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Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2015.08.003

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

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ARTICLE INFO

Article history:
Received 29 Apr 2015
Received in revised form 24 Jun, 2nd revised form 3 Jul, 3rd revised form 4 Jul 2015
Accepted 20 Aug 2015
Available online 10 Nov 2015

Keywords:
Medicinal plants
Antibacterial activity
Minimum inhibitory concentration
Resistance bacteria
Secondary metabolites

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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Peer review under responsibility of Hainan Medical University.

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, alcaloid, 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 (%)
K. pandurata	Rhizome	Ethanol (96%)	26.43
C. xanthorrhiza	Rhizome	Ethanol (96%)	22.35
Z. officinale	Rhizome	Ethanol (96%)	13.53
O. sanctum	Leaf	Ethanol (96%)	6.19
S. alata	Leaf	Ethanol (96%)	21.66
M. oleifera	Leaf	Ethanol (96%)	11.12
P. edule	Fruit	Aqua dest	6.28
T. indica	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
O. sanctum	> 8 192	> 8 192	> 8 192
Z. officinale	> 8 192	> 8 192	> 8 192
M. oleifera	> 8 192	> 8 192	> 8 192
S. alata	512	> 8 192	> 8 192
K. pandurata	256	4 096	> 8 192
C. xanthorrhiza	512	> 8 192	> 8 192
P. edule	> 8 192	> 8 192	> 8 192
T. indica	> 8 192	> 8 192	> 8 192
Tetracycline HCl	32		
Vancomycin HCl	1		
Cefotaxime		64	128
Meropenem		0.5	2