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Original Article

Antibacterial and antioxidant activity of *Rafflesia kerrii* extract against multidrug-resistant bacteria

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Abstract

Rafflesia kerrii (RK) has been used as a folk medicine for the treatment of many diseases. Bactericidal activity of its ethanol extract against multidrug-resistant (MDR) bacteria including methicillin resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia* was investigated using the microbroth dilution method and time-kill assay. Results showed broad spectrum antibacterial activity against all test bacteria with minimum bactericidal concentrations (MBCs) in the range of 0.78 to 6.25 mg/ml. The extract with a concentration of 2xMBC completely killed the MRSA after 24 h-exposure, whereas it completely killed *A. baumannii*, *P. aeruginosa* and *S. maltophilia* after 3 h-exposure. It contained a high total phenolic content (669.66±38.60 mg TAE/g) and exhibited stronger antioxidant activity than the ascorbic acid standard (EC50 at 1.79±0.02 µg/ml). These results indicate that the RK extract is a potential candidate to be used as an antioxidant and antibacterial agent for MDR bacteria.

Keywords: *Rafflesia kerrii*, minimum bactericidal concentration, antioxidant activity, multidrug-resistant bacteria

1. Introduction

The emergence of multidrug-resistant (MDR) bacteria is a major public health problem worldwide, as resistance affects doctors' ability to treat the infection as well as increasing the cost and duration of treatment (Karisetty *et al.*, 2013). Major problematic MDR bacteria causing nosocomial infections include non-fermentative gram negative bacilli (such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia*) and methicillin-resistant *Staphylococcus aureus* (MRSA) (Levy & Marshall, 2004; McGowan, 2006). In Thailand, the incidence of these MDR bacteria has also increased (Dejsirilert *et al.*, 2009a; Dejsirilert *et al.*, 2009b; Mootsikapun *et al.*, 2009). Plants are a valuable source of antibacterial agents in combating MDR bacteria (Cowan, 1999; Gibbon, 2005). In addition, it has been demonstrated that there is an increase in the antimicrobial activity of pure compounds when they are combined with antioxidants

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(Belofsky *et al.*, 2004). Therefore, alternative antimicrobial agents from plants are needed to discover and develop for the treatment of infectious diseases caused by MDR bacteria. *Rafflesia kerrii* Meijer, commonly known as *Bua-phut* in Thai, belongs to the *Rafflesiaceae* family and has been found in southern part of Thailand. It is a parasitic flowering plant (Figure 1) on plants of the genus *Tetrastigma*. A decoction of the flower buds has been traditionally used by folk medicinal practitioners to restore the female uterus after giving birth, to treat fever and to prolong life (Viriyarattanaporn, 2004). Kanchanapoom *et al.* (2007) have reported the presence of hydrolysable tannins and a phenylpropanoid glycoside in the RK flower extract. Several biological effects of RK extract, such as anticancer activity against epidermoid carcinoma cells (Tancharoen *et al.*, 2013), antimutagenicity and antityrosinase activities (Nittayajaiprom *et al.*, 2014), have been previously reported. Furthermore, *Rafflesia* species have been shown to have antibacterial activity against *Bacillus cereus*, *B. subtilis*, *P. aeruginosa* and *S. aureus* (Wiart *et al.*, 2004). However, the antibacterial activity of RK extract against MDR bacteria has not been reported. Therefore, we aimed to investigate antibacterial potentials of RK extracts against MDR bacteria and its antioxidant activity in this study.

2. Materials and Methods

2.1 Plant material and extraction

The flower buds of RK (Figure 1) were collected in October, 2010 from Chumporn, Thailand. The RK voucher specimen was kept at the herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand. The whole flower buds were washed, cut into small pieces and dried in an oven (Memmert, Germany) at 45°C. The dried powder of RK was macerated with 50% ethanol (Lab-Scan Analytical Science, Ireland) for seven days at room temperature. The supernatant was separated, filtrated and then concentrated using a rotary evaporator (Ika, Germany). The concentrated extract was dried using a freeze dryer (Christ, UK). The yield of RK extract was 9.03 %. To prepare the test sample, the extract was dissolved in 50% ethanol, which was the extract solvent, to obtain a 100 mg/ml concentration and then filtered through a 0.45 µm syringe filter (Sartorius, Germany).

2.2 Determination of antibacterial activity

2.2.1 Bacterial cultures

Multidrug-resistant clinical isolates in this study including five strains of methicillin-resistant *S. aureus* (MRSA), *P. aeruginosa* ($n = 5$), *A. baumannii* ($n = 5$), and *S. maltophilia* ($n = 5$) and a single reference strain of *S. aureus* ATCC 25923, *A. baumannii* ATCC 19606, and *P. aeruginosa* ATCC 27853 were used. Clinical isolates of MRSA were

Figure 1. Flower bud of *R. kerrii* Meijer. Diameter is about 20 cm.

obtained from Srinakarind hospital, Thailand and the other species were obtained from Siriraj hospital, Thailand. Bacterial strains were grown in Mueller Hinton broth (MHB) (Difco, USA) at 37°C for 18-20 h and diluted to 1:100 in normal saline solution to 10^6 cfu/ml.

2.2.2 Antibiotic sensitivity

Antibiotic sensitivity of test strains was determined using the disc diffusion method (Jorgensen & Turnidge, 2007). An overnight bacterial culture was diluted to 10^6 cfu/ml and then spread on the surface of Mueller-Hinton agar (MHA) (Difco, USA) using a sterile swab. Antibiotic discs (Oxoid, UK) were placed on the inoculated MHA surface and then incubated at 37°C for 24 h. All tests were performed in duplicate. The diameter of inhibition zones (mm) were measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2011). The antibiotic discs used were piperacillin (100 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefpirome (30 µg), cefoxitin (30 µg), cephalothin (30 µg), imipenem (10 µg), meropenem (30 µg), ampicillin/sulbactam (10/10 µg), piperacillin/tazobactam (100/10 µg), amikacin (30 µg), gentamicin (10 µg), netilmicin (30 µg), ofloxacin (5 µg), levofloxacin (10 µg), ciprofloxacin (5 µg), co-trimoxazole (1.25/23.75 µg), minocycline (30 µg), erythromycin (15 µg), clindamycin (2 µg), and fosfomycin (200 µg).

2.2.3 Antibacterial activity of the extract

Broth microdilution assay was used to determine minimum bactericidal concentrations (Jorgensen & Turnidge, 2007). The 50 µl of bacterial culture (5×10^5 cfu/ml) in MHB was added to the wells of a 96-well microtitre plate (Nunc, USA) which contained 50 µl of serial two-fold diluted plant extract (0.78 to 12.5 mg/ml) in MHB. The plates were incubated at 37°C for 24 h. Then, 5 µl from each well was spotted onto Nutrient agar containing with 5% Tween 80 (Sigma chemical Co., USA) and incubated overnight at 37°C. The minimum bactericidal concentration (MBC) was determined as the lowest concentration that showed no bacterial growth in the wells. Colistin and vancomycin were used as the reference antibiotics for Gram-negative bacilli and Gram- positive bacteria, respectively.

2.2.4 Time-kill assay

The time-kill assay was used to determine the killing rate of the plant extract (Jorgensen & Turnidge, 2007). The extract was prepared at a final concentration of 1 and 2 times of MBC value, inoculated with the test strains (5×10^5 cfu/ml) and incubated at 37°C. Aliquots of the culture (100 µl) at 0, 1, 3, 6 and 24 hours of incubation were taken and diluted in tenfold series by using 0.9 % normal saline solution containing 3 % Tween 80 to determine viable counts using the drop plate technique (Herigstad *et al.*, 2001). After incubation at 37°C for 24 hrs, the colonies were counted. Data from duplicate runs were analyzed by expressing growth as the $\log_{10}$ colony forming unit per milliliter (cfu/ml). The results of time-kill assay were expressed as bactericidal activity when log reduction in viable cell count was more than 3 $\log_{10}$ cfu/ml (99.9 %), and bacteriostatic activity when log reduction in viable cell count was 0-3 $\log_{10}$ cfu/ml, compared with the control (National Committee for Clinical Laboratory Standards, 1992; Pankey & Sabath, 2004).

2.3 Determination of total phenolic content

Total phenolic content of the extract was determined by using Folin-Ciocalteu reagent (Singleton *et al.*, 1999). The various concentrations of the plant extract were dissolved in water (total volume of 0.5 ml) and then mixed with 250 µl of Folin-Ciocalteu reagents (Merck, Germany). After static incubation for 3 min, 1.25 ml of 0.188 mol/l Na_2CO_3 (Sigma chemical Co., USA) was added. The mixture was kept in the dark for 40 min and then the absorbance was measured at 725 nm using a UV spectrophotometer (Shimadzu, Japan). The contents of total phenolics were calculated using a calibration curve from tannic acid standard solution and expressed as mg tannic acid equivalents (TAE) per gram of sample (mg/g). All determinations were conducted in triplicate.

2.4 Determination of antioxidant activity

Antioxidant activity of the plant extract was evaluated using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay (Brand-Williams *et al.*, 1995). Briefly, the extract was dissolved in 95% methanol (BDH Laboratory, UK) to various concentrations and then 2.8 ml of the extract was added with 200 µl of freshly prepared DPPH solution (1 mM in methanol) (Sigma chemical Co., USA). The mixture was incubated at room temperature in the dark for 15 min. The mixture absorbance was measured at 515 nm using a UV spectrophotometer (Shimadzu, Japan). The DPPH solution alone in methanol was used as a control. All the tests were performed in triplicate. L-ascorbic acid (Sigma chemical Co., USA), a positive control for the DPPH method, was used as a standard antioxidant to check that the procedures were working correctly (Molyneux, 2004). DPPH scavenging activity was calculated using the following equation:

$$\% \text{ scavenging activity} = [(A_{control} - A_{extract})/ A_{control}] \times 100 \quad (1)$$

The percentage of scavenging activity was plotted against concentration, EC50 of RK extract and ascorbic acid calculated using linear regression analysis from the graph. The antioxidant activity was expressed as EC50, which is the effective concentration of the extract required to scavenge 50% of DPPH radicals.

2.5 Statistical analysis

The results were expressed as mean ± standard deviation. Statistical analysis of the differences between mean values obtained for experimental groups was conducted using Student's *t*-test. *P*-values of 0.05 or less were considered significant.

3. Results and Discussion

The antibiotic susceptibility profiles of the bacterial isolates used in this study were shown in Table 1. All the test bacteria were multidrug-resistant bacteria that were resistant to at least three classes of antibacterial agents. All five isolates of MRSA were resistant to cefoxitin, cephalothin, gentamicin, ofloxacin, clindamycin, erythromycin, and trimethoprim/ sulfomethoxazole. All *A. baumannii* isolates were resistant to cefotaxime, ceftazidime, imipenem, meropenem, ampicillin/sulbactam, piperacillin/tazobactam, amikacin, netilmicin, levofloxacin and ciprofloxacin. Three isolates of *P. aeruginosa* were resistant to ceftazidime, imipenem, meropenem, piperacillin/tazobactam, amikacin, gentamicin, netilmicin, levofloxacin and ciprofloxacin, while isolate 4 was sensitive to amikacin and isolate 3 was resistant to imipenem, meropenem, levofloxacin and ciprofloxacin. Five isolates of *S. maltophilia* were resistant to piperacillin/tazobactam, netilmicin, and trimethoprim/ sulfamethoxazole. However isolates 1, 2, 4, and 5 remained sensitive to levofloxacin and isolate 3 was resistant to levofloxacin. The standard bacterial strains showed sensitivity to all test antibiotics.

As shown in Table 2, colistin and vancomycin exhibited strong antibacterial activity against all tested MDR and reference bacteria with MICs of 0.25 to 4.00 µg/ml. Although antibacterial activities of both standard antibiotics against the tested bacteria were stronger than RK extract, the RK extract was also found to exhibit potent bactericidal activity. RK extract exhibited broad spectrum antibacterial activities which were effective against both Gram positive and Gram negative bacteria in a similar fashion as has been describe in previous reports on *R. kerrii* and other *Rafflessia* species (Wiart *et al.*, 2004; Wichantuk, 2012). The MBCs of the RK extract against MRSA, *A. baumannii*, *P. aeruginosa*, and *S. maltophilia* isolates were in the range of 1.56-2.34, 1.56, 3.12-6.25, and 0.78-2.34 mg/ml, respectively. The MDR *A. baumannii* isolates showed higher sensitivity to RK extract than those of MDR *S. maltophilia*, MRSA and *P. aeruginosa* (Table 2). These results indicate that RK extract has potential

Table 1. Antibiotic susceptibility profiles of the bacterial isolates used in this study.

Bacterial strains	Antibacterial susceptibility profile
S. aureus ATCC 25923	FOX^S, KF^S, CN^S, OFX^S, DA^S, E^S, SXT^S
P. aeruginosa ATCC 27853	CAZ^S, IPM^S, MEM^S, TZP^S, AK^S, CN^S, NET^S, LEV^S, CIP^S
A. baumannii ATCC 19606	CTX^S, CAZ^S, IPM^S, MEM^S, SAM^S, TZP^S, AK^S, CN^S, LEV^S, CIP^S
	Clinical MDR isolates
MRSA 1	FOX^R, KF^R, CN^R, OFX^R, DA^R, E^R, SXT^R
MRSA 2	FOX^R, KF^R, CN^R, OFX^R, DA^R, E^R, SXT^R
MRSA 3	FOX^R, KF^R, CN^R, OFX^R, DA^R, E^R, SXT^R
MRSA 4	FOX^R, KF^R, CN^R, OFX^R, DA^R, E^R, SXT^R
MRSA 5	FOX^R, KF^R, CN^R, OFX^R, DA^R, E^R, SXT^R
A. baumannii 1	CTX^R, CAZ^R, IPM^R, MEM^R, SAM^R, TZP^R, AK^R, CN^R, LEV^R, CIP^R
A. baumannii 2	CTX^R, CAZ^R, IPM^R, MEM^R, SAM^R, TZP^R, AK^R, CN^R, LEV^R, CIP^R
A. baumannii 3	CTX^R, CAZ^R, IPM^R, MEM^R, SAM^R, TZP^R, AK^R, CN^R, LEV^R, CIP^R
A. baumannii 4	CTX^R, CAZ^R, IPM^R, MEM^R, SAM^R, TZP^R, AK^R, CN^R, LEV^R, CIP^R
A. baumannii 5	CTX^R, CAZ^R, IPM^R, MEM^R, SAM^R, TZP^R, AK^R, CN^R, LEV^R, CIP^R
P. aeruginosa 1	CAZ^R, IPM^R, MEM^R, TZP^R, AK^R, CN^R, NET^R, LEV^R, CIP^R
P. aeruginosa 2	CAZ^R, IPM^R, MEM^R, TZP^R, AK^R, CN^R, NET^R, LEV^R, CIP^R
P. aeruginosa 3	CAZ^S, IPM^R, MEM^R, TZP^S, AK^S, CN^S, NET^S, LEV^R, CIP^R
P. aeruginosa 4	CAZ^R, IPM^R, MEM^R, TZP^R, AK^S, CN^R, NET^R, LEV^R, CIP^R
P. aeruginosa 5	CAZ^R, IPM^R, MEM^R, TZP^R, AK^R, CN^R, NET^R, LEV^R, CIP^R
S. maltophilia 1	TZP^R, NET^R, LEV^S, SXT^R
S. maltophilia 2	TZP^R, NET^R, LEV^S, SXT^R
S. maltophilia 3	TZP^R, NET^R, LEV^R, SXT^R
S. maltophilia 4	TZP^R, NET^R, LEV^S, SXT^R
S. maltophilia 5	TZP^R, NET^R, LEV^S, SXT^R

FOX, Cefoxitin; KF, Cephalothin; CN, Gentamicin; OFX ,Ofloxacin ; DA,Clindamycin; E, Erythromycin; SXT, trimethoprim/sulfamethoxazole; CTX,Cefotaxime; CAZ,Ceftazidime; IPM, Imipenem; MEM, Meropenem; SAM, Ampicillin/sulbactam; TZP, Piperacillin/tazobactam; AK, Amikacin; NET, Netilmicin; LEV, Levofloxacin; CIP, Ciprofloxacin; S, sensitive; R, resistant

bactericidal activity on MDR bacteria compared with the standard antibiotics, colistin and vancomycin.

Time-kill assay is a method for determining the killing rate of the extract by comparing the viable count of tested bacterial isolates between control and extract treatment at each time interval. The time kill profiles of the RK extract against MRSA, *A. baumannii*, *S. maltophilia* and *P. aeruginosa* isolates are presented in Table 3 in the terms of the $\log_{10}$ cfu/ml reduction. The extract at MBC (1.56-2.34 mg/ml) and 2xMBC (3.12-4.68 mg/ml) did not kill all MRSA isolates after 6 h-exposure, but decreased the viable cell counts in ranges of 0.34 Log_{10} to 1.85 Log_{10} cfu/ml. However, all tested MRSA isolates were killed at 2xMBC after 24 h exposure. Interestingly, the extract rapidly reduced the viable counts and completely killed all tested Gram negative bacilli within 6 hrs (Table 3). For MDR *A. baumannii*, log reduction in viable cell counts was in the range of 2.89 Log_{10} – 6.37 Log_{10} within 3 h of interaction with MBC (1.56 mg/ml) and complete killing occurred within 3-6 h after exposure to 2xMBC (3.12 mg/ml). For MDR *S. maltophilia*, log reduction in viable cell counts was in the range of 2.61 Log_{10} – 5.49 Log_{10} within 3 h-interaction to MBC (0.78-2.34 mg/ml) and complete killing occurred within 1-6 h after exposure to 2xMBC. In the case of MDR *P. aeruginosa*, the MBC of the RK extract against *P. aeruginosa* was high (6.25 mg/ml). Therefore, only a standard strain and 1 isolate of MDR *P. aeruginosa* were selected to determine the bactericidal activity. The extract at 2xMBC (12.5 mg/ml) was able to kill the MDR *P. aeruginosa* isolate within 6 hrs. The effect of the crude ethanol extract of RK on the test bacteria in this study is dose and time dependent manners. With regard to overall killing activity, the RK extract was able to reduce the viable cell count of MDR *A. baumannii* isolates greater than those of MDR *P. aeruginosa* and MRSA isolates (Table 3). Based on Table 3, bactericidal activity of the RK extract against MDR *A. baumannii* isolates is similar to that against MDR *S. maltophilia* isolates. The differences in the antimicrobial effect of the compound against Gram positive and Gram negative bacteria may be due to the differ-

Table 2. Antibacterial activity of *R. kerrii* extract against reference standard strains and clinical MDR isolates.

Bacterial strains	Antibacterial activity		
	MBC of *R. kerrii* (mg/mL)	MIC of Colistin (µg/ml)	MIC of Vancomycin (µg/ml)
S. aureus ATCC 25923	1.56±0.00	NT	0.25±0.00
P. aeruginosa ATCC 27853	6.25±0.00	2.0±0.00	NT
*A. baumann*ii ATCC 19606	1.56±0.00	2.0±0.00	NT
Clinical MDR isolates			
MRSA 1	1.56±0.00	NT	0.25±0.00
MRSA 2	2.34±0.78	NT	0.25±0.00
MRSA 3	2.34±0.78	NT	0.25±0.00
MRSA 4	2.34±0.78	NT	0.25±0.00
MRSA 5	2.34±0.78	NT	0.25±0.00
A. baumannii 1	1.56±0.00	2.00±0.00	NT
A. baumannii 2	1.56±0.00	0.50±0.00	NT
A. baumannii 3	1.56±0.00	2.00±0.00	NT
A. baumannii 4	1.56±0.00	4.00±0.00	NT
A. baumannii 5	1.56±0.00	4.00±0.00	NT
P. aeruginosa 1	6.25±0.00	2.00±0.00	NT
P. aeruginosa 2	6.25±0.00	2.00±0.00	NT
P. aeruginosa 3	3.12±0.00	2.00±0.00	NT
P. aeruginosa 4	6.25±0.00	2.00±0.00	NT
P. aeruginosa 5	3.12±0.00	1.00±0.00	NT
S. maltophilia 1	2.34±0.78	NT	NT
S. maltophilia 2	0.78±0.00	NT	NT
S. maltophilia 3	2.34±0.78	NT	NT
S. maltophilia 4	1.56±0.00	NT	NT
S. maltophilia 5	1.56±0.00	NT	NT

ences in the permeability barriers or compound diffusion properties (Cushnie & Andrew, 2005). Generally, plant extracts have been reported to be more effective against Gram positive bacteria than Gram negative bacteria, which may be due to their differences in cell wall composition (Ahmad & Beg, 2001). In the present study, the RK extract exhibited broad spectrum activity and was more effective against Gram negative bacteria than Gram positive bacteria. These findings suggest that the RK extract can be used as an alternative source of antimicrobial agent for the treatment of bacterial infections, especially those due to MDR bacteria.

The antibacterial activity of plant extracts has been attributed to the presence of some constituents in the extract such as phenolic compounds (Cowan, 1999). The total phenolic content of the RK extract was 669.66±38.6 mg TAE/g indicating that it contains a high content of phenolic compounds. The mechanisms responsible for the antimicrobial activity of phenolics include an adsorption and a disruption of microbial membranes, interaction with enzymes and substrates and metal ion deprivation (Cowan, 1999; Daglia, 2012). Therefore, it is possible that high phenolic content in the extract may play an important role in antibacterial activity in this study. Kanchanapoom *et al.* (2007) reported that several hydrolysable tannins were isolated from RK flower extract. This may be the reason why RK extract was more effective against Gram negative bacteria than Gram positive bacteria. As the cell walls of Gram-negative bacteria are thinner than those of Gram-positive bacteria, tannins are able to more easily attack the proteins found in the cell wall and cell membrane of the bacterium resulting in damage and cell death (Doss *et al.*, 2009; Scalbert, 1991). Helander *et al.* (1998) reported that the phenolic compounds were both bactericidal and had outer membrane-disintegrating properties on Gram negative bacteria. However, it is necessary to investigate further in order to understand the mechanisms of action of the chemical constituents of the RK extract.

Antioxidant activity of RK extract compared with the standard antioxidant, ascorbic acid is shown in Figure 2. It was able to reduce DPPH radical in a dose-dependent manner. Interestingly, the extract had strong antioxidant

Table 3. Time kill profiles of the *R. kerrii* extract against multidrug-resistant bacteria.

Bacteria	Log_{10} reduction at MBC				Log_{10} reduction at 2MBC			
	1 h	3 h	6 h	24 h	1 h	3 h	6 h	24 h
MRSA 1	0.12	1.49	1.74	5.92**	0.46	1.59	1.85	5.92**
MRSA 2	0.03	0.05	0.38	5.86**	0.00	0.00	0.90	5.86**
MRSA 3	0.09	0.14	0.34	2.85	0.00	0.54	0.73	5.94**
MRSA 4	0.27	0.41	0.86	6.25**	0.33	0.74	0.94	6.25**
MRSA 5	0.09	0.84	1.60	5.87**	0.36	1.04	1.80	5.87**
A.baumannii 1	1.17	6.37**	6.98**	7.60**	1.61	5.97**	6.12**	6.95**
A.baumannii 2	1.09	2.89	6.73**	8.00**	1.85	6.09**	6.73**	8.00**
A.baumannii 3	0.47	2.95	6.64**	7.32**	1.04	6.62**	6.64**	7.32**
A.baumannii 4	1.06	5.68**	5.97**	7.61**	2.02	5.68**	5.97**	7.61**
A. baumannii 5	1.32	3.45*	6.93**	7.93**	2.13	6.15**	6.93**	7.93**
S. maltophilia 1	5.24**	5.28**	5.32**	5.54**	5.24**	5.28**	5.32**	5.54**
S. maltophilia 2	0.42	2.61	5.82**	5.85**	1.27	5.81**	5.82**	5.85**
S .maltophilia 3	1.33	5.49**	5.51**	5.53**	5.48**	5.49**	5.51**	5.53**
S. maltophilia 4	1.00	5.48**	5.50**	5.55**	1.81	5.48**	5.50**	5.55**
S. maltophilia 5	0.34	2.71	5.68**	5.71**	0.76	5.67**	5.68**	5.71**
P. aeruginosa ATCC	0.47	1.00	1.56	6.24**	0.67	1.02	3.26*	6.24**
P. aeruginosa 1	0.04	1.56	6.28**	6.28**	1.07	1.75	6.28**	6.28**

* represents bactericidal effect, ** represents completely kill.

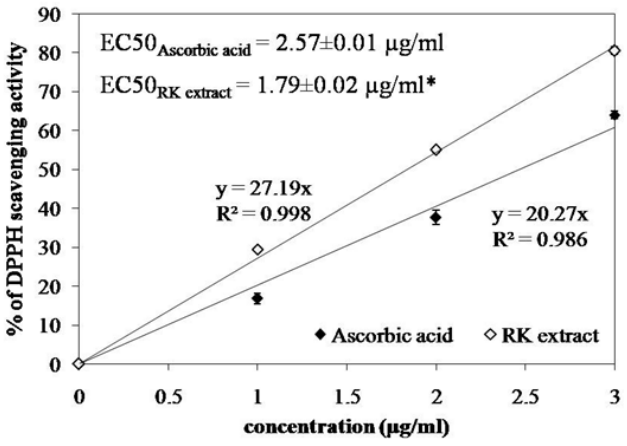

Figure 2. DPPH scavenging activity and EC50 values of RK extract. *Significant difference between RK extract and Ascorbic acid ($p<0.05$).

activity with EC50 of 1.79±0.02 µg/ml which was significantly ($p< 0.05$) stronger than that of ascorbic acid (2.57±0.01 µg/ml). The strong antioxidant activity of the RK extract may be due to the high total phenolic content. This result was similar to a recent report that a methanolic extract of the RK flower possessed high antioxidant activity and high quantities of phenolic content (Puttipan & Okonogi, 2014). In addition, a major component of this methanolic extract was gallic acid which correlated to its high antioxidant properties. These data may support the traditional use of RK for prolonging life (Kanchanapoom *et al.*, 2007) via the anti-aging effect of the high content of antioxidative agents.

4. Conclusions

The ethanol extract of RK possessed antibacterial potential and bactericidal activity against MDR bacteria. The antibacterial activity of the extract corresponded to its phenolic contents and antioxidant activity. These findings indicate that RK extract has potential in the further development of a novel antibacterial agent for treatment of MDR bacteria. However, the toxicity of the extract should be tested to confirm its safety. Further studies are needed to identify the active compounds and their mode of action.

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