Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

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

Document heading

Antibacterial activities of some plant extracts alone and in combination with different antimicrobials against multidrug-resistant *Pseudomonas aeruginosa* strains

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ARTICLE INFO

Article history:
Received 21 January 2010
Received in revised form 7 March 2010
Accepted 10 March 2010
Available online 20 April 2010

Keywords:
Synergism
Rhus coriaria
Sacropoterium spinosum
Rosa damascena
Medicinal plants
Antimicrobial agents
Pseudomonas aeruginosa
Palestine

ABSTRACT

Objective: To evaluate the possible *in vitro* interaction between ethanolic extracts of *Rhus coriaria* (*R. coriaria*) (seed), *Sacropoterium spinosum* (*S. spinosum*) (seed), *Rosa damascena* (*R. damascene*) (flower) and certain known antimicrobial drugs including oxytetracycline HCl, penicillin G, cephalexin, sulfadimethoxine as sodium, and enrofloxacin. This synergy study was carried out against 3 clinical strains of multidrug-resistant *Pseudomonas aeruginosa* (*P. aeruginosa*). **Methods:** Evaluation of synergy interaction between plant extracts and antimicrobial agents was carried out using microdilution method. **Results:** The results of this study showed that there is a decrease in the MIC in case of combination of ethanolic plant extracts and test antimicrobial agents. The most interesting result was that the combination between *R. coriaria* and these antibiotics, showed a high decrease in minimum inhibitory concentration (MIC), and a strong bactericidal activity against these strains. **Conclusions:** These results may indicate that combinations between *R. coriaria* extract and these antibiotics could be useful in fighting emerging drug-resistance *P. aeruginosa*, which may due to that *R. coriaria* extract contain natural inhibitors working by different mechanisms or inhibiting efflux pumps. Now we have experiments underway leading to the identification of the active molecules present in *R. coriaria*. Further, *in vivo* experiments are needed to confirm pseudomonal protection.

1. Introduction

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases, appearance of undesirable side effects of certain antibiotics, as well as the increasing development of resistance to the antibiotics in current clinical use[1]. Therefore, actions must be taken to control the use of antibiotic, to better understand the genetic mechanisms of resistance, and to continue studies of developing new drugs. There are different approaches to cure and control the infection caused by the multidrug-resistant (MDR) strains of bacteria, one of which is by isolation of active phytochemicals that can help to prevent the spread of infection. Another method is to formulate new synergistic combinations using different commercially available antibiotics, or to combine an antibiotic with active phytochemicals that have antimicrobial properties. Several *in vitro* studies have reported synergistic effects with significant reduction in the minimum inhibitory concentrations (MIC) of the antibiotics, resulting from the combination of different antibiotics with different crude plant extracts against *Staphylococcus aureus (S. aureus)* strains[2-7], and emerge as the real sources of potential resistance modifying agents[8,9]. In addition to that, synergistic effects have been reported against Gram-negative bacteria [10-15]. The ability of plant extracts to potentiate antibiotics has not been well explained. It is predicted that inhibition of drug efflux, and alternative mechanisms of action could be responsible for the synergistic interactions between plant extracts and antibiotics [16,17].

Pseudomonas aeruginosa (P. aeruginosa) causes nosocomial infections as a result of its ubiquitous nature,

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ability to survive in moist environments, and resistance to many antibiotics and antiseptics. A main problem is the emergence of multidrug-resistant *P. aeruginosa* strains resistant to different antimicrobial agent classes. Infections caused by this microorganism are often severe, life threatening and difficult to be treated because of the high frequency of antibiotic resistance during therapy[18]. This high degree multidrug resistance may relate to the presence of antibiotic efflux systems which provide resistance to multiple antimicrobial agents[19].

There is little data on synergy between extracts of *Rhus coriaria (R. coriaria), Sacropoterium spinosum (S. spinosum), Rosa damascena (R. damascena)* and antibiotics[7,20]. The purpose of the present work was to establish synergy between ethanolic plant extracts of *Rhus coriaria (R. coriaria)* (seed), *Sacropoterium spinosum*(*S. spinosum)* (seed), and *Rosa damascena (R. damascena)* (flower) and certain known antimicrobial drugs such as oxytetracycline HCl, penicillin G, cephalexin, sulfadimethoxine as sodium, and enrofloxacin using microdilution method against 3 multidrug-resistant *P. aeruginosa* strains; thereby, throw light on the potential role of the phytochemicals in increasing the effectiveness of antibiotics.

2. Materials and methods

2.1. Plant material and extract preparation

The plant materials used in this study consisted of *R. coriaria* (seed), *S. spinosum* (seed), and *R. damascena* (flower), which are growing in Palestine. The fresh plant materials were dried in open air protected from direct exposure to sunlight. Approximately 30-50 g of dried plant materials was separately powdered, and extracted with 200-300 mL of 80% ethanol as describe previously[21]. Extracts were filtered through Whatman No. 2 filter paper under vacuum and concentrated to dryness at 37 ℃. Then, 100 mg of the dry residue was dissolved in 1 mL of sterile distilled water.

2.2. Bacterial strains

Three strains of multidrug-resistant *P. aeruginosa* were isolated from clinical samples (urine, surgical wound, and ear swab) have been used in this study. These strains were resistant to different antibiotics such as ampicillin, cefuroxime, cefotaxime, gentamicin, amikacin, erythromycin, clindamycin, ofloxacin, nalidixic acid, norfloxacin, ciprofloxacin and amoxicillin-clavulanic. In addition, *Bacillus subtilis* ATCC 6633 was included as a reference strain.

2.3. Antimicrobial drugs

Five drugs were evaluated for synergism assays including oxytetracycline HCl (10%), enrofloxacin (10%), sulfadimethoxine as sodium (40%), cephalexin (0.15%) and penicillin G (penicillin G procaine 900 000 and penicillin G sodium 300 000 U). All these drugs were produced by Jerusalem Pharmaceutical CO. Balsam branch except penicillin G was produced by Birzeit-Palestine Pharamaceutical Co. These drugs were diluted to a final concentration of 200 U/mL for penicillin G; 50 μg/mL for oxytetracycline HCl, cephalexin and enrofloxacin; and 100 μg/mL for sulfadimethoxine.

2.4. Antimicrobial tests

Minimum inhibitory concentration (MIC) of antibiotics as well as plant extracts were determined by the microdilution method as described by Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards, NCCLS) [22]. The antibiotic was serially diluted in Mueller Hinton broth. Plant extracts solution were separately added into wells in a final concentration of 1.5 mg/mL, then bacterial inoculum size of 105 CFU/mL was added to each well. Controls without plant extracts, without bacterial inoculum or with plant extracts only were also included in the experiment. Each plant extract was run in duplicate. The test plates were incubated at 37 ℃ for 18 h. The MIC was taken as the minimum concentration of the dilutions that inhibited the growth of the test microorganism. For bactericidal activity detection, 100 μL were spread on agar plate and incubated at 37 ℃ for 18 h.

3. Results

Our results showed that there is a decrease in the MIC in case of combination between ethanolic plant extracts of *R. coriaria*, *S. spinosum*, and *R. damascena* and different antimicrobial agents (oxytetracycline HCl, penicillin G, cephalexin, sulfadimethoxine as sodium, and enrofloxacin) against 3 test strains of *P. aeruginosa* using microdilution method. This implies that these plant extracts increased the antibacterial activity of the antibiotics against the test strains of *P. aeruginosa*, and showed synergistic interaction. The most interesting result is shown by the combination between *R. coriaria* and these antibiotics, which showed a high decrease in MIC, and a strong bactericidal activity against these strains. Minimum fold reduction of inhibitory concentration and change in MIC of antimicrobial agents are presented in Table 1.

4. Discussion

Many studies have shown that active efflux can be a mechanism of resistance for almost all antibiotics[23]. The majority of the efflux systems in bacteria are non-drug-specific proteins that can recognize and export a broad range of chemically and structurally unrelated compounds from bacteria without drug alteration or degradation[24]. Antibiotic efflux is a major mechanism of antibiotic resistance in *P. aeruginosa* due to Mex efflux proteins. Resistance to β-lactams and non- β-lactam antibiotics

Table 1

Minimum inhibitory concentration of antibiotics alone, plant extracts alone, and in combination against 3 clinical isolates of *P. aeruginosa* using microdilution method.

Antibiotic [a]/plant extrat	MIC (μ g/mL) [b]			Minimum fold reduction of inhibitory concentration
	Strain 1	Strain 2	Strain 3	
R. coriaria	3.125×10^3	$(3.125–1.563) \times 10^3$	1.563×10^3	
S. spinosum	6.5×10^3	$(12.5–6.5) \times 10^3$	12.5×10^3	
R. damascene	25×10^3	$(25–12.5) \times 10^3$	12.5×10^3	
ENR	>25	>25	>25	
R. coriaria + ENR	<0.012 2	<0.012 2	<0.012 2	>2 000
S. spinosum + ENR	1.563	0.390	0.195	>64
R. damascene + ENR	3.125	3.125	3.125	>64
OT	>25	>25	>25	
R. coriaria + OT	<0.024 4	<0.024 4	<0.024 4	>1 024
S. spinosum + OT	6.25	6.25	<0.024 4	>4
R. damascene + OT	6.25	6.25	6.25	>4
CL	>25	>25	>25	
R. coriaria + CL	<0.012 2	<0.012 2	<0.012 2	>2 000
S. spinosum +CL	0.048 8	0.195 0	1.563 0	>16
R. damascene CL	0.048 8	0.195 0	1.563 0	>16
P (Unit)	>100	>100	>100	
R. coriaria + P	<0.048 8	<0.048 8	<0.048 8	>2 000
S. spinosum + P	12.500	1.563	0.195	>8
R. damascene + P	12.500	3.125	1.563	>8
SDM	>50	>50	>50	
R. coriaria + SDM	<0.195 0–0.097 7	<0.024 4	<0.024 4	>256
S. spinosum + SDM	6.250 0	6.250 0	<0.024 4	>8
R. damascene + SDM	6.250	0.780	3.125	>8

[a]P, Penicillin G; CL, Cephalexin; SDM, sulfadimethoxine as sodium; ENR, Enrofloxacin; OT, Oxytetracycline HCl. [b]Concentration of penicillin G in units (U).

has been attributed to efflux by the MexAB–OprM pump[25]. Other Mex efflux proteins mediating multidrug resistance have also been identified in *P. aeruginosa*[26]. Efflux pump inhibitors combined with antibiotics strategy is an effective way to solve the problem caused by resistant bacteria[27]. The majority of plant derived antimicrobial compounds generally have higher MICs than bacterial or fungal produced antibiotics, thus limiting their therapeutic potential[28].

The plant extracts tested in this study, especially *R. coriaria* extract with oxytetracycline HCl, penicillin G, cephalexin, sulfadimethoxine as sodium, or enrofloxacin showed a powerful bactericidal activity to three test strains of *P. aeruginosa* and combinations have obvious synergistic activity. These results may indicate that *R. coriaria* extract contain natural inhibitors working by different mechanisms or inhibiting efflux pumps.

In conclusion, the results of this study were encouraging, although clinical controlled studies are needed to define the real efficacy and possible toxic effects *in vivo*. However, it is hard to predict synergistic effects *in vivo* on the basis of the presented *in vitro* evidence alone because it is difficult to estimate the *in vivo* concentration of active ingredients. Now we have experiments underway leading to the identification of the active molecules present in *R. coriaria*. Here we recommended the evaluation of the exact drug–plant ratio at which the interaction in maximal between the plant extract and antimicrobial drug. A wider study with increase in the number of drugs, increase number of clinical isolates, are also necessary in order to establish the mode of action against the *P. aeruginosa* isolates and the mechanism of synergy, which is fundamental to development of pharmacological agents to treat diseases by

P. aeruginosa using medicinal plants. Our results revealed that the combined use of plant extracts and antibiotics could be useful in fighting emerging drug-resistance problem and *in vivo* experiments are needed to confirm pseudomonal protection using these combinations.

References

[1]Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev* 1999; **12**: 564-82.

[2]Yam TS, Hamilton-Miller JM, Shah S. The effect of a component of tea (Camellia sinensis) on methicillin resistance, PBP2′ synthesis, and beta-lactamase production in *Staphylococcus aureus*. *J Antimicrob Chemother* 1998; **42**: 211-16.

[3]Aqil F, Khan MSA, Owais M, Ahmad I. Effect of certain bioactive plant extracts on clinical isolates of β-lactamase producing methicilin resistant *Staphylococcus aureus*. *J Basic Microbiol* 2005; **45**:106-14.

[4]Braga LC, Leite AAM, Xavier KGS, Takahashi JA, Bemquerer MP, Chartone-Souza E, et al. Synergic interaction between pomegranate extract and antibiotics against *Staphylococcus aureus*. *Can J Microbio* 2005; **51**: 541-7.

[5]Betoni JE, Mantovani RP, Barbosa LN, Di Stasi LC, Junior AF. Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. *Mem Inst Oswaldo Cruz* 2006; **101**: 387-90.

[6]Esimone CO, Iroha IR, Ibezim EC, Okeh CO, Okpana EM. *In vitro* evaluation of the interaction between tea extracts and penicillin G against *Staphylococcus aureus*. *Afr J Biotechnol* 2006; **5**: 1082-6.

[7]Adwan GM, Abu-Shanab BA, Adwan K. *In vitro* activity of certain drugs in combination with plant extracts against *Staphylococcus aureus* infections. *Pak J Med Sci* 2008; **24**(4): 541-4.

[8]Dickson RA, Houghton PJ, Hylands PJ, Gibbons S. Antimicrobial, resistance-modifying effects, antioxidant and free radical scavenging activities of *Mezoneuron benthamianum* Baill, *Securinega virosa* Roxb. and Wlld. and *Microglossa pyrifolia*. *Lam Phytother Res* 2006; **20**: 41-5.

[9]Sibanda T, Okok AI. The challenges of overcoming antibiotic resistance: plant extracts as potential sources of antimicrobial and resistance modifying agents. *Afr J Biotechnol* 2007; **6**: 2886-96.

[10]Nascimento GGF, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Braz J Microbiol* 2000; **31**:247-56.

[11]Tiwari RP, Bharti SK, Kaur HD, Dikshit RP, Hoondal GS, Synergistic antimicrobial activity of tea and antibiotics. *Indian J Med Res* 2005; **122**: 80-4.

[12]Ibezim EC, Esimone CO, Nnamani PO, Onyishi IV, Brown SA, Obodo CE. *In vitro* study of the interaction between some fluoroquinolones and extracts of kola nitida seed. *Afr J Biotechnol* 2006; **5**: 1781-4.

[13]Ahmad I, Aqil F. *In vitro* efficacy of bioactive extracts of 15 medicinal plants against ESβL-producing multidrug-resistant enteric bacteria. *Microbiol Res* 2007; **162**: 264-75.

[14]Ali NH, Kazmi SU, Faizi S. Activity of synergistic combination amoxy-cassia against Salmonella. *Pak J Pharm Sci* 2007; **20**: 140-5.

[15]Stefanovic O, Comic L, Stanojevic D, Solujic-Sukdolak S. Antibacterial activity of *Aegopodium podagraria* L. extracts and interaction between extracts and antibiotics. *Turk J Biol* 2009; **33**: 145-50.

[16]Zhao WH, Hu ZQ, Okubo S, Hara Y, Shimamura T. Mechanism of synergy between epigallochatechin gallate and β-Lactams against methicillin resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2001; **45**: 1737-42.

[17]Lewis K, Ausubel FM. Prospects for plant-derived antibacterials. *Nat Biotechnol* 2006; **24**:1504-7.

[18]Carmeli Y, Troillet N, Eliopoulos G, Samore MH. Emergence of antibiotic-resistant P*seudomonas aeruginosa*: comparison of risks associated with different antipseudomonal agents. *Antimicrob Agents Chemother* 1999; **43**: 1379-82.

[19]Poole K. Multidrug efflux pumps and antimicrobial resistance in *Pseudomonas aeruginosa* and related organisms. *J Mol Microbiol Biotechnol* 2001; **3**: 255-64.

[20]Abu-Shanab B, Adwan G, Jarrar N, Abu-Hijleh A, Adwan K. Antibacterial activity of four plant extracts used in Palestine in folkloric medicine against methicillin-resistant *Staphylococcus aureus*. *Turk J Biol* 2006; **30**: 195-8.

[21]Adwan G, Abu-Shanab B, Adwan K, Abu-Shanab F. Antibacterial effects of nutraceutical plants growing in Palestine on *Pseudomonas aeruginosa*. *Turk J Biol* 2006; **30**: 239-42.

[22]National Committee for Clinical Laboratory Standards (NCCLS). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, M7-A5*. Pennsylvania: NCCLS; 2000.

[23]Lin J, Michel LO, Zhang Q. Cme ABC functions as a multidrug efflux system in *Campylobacter jejuni*. *Antimicrob Agents Chemother* 2002; **46**: 2124-31.

[24]Kumar A, Schweizer HP. Bacterial resistance to antibiotics: active efflux and reduced uptake. *Adv Drug Deliv Rev* 2005; **57**: 1486-513.

[25]Ziha-Zarifi I, Llanes C, Kohler T, Pechere JC, Plesiat P. *In vivo* emergence of multidrug-resistant mutants of *Pseudomonas aeruginosa* overexpressing the active efflux system MexA-MexBOprM. *Antimicrob Agents Chemother* 1999; **43**: 287-91.

[26]Mine T, Morita Y, Kataoka A, Mizushima T, Tsuchiya T. Expression in *Escherichia coli* of a new multidrug efflux pump, MexXY from *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1999; **43**: 415- 7.

[27]Lomovskaya O, Bostian KA. Practical applications and feasibility of efflux pump inhibitors in the clinic - A vision for applied use. *Biochem Pharmacol* 2006; **7**: 910-8.

[28]Gibbons S. Anti-staphylococcal plant natural products. *Nat Prod Rep* 2004; **21**: 263-77.