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In vitro assessment of antibiotic-resistance reversal of a methanol extract from *Rosa canina* L

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ABSTRACT

The crude methanol extract of *Rosa canina* (RC) fruit was tested against multidrug-resistant (MDR) bacterial strains, including methicillin-resistant *Staphylococcus aureus* SA1199B, EMRSA16 and XU212 harbouring NorA, PBP2a and TetK resistance mechanisms, respectively, as well as *S. aureus* ATCC25923, a standard antimicrobial susceptible laboratory strain. The inhibition of the conjugal transfer of plasmid PKM101 and TP114 by the RC extract was also evaluated. The RC extract demonstrated a mild to poor antibacterial activity against the panel of bacteria having MIC values ranging from 256 to >512 μg/mL but strongly potentiated tetracycline activity (64-fold) against XU212, a tetracycline-effluxing and resistant strain. Furthermore, the extract showed moderate capacity to inhibit the conjugal transfer of TP114 and PKM101; transfer frequencies were between 40% and 45%. Cytotoxicity analysis of the RC extract against HepG2 cells line showed the IC_{50} > 500 mg/L and, thus, was considered non-toxic towards human cells. Phytochemical characterisation of the extracts was performed by the assessment of total phenolic content (RC: 60.86 mg TAE/g) and HPLC fingerprints with five main peaks at 360 nm. The results from this study provide new mechanistic evidence justifying, at least in part, the traditional use of this extract. However, the inhibition of bacterial plasmid conjugation opens the possibility of combination therapies to overcome antibiotic resistance.

1. Introduction

The rapid spread of multidrug-resistant (MDR) bacteria has posed a serious global health threat over the last decades and continues to constitute a major problem in the treatment of hospital- and community-acquired infections (Annan et al., 2009). As a consequence, treatment of infections caused by MDR bacteria strains is becoming increasingly difficult to manage with existing antibiotics. This is in part due to the ability of bacteria to evolve mechanisms that thwart antimicrobial action (Martinez, 2012). Examples include strains of *Staphylococcus aureus* such as SA1199B (NorA), XU212 (TetK and PBP2a), RN4220 (MsrA), EMRSA15 and EMRSA16 (PBP2a) that are prominent for their high level of resistance to antibiotics due to their encoded efflux mechanism (Richardson and Reith, 1993; Cox et al., 1995). Recent studies have suggested the horizontal transmission of genes as a major part of the growing problem of antibiotic resistance (Dantas and Sommer, 2014). The dissemination of extended-spectrum beta-lactamase (ESBL) genes, conferring resistance to third-generation cephalosporin and other β-lactams, are promoted bacteria such as *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter* spp. (Paterson and Bonomo, 2005). These plasmid-mediated multidrug resistance phenotypes are responsible for compromised treatment of infectious human diseases (Kumar et al., 2013). Unfortunately, synthetic plasmid-curing agents such as acridine orange, ethidium bromide and sodium dodecyl sulfate are unsuitable for therapeutic application owing to their toxic nature. There is therefore a need to develop new antibiotics with alternative mechanisms to overcome bacterial resistance, as resistance-modifying and efflux inhibitors or plasmid-curing compounds (Spengler et al., 2006; Stavri et al., 2007). In this context, plant-derived products have been proposed as potential candidates of drugs leads to combat bacterial resistance mechanisms (Hollander et al., 1998) through the reversal of MDR phenotypes facilitating the re-establishment of use of existing antibiotics.

Rosa canina L (Rosaceae; RC) known as rose hip or dog rose is mostly used traditionally as a dietary supplement and a herbal remedy for the prevention and treatment of diverse human diseases such as diabetes, kidney disorders, inflammation, ulcer and cancer, but only a few of these have any in vitro scientific data to support these indications (Chrubasik et al., 2008). In the traditional medicine, 2–5 g of the *R. canina* fruits is prepared and used internally as tea (a cup taken 3 to 4 times per day) to strengthening the body immune system against infections such as common colds, influenza, scurvy, diarrhoea, gastritis, cough, vaginitis and urinary tract infections (Sen and Gunes, 1996). The strong antimicrobial potential of *R. canina* against certain microorganisms has been documented (Horvath et al., 2012). The extracts

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from RC fruits have been reported as a significant source of ascorbic acid, tocopherol, tannins, pectin, minerals, fatty acids, carotenoids and phenolic compounds (Chevallier, 1996; Ozturk and Sezai, 2011). It has previously been reported that tellimagrandin I isolated from RC reduced the MIC of oxacillin and tetracycline against four MRSA strains (Shiota et al., 2000). Presently, there is no scientific data on the antibacterial properties of RC fruits against strains with characterised efflux-mediated mechanisms of resistance or the capacity to inhibit plasmid transfer. This study therefore aimed to investigate the antibacterial; plasmid conjugation inhibition and antibiotic modulation potential of the methanol extract of *R. canina* fruit against selected MDR *S. aureus* strains. In addition, their differential cytotoxicity against mammal cells expressing efflux pumps was also determined using the sulforhodamine B (SRB) assay with human liver carcinoma cells (HepG2).

2. Materials and methods

2.1. Plant extracts collection and preparation

R. canina L (RC) fruits were obtained in fully dried and powdered form from Herbs in a Bottle Ltd. UK, and a voucher specimen (No. RC-001) was deposited at the herbarium in the School of Pharmacy, University College London (UCL). Fifty grams of the dried powdered materials was extracted in 300 mL of methanol, at room temperature by constant mechanised agitation in an ultrasonic bath, for 45 min. The extract was filtered using a Buchner funnel and Whatman No. 1 filter paper. The volume was concentrated under vacuum at 40 °C to recover the methanol, air-dried, weighed and stored in a refrigerator at 6 °C for future use.

2.2. Bacterial strains

The antibacterial assay was performed against strains of *Staphylococcus aureus*: SA1199B and XU212 are MDR strains that overexpress the NorA and TetK efflux transporter proteins with a high level of resistance to norfloxacin and tetracycline, respectively. EMRSA15 and EMRSA16 are the major epidemic methicillin-resistant *S. aureus* strains in UK hospitals site (Richardson and Reith, 1993; Cox et al., 1995). *S. aureus* ATCC25923, *E. coli* NCTC 10418 and *Pseudomonas aeruginosa* NCTC10662 are standard susceptibility testing control strains. *S. aureus* RN4220 is a macrolide-resistant strain, while *K. pneumoniae* 342 and *Proteus spp.* P10830 are MDR clinical isolates. The plasmid conjugal inhibitory potential of RC extract was tested on a conjugal transfer system of *E. coli* bearing the TP114 plasmid, which encodes for kanamycin resistance and *E. coli* harbouring the PKM 101, which codes for ampicillin resistance. The recipient did not harbour plasmids but had chromosomally encoded resistance to streptomycin. All the bacteria and plasmids tested were obtained from Dr. Paul Stapleton of the Research Department of Pharmaceutical and Biological Chemistry, School of Pharmacy, University College London (UCL).

2.3. Cell culture and maintenance

The HepG2 cell lines (American Type Culture Collection) were obtained from Prof. Andreas Kortenkamp formerly at the Research Department of Pharmaceutical and Biological Chemistry, School of Pharmacy, University College London (UCL). HepG2 cells were replenished with new growth medium every 2–3 days and sub-cultured every 3–4 days when they were 80% confluent. Cells were maintained in DMEM (Invitrogen, UK) containing 10% foetal bovine serum (GIBCO 10010) and antibiotics [penicillin (100 IU/mL) and streptomycin (100 mg/L)], incubated at 37 °C in a 5% CO_2 humidified atmosphere and split when confluent. The cell density was adjusted to 7.5×10^4 cells/mL before exposure to different concentrations of plant extracts prepared in 1% *w/v* DMSO.

2.4. Total phenolic content (TPC)

The total phenolic content of the methanol extract from RC was determined with Folin–Ciocalteu reagent using the method of Lister and Wilson (2001) with slight modification. A volume of 0.5 mL of the plant extract (1000 mg/L) was added to 2.5 mL of 10% *v*/v Folin–Ciocalteu reagent (FCR) and 2 mL of 20,000 mg/L Na_2CO_3. The resulting mixture was incubated at 45 °C with shaking for 15 min, followed by measuring the absorbance at 765 nm. The calibration curve of the standard tannic acid (1 mL; 25–400 mg/L) was prepared following the same method. The experiment was conducted in triplicate, and the results were reported as mean ± SD values. Tannic acid equivalents (TAE) in the plant extract was calculated by the following formula: $T = CV/M$, where T is the TPC (mg/g) of the extract in TAE, C is the concentration of tannic acid established from the calibration curve, V is the volume of the extract (mL) and M is the weight of the extract (g).

2.5. Minimum inhibitory concentration (MIC) assay

The minimum inhibitory concentrations (MICs) of the antibiotics and plant extract were determined against resistant bacteria using the method described by Shiu and Gibbons (2006). The bacterial strains and isolates were grown at 37 °C overnight on nutrient agar and inocula of the test organisms were prepared in normal saline (9 g/L) and compared with a 0.5 McFarland standard and diluted to achieve 5×10^5 CFU/mL. A volume of 100 μL of Mueller Hinton broth (MHB) was dispensed into 96 wells of microtitre plate. A stock solution of antibiotics or drug extracts was prepared in dimethyl sulfoxide (DMSO) (Sigma) and further diluted in MHB (1.5 mL) to reduce DMSO concentration to 1%. One hundred microliters of stock solution of 2048 mg/L was dispensed into the first well and serially diluted into each well, added with 100 μL of standardised bacterial inoculum to give a final concentration that ranged between 512 and 1 mg/L for the extract and 128 to 0.03 mg/L for the antibiotics. All procedures were performed in duplicate; the plates were incubated at 37 °C for 18 h. A volume of 20 μL of a 5 g/L methanol solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma) was added to each well and incubated for 30 min. A blue coloration indicated bacterial growth while the MIC was recorded as the lowest concentration at which no visible growth was observed.

2.6. Bacterial resistance modulation assay

For the evaluation of extracts as antibiotic resistance modulators, MICs of norfloxacin and tetracycline were determined in the presence or absence of sub-inhibitory concentrations (SIC) of the extract (64–512 mg/L) against susceptibility testing control strain *S. aureus* ATCC 25693 and three resistant *S. aureus* isolates: XU212, SA1199B and EMRSA16 following the procedure described by Oluwatuyi et al. (2004) with slight modification. A volume of 100 μL of MHB was dispensed in each well except wells in column 1 that contained 100 μL of the extract. The samples at the stock concentrations (256–2048 mg/L) were chosen to obtain one-quarter MICs and were introduced into wells 2–10, while 100 μL of the appropriate antibiotic (MICs) was added into well 1 and serially diluted across the plate leaving well 11 empty for final growth control and well 12 as sterility controls. To the wells in rows four to six, 100 μL of 20 mg/L reserpine (a modulatory agent) was added. All strains were cultured on nutrient agar slope before MIC determination. Overnight cultures of each strain were prepared in 9 g/L saline to an inoculums density of 5×10^5 CFU/mL. To the wells, 100 μL of a standardised inoculum was added, but wells in rows seven and eight were maintained free from the extracts and reserpine. All experiments were performed in triplicate under aseptic conditions. The MIC was determined as mentioned above.

2.7. Plasmid conjugation inhibition assay

The plasmid conjugal transfer inhibition assay was performed by the broth mating method described by Rice and Bonomo (2007) with some modifications. Mating between the plasmid-containing donors strains *E. coli* K12J53 and the recipients *E. coli* ER1793 and JM 109 were performed in Luria–Bertani (LB) broth. The independent overnight cultures of the plasmids were inoculated into 5 mL of fresh LB broth and incubated overnight with shaking at 37 °C. Donor and recipient cultures were mixed 1:1 in 100 μL of LB with 100 mg/L of each of the extracts or 10 mg/L of standard novobiocin to a final volume of 200 μL and incubated overnight at 37 °C. For time course experiments, overnight cultures were mixed and mated in the presence of the drug or without drug (negative control) and incubated for the time periods stated. Only the bacteria that had successfully taken up the resistance gene and become resistant grew indicating plasmid conjugation.

2.8. Sulforhodamine B (SRB) assay

The method described by Houghton et al. (2007) was adopted to test for the toxicity of the plant extract using the HepG2 cell line. A volume of 100 μL cell suspension of optimum density was introduced into each well of a 96-well plate. Plant extract concentrations that ranged between 15.625 and 500 μg/mL were prepared in the culture medium, and 100 μL of each extract concentration was added to the cell monolayer and control well. The range of the concentrations was chosen to determine if the plant extract within this concentration could raise concern in the treatment of chronic diseases and if it is responsible for the MIC values against the tested bacterial strains. Monolayer cells were incubated with the drug extracts or standard doxorubicin (positive control) or without treatment (negative control) for 48 h, and cells were fixed with ice-cold TCA for 1 h at 4 °C. After incubation, the plates were washed five times in sterilised distilled water and then air-dried. A volume of 50 μL of sulforhodamine B (0.4%*w*/*v* in 1%*v*/v acetic acid) solution was added to each well of the dry 96-well plates and allowed to stain at room temperature for 30 min. The SRB unbound dye solution was removed after staining by washing the plates five times with 1% *v*/v acetic acid. The bound SRB dye was solubilised by adding 100 μL of 10 mM non-buffered Tris base (pH 10.5) to each well, shaking for 5 min. The plates were read in a 96-well plate reader at 492 nm. The mean background absorbance was subtracted, and the mean values of each drug concentration were calculated. The IC_{50} value of tested extract was then calculated.

2.9. HPLC/DAD analysis for phenolic compounds

The phenolic compounds in the fruit methanol extracts from RC were identified and characterised by HPLC-DAD according to the method described by Giner et al. (1993). All analyses were performed using a Hewlett-Packard Chemstation series 1100 chromatograph (Agilent, Palo Alto, CA, USA), coupled with a diode array detector. Ten milligrammes of the plant samples were sonicated in HPLC grade methanol (1 mL) for 30 min. The resultant mixtures were centrifuged at 10,000*g* for 10 min and the supernatants were used for the HPLC/DAD analysis. The resulting solutions were filtered through a 0.22 μm filter and 10 μL aliquots of the filtrates were injected onto the HPLC system. This system comprised a RP Nova Pack C18 column (300 × 3.9 mm) packed with 4 μm particles and a pre-column containing the same packing material. The columns were eluted in two solvent systems: Solvent A (water/phosphoric acid, 0.1%) and solvent B (methanol). The solvent gradient was composed of A (75–0%) and B (25–100%) for 20 min, then 100% B for 4 min, then again at the initial conditions (75% A and 25% B) for 10 min. A flow rate of 1.0 mL/min was used at 30 °C. The UV spectral data for all peaks of different compounds were accumulated in 285 nm and recorded using DAD. These compounds were identified by comparison of their retention times and spectra of each peak with those of known standards analysed in the same conditions.

2.10. Statistics

Data analysis was done with Microsoft Excel to obtain descriptive statistics. Means were separated by the Duncan multiple test using SAS. The different levels of significance within the separated groups were analysed using one way analysis of variance (ANOVA). Values were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Total phenolic content (TPC)

The TPC of the RC extract was expressed as tannic acid equivalents (TAE). The TPC value was found to be 60.86 ± 0.04 mg TAE/g for RC, extrapolated from the standard tannic acid curve: $Y = 0.1216\times$; $R^2 = 0.93651$. The data recorded for RC fruits was contrary to that of Montazeri et al. (2011) who reported 424.6 ± 1.8 mg/gallic acid equivalent (GAE)/g but corroborated with the findings (59.69 ± 0.89 mg GAE/g dry matter) of Ilbay et al. (2013). The observed inconsistency could be attributed to different climatic conditions, the standard equivalent used, solvent system and/or processing methods. Moreover, the Folin–Ciocalteu reagent (FCR) has been used to measure other compounds and hence may not give a concise amount of the phenolic compounds present. However, a HPLC/DAD chromatogram with detection at 360 nm showed the presence of five major peaks of phenolic compounds for the RC extract between a retention time of 2 and 25 min.

3.2. Antibacterial activity of RC extract

The methanol extract showed a mild to poor antibacterial activity with minimum inhibitory concentration (MIC) values ranging between 256 and >512 mg/L, whereas the standard antibiotics used as controls ranged from ≤0.03 to 0.5 mg/L as summarised in Table 1. According to Rios and Recio (2005), extracts can be classified as significant (MIC <100 mg/L), moderate (100 < MIC = 512 mg/L) or weak (MIC > 512 mg/L) depending on their respective activities against the corresponding pathogens. The same criterion was used elsewhere to classify the antimicrobial activities of plant extracts (Kuete et al., 2011; Fouotsa et al., 2013). This scheme was further supported by Gibbons (2008) and Van Vuuren (2008) who considered crude extracts with MICs greater than 512 mg/L, being poorly active and isolated compounds with MIC less than 10 mg/L as being interest to Pharma for drug formulation. Utilising this scheme, the RC extract demonstrated a

Table 1
Antibacterial activities of methanol crude extracts from *Rosa canina* fruits against MRSA and MDR bacterial strains.

MIC of plant extracts/antimicrobial agents (mg/L)					
Bacteria	RC	CIPR	Tet	Norx	Ery
Proteus mirabilis (P10830)	512	32			
Klebsiella pneumoniae (342)	>512	≤0.03			
Pseudomonas aeruginosa (10,662)	256	≤0.03			
Escherichia coli (NCTC 10418)	>512	≤0.06			
¶EMRSA15	256		0.25		
¶EMRSA16	512		0.25		
¶XU212	>512		16		
¶SA1199B	>512			32	
*ATCC25923	>512			0.5	
¶RN4220	>512				32

Norx stands for norfloxacin; Tet: tetracycline; CIPR; ciprofloxacin; and Ery: erythromycin, which was used as control. Bacteria culture without drug treatment was used as negative control. RC: *Rosa canina*.¶ denotes bacteria with efflux protein transporter, SA1199B possesses the NorA efflux transport protein, RN4220 (MsrA) XU212 (TetK), EMRSA15 and 16 (mecA) and* ATCC25923 a standard laboratory strain.

moderate antibacterial activity against EMRSA15 and EMRSA16 (Richardson and Reith, 1993; Cox et al., 1995), the major epidemic methicillin-resistant *S. aureus* strains occurring in UK hospital, as well as *Proteus spp.* P10830 and *P. aeruginosa* 10,662 with MIC values ranging between 256 and 512 mg/L, but was less active towards other MDR organisms (MIC > 512 mg/L). The poor antibacterial activity of RC fruit is consistent with the report of Yilmaz and Ercisli (2011) against certain bacteria. Although the MIC values obtained from extracts against the test organisms were much higher compared to the antimicrobial agents, this is likely to be due to the complex mixture of the phytochemicals in the sample.

3.3. Antibiotic resistance-modifying activity

The bacterial resistance-modifying activity of the RC extract in the presence of either norfloxacin or tetracycline against four bacterial strains (SA1199B, XU212, ATCC25923 and EMRSA16) is depicted in Table 2. The combination of RC extract with norfloxacin had no effect on the activities towards EMRSA16 and ATCC 25923. Interestingly, an increase in the MIC of norfloxacin was recorded against SA1199B, suggesting antagonism. Moreover, when tested against the tetracycline-resistant strain (XU212) expressing the TetK efflux transporter, a sixty-four-fold reduction in the MIC was noted at a sub-inhibitory concentration (SIC). When reserpine, a known efflux inhibitor, was combined with norfloxacin or tetracycline, a four-fold potentiation was observed towards EMRSA16, ATCC25923 and XU212 while eight fold reductions in the MIC of norfloxacin was displayed against SA1199B. The development of efflux pump inhibitors has been proposed to be used in conjunction with existing antibiotics to improve therapeutic efficacy and suppress the emergence of resistant variants that may arise during treatment (Gibbons et al., 2003). Although the plant extract showed promising resistance-modifying properties against XU212 and appeared to overcome the efflux mechanisms present, the isolation of their active principles could provide useful drug leads to reverse antibiotic resistance mechanisms in clinically relevant pathogens.

3.4. Plasmid conjugal inhibition activity

The search for inhibitors of plasmid-mediated resistance is currently gaining new ground, with only a small number of synthetic compounds that are identified (Hooper, 2005) but are unsuitable for clinical application due to their neurotoxicity. Consequently, recent studies have concentrated on natural products in order to identify natural inhibitors of bacterial conjugation (Lakshmi et al., 1987). Here, the ability of RC extract to reduce the transfer frequency of the plasmids TP114 and PKM 101 was evaluated. TP114 is a self-transmissible plasmid belonging to $IncI_2$ compatibility group. It is isolated from *E. coli* (Carattolia and Bertinia, 2005) that encodes a kanamycin resistance determinant. PKM 101 belongs to the IncN compatibility group, which is a variant of the parent plasmid R46 (Langer and Walker, 1981), and confers ampicillin resistance via β-lactamase production. Moderate inhibitory activity against the transfer of TP114 and PKM101 was demonstrated

Table 2
Minimal inhibitory concentrations (mg/L) of selected antimicrobial agents in the presence and absence of plant extracts (MIC/4) and reserpine (20 mg/L).

Treatment	EMRSA16 (mecA)	SA1199B (norA)	ATCC25923	XU212 (TetK)
Norfloxacin	128	32	0.5	–
Tetracycline	–	–	–	128
RC + Norfloxacin	128 (0)[a]	64	0.5 (0)[a]	–
Reserpine + Norfloxacin	128 (4)[a]	4 (8)[a]	0.125 (4)[a]	–
RC + Tetracycline	–	–	–	2 (64)[a]
Reserpine + Tetracycline	–	–	–	32 (4)[a]

RC, *Rosa canina*; Reserpine + Tetracycline; and Reserpine + Norfloxacin were used as positive controls.
[a] Potentiation values.

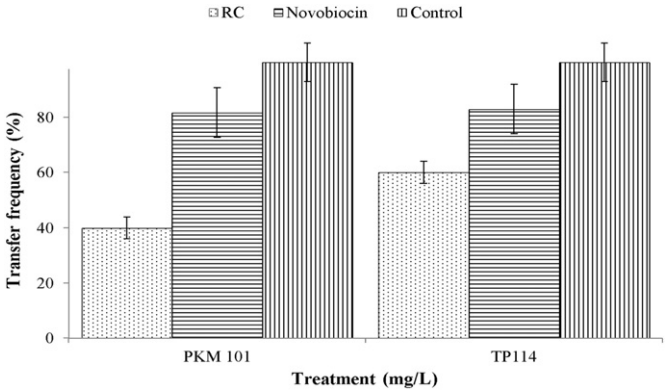

Fig. 1. Percentage (%) plasmid transfer frequency of PKM 101 and TP114 in the presence and absence of *Rosa canina* extracts (100 mg/L). $^{**}P < 0.05$ compared to novobiocin (10 mg/L) treatment. Novobiocin was used as positive control while plasmid culture without drug treatment was used as negative control.

by RC despite a weak antibacterial and resistance-modifying effect, signifying a different mechanism (Fig. 1). *R. canina* is notable for its phenolic constituents and this class of compound are typified by bharangin isolated from *Pygmacopremna herbacea* (Roxb), together with gossypetin and gossypin, known to cause the loss of the TP181 plasmid as well as a penicillinase-conferring plasmid in *E. coli* 46R41 (Skehan et al., 1990). Novobiocin was used as the reference control at 10 mg/L, having 81.7% and 83% plasmid conjugal transfer frequency for PKM 101 and TP114, respectively. Synergy in whole plant medicines or crude extract treatments is very commonly responsible in bioactive extracts, especially given that many folklore medicines are co-administered with one or more preparations (Hollander et al., 1998). In RC, the combinatorial effect of the crude extract may possibly facilitate transport across the bacterial cell wall, which could aid maximum absorption of the drug to assist the inhibition of the conjugation process. However, further bioassay-guided isolation of the active crude extract is recommended to identify individual compounds.

3.5. SRB cytotoxicity potential of the extracts

Numerous assays such as MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide sodium salt) and SRB (sulforhodamine B) have been employed to evaluate toxic effects of chemicals on the membrane integrity, mitochondrial function or protein synthesis in mammalian cells (Fricker and Buckley, 1996). The

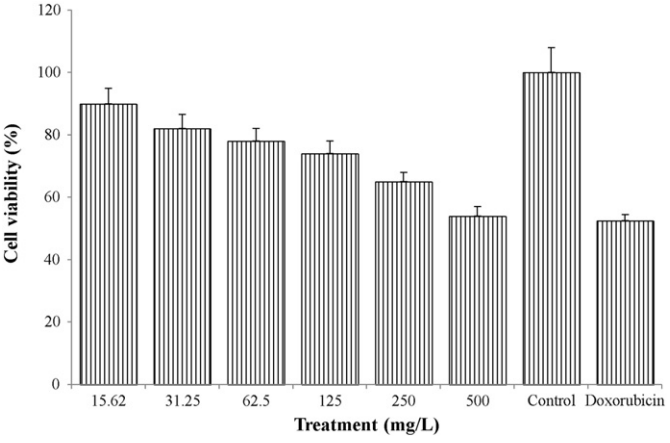

Fig. 2. Sulforhodamine B viability assay of HepG2 cells treated with methanol extracts from *Rosa canina* (RC) fruits. Confluent cultures were treated for 48 h prior to SRB assay. Data are expressed as % of control ± SD ($n = 6$). Doxorubicin (0.5 mg/L) was used as postive control and HepG2 cells with 1% DMSO was used as negative control.

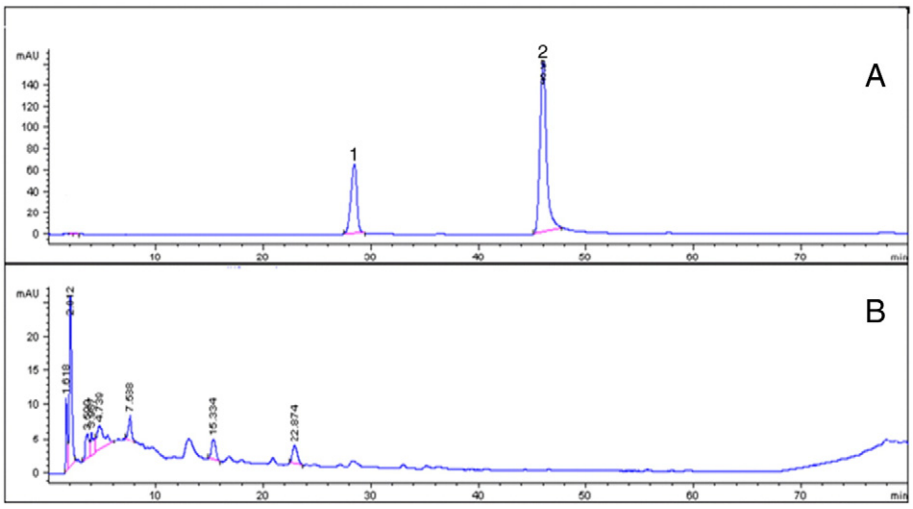

Fig. 3. HPLC-DAD chromatograms of standard phenolic acids (A) commonly found in plants, the peaks correspond to gallic acid (1), gallic acid (2) rutin. (B) The chromatograms of methanol extracts (1 mg/mL) from powdered *Rosa canina* fruits at 360 nm.

sulforhodamine B assay is considered the preferable approach based on the ability to bind to protein basic amino acid residues of trichloroacetic acid (TCA) fixed cells (Keepers et al., 1991). It is sensitive, simple, reproducible and more rapid than the formazan-based assays with better linearity without a time-sensitive measurement as regarding the MTT or XTT assays (Marie-Magdeleine et al., 2010). The effect of the RC (IC_{50} > 500 mg/L) extract on HepG2 cell viability was assessed using a colorimetric assay that evaluates cell number indirectly by staining total cellular protein with the SRB dye (Marie-Magdeleine et al., 2010) as presented in Fig. 2. The RC extracts did not impair lysosomes as compared with the standard doxorubicin (0.5 mg/L: 52.45%) a chemotherapeutic agent used for the treatment of many different cancer types. A similar observation was reported by Cagle et al. (2012) and thus supports the safe use of this botanical as complementary and alternative therapy in the prevention or treatment of certain health problems. The weak toxic effect could be linked to its hydrophilic nature inhibiting the interaction with the cell membranes where signal transduction pathways occur (Atmani et al., 2011).

3.6. HPLC-DAD analysis for phenolic fingerprints

The chromatogram at 360 nm showed the presence of 5 peaks of phenolic compounds with retention times (RT) between 4 and 25 min. Due to limited amount of the extract, the HPLC fingerprints were determined, which showed major peaks at the RTs (min) of 4.75, 7.538, 13.2, 15.334 and 22.874 as shown in Fig. 3. Here, none of the peaks identified based on their RT and UV/DAD absorption spectra corresponded with those of the standards (gallic acid and rutin). However, the peaks at 15.334 and 22.87 were somewhat similar to methyl gallate and quercetin-3-galactoside, respectively, as reported by Hvattum (2002). Other compounds identified by the same author are missing in this work, conceivably due to the difference in methodology and solvent system used. Further studies would be carried out to identify and quantify the major peaks of these compounds.

4. Conclusion

We have shown that while the RC extract had mild to poor antibacterial activity, the sample was able to potentiate tetracycline activity against tetracycline-resistant strain XU212 possessing a multidrug efflux mechanism and had a moderate inhibitory effect on plasmid conjugation. Encouragingly, the RC extract did not exhibit any significant toxic effect to mammalian cells and thus substantiate its safe use as a complementary and alternative therapy for the treatment or prevention of human diseases. Further research is ongoing to identify active ingredient and the mechanism of interaction with antibiotic against resistant bacterial strains.

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Author Contributions

S.O.O. and B.O.O. designed the experiments; S.O.O. and B.O.O. performed the experiment; S.O.O. and B.O.O. wrote the paper; S.G., P.S. and J.M.P. edited the paper; P.S., J.M.P. and S.G. provided the lab reagents; R.M.C. provided the funding; S.O.O. and B.O.O. analysed the data.

Conflicts of Interest

The authors declare no conflict of interest.

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