

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Activity of *Kaempferia pandurata* (Roxb.) Rhizome Ethanol Extract Against MRSA, MRCNS, MSSA, *Bacillus subtilis* and *Salmonella typhi*

Elin Yulinah Sukandar, Nethiyakalyani Sunderam and Irda Fidrianny
School of Pharmacy, ITB, Jl. Ganesha 10 Bandung, Indonesia

Abstract: Temu kunci (*Kaempferia pandurata* (Roxb.)) has a number of benefits and one of these is antibacterial. The rhizome is said to have antibacterial activity against *Streptococcus mutans*, *Lactocillus* sp. and *Candida albicans*. The aim of the study is to test the antibacterial activity of *Kaempferia pandurata* (Roxb.) rhizome ethanol extract on methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant coagulase negative Staphylococci (MRCNS), methicillin-sensitive *Staphylococcus aureus* (MSSA), *Bacillus subtilis* and *Salmonella typhi*. Antimicrobial activity of the extract was assayed by the microdilution method using Mueller Hinton Broth with sterilized 96 round-bottomed microwells to determine the Minimum Inhibitory Concentration (MIC) as well as to determine the time-kill activity. The MIC of the extract was 16 ppm for both *Bacillus subtilis* and MRSA; 8 ppm for both MSSA and *Salmonella typhi* and 4 ppm for MRCNS. Ethanol extract of *Kaempferia pandurata* (Roxb.) showed antibacterial activity against all the tested bacteria and was the most potent against MRCNS, with MIC 4 ppm. The killing profile test of the extract displayed bactericidal activity at 8-16 ppm against MRSA, MSSA, *Bacillus subtilis* and *Salmonella typhi* and bacteriostatic activity at 4 ppm towards MRCNS.

Key words: Antibacterial, *Kaempferia pandurata* (Roxb.), temu kunci, MRSA, MRCNS, MSSA, *Bacillus subtilis*, *Salmonella typhi*

INTRODUCTION

The prevalence increase of microbial resistance to antibiotics, especially multidrug resistance causing a difficulty to eradicate infectious disease by antibiotics, this adds the urgency to search for alternative agents. Much effort has been taken to solve this rising problem as one example is by using plants. Plants have been used for centuries as folk medicines. Using systematic screening of plants could lead to the discovery of potential herbal medicine and new active compounds. Furthermore, medicines from natural products especially plants have been progressing from time to time. The advantage of plant as a holistic medicine gives lesser side effects over modern medication produced by synthetic chemicals. Significant antimicrobial effect have been shown by many herbs with low MICs against many pathogenic microbes including resistant microbes to conventional antibiotics (Watson and Preedy, 2008).

Kaempferia pandurata (Roxb.) with synonym *Boesenbergia pandurata* and with Indonesian local name temu kunci is a perennial herb of the family Zingiberaceae. The fresh rhizome is used as a spice and in folk medicine for the treatment of several disorders. Prior to this research, many researcher reported the constituents of the

Kaempferia pandurata rhizomes namely cardamonin, pinostrobin, pinocembrin, 2',6'-dihydroxy-4'-methoxychalcone and a new chalcone boesenbergin A (Jaipetch *et al.*, 1982, 1983), boesenbergin B (Mahidol *et al.*, 1984), 5,7-dimethoxyflavone, 1,8-cineole and pandurantin (Pancharoen *et al.*, 1987).

Studies has previously been reported to have carried research about its antibacterial effect of rhizome extract of *Kaempferia pandurata*. Hwang *et al.* (2004) found that isopanduratin active against *Streptococcus mutans*. The Essential oil and 95% ethanol extracts of *Kaempferia pandurata* rhizomes showed potent activity against *Candida albicans* and active against *Lactobacillus* sp., *Streptococcus mutans* and *Actinobacillus actinomycetemcomitans* (Taweekaisupapong *et al.*, 2010). The similar study was done by Sroisiri and Boonyanit, 2010 that proved the activity of the extract on *Candida albicans*. Taweekaisupapong *et al.* (2010) also reported that the essential oil was effective against three filamentous fungi (*Aspergillus niger*, *Aspergillus fumigatus* and *Mucor* sp.) and five strains of yeast (*Saccharomyces cerevisiae*, *Cryptococcus neoformans*, *Candida albicans*, *Candida tropicalis* and *Torulopsis glabrata*). Panduratin A showed activities against multi-species oral biofilms

consisting of *Streptococcus mutans*, *Streptococcus sanguis* and *Actinomyces viscosus*. Panduratin A has also anti-inflammatory activity (Yun *et al.*, 2003). Another researcher also reported that the essential oil possessed activities against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Listeria monocytogenes* (Natta *et al.*, 2008).

The detection of decreased penicillin (methicillin) susceptibility in *Staphylococcus aureus* has become important with the increased prevalence MRSA (Dipiro, 2008), this condition lead to the higher needs of new antibiotic and as an alternative we can search antibacterial substances from plants. MRSA is crucial to ensure appropriate therapy, it cause outbreaks in many hospitals and nursing homes and are endemic in others. Some MRSA strains are resistant to all antibiotics except glycopeptide vancomycin and isolates with mutations conferring low-level resistance to glycopeptides have already been reported (Greenwood, 2000).

Coagulase-negative staphylococci is gaining importance as multi-resistant pathogens causing infections that are difficult to treat (Greenwood, 2000).

This study was to do an advance study about the potency of *Kaempferia pandurata* (Roxb.) ethanol extract in having antibacterial effect towards resistant strain bacteria and selected gram positive and gram negative bacteria. The bacteria selected were methicillin resistant *Staphylococcus aureus* (MRSA), methicillin resistant coagulase negative *Staphylococci* (MRCNS), methicillin-sensitive *Staphylococcus aureus* (MSSA), *Bacillus subtilis*, *Salmonella typhi*. The plant's ethanol extract which shows positive activity was compared to the standard antibacterial drug with broad spectrum activity tetracycline HCl. In order to determine the MIC, microdilution method was carried out by using sterile plates with 96 round bottomed-microwells. The medium Mueller Hinton Broth was used in this study. Then to determine the total colony count for time kill assay as well as equivalency test Mueller Hinton Agar was used.

MATERIALS AND METHODS

Materials: Dried temu kunci (*Kaempferia pandurata* (Roxb.)) rhizome was purchased from Manoko farm in Lembang, West Java, Indonesia at the end of January 2012 and identified by expert in Herbarium Bandungense, School of Biological Science and Technology, Bandung Institute of Technology. Mueller Hinton Agar (MHA) and Mueller-Hinton-Broth (MHB) Sigma-Aldrich were used as growth media.

Tests bacteria: Tests bacteria include Methicillin Resistant *Staphylococcus aureus* (MRSA), Methicillin resistant *Coagulase Negative Staphylococci* (MRCNS), *Methicillin-sensitive Staphylococcus aureus* (MSSA), *Bacillus subtilis*, *Salmonella typhi* were obtained from culture collection of School of Pharmacy ITB.

To culture and grow the bacteria, agar slant using MHA was used, while to prepare bacteria suspension and for dilution MHB was used.

Sample preparation: The temu kunci rhizomes were first washed and dried under the shade then chopped into little pieces using a chopper blender, then it was dried in the oven under 40-42°C for a few days. The dried rhizomes which is grinded to a mash 20 until crude drug powder is obtained.

Preparation of ethanol extract: The powdered of temu kunci was extracted with ethanol using Soxhlet apparatus. The ethanol extract was evaporated to remove the solvent to obtain a thick concentrated extract. The stock solution was prepared by 1024 ppm of extract with 10% DMSO.

Characterization of temu kunci rhizomes ethanol extract: Characterization of the plant was done through general test which was the phytochemical screening (determine alkaloid, flavonoid, quinine, tannin, saponin, steroid/ triterpenoid), total ash, water soluble and acid insoluble ash, loss on drying, water and ethanol extractable matter, water content, yield of extract, density and volatile oil content (WHO, 1998).

Preparation of suspended microbe: After the agar slant was scratched using an Oese needle with the microbe of interest it is kept in the incubator at 37°C for 24 h. Then the following day, the microbe that has grown on the surface of the agar slant is slowly taken using the Oese needle again and suspended in the MHB and incubated for 18-24 h at 37°C. The next day, the suspended microbe is diluted till it reaches the 0.5 McFarland standards or the UV-Vis spectrophotometer absorbance range between 0.08-0.13. After it has fulfilled the absorbance requirement, the 0.5 McFarland suspensions is diluted again with MHB to a ratio of 1:20. This new suspension is used to test on the 96 round bottomed microwells by micro dilution method, the microbe that is estimated to be in this 1:20 suspension is about 1×10^6 . Therefore, the final amount of microbe in one microwell would be 5×10^5 (CSLI, 2009).

Determination of total colony forming unit: The microbe suspension 1:20 is the used to determine the total colony

forming unit by means of dilution till 10^{-4} or 10^{-5} . A 100 μ L from this suspension is pipetted and placed on the liquid form of MHA 20 mL in a petri dish. It is then gently shaken so the bacteria will disperse evenly to the bottom and in the middle of the medium. It is kept in the incubator for 24 h, then the colony forming unit is recorded using the Colony Counter.

Testing of antibacterial effect of temu kunci ethanol extract towards bacteria and mic determination: The method that was used is microdilution with 96 round-bottomed-microwell plates. A 100 μ L of MHB (which is sterilized by autoclave) is added into each of the microwells. Then, 100 μ L of the extract is added at the 12 position (1st row, 12column) on the microdilution plate. Next it is mixed till homogenized and 100 μ L is removed from that and added into the next microwell at the 11 position (it goes from right to left on the plate) the previous procedure is repeated. After the microdilution is performed on the entire plate, finally the 100 μ L of the bacteria suspension (1:20) is added filling up each microwell to 200 μ L at total. The entire 1 column is filled with 200 μ L of MHB (negative control) and 2nd column is filled with 100 μ L of bacteria and 100 μ L of MHB (positive control without extract present). It is finally incubated for 18-24 h. MIC is observed at the smallest concentration where no microbe precipitate is sediment at the bottom of the microwell based on CLSI (clinical and laboratory standard institute) method (CSLI, 2009).

The sample of the plant extract activity was tested. The initial concentration was 1024 ppm (CSLI, 2009). After it is diluted with the media the concentration is half of the initial 512 ppm, then the addition of the microbe suspension makes it half again, so the highest concentration would be 256 ppm at the 12 position this process continues to 128, 64, 32, 16, 8, 4, 2, 1 and 0.5 ppm. This is also done for the two reference antimicrobial agents, tetracycline HCl. It is done duplo (2 \times) and extract is done triplo (3 \times).

Equivalency antibacterial activity determination of temu kunci ethanol extract with standard antibacterial agent (tetracycline HCl): The antibacterial activity of extract was then compared with tetracycline HCl. The equivalency antibacterial activity was determined based on finding the concentration of extract that had inhibition diameter similar to or near to that of tetracycline HCl. The procedure is done by using the same bacterial suspension as described earlier. Then from that 100 μ L is taken from the bacterial suspension of 1:20 and pipette out on the semi-liquid MHA plate. It is allowed to solidify then using the cylinder holes are punched, 10 holes in one petri plate. Then the extract and tetracycline concentration is pipette (20-23 μ L) is placed in those holes. It is incubated at 37°C

for 24 h. For each bacterium, the test was done triplo and the average of inhibition was calculated. Then a standard curve of inhibition diameter versus log concentration of tetracycline HCl was drawn for each bacterium. The equivalency activity was gained from linear regression of the standard curve for each tested bacteria.

Killing profile test: Killing profile test was performed to evaluate the bactericidal reduction by the extract at concentration of MIC against the tested bacteria at six different incubation time; 0, 30,60, 90, 120 and 150 min. The concentration of MIC (different MIC for different tested bacteria). The same ratio from the microdilution well is taken and calculated for a 5 mL ratio to be done in the sterilized test tube. According to the MIC, the concentration for 5 mL is calculated; therefore, 200 μ L of extract as well as 200 μ L of bacteria suspension and 4600 μ L of MHB is added to the test tube. Then it is immersed into a shaker filled with aquades and the temperature is constant to 37°C. Four tests are done here, the positive control positive (containing bacteria suspension (400 μ L and MHB), extract, tetracycline HCl. For the first 0 min, 100 μ L is pipette and inoculated on the 20 mL of liquid MHA then it is shaken gently. It is left to solidify, after which it is placed in the incubator at 37°C, for 18-24 h. Then the viable colony forming units are counted using Colony Counter. This test was done triplo. The log viable colony number was plotted versus time to construct the time killed profile of the extract for each bacteria. The time kill profiles were compared.

RESULTS AND DISCUSSION

The crude drug contains the same phytochemical groups as in the extract which is tannin, saponin, flavonoid and steroid/triterpenoid. Phytochemical screening was done to determine the nature of the crude drug itself. This is because of plants that are sourced from different regions and environment posses varying characteristic. Phytochemical screening result of the crude drug as well as the extract can be seen in Table 1. As seen in Table 1, both crude drug and extract contains tannin, saponin, flavonoid and steroid/triterpenoid. It is important to assess their quality because it indirectly affects the antimicrobial activity on the test microbes. The characterization of crude drug were total ash, water soluble ash, acid soluble ash, loss on drying and water content and for extract were water extractable matter, ethanol extractable matter, water content, extract yield, density and volatile oil. The results of the characterization are listed in Table 2 and 3.

The water content of *Kaempferia pandurata* (Roxb.) was 6.0% for the crude drug but 2.2 for the extract. The Farmakope Herbal Indonesia stated that water content

Table 1: Class of chemical compound in temu kunci crude drug and ethanol extract

Phytochemical group	Crude drug	Extract
Tannin	+	+
Saponin	+	+
Flavonoid	+	+
Alkaloid	-	-
Quinone	-	-
Steroid/triterpenoid	+	+

+: Detected and -: Not detected

Table 2: Characterization results of temu kunci crude drug

	Result (% w/w)
Total ash	5.06
Water soluble ash	2.67
Acid soluble ash	1.81
Loss on drying	6.50
Water content	6.00*

* = % v/w

Table 3: Characterization results of *Kaempferia pandurata* (Roxb.) Ethanol extract

	Results (% w/w)
Water extractable matter	5.82
Ethanol extractable matter	6.75
Water content	2.20*
Extract Yield	19.1
Density	0.80 g mL ⁻¹
Volatile oil	0.5*

* = % v/w

from crude drug should be less than 10%. The crude drug has higher percentage of ethanol soluble extractable matter compared to water soluble extractable matter. This shows that compounds in crude drug have higher solubility in ethanol than water. Volatile oil is one of the important compounds that have antibacterial activity; its content in the extract was determined by distillation method. The extract contained 0.5% volatile oil.

For MIC (Minimum Inhibitory Concentration) determination of the extract, in vitro susceptibility test was performed in a sterilized 96 round bottomed microwells in a titre plate. The standard used was Muller Hinton Broth (MHB), with bacterial inoculums of 5×10^5 CFU mL⁻¹, according to guidelines stated by CSLI standards. A twofold dilution of *Kaempferia pandurata* (Roxb.) ethanol extract was mixed with the test microbe (5×10^5 CFU mL⁻¹ in Muller Hinton Broth (MHB)) medium. Firstly, the suspended bacteria in the MHB are checked for its absorbance under the UV-Visible spectrophotometer (wavelength 625 nm). This should be in the range of 0.08–0.13 for absorbance; if it is higher further dilution is carried by adding MHB. After in the range, it was pipette 1 mL from the suspended microbe and diluted at a ratio of 1:20 with MHB. The absorbance of each test bacteria can be seen in Table 4.

The bacterial suspension that was made is required to meet the colony forming unit of 1×10^6 . This was done with the method of counting “Total Number of Plate counts”, the result can be seen in Table 5.

Table 4: Absorbance range for each tested bacteria

Microbe	Absorbance ($\lambda = 625$ nm)
MRSA	0.096
MRCNS	0.087
MSSA	0.101
<i>Bacillus subtilis</i>	0.111
<i>Salmonella typhi</i>	0.098

Table 5: Colony forming units for total plate counts

Microbe	Range of total colony counts
MRSA	6.7×10^5 – 1.03×10^6
MRCNS	7.2×10^5 – 1.16×10^6
<i>Bacillus subtilis</i>	1.6×10^6 – 2.3×10^6
MSSA	8.4×10^5 – 1.15×10^6
<i>Salmonella typhi</i>	8.4×10^5 – 1.63×10^6

Table 6: The Minimum Inhibitory Concentration (MIC) of Extract and Tetracycline HCl Against All Tested Bacteria

Microbes	MIC (ppm) of extract	MIC (ppm) of tetracycline
MRSA	8-16	16
MRCNS	2-4	4
MSSA	4-8	2
<i>Bacillus subtilis</i>	8-16	2
<i>Salmonella typhi</i>	4-8	2

As stated previously, the MIC determination was done using the microdilution method, whereby, the smallest concentration which inhibits the microbe growth was recorded. From the observation, MIC was the reported when the microwell free from precipitation sediment at the bottom of the well. We can compare the results to the reference drug used which is tetracycline HCl as it has a broad spectrum antimicrobial activity but tetracycline is not a drug of choice for MRSA, therefore the MIC of tetracycline is high i.e., 16 ppm (Table 6).

Temu kunci ethanol extract showed antibacterial activity maybe due to the presence of major compounds such as flavonoid, tannin, saponin and steroid/triterpenoid as well as volatile oil. The phenolic group of this compound plays an important role in killing bacteria by binding to extracellular protein to form complex and thus disrupt the integrity of the bacterial cell membrane (Yanti *et al.*, 2009).

Flavonoid has been found to have a significant antibacterial effect. The type of flavonoids found in temu kunci are flavones and flavanoids (pinostrobin, alpinetin, pinocembrin), chalcones (cardamomin) and dihydrachalcones (pandurantin A). The chalcone is one of the flavonoid types, has been discovered to inhibit the activity of beta lactamase and had intrinsic antibacterial activity (Jaipetch *et al.*, 1982, 1983; Mahidol *et al.*, 1984; Pancharoen *et al.*, 1987).

Tannin has phenol group that can precipitate bacterial membrane protein that will disrupt the cell permeability and cause cell death, its other mechanism to kill the bacteria is by inactivating microbial adhesion, enzymes, cell envelope transport protein. They also form complex with polysaccharides. The volatile oil content in *Kaempferia pandurata* (Roxb.) ethanol extract gives an

Table 7: Inhibition diameter of extract for each tested bacteria

	Inhibition diameter for extract concentration (% w/v) (Mean±SD) mm						
Tested bacteria	512	256	128	64	32	16	8
MRSA	21.2±0.05	14.4±0.08	8.5±0.03	-	-	-	-
MRCNS	25.12±0.03	17.2±0.07	11.9±0.02	8.22±0.05	-	-	-
MSSA	19.06±0.06	12.46±0.07	10.51±0.03	9.75±0.05	-	-	-
<i>B. subtilis</i>	21.16±0.06	18.79±0.01	11.31±0.04	8.21±0.03	-	-	-
<i>S. typhi</i>	24.3±0.02	18.31±0.03	15.18±0.03	9.28±0.03	-	-	-

Table 8: Inhibition diameter of tetracycline HCl for each tested bacteria

Tetracycline HCl ($\mu\text{g mL}^{-1}$)	Inhibition diameter \pm SD (mm)	Tetracycline HCl ($\mu\text{g mL}^{-1}$)	Inhibition diameter \pm SD (mm)
<i>Bacillus subtilis</i>			
35	14.12±0.35	85	12.54±0.13
30	14.09±0.21	80	12.25±0.08
25	13.45±0.53	75	12.05±0.10
20	13.08±0.32	70	11.65±0.35
15	12.09±0.52	65	11.52±0.67
MRSA			
100	9.75±0.13	100	11.26±0.25
95	9.36±0.07	95	11.02±0.9
90	9.1±0.24	90	10.79±0.36
85	8.92±0.17	85	10.57±0.07
80	8.56±0.82	80	10.26±0.25
<i>S. typhi</i>			
110	21.15±0.45		
105	19.28±0.35		
100	18.27±0.17		
95	17.65±0.31		
90	16.48±0.62		

n = 3

Table 9: Linear regression of standard curve for each tested bacteria

Linear regression of standard curve inhibition		
Tested bacteria	diameter vs log of tetracycline HCL concentration	R ²
<i>Bacillus subtilis</i>	y = 5.86x+5.30	0.98
MSSA	y = 9.074x-4.99	0.98
MRSA	y = 11.57x- 13.4	0.98
MRCNS	y = 10.08x-8.81	0.96
<i>S. typhi</i>	y = 50.59x-82.5	0.99

Table 10: Equivalency Antibacterial Activity of Extract Compared to Tetracycline HCl

Tested bacteria	Extract ($\mu\text{g mL}^{-1}$)	Tetracycline HCl ($\mu\text{g mL}^{-1}$)	1 mg of extract to tetracycline HCl (μg)
<i>Bacillus subtilis</i>	128	10.61	82.9
MSSA	128	51.07	398.9
MRSA	128	78.13	610.3
MRCNS	128	113.39	885.8
<i>S. typhi</i>	256	98.33	384.1

antibacterial activity where its mechanism is by disturbing the process of membrane or cell wall formation which results in incomplete structure of cell wall leading to death of bacterial cell. This was done by action of the hydroxyl and carbonyl functional group of volatile oil that interacts with bacterial protein by hydrogen bond. The interaction produces complex which causes denaturation of protein and membrane cell lysis (Lattanzio *et al.*, 2006).

The antibacterial activity of temu kunci ethanol extract was compared to tetracycline HCl standard. The susceptibility test was done by agar diffusion method using metal cylinder to punch holes in the solidified agar containing suspended bacteria. In performing the test, factors that influences the inhibition diameter was made constant such as amount of bacteria used, type, volume of agar medium, size of Petri dish, the incubation time and condition and volume of sample; only the concentration of tetracycline HCl and temu kunci ethanol extract was not made constant. Serial concentration of extract and tetracycline HCl were tested against each bacteria. Finally, the inhibition diameter with standard deviation was calculated (Table 7, 8).

A standard curve of inhibition diameter is drawn versus log tetracycline HCl concentration for each bacteria that was tested and the linear regression was taken (Table 9). From the linear regression of the standard curve, equivalency of antibacterial activity of *Kaempferia pandurata* (Roxb.) ethanol extract was determined by substituting the inhibition diameter of extract with the value near to that of tetracycline HCl. The equivalency of antibacterial activity of tetracycline HCl in μg was compared with that of 1 mg of extract.

The result showed that antibacterial activity of temu kunci ethanol extract compared to tetracycline HCl was lowest against *Bacillus subtilis* where 1 mg of extract is equivalent to the smallest amount of tetracycline which is 82.9 μg . On the other hand, antibacterial activity of the extract compared to tetracycline HCl was highest against MRCNS where 1 mg equivalent to 885.8 μg of tetracycline HCl, because tetracycline is not drug of choice for *Staphylococcus aureus* (Table 10).

The time kill curve shows the activity of temu kunci extract towards the tested bacteria. Based on bacterial

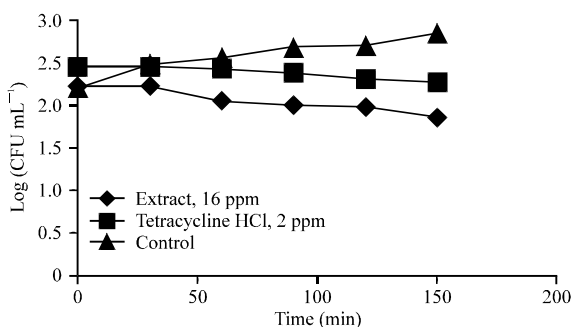


Fig. 1: Number of *Bacillus subtilis* colony in log CFU mL⁻¹ against time when tested with extract, tetracycline HCl and control

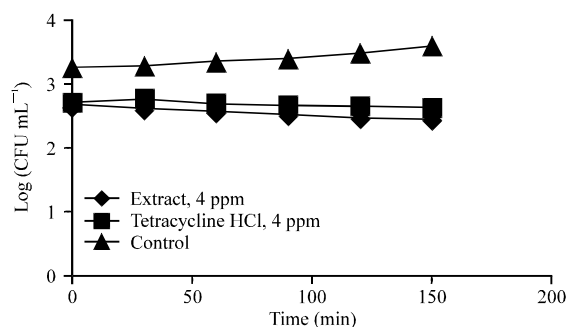


Fig. 4: Number of MRCNS colony in log CFU mL⁻¹ against time when tested with extract, tetracycline HCl and control

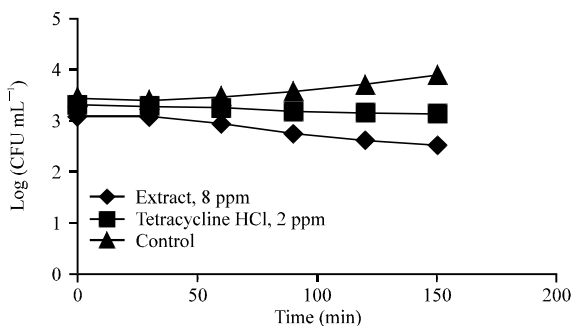


Fig. 2: Number of MSSA colony in log CFU mL⁻¹ against time when tested with extract, tetracycline HCl and control

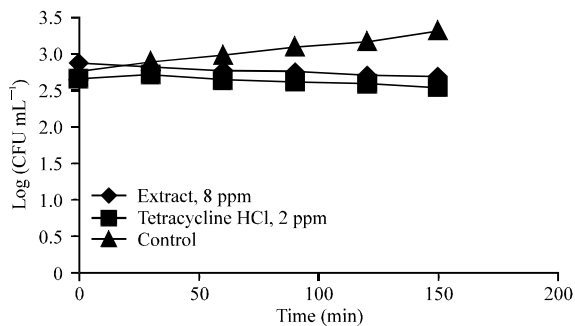


Fig. 5: Number of *Salmonella typhi* colony in log CFU mL⁻¹ against time when tested with extract, tetracycline HCl and control

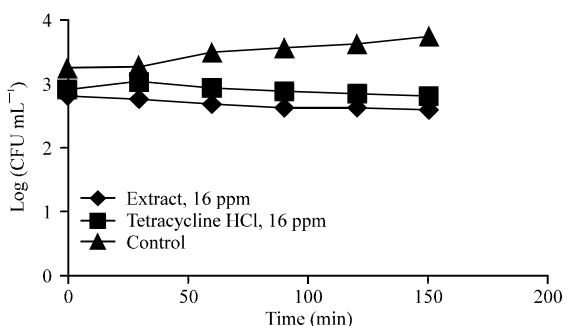


Fig. 3: Number of MRSA colony in log CFU mL⁻¹ against time when tested with extract, tetracycline HCl and control

growth from the Fig. 1, the extract showed a clear decrease in the CFU. The extract shows bactericidal activity against *Bacillus subtilis*, compared to tetracycline HCl which showed bacteriostatic activity. It is the same in Fig. 2 for MSSA bacteria as well. In Fig. 3, MRSA colonies reduced in a bacteriostatic action by tetracycline HCl as for the extract it was bactericidal action. Fig. 4, MRCNS showed

similar activity with tetracycline HCl, bacteriostatic. Finally, Fig. 5 for *Salmonella typhi*, the extract has a better bactericidal activity at 16 ppm than the tetracycline HCl which has a bacteriostatic curve. The killing profile for temu kunci extract displayed bactericidal activity against *Bacillus subtilis*, MSSA, MRSA, *Salmonella typhi* and bacteriostatic towards MRCNS. As the incubation time increases, bactericidal colony decreases gradually. In longer incubation time, the contact time of bacteria with extract was higher antibacterial effect of extract towards bacteria. Time is the factor for active compounds present in extract to take action and exert different mechanism to kill the bacteria. The timed kill curve for tetracycline HCl displayed bacteriostatic at 2 ppm and 4 ppm and bactericidal at 8 ppm - 16 ppm.

CONCLUSION

Ethanol extract of *Kaempferia pandurata* (Roxb.) showed antibacterial activity against all the tests bacteria used in this study including resistant bacteria and the most potent against methicillin resistant *coagulase*

negative *Staphylococci* (MRCNS) with MIC of 4 ppm. Minimum inhibitory concentration of the extract was 16 ppm against *methicillin resistant Staphylococcus aureus* (MRSA), 8 ppm against *methicillin sensitive Staphylococcus aureus* (MSSA), 16 ppm against *Bacillus subtilis* and 8 ppm against *Salmonella typhi*.

The killing profile test of extract displayed bactericidal activity at > 8 ppm (against *Bacillus subtilis*, MSSA, MRSA, *Salmonella typhi*) and bacteriostatic at 4 ppm (against MRCNS).

REFERENCES

- CSLI, 2009. Methods for Dilution Antimicrobial Susceptibility Test for Bacterial That Grow Aerobically& Anaerobically; Approved Standard M7-A6. 6th Edn., CLSI, Wayne, PA., Pages: 23.
- Dipiro, J.T., 2008. Pharmacotherapy: A Pathophysiologic Approach. 7th Edn., McGraw-Hill, New York, USA., Pages: 1901.
- Greenwood, D., 2000. Antimicrobial Chemotherapy. 4th Edn., Oxford University Press, Oxford, UK., pp: 143.
- Hwang, J.K., J.Y. Chung, N.I. Baek and J.H. Park, 2004. Isopanduratin A from *Kaempferia pandurata* as an active antibacterial agent against cariogenic *Streptococcus mutans*. Int. J. Antimicrob. Agents, 23: 377-381.
- Jaipetch, T., S. Kanghae, O. Pancharoen, V. Patrick, V. Reutrakul, P. Tuntiwachwuttikul and A. White, 1982. Constituents of *Boesenbergia pandurata* (syn *Kaempferia pandurata*): Isolation, crystal structure and synthesis of (±)-Boesenbergin A. Aust. J. Chem., 35: 351-361.
- Jaipetch T., V. Reutrakul, P. Tuntiwachwuttikul and T. Santisuk, 1983. Flavonoids in black rhizomes of *Boesenbergia pandurata*. Phytochemical, 22: 625-626.
- Lattanzio, V., V.M.T. Lattanzio and A. Cardinali, 2006. Role of Phenolics in the Resistance Mechanisms of Plants Against Fungal Pathogens and Insects. In: Phytochemistry: Advances in Research, Imperato, F. (Ed.). Research Signpost, Trivandrum, Kerala, India, pp: 23-67.
- Mahidol C., P. Tuntiwachwuttikul, V. Reutrakul and W.C. Taylor, 1984. Constituents of *Boesenbergia pandurata* (syn. *Kaempferia pandurata*). III. Isolation and synthesis of (±)-boesenbergin B. Aust. J. Chem., 37: 1739-1745.
- Natta, L., K. Orapin, N. Krittika and B. Pantip, 2008. Essential oil from five *Zingiberaceae* for anti food-borne bacteria. Int. Food Res. J., 15: 337-346.
- Pancharoen, O., P. Kelvin, V. Reutrakul, W.C. Taylor and P. Tuntiwachwuttikul, 1987. Constituents of the *Zingiberaceae*. Aust. J. Chem., 40: 455-459.
- Sroisiri, T. and T. Boonyanit, 2010. Inhibition of candida adhesion to denture acrylic by *Boesenbergia pandurata*. Asian Pac. J. Trop. Med., 3: 272-275.
- Taweekhaisupapong, S., S. Singhara, P. Lertsatitthanakorn and W. Khunkitti, 2010. Antimicrobial effects of *Boesenbergia pandurata* and *Piper sarmentosum* leaf extracts on planktonic cells and biofilm of oral pathogens. Pak. J. Pharm. Sci., 23: 224-231.
- WHO, 1998. Quality Control Methods for Medicinal Plant Materials. World Health Organization, Geneva, Switzerland, pp: 28-37.
- Watson, R.R. and V.R. Preedy, 2008. Botanical Medicine in Clinical Practice. CABI International, London, UK., Pages: 224.
- Yanti, Y.R., K. Lee, S. Han and J.K. Hwang, 2009. Activity of panduratin A isolated from *Curcuma xanthorrhiza* Roxb. against multi-species oral biofilms. J. Oral Sci., 51: 87-95.
- Yun, J.M., H. Kwon and J.K. Hwang, 2003. *In vitro* anti-inflammatory activity of panduratin A isolated from *Kaempferia pandurata* in RAW264.7 cells. Planta Medica, 69: 1102-1108.