International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 1, 2016

Original Article

IN VITRO ANTIBACTERIAL ACTIVITY OF *KAEMPFERIA PANDURATA* ROXB. AND *CURCUMA XANTHORRHIZA* ROXB. EXTRACTS IN COMBINATION WITH CERTAIN ANTIBIOTICS AGAINST MSSA AND MRSA

ELIN YULINAH SUKANDAR[1], NENG FISHERI KURNIATI[1], KUSNANDAR ANGGADIREDJA[1], ANNISA KAMIL[1]

[1]School of Pharmacy, Pharmacology and Clinical Pharmacy Research Group, Bandung Institute of Technology, Ganesha 10, Bandung, Indonesia
Email: elin@fa.itb.ac.id

Received: 09 Sep 2015 Revised and Accepted: 18 Nov 2015

ABSTRACT

Objective: The aim of this study was to find an alternative treatment of MRSA infection. We evaluated the interaction of *in vitro* antibacterial activity of *Kaempferia pandurata* Roxb. (rhizome) or *Curcuma xanthorrhiza* Roxb. (rhizome) ethanolic extracts combined with commonly used antibiotics, namely penicillin V, ampicillin, ciprofloxacin HCl, and tetracycline HCl.

Methods: Interaction between the plant extracts and antibiotics against *Methicillin-sensitive Staphylococcus aureus* (MSSA) and *Methicillin-resistant Staphylococcus aureus* (MRSA) were tested by checkerboard method and interpreted as Fractional Inhibitory Concentration (FIC) index.

Results: *Kaempferia pandurata* showed a synergistic effect on all antibiotics tested against MSSA. Against MRSA, however, only combinations of *Kaempferia pandurata* and penicillin V or ampicillin which showed synergism, while others were antagonisms. Meanwhile, with *Curcuma xanthorrhiza*, only its combination with ampicillin which resulted in synergistic effect against both MSSA and MRSA, others resulted in various interaction responses against MSSA and MRSA.

Conclusion: Interaction between *Kaempferia pandurata* and penicillin V or ampicillin and that between *Curcuma xanthorrhiza* and ampicillin resulted in synergistic effect against MRSA. These combinations are promising to be developed as an alternative strategy to treat MRSA.

Keywords: Antibacterial activity, Plants extract–drug interaction, MSSA, MRSA.

INTRODUCTION

Methicillin-sensitive Staphylococcus aureus (MSSA) has developed to become resistant bacteria, known as *Methicillin-resistant Staphylococcus aureus* (MRSA) [1]. The current treatments for MSSA are limited. MRSA has been reported to be resistant to beta-lactams, aminoglycosides, fluoroquinolones and macrolides [2]. Therefore, the need in finding a new drug to treat bacterial infection is increased due to the occurrence of resistant bacteria. Finding a new compound derived from the plant as an antibacterial drug might be one alternative. An earlier study has reported that by combining the plant-derived drug with certain antibiotics reduced the minimum inhibitory concentrations of the antibiotics [3]. Such combination strategy could be a new approach to treating bacterial infection and reduce the occurrence of bacterial resistance.

Among medicinal plants well-known to have potent antibacterial activities are *Kaempferia pandurata* Roxb. and *Curcuma xanthorrhiza* Roxb. These plants are, therefore, expected to have beneficial effects when combined with antibiotics. *Kaempferia pandurata* Roxb or fingerroot, belonging to the family Zingiberaceae, is a ginger species that is widely found in Southeast Asia, Sri Lanka, India, and Southern China. The fresh rhizomes are used to treat illnesses such as swelling, wounds, dental caries, dermatitis, dry cough and cold, tooth and gum disease, diarrhea, dysentery, and as diuretic. Fingerroot rhizomes contain many compounds, ranging from the chalcone derivatives, flavonoid derivatives, kawains, terpenes, esters, and terpenoids. These compounds have been shown to exhibit high medicinal potential. Previous studies have reported that *K. pandurata* showed antimicrobial, antiparasitic, antioxidant, antiulcer, antiobesity, antimutagenic, antitumor, antifungal, anti-inflammatory, antiviral, and wound healing activities [4]. Furthermore, an early work reported that the ethanol extract of fingerroot rhizome had antibacterial activity against MSSA, MRSA, Methicillin-resistant coagulase-negative Staphylococci (MRCNS), *Bacillus subtilis*, and *Salmonella typhi*, with MIC of 2-16 µg/ml [5]. Panduratin A, a natural chalcone compound isolated from the fingerroot rhizomes was also found to possess strong *in vitro* antimicrobial activity against clinical *Staphylococcus* and *Enterococci* strains [6, 7].

Curcuma xanthorrhiza Roxb., commonly known as temulawak or Javanese turmeric is one type of herbs derived from family Zingiberaceae. It is used in Southeast Asian countries for food and medicinal purposes. *C. xanthorrhiza* has been traditionally used to treat stomach disease, liver disorder, constipation, dysentery, diarrhea, children's fever, and hemorrhoids [8]. The rhizomes contain two classes of characteristic constituents. The main substances are curcuminoids (1-2%) such as curcumin, desmethoxycurcumin, and bisdemethoxycurcumin, and volatile oil (3-12%) composed mainly of sesquiterpenes, xanthorrhizol, and a small amount of camphor. Curcumin and xanthorrhizol are the principal components of *C. xanthorrhiza* that have a wide range of pharmacological activities including hepatoprotective, antioxidant, antiinflammatory, immunomodulatory, antimutagenic, anticarcinogenic, hypolipidaemic, antimicrobial, antifungal, insecticidal, anti aging, and anticoagulation [9]. An ethanol extract of *C. xanthorrhiza* has been demonstrated to inhibit the growth of *Staphylococcus aureus*, *Streptococcus mutans*, and *Bacillus cereus* with a MIC of 0.1-2.0% (w/v) [10]. Xanthorrhizol, a natural sesquiterpenoid compound isolated from the turmeric rhizomes have been known to have powerful antibacterial activity against *S. mutans* and oral pathogens [8, 11].

In the present study, we evaluated the interaction of *in vitro* antibacterial activity of *Kaempferia pandurata* Roxb. (rhizome) or *Curcuma xanthorrhiza* Roxb. (rhizome) ethanolic extracts combined with commonly used antibiotics, namely penicillin V, ampicillin, ciprofloxacin HCl, and tetracycline HCl, in an attempt to find an alternative treatment of MRSA infection.

MATERIALS AND METHODS

Chemicals

Mueller Hinton Agar (MHA) and Mueller-Hinton Broth (MHB) (Oxoid Ltd., Hampshire, England) were used as growth media for bacteria.

96% ethanol and dimethylsulfoxide (DMSO, Merck, Germany) were obtained from School of Pharmacy, Bandung Institute of Technology. Penicillin V, ampicillin, ciprofloxacin HCl, and tetracycline HCl were obtained from Brataco Chemical, Indonesia.

Plant collection and identification

Fresh rhizomes of *Kaempferia pandurata* were collected from the garden in Tanjungkerta village, Sumedang, Indonesia. *Curcuma xanthorrhiza* rhizome was freshly harvested and collected in the garden of Cipendey village, Majalengka, Indonesia. Identification of plants was performed in Herbarium Bandungense, School of Life Sciences and Technology, Bandung Institute of Technology. Rhizomes and leaves were sorted, washed, chopped, and dried using the oven (WTB binder) at 40-45 °C for several days. The dried rhizomes then were grounded to a fine powder.

Microorganism tested and culture

Methicillin-sensitive Staphylococcus aureus (MSSA) was obtained from the culture collection of School of Pharmacy, Bandung Institute of Technology. Methicillin-resistant *Staphylococcus aureus* (MRSA) was obtained from clinical patient isolate at Hasan Sadikin Hospital, Bandung. All of bacteria strains were cultured aerobically in MHA and MHB medium.

Extraction

The rhizome of *Kaempferia pandurata* and *Curcuma xanthorrhiza* were extracted by reflux using 96% ethanol as solvent. The ethanol extracts were filtered through filter paper (Whatman No. 1) and concentrated by a rotary evaporator (Buchi R-125) to obtain viscous extracts. The viscous extracts were stored at room temperature and protected from sunlight.

Determination the susceptibility of bacteria

In vitro susceptibility test was performed in a 96-well microtiter plate to determine minimum inhibitory concentrations (MICs) of plant extracts and antibiotics against tested bacterial strains using standard Broth microdilution methods with an inoculum of 5 x 10^5 CFU/ml, according to the guidelines of CLSI standard M7-A8 [12]. Briefly, the extracts and antibiotics were first diluted in 20% DMSO, followed by two-fold dilutions in the test well and tested over a range of concentrations from 0.5 to 4096 µg/ml against overnight broth cultures of MSSA and MRSA. There was positive control (only medium and inoculum) and negative control (only medium) for all samples in each plate. Microtiter plates were incubated aerobically at 37 °C for 24 h. The MIC was defined as the lowest concentration of antimicrobial agent that resulted in the complete inhibition of visible growth. Minimum bactericidal concentrations (MBCs) were established for each test sample. Briefly, medium (approximately 100 µl) from each well showing no visible growth was spread onto MHA plates. Plates were incubated at 37 °C for 24 h or until growth was seen in the growth-positive control plates. MBC was defined as the lowest concentration of antimicrobial agent at which all bacteria in the culture are killed or the lowest concentration of drug that kills 99.9% of the total initially viable cells [13]. Experiments were conducted in triplicate.

Antimicrobial combination testing of plant extracts and antibiotics

The effects of interactions between plant extracts and antibiotics against bacterial strains were evaluated using the modification and simplified checkerboard technique. The test for the antimicrobial combination was performed using a 96-well microtiter plate, round bottom microdilution plate with MHB. Antimicrobial activity of each plant extract in combination with antibiotic were tested (in quadruplicate) against each microorganism. The highest concentration of extract A and antibiotic B used in combination was 2 times of each MIC. Furthermore, dilutions of plant extract and antibiotic were made with a series of twofold dilution. The extract A and antibiotic B were diluted twofold along the x-axis and y-axis of the plate, respectively. Overnight broth cultures of each strain (MSSA and MRSA) were adjusted to10^6 CFU/ml and subsequently were added to the wells. Negative control (only MHB) and growth control (MHB and inoculum) were also included in each plate. The plates were then incubated at 37 °C for 24 h and monitored for growth. At the end of incubation period, the MICs of each extract and antibiotic alone and in the combination were recorded. The MIC was defined as the lowest concentration of the plant extract or antibiotic at which visible growth is inhibited. To evaluate the effect of combinations, the fractional inhibitory concentration index (FIC index) was calculated for each of the combinations according to the formula below.

$$\text{FIC index} = \text{FICa} + \text{FICb}$$

$$= \frac{\text{MICa in combination}}{\text{MICa alone}} + \frac{\text{MICb in combination}}{\text{MICb alone}}$$

The results were interpreted as synergism (FIC index ≤ 0.5), additive (FIC index 1), and antagonism (FIC index>2) [14].

RESULTS AND DISCUSSION

Antimicrobial activity of plant extracts and antibiotics alone against MSSA and MRSA

In vitro antibacterial activity of plant extract and antibiotics was quantitatively investigated in terms of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) and results are represented in table 1. The MIC of plant extracts and antibiotic was varied, depending on the type of plant extract and antibiotic and the strain of bacteria tested. In general, all the plant extracts and antibiotics exerted antibacterial effects.

The strongest antibacterial activity against MSSA for plant extract was shown by ethanolic extract of *Kaempferia pandurata* (MIC = 128 µg/ml), while, for antibiotics, ciprofloxacin HCl showed the strongest activity (MIC = 0.5 µg/ml). Furthermore, the MIC for all tested plant extracts and antibiotics against MRSA had higher MIC level compared to MSSA, suggesting that the antimicrobial activity of the plant extracts and antibiotics were lower against MRSA than MSSA.

Table 1: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts and antibiotics against MSSA and MRSA

Plant extract and antibiotic	MSSA		MRSA	
	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
Kaempferia pandurata extract	128	256	2048	4096
Curcuma xanthorrhiza extract	256	512	2048	4096
Penicillin V	8	16	64	64
Ampicillin	2	4	8	8
Ciprofloxacin HCl	0.5	0.5	64	64
Tetracycline HCl	8	16	32	64

Note: Experiments were conducted in triplicate.

Minimum bactericidal concentrations (MBCs) were also determined for each plant extracts and antibiotics. The results showed that in general, the MBC values were equal to (MBC = MIC) or two times greater than the MIC (MBC= 2 x MIC). The MIC and MBC values of the ethanolic plant extracts were higher than the antibiotics.

Effects of combination between plant extracts and antibiotics in antimicrobial activity

The antibacterial activity of the combination of each plant extract and antibiotics against MSSA and MRSA are shown in table 2 and table 3.

Against MSSA, the interaction between *Kaempferia pandurata* with each antibiotic tested showed a synergistic effect. The MIC value of *Kaempferia pandurata* and antibiotic in combination was reduced up to 1/4-1/8 MIC. Moreover, for *Curcuma xanthorrhiza*, only interaction with ciprofloxacin HCl and tetracycline HCl that showed a synergistic effect. While the interaction between *Curcuma xanthorrhiza* and penicillin V or tetracycline HCl showed additive and antagonistic effect, respectively. Against MRSA, a combination of *Kaempferia pandurata* or *Curcuma xanthorrhiza* with penicillin V or ampicillin gave a synergistic effect. An additive effect was found when *Curcuma xanthorrhiza* was combined with tetracycline HCl.

Moreover, the antagonistic effect was observed when ciprofloxacin HCl was combined with ethanolic extracts of *Kaempferia pandurata* or *Curcuma xanthorrhiza,* and when tetracycline HCl was combined with extracts of *Kaempferia pandurata*. According to FIC index, MRSA exerted the greatest resistance to most of the combinations between plant extract and antibiotic.

Table 2: Fractional inhibitory concentration (FIC) index of combinations of plant extract and antibiotic against MSSA

Combinations		MIC	FIC index	Interactions
Plant extracts	**Antibiotics**			
Kaempferia pandurata	Penicillin V	1/8+1/8	0.25	Synergism
	Ampicillin	1/8+1/8	0.25	Synergism
	Ciprofloxacin HCl	1/8+1/8	0.25	Synergism
	Tetracycline HCl	1/4+1/4	0.50	Synergism
Curcuma xanthorrhiza	Penicillin V	1/2+1/2	1.00	Additive
	Ampicillin	1/8+1/8	0.25	Synergism
	Ciprofloxacin HCl	1/8+1/8	0.25	Synergism
	Tetracycline HCl	1+1	2.00	Antagonism

Note: Experiments were conducted in quadruplicate.

Table 3: Fractional inhibitory concentration (FIC) index of combinations of plant extract and antibiotic against MRSA

Combinations		MIC	FIC index	Interactions
Plant extracts	**Antibiotics**			
Kaempferia pandurata	Penicillin V	1/8+1/8	0.25	Synergism
	Ampicillin	1/8+1/8	0.25	Synergism
	Ciprofloxacin HCl	1+1	2.00	Antagonism
	Tetracycline HCl	2+2	4.00	Antagonism
Curcuma xanthorrhiza	Penicillin V	1/8+1/8	0.25	Synergism
	Ampicillin	1/8+1/8	0.25	Synergism
	Ciprofloxacin HCl	2+2	4.00	Antagonism
	Tetracycline HCl	1/2+1/2	1.00	Additive

Note: Experiments were conducted in quadruplicate.

In this study, the synergistic effects resulted from the combination of antibiotics with plant extracts varied against different bacteria. These results were in agreement with a previous report [15]. In that report, it demonstrated the synergistic property of combination of doxycycline with ethanolic leaf extract of *Vangueria spinosa* against *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumonia* [15]. Such combination, however, showed additive interaction against *Pseudomonas aeruginosa* [15].

This synergistic action can occur due to the favorable interactions between the active component of plant extracts and antibiotics. The synergistic effect that was detected in this study suggests that plant extract is a mixture of compounds [16] that can enhance the activity of different antibiotics. One of the mechanisms of action of the synergistic interaction that has been reported was increasing the permeability of the cell membrane which leads to the increased penetrability of antibiotics [16]. These results revealed that the combined use of plant extracts and antibiotics could be useful in the treatment of infectious bacterial disease and useful in overcoming drug resistance problem although clinical controlled studies are needed to confirm the real efficacy and possible toxic effects *in vivo*. Therefore, understanding the mechanism of action of combination of antibiotics with plant extracts against bacteria is fundamental to the development of pharmacological agents to treat bacterial infections using the medicinal plant.

CONCLUSION

In conclusion, the results presented in this study showed that there were synergistic antibacterial effects between ethanolic plant extracts and antibiotics against MSSA and MRSA bacteria. The interaction between *Kaempferia pandurata* and penicillin V or ampicillin and interaction between *Curcuma xanthorrhiza* and ampicillin was shown to be synergistic against MRSA. These combinations can be used as an alternative strategy to treat MRSA.

ACKNOWLEDGEMENT

This research was supported by ITB-DIKTI Research 2015.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Chambers HF. The changing epidemiology of staphylococcus aureus? Emerging Infect Dis 2001;7:178-82.
2. Michel M, Gutmann L. Methicillin-resistant staphylococcus aureus (MRSA) and vancomycin-resistant enterococci: therapeutic realities and possibilities. Lancet 1997;349:1901-6.
3. Chanda S, Rakholiya K. Combination therapy: synergism between natural plant extracts and antibiotics against infectious diseases. In: Méndez-Vilas A. editors. Science against microbial pathogens: communicating current research and technological advances. Spain: Formatex; 2011. p. 520-29.
4. Chong TE, Kee LY, Fei CC, Han HC, Ming WS, Ping CT, *et al. Boesenbergia rotunda*: from ethnomedicine to drug discovery. a review. J Evidence-Based Complementary Altern Med 2012:1-25. doi.org/10.1155/2012/473637. [Article in Press]
5. Sukandar YS, Sunderam N, Fidrianny I. Activity of *Kaempferia pandurata* (Roxb.) rhizome ethanol extract against MRSA,

MRCNS, MSSA, *Bacillus subtilis*, and *Salmonella typhi*. Pak J Biol Sci 2014;17:49-55.

6. Hwang JK, Rukayadi Y, Han S, Yong D. *In vitro* antibacterial activity of panduratin an against *Enterococci* clinical isolates. Biol Pharm Bull 2010;33:1489-93.
7. Hwang JK, Rukayadi Y, Lee K, Han S, Yong D. *In vitro* activities of panduratin an against clinical *staphylococcus* strains. Antimicrob Agents Chemother 2009;53:4529-32.
8. Hwang JK, Shim JS, Pyun YR. Antibacterial activity of xanthorrhizol from *Curcuma xanthorrhiza* against oral pathogens. Fitoterapia 2000;71:321-3.
9. Committee on Herbal Medicine Products (HPMC). Assessment Report on *Curcuma xanthorrhiza* Roxb. (*C. xanthorrhiza* D. Dietrich)., Rhizoma. London: European Medicines Agency (EMA); 2012.
10. Mangunwardoyo W, Deasywaty Usia T. Antimicrobial and Identification of active compound *Curcuma xanthorrhiza* Roxb. Int J Basic Appl Sci 2012;12:69-78.
11. Kim JU, Kim HE, Lee HJ, Kwon HK, Kim BI, Hwang JK. Antibacterial characteristics of *Curcuma xanthorrhiza* extract on *Streptococcus mutans* biofilm. Chin J Microbiol 2008;46:228-32.
12. Clinical and Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. Approved Standard M07-A8. 8th ed. Wayne, Philadelphia: National Committee for Clinical Laboratory Standards; 2010. p. 15-8.
13. Michael JR, Jeffrey RA. Laboratory tests to direct antimicrobial pharmacotherapy. In: Dipiro JT, Talber RL, Yee GC, Matzke GR, Wells BG, Posey LM. Pharmacotherapy: a pathophysiologic approach. 7th ed. United States: The McGraw-Hill Companies Inc; 2008. p. 1715-25.
14. Garrod LP, Waterworth PM. Methods of testing combined antibiotics bactericidal action and the significance of the results. J Clin Pathol 1962;15:328-38.
15. Chatterjee SK, Bhattacharjee I, Chandra G. *In vitro* synergistic effect of doxycycline and ofloxacin in combination with ethanolic leaf extract of *Vangueria spinosa* against four pathogenic bacteria. Indian J Med Res 2009;130:475-8.
16. Moghadam SM, Maleki S, Darabpour E, Motamedi H, Nejad MS. Antibacterial activity of eight Iranian plants extracts against methicillin and cefixime-resistant Staphylococcus aureus strains. Asian Pac J Trop Med 2010;3:262-5.