American Journal of Infectious Diseases

Original Research Paper

Pterostilbene Enhanced Anti-Methicillin Resistant *Staphylococcus aureus* (MRSA) Activity of Oxacillin

Siti Fairuz Ishak, Ahmad Rohi Ghazali, Noraziah Mohamad Zin and Dayang Fredalina Basri

School of Diagnostic and Applied Health Sciences, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

Article history
Received: 16-11-2015
Revised: 17-11-2015
Accepted: 08-01-2016

Corresponding Author:
Dayang Fredalina Basri
School of Diagnostic and Applied Health Sciences, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia
Fax: +60326929032
Email: dayang@ukm.edu.my

Abstract: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a deadly pathogen that initially was limited to hospital and healthcare facilities but has gradually became a growing problem in healthy children and adults. Pterostilbene belongs to the phenylpropanoid phytoalexin which is involved in plant response to various pathogen and herbivores attack. The aim of this study is to evaluate the anti-MRSA action of pterostilbene in combination with selected antibiotics; vancomycin, linezolid and oxacillin against ATCC 43300 and ATCC 33591. The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and fractional inhibitory concentration (FIC) index values were determined. Microbroth dilution technique and microdilution checkerboard (MDC) assay were employed. The MIC and MBC of pterostilbene against ATCC 33591 was 31.25 and 62.50 μg mL^{-1}, respectively. While for ATCC 43300, the MBC value was also twice (62.50 μg mL^{-1}) its MIC value of 31.25 μg mL^{-1}. This indicated that pterostilbene was bacteriostatic against both MRSA strains. Our MIC/MBC study also showed that linezolid exhibited bacteriostatic action but, oxacillin and vancomycin were bactericidal. MDC study showed that pterostilbene-oxacillin combination exhibited lowest FIC value (0.56) against both MRSA strains which indicated partial synergistic interaction. On the other hand, pterostilbene was additive (FIC 1.00) in combination with vancomycin whereas pterostilbene-linezolid combination displayed indifference effect with FIC of 1.25 against both MRSA strains. Pterostilbene in combination with oxacillin partially enhanced anti-MRSA activity with twofold reduction in MIC of oxacillin by acting at different site at the bacterial cell wall from that of oxacillin but more specific to the site of action of vancomycin.

Keywords: Pterostilbene, MRSA, Synergism, MIC, FIC, Bacteriostatic

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a human pathogen that causes both nosocomial and community-acquired infection (Boucher *et al*., 2010; Grundmann *et al*., 2006; McCarthy *et al*., 2010). The emergence of MRSA which has caused an increase in incidence and prevalence rate in many country, is widely known for causing endocarditis, meningitis, bacteremia, osteomyelitis and toxic shock syndrome (Lowy, 2003). As resistance toward antibiotic arises, cure towards bacterial infection becomes more difficult (Alexander and Wang, 2015). The impact of MRSA infection will increase burden to patient due to longer duration of hospital stay and also higher cost (Cosgrove *et al*., 2005; Shorr *et al*., 2006).

Vancomycin is currently the last defense against MRSA infection due to the fact that MRSA has shown resistance to most β-lactam antibiotics including penicillin, cephalosporin and carbapenem. In addition to its parental indication, the side effects associated with vancomycin are nephrotoxicity, hearing impairment and 'red man syndrome'. The latter is known to cause skin redness and itching resulting from histamine reaction (Alexander and Greenberger, 1996). To complicate the situation, uncontrolled spreading of MRSA and the increased usage of vancomycin make the drug more susceptible to failure due to emergence of vancomycin-resistant organism (Hiramatsu, 2001).

Science Publications

© 2016 Siti Fairuz Ishak, Ahmad Rohi Ghazali, Noraziah Mohamad Zin and Dayang Fredalina Basri. This open access article is distributed under a Creative Commons Attribution (CC-BY) 3.0 license.

Thus, the problem of bacterial resistance has triggered many researchers to seek alternative treatment and overcome this phenomenon from becoming more serious. One approach using combination of antimicrobial therapy that consists of existing antibiotic and plant extract is the best alternative therapeutic weapon to combat superbug pathogen (Cowan, 1999; Kyaw and Lim, 2012).

Pterostilbene is the component that belongs to a phenolic group known as stilbene (Pan *et al*., 2008). Stilbene is a secondary plant metabolite formed from the flavonoid biosynthesis pathway and belongs to the phenylpropanoid family (Chong *et al*., 2009). Pterostilbene is found in the heartwood of sandalwood *Pterocarpus marsupium* (Maurya *et al*., 1984), leaves of *Vitis vinifera* (Langcake *et al*., 1979) and in some species of *Vaccinium* berries (Pezet and Pont, 1988). Pterostilbene is the analog of resveratrol, found in grapes and blueberries (Rimando *et al*., 2004). This component is biologically classified as phytoalexin. Phytoalexin has antimicrobial property which serves as part of the plant defense system against pathogen and herbivore (Chong *et al*., 2009). Additionally, pterostilbene has demonstrated potential antioxidant (Rimando *et al*., 2002), anti-fungal (Li *et al*., 2014), anti-glycemic (Grover *et al*., 2005), anti-mutagenic and anti-carcinogenic properties (Chio *et al*., 2011; Ghazali *et al*., 2012; McCormack and McFadden, 2012) which indicated the potential of pterostilbene in the prevention of certain illnesses and infectious diseases.

However, there is no report on the antimicrobial activity of pterostilbene to date, which triggers the present study to be carried out. As such, the present study could also be assumed as the first report for the antibacterial activity of pterostilbene and hence, evaluation of the anti-MRSA action of pterostilbene in combination with selected antibiotics may lead to discovery of a novel, more effective treatment for combating MRSA infection.

Materials and Methods

Preparation of Antimicrobial Agents

The phytochemical used in this study is pterostilbene purchased commercially from EMD Biosciences/Calbiochem (USA). Stock solutions of antibiotic and phytochemical were prepared according to the manufacturer's recommendations. The chemicals were dissolved in their respective solvents by using auto vortex mixer prior to the experiments.

Preparation of Bacterial Inoculum

The bacteria used was the reference strains of Methicillin-Resistant *Staphylococcus aureus* (MRSA) ATCC 43300 and ATCC 33591 obtained from the American Type Culture Collection (ATCC), grown and maintained on nutrient agar slant. Three to five colonies that grew on Mueller Hinton Agar (MHA) was cultured and transferred into Mueller Hinton broth (MHB). After incubation for 24 h at 37°C, the turbidity of the bacterial inoculum was adjusted to optical density reading of between 0.08-0.1 using spectrophotometer at a wavelength of 625 nm.

Determination of Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

Minimum inhibitory concentration (MIC) was determined using microbroth serial dilution method in a sterile 96-well microtiter plate. This assay was performed in triplicate for consistency in accordance with guidelines provided by CLSI (2014). MIC value is the lowest concentration of antimicrobial agent in inhibiting the visible growth of bacteria after overnight incubation at 37°C. The concentration of the tested antimicrobial agents used in the study against MRSA ATCC 33591 and ATCC 43300 ranged from 500 to 0.98 μg mL^{-1} for pterostilbene, 125 to 0.24 μg mL^{-1} for vancomycin, 100 to 0.19 μg mL^{-1} for linezolid whereas the concentration of oxacilin was from 2, 000 to 3.91 μg mL^{-1}. The sterile MHB was enriched with 2% NaCl before the tested antimicrobial agents were inserted into the well at concentration gradient in a serial dilution. Then the diluted bacterial suspension at final inoculum of 10^6CFU mL^{-1} was added. The tested compound in MHB was used as negative control to ensure medium sterility while the inoculum in MHB served as positive control to ensure the adequacy of the broth for bacterial growth. To facilitate the observation of the growth of bacteria in each well, 20 μL of 2,3,5-triphenyltetrazolium chloride (TTC) at 2 mg mL^{-1} was added into each well. The minimum bactericidal concentration (MBC) was determined by culturing the sample from each clear well from the 96-microtiter plate onto the MHA plate. Then, the plates were incubated at 37°C overnight. The MBC value was determined as the least concentration of an antibacterial agent showing no visible growth (either totally inhibiting growth or killing 99.9% of the bacterial inoculum) on agar plates after overnight incubation (Pankey and Sabath, 2004).

Determination of Fractional Inhibitory Concentration (FIC)

FIC index value of combination between pterostilbene with each of the respective standard antibiotics; oxacillin, vancomycin and linezolid against MRSA ATCC 33591 and 43300 were obtained using microdilution checkerboard (MDC) method. The concentration of individual compound in the combination of pterostilbene with the selected antibiotics which inhibited visible bacterial growth was recorded as

the MIC of the individual compound in the respective combination. The effects of each combination were evaluated by calculating the FIC index using the following formula (Drago *et al*., 2007):

$$FIC_{index}\left(\sum FIC\right) = FIC_A + FIC_B = A / MIC_A + B / MIC_B$$

Where:
A = MIC of drug A in combination
B = MIC of drug B in combination
MIC_A = MIC of drug A alone
MIC_B = MIC of drug B alone

FIC index by checkerboard method is interpreted as follows: Synergistic effect if FICI of ≤ 0.5; partial synergism as FICI > 0.5 < 1; additive effect as FICI = 1; indifference as FICI > 1 ≤ 4; and antagonism as FICI of more than 4 (Drago *et al*., 2007).

Results

The MIC and MBC values of pterostilbene against MRSA ATCC 33591 and ATCC 4330 were shown in Table 1. The MIC value of pterostilbene was 31.25 µg mL^{-1} whereas its MBC was twice the MIC value against both MRSA strains. As far as linezolid is concerned, the MIC value is lower than pterostilbene at 1.56 and 0.78 µg mL^{-1}, respectively against ATCC 33591 and ATCC 43300 as shown in Table 2. The MBC value of linezolid indicated that it was bacteriostatic against MRSA strains as it was fourfold and sixteen fold higher than its MIC values against ATC 33591 and ATCC 43300 strains. The standard antibiotics, vancomycin (Table 3) and oxacillin (Table 4) was shown to have MIC values of 0.98 and 62.5 µg mL^{-1} respectively against ATCC 33591 whereas ATCC 43300 strain was more susceptible to the effect of vancomycin and oxacillin at MIC values of respectively, 0.49 and 31.25 µg mL^{-1}. From the MBC values of vancomycin and oxacillin, it can be seen from Table 3 and 4 that both antibiotics were bactericidal against MRSA strains with MBC values that equaled their MIC values. In other words, the MIC and MBC values of vancomycin were 0.98 µg mL^{-1} against ATCC 3591 whereas for ATCC 43300, both the values were 0.49 µg mL^{-1} (Table 3). Oxacillin against ATCC 33591 also showed the same MIC/MBC value which was 62.5 µg mL^{-1} whereas, it was 31.25 µg mL^{-1} against ATCC 43300 (Table 4).

Table 1. Determination of MIC and MBC values of pterostilbene against MRSA ATCC 33591 and ATCC 43300

Concentration	ATCC 33591		ATCC 43300		Control	
(µg/mL)	MIC	MBC	MIC	MBC	Positive	Negative
500	-	-	-	-	+	-
250	-	-	-	-	+	-
125	-	-	-	-	+	-
62.5	-	-	-	-	+	-
31.25	-	+	-	+	+	-
15.63	+	+	+	+	+	-
7.81	+	+	+	+	+	-
3.91	+	+	+	+	+	-
1.95	+	+	+	+	+	-
0.98	+	+	+	+	+	-

+ represents presence of growth (turbid well)
- represents absence of growth (clear well)
Positive control: Bacterial suspension and Mueller-Hinton broth
Negative control: Pterostilbene/solvents used and Mueller-Hinton broth

Table 2. Determination of MIC and MBC values of linezolid against MRSA ATCC 33591 and ATCC 43300

Concentration	ATCC 33591		ATCC 43300		Control	
(µg/mL)	MIC	MBC	MIC	MBC	Positive	Negative
100	-	-	-	-	+	-
50.0	-	-	-	-	+	-
25.0	-	-	-	-	+	-
12.5	-	-	-	-	+	-
6.25	-	-	-	+	+	-
3.125	-	+	-	+	+	-
1.56	-	+	-	+	+	-
0.78	+	+	-	+	+	-
0.39	+	+	+	+	+	-
0.19	+	+	+	+	+	-

+ Represents presence of growth (turbid well)
- Represents absence of growth (clear well)
Positive control: Bacterial suspension and Mueller-Hinton broth
Negative control: Linezolid/solvents used and Mueller-Hinton broth

Table 3. Determination of MIC and MBC values of vancomycin against MRSA ATCC 33591 and ATCC 43300

Concentration (µg/mL)	ATCC 33591		ATCC 43300		Control	
	MIC	MBC	MIC	MBC	Positive	Negative
125	-	-	-	-	+	-
62.5	-	-	-	-	+	-
31.25	-	-	-	-	+	-
15.63	-	-	-	-	+	-
7.81	-	-	-	-	+	-
3.91	-	-	-	-	+	-
1.95	-	-	-	-	+	-
0.98	-	-	-	-	+	-
0.49	+	+	-	-	+	-
0.24	+	+	+	+	+	-

+ Represents presence of growth (turbid well)
- Represents absence of growth (clear well)
Positive control: Bacterial suspension and Mueller-Hinton broth
Negative control: Vancomycin /solvents used and Mueller-Hinton broth

Table 4. Determination of MIC and MBC values of oxacillin against MRSA ATCC 33591 and ATCC 43300

Concentration (µg/mL)	ATCC 33591		ATCC 43300		Control	
	MIC	MBC	MIC	MBC	Positive	Negative
2000	-	-	-	-	+	-
1000	-	-	-	-	+	-
500	-	-	-	-	+	-
250	-	-	-	-	+	-
125	-	-	-	-	+	-
62.50	-	-	-	-	+	-
31.25	+	+	-	-	+	-
15.63	+	+	+	+	+	-
7.81	+	+	+	+	+	-
3.91	+	+	+	+	+	-

+ Represents presence of growth (turbid well)
- Represents absence of growth (clear well)
Positive control: Bacterial suspension and Mueller-Hinton broth
Negative control: Oxacillin/solvents used and Mueller-Hinton broth

Table 5. Determination of FIC values and interaction effects with three antibiotics against MRSA ATCC 33591

Antimicrobial agents	MIC (µg/mL)		FIC (µg/mL)		Outcome
	Alone	Combination	FICI	FICI	
Oxacillin	62.50	31.25	0.500		Partial Synergism
Pterostilbene	31.25	1.95	0.062	0.56	
Vancomycin	0.98	0.49	0.500		
Pterostilbene	31.25	15.62	0.500	1.00	Additive
Linezolid	1.56	0.39	0.250		
Pterostilbene	31.25	31.25	1.000	1.25	Indifference

FICI > 0.5 < 1 denotes partial synergism; FICI > 1 ≤ 4 denotes indifference and FICI = 1 denotes additivity

Table 6. Determination of FIC values and interaction effects with three antibiotics against MRSA ATCC 43300

Agents	MIC (µg/mL)		FIC (µg/mL)		Outcome
	Alone	Combination	FIC	FICI	
Oxacillin	31.25	15.620	0.500		Partial synergism
Pterostilbene	31.25	1.950	0.062	0.56	
Vancomycin	0.49	0.240	0.500		
Pterostilbene	31.25	15.620	0.500	1.00	Additive
Linezolid	0.78	0.195	0.250		
Pterostilbene	31.25	31.250	1.000	1.25	Indifference

FICI > 0.5 < 1 denotes partial synergism; FICI > 1 ≤ 4 denotes indifference and FICI = 1 denotes additivity

The results for combination studies between pterostilbene and three selected antibiotics against ATCC 33591 and ATCC 43300 were presented in Table 5 and 6, respectively as well as in Figure 8. Out of the three standard antibiotics, oxacillin showed the lowest FIC index value of 0.56 in combination with pterostilbene against both MSRA strains. An FIC index value of greater than 0.5 but less than 1 denotes partial synergism hence, combination of pterostilbene and oxacillin exhibited partial synergism interaction against both ATCC strains. In order words, pterostilbene was capable of reducing the MIC value of oxacillin by twofold from 62.50 to 31.25 μg mL^{-1} against ATCC 33591 while for ATCC 43300, the twofold reduction was from 31.25 to 15.62 μg mL^{-1}. Pterostilbene in combination with vancomycin exhibited additivity (FIC 1.00) against both ATCC strains indicating that pterostilbene could well act at the same site of antibacterial action as vancomycin. Linezolid, on the other hand, showed indifference against ATCC 33591 and ATCC 43300 at FIC value of 1.25. This indicates that the combined action of pterostilbene-linezolid was the same as with either pterostilbene or linezolid alone. There is lack of interaction between pterostilbene and linezolid because there is no change in the MIC value of the individual agents in the combination treatment. As such, we can postulate that pterostilbene could target the cell wall different from where oxacillin acts but more specific to the site of action of vancomycin.

Discussion

The current study provides lead that pterostilbene possessed stronger antibacterial property compared with oxaciilin against MRSA ATCC 33591 and comparable anti-MRSA potency as oxacillin against ATCC 43300 strain. Pterostilbene is a phytoalexin which belongs to the phenylpropanoid family and is involved in plant response to various pathogens and herbivore attack (Chong *et al*., 2009). In comparison with antibiotics used as current therapy against MRSA infection, pterostilbene still showed 20 to 31 times lower antimicrobial activity than linezolid and vancomycin against ATCC 33591 and 40 to 60 times less inhibitory potency against ATCC 43300. Despite the observation that ATCC 33591 strain is less susceptible towards linezolid and vancomycin, it is interesting to note that both MRSA reference strains were equally susceptible towards the effect of pterostilbene. This mean to say that whilst ATCC 33591 has developed resistance towards the last line of defense antibiotics, pterostilbene has not induced resistance in this particular strain. The same phenomenon was seen in the anti-MRSA effect of oxacillin whereby the susceptibility of ATCC 33591 was less than that of ATCC 43300. Pterostilbene could possibly reduce the problem of microbial resistance as supported by (Monte *et al*., 2014) that phytochemical have the ability to demonstrate significant potential to reverse antibiotic resistance. Despite the comparable antimicrobial efficacy of pterostilbene with oxacillin, pterostilbene acts bacteriostatic in the same manner as linezolid with MBC exceeding their MIC values. This is in agreement with Basri *et al*. (2012) that stilbenoid compounds belonging to the phenylpropanoid family were reported to exhibit bacteriostatic action against MRSA strains. In general, phytochemicals or secondary metabolites from the plants are capable of inhibiting or slowing the growth of bacteria rather than killing the pathogen (Nazzaro *et al*., 2013).

Individually, pterostilbene and linezolid were bacteriostatic but pterostilbene in combination with linezolid actually produced indifference effect. This is in line with Kyaw and Lim (2012) which reported that eugenol which is a bacteriostatic phytochemical (Rastogi *et al*., 2008; Yadav *et al*., 2015) in combination with a bacteriostatic antibiotic minocycline produced indifference interaction. According to Qin *et al*. (2013), indifference is when the combined action is the same as with either component alone. Previous study (Sopirala *et al*., 2010) reported that, if the MIC of an antibiotic changed within onefold dilution, the resulting effect is indifference. Ironically, although the FIC index was interpreted as indifference, but it was conflicting to note that there was a marked reduction of MIC value of linezolid by fourfold by pterostilbene which indicated synergism. This is because synergy occurs when a combination of two drugs causes inhibition or killing by fourfold lower concentration than that of either component drug used separately (Qin *et al*., 2013).

As far as oxacillin is concerned, pterostilbene displayed partial synergism in combination with this bactericidal agent against MRSA in spite of their comparably similar anti-MRSA potency. When the interaction is synergistic, it indicates that their mechanisms of antibacterial action might be different (Bassolé and Juliani, 2012). The MDC analysis in this study demonstrated that the interaction between pterostilbene with oxacillin partially enhanced the activity of oxacillin by reducing the MIC of oxacillin by twofold. In other words, the significant finding in this study was that pterostilbene partially enhanced anti-methicillin resistant *Staphylococcus aureus* (MRSA) activity of oxacillin by acting at partially different target as that of oxacillin action at the bacterial cell wall and at site that does not involved site of linezolid action at protein level. This is further supported by the present FIC study that pterostilbene with vancomycin showed additive effect against MRSA. Previous study (Jayaraman *et al*., 2010) reported that additivity was observed in combination of ellagic acid and gallic acid with β-lactam antibiotics possibly due to their action at the same target site in the cytoplasmic membrane Hence, this could well indicated that pterostilbene acts at the

same target as vancomycin at the bacterial cell wall but different with that of oxacillin. This additivity in pterostilbene-vancomycin combination was disputed by Basri *et al*. (2014) as synergism was portrayed by the combination of ε-viniferin and vancomycin. This is probably because ε-viniferin and pterostilbene differs in their chemical structure (Fig. 1-7) whereby ε-viniferin lacks two methoxy groups on the benzene ring which could account for the same site of action of pterostilbene and vancomycin.

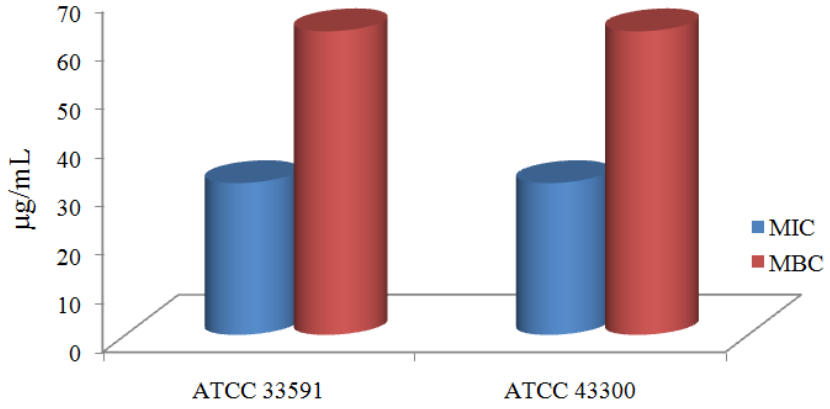


Fig. 1. Determination of MIC and MBC values of pterostilbene against MRSA ATCC 33591 and ATCC 43300

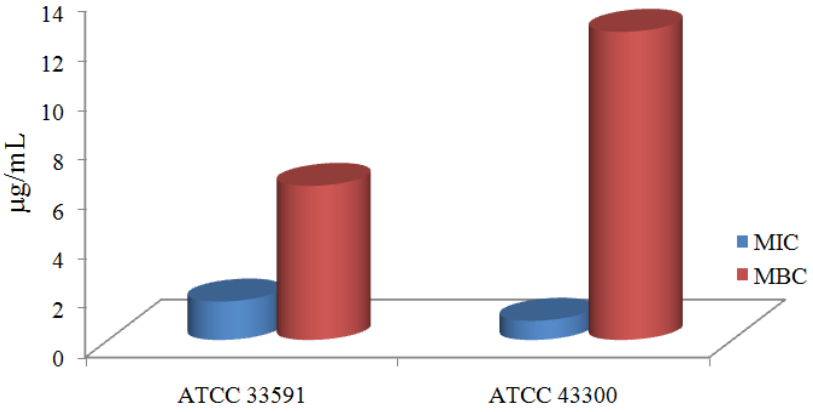


Fig. 2. Determination of MIC and MBC values of linezolid against MRSA ATCC 33591 and ATCC 43300

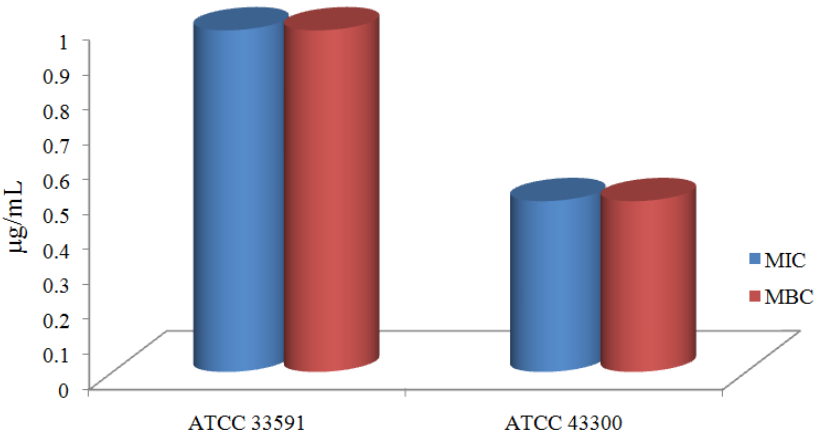


Fig. 3. Determination of MIC and MBC values of vancomycin against MRSA ATCC 33591 and ATCC 43300

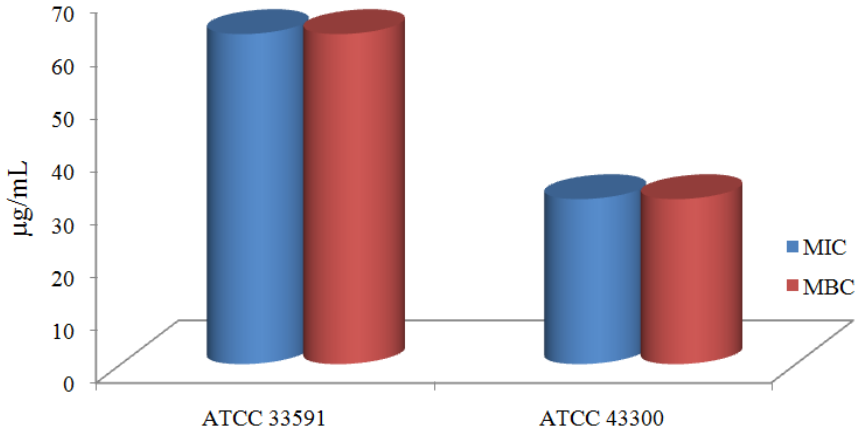


Fig. 4. Determination of MIC and MBC values of oxacillin against MRSA ATCC 33591 and ATCC 43300

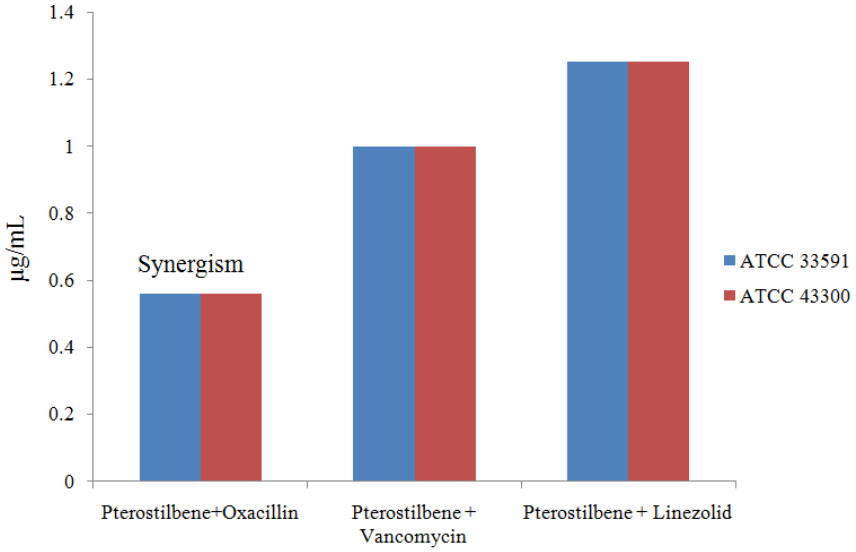


Fig. 5. Determination of FIC values and interaction effects with three antibiotics against MRSA ATCC 43300 and ATCC 33591

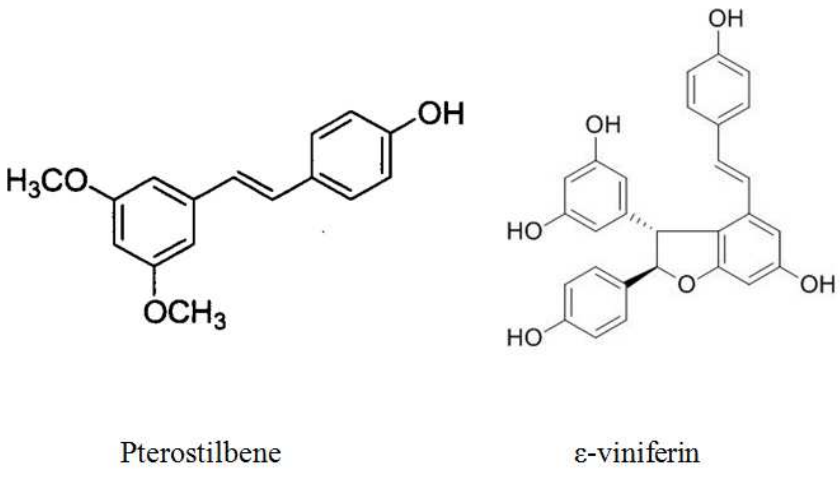
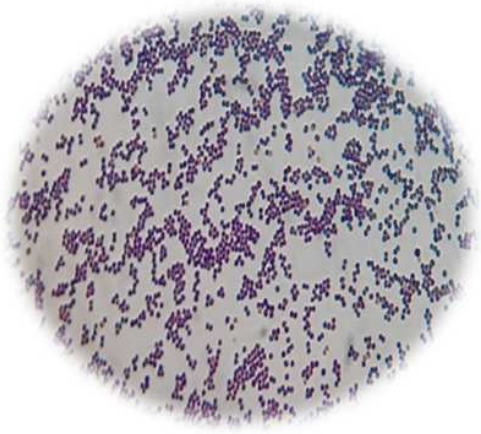


Fig. 6. Chemical structures of pterostilbene and ε-viniferin



Methicillin-resistant *S.aureus* (MRSA)

Fig. 7. Gram stain of Methicillin-resistant *S. aureus* (MRSA)

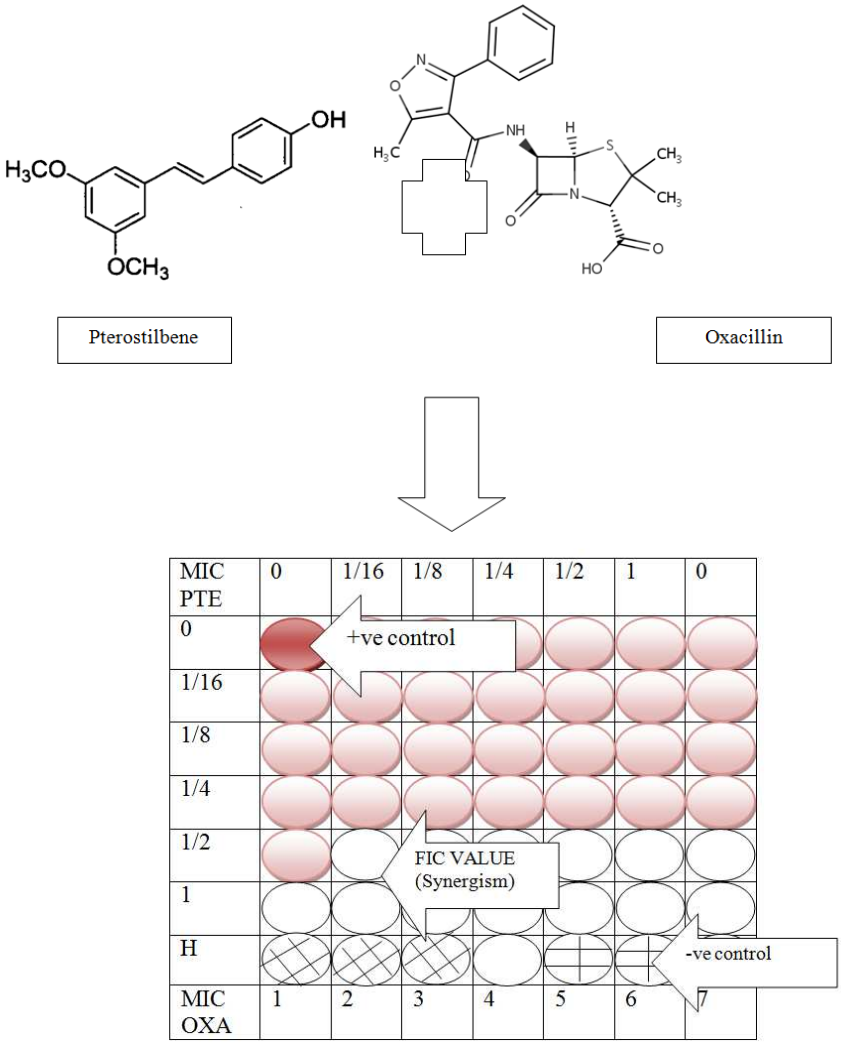


Fig. 8. Flowchart of combination drugs and the effect of their combination

Conclusion

In summary, it can be concluded that pterostilbene have an antibacterial activity against MRSA with partial synergistic interaction in combination with oxacillin by reduction of MIC value by twofold and reduction of MIC value of linezolid by fourfold despite indifference effect in FIC analysis. It can also be postulated that pterostilbene acts at different site of target as oxacillin at the bacterial cell wall but more specific to that of vancomycin. Therefore further studies involving time-kill assay and postantibiotic effect (PAE) are currently ongoing to validate these interactions. This is because time-kill assay can confirm the partial synergism between pterostilbene-oxacillin combination as well as indifference effect between pterostilbene-linezolid as displayed by the FIC index values. These combinations have huge potential to be further studied and can be developed as an alternative treatment in combating MRSA infection.

Acknowledgement

The authors wish to extend their gratitude to Toxicology Research Laboratory, Universiti Kebangsaan Malaysia for supplying pterostilbene for the studies and Novel Antibiotic Laboratory, Universiti Kebangsaan Malaysia for providing research facilities.

Funding Information

This project is funded by the Malaysian Government under the Fundamental Research Grant Scheme FRGS /2/2014/SG05/UKM/02/3.

Author's Contributions

Siti Fairuz Ishak: Performed the experiments and drafting the article.

Ahmad Rohi Ghazali: Cosupervised the project and contributing to context.

Noraziah Mohamad Zin: Provide the laboratory facilities for the study.

Dayang Fredalina Basri: Design the experiments and edit the manuscript.

Conflicts of Interest

The authors wish to declare that there are no conflicts of interest. This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References

Alexander, C.A. and L. Wang, 2015. Infection control: Methicillin resistant *Staphylococcus aureus.* Am. J. Inf. Dis., 11: 74-82.

Alexander, I.I. and P.A. Greenberger, 1996. Vancomycin-induced Stevens-Johnson syndrome. Allergy Asthma Proc., 17: 75-78. DOI: 10.2500/108854196778645029

Basri, D.F., K.L. Chan, A.M. Azmi and L. Jalifah, 2012. Evaluation of the combined effects of stilbenoid from *Shorea gibbosa* and vancomycin against methicillin-resistant *Staphylococcus aureus* (MRSA). Pharmaceuticals, 5: 1032-1043. DOI: 10.3390/ph5091032

Basri, D.F., L.W. Xian, N.I. Abdul Shukur and J. Latip, 2014. Bacteriostatic antimicrobial combination: Antagonistic interaction between epsilon-viniferin and vancomycin against methicillin-resistant *Staphylococcus aureus*. Bio. Med. Res. Int. DOI: 10.1155/2014/461756

Bassolé, I.H.N. and H.R. Juliani, 2012. Essential oils in combination and their antimicrobial properties. Molecules, 17: 3989-4006. DOI: 10.3390/molecules17043989

Boucher, H., L.G. Miller and R.R. Razonable, 2010. Serious infections caused by methicillin-resistant *Staphylococcus aureus*. Clin. Infect. Dis., 51: S183-S197. DOI: 10.1086/653519

Chio, Y.S., M.L. Tsai, K. Nagabhushanam, Y.J. Wang and C.H. Wu *et al*., 2011. Pterostilbene is more potent than resveratrol in preventing azoxymethane (AOM)-induced colon tumorigenesis via activation of the NF-E2-related factor 2 (Nrf2)-mediated antioxidant signaling pathway. J. Agri. Food Chem., 59: 2725-33. DOI: 10.1021/jf2000103

Chong, J., A. Poutaraud and P. Hugueny, 2009. Metabolism and roles of stilbenes in plants. Plant Sci., 177: 143-155. DOI: 10.1016/j.plantsci.2009.05.012

CLSI, 2014. Performance standards for antimicrobial susceptibility testing; twenty-fourthinformation supplement. Clinical and Laboratory Standard Institute, CLSI.

Cosgrove, S.E., Y. Qi, K.S. Kaye, S. Harbarth and A.W. Karchmer *et al*., 2005. The impact of methicillin resistance in *Staphylococcus aureus* bacteremia on patient outcomes: Mortality, length of stay and hospital charges. Infect. Control Hosp. Epidemiol., 26: 166-174. DOI: 10.1086/502522

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564-582. PMID: 10515903

Drago, L., E. De Vecchi, L. Nicola and M.R. Gismondo, 2007. *In vitro* evaluation of antibiotics combinations for empirical therapy of suspected methicillin-resistant *Staphylococcus aureus* severe respiratory infections. BMC Infect. Dis., 7: 111-111. DOI: 10.1186/1471-2334-7-111

Ghazali, A.R., O.S. Ki, H.A. Hamid, N.M. Room and F. Kamarulzaman, 2012. Effects of pterostilbene on O-deethylation and glutathione conjugation of drug metabolizing enzyme activities. Pharmacologia, 3: 456-461. DOI: 10.5567/pharmacologia.2012.456.461

Grover, J.K., V. Vats and S.S. Yadav, 2005. *Pterocarpus marsupium* extract (Vijayasar) prevented the alteration in metabolic patterns induced in the normal rat by feeding an adequate diet containing fructose as sole carbohydrate. Diabetes Obes. Metab., 7: 414-420. DOI: 10.1111/j.1463-1326.2005.00414.x

Grundmann, H., M. Aires-De-Sousa, J. Boyce and E. Tiemersma, 2006. Emergence and resurgence of methicillin-resistant *Staphylococcus aureus* as a public-health threat. Lancet, 368: 874-885. DOI: 10.1016/S0140-6736(06)68853-3

Hiramatsu, K., 2001. Vancomycin-resistant *Staphylococcus aureus*: A new model of antibiotic resistance. Lancet Infect. Dis., 1: 147-155. DOI: 10.1016/S1473-3099(01)00091-3

Jayaraman, P., M.K. Sakharkar, C.S. Lim, T.H. Tang and K.R. Sakharkar, 2010. Activity and interactions of antibiotic and phytochemical combinations against *Pseudomonas aeruginosa in vitro*. Int. J. Biol. Sci., 6: 556-568. DOI: 10.7150/ijbs.6.556

Kyaw, B.M. and C.S. Lim, 2012. Bactericidal antibiotic-phytochemical combinations against methicillin-resistant *Staphylococcus aureus*. Braz. J. Microbiol., 43: 938- 945. DOI: 10.1590/S1517-83822012000300013

Langcake, P., C.A. Cornford and R.J. Pryce, 1979. Identification of pterostilbene as a phytoalexin from *Vitis vinifera* leaves. Phytochemistry, 18: 1025-1027. DOI: 10.1016/S0031-9422(00)91470-5

Li, D.D., L.X. Zhao, E. Mylonakis, G.H. Hu and Y. Zou *et al*., 2014. *In vitro* and *in vivo* activities of pterostilbene against *Candida albicans* biofilms. Antimicrob. Agents Chemother., 58: 2344-2355. DOI: 10.1128/AAC.01583-13

Lowy, F.D., 2003. Antimicrobial resistance: The example of *Staphylococcus aureus*. J. Clin. Invest., 111: 1265-1273. DOI: 10.1172/JCI18535

Maurya, R., A.B. Ray, F.K. Duah, D.J. Slatkin and P.L. Schiff, 1984. Constituents of *Pterocarpus marsupium*. J. Nat. Prod., 47: 179-181. DOI: 10.1021/np50031a029

McCarthy, N.L., P.S. Sullivan, R. Gaynes and D. Rimland, 2010. Healthcare-associated and community-associated methicillin-resistant *Staphylococcus aureus* infections: A comparison of definitions. Am. J. Infect. Control, 38: 600-606. DOI: 10.1016/j.ajic.2010.01.006

McCormack, D. and D. McFadden, 2012. Pterostilbene and cancer: Current review. J. Surg. Res., 173: 53-61. DOI: 10.1016/j.jss.2011.09.054

Monte, J., A.C. Abreu, A. Borges, L.C. Simões and M. Simões, 2014. Antimicrobial activity of selected phytochemicals against *Escherichia coli* and *Staphylococcus aureus* and their biofilms. Pathogens, 3: 473-498. DOI: 10.3390/pathogens3020473

Nazzaro, F., F. Fratianni, L. De Martino, R. Coppola and V. De Feo, 2013. Effects of essential oils on pathogenic bacteria. Pharmaceuticals, 6: 1451-1474. DOI: 10.3390/ph6121451

Pan, Z., A.K. Agarwal, T. Xu, Q. Feng, S.R. Baerson and S.O. Duke *et al*., 2008. Identification of molecular pathways affected by pterostilbene, anatural dimethyletheranalog of resveratrol. BMC Med. Genomics, 1: 1-13. DOI: 10.1186/1755-8794-1-7

Pankey, G. and L. Sabath, 2004. Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of gram-positive bacterial infections. Clin. Infect. Dis., 38: 864-870. DOI: 10.1086/381972

Pezet, R. and V. Pont, 1988. Identification of pterostilbene in grape berries of *Vitis vinifera*. Plant Physiol. Biochem., 26: 603-607.

Qin, R., K. Xiao, B. Li, W. Jiang and W. Peng *et al*., 2013. The combination of catechin and epicatechin gallate from *Fructus crataegi* potentiates β-lactam antibiotics against methicillin-resistant *Staphylococcus aureus* (MRSA) *in vitro* and *in vivo*. Int. J. Mol. Sci., 14: 1802-1821. DOI: 10.3390/ijms14011802

Rastogi, N., P. Domadia, S. Shetty and D. Dasgupta, 2008. Screening of natural phenolic compounds for potential to inhibit bacterial cell division protein FtsZ. Ind. J. Exp. Biol., 46: 783-787. PMID: 19090350

Rimando, A.M., M. Cuendet, C. Desmarchelier, R.G. Mehta and J.M. Pezzuto *et al*., 2002. Cancer chemopreventive and antioxidant activities of pterostilbene, a naturally occurring analogue of resveratrol. J. Agri. Food Chem., 50: 3453-3457. DOI: 10.1021/jf0116855

Rimando, A.M., W. Kalt, J.B. Magee, J. Dewey and J.R. Ballington, 2004. Resveratrol, pterostilbene and piceatannol in *vaccinium* berries. J. Agri. Food Chem., 52: 4713-4719. DOI: 10.1021/jf040095e

Shorr, A.F., A. Combes, M.H. Kollef and J. Chastre, 2006. Methicillin-resistant *Staphylococcus aureus* prolongs intensive care unit stay in ventilator-associated pneumonia, despite initially appropriate antibiotic therapy. Crit. Care Med., 34: 700-706. DOI: 10.1097/01.CCM.0000201885.57697.21

Sopirala, M.M., J.E. Mangino, W.A. Gebreyes, B. Biller and T. Bannerman *et al*., 2010. Synergy testing by E-test, microdilution checkerboard and time-kill methods for pan-drug-resistant *Acinetobacter baumannii*. Antimicrob. Agents Chemother., 54: 4678-468. DOI: 10.1128/AAC.00497-10

Yadav, M.K., S.W. Chae, G.J. Im, J.W. Chung and J.J. Song, 2015. Eugenol: A phyto-compound effective against methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* clinical strain biofilms. PLoS ONE, 10: e0119564-e0119564. DOI: 10.1371/journal.pone.0119564