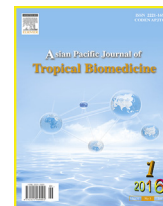




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The antibacterial activity of selected plants towards resistant bacteria isolated from clinical specimens



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ABSTRACT

Objective: To evaluate the antibacterial activity of eight plants against methicillin-resistant *Staphylococcus aureus* (MRSA), extended spectrum beta-lactamase and carbapenemase-resistant Enterobacteriaceae, which are the most prevalent causes of infections in inpatients.

Methods: The antibacterial activity was calculated based on the minimum inhibitory concentration using Mueller–Hinton broth in a microdilution method.

Results: The best antibacterial activity, calculated as minimum inhibitory concentration values, against MRSA was shown by the *Kaempferia pandurata* (Roxb) (*K. pandurata*) extract (256 µg/mL) and the *Senna alata* (*S. alata*) extract (512 µg/mL). Phytochemical screening of dried *S. alata* leaf and its extract showed the presence of flavonoids, alkaloids, saponins, quinones, tannins and sterols, while dried *K. pandurata* and its extract only showed the presence of flavonoids and sterols/triterpenoids.

Conclusions: *K. pandurata* and *S. alata* have the potential to be developed as antibacterial agents, especially against MRSA strain, but further *in vivo* research and discovery of the mode of its action are still needed to shed light on the effects.

1. Introduction

Antibiotic resistance has become a serious and widespread problem in developing countries, both in hospitals and the community, causing high mortality each year [1]. Inappropriate usage of antibiotics is the most influential factor of antibiotic resistance and the global emergence of multi-drug resistant bacteria is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure [2]. Antibiotic resistance results in reduced efficacy of antibacterial drugs, making the treatment of patients difficult, costly, or even impossible. The impact on particularly vulnerable patients is most obvious, resulting in prolonged illness and increased mortality [3]. New therapy classes of antibiotics have become a popular choice to reduce antibiotic resistance. However, antibiotic resistance is difficult to reduce. One strategy to avoid this is by using alternative therapeutic agents from plants that are effective against antibiotic resistant bacteria,

safe and have low cost. Consequently, one of the objectives of our research group is to investigate the potential antibacterial properties of traditional plants. In the present study, we used eight plants that have the potential to be used as antibacterial agents against non-resistant bacteria [4–11], to conduct antibacterial activity assays against resistant bacteria isolated from inpatients, such as methicillin-resistant *Staphylococcus aureus* (*S. aureus*) (MRSA), extended spectrum beta lactamase (ESBL)-producing bacteria and carbapenemase-resistant Enterobacteriaceae (CRE). The eight plants used in this study were *Curcuma xanthorrhiza* (*C. xanthorrhiza*), *Ocimum sanctum* (*O. sanctum*), *Senna alata* (*S. alata*), *Kaempferia pandurata* (Roxb) (*K. pandurata*), *Zingiber officinale* (*Z. officinale*), *Moringa oleifera* (*M. oleifera*), *Tamarindus indica* (*T. indica*) and *Pangium edule* (*P. edule*).

2. Materials and methods

2.1. Plants

Fresh plant parts were collected from the Manoko field in Bandung at winter season. The collected plants were identified and classified according to the Herbarium Bandungense at the

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School of Life Sciences and Technology research centre. They were then dried under sunlight for two days. The dried plants were then milled into fine powder using a milling machine.

2.2. Materials

The used materials were ninety-six microwell plates, autoclave, shaker, laminar air flow, Eppendorf, micropipette, separation funnel, glass set, chromatography set and MRSA collected from hospital, ethanol, *n*-hexane, ethyl acetate, water, dimethyl sulfoxide, Mueller–Hinton broth and Mueller–Hinton agar, curved.

2.3. Preparations of extracts

Dried *C. xanthorrhiza*, *O. sanctum*, *S. alata*, *K. pandurata*, *Z. officinale* and *M. oleifera* were extracted by the reflux method using ethanol 96% while *T. indica* and *P. edule* were extracted by maceration using distilled water (aqua dest). The solvent in the extracts was completely removed by using a rotary evaporator to obtain a semi-solid mass and the yield was calculated based on the weight of the dried plants. A portion of the resulting crude extract was fractioned by a separation funnel using *n*-hexane, ethyl acetate, and water as solvents. Eluates were collected in 1 L Erlenmeyer flasks and each fraction was subjected to evaporation under reduced pressure in a rotary evaporator. Fractions were stored at 4 °C until assayed.

2.4. Bacterial preparation

The MRSA, ESBL-producing bacteria and CRE were taken from isolated specimens which exhibited resistance to some antibiotics in hospitalized patients. They were taken based on ethical clearance approval from the ethical committee in the hospital. The bacteria were cultured over night (18–24 h) at 37 °C on nutrient broth for the preparation of cell suspensions. The bacteria cell suspensions were homogenized and adjusted to 0.5 McFarland standards (5×10^5 CFU/mL) using spectrophotometry.

2.5. Antimicrobial susceptibility assays

The minimum inhibitory concentrations (MICs) of plant extracts were initially determined using Mueller–Hinton broth microdilution [12]. MIC determination was performed by a serial dilution technique using 96-well microtiter plates. Plant extract (100 µL) was placed into the well/plate. Then, 100 µL bacterial cell suspensions were placed in each well/plate. Microplates were incubated for 24 h at 37 °C. The lowest concentrations without visible growth completely inhibited the bacteria (MICs). Dimethyl sulfoxide was used as a control and Mueller–Hinton broth as negative control. Tetracycline and vancomycin were used as positive controls for MRSA, while cefotaxime and meropenem were used as positive controls for ESBL-producing bacteria and CRE. The assay was repeated twice with three replicates per assay.

2.6. Phytochemical screening

The selected plants which showed the MIC were screened for the presence of different classes of secondary metabolites,

including alkaloids, flavonoids, alkaloid, saponins, tannins, quinones and sterols/triterpenes using previously described methods [13].

3. Results

3.1. Extracts yield

The ethanolic extracts of eight plants and the water extracts of two plants were calculated for the yield. *T. indica* showed the highest yield in water solvent, which showed that its constituents were relatively polar (Table 1).

3.2. The antibacterial activity

The antibacterial activity of the eight extracts were assayed *in vitro* by the agar microdilution method against three resistant bacteria. The antibacterial activity against each bacterium was observed to be varied. Table 2 shows that among the eight plants, *K. pandurata* exhibited the smallest value of MIC against MRSA (256 µg/mL) (Figure 1), while *S. alata* and *C. xanthorrhiza* also had the same activity against MRSA (Figures 2 and 3). This showed that *K. pandurata*, *S. alata* and *C. xanthorrhiza* had the potential to be developed as antibacterial agents for MRSA strains.

3.3. Phytochemical analysis

The screening of the phytochemical composition was conducted only for the two dried plants, *S. alata* and *K. pandurata*, as well as their extracts, that showed the lowest MIC, because they have the potential to be developed as antibacterial agents. The secondary metabolites are shown in Table 3. All tested

Table 1

The extract yield.

Plants	Part of plants	Solvent	Yield (%)
<i>K. pandurata</i>	Rhizome	Ethanol (96%)	26.43
<i>C. xanthorrhiza</i>	Rhizome	Ethanol (96%)	22.35
<i>Z. officinale</i>	Rhizome	Ethanol (96%)	13.53
<i>O. sanctum</i>	Leaf	Ethanol (96%)	6.19
<i>S. alata</i>	Leaf	Ethanol (96%)	21.66
<i>M. oleifera</i>	Leaf	Ethanol (96%)	11.12
<i>P. edule</i>	Fruit	Aqua dest	6.28
<i>T. indica</i>	Fruit	Aqua dest	63.72

Table 2

The antibacterial activity of eight plants towards MRSA, ESBL and CRE.

Plant extracts	MIC (µg/mL)		
	MRSA	ESBL	CRE
<i>O. sanctum</i>	> 8 192	> 8 192	> 8 192
<i>Z. officinale</i>	> 8 192	> 8 192	> 8 192
<i>M. oleifera</i>	> 8 192	> 8 192	> 8 192
<i>S. alata</i>	512	> 8 192	> 8 192
<i>K. pandurata</i>	256	4096	> 8 192
<i>C. xanthorrhiza</i>	512	> 8 192	> 8 192
<i>P. edule</i>	> 8 192	> 8 192	> 8 192
<i>T. indica</i>	> 8 192	> 8 192	> 8 192
Tetracycline HCl	32		
Vancomycin HCl	1		
Cefotaxime		64	128
Meropenem		0.5	2

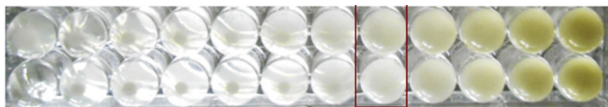


Figure 1. MIC value of *K. pandurata* against MRSA at 256 µg/mL.

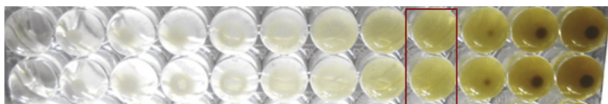


Figure 2. MIC value of *S. alata* against MRSA at 512 µg/mL.

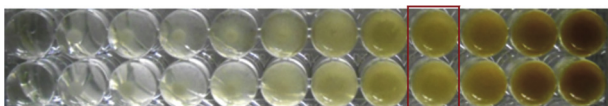


Figure 3. MIC value of *C. xanthorrhiza* against MRSA at 512 µg/mL.

secondary metabolites were present in the *S. alata* extract. In *K. pandurata*, only flavonoids and steroid/triterpenoids were present.

Table 3

Results of phytochemical analysis of dried *S. alata* and its extracts.

Secondary metabolites	<i>S. alata</i>		<i>K. pandurata</i>	
	Dried	Extract	Dried	Extract
Flavonoid	+	+	+	+
Quinnon	+	+	–	–
Saponin	+	+	–	–
Tannin	+	+	–	–
Alcaloid	+	+	–	–
Steroid/triterpenoid	+	+	+	+

+: Present; -: Absent.

4. Discussion

The research for new antibacterial agents has become a very important endeavor, especially in recent times, considering the escalating levels of antibiotic resistance among pathogenic bacteria. One of the efforts in this research is focused on the use of medicinal plants, which are widely available resources, less if no side effects, less expensive and have shown antimicrobial properties [14]. Also, the therapeutic properties of medicinal plants are well recognized at a global level, especially for antibiotic development. Thus, the research of alternative and effective medicines from plants against such resistant bacteria has become an important concern all over the world [15]. Antibiotic misuse has been considered as a major cause of antibiotic resistant bacteria. As a result, bacteria become resistant to antibiotics, which are in turn less effective after extended periods of use. Pharmaceutical companies whose efforts are focused on the production and manufacture of antibiotics strive to manufacture new generations of antibiotics which are capable of treating such antibiotic-resistant bacterial strains [16].

The evolution of antibiotic-resistant bacteria has left researchers scrambling to develop new and stronger antibiotics. MRSA, ESBL-producing bacteria and CRE are commonly occurring drug resistant bacteria in hospitals. MRSA is a major pathogen causing nosocomial and community acquired infection throughout the

world. It is also one of the leading causes of skin, soft tissue, bone, joint, abscess and normal heart valve infections and its infections in humans have been associated with excess morbidity, mortality and increased length of hospitalization [14]. ESBL is an enzyme that mediates resistance to third generation cephalosporins (e.g., ceftazidime, cefotaxime and ceftriaxone) and monobactams (aztreonam), but do not affect cephamycins (cefoxitin and cefotetan) or carbapenems (meropenem and imipenem). *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* are the most common ESBL-producing pathogens [17].

The microdilution method was used in the present study because it is a quantitative reference method routinely used in clinical laboratories. In this method, susceptibility panels in 96-well microtiter plates contained various concentrations of antimicrobial agents. Then, standardized numbers of bacteria were inoculated into the wells of the microtiter plates and incubated overnight at 35 °C. The MIC value was observed as the lowest concentration where no viability was observed in the wells after incubation. Compared with agar-based methods, broth microdilution can decrease much labor and time [18].

Each of the tested extracts displayed antibacterial activity against MRSA, ESBL-producing bacteria and CRE. Furthermore, all extracts showed highly varying MIC values against those resistant bacteria, but the lowest MIC value belonged to *S. alata* leaf extract (512 µg/mL) and *K. pandurata* (256 µg/mL) against MRSA. A previous study has shown that *S. alata* is susceptible to *Streptococcus pyogenes* and *S. aureus*, and the ethanolic extract of *K. pandurata* had antibacterial activity against MRSA, methicillin-susceptible *S. aureus* and *Salmonella typhi* at varying values of MIC [7]. MRSA has the ability to grow in the presence of beta-lactams and its derivatives, including cephalosporin and penicillin. This resistance is intrinsic and can be transferred to susceptible strains through horizontal transfer of the *mecA* gene [16]. All plant extracts showed no antibacterial activity with their high MIC values against ESBL-producing bacteria and CRE.

The results obtained from the present study provide evidence that ethanolic extracts of *S. alata* and *K. pandurata* exhibit antibacterial activities against isolated MRSA strain, which suggests that they may be clinically useful. Further research in respect of these findings are needed and are promising.

Preliminary phytochemical analyses revealed that *K. pandurata* only consisted of flavonoids and sterols/triterpenoids while *S. alata* contained flavonoids, alkaloids, saponins, tannins, quinones, and sterols/triterpenoids. These bioactive compounds have been reported to be used by plants for protection against bacterial and are responsible for antimicrobial activity [7,19,20].

K. pandurata and *S. alata* were shown to be potentially developed as antibacterial agents, especially for MRSA strain. Further *in vivo* research and discovery of mode action are needed to shed light on their antibacterial effects.

Conflict of interest statement

We declare that we have no conflict of interest.

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