5 and 4 was interpreted as additive or indifferent and a value of >4 was interpreted as antagonistic[14].

Animals: Six to seven months old male New Zealand albino rabbits (2-3 kg b.wt.,) were obtained from a rabbit husbandry in Lembang, Indonesia. Rabbits were housed individually and were fed a standard pellet diet, fresh vegetables (carrots and cabbage) and tap water. All of the procedures were performed according to the rules of animal handling experiments using animals, of the School of Pharmacy, Bandung Institute of Technology, adopted from the guidelines of "Institutional Animal Care and Use Committee Guidebook"[15].

Curcumin and tetracycline combination ointment: A curcumin and tetracycline combination ointment was made using a base consisting of 90% white vaseline and 10% liquid paraffin. The base was sterilized at 170°C for 1 h. Tetracycline and curcumin were then added to the sterile ointment base and the mixture homogenized.

Acute skin irritation study: An acute skin irritation study was performed for the 1% tetracycline and 2% curcumin combination ointment according to the Organization for Economic Cooperation and Development methodology used for determining the degree of acute dermal irritation/corrosion[16]. Half a gram of ointment was applied to the skin of each animal at a shaved, dorsal area of approximately 6 cm^2. Both of the treated sites on each rabbit were covered by sterile gauze and the backs of the rabbits were wrapped with non-occlusive bandages. After 24 h, the bandage and the test materials were removed and 1 h later the sites were examined for abnormal skin responses, including irritation, redness, itching, inflammation and other related symptoms[17].

Ocular irritation test: At least three albino rabbits were used for the ocular irritation test. One Hundred milligram of the combination ointment was placed in the conjunctival sac of the right eye of each rabbit, the left eye of the rabbits was used as a negative control. The eyelids were then gently held together for about 1 sec in order to prevent loss of the test material. Observations were performed 1, 24, 48 and 72 h after application of the drug. The ocular response was assessed by calculating the score of the cornea, iris, conjunctiva and chemosis[17].

Determination of *in situ* antibacterial activity of a combination of curcumin and tetracycline HCl topically on the back of each rabbit: An area of approximately 6 cm^2 on the back of each rabbit was shaved and the skin divided into six marked areas designated for the positive control, ointment base control, tetracycline HCl 3% (Trifacyclin® as a reference) and three combinations of tetracycline HCl and curcumin (1:2, 1.5:1.5 and 2:1%). Each area of shaved skin was inoculated with bacteria by an intracutaneous injection of 0.1 mL *S. aureus* suspension (10^8 CFU mL^{-1} of bacteria) and was then left for 24 h. The areas were then treated based on their groups by applying half a gram of each sample. After treatment, the treated sites were covered by sterile gauze and wrapped with non-occlusive bandages. The samples were applied every day for 29 days. Observation of skin reactions, including erythema, edema and pus, was carried out every 3 days for 30 days. Evaluation of the skin reactions was performed by calculating the dermal irritation score (DIS)[17].

Scanning electron microscopy: Scanning electron microscopy (SEM) was performed on *S. aureus* treated with curcumin, tetracycline and a combination of curcumin and tetracycline at MIC. *Staphylococcus aureus* was cultured to reach the mid-log growth phase in MHB before use. The control and treated cells were prepared for morphological observation. The bacterial samples were washed 5 times with fresh media and then fixed with 2.5% glutaraldehyde in phosphate buffer (pH 7.2) at 4°C for 1 h, washed 3 times with phosphate buffer for 10 min and fixed with 1% osmium tetroxide for 2 h. This was followed by three washings in the phosphate buffer for 10 min and subsequently dehydrated in a series of ethanol concentrations (30, 50, 70, 90 and 95%), for 15 min each. The samples were subjected to 100% ethanol and CO_2 to achieve the critical point and then coated with gold ion in a pressure metallic chamber. At the end of the process, the samples were submitted for analysis by SEM (Hitachi SU3500, Japan).

Transmission electron microscopy: Bacterial cells treated with a combination of curcumin and tetracycline was observed using transmission electron microscopy (TEM). The cells were harvested after 18 h of incubation and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer for 2 h. The cells were then washed 3 times with 0.05 M phosphate buffer (pH 7.2) and post fixed for 2 h with 1% osmium tetroxide in a 0.1 M phosphate buffer (pH 7.2) at room temperature. After being washed twice in phosphate buffer, the cells were dehydrated through serial graded concentrations of ethanol

(35, 70, 95 and 100%, respectively) for 15 min, then infiltrated and embedded in Spurr's resin. Ultrathin sections were cut with a diamond knife using an ultramicrotome and then mounted on bare copper grids. Finally, specimens were counterstained with 2% (w/v) for 3 min and then with 0.25% (w/v) lead citrate solution for 2 min and examined with a Tecnai G2 electron microscope (FEI, USA) operated at 120 kV.

Statistical analysis: Statistical analysis of the results was performed using one-way ANOVA followed by LSD and Tukey *post hoc* analysis using SPSS 16.00 software (SPSS Inc., Chicago, IL, USA). The p-values of <0.05 were considered to be statistically significant.

RESULTS

***In vitro* antibacterial activity of curcumin against *S. aureus* and *E. coli*:** The antibacterial activity against *S. aureus* and *E. coli* of curcumin, tetracycline and ciprofloxacin were determined by measuring its MIC and MBC. The lowest antibacterial activity against *S. aureus* and *E. coli* was shown by curcumin, with an MIC value of 64 μg mL^{-1} for both bacteria (Table 1). On the other hand, ciprofloxacin showed the best antibacterial activity against *S. aureus* and *E. coli*, with MIC values of 0.125 and 0.0625 μg mL^{-1} and MBC values of 0.5 and 0.0625 μg mL^{-1}, respectively. The antibacterial activity of curcumin in combination with tetracycline or ciprofloxacin was further evaluated.

***In vitro* antibacterial activity of a combination of curcumin and tetracycline or ciprofloxacin against *S. aureus* and *E. coli*:** This experiment was conducted in order to analyze the potency of curcumin in increasing the antibacterial activity of tetracycline or ciprofloxacin against *S. aureus* and *E. coli*. The results showed that the antibacterial activity of curcumin in combination with tetracycline or ciprofloxacin against *S. aureus* has an FIC index below 0.5, suggesting a synergistic interaction (Table 2). On the other hand, its antibacterial activity against *E. coli* showed a synergistic activity only in combination with tetracycline (Table 2). Therefore, the combination of curcumin and tetracycline was chosen for further *in vivo* studies.

Acute skin and dermal irritation study of a combination of curcumin and tetracycline: The study continued evaluating the safety of a combination of curcumin and tetracycline when used topically. The experiment was performed based on the Organization and Economic Cooperation Development (OECD) standard protocol. After 24 h of exposure to the combination ointment, there were no dermal or ocular irritations observed on rabbit skin or eyes. Similar results were observed at 48 and 72 h after ointment exposure (Table 3 and 4). This data show that the combination of 2% curcumin and 1% tetracycline does not cause acute dermal and ocular irritation, suggesting that it is safe to be used topically.

***In situ* antibacterial activity of curcumin and tetracycline combination ointment against *S. aureus*-infected rabbit skin:** In this study, the *in situ* antibacterial activity of the curcumin-ciprofloxacin combination was not evaluated because it had less antibacterial activity than the curcumin-tetracycline combination. Moreover, there is no comparable ciprofloxacin ointment dosage form available in the Indonesian market. Therefore, the combination of curcumin and tetracycline was chosen as a further *in situ* antibacterial activity study. Various concentrations of curcumin-tetracycline combinations were tested on *S. aureus* infected rabbit skin.

Table 1: MIC and MBC of curcumin, tetracycline and ciprofloxacin against *S. aureus* and *E. coli*

	MIC and MBC of a compound against certain bacteria			
	Staphylococcus aureus		*Escherichia coli*	
Compounds	MIC (μg mL^{-1})	MBC (μg mL^{-1})	MIC (μg mL^{-1})	MBC (μg mL^{-1})
Curcumin	64	>2048	64	>2048
Tetracycline	2	32	16	128
Ciprofloxacin	0.125	0.5	0.0625	0.0625

The data are expressed as the means of three independent experiments, MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

Table 2: FIC index of combination of curcumin with tetracycline or ciprofloxacin against *S. aureus* and *E. coli*

Bacteria	Antibiotic	FIC	FIC index	Combination
Staphylococcus aureus	Tetracycline	0.25 MIC_{Cu}+0.16 MIC_{T}	0.41	Synergism
	Ciprofloxacin	0.06 MIC_{Cu}+0.38 MIC_{Ci}	0.44	Synergism
Escherichia coli	Tetracycline	0.25 MIC_{Cu}+0.03 MIC_{T}	0.28	Synergism
	Ciprofloxacin	0.50 MIC_{Cu}+0.19 MIC_{Ci}	0.69	Additive

MIC_{Cu}: MIC of curcumin, MIC_{T}: MIC of tetracycline: MIC; MIC of ciprofloxacin

Table 3: Dermal irritation index of combination 2% curcumin: 1% tetracycline

Rabbit	Time of observation (h)	Response	
		Erythema	Oedema
1	1	0	0
	24	0	0
	48	0	0
	72	0	0
2	1	0	0
	24	0	0
	48	0	0
	72	0	0
3	1	0	0
	24	0	0
	48	0	0
	72	0	0
Average		0	0
Total		0	

Table 4: Ocular irritation index of combination of 2% curcumin: 1% tetracycline

Rabbit	Day of observation (h)	Lesion			
		Cornea	Iris	Conjunctive	Chemosis
1	1	0	0	0	0
	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	0
2	1	0	0	0	0
	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	0
3	1	0	0	0	0
	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	0
Average		0	0	0	0
Total			0		

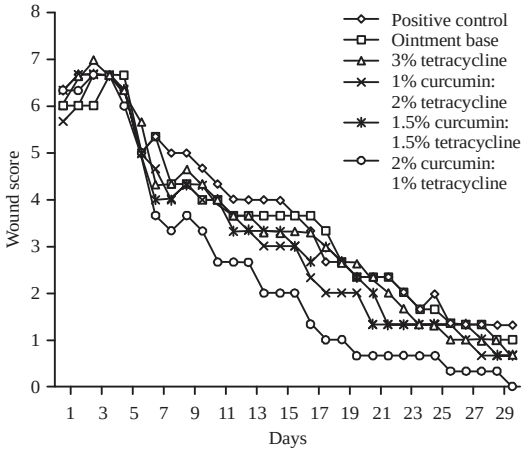

Fig. 1: Wound score after curcumin-tetracycline combination treatment against *S.aureus* infections

The results (Fig. 1) show that the positive control, ointment base and the combination of 1.5% curcumin and 1.5% tetracycline resulted in the highest DIS on the last day of observation and the combination of 2% curcumin and 1% tetracycline resulted in the lowest. This suggests that the latter was able to induce faster healing of *S. aureus* infected skin. All of the animals used in this study showed the same wound score at day 0 of observation, a slight increase in wound score was observed for all groups at day 3. Furthermore, all of the animal groups showed an overall decrease in wound score, including the positive control group, suggesting that there is a self-recovery mechanism in the animals. However, the group treated with the combination of 2% curcumin and 1% tetracycline showed the lowest wound score, suggesting that this combination has the fastest recovery among the groups (Fig. 1).

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) observations at 18 h: The effects of the curcumin-tetracycline combination on the surface morphology of *S. aureus* during its logarithmic phase of growth are presented in Fig. 2 and 3. The antimicrobial activity

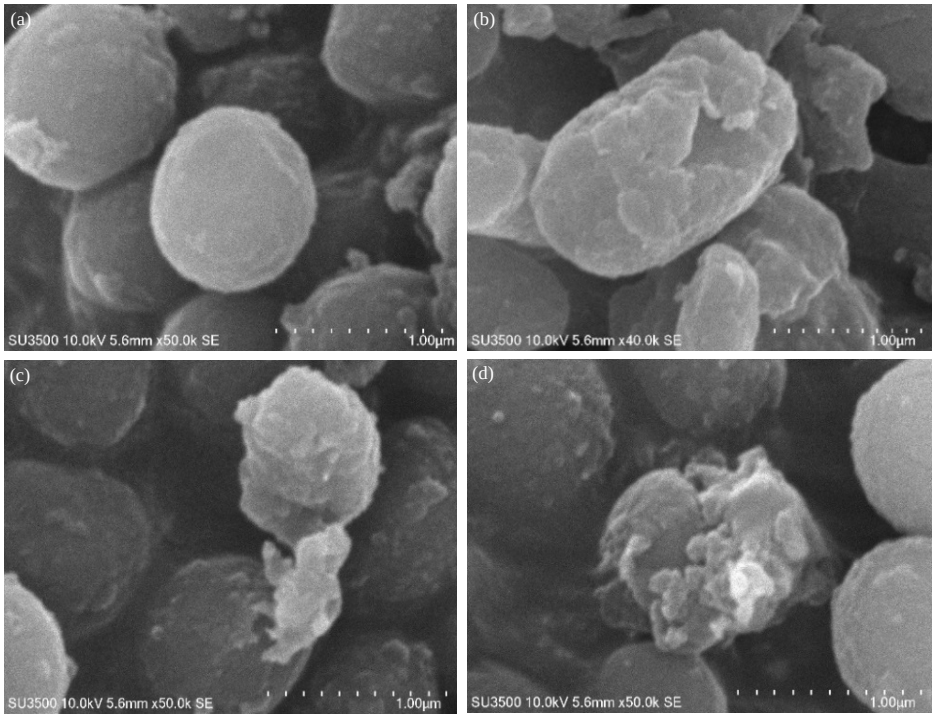

Fig. 2(a-d): Scanning electron micrograph of *S. aureus* treated with combination of curcumin and tetracycline. The swollen cells were observed after treated for 18 h with regular shape of (a) Control, (b) Curcumin, (c) Tetracycline and (d) Combination of curcumin and tetracycline, Enlargement: For a, c, d 50,000× and for b 40,000×

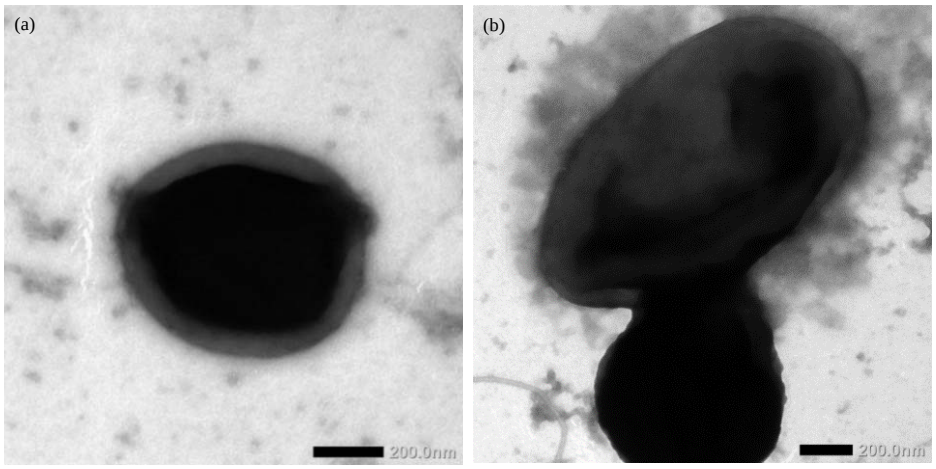

Fig. 3(a-b): Transmission electron micrograph of *S. aureus* treated with combination of curcumin and tetracycline. Damaged cell wall was observed after 18 h of incubation (a) Control and (b) Combination of curcumin and tetracycline, Enlargement: 40,000×

on the surface morphology of individual cells was visualized. It can be observed that exposure to the combination resulted in distortion and disruption of the bacterial wall, resulting in deformation of the cell, while the untreated cells appeared round-shaped.

DISCUSSION

The present data suggest that the combination of 2% curcumin and 1% tetracycline has *in situ* antibacterial activity against skin infections caused by *S. aureus*. This combination

showed the fastest healing time among various concentrations of curcumin-tetracycline combinations and is safe to be used topically.

S. aureus contributes to a majority of skin and soft tissue infections (SSTIs),[18] while *E. coli* infections occur exclusively in immunodeficient patients[19]. Moreover, Meulemans *et al.*[20] showed that the injection of *S. aureus* into rabbit skin resulted in similar skin infections to those seen in the field. Therefore, for an *in situ* study, an *S. aureus* suspension was chosen to be injected into the skin of rabbits to induce SSTIs.

Curcumin has been reported to inhibit the growth of antibiotic-sensitive and resistant bacteria, including *S. aureus* and methicillin-resistant *S. aureus*[21]. *In vitro* synergistic antibacterial activities of curcumin with various antibiotics and bacteria have also been previously reported[9-10]. Furthermore, combinations of curcumin with gentamicin, tetracycline and ciprofloxacin against *S. aureus* showed synergistic antibacterial activity,[9-10] which is in line with the present study. In this study, it is demonstrated that a combination of 2% curcumin and 1% tetracycline resulted in the best antibacterial effectiveness against *S. aureus* infection through the disruption of the bacterial cell wall. Tetracycline was chosen to be combined with curcumin because a tetracycline ointment product is available in the market, while a ciprofloxacin ointment is not. Several studies have reported the mechanism of action of curcumin, Tyagi *et al.*[22] demonstrated that curcumin at higher concentrations can have antibacterial activity against Gram-negative and Gram-positive bacteria by causing membrane permeabilization. Other studies demonstrated that curcumin inhibits the assembly of FtsZ (prokaryotic homologue of eukaryotic cytoskeletal protein tubulin) protofilaments which leads to the inhibition of bacterial cell proliferation[23-25]. The synergistic interaction observed in the present study is probably due to an increase of tetracycline influx into the bacteria cells caused by membrane hyper permeabilization.

This study implies a pharmacological and toxicological knowledge of a curcumin-tetracycline combination ointment for the development of clinical studies to treat skin infections. The therapeutic use of a curcumin-tetracycline combination can be explored further for other type of infections as it has a wide spectrum of antibacterial activity. However, one of the limitations of this study is that the antibacterial activity of the curcumin-tetracyclin combination ointment was only tested on rabbit skin, it is unknown whether the results can be extrapolated to humans. Moreover, the antibacterial activity was only tested on *S. aureus* induced skin infections; its activity on other bacterial infections should be investigated further.

CONCLUSION

In an *in vitro* study, curcumin has shown antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* and has synergistic interaction in combination with tetracycline. The combination of curcumin and tetracycline accelerates the healing process of *S. aureus*-caused skin infections as observed on rabbit skin; its mechanism of action is by disrupting the bacterial wall. A combination of 2% curcumin and 1% tetracycline does not induce dermal or ocular irritation. Therefore, this combination has the potential to be developed as an alternative drug in the treatment of skin infections.

SIGNIFICANCE STATEMENT

This study discovered that the combination of 2% curcumin and 1% tetracycline had antibacterial activity against *Staphylococcus aureus* that can be beneficial for *S. aureus*-induced skin infection on rabbit. This investigation will facilitate the researcher to obtain an alternative drug for skin infection that many researchers were not able to explore.

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REFERENCES

1. Lister, P.D., 2000. β-lactamase inhibitor combinations with extended-spectrum penicillins: Factors influencing antibacterial activity against Enterobacteriaceae and *Pseudomonas aeruginosa*. Pharmacotherapy, 20: 213S-218S.
2. Sukandar, E.Y., N.F. Kurniati, K. Anggadiredja and A. Kamil, 2016. *In vitro* antibacterial activity of *Kaempferia pandurata* roxb. and *Curcuma xanthorrhiza* roxb. extracts in combination with certain antibiotics against MSSA and MRSA. Int. J. Pharm. Pharm. Sci., 8: 108-111.
3. Adwan, M.G., A.B. Abu-Shanab and M.K. Adwan, 2008. *In vitro* activity of certain drugs in combination with plant extracts against *Staphylococcus aureus* infections. Pak. J. Med., 24: 541-544.
4. Chang, P.C., H.Y. Li, H.J. Tang, J.W. Liu, J.J. Wang and Y.C. Chuang, 2007. *In vitro* synergy of baicalein and gentamicin against vancomycin-resistant *Enterococcus*. J. Microbiol. Immunol. Infect., 40: 56-61.
5. Chattopadhyay, I., K. Biswas, U. Bandyopadhyay and R.K. Banerjee, 2004. Turmeric and curcumin: Biological actions and medicinal applications. Curr. Sci., 87: 44-53.

6. Gupta, S.C., S. Prasad, J.H. Kim, S. Patchva, L.J. Webb, I.K. Priyadarsini and B. Aggarwal, 2011. Multitargeting by curcumin as revealed by molecular interaction studies. Nat. Prod. Rep., 28: 1937-1955.
7. Na, H.S., M.H. Cha, D.R. Oh, C.W. Cho, J.H. Rhee and Y.R. Kim, 2011. Protective mechanism of curcumin against *Vibrio vulnificus* infection. FEMS Immunol. Med. Microbiol., 63: 355-362.
8. De, R., P. Kundu, S. Swarnakar, T. Ramamurthy, A. Chowdhury, G.B. Nair and A.K. Mukhopadhyay, 2009. Antimicrobial activity of curcumin against *Helicobacter pylori* isolates from India and during infections in mice. Antimicrob. Agents Chemother., 53: 1592-1597.
9. Moghaddam, K.M., M. Iranshahi, M.C. Yazdi and A.R. Shahverdi, 2009. The combination effect of curcumin with different antibiotics against *Staphylococcus aureus*. Int. J. Green Pharm., 3: 141-143.
10. Teow, S.Y. and S.A. Ali, 2015. Synergistic antibacterial activity of Curcumin with antibiotics against *Staphylococcus aureus*. Pak. J. Pharm. Sci., 28: 2109-2114.
11. CLSI., 2009. M07-A08–Methods Dilution Antimicrobial Succeptibility Tests for Bacteria That Grow Aerobically Approve Standard. 8th Edn., Clinical and Laboratory Standards Intitute, Pennsylvania, USA.
12. CLSI., 2007. M100-S17-Performance Standards for Antimicrobial Susceptibility Testing Seventeenth Informational Supplement. Clinical and Laboratory Standards Intitute, Wayne, PA.
13. Lorian, V., 1991. Antibiotics in Laboratory Medicine. 3rd Edn., Williams Wilkins, Baltimore, Md., USA.
14. Odds, F.C., 2003. Synergy, antagonism and what the chequerboard puts between them. J. Antimicrob. Chemother., 52: 1-1.
15. OLAW., 2002. Animal care and use committee guidebook. 2nd Edn. National Institutes of Health: Applied Research Ethics National Association (ARENA).
16. Dutok, C.M.S., C.A. Berenguer-Rivas, E. Rodriguez-Leblanch, L. Perez-Jackson and I. Chil-Nunez *et al*., 2015. Acute toxicity and dermal and eye irritation of the aqueous and hydroalcoholic extracts of the seeds of "zapote" *Pouteria mammosa* (L.) cronquist. Sci. World J. 10.1155/2015/642906.
17. Vinardell, M.P. and M. Mitjans, 2008. Alternative methods for eye and skin irritation tests: An overview. J. Pharm. Sci., 97: 46-59.
18. Ki, V. and C. Rotstein, 2008. Bacterial skin and soft tissue infections in adults: A review of their epidemiology, pathogenesis, diagnosis, treatment and site of care. Can. J. Infect. Dis. Med. Microbiol., 19: 173-184.
19. Sunder, S., E. Haguenoer, D. Bouvet, S. Lissandre and A. Bree *et al*., 2012. Life-threatening *Escherichia* coli cellulitis in patients with haematological malignancies. J. Med. Microbiol., 61: 1324-1327.
20. Meulemans, L., K. Hermans, L. Duchateau and F. Haesebrouck, 2007. High and low virulence *Staphylococcus aureus* strains in a rabbit skin infection model. Vet. Microbiol., 125: 333-340.
21. Teow, S.Y., K. Liew, S.A. Ali, A.S.B. Khoo and S.C. Peh, 2016. Antibacterial action of curcumin against *Staphylococcus aureus*: A brief review. J. Trop. Med. 10.1155/2016/2853045.
22. Tyagi, P., M. Singh, H. Kumari, A. Kumari and K. Mukhopadhyay, 2015. Bactericidal activity of curcumin I is associated with damaging of bacterial membrane. PLoS One, Vol. 10, No. 3. 10.1371/journal.pone.0121313.
23. Rai, D., J.K. Singh, N. Ray and D. Panda, 2008. Curcumin inhibits FtsZ assembly: An attractive mechanism for its antibacterial activity. Biochem. J., 410: 147-155.
24. Matsui, T., J. Yamane, N. Mogi, H. Yamaguchi, H. Takemoto, M. Yao and I. Tanaka, 2012. Structural reorganization of the bacterial cell-division protein FtsZ from *Staphylococcus aureus*. Acta Crystallogr., D68: 1175-1188.
25. Singh, P. and D. Panda, 2010. FtsZ inhibition: A promising approach for antistaphylococcal therapy. Drug News Perspect., 23: 295-304.