Sains Malaysiana 47(10)(2018): 2429–2435
http://dx.doi.org/10.17576/jsm-2018-4710-19

Graveoline from *Ruta angustifolia* (L.) Pers. and Its Antimicrobial Synergistic Potential in Erythromycin or Vancomycin Combinations

(Graveolin daripada *Ruta angustifolia* (L.) Pers. dan Potensi Sinergistik Antimikrobnya dalam Gabungan Erithromicin atau Vancomicin)

LAINA ZARISA MOHD KAMAL, NORAZIAN MOHD HASSAN*, NURHAYA MD TAIB & MAY KHIN SOE

ABSTRACT

Ruta angustifolia *(L.) Pers. is a Rutaceous species which contains various anthranilic acid derived alkaloids including the bioactive quinolones. This study is aimed at identifying the antimicrobial active alkaloids of* R. angustifolia *and evaluating their potential as synergistic enhancers in alkaloid-antibiotic combinations. Antimicrobial bioautography-guided isolation of alkaloidal fractions of* R. angustifolia *leaves has led to the identification of 2,3-dimethoxy-1-hydroxy-10-methylacridone [arborinine]; and 4,7,8-trimethoxyfuro[2,3-b]quinoline [skimmianine]; together with the major active alkaloid, 1-methyl-2-[3',4'-methylenedioxyphenyl]-4-quinolone [graveoline]. Graveoline showed Minimum Inhibitory Concentration (MIC) values ranging from 500 to 1000 µg/mL against* Staphylococcus aureus *ATCC 25923,* Enterococcus faecalis *ATCC 29212 and* Escherichia coli *ATCC 25922. Checkerboard assay for antimicrobial combination effects between graveoline with either erythromycin or vancomycin showed enhancement of the antimicrobial activity of both antibiotics with Fractional Inhibitory Concentration Indices (FICI) ranged from 0.37 to 1.50. Synergistic effect with FICI of 0.37 was observed for graveoline-erythromycin combination against* S. aureus *compared to FICI of 1.00 for ciprofloxacin-erythromycin additive effect. Graveoline was a potential candidate for antimicrobial combination agent especially against* S. aureus. *The result supports the idea of using plant metabolites as antimicrobial synergistic agents.*

Keywords: Antimicrobial; graveoline; Ruta angustifolia*; synergistic enhancer*

ABSTRAK

Ruta angustifolia *(L.) Pers. adalah spesies Rutaceae yang mengandungi pelbagai alkaloid yang hasilkan daripada asid anthranilik seperti alkaloid quinolon. Kajian ini bertujuan untuk mengenal pasti alkaloid aktif daripada* R. angustifolia *dan potensinya sebagai penggalak kesan sinergi bersama antibiotik terpilih. Pemencilan alkaloid aktif berpandukan bioautografi antimikrob daripada pecahan ekstrak daun* R. angustifolia *berjaya mengenal pasti 2,3-dimetoksi-1-hidroksi-10-metilakridon [arborinin]; dan 4,7,8-trimetoksifuro[2,3-b]quinolin [skimmianin]; dan akaloid aktif utama 1-metil-2-[3',4'-metilenedioksifenil]-4-quinolon [graveolin]. Graveolin menunjukkan Kepekatan Perencatan Minima (MIC) pada julat antara 500 dan 1000 µg/mL terhadap* Staphylococcus aureus *ATCC 25923,* Enterococcus faecalis *ATCC 29212 dan* Escherichia coli *ATCC 25922. Gabungan antara graveolin dan erithromicin atau vankomicin yang dinilai melalui Assay Checkerboard menunjukkan bahawa graveolin meningkatkan aktiviti kedua-dua antibiotik dengan Indeks Pecahan Kepekatan Perencatan (FICI) antara 0.37 dan 1.50. Kesan sinergi dengan nilai FICI 0.37 ditunjukkan oleh gabungan graveolin-erithromicin terhadap* S. aureus *berbanding kesan tambahan dengan nilai FICI 1.00 oleh gabungan ciprofloxacin-erithromicin. Graveolin mempunyai potensi sebagai agen gabungan antimikrob terutama terhadap* S. aureus. *Keputusan kajian ini menyokong penggunaan metabolit daripada tumbuhan sebagai agen sinergi antimikrob.*

Kata kunci: Antimikrob; graveolin; penggalak sinergistik; Ruta angustifolia

INTRODUCTION

The resistance in current antibiotics which restricts their effectiveness in the standard treatment of infections has dramatically increasing due to global emergence of multi-drug resistant bacterial strains. The global concern on the present updates on widespread antibiotic resistance include treatment failure of *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and Enterobacteriaceae infections to fluroquinolones, carbapenem, the first line drugs treatment and colistin, respectively. Erythromycin, vancomycin and ciprofloxacin are among the antibiotics which are prone to be degraded by the bacterial enzymatic resistance mechanism (WHO 2017). One of the strategies employed in overcoming limited number of antimicrobial agents that are currently available in fighting the highly resistant strains is antibiotic combination therapy (Tamma et al. 2012).

Plant-based natural product is one of the ideal candidates in discovering new class of antimicrobial agent and has contributed in surviving the increasing number of bacterial resistance towards currently existing antibiotics (Abreu et al. 2012). These phytochemicals are potential

antimicrobial combination agents since several compounds have also been found to be synergistic enhancers (Kyaw et al. 2012; Linda et al. 2011). Although they may possessed weak inhibitory activity alone but when combined with certain antibiotics they enhanced the activity of the later (Kyaw et al. 2012). The combination also has been recognised as important approach for delaying the emergence of bacterial resistance and may also eventually reduced the undesirable side effects of the antibiotics (Hemaiswarya & Doble 2010).

Rutaceae has been a source of interest for its novel anthranillic acid derived alkaloids with biologically active antibacterial and antifungal properties (da Silva et al. 2013). *Ruta angustifolia* (Garuda or Aruda in Malay) of the Rutaceae is one of the rich sources of anthranilic acid derived alkaloids including acridone, quinoline, quinolone and furoquinoline alkaloids (El Sayed et al. 2003). It is a small shrub with strong and unpleasant smell and used traditionally for treating ear infection, boils and bruised (Shamsul et al. 2003). Alkaloids are among the bioactive phytochemicals which are responsible for the antimicrobial activity possessed by *Ruta* species (Raj et al. 2013). The reported alkaloids of *R. angustifolia* include rutaverine, arborinine, fagarine, graveolinine, graveoline and skimmianine (Koh et al. 2009). The present study was undertaken to isolate and identify the antimicrobial active alkaloids of *R. angustifolia,* and evaluating their potential as synergistic enhancers in alkaloid-antibiotic combinations with either erythromycin or vancomycin.

Materials and Methods

Instrumentations

Spots on TLC chromatograms were visualized under UV lights at 254 and 365 nm by using Fluorescent Analysis Cabinet (SPECTROLINE, CM-10). Alkaloid purification was performed using chromatotron (Model 7924T, Harrison Research U.S.A) with silica gel containing gypsum Kieselgel 60 PF_{254} (Merck, 7749) as the stationary phase. Melting point was determined using digital melting point apparatus (Stuart's SMP20) equipped with microscope. Ultraviolet spectra were recorded in methanol using HITACHI, U-2900 Spectrophotometer. The infrared spectra were recorded on Perkin Elmer FTIR Spectrum 2000 Spectrometer with chloroform as solvent. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker, 500 MHz (Avance III, Ultrashield Plus) Spectrometer. MS were recorded by direct probe method using Thermofinnigan Trace GC-Polaris-Q GCMS.

Plant Materials

Ruta angustifolia (L.) Pers. was purchased at Muar, Johor, Malaysia. The plant species was verified by Dr. Shamsul Khamis, the botanist of Herbarium, Universiti Kebangsaan Malaysia (UKMB). A voucher specimen (PIIUM 0002-1) was deposited at the Herbarium, Kulliyyah of Pharmacy, International Islamic University Malaysia.

Extraction

The dried leaves (400 g) were defatted with hexane and then extracted with acetone (4 L) using soxhlet apparatus. The extract was concentrated to 1/8 of its original volume and extracted exhaustively with 2% hydrochloric acid until Mayer's test became negative. The acidic aqueous solution was basified with 0.5 M sodium hydroxide to pH8-9 and extracted with $CHCl_3$ until extinction. The crude alkaloidal extract was then dried using sodium sulphate anhydrous, filtered and evaporated to dryness (7.1 g).

Fractionation and Isolation

The extract (2.6 g) was fractionated by column chromatography on silica gel (100 g, 70-230 mesh) (column 3 × 100 cm) eluting successively with hexane/CH_2Cl_2 (4:6 - 0:1, 5% increment of CH_2Cl_2), then CH_2Cl_2/EtOAc (9:1 - 1:1, 10% increment of EtOAc) and a gradient of CH_2Cl_2/EtOAc/Me_2CO (10:9:1-2:7:1, 10% and 1% increment of EtOAc and Me_2CO, respectively, to furnished 10 fractions (F1 - F10). Fractions F3, F4 and F10 were subjected to bioautography agar overlay assay which showed RA2, RA3 and RA9 as the antimicrobial active alkaloids. Fraction F3 (50 mg) was rechromatographed by column chromatography (40 g) (column 2 × 40 cm) with hexane/CH_2Cl_2 (4:6-2:8, 1% increment of CH_2Cl_2 then 1-2% of EtOAc) to give 3 fractions (F3-1 - F3-3). Fraction F3-3 (150 mg) was further purified by chromatotron (1 mm thickness) with 100% pet-ether then a gradient of pet-ether/CH_2Cl_2 (99:1-8:2, 1% increment of CH_2Cl_2 then 1% increment of EtOAc with subsequent 1% decrement of CH_2Cl_2. RA2 or arborinine (1) was eluted with pet-ether/CH_2Cl_2/EtOAc (80:6:14) (30 mg, 1.2%). A total of 20 mg Fraction F4 was separated by chromatotron (1 mm thickness) with mixture of RA3 or skimmianine (2) (0.8 mg, 0.03%) was purified from fraction F4 (20 mg) by using chromatotron (1 mm thickness) eluted with pet-ether/CH_2Cl_2/EtOAc (80:11:9) at the flow rate of 2 to 3 mL/min. Fraction F10 (120 mg) was rechromatographed on silica gel (15 g) (column 2 × 20 cm) eluting consecutively with pet-ether/CH_2Cl_2 (1:1 - 0:1, 10% increment of CH_2Cl_2) and CH_2Cl_2/MeOH (99:1 and 98:2). RA9 or graveoline **(3)** was eluted with CH_2Cl_2/MeOH (98:2) (80 mg, 3.1%).

Antimicrobial Activity

Test Microorganisms

Three bacterial strains of American Type Culture Collection (ATCC), namely *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 were used in this study.

Inocula for Antimicrobial Tests

The inocula for antimicrobial testing were prepared according to Rahalison et al. (1991). The inoculum size of 18 h culture incubated at 37°C was adjusted by using

UV spectrophotometer to an absorbance of 0.11 to 0.12 at 600 nm.

BIOAUTOGRAPHY AGAR OVERLAY ASSAY

This assay was performed according to the bioautographic procedures (Rahalison et al. 1991) with a few modifications. All fractions were chromatographed on 4 cm × 10 cm TLC commercial aluminium sheets Silica gel $60F_{254}$ of layer thickness 0.2 mm. The chromatogram was rapidly distributed with 5 mL of inoculated molten agar at 35°C. After solidification of the agar, the TLC plates were kept in sterile petri dishes lined with moist filter papers and incubated at 37°C for 24 h. The plates were sprayed with an aqueous solution of 0.5% 2-[4-iodo-phenyl]-3-[4-nitrophenyl]-5-phenyl-tetrazolium chloride (INT) and reincubated for 4 h. The active alkaloids were detected as the clear zones against a pink background of viable microbes. The reference chromatograms were visualized under UV lights and sprayed with Dragendorff's reagent.

BROTH MICRODILUTION ASSAY

The minimum inhibitory concentrations (MICs) of graveoline and selected antibiotics were determined in triplicate against the test microorganisms by broth microdilution assay following the Clinical and Laboratory Standards Institute (CLSI) 2006 method with some modifications. Stock solution of gaveoline was two-fold serially diluted in 96 well microtiter plate to obtain concentrations ranging from 1.56 to 1000 µg/mL. Standard antibiotics were prepared at a few ranges of concentrations from 0.48 to 500 µg/mL by two-fold serial dilution of two, five or ten times diluted stock solution of 1000 µg/mL. The microbial inocula were diluted hundred times to an approximate concentration of 1×10^5 colony forming unit (CFU)/mL with sterile broth. Each well contained a final volume of 200 µL of 180 µL of inoculated broth and 20 µL of tested solution. The assay was repeated with diluents to check their effects on bacterial growth. Wells with bacterial suspension and uninoculated broth are included as normal growth and sterility control, respectively. The plates were incubated for 18 h at 37°C. 10 µL of 0.25% (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) was added to each well subsequent to incubation for another 30 min. A colour change from yellow to blue indicated the presence of viable bacteria. MIC was recorded as the lowest concentration with no colour changes. The minimum bactericidal concentration (MBC) was determined by streaking a loopful of mixture from each well with no colour changes onto agar and incubated at 37°C for 24 h. MBC was recorded as the lowest concentration with no bacterial growth.

CHECKERBOARD ASSAY

The antimicrobial combination effects between graveoline and either erythromycin or vancomycin were studied following the CLSI 2006 guideline with some modifications. Two-fold serial dilutions of graveoline and antibiotic alone were performed in a volume of 20 µL per well in the first column and row of a 96-well microtiter plate, respectively. For graveoline-antibiotic combination, 10 uL of each concentration of a serially diluted antibiotic solution was put in each well of the same remaining row of the plate. Then, each well was added with 180 µL of 100 fold diluted inoculum. After 60 min incubation at room temperature, 10 µL of each concentration of a serially diluted graveoline solution was added in each well of the same column, so that each row contained a fixed amount of antibiotic while decreasing amounts of graveoline. All plates were incubated at 37°C for 18 h and the MIC values were confirmed by 0.25% MTT. The fractional inhibitory concentration (FIC) and the FIC indices (FICI) were calculated and interpreted following Orhan et al. (2005). The results were determined from the majority of three independent tests. The procedure was repeated for ciprofloxacin-antibiotic combination where ciprofloxacin

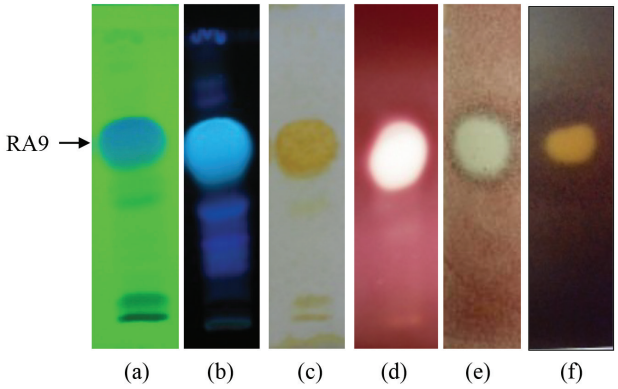


FIGURE 1. Antimicrobial bioautographic profile of fraction R10 of *R. angustifolia* leaves alkaloidal extract showing RA9 or graveoline as the active alkaloid of the fraction, (a) and (b) reference chromatograms viewed under UV_{254} and UV_{365} light, respectively, (c) reference chromatogram sprayed with Dragendorff's reagent (d), (e) & (f) bioautograms against *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212 and *E. coli* ATCC 25922, respectively. Chromatographic conditions: pre-coated aluminium silica gel 60 F_{254} of 0.2 mm thickness, solvent system, CH_2Cl_2:MeOH (9:1)

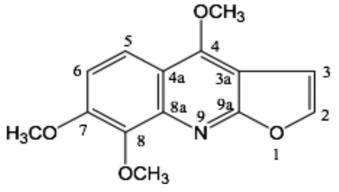
was used as the reference for quinolone antimicrobial agent.

Results and Discussion

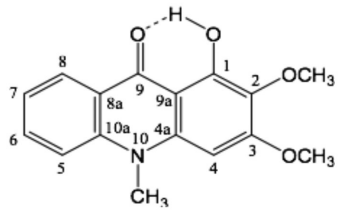
Antimicrobial bioautography-guided isolation of alkaloidal fractions of *R. angustifolia* leaves has led to the identification of a major active alkaloid identified as graveoline **(3)** together with arborinine **(1)** and the minor alkaloid, skimmianine **(2)** (Figures 1 & 2). Graveoline possessed MIC values of 500 µg/mL against *E. faecalis* and 1000 µg/mL for *S. aureus* and *E. coli* (Table 1). The susceptibility values are within the range regarded for antimicrobial active phytochemicals (Monte et al. 2014). Antimicrobial active alkaloids may possess different mechanism of action than those of antibiotics which might potentiate the activity of the later when used in combination (Cushnie et al. 2014). Therefore, combination of graveoline, a natural 4-quinolone alkaloid with either erythromycin, a macrolide or vancomycin, a glycopeptide was performed with the objective of achieving higher efficacy in their activity with desirable synergistic effect possible for preventing drug resistance in the future. The combination effects were compared to that of ciprofloxacin which is a synthetic quinolone antimicrobial agent.

Graveoline possesses a structural characteristic of quinolone antimicrobial agent by bearing completely aromatic 4-quinolone ring with specific substituents at C-1, C-5 and C-8 but lacking in a 4-pyridone ring with 3-carboxyl group which is the essential pharmacophore for exerting significant antibacterial activity (Chung et al. 2015; Emami et al. 2005). Nevertheless, it could be presumed that graveoline might possesses a few characteristics mechanism of action of the quinolones although at weaker activity. The graveoline-antibiotic interactions against *S. aureus*, *E. faecalis* and *E. coli* were recorded as synergy, partial synergy, additive and indifference, respectively, based on their FICI values (Orhan et al. 2005) (Table 2). The MICs of both graveoline and antibiotics were substantially reduced although to a variable extent as compared to the values when tested alone against *S. aureus* and *E. coli*. *S. aureus* was the most susceptible microbe towards graveoline-antibiotic interactions.

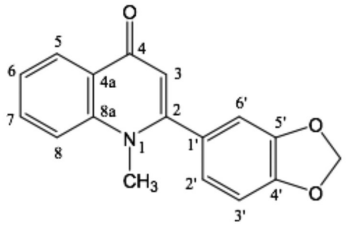
Graveoline-erythromycin combination resulted in synergy effect with the FICI of 0.37 and reduced MIC by



1



2



3

FIGURE 2. Antimicrobial active alkaloids of *Ruta angustifolia*

6-folds and 4-folds for graveoline and erythromycin, respectively. Graveoline-vancomycin interaction produced partial synergy effect with FICI value of 0.5. Erythromycin and vancomycin are clinically used antibiotics particularly against Gram-positive bacterial infections. Erythromycin disrupts protein synthesis by inhibiting peptide elongation on the ribosome (Gaynor & Mankin 2003) whereas vancomycin is an inhibitor to cell wall peptidoglycan synthesis (Kang & Park 2015). The combination effects could be attributed to the dual actions of both agents at different target sites of the bacterial cells. Indifference effect with 2-folds reduction to the MIC of erythromycin

TABLE 1. Quantitative antimicrobial activity of graveoline from *R. angustifolia* and selected antibiotic standards

Test compound	Antimicrobial Activity (µg/mL)					
	S. aureus		*E. faecalis*		*E. coli*	
	MIC	MBC	MIC	MBC	MIC	MBC
Graveoline	1000	>1000	500	1000	1000	>1000
Ciprofloxacin	0.195	0.195	1.562	1.562	0.039	0.039
Erythromycin	1.098	1.098	0.78	0.78	50	50
Vancomycin	2.50	2.50	3.13	3.13	250	500

TABLE 2. Antimicrobial combination effects between graveoline and either erythromycin or vancomycin against *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212 and *E. coli* ATCC 25922 with comparison to that of ciprofloxacin

Bacteria	Combination	MIC alone (μg/mL)	MIC in combination (μg/mL)	FIC	FICI	Combination effect
S. aureus ATCC 25923	Graveoline Erythromycin	1000 1.098	125 0.274	0.12 0.25	0.37	Synergy
	Graveoline Vancomycin	1000 2.50	250 0.625	0.25 0.25	0.50	Partial synergy
	Ciprofloxacin Erythromycin	0.195 1.098	0.098 0.547	0.50 0.50	1.00	Additive
	Ciprofloxacin Vancomycin	0.195 2.50	0.098 1.25	0.50 0.50	1.00	Additive
E. faecalis ATCC 29212	Graveoline Erythromycin	500 0.781	500 0.39	1.00 0.50	1.50	Indifference
	Graveoline Vancomycin	500 3.13	500 3.13	1.00 1.00	2.00	Indifference
	Ciprofloxacin Erythromycin	1.562 0.781	0.781 0.39	0.50 0.50	1.00	Additive
	Ciprofloxacin Vancomycin	1.562 3.125	0.781 1.562	0.50 0.50	1.00	Additive
E. coli ATCC 25922	Graveoline Erythromycin	1000 50	250 25	0.25 0.50	0.75	Partial synergy
	Graveoline Vancomycin	1000 250	250 125	0.25 0.50	0.75	Partial synergy
	Ciprofloxacin Erythromycin	0.039 50	0.019 1.562	0.50 0.03	0.53	Partial synergy
	Ciprofloxacin Vancomycin	0.039 250	0.002 7.813	0.05 0.13	0.18	Synergy

FICI < 0.5, synergy; 0.5 - 0.75, partial synergy; > 0.75 to 1.0, additive effect; > 1.0 to 4.0 indifference and > 4, antagonism (Orhan et al. 2005)

was produced in graveoline-antibiotic combinations against *E. faecalis*. The results showed that graveoline-antibiotic actions occurred at a different susceptibility degree against different bacteria.

Both graveoline-antibiotic combinations against *E. coli* indicated a partial synergy with 4-folds reduction in the MIC of graveoline and 2-folds reduction of both erythromycin and vancomycin. Although vancomycin is considered inactive against Gram-negative bacteria due to variety of mechanisms involve in its cell wall synthesis and factors that affect membrane permeability (Kang & Park 2015), the resulted partial synergy effect for its combinations with graveoline suggested for effective combination for glycopeptide against Gram-negative *E. coli*. The partial synergy could be attributed to the actions of both agents at different targets consisting of disruption of cell wall synthesis by vancomycin which increase membrane permeability of graveoline to its target sites while inhibition of protein synthesis by erythromycin enhanced graveoline activity.

Combinations involving ciprofloxacin have resulted in additive interaction by which in every combination, both combined antibiotics exhibited 2-folds of MIC reduction and thus gave the FIC index of 1.00 against both Gram-positive *S. aureus* and *E. faecalis*. The result is in agreement to the previous finding on the influence of non-quinolone antimicrobial agents such as macrolides (Gradelski et al. 2001) and glycopeptides (Noviello et al. 2001) which demonstrated additive interactions with ciprofloxacin by all dual drug combinations. In *E. coli*, ciprofloxacin-erythromycin combination achieved partial synergy by 32-folds reduction in the MIC of erythromycin. A synergistic interaction was achieved for ciprofloxacin and vancomycin combination with 16-folds reduction of the MIC of the former and enhancement of the antimicrobial activity of the later by 32-folds. Ciprofloxacin is a broad spectrum antibiotic which is more susceptible to Gram-negative bacteria than Gram-positive bacteria. It rapidly inhibits bacterial growth by primarily interfering with the DNA synthesis in combination with its efficient membrane permeability (Cramariuc et al. 2012) which resulted to a noticeable reduction of erythromycin and vancomycin. The findings showed that graveoline was a synergistic enhancer to erythromycin which is superior combination

agent than ciprofloxacin against *S. aureus* whereas it was inferior agent than ciprofloxacin against *E. coli*.

The identification details of the isolated antimicrobial alkaloids are shown as follows:

Compound 1 2,3-dimethoxy-1-hydroxy-10-methylacridone [Arborinine]; $C_{16}H_{15}NO_4$; MW: 285 g/mol; bright yellow fine needle-shaped crystals; MP: 175-176°C, Rf: 0.67 ($CHCl_3$); IR ($CHCl_3$) cm^{-1}: 1641, 1591, 1556, 1463, 1322, 1251, 1188, 1139, 1106, 1058, 989, 918, 849, 784, 753; UV/Vis λ_{max} (MeOH) nm (log ε): 230 (3.79), 274 (4.24), 399 (3.41); ^{1}H NMR (500 MHz, $CDCl_3$): 14.73 (1H, s, 1-OH), 8.39 (1 H, dd, *J* = 1.7 and 8.2 Hz, H-8), 7.70 (1H, m, H-6), 7.47 (1H, d, *J* = 8.6 Hz, H-5), 7.26 (1H, t, *J* = 7.4 Hz, H-7), 6.24 (1H, s, H-4), 4.00 (3H, s, 2-OMe), 3.92 (3H, s, 3-OMe), 3.82 (3H, s, N-Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 180.6 (C-9), 159.2 (C-3) 155.9 (C-1), 141.8 (C-10a), 140.3 (C-4a), 133.9 (C-6), 130.0 (C-2), 126.4 (C-8), 121.4 (C-7), 120.50 (C-8a), 114.6 (C-5), 105.6 (C-9a), 86.7 (C-4), 60.8 (2-OCH_3), 55.9 (3-OCH_3), 34.0 (*N*-CH_3); MS (EI, 70 eV): *m/z* (%) = 285 [M + H^+] (52), 270 (100), 256 (15), 242 (85), 227 (5), 212 (12), 199 (59), 171 (12), 143 (9), 115 (10).

Compound 2 4,7,8-trimethoxyfuro[2,3-b]quinoline [Skimmianine]; Formula: $C_{14}H_{13}NO_4$ MW: 259 g/mol, colorless rhombohedral prisms; MP: 175-176°C; Rf: 0.52 ($CHCl_3$); IR ($CHCl_3$) cm^{-1}: 1616, 1575, 1389, 1364, 1266, 1088, 950; UV/Vis λ_{max} (MeOH) nm (log ε): 250 (4.30), 332 (3.39); ^{1}H NMR (500 MHz, $CDCl_3$): 8.03 (1 H, d, J = 9.5 Hz, H-5), 7.61 (1H, d, J = 2.5 Hz, H-2), 7.25 (1 H, d, J = 9.5 Hz, H-6), 7.06 (1 H, d, J = 2.5 Hz, H-3), 4.45 (3 H, s, 4-OMe), 4.14 (3 H, s, 8-OMe), 4.05 (3 H, s, 7-OMe); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.6 (C-9a), 157.5 (C-4), 152.4 (C-8a), 143.1 (C-2), 142.1 (C-7), 141.2 (C-8), 118.3 (C-5), 115.1 (C-3a), 112.1 (C-6), 104.7 (C-3), 102.1 (C-4a), 61.7 (8-OCH_3), 59.1 (4-OCH_3), 56.8 (7-OCH_3); MS (EI, 70 eV): m/z (%) = 259 [M + H^+] (64), 230 (5), 216 (4), 172 (2).

Compound 3 1-methyl-2-[3',4'-methylenedioxyphenyl]-4-quinolone [Graveoline]; Formula: $C_{18}H_{17}O_3N$; MW: 279 g/mol; white amorphous powder; MP: 185-186°C; Rf: 0.58 ($CHCl_3$: MeOH, 9:1); IR ($CHCl_3$) cm^{-1}: 1621, 1597, 1563, 1486, 1446, 1250, 1036, 827, 760; UV/Vis λ_{max} (MeOH) nm (log ε): 243 (4.18), 324 (3.89), 336 (3.90); ^{1}H NMR (500 MHz, $CDCl_3$): 8.50 (1 H, dd, *J* = 1.5 and 8.0 Hz, H-5), 7.74 (1 H, t, *J* = 8.5 Hz, H-7), 7.57 (1 H, d, *J* = 8.5 Hz, H-8), 7.40 (1H, t, *J* = 8.0 Hz, H-6), 6.88 (1H, d, *J* = 1.5 Hz, H-2′), 6.91 (1H, s, H-3′), 6.88 (1H, d, *J* = 1.5, H-6′), 6.37 (1H, s, H-3), 6.09 (2H, s, OCH_2O), 3.70 (3H, s, N-Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 177.3 (C-4), 154.7 (C-2), 148.8 (C-4′), 147.9 (C-5′), 141.9 (C-8a), 132.5 (C-2′), 129.3 (C-1′), 126.7 (C-4a), 126.6 (C-7), 123.9 (C-6), 122.8 (C-5), 116.1 (C-8), 112.5 (C-3), 109.0 (C-3′), 108.7 (C-6′), 101.8 (OCH_2O), 37.5 (*N*-CH_3); MS (EI, 70 eV): *m/z* (%) = 279 [M + H^+] (60), 251 (100), 192 (17).

Conclusion

The synergistic action of the combination between graveoline, a natural quinolone alkaloid and erythromycin, a macrolide or vancomycin, a glycopeptide possesses a potential clinical significance as an alternative antimicrobial combination agent to be further researched for future benefit in overcoming or at least delaying the emergence of resistance in bacteria particularly against *S. aureus*.

Acknowledgements

A special gratitude is acknowledged to the Ministry of Higher Education Malaysia (MOHE) for funding the research through Fundamental Research Grant Scheme (FRGS0207-60) and International Islamic University Malaysia for the publication support (RIGS16-123-0287).

References

Abreu, A.C., McBain, A.J. & Simões, M. 2012. Plants as sources of new antimicrobials and resistance-modifying agents. *Natural Product Reports* 29(9): 1007-1021.

Chung, P.Y., Bian, Z.X., Pun, H.Y., Chan, D., Chan, A.S.C., Chui, C.H., Tang, J.C.O. & Lam, K.H. 2015. Recent advances in research of natural and synthetic bioactive quinolines. *Future Medicinal Chemistry* 7(7): 947-967.

CLSI. 2006. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard, 7th edition, document M7-A7 [ISBN 1-56238-587-9]*. Wayne, Clinical and Laboratory Standards Institute.

Cramariuc, O., Rog, T., Javanainen, M., Monticelli, L., Polishchuk, A.V. & Vattulainen, I. 2012. Mechanism of translocation of fluoroquinolones across lipid membranes. *Biochimica et Biophysica Acta* 1818: 2563-2572.

Cushnie, T.P., Cushnie, B. & Lamb, A.J. 2014. Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *Int. J. Antimicrob. Agents* 44(5): 377-386.

da Silva, M.F.G.F., Fernandes, J.B., Forim, M.R., Vieira, P.C. & de Sa, I.G.C. 2013. Alkaloids derived from anthranilic acid: Quinoline, acridone, and quinazoline in *Natural Product*, edited by Ramawat, K.G. & Merillon, J.M. Berlin Heidelberg: Springer-Verlag. pp: 715-858.

El Sayed, K., Al-Said, M.S., El-Feraly, F.S. & Ross, S.A. 2003. New quinoline alkaloid from *Ruta chalepensis*. *Journal of Natural Product* 63: 995-997.

Emami, S., Shafiee, A. & Foroumadi, A. 2005. Quinolones: Recent structural and clinical development. *Iranian Journal of Pharmaceutical Research* 3: 123-136.

Gaynor, M. & Mankin, A.S. 2003. Macrolide antibiotics: Binding site, mechanism of action, resistance. *Current Topics in Medicinal Chemistry* 3: 949-961.

Gradelski, E., Kolek, B., Bonner, D.P., Valera, L., Minassian, B. & Fung-Tomc, J. 2001. Activity of gatifloxacin and ciprofloxacin in combination with other antimicrobial agents. *International Journal Antimicrobial Agents* 17: 103-107.

Hemaiswarya, S. & Doble, M. 2010. Synergistic interaction of phenylpropanoids with antibiotics against bacteria. *Journal of Medical Microbiology* 59: 1469-1476.

Kang, H.K. & Park, Y. 2015. Glycopeptide antibiotics: Structure and mechanisms of action. *Journal of Bacteriology and Virology* 45(2): 67-78.

Koh, H.L., Chua, T.K. & Tan, C.H. 2009. *A Guide to Medicinal Plants: An Illustrated, Scientific and Medicinal Approach*. Singapore: World Scientific. p. 135.

Kyaw, B.M., Shuchi, A.S. & Lim, C.S. 2012. Bactericidal antibiotic-phytochemical combinations against methicillin resistant *Staphylococcus aureus*. *Brazilian Journal of Microbiology* 43(3): 938-945.

Linda, E., Maya, A.F., Shannon, B.F., Jan, W., Brian, K.C., Mike, T., Eric, D.B. & Gerard, D.W. 2011. Combinations of antibiotic and non-antibiotic drugs enhance antimicrobial efficacy. *Nature Chemical Biology* 7: 348-350.

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

Noviello, S., Ianniello, F. & Esposito, S. 2001. *In vitro* activity of LY333328 (oritavancin) against Gram-positive aerobic cocci and synergy with ciprofloxacin against enterococci. *J. Antimicrobial Chemotherapy* 48: 283-286.

Orhan, G., Bayram, A., Zer, Y. & Balci, I. 2005. Synergy tests by E test and checkerboard methods of antimicrobial combinations against *Brucella melitensis*. *Journal of Clinical Microbiology* 43(1): 140-143.

Rahalison, L., Hamburger, M. & Hostettman, K.A. 1991. Bioautographic agar overlay method for the detection of antifungal compounds from higher plants. *Phytochemical Analysis* 2: 199-203.

Raj, M.P., Arora, L. & Kumar, N.A. 2013. Phytochemicals extraction of alkaloids from *Ruta graveolens* (Snake repellent plant) and evaluation of its antibacterial and antifungal activity. *International Journal of Biological Research* 1(1): 1-10.

Shamsul, K., Tajuddin, A.M. & Mazina, M.Y. 2003. *Tumbuhan Ubatan Tradisional Malaysia*. Serdang: Institut Biosains Universiti Putra Malaysia. p. 87.

Tamma, P.D., Cosgrove, C.E. & Finch, R. 2012 Combination therapy for treatment of infections with Gram-negative bacteria. *Clinical Microbiology Review* 25(3): 450-470.

World Health Organization (WHO). 2017. *Antimicrobial Resistance. Fact Sheet No. 194*. http://www.who.int/mediacentre/factsheets/fs194/en/. Accessed on January 7, 2018.

Laina Zarisa Mohd Kamal, Norazian Mohd Hassan*
& Nurhaya Md Taib
Department of Pharmaceutical Chemistry
Kulliyyah of Pharmacy
International Islamic University Malaysia
25200 Kuantan, Pahang Darul Makmur
Malaysia

May Khin Soe
Department of Basic Medical Sciences
Kulliyyah of Pharmacy
International Islamic University Malaysia
25200 Kuantan, Pahang Darul Makmur
Malaysia

*Corresponding author; email: norazianmh@iium.edu.my

Received: 9 April 2017
Accepted: 7 June 2018