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Antibacterial properties of compounds isolated from *Carpobrotus edulis*

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ABSTRACT

Several compounds isolated from the plant *Carpobrotus edulis* were evaluated for their activity against multidrug-resistant (MDR) bacteria and their efflux pump systems. Amongst the compounds isolated, six compounds were tested, namely uvaol, β-amyrin, oleanolic acid, catechin, epicatechin and monogalactosyldiacylglycerol. Oleanolic acid presented high antibacterial activity against a large number of bacterial strains. The triterpene uvaol was the most active compound for modulation of efflux activity by MDR Gram-positive strains.

1. Introduction

The plant *Carpobrotus edulis* is used in sub-Saharan Africa as traditional medicine for symptoms of tuberculosis (TB), throat infections, dysentery, diarrhoea, stomach ailments, burns, chilblains, mouth ulcers, throat infections, sinusitis, toothache, oral and vaginal thrust, etc. [1–5]. This succulent plant is very common in Portugal [6], the Mediterranean countries [5,7], California, USA [8] and South Africa [5].

Extracts of *C. edulis* were previously tested for in vitro and ex vivo activity against *Mycobacterium tuberculosis* [6,9]. Whereas the methanolic extract of *C. edulis* increased the killing activity of non-killing macrophages infected with *Staphylococcus aureus* [6], meticillin-resistant *S. aureus* (MRSA) [9] and *M. tuberculosis* [9], it had no activity against these organisms in vitro [9]. This same extract was also shown to reverse the resistance of mouse lymphoma cells that carry the human *mdr1* gene to chemotherapeutic agents [6], supposedly by inhibiting P-glycoprotein 1 (P-gp1) of the ABCC1 efflux pump of cancer cells [10]. In our previous study, the compounds uvaol, β-amyrin, oleanolic acid, catechin, epicatechin and monogalactosyldiacylglycerol (MGDG) isolated from *C. edulis* were evaluated for their anticancer activity and their ability to modulate efflux by the efflux pump ABCC1 [10]. Amongst these compounds, uvaol was demonstrated to have the greatest capacity to modulate efflux of the efflux pump substrates rhodamine 123 and ethidium bromide (EtBr) by cancer cells as well as acting synergistically with doxorubicin, the anticancer agent to which the cancer cells were initially resistant [10].

Phenolic compounds such as catechin, epicatechin and their derivatives, mainly found in green tea, have been shown to act as antioxidants and to provide protection from congestive heart failure [11], to exhibit anti-atherosclerotic [12] and anti-inflammatory properties [13] and to inhibit the secretion and production of gastric H^+, K^+ and ATPases [14] and have therefore been considered to act as chemopreventives [15]. Simple phenolic compounds such as epicatechin have also been shown to have antimicrobial properties via a mechanism that disrupts the cell envelope [16]. Catechin has also been identified as an antimicrobial agent with minimum inhibitory concentrations (MICs) between 2 mg/L and 78 mg/L against a wide range of Gram-negative bacteria and between 10 mg/L and 20 mg/L against Gram-positive bacteria [17]. Catechin has been described to potentiate the action of streptomycin against *M. tuberculosis* infection in mice and to decrease the incidence of pulmonary TB four-fold; this effect has been attributed to their inhibitory effects

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on fatty acid and mycolic acid biosynthesis [18]. These are important observations, since *C. edulis* juice is used in traditional medicine for symptoms of pulmonary TB infections. Other flavonoids have been shown to potentiate the action of isoniazid, supposedly by inhibition of the organism's efflux pump system [18], and to inhibit efflux by NorA, the main efflux pump of MRSA strains [19].

Triterpenes such as uvaol, oleanolic acid and β-amyrin have been shown to exhibit antimycobacterial activity against antibiotic-susceptible and -resistant strains of *M. tuberculosis* [20–24]. These compounds and others from oleane and ursane skeleton triterpenes have been shown to have anti-ulcer, anti-inflammatory, anti-allergic, antinociceptive, antitumour and antiviral properties [25,26]. In 1995, Liu [27] suggested the potential of non-toxic oleanolic acid for therapy of liver failure and systemic inflammatory disorders.

Because plants employed in traditional medicine that alleviate symptoms of infection have been proven to contain compounds with activity against the very organisms that promote symptoms of infection, compounds isolated from *C. edulis* were evaluated for their in vitro activity against common bacterial pathogens and for activity against the efflux pump system of these same pathogens.

2. Experimental procedures

2.1. Isolation procedures

Isolation of compounds from *C. edulis* employed in this study has been described in detail previously [10]. The following purified compounds were tested for activity against the efflux pump system of Gram-negative and Gram-positive bacterial pathogens: uvaol; β-amyrin; oleanolic acid; catechin; epicatechin; and MGDG.

2.2. Bacterial strains

Strains used in this study were: *S. aureus* ATCC 25923; an MRSA clinical strain; *S. aureus* HPV 107 and MRSA COL strains (generously provided by Prof. Dr H. de Lencastre); MRSA COL adapted to 1600 mg/L oxacillin (named MRSA COL_{OXA}) [28]; *Enterococcus faecalis* ATCC 29212; *Escherichia coli* K-12 AG100 strain [*argE3 thi-1 rpsL xyl mtl* Δ(*gal-uvrB*) supE44] (generously provided by Prof. Dr H. Nikaido) [29]; *E. coli* AG100 strain exposed to increasing concentrations of tetracycline [30] leading to an efflux pump-overexpressing strain (named *E. coli* $AG100_{TET8}$); *Salmonella enterica* serotype Enteritidis; *S.* Enteritidis 5408; *S.* Enteritidis 104_{CIP} and *S.* Enteritidis 5408_{CIP} (*Salmonella* strains provided by Prof. S. Fanning, adapted to 4 mg/L and 16 mg/L ciprofloxacin, respectively, and shown to have an overexpressed AcrB transporter [31]); and *M. tuberculosis* H37Rv strain that is susceptible to rifampicin, isoniazid, streptomycin and ethambutol.

2.3. Cultures

With the exception of any change specified during each protocol, *E. coli* strains were grown in Luria–Bertani (LB) broth and LB agar purchased in powder form from Merck (Darmstadt, Germany). *Salmonella*, *Enterobacter*, *Enterococcus* and *Staphylococcus* strains were grown in tryptone soya broth and tryptone soya agar, both purchased from Oxoid Ltd. (Basingstoke, UK) in powder form. *Mycobacterium tuberculosis* was grown in Middlebrook 7H9 broth media and Middlebrook 7H11 solid media purchased from Difco (Sparks, MD).

2.4. Determination of minimum inhibitory concentrations

MIC determination of the compounds used in the different assays was conducted by the broth microdilution method in Muller–Hinton broth (Oxoid Ltd.) according to Clinical and Laboratory Standards Institute (CLSI) recommendations [32]. The MIC, defined as the lowest concentration of compound that completely inhibits growth as evidenced by absence of turbidity in the medium, was determined after 16 h of incubation at 37 °C. Each compound was tested to a maximum concentration of 200 mg/L.

Susceptibility of *M. tuberculosis* H37Rv to the pure compounds was tested with a BACTEC 460TB system (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD) using BACTEC 12B medium supplemented with 0.1 mL of PANTA™ (Quilaban, Sintra, Portugal). Cultures were maintained at 37 °C until the first control reached a maximum growth index (GI) of 999 and the second control reached a GI of 30 [33]. An aliquot of each vial was plated on 7H11 agar medium and the plates were incubated at 37 °C for up to 4 weeks and were subjected to colony-forming unit counts. Details of the above procedures have been described previously [33,34].

2.5. Evaluation of the effects of compounds isolated from *Carpobrotus edulis* on the minimum inhibitory concentration of a given antibiotic to which the strain was made resistant

The MIC of each antibiotic to which the bacterium was resistant was first determined. The MIC assay for each antibiotic was then performed in the presence and absence of compounds isolated from *C. edulis* at final concentrations of 0.5× and 0.25× MIC, if any, or at 50 mg/L if there was no detectable MIC at concentrations as high as 200 mg/L.

2.6. Evaluation of efflux of ethidium bromide (EtBr) by a semi-automated EtBr method

The modulating activity of each compound on accumulation and efflux of EtBr was assessed by a semi-automated method using a Rotor-Gene 3000™ thermocycler with real-time analysis software (Corbett Research, Sydney, Australia) [35]. Briefly, bacteria were grown to an optical density at 600 nm (OD_{600}) of 0.6 and were washed twice in phosphate-buffered saline (PBS) with centrifugation at 3000 × *g*. Pellets were suspended in PBS to yield a final OD_{600} of 0.6 and 50 μL aliquots of this suspension were distributed into microtubes containing 50 μL of PBS (pH 7) containing 1 mg/L EtBr, with and without a source of metabolic energy (0.4% glucose) and a milligram quantity of each compound. The concentration of each compound evaluated for effects on the efflux system of a given bacterium was previously determined to have no in vitro activity against that bacterium. The effect on the efflux of EtBr by any compound was evidenced by an increase in the amount of fluorescence of EtBr accumulated in the cell above that of the compound-free controls. Details of the EtBr assay have been previously presented in detail [35,36].

3. Results

3.1. In vitro activity of the isolated compounds against bacteria

The MIC of each compound against pathogenic bacteria was determined in order to define the antibacterial activity of the isolated compounds. As shown in Table 1, the majority of the bacteria tested were resistant to >200 mg/L of each compound. Higher concentrations were not tested because higher concentrations of these compounds would not be expected to have clinical significance, as shown by other studies [37].

The compound oleanolic acid was very active against *E. faecalis*, with a MIC of 6.25 mg/L, and was moderately active against the *S. aureus* strains, which differed with respect to their antibiotic susceptibility pattern. The reference MRSA strain was more resistant to all of the compounds than MRSA COL_{OXA} and HPV 107 strains.